Marijuana and Madness

Second Edition

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Edited by

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Preface

Since the first edition of *Marijuana and Madness* in 2004, interest in the topic has continued to grow. For example, in the seven years since the first edition in 2004, almost as many papers have been published on this topic (400) as in the time period between 1962 and 2004 (462). This interest has been driven by a number of factors, including advances in our understanding of the brain cannabinoid system, and recognition that cannabinoids other than Δ^9-tetrahydrocannabinol (THC) may be important. The second edition of this book provides an opportunity to update the core chapters and to add several entirely new chapters that focus on these advances.

The book begins with an overview by Iversen on how cannabis works in the brain, followed by reviews of cannabioids other than THC (Mechoulam *et al.*) and of the cannabionoid system (Cascio and Pertwee). ElSohly and colleagues consider the evidence as to whether cannabis is increasing in potency, an important issue that has often been obscured by the debate about the legal status of cannabis. Then Hall and Degenhardt discuss the implications of the negative effects of cannabis on mental health services, health education and public policy.

These chapters set the scene for a detailed discussion of the most pressing issues in the field of cannabis and psychiatric disorders.

If exposure to cannabis being associated with negative health consequences is to have biological plausibility, there needs to be a biological mechanism/s to explain the association. Galve-Roperh reviews the evidence that the endocannabinoid system constitutes a novel extracellular signaling system involved in the regulation of nervous system formation, and the possible effects of perturbation of this system at crucial periods of brain development. Schneider presents the animal research showing that pubertal development, during which the endocannabinoid system appears to be very active, seems to represent the period most susceptible toward possible lasting negative cannabinoid effects. Solowij and Pesa review the evidence suggesting long term effects of cannabis on brain structure and neuropsychological function in humans. Skosnik proposes the cerebellum as a point of convergence through which alterations in the cannabinoid system may mediate processes involved in the generation of psychosis.

One of the vexing clinical conundrums is the discrepancy between the "benefits" of cannabis reported by users, and the negative consequences on the course and expression of schizophrenia observed by clinicians. Henquet *et al.* review the acute effects of cannabis and cannabinoids in people with psychotic illness, whereas Di Forti *et al.* explore genetic factors that may moderate the psychomometic effects of cannabis and Bhattacharyya and McGuire address the effects of cannabis on learning and psychosis. Turning to other psychiatric disorders, Silberberg *et al.* review the literature on cannabis and bipolar disorder, and Degenhardt *et al.* do likewise for depression.

The rest of the book concentrates on the impact of cannabis on schizophrenia, with a review of the evidence as to whether cannabis might be a causal factor in schizophrenia (Zammit *et al.*), studies of the cannabinoid system in schizophrenia (Sundram *et al.*, and Morrison), and of the impact of cannabis on the course of schizophrenia (Linszen *et al.*). The concluding chapters address the motives that maintain cannabis use among people with schizophrenia (Hides *et al.*) and treatment interventions for cannabis use in schizophrenia (James and Castle).

As editors, we are excited at the richness of the material provided to us by the contributors, all leaders in their field. We hope that readers will be likewise impressed at the progress that has been made in our understanding of the relationship between marijuana and madness.

Chapter 1

How cannabis works in the brain

Leslie Iversen

This book is about cannabis and mental illness. Crucial to our understanding of this complex area is an appreciation of how cannabis affects the brain. Important advances have been made in this regard over the last few years. As with morphine thirty years earlier, research on the psychopharmacology of a plant-derived drug led to the discovery of a naturally occurring cannabinoid system in the brain, the functions of which are only now beginning to be understood. This chapter reviews what is known about the interactions of cannabis with the cannabinoid system in the brain, and how the drug affects psychomotor, cognitive, perceptual and appetitive functions. There is also speculation on what brain mechanisms may underlie the intoxicant effects of cannabis, and a review of its addictive properties.

Cannabinoid receptors

Δ^9-Tetrahydrocannabinol (THC) is the principal active component in the complex mixture of cannabinoids present in extracts of the plant *Cannabis sativa*. The other cannibinoids are reviewed by Mechoulam and Hanus in Chapter 2 of this book, while the rapidly growing field of endocannabinoid research is reviewed by Cascio and Pertwee in Chapter 3. A series of synthetic cannabinoids, some of which are more potent and more water soluble than THC, is also available (Pertwee, 1999, 2006) (Figure 1.1). All of these compounds act as agonists at the cannabinoid CB1 cannabinoid receptor (Matsuda *et al*., 1990), which is the predominant receptor subtype expressed in the brain. A second cannabinoid receptor, CB2, is expressed mainly in peripheral tissues, principally in the immune system (Munro *et al*., 1993; Felder and Glass, 1998; Pertwee, 1999, 2006), although it is also expressed at lower levels in neurons and microglial cells in the brain, where it may be upregulated in conditions of inflammation or neurodegeneration (Onaivi *et al*., 2008; Palazuelos *et al*., 2009). Δ^9-Tetrahydrocannabinol and some of the synthetic cannabinoids act to some extent as agonists at the CB2 receptor. A series of synthetic drugs is also now available that act as selective agonists or antagonists at CB1 or CB2 receptors (D'Souza and Kosten, 2001; Pertwee, 2006); one of these compounds, rimonabant (SR141716A), which acts selectively to block CB1 receptors (Rinaldi-Carmona *et al*., 1994; Compton *et al*., 1996), has been widely used in studies of the actions of cannabinoids in the central nervous system (CNS). The availability of the synthetic cannabinoid agonists and antagonists has been supplemented also in recent years by the generation of genetically engineered strains of mice that do not express CB1 or CB2 receptors ("knockout mice").

There has been interest in the possibility that further cannabinoid receptors may exist. The most thoroughly characterized so far has been the G-protein coupled receptor, GPR55, discovered by genomic searches for proteins with homology to either CB1 or CB2 receptors (Pertwee, 2007; Ross, 2008). GPR55 has only 13–14% homology with CB1 or CB2, and levels of expression in the brain are about tenfold lower than those of CB1 (Ross, 2008). The first detailed description of the pharmacology of GPR55 indicated some unusual properties (Ryberg *et al*., 2007). Δ^9-Tetrahydrocannabinol acted as a highly efficacious agonist with nanomolar affinity, and the synthetic cannabinoid CP55 940 was also a potent agonist, but WIN55 212-2, another potent agonist at CB1 sites, was inactive. GPR55 has a distribution in the brain similar to that of the CB1 receptor, with the highest levels in striatum. However, not all reports have agreed that THC is an effective agonist at GPR55 (Ross, 2008), and it is not clear what role, if any, it plays in mediating the CNS effects of THC. Knockout mice lacking expression

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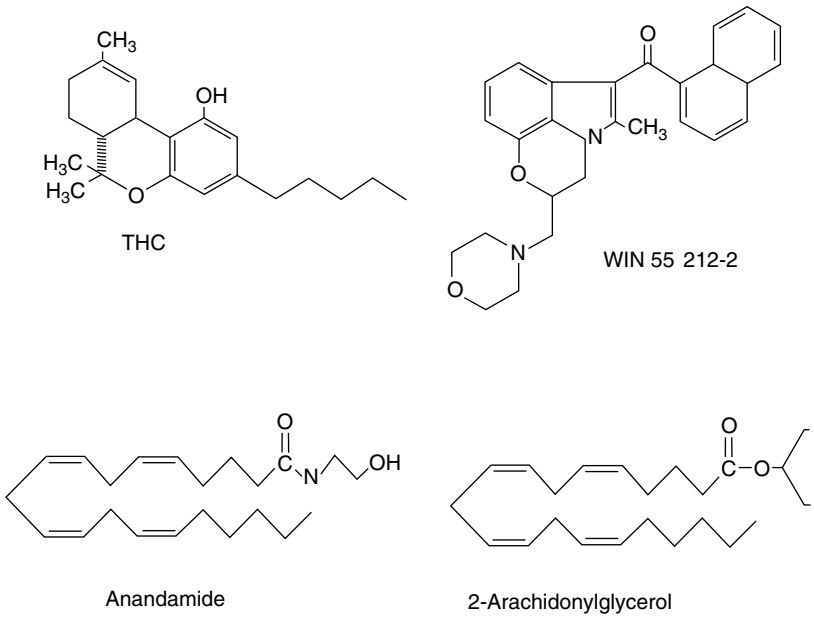

Figure 1.1. Chemical structures of THC (Δ^9-tetrahydrocannabinol), the synthetic CB1 receptor agonist WIN55 212-2 and the endocannabinoids. Reproduced with permission from Murray *et al.*, 2007.

of GPR55 appeared normal but failed to develop mechanical hyperalgesia in experimental models of inflammatory or neuropathic pain (Staton *et al.*, 2008); a role in sensory pain mechanisms is also suggested by the high levels of expression of GPR55 in primary sensory neurons (Laukner *et al.*, 2008).

Neuroanatomical distribution of CB1 receptors in the brain

The distribution of cannabinoid receptors was first mapped in rat brain in autoradiographic studies, using the radioligand [H^3]CP55 940, which binds with high affinity to CB1 sites (Herkenham *et al.*, 1991) (Figure 1.2). Antibodies that target the C-terminal or N-terminal regions of the CB1 receptor protein have also been used for immunohistochemical mapping studies (Ergotová *et al.*, 1998; Pettit *et al.*, 1998; Ergotová and Elphick, 2000). Immunohistochemistry provides a superior degree of spatial resolution than autoradiography, but the overall pattern of distribution of CB1 receptors revealed by the two approaches is very similar (Elphick and Ergotová, 2001). Another way of imaging CB1 receptors in the intact brain is to use selective radioligands and positron emission tomography (PET). Burns *et al.* (2007) described [^{18}F]MK-9470 as a suitable PET ligand, and showed that pretreatment with the CB1-selective inverse agonist MK-0364 led to a dose-dependent reduction in radioligand binding in both monkey and human brain. Other potential PET ligands have been described (Finnema *et al.*, 2009), opening a new way of using brain imaging to study CB1 pharmacology in the intact brain.

The mapping studies in rat brain showed that CB1 receptors are mainly localized to axons and nerve terminals, and are largely absent from the neuronal soma or dendrites. The finding that cannabinoid receptors are predominantly presynaptic rather than postsynaptic is consistent with the postulated role of cannabinoids in modulating neurotransmitter release (see below). The presynaptic location of the CB1 receptor can be confirmed by immunocytochemical studies at the electron microscope level. For example, Oropeza *et al.* (2007) examined the ultrastructural localization of CB1 receptors and the enzyme dopamine beta-hydroxylase (DBH) in rat frontal cortex. Using DBH as a marker for noradrenergic nerve terminals, they found that one-third of the CB1-positive terminals were also DBH positive, although not all noradrenergic terminals showed such coexistence. Pickel *et al.* (2006) studied the ultrastructural localization of CB1 receptors and dopamine D_2 receptors in rat nucleus accumbens and found many examples of overlapping distributions, with CB1-positive terminals contacting D_2-positive dendrites or soma.

In both animals and humans the cerebral cortex, particularly the frontal regions, contains high densities of CB1 receptors. There are also very high densities in

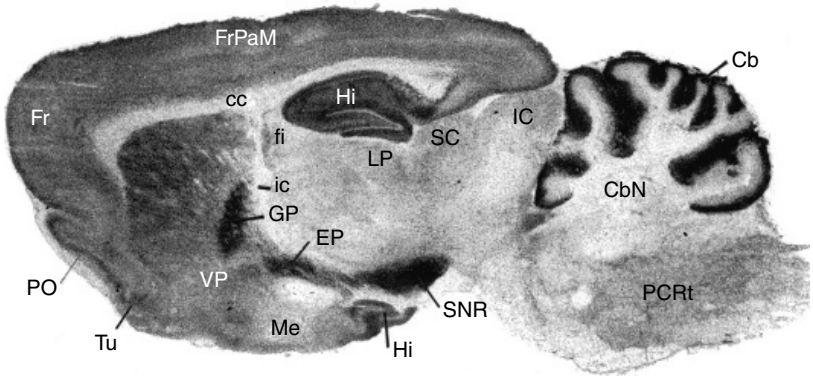

Figure 1.2. Distribution of cannabinoid CB1 receptors in rat brain revealed by an autoradiograph of the binding of radioactively labeled CP55 940 (a high-affinity agonist ligand) to a sagittal brain section. The brain regions labeled are: Cb, cerebellum; CbN, deep cerebellar nucleus; cc, corpus callosum; EP, entopeduncular nucleus; fi, fimbria hippocampus; Fr, frontal cortex; FrPaM, frontoparietal cortex motor area; GP, globus pallidus; Hi, hippocampus; IC, inferior colliculus; LP, lateral posterior thamalmus; Me, medial amygdaloid nucleus; PO, primary olfactory cortex; PCRt, parvocellular reticular nucleus; SNR, substantia nigra reticulate; Tu, olfactory tubercle; VP, ventroposterior thamalus. Photograph kindly supplied by Miles Herkenham, National Institute of Mental Health, USA.

the basal ganglia and in the cerebellum (Figure 1.2). In the limbic forebrain CB1 receptors are found particularly in the hypothalamus and in the anterior cingulate cortex. The hippocampus is also rich in CB1 receptors. The relative absence of cannabinoid receptors from brainstem nuclei may account for the low toxicity of cannabinoids when given in overdose. A meta-analysis of more than 100 autoradiographic, immunohistochemical and in-situ hybridization studies showed that the distribution of CB1 receptors in the human brain showed denser expression in cognitive regions (cerebral cortex) compared with the rat brain, in which CB1 receptor expression was relatively richer in movement-associated areas (cerebellum, caudate-putamen) (McPartland *et al*., 2007).

Effects of cannabinoids on synaptic function

Regulation of neurotransmitter release

The presynaptic localization of CB1 receptors suggests a role for cannabinoids in modulating the release of neurotransmitters from axon terminals, and this has been confirmed by a substantial body of experimental data. Early reports (Gill *et al*., 1970; Roth, 1978) showed that THC-inhibited acetylcholine release from electrically stimulated guinea-pig ileum. Similar inhibitory effects of THC and other cannabinoids on the release of a variety of neurotransmitters from CNS neurons have been observed in many subsequent studies (Schlicker and Kathmann, 2001). The neurotransmitters involved include L-glutamate, gamma-aminobutyric acid (GABA), noradrenaline, dopamine, 5-hydroxytryptamine (5-HT) and acetylcholine. The brain regions most often studied in vitro, usually in tissue slice preparations, have been cerebellum, hippocampus and neocortex. Neurotransmitter release has been studied directly in superfused preparations, or indirectly by measuring postsynaptic currents. Although most of these studies involved rat or mouse brain, a few studies have shown similar results using human-brain tissue (Katona *et al*., 2000; Schlicker and Kathmann, 2001). Because THC is only poorly water soluble, the more soluble synthetic CB1 receptor agonists WIN55 212–3, HU210 or CP55 940 were most commonly used in these in-vitro studies. The specificity of the cannabinoid effects were confirmed by demonstrating that the inhibitory effects of the agonists were completely blocked by the CB1-selective antagonist, rimonabant. Not all presynaptic actions of CB1 agonists are inhibitory. In rat frontal cortex, for example, activation of CB1 receptors stimulates noradrenaline release (Oropeza *et al*., 2007).

Endogenous cannabinoids act as retrograde signal molecules at synapses

Important new insights into the physiological role of cannabinoids emerged from neurophysiological studies in 2001. A phenomenon known as "depolarization-induced suppression of inhibition" (DSI) has been known to neurophysiologists for some years (Alger and Pitler, 1995). It is a form of fast retrograde signaling

from postsynaptic neurons back to inhibitory cells that innervate them, and is particularly prominent in the hippocampus and cerebellum. Three properties of DSI were suggested to Wilson and Nicoll (2001) that a cannabinoid mechanism might be involved. First, DSI, like endocannabinoid synthesis, requires Ca^{2+} influx into the postsynaptic neuron (Lenz *et al*., 1998). Second, DSI is probably presynaptic because the sensitivity of the postsynaptic cell to GABA is unaffected (Pitler and Alger, 1992). Finally, DSI is blocked by pertussin toxin, which interacts with the $G_{i/o}$ protein to which the CB1 receptor is coupled (Pitler and Alger, 1994). Wilson and Nicoll (2001) used slice preparations of rat hippocampus and induced DSI by brief depolarization of CA1-pyramidal neurons. They found that DSI was completely blocked by the cannabinoid CB1 receptor antagonists, AM-251 or rimonabant. Depolarization-induced suppression of inhibition could be mimicked by application of the CB1 receptor agonist WIN55 212-2, but the continued presence of the agonist prevented DSI by occlusion. Wilson and Nicoll (2001) were also able to show by recording from pairs of nearby CA1 neurons that depolarizing one of these neurons caused DSI to spread and affect adjacent neurons up to 20 μm away. They suggested that the small, lipid-soluble, freely diffusible endocannabinoids act as retrograde synaptic signals that can affect axon terminals in a sphere of influence some 40 μm in diameter.

Ohno-Shosaku *et al*. (2001) came to a similar conclusion using a different experimental paradigm. Recording from pairs of cultured hippocampal neurons with inhibitory synaptic connections, they found that depolarization of the postsynaptic neurons led to DSI in approximately two-thirds of the neuron pairs, and showed that this was due to inhibition of GABA release. Those that exhibited DSI, but not the others, proved to be sensitive to the CB1 receptor agonist WIN55 212-2, which mimicked the inhibitory effect of GABA on DSI. Both DSI and the cannabinoid effect could be blocked by the CB1 receptor antagonists, AM-281 or rimonabant.

Further support for the conclusion that a cannabinoid-mediated mechanism underlies DSI came from Varma *et al*. (2001), who found that DSI was completely absent in hippocampal slices prepared from CB1 receptor-knockout mice (Ledent *et al*., 1999).

Retrograde signaling by endocannabinoids is not restricted to the inhibitory inputs to postsynaptic neurons. Kreitzer and Regehr (2001) showed that depolarization of rat cerebellar Purkinje cells led to a transient inhibition of excitatory inputs from parallel-fiber and climbing-fiber inputs, a phenomenon described as "depolarization-induced suppression of excitation," or DSE. They found that DSE was triggered by calcium influx into the Purkinje cells, and it could be completely blocked by the CB1 antagonist AM-251, and mimicked and occluded by the CB1 receptor agonist WIN55 212-2. Kreitzer and Regehr (2001) went on to show that inhibitory inputs to rat-cerebellar Purkinje cells from basket cells and stellate cells were subject to DSI, and that this was also blocked by AM-251 and occluded by WIN55 212-2. The DSE phenomenon in the cerebellum is also linked to mGlu receptors. Maejima *et al*. (2001) reported that mGlu agonists acting on mouse Purkinje cells mimicked DSE, and the effects could be blocked by CB1 antagonists.

These findings suggest that endocannabinoids are involved in the rapid modulation of synaptic transmission in CNS by a retrograde signaling system capable of causing inhibitory effects on both excitatory and inhibitory neurotransmitter release that persist for tens of seconds. Retrograde cannabinoid signaling has been likened to a "molecular coincidence detector" activated by the temporal and spatial convergence of multiple neurochemical signals (Gerdeman *et al*., 2002). Principal output neurons such as Purkinje cells in the cerebellum, pyramidal cells in the hippocampus and cortex, medium spiny cells in the striatum, and dopaminergic neurons in the mid-brain fine tune their excitatory and inhibitory synaptic inputs in part by releasing endocannabinoids (Figure 1.3A) (see Cascio and Pertwee, Chapter 3).

The mechanisms underlying synaptic plasticity have been studied more intensely in the hippocampus than in any other brain region (see above). In particular the electrophysiological phenomena of long-term potentiation (LTP) and long-term depression (LTD) are thought to be involved in memory formation at glutamatergic synapses in the hippocampus. A number of studies have shown that exogenously administered cannabinoids inhibit the induction of both LTP and LTD in the hippocampus (for review see Elphick and Egertová, 2001). Exogenously administered cannabinoids appear to work by reducing glutamate release below the level needed to activate N-methyl D-aspartate (NMDA) receptors, a requirement for LTP and LTD (Shen *et al*., 1996; Misner and Sullivan, 1999). Although the actions of cannabinoids in reducing GABA release from hippocampal interneurons

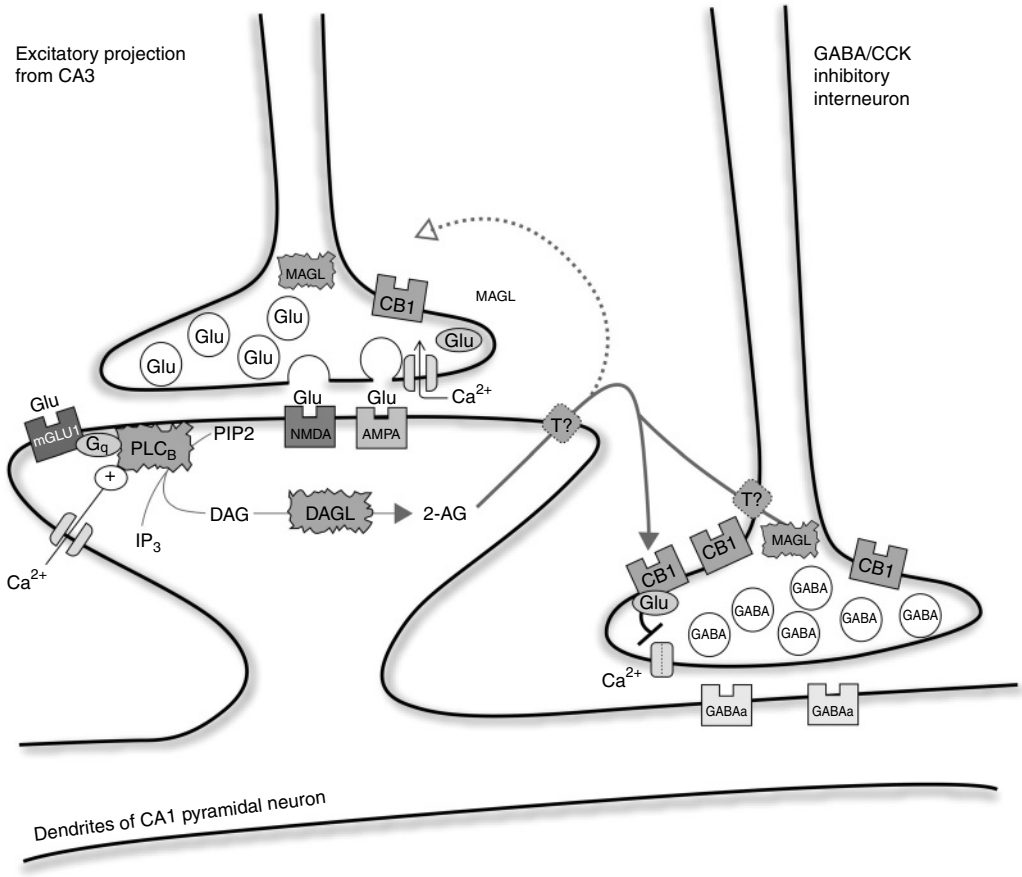

Figure 1.3. (A) Cannabinoids "fine tune" neurotransmission in the hippocampus. In area CA1 of the hippocampus, activation of pyramidal neurons stimulates the synthesis and release of the endocannabinoid 2-AG, which acts at CB1 receptors on adjacent GABAergic nerve terminals to suppress GABA release. Synthesis of 2-AG is driven by stimulation of metabotropic glutamate receptors (mGlu1) or by Ca^{2+} entry via voltage-gated channels. In CA1, locally released 2-AG depresses GABAergic inhibitory tone, thereby facilitating long-term potentiation at adjacent glutamatergic excitatory synapses. CB1 receptors present on glutamatergic terminals may serve to limit the extent of 2-AG synthesis and release, and prevent excessive excitation leading to seizures. **(B)** By contrast, exogenous cannabinoids, such as THC, disrupt the endocannabinoid system. Δ^9-Tetrahydrocannabinol occupies and activates CB1 receptors indiscriminately, and inhibits long-term potentiation of hippocampal synapses, leading to impairments in learning and memory. 2-AG, 2-arachidonylglycerol; AMPA, 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid; CCK, cholecystokinin; DAG, diacylglycerol; DAGL, sn-1 diacylglycerol lipase; GABA, gamma-aminobutyric acid; $G_{i/o}$ and Gq, G-proteins; IP3, inositol triphosphate; LTP, long-term potentiation; MAGL, monoacylglycerol lipase; mGlu1, metabotropic glutamate receptors; NMDA, N-methyl-D-aspartate receptor; PIP2, phosphatidyl inositol; PLC-β, phospholipase C-β; THC, Δ^9- tetrahydrocannabinol.

might have been expected to increase the level of excitability of hippocampal pyramidal cells, it seems that a reduction in glutamate release predominates in response to exogenous cannabinoids. The administration of exogenous cannabinoids is, of course, wholly unphysiological and cannot mimic the effects of endocabinnoids that are released in discrete local regions in response to particular patterns of afferent inputs (Figure 1.3B). CB1 receptors are capable of regulating both inhibitory and excitatory neurotransmitter release in the hippocampus, and are thus capable of subtle control of synaptic plasticity. The CB1-containing GABAergic interneurons are thought to control oscillatory electrical activity in the hippocampus in the theta and gamma frequencies, which plays a role in synchronizing pyramidal cell activity (Hoffman and Lupica, 2000; Chevaleyre *et al.*, 2006). CB1 agonists decrease the power of such oscillations in hippocampal slices (Hájos *et al.*, 2000) and may thus influence the synchronous activity of pyramidal cells. The physiological importance of cannabinoid-mediated DSI may be to decrease GABAergic inhibition of these cells and thus facilitate learning when hippocampal inputs are active (Wilson and Nicoll, 2001).

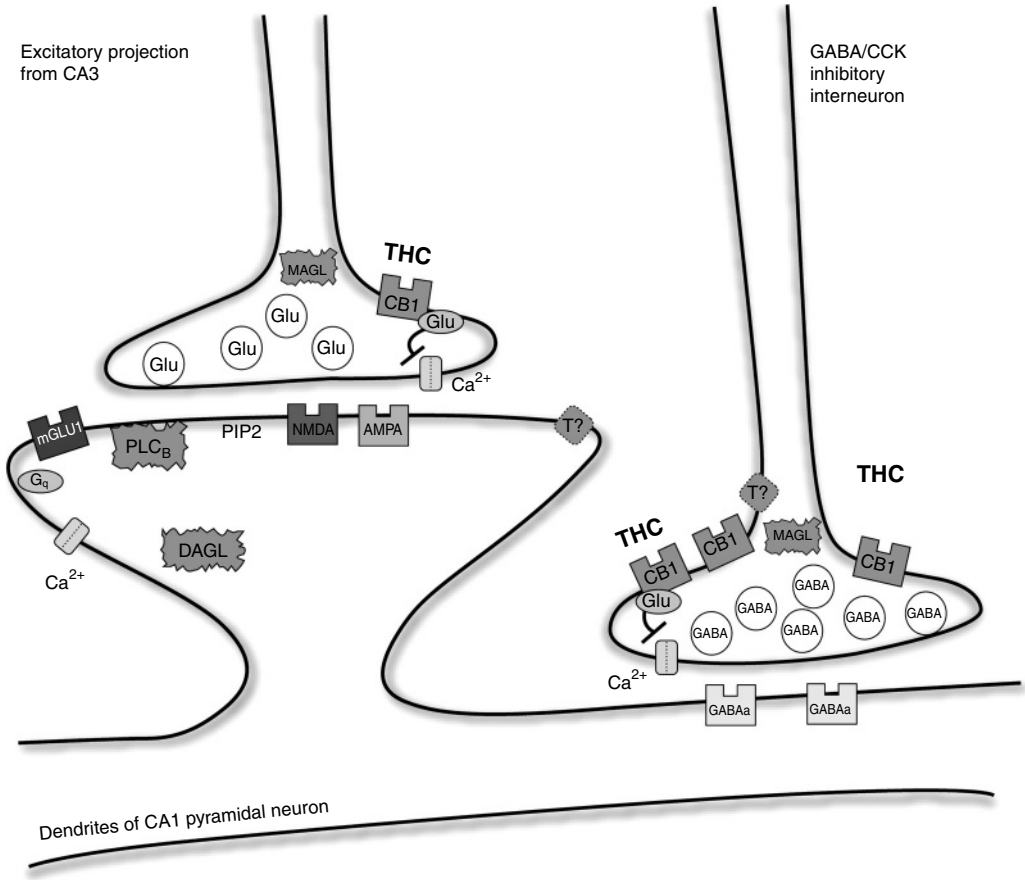

Figure 1.3. (*cont.*)

Effects of cannabinoids on psychomotor control

CB1 receptors are expressed at particularly high densities in the basal ganglia and cerebellum so it is not surprising that cannabinoids have complex effects on psychomotor function (reviewed by Rodriguez de Fonseca *et al*., 1998). One of the earliest reports of the effects of cannabis extracts in experimental animals described the awkward swaying and rolling gait caused by the drug in dogs, with periods of intense activity provoked by tactile or auditory stimuli, and followed eventually by catalepsy and sleep (Dixon, 1899). In rodents cannabinoids tend to have a triphasic effect. Thus, in rats, low doses of THC (0.2 mg/kg) decreased locomotor activity, while higher doses (1–2 mg/kg) stimulated movements, and catalepsy emerged at doses of 2.5 mg/kg (Saňudo-Peňa *et al*., 1999). Similarly in mice, Adams and Martin (1996) described a "popcorn effect" in animals treated with THC. Groups of mice were sedated by the drug, but jumped in response to auditory or tactile stimuli, as they fell into other animals these in turn jumped, resembling corn popping in a popcorn machine. Interestingly the CB1 receptor antagonist rimonabant stimulated locomotor activity in mice, suggesting that there is tonic activity in the endocannabinoid system that contributes to the control of spontaneous levels of activity (Compton *et al*., 1996).

These effects of cannabinoids may be because, in part, of actions at cerebellar or striatal receptors. Patel and Hillard (2001) used tests of specific cerebellar functions to show that cannabinoids caused increased gait width and the number of slips on a bar cross test. DeSanty and Dar (2001) observed rotorod impairments in mice after direct injection of synthetic cannabinoids into the cerebellum. These defects were no longer seen in

animals pretreated with cerebellar injections of an antisense oligonucleotide, directed to a sequence in the CB1 receptor to locally suppress CB1 receptor expression. Local cerebellar microinjection of the CB1-antagonist rimonabant into mice treated chronically with the agonist WIN55 212-2 precipitated severe withdrawal signs, including wet dog shakes, body tremor, paw tremor, piloerection, mastication, genital licks and sniffing. Microinjection of rimonabant into the striatum of these animals, however, elicited no signs of abstinence. This seems to show that cerebellar CB1 receptors play a key role in this series of behaviors (Castaňé *et al*., 2004).

In human subjects it is also possible to demonstrate that cannabis causes impaired performance in test of balance (Greenberg *et al*., 1994), or in tests that require fine psychomotor control, for example tracking a moving point of light on a screen (Manno *et al*., 1970). Human cannabis users may also seek isolation and remain immobile for long periods, a condition resembling catalepsy in animals. Monory *et al*. (2007) found that the selective deletion of CB1 receptor expression from striatal neurons and a subpopulation of cortical glutamatergic neurons in conditional mutant mice blocked the cataleptic effects of THC.

A number of authors have attempted to combine what is known of the neuroanatomical distribution of the cannabinoid system, and the results of behavioral and electrophysiological studies, to speculate on the mechanisms underlying cannabinoid modulation of psychomotor function (Brievogel and Childers, 1998; Sañudo-Peña *et al*., 1999; Giuffrida and Piomelli, 2000; Elphick and Egertová, 2001). The CB1 receptor is expressed particularly by the main output cells of the striatum, GABAergic medium-spiny projection neurons. The receptor is abundant in regions containing the axon terminals of these cells (globus pallidus, entopeduncular nucleus and substantia nigra reticulata, and in axon collaterals feeding back to medium-spiny projection neurons in striatum).

CB1 receptors are also abundant on the terminals of glutamatergic projection neurons from the subthalamic nucleus to globus pallidus, entopeduncular nucleus and substantia nigra reticulata. Cannabinoids might thus be expected to inhibit GABA release in striatum, and GABA and glutamate release in the other nuclei. High-frequency activation of cortical inputs to medium-spiny neurons in the striatum leads to LTD of excitatory synaptic transmission. This form of synaptic plasticity appears to be dependent on cannabinoid signaling; it is absent in CB1 receptor-knockout mice and enhanced by anandamide loading (Gerdeman *et al*., 2002). Studies of LTD in the lateral amygdala of the mouse brain found that it was abolished in conditional mutants lacking CB1 receptor expression in GABAergic neurons, but remained intact in mutants where CB1 expression was lacking in forebrain principal neurons (Azad *et al*., 2008).

Effects of cannabinoids on memory

One of the well-established effects of acute intoxication with cannabis in humans is an impairment of short-term memory. The extensive literature on human studies is reviewed by Jones (1978), Miller and Branconnier (1983), Solowij (1998) and Earleywine (2002) (see also Chapter 9). Many studies have shown significant effects on short-term memory, particularly when tests were used that depend heavily on attention (Abel, 1971; Mendelson *et al*., 1976). Animal studies have also found that THC, synthetic cannabinoids and anandamide cause deficits in short-term memory in spatial learning tasks (for review see Hampson and Deadwyler, 1999). These include delayed matching or non-matching tests in rodents (Hampson and Deadwyler, 1999; Mallet and Beninger, 1998), performance in a radial arm maze (Stiglick and Kalant, 1985; Lichtman and Martin, 1996) and a fixed-ratio, food-acquisition task in squirrel monkeys (Nakamura-Palacios *et al*, 2000). The effects of both cannabinoids (Lichtman and Martin 1996) and anandamide (Mallet and Beninger, 1998) were reversed by rimonabant, indicating that they are mediated by the CB1 receptor.

A likely site for these effects is the hippocampus. Hampson and Deadwyler (1999) claimed that the effects of treatment of rats with cannabinoids on short-term memory in a delayed non-matching to sample test were equivalent to the effects seen after surgical removal of the hippocampus. In each case the animals were unable to segregate information between trials in the task because of disruptions to the processing of sensory information in hippocampal circuits. CB1 receptors are expressed at high densities in the rat hippocampus. They are particularly abundant on the terminals of a subset of GABAergic basket cell interneurons, which also contain the neuropeptide cholecystokinin (Katona *et al*., 1999), and this is also the case in the human hippocampus (Katona *et al*., 2000). These are presumably the GABAergic neurons involved in the endocannabinoid-mediated DSI phenomenon described above. The terminals of these cells

surround large pyramidal-neuron somata in the CA1–CA4 fields. In addition CB1 receptors are expressed, at a lower level, in the glutamatergic pyramidal cells and their terminals. Cannabinoids can thus inhibit both the release of GABA and glutamate in hippocampal circuits, as discussed previously.

A novel role for cannabinoids in the extinction of aversive memories was suggested by the finding that CB1 receptor-knockout mice showed selectively impaired extinction of auditory fear-conditioned tests (Marsicano *et al*., 2002). This can also be seen in mice treated with rimonabant, which selectively disrupted extinction learning of fear-motivated tasks, while having no such effect on the extinction of a reward-motivated task (Niyuhire *et al*., 2007). The formation of fear memory is an important adaptive response in animals and humans to potentially dangerous environmental cues. The ability to forget such memories when danger has past is also an important adaptive response, and this seems to involve a cannabinoid mechanism.

Effects of cannabinoids on control of appetite and body weight

Many subjective reports suggest that cannabis intoxication is associated with an increased appetite, particularly for sweet foods, even in subjects who were previously satiated. This effect can be confirmed under laboratory conditions (Hollister, 1971; Mattes *et al*., 1994) although results from studies in human subjects have tended to be variable, perhaps because the increased appetite is focused on certain types of food (see also Chapter 3). Nevertheless, controlled clinical trials showed that THC (dronabinol) had significant beneficial effects in counteracting the loss of appetite and reduction in body weight in patients suffering from AIDS-related wasting syndrome (Beal *et al*., 1995), and this is one of the medical indications for which the drug has official approval in the USA.

Δ^9-Tetrahydrocannabinol also stimulates food intake in experimental animals, again the effect is specific for high-fat or sweet high-fat diets, and is not seen in animals offered standard rat chow (Koch, 2001). The endocannabinoid anandamide also stimulates food intake in rats, and the effect is blocked by rimonabant (Williams and Kirkham, 1999). These results suggest that cannabinoids may play a role in the regulation of food intake and body weight (Mechoulam and Fride, 2001). At certain stages during development these effects of endocannabinoids may be of crucial importance. Fride *et al*. (2001) found that administration of the CB1 antagonist rimonabant to new born mouse pups had a devastating effect in decreasing milk ingestion and growth; continuing treatment with the antagonist led to death in 4–8days. The effect of rimonabant could be almost fully reversed by co-administering THC.

Whereas cannabinoids increase food intake, the CB1 antagonist rimonabant given on its own suppresses food intake and leads to reduced body weight in adult non-obese rats (Colombo *et al*., 1998). A number of studies have shown that rimonabant caused a marked reduction in daily food intake and significant reductions in body weight when given to normal or obese rats and mice given unlimited access to normal or high-fat diets. These effects were clearly linked to a blockade of CB1 receptors, as the CB2-selective antagonist SR144528 failed to affect food intake, and rimonabant was ineffective in CB1 receptor-knockout mice (Wiley *et al*., 2005). The effects of rimonabant on food intake diminished with repeated dosing, and were no longer seen after the first week. Despite this, the drug continued to cause reductions in body weight, even though food intake had recovered to near normal levels. This could be explained by the finding of increased energy expenditure in the treated animals. A key target seems to be peripheral-fat tissue, the cells of which carry CB1 receptors. Blockade of these receptors led to increased metabolism of fatty acids (otherwise deposited as fat). CB1receptors in the liver also seem to be involved, as activation of these receptors stimulates fatty acid synthesis and promotes diet-induced obesity (Osei-Hyiaman *et al*., 2005). In the brain rimonabant acts on the hypothalamus to cause a reduction in food intake, as part of the complex mechanisms whereby the brain helps to control food intake and body weight (Morton *et al*., 2006). These findings from animal experiments formed a valuable translational bridge to guide subsequent clinical studies.

The results of three large-scale randomized, double-blind, placebo-controlled clinical trials in obese subjects have been reported (reviewed by Carai *et al*., 2006). The results were remarkable. After 1 year, patients receiving 20 mg rimonabant lost 6.3–6.9 kg, compared with a loss of l.5–1.8 kg in the placebo groups. The weight loss was accompanied by significant decreases in plasma glucose and fat levels; and elevations in "good" HDL cholesterol, indicating protective effects against a number of known risk factors for heart disease. Rimonabant appeared to be well

tolerated and safe, although episodes of dizziness, nausea, anxiety and depression were seen more frequently in patients receiving 20 mg rimonabant than in the placebo group.

Rimonabant was approved for sale in Europe, and for a short time enthusiasm grew for this new approach to the treatment of obesity and the associated "metabolic syndrome" that often leads to type 2 diabetes. Several other major pharmaceutical companies launched clinical trials of their own CB1 antagonists. However, growing concern about the occurrence of psychiatric side effects led the Food and Drug Administration in the USA to refuse approval, and in 2009 the European Medicines Agency, concerned about possible drug-induced suicides, also withdrew approval of the drug (Janero and Makiyannis, 2009; Le Fall *et al.*, 2009).

Cannabis as an intoxicant and drug of dependence

Cannabis intoxication

There have been many subjective accounts of the cannabis "high" (see Earleywine, 2002; Iversen, 2008). The experience is highly variable, depending on the dose of drug, the environment and the experience and expectations of the drug user. A typical "high" is preceded initially by a transient stage of tingling sensations felt in the body and head accompanied by a feeling of dizziness or lightheadedness. The "high" is a complex experience, characterized by a quickening of mental associations and a sharpened sense of humour, sometimes described as a state of "fatuous euphoria." The user feels relaxed and calm, in a dreamlike state disconnected from the real world. The intoxicated subject often has difficulty in carrying on a coherent conversation, and may drift into daydreams and fantasies. Drowsiness and sleep may eventually ensue.

Studies of the effects of cannabis on perceptual abilities have yielded a variety of often conflicting results. While users often report a subjective enhancement of visual and auditory perception, sometimes with synesthesia (sounds take on visual colourful qualities), laboratory studies have usually not shown marked changes in visual or auditory perception. One subjective effect that has been confirmed, is the sensation that cannabis users experience time as passing more quickly relative to real time. In laboratory tests subjects overestimate the amount of elapsed time when asked to estimate, or produce shorter than required intervals when asked to signal a period of elapsed time (Hicks *et al.*, 1984; Matthew *et al.*, 1998). This curious effect can also be seen in animals. Han and Robinson (2001) trained rats to respond for a food reward using a fixed interval schedule. When treated with THC or WIN55 212-2 the animals shortened their response interval, whereas the antagonist rimonabant lengthened this interval.

As with other intoxicant drugs, little is known about the brain mechanisms that underlie the cannabis "high." The intoxicant effects are clearly mediated via CB1 receptors. Huestis *et al.* (2001) carried out a well-controlled study in 63 healthy cannabis users, who received either rimonabant or placebo, and smoked either a THC-containing or placebo marijuana cigarette. The CB1 antagonist blocked the acute psychological effects of the active cigarettes. Interestingly, rimonabant itself when given alone (with placebo cigarette) produced no significant psychological effects. The CB1 receptor in the brain also mediates the subjective effects of THC in animals. In rats trained to recognize oral THC as a discriminative cue (ED50 = 0.64 mg/kg), the antagonist rimonabant blocked this behavior (Perio *et al.*, 1996; Jarbe *et al.*, 2006). Similar results have been reported in mice (Vann *et al.*, 2009).

Human subjects can also be trained to self-administer smoked cannabis; cannabis has been chosen significantly more than placebo, and cannabis with a higher THC content was preferred over that with a lower THC content (Haney *et al.*, 1997; Ward *et al.*, 1997; Haney, 2008). A topical question is how cannabis users adapt their smoking behavior in response to the higher potency cultivated cannabis now commonly available. This may contain three to four times more THC than traditional imported cannabis resin (see Chapter 5). There has been little scientific study of this question, but Korf *et al.* (2007), in a survey of Dutch coffee shop users, found that at least some compensated for stronger cannabis by inhaling less deeply and smoking less.

Another procedure used to determine the rewarding properties of drugs is intracranial self-stimulation (ICSS). Electrical stimulation of ascending fibers of the mesolimbic pathway is reinforcing in rats, and drugs that increase sensitivity to ICSS suggest that they have rewarding actions. Δ^9-Tetrahydrocannabinol and other cannabinoids decrease the threshold for ICSS, and this effect is blocked by rimonabant (Vlachou *et al.*, 2005).

A different way of demonstrating the rewarding effects of drugs in animals is the conditioned-place-preference paradigm, in which an animal learns to

approach an environment in which it had previously received a rewarding stimulus. Rats demonstrated a positive THC place preference after doses as low as 1 mg/kg (Lepore *et al*., 1995).

In common with other euphoriant drugs, THC selectively activates dopaminergic neurons in the ventral-tegmental area, and this is believed to be a key feature in explaining the effects of cannabinoids on brain reward circuits (Lupica *et al*., 2004; Solinas *et al*., 2008; Cooper and Haney, 2009). In an electrophysiological study, French *et al*. (1997) reported that low doses of THC increased the firing of these cells. Tanda *et al*. (1997) used microdialysis probes to show that low doses of THC (0.15 mg/kg iv) caused an increase in the release of dopamine from the shell region of the nucleus accumbens, an effect that is also seen after administration of heroin, cocaine, D-amphetamine and nicotine. Electrophysiological studies showed that the cannabinoid WIN55 212-2 depressed the inhibitory GABAergic input to dopamine neurons in the ventral tegmental area in rat brain slice preparations in vitro, suggesting a mechanism that may underlie their increased firing rate in vivo (Szabo *et al*., 2002).

There is increasing preclinical evidence that some of the rewarding effects of THC may involve an overlap with opioid mechanisms in brain (Robledo *et al*., 2008; Cooper and Haney, 2009). Tanda *et al*. (1997) found that the increased release of dopamine in rat nucleus accumbens provoked by THC could be blocked by administration of the mu-opiate receptor antagonist naloxonazine, suggesting the involvement of an opioid mechanism. There is other evidence for an interaction between cannabinoid and opioid mechanisms. In tests of acute pain (Fuentes *et al*., 1999) and chronic inflammatory pain (Welch and Stevens, 1992; Smith *et al*., 1998), THC and morphine acted synergistically – one potentiated the anti-nociceptive actions of the other. This potentiation could be blocked by either rimonabant or naloxone, indicating that both CB1 and opiate receptors were involved (Fuentes *et al*., 1999). An electrophysiological analysis of the effects of cannabinoids on single-cell firing patterns in the rostral ventromedial medulla revealed that the effects of cannabinoids were similar to those elicited by morphine. The authors concluded that cannabinoids may produce analgesia through activation of a brainstem circuit that is also required for opiate analgesia, although the two mechanisms are pharmacologically distinct (Meng *et al*., 1998).

Studies of the effect of THC in the place preference model in mice lacking mu- or kappa-opioid receptors also suggest that opioid mechanisms may play a key role in the rewarding effects of THC. While the effects of THC on body temperature, pain sensitivity and reducing motor activity were unaffected in either of the opioid-receptor-knockout strains, the rewarding effects of THC, assessed by place preference, were abolished in the mu-knockout mice, and enhanced in the kappa-knock out animals (Ghozland *et al*., 2002). Δ^9-Tetrahydrocannabinol-induced place preference was blocked by the mu-opiate antagonist naloxone (Braida *et al*., 2004). Opioid antagonists also diminished self-administration of CB1 agonists in both rodents (Navarro *et al*., 2001) and monkeys (Justinova *et al*., 2004).

Tolerance and dependence

Many animal studies showed that tolerance develops to most of the behavioral and physiological effects of THC (for review see Pertwee, 1991; Lichtman and Martin, 2005). The earlier clinical literature also suggested that tolerance occurs after repeated administration of THC in humans, although many of these studies were poorly controlled (for reviews see Jones, 1978, 1987; Hollister, 1986,1998). But for many years cannabis was not considered to be a drug of addiction. Withdrawal of the drug did not lead to any obvious physical withdrawal symptoms either in people or in animals, and animals failed to self-administer the drug, a behavior usually associated with drugs of addiction.

Attitudes have changed markedly in recent years. The DSM-IV (American Psychiatric Association, 1994) defines "substance dependence" and "substance abuse" rather than "addiction." When the DSM-IV criteria are applied to populations of regular cannabis users, surprisingly high proportions appear positive by these definitions (Anthony *et al*., 1994; Swift *et al*., 2001). More carefully controlled studies have also shown that a reliable and clinically significant withdrawal syndrome does occur in human cannabis users when the drug is withdrawn. The symptoms include craving for cannabis, decreased appetite, sleep difficulty and weight loss and may sometimes be accompanied by anger, aggression, increased irritability, restlessness and strange dreams (Haney *et al*., 1999; Budney *et al*., 2001,2004). There is some evidence that genetic factors may increase or decrease the risk of dependence. In a genome-wide survey, evidence for a linkage between symptoms of cannabis dependence was found

on chromosomes 3q21 and 9q34 (Hopfer *et al*., 2007). Certain polymorphisms of the CB1 receptor protein may also confer greater or lower risk of cannabis dependence (Hopfer *et al*., 2006).

The existence of dependence on cannabinoids in animals is also much more clearly observable because of the availability of CB1 receptor antagonist drugs that can be used to precipitate withdrawal. Rimonabant-precipitated withdrawal has been extensively documented in animals (Cooper and Haney, 2009). Thus, Aceto *et al*. (1996) described a behavioral withdrawal syndrome precipitated by rimonabant in rats treated for only 4 days, with doses of THC as low as 0.5–4.0 mg/kg per day. The syndrome included scratching, face rubbing, licking, wet-dog shakes, arched back and ptosis, many of the same signs are seen in rats undergoing opiate withdrawal. Similar withdrawal signs could be elicited by rimonabant in rats treated chronically with the synthetic cannabinoids, CP55 940 (Rubino *et al*., 1998) or WIN55 212-2 (Aceto *et al*., 2001). Rimonabant-induced withdrawal in rats was accompanied by marked elevations of release of the stress-related neuropeptide corticotropin-releasing factor in the amygdala, a result also seen in animals undergoing heroin withdrawal (Rodriguez de Fonseca *et al*., 1997). An electrophysiological study showed that precipitated withdrawal was also associated with reduced firing of dopamine neurons in the ventral tegmental area of rat brain (Diana *et al*., 1998). These data clearly indicate that chronic administration of cannabinoids leads to adaptive changes in the brain, some of which are similar to those seen with other drugs of dependence. The ability of THC to cause a selective release of dopamine from the nucleus accumbens (Tanda *et al*., 1997) also suggests some similarity between THC and other drugs in this category.

Furthermore, although many earlier attempts to obtain reliable self-administration behavior with THC were unsuccessful (Pertwee, 1991), success has subsequently been obtained. The potent synthetic cannabinoids are more water soluble than THC, which makes intravenous administration easier, and there are well-documented reports that both rodents and monkeys self-administer CB1 agonists in a dose-dependent manner (Cooper and Haney, 2009), although CB1 receptor-knockout mice fail to exhibit this behavior.

A number of studies have suggested that there may be links between the development of dependence to cannabinoids and to opiates. Some of the behavioral signs of rimonabant-induced withdrawal in THC-treated rats can be mimicked by administration of the opiate antagonist naloxone (Kaymakçalan *et al*., 1977). Conversely, the withdrawal syndrome precipitated by naloxone in morphine-dependent mice can be partly relieved by administration of THC (Hine *et al*., 1975) or by endocannabinoids (Yamaguchi *et al*., 2001). Rats treated chronically with the cannabinoid WIN55 212-2 became sensitized to the behavioral effects of heroin (Pontieri *et al*., 2001). Such interactions can also be demonstrated acutely. A synergy between cannabinoids and opiate analgesics has already been described above. Δ^9-Tetrahydrocannabinol also facilitated the anti-nociceptive effects of RB101, an inhibitor of enkephalin inactivation (Valverde *et al*., 2001). These authors found that acute administration of THC caused an increased release of Met-enkephalin into microdialysis probes placed into the rat nucleus accumbens.

The availability of receptor-knockout animals has also helped to illustrate cannabinoid/opioid interactions. CB1 receptor-knockout mice exhibited greatly reduced morphine self-administration behavior and less severe naloxone-induced withdrawal signs than in wild-type animals, although the anti-nociceptive actions of morphine were unaffected in the knockout animals (Ledent *et al*., 1999). The rimonabant-precipitated withdrawal syndrome in THC-treated mice was significantly attenuated in animals with knockout of the pro-enkephalin gene (Valverde *et al*., 2001). Knockout of the mu-opioid receptor also reduced rimonabant-induced withdrawal signs in THC-treated mice, and there was an attenuated naloxone withdrawal syndrome in morphine-dependent, CB1 receptor-knockout mice (Lichtman *et al*., 2001a, 2001b).

These preclinical findings point clearly to interactions between the endogenous cannabinoid and opioid systems in CNS, although the neural circuitry involved remains unknown. It is possible that the involvement of opioid mechanisms in mediating at least some of the effects of cannabinoids is relevant to understanding the euphoriant and addictive properties of these drugs. However, it has proved difficult to demonstrate opioid modulation of cannabinoid effects in humans. Although some studies showed that naloxone blunted the subjective effects of THC, other studies with another opioid antagonist naltrexone have shown either no effect or an enhancement of subjective effects of THC (Haney, 2007). There is also no evidence that naloxone precipitates withdrawal in cannabis smokers (Haney, 2007).

Conclusions

Although we are begining to understand some of the effects of cannabis on brain function there is much still to be learned. In both animals and humans, most of the CNS effects of the drug, including its intoxicant and rewarding properties, appear to be due to its interactions with the cannabinoid CB1 receptor. The availability of CB1 receptor-knockout mice and CB1 receptor-antagonist drugs has provided powerful new tools for research on the central actions of cannabis. The interaction of the cannabinoid and opioid systems in CNS, although well documented in animals, remains to be demonstrated convincingly in humans.

References

Abel, E.L. (1971) Marihuana and memory: acquisition or retrieval? *Science,* **173**:1038–41.

Aceto, M.D., Scates, S.M. and Martin, B.R. (2001) Spontaneous and precipitated withdrawal with a synthetic cannabinoid, WIN 55212–2. *Eur J Pharmacol*, **416**:75–81.

Aceto, M.D., Scates, S.M., Lowe, J.A. and Martin, B.R. (1996) Dependence on Δ^9-tetrahydrocannabinol: studies on precipitated and abrupt withdrawal. *J Pharmacol Exp Ther,* **278**:1290–95.

Adams, I.B. and Martin, B.R. (1996) Cannabis: pharmacology and toxicology in animals and humans. *Addiction*, **91**:1585–1614.

Alger, B.E. and Pitler, T.A. (1995) Retrograde signaling at $GABA_A$-receptor synapses in the mammalian CNS, *Trends Neurosci*, **18**:333–40.

American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), 4th edn*, Washington DC, American Psychiatric Association.

Anthony, J.C., Warner, L.A. and Kessler, R.C. (1994) Comparative epidemiology of dependence on tobacco, alcohol, controlled substances and inhalants. Basic findings from the National Comorbidity Survey. *Exp Clin Psychopharmacol*, **2**:244–68.

Azad, S.C., Kurz, J., Marsicano, G., Lutz, B., Ziegelgänsberger, W. and Razmmes, G. (2008) Activation of CB1 specifically located on GABAergic interneurons inhibits LTD in the lateral amygdale. *Learn Mem*, **15**:143–52.

Beal, J.A., Olson, R., Laubenstein, L., *et al.* (1995). Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *J Pain Symptom Manag,* **10**:89–97.

Braida, D., Iosue, S., Pegorini, S. and Sala, M. (2004) Delta-9-tetrahydrocannabinol-induced conditioned place preference and introcerebroventricular self-administration in rats. *Eur J Pharmacol,* **506**:63–9.

Breivogel, C.S. and Childers, S.R. (1998) The functional neuroanatomy of brain cannabinoid receptors. *Neurobiol Dis*, **5**:417–31.

Budney, A.J., Hughes, J.R., Moore, B.A. and Novy, P.L. (2001) Marijuana abstinence effects in marijuana smokers maintained in their home environment. *Arch Gen Psychiatry*, **58**:917–24.

Budney, A.J., Hughes, J.R., Moore, B.A. and Vandrey, R. (2004) Review of the validity and significance of cannabis withdrawal syndrome. *Am J Psychiatry*, **161**:1967–77.

Burns, H.D., Van Laere, K., Snabria-Bohórquez, S., *et al.* (2007) [^{18}F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. *Proc Nat Acad Sci US*, **104**: 9800–805.

Carai, M., Colombo, G., Maccioni, P. and Gessa, G.L. (2006) Efficacy of rimonabant and other cannabinoid CB-1 receptor antagonist in reducing food intake and body weight: preclinical and clinical data. *CNS Drug Rev*, **12**: 91–9.

Castañé, A., Maldonado, R. and Valverde, O. (2004) Role of different brain structures in the behavioral expression on WIN 55,212–2 withdrawal in mice. *Br J Pharmac*, **142**:1309–17.

Chevaleyre, V., Takahashi, K.A. and Castillo, P.E. (2006) Endocannabinoid-mediated synaptic plasticity in the CNS. *Ann Rev Neurosci*, **29**: 37–76.

Colombo, G., Agabio, R., Diaz, G., Lobina, C., Reali, R. and Gessa, G.L. (1998) Appetite suppression and weight loss after the cannabinoid antagonist SR141716A. *Life Sciences*, **63**:PL113–PL7.

Compton, D.R., Aceto, M.D., Lowe, J. and Martin, B.R. (1996) In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A) inhibition of Δ^9-tetrahydocannabinol-induced responses and apparent agonist activity. *J Pharmacol Exp Ther,* **277**:586–94.

Cooper, Z.D. and Haney, M. (2009) Actions of delta-9-tetrahydrocannabinol in cannabis. *Int Rev Psychiatry,* **21**:104–12.

DeSanty, K.P. and Dar, M.S. (2001) Cannabinoid-induced motor incoordination through the cerebellar CB1 receptor in mice. *Pharmacol Biochem Behav*, **69**:251–9.

Diana, M., Melis, M., Muntoni, A.L. and Gessa, G.L. (1998) Mesolimbic dopaminergic decline after cannabinoid withdrawal. *Proc Natl Acad. Sci USA*, **95**:10269–73.

Dixon, W.E. (1899) The pharmacology of *Cannabis indica*. *Br Med J*, **11**:1354–7.

D'Souza, D.C. and Kosten, T.R. (2001) Cannabinoid antagonists. *Arch Gen Psychiatry,* **58**:330–1.

Earleywine, M. (2002) *Understanding Marijuana,* New York: Oxford University Press.

Egertová, M. and Elphick, M.R. (2000) Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB1. *J Comp Neurol*, **422**:159–71.

Egertová, M., Giang, D.K., Cravatt, B.F. and Elphick, M.R. (1998) A new perspective on cannabinoid signaling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in the rat brain. *Proc R Soc Lond B*, **265**:2081–5.

Elphick, M.R. and Egertová, M. (2001) The neurobiology and evolution of cannabinoid signaling. *Phil Trans R Soc Lond B*, **356**:381–408.

Felder, C.C. and Glass, M. (1998) Cannabinoid receptors and their endogenous agonists. *Ann Rev Pharmacol Toxicol*, **38**:179–200.

Finnema, S.J., Donohue, S.R., Zoghbi, S.S., *et al.* (2009) Evaluation in monkey of [^{11}C]PipISB and [^{18}F]PipISB as candidate radioligands for imaging brain cannabinoid type-1 (CB1) receptors in vivo. *Synapse,* **63**: 22–30.

French, E.D., Dillon, K. and Wu, X. (1997) Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *NeuroReport*, **8**:649–52.

Fride, E., Ginzburg, Y., Breuer, A., Bisogno, T., Di Marzo, V. and Mechoulam, R. (2001) Critical role of the endogenous cannabinoid system in mouse pup suckling and growth. *Eur J Pharmacol*, **419**:207–14.

Fuentes, J.A., Ruiz-Gayo, M., Manzanares, J., Vela, G., Reche, I. and Corchero, J. (1999) Cannabinoids as potential new analgesics. *Life Sci*, **65**:675–85.

Gerdeman, G.L., Ronesi, J. and Lovinger, D.M. (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nature Neurosci,* **5**:446–51.

Ghozland, S., Matthes, H.W., Simonin, F., Filliol, D., Kieffer, B.L. and Maldonado, R. (2002) Motivational effects of cannabinoids are mediate by mu-opioid and kappa-opioid receptors. *J Neurosci,* **22**:1146–54.

Gill, E.W., Paton, W.D.M. and Pertwee, R.G. (1970) Preliminary experiments on the chemistry and pharmacology of cannabis. *Nature,* **229**:134–6.

Giuffrida, A. and Piomelli, D. (2000) The endocannabinoid system: a physiological perspective on its role in psychomotor control. *Chem Physics Lipids,* **108**:151–8.

Greenberg, H.S., Werness, S.A.S., Pugh, J.E., Andrus, R.O., Anderson, D.J. and Domino, E.F. (1994) Short-term effects of smoking marijuana on balance in patients with multiple sclerosis and normal volunteers. *Clin Pharmacol Ther,* **55**:324–8.

Hájos, N., Katona, I., Naiem, S.S., *et al.* (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur J Neurosci*, **12**:3239–49.

Hampson, R.E. and Deadwyler, S.A. (1999) Cannabinoids, hippocampal function and memory. *Life Sci,* **65**:715–23.

Han, C.J. and Robinson, J.K. (2001) Cannabinoid modulation of time estimation in the rat. *Behav Neurosci*, **115**:243–6.

Haney, M. (2007) Opioid antagonism of cannabinoid effects: differences between marijuana smokers and nonmarijuana smokers. *Neuropsychopharmacology,* **32**:1391–403.

Haney, M. (2008) Self-administration of cocaine, cannabis and heroin in the human laboratory: benefits and pitfalls. *Addiction Biol*, **14**:9–21.

Haney, M., Comer, S.D., Ward, A.S., Foltin, R.W. and Fischman, M.W. (1997) Factors influencing marijuana self-administration by humans. *Behav Pharmacol,* **8**:101–12.

Haney, M., Ward, A.S., Comer, S.D., Foltin, R.W. and Fischman, M.W. (1999) Abstinence symptoms following oral THC administration to humans. *Psychopharmacology (Berl),* **141**:385–94.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R. and Rice, K.C. (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J Neurosci*, **11**:563–83.

Hicks, R.E., Gualtieri, C.T., Mayo, P. Jr. and Perez-Reyes, M. (1984) Cannabis, atropine and temporal information processing. *Neuropsychobiology*, **12**:229–37.

Hine, B., Friedman, E., Torrelio, M. and Gershon, S. (1975) Morphine-dependent rats: blockade of precipitated abstinence by tetrahydrocannabinol. *Science,* **187**:443–5.

Hoffman, A.F. and Lupica, C.R. (2000) Mechanisms of cannabinoid inhibition of $GABA_A$ synaptic transmission in the hippocampus. *J Neuroscience,* **20**:2470–9.

Hollister, L.E. (1971) Hunger and appetite after single doses of marihuana, alcohol and dextroamphetamine. *Clin Pharmacol Ther,* **12**:44–9.

Hollister, L.E. (1986) Health aspects of cannabis. *Pharmacol Rev,* **38**:1–20.

Hollister, LE. (1998) Health aspects of cannabis: revisited. *Int J Neuropsychopharmacology*, **1**:71–80.

Hopfer, C.J., Lessem, J.N., Hartman, C.A., *et al.* (2007) A genome-wide scan for loci influencing adolescent cannabis dependence symptoms: evidence for linkage on chromosomes 3 and 9. *Drug Acohol Depend*, **89**:34–41.

Hopfer, C.J., Young, S.E., Purcell, S., *et al.* (2006) Cannabis receptor haplotype associated with fewer cannabis dependence symptoms in adolescents. *Am J Med Genet B*, **141B**:895–901.

Huestis, M.A., Gorelick, D.A., Heishman, S.J., *et al.* (2001) Blockade of effects of smoked marijuana by the

CB1-selective cannabinoid receptor antagonist SR141716. *Arch Gen Psychiatry*, **58**:322–8.

Iversen, L.L. (2008) *The Science of Marijuana, 2nd edn,* New York: Oxford University Press.

Janero, D.R. and Makiyannis, A. (2009) Cannabinoid receptor antagonists: pharmacological opportunities, clinical experience, and translational prognosis. *Expert Opin Emerg Drugs*, **14**:43–65.

Jarbe, T.U., Liu, Q. and Makriyannis, A. (2006) Antagonism of discriminiative stimulus effects of Δ^9-THC and (R)-methanandamide in rats. *Psychopharmacology (Berl),* **184**:36–45.

Jones, R.T. (1978) Marihuana: human effects. In Iversen, L. L., Iversen, S. D., Snyder, S. M. (eds), *Handbook of Psychopharmacology, Vol 12*, New York: Plenum Press, pp. 373–412.

Jones, R.T. (1987) Drug of abuse profile: cannabis. *Clin Chem,* **33**:72B–81B.

Justinova, Z., Tanda, G., Munzar, P. and Goldberg, S.R. (2004) The opioid antagonist naltrexone reduces the reinforcing effects of delta 9 tetrahydrocannabinol (THC) in squirrel monkeys. *Psychopharmacology (Berl),* **173**:186–94.

Katona, I., Sperlagh, B., Sik, A., *et al*. (1999) Presynaptically located CB1 receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci,* **19**:4544–58.

Katona, I., Sperlagh, B., Maglóczky, Z., *et al*. (2000) GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neurosci*,**100**:797–804.

Kaymakçalan, S., Ayhan, I.H. and Tulunay, F.C. (1977) Naloxone-induced or postwithdrawal abstinence signs in Δ^9-tetrahydrocannabinol-tolerant rats. *Psychopharmacology*, **55**:243–9.

Koch, J.E. (2001) Δ^9-THC stimulates food intake in Lewis rats. Effects on chow, high-fat and sweet high-fat diets. *Pharmacol Biochem Behav,* **68**:539–43.

Korf, D.J., Benschop, A. and Wouters, M. (2007) Differential response to cannabis potency: a typology of users based on self-reported consumption behavior. *Int J Drug Policy,* **18**:168–76.

Kreitzer, A.C. and Regehr, W.G. (2001) Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. *J Neurosci,* **21**:RC174–79.

Laukner, J.E., Jensen. J.B., Chen, H-Y., Lu, H-C., Hille, B. and Mackie, K. (2008) GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Nat Acad Sci US,* **105**:2699–704.

Le Fall, B., Gorelik, D.A. and Goldberg, S.R. (2009) The future of encocannabinoid-oriented clinical research after CB1 antagonists. *Psychopharmacology (Berl),* **205**:171–4.

Ledent, C., Valverde, O., Cossu, G., *et al.* (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science,* **283**:401–4.

Lenz, R.A., Wagner, J.J. and Alger, B.E. (1998) N- and L-type calcium channel involvement in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. *J Physiol (Lond),* **512**:61–73.

Lepore, M., Vorel, S.R., Lowinson, J. and Gardner, E.L. (1995) Conditioned place preference induced by Δ^9-tetrahydrocannabinol: comparison with cocaine, morphine and food reward. *Life Sci,* **56**:2073–80.

Lichtman, A.H. and Martin, B.R (1996) Δ^9-Tetrahydrocannabinol impairs spatial memory through a cannabinoid mechanism. *Psychopharmacology,* **126**:125–31.

Lichtman, A.H. and Martin, B.R. (2005) Cannabinoid tolerance and dependence. *Handbook Exp Pharmacol*, **168**:691–717.

Lichtman, A.H., Fisher, J. and Martin, B.R. (2001a) Precipitated cannabinoid withdrawal is reversed by Δ^9-tetrahydrocannabinol or clonidine. *Pharmacol Biochem Behav*, **69**:181–8.

Lichtman, A.H., Sheikh, S.M., Loh, H.H. and Martin, B.R. (2001b) Opioid and cannabinoid modulation of precipitated withdrawal in Δ^9-tetrahydrocannabinol and morphine-dependent mice. *J Pharmacol Exp Ther*, **298**:1007–14.

Lupica, C.R., Riegel, A.C. and Hoffman, A.F. (2004) Marijuana and cannabinoid regulation of brain reward circuits. *Br J Pharmacol*, **143**:227–34.

Maejima, T., Hashimoto, K., Yoshida, T., Aiba, A. and Kano, M. (2001) Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron*, **31**:463–75.

Mallet, P.E. and Beninger, R.J. (1998) The cannabinoid CB1 receptor antagonist SR141716A attenuates the memory impairment produced by Δ^9-tetrahydrocannabinol or anandamide. *Psychopharmacology,* **140**:11–19.

Manno, J.E., Glenn, M.S., Kiplinger, G.F., Haine, S.E., Bennett, I.F. and Forney, R.B. (1970) Comparative effects of smoking marihuana or placebo on human motor and mental performance. *Clin Pharmacol Ther,* **11**:808–15.

Marsicano, G., Wotjak, C.T., Azad, S.C., *et al*. (2002) The endogenous cannabinoid system controls extinction of aversive memories. *Nature*, **418**:530–4.

McPartland, J.M., Glass, M. and Pertwee, R.G. (2007) Meta-analysis of cannabinoid ligand binding affinity and receptor distribution: interspecies differences. *Br J Pharmac*, **152**: 583–93.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C. and Bonner, T.I. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature,* **346**:561–4.

Mattes, R.D., Engelman, K., Shaw, L.M. and Elsohly, M.A. (1994) Cannabinoids and appetite stimulation. *Pharmacol Biochem Behav*, **49**:187–94.

Matthew, R.J., Wilson, W.H., Turkington, T.G. and Coleman, R.E. (1998) Cerebellar activity and disturbed time sense after THC. *Brain Res*, **797**:183–9.

Mechoulam, R. and Fride, E. (2001) A hunger for cannabinoids. *Nature,* **410**:763–5.

Mendelson, J.H., Babor, T.F., Kuehnle, J.C., *et al.* (1976) Behavioral and biological aspects of marijuana use. *Annal NY Acad Sci,* **282**:186–210.

Meng, I.D., Manning, B.H., Martin, W.J. and Fields, H.L (1998) An analgesia circuit activated by cannabinoids. *Nature,* **395**:381–3.

Miller, L.L. and Branconnier, R.J. (1983) Cannabis: effects on memory and the cholinergic limbic system. *Psychol Bull*, **93**:441–56.

Misner, D.L. and Sullivan, J.M. (1999) Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. *J Neurosci,* **19**:6795–805.

Monory, K., Blaudzun, H., Massa, F., *et al.* (2007) Genetic dissection of behavioral and autonomic effects of Δ^9-tetrahydrocannabinol in mice. *PLoS Biology* 2007 October 9, online, **5**:e269.

Morton, G.J., Cummings, D.E., Baskin, D.G., *et al.* (2006) Central nervous system control of food intake and body weight. *Nature,* **443**:289–95.

Munro, S., Thomas, K.L. and Abu-Shaar, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature,* **365**:61–5.

Murray, R.M., Morrison, P.D., Henquet, C. and Di Forti, M. (2007) Cannabis, the mind and society: the harsh realities. *Nature Rev Neurosci*, **8**:885–95.

Nakamura-Palacios, E.M., Winsauer, P.J. and Moerschbaecher, J.M. (2000) Effects of the cannabinoid ligand SR 141716A alone or in combination with delta-9-tetrahydrocannabinol or scopolamine on learning in squirrel monkeys. *Behav Pharmacol*, **11**:377–86.

Navarro, M., Crrera, M.R., Fratta, W., *et al.* (2001) Functional interaction between opioid and cannabinoid receptors in drug self-administration. *J Neuroscience,* **21**:5344–50.

Niyuhire, F., Varvel, S.A., Thorpe, A.J., Stokes, R.J., Wiley, J. and Lichtman, A.H. (2007) The disruptive effects of the CB1 receptor antagonist rimonabant on extinction learning in mice are task-specific. *Psychopharmacology (Berl),* **19**:221–31.

Ohno-Shosaku, T., Maejima, T. and Kano M. (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron,* **29**:729–38.

Onaivi, E.S., Ishiguro, H., Gong, J-P., *et al.* (2008) Brain neuronal CB2 cannabinoid receptor in drug abuse and depression: from mice to human subjects. *PloS ONE*, **3**:online, February 20, 2008.

Oropeza, V.C., Mackie, K. and Van Bockstaele, E.J. (2007) Cannabinoid receptors are localized to noradrenergic axon terminals in the rat frontal cortex. *Brain Res,* **1127**:35–44.

Osei-Hyiaman, D., DePetrillo, M., Pacher, P., *et al.* (2005) Endocannainboid activation of hepatic CB-1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest,* **115**:1298–305.

Palazuelos, J., Aguado, T., Pazos, M.R., *et al.* (2009) Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease. *Brain*, **132**:3152–64.

Patel, S. and Hillard, C.J. (2001) Cannabinoid CB1 receptor agonists produce cerebellar dysfunction in mice. *J Pharmacol Exp Ther,* **297**:629–37.

Perio, A.A., Rinaldi-Carmona, M.M., Maruani, J.J., *et al.* (1996) Central mediation of the cannabinoid cue: activity of a selective CB1 antagonist SR 141716A. *Behav Pharmacol,* **7**:65–71.

Pertwee, R.G. (1991) Tolerance to and dependence on psychotropic cannabinoids. In Pratt, J. (ed) *The Biological Basis of Drug Tolerance*, London: Academic Press Ltd, pp 232–65.

Pertwee. R.G. (1999) Pharmacology of cannabinoid receptor ligands. *Current Med Chem*, **6**:635–64.

Pertwee, R.G. (2006) Cannabinoid pharmacology: the first 66 years. *Br J Pharmacol,* **147(S1)**:S163–71.

Pertwee, R.G. (2007) GPR55: a new member of the cannabinoid receptor clan*? Br J Pharmac*, **152**:984–6.

Pettit, D.A.D., Harrison, M.P., Olson, J.M., Spencer, R.F. and Cabral, G.A. (1998) Immunohistochemical localization of the neural cannabinoid receptor in rat brain. *J Neurosci Res*, **51**:391–402.

Pickel, V.M., Chan, J., Kearn, C.S. and Mackie, K. (2006) Targeting dopamine D2 and cannabinoid-1 (CB1) receptors in rat nucleus accumbens. *J Comp Neurol,* **495**:299–313.

Pitler, T.A. and Alger, B.E. (1992) Postsynaptic spike firing reduces synaptic $GABA_A$ responses in hippocampal pyramidal cells. *J Neurosci,* **12**:4122–32.

Pitler, T.A. and Alger, B.E. (1994) Depolarization-induced suppression of GABAergic inhibition in rat hippocampal pyramidal cells: G protein involvement in a presynaptic mechanism. *Neuron,* **13**:1447–55.

Pontieri, F.E., Monnazzi, P., Scontrini, A., Buttarelli, F.R. and Patacchioli, F.R. (2001) Behavioral sensitization to heroin by cannabinoid pretreatment in the rat. *Eur J Pharmacol,* **421**:R1–R3.

Rinaldi-Carmona, M. [and 13 others]. (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett,* **350**:240–4.

Robledo, P., Berrendero, F., Ozaita, A., and Maldonado, R. (2008) Advances in the field of cannabinoid-opioid cross talk. *Addiction,* **13**:213–24.

Rodríguez de Fonseca, F., Rocio, M., Carrera, A., Navarro, M., Koob, G.F. and Weiss, F. (1997) Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science*, **276**:2050–4.

Rodríguez de Fonseca, F., Del Arco, I., Martín-Calderón, J.L., Gorriti, M.A. and Navarro, M. (1998) Role of the endogenous cannabinoid system in the regulation of motor activity. *Neurobiol Dis*, **5**:483–501.

Ross, R.A. (2008) The enigmatic pharmacology of GPR55. *Trends Pharmacol Sci*, **30**:156–63.

Roth, S. (1978) Stereoselective presynaptic inhibitory effect of delta-9-tetrahydrocannabinol on cholinergic transmission in the myenteric plexus of the guinea pig. *Can J Physiol Pharmacol*, **56**:968–75.

Rubino, T., Patrini, G., Massi, P., *et al*. (1998) Cannabinoid-precipitated withdrawal: a time course study of the behavioral aspect and its correlation with cannabinoid receptors and G protein expression. *J Pharmacol Exp Ther*, **285**:813–9.

Ryberg, E., Larsson, N., Sjőgren, S., *et al*. (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmac*, **152**:1092–101.

Sañudo-Peña, M.C., Tsou, K. and Walker, J.M. (1999) Motor actions of cannabinoids in the basal ganglia output nuclei. *Life Sci*, **65**:703–13.

Schlicker, E. and Kathmann, M. (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci*, **22**:565–72.

Shen, M., Piser, T.M., Seybold, V.S. and Thayer, S.A. (1996) Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J Neurosci,* **16**:4322–34.

Smith, F.L., Cichewicz, D., Martin, Z.L. and Welch, S.P. (1998) The enhancement of morphine antinociception in mice by delta-9-tetrahydrocannabinol. *Pharmacol Biochem Behav*, **60**:559–66.

Solinas, M., Goldberg, S.R. and Piomelli, D. (2008) The endocannabinoid system in brain reward processes. *Br J Pharmacol*, **154**:369–83.

Solowij, N. (1998*) Cannabis and Cognitive Functioning*, Cambridge: Cambridge University Press.

Staton, P.C., Hatcher, J.P., Walker, D.J., *et al*. (2008) The putative cannabinoid receptor GPR55 plays a role in mechanical hyperalgesia associated with inflammatory and neuropathic pain. *Pain,* **152**:559–61.

Stiglick, A. and Kalant, H. (1985) Residual effects of chronic cannabis treatment on behavior in mature rats. *Psychopharmacology,* **85**: 436–9.

Swift, W., Hall, W. and Teesson, M. (2001) Cannabis use and dependence among Australian adults: results from the National Survey of Mental Health and Wellbeing. *Addiction*, **96**:737–48.

Szabo, B., Siemes, S. and Wallmichrath, I. (2002) Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. *Eur J Neurosci,* **15**:2057–61.

Tanda, G., Pontieri, F.E. and Di Chiara, G. (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ_1 opioid receptor mechanism. *Science,* **276**:2048–50.

Valverde, O., Noble, F., Beslot, F., Daugé, V., Fournié-Zaluski, M.C. and Roques, B.P (2001) Δ^9-Tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. *Eur J Neurosci*, **13**:1816–24.

Vlachou, S., Nomikos, G.G., and Panagis, G. (2005) CB1 Cannabinoid receptor agonists increase self-stimulation thresholds in the rat. *Psychopharmacology (Berl),* **179**:498–508.

Vann, R.E., Warner, J.A., Bushell, K., Huffman, J.W., Martin B.R. and Wiley, J.L. (2009) Discriminative stimulus properties of Δ^9-tetrahydrocannabinol (THC) in C57Bl/6J mice. *Eur J Pharmacol,* **615**:102–7.

Varma, N., Calrson, G.C., Ledent, C. and Alger, B.E. (2001) Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J Neurosci,* **21**:RC188–93.

Ward, A.S., Comer, S.D., Haney, M., Foltin, R.W. and Fischman, M.W. (1997) The effects of a monetary alternative in marijuana self-administration. *Behav Pharmacol*, **8**:275–86.

Welch, S.P. and Stevens, D.L. (1992) Antinociceptive activity of intrathecally administered cannabinoids alone, and in combination with morphine, in mice. *J Pharmacol Exp Ther*, **262**:10–18.

Wiley, J.L., Burston, J.J., Leggett, D.C., *et al*. (2005) CB1 cannabinoid receptor-mediated modulation of food intake in mice. *Br J Pharmacol*, **145**:293–300.

Williams, C.M. and Kirkham, T.C. (1999) Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. *Psychopharmacology,* **143**:315–7.

Wilson, R.I. and Nicoll, R.A. (2001) Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. *Nature,* **410**:588–92.

Yamaguchi, T., Hagiwara, Y., Tanaka, H., *et al*. (2001) Endogenous cannabinoid 2-arachidonoylglycerol, attenuates naloxone-precipitated withdrawal signs in morphine-dependent mice. *Brain Res*, **909**:121–6.

Chapter 2

Other cannabinoids

Raphael Mechoulam and Lumir Hanus

Today over 100 compounds called cannabinoids, typical for *Cannabis sativa*, have been identified in the plant. The levels of the individual constituents in the plant differ depending on the soil, weather conditions and genetics of the plant. Over the last few years cannabis plants with up to 20–30% (by dry weight) Δ^9-tetrahydrocannabinol (THC) have been grown by illegal cultivation, leading to high-potency marijuana (ElSohly and Slade, 2005; Ross *et al*., 2005; Ahmed *et al*., 2008a, 2008b; Radwan *et al*., 2008, 2009; Appendino *et al*., 2008; and see Chapter 4).

The pharmacology of only a few of the plant cannabinoids has been studied so far – mostly THC, cannabidiol (CBD) and Δ^9-tetrahydrocannabivarin (Δ^9-THCV) – hence today we have no information about the pharmacology of the overwhelming majority of these compounds. In view of the interesting properties of some of the constituents investigated over the last few years, the cannabis plant may actually represent a pharmacological treasure trove (Mechoulam, 2005).

Fifty two minor compounds isolated from a high potency *Cannabis sativa* were tested by radioligand binding assay for CB1 and/or CB2 affinity (Ross *et al*., 2009). The CB1 receptor binding assays revealed two compounds with significant binding affinities (0.8 ± 0.1 nM and 5.5 ± 1.0 nM, respectively) in comparison with THC (88.6 ± 6 nM). Seven compounds had affinities (46.2–141.6 nM) comparable with that of THC. Five compounds showed significant binding affinities (5.9–116.0 nM) for CB2 receptors; two of them were very selective as they showed no affinities for CB1 receptors. One compound showed very high affinity for both CB1 and CB2 receptors (0.8 ± 0.1 nM and 6 ± 2 nM, respectively), exceeding the synthetic positive control, CP55 940 (12 and 15 nM, respectively). The structures of the active compounds have not yet been reported.

Cannabidiol

Several overviews on CBD, with an emphasis on the biochemical and pharmacological advances, have been published (Mechoulam *et al*., 2007a, 2009; Scuderi *et al*., 2009). Here we address other aspects of CBD action.

As it was assumed that non-cannabinoid receptor mechanisms of CBD might contribute to its anti-inflammatory and neuroprotective effects, Ahrens *et al*. (2009) investigated the interaction of CBD with heterologously expressed α_1-homomeric and $\alpha_1\beta$-heteromeric strychnine-sensitive glycine receptors, expressed in HEK 293 cells. This in-vitro study showed that CBD has a positive allosteric modulating effect (EC_{50}: $\alpha_1 = 12.3 \pm 3.8$ μmol/l and $\alpha_1\beta = 18.1 \pm 6.2$ μmol/l). Direct activation of glycine receptors was observed at concentrations above 100 μmol/l (EC_{50}: α_1 = 132.4 ± 12.3 μmol/l and $\alpha_1\beta$ = 144.3 ± 22.7 μmol/l). These in-vitro results suggest CBD may mediate some of its anti-inflammatory and neuroprotective properties by activation of strychnine-sensitive glycine receptors.

Cannabidiol ameliorates cognitive and motor impairement in mice with bile duct ligation, a model of hepatic encephalopathy (Magen *et al*., 2009). The mechanism seems to involve the A(2) adenosine receptor, as the effects were blocked by a suitable antagonist. Cannabidiol also upregulated brain-derived neurotrophic-factor expression through a non-A(2) adenosine receptor mechanism. Cannabidiol administration may thus represent an adjunct treatment dealing with the central nervous system symptoms secondary to liver disease, along with other drugs improving liver function.

The serotonin 1A (5-HT_{1A}) receptor also seems to be involved in the activity of cannabidiol. Zanelati *et al*. (2009) have reported that the antidepressant-like effects of cannabidiol in mice possibly involve this

Marijuana and Madness, Second Edition, ed. David Castle, Robin M. Murray and Deepak Cyril D'Souza. Published by Cambridge University Press.

receptor. Mato *et al.* (2009) have reported that chronic fluoxetine modulates CB1-receptor-mediated inhibition of adenylyl cyclase in the rat prefrontal cortex through a 5-HT_{1A}-receptor-dependent mechanism.

Cannabidiol is also an inhibitor of ID-1 gene expression in aggressive breast cancer cells (McAllister *et al.*, 2007).

Δ^9-Tetrahydrocannabivarin

Δ^9-Tetrahydrocannabivarin (Δ^9-THCV), an analog of THC, which has a 3-carbon side chain rather than a 5-carbon side chain, as in THC, binds to both CB1 (mouse brain synaptosomes; K_i = 75.4 nM) and CB2 (CHO-hCB2 cell membranes; K_i = 62.8 nM) receptors. Unexpectedly, Δ^9-THCV behaves as a competitive CB1- and CB2-receptor antagonist (Adele *et al.*, 2005; Pertwee *et al.*, 2007). On this basis Δ^9-THCV was patented for treatment of diseases and conditions benefiting from neutral antagonism of the CB1 cannabinoid receptor: obesity, schizophrenia, epilepsy, cognitive disorders such as Alzheimer's, bone disorders, bulimia, obesity associated with non-insulin dependent diabetes and in the treatment of drug, alcohol and nicotine abuse or dependency (Guy and Pertwee, 2006). The pharmacology of Δ^9-THCV has recently been reviewed (Pertwee, 2008a).

Cannabigerol

Cannabigerol (CBG) was isolated from hashish in the 1960s (Gaoni and Mechoulam, 1964), but due to its apparent lack of psychoactivity (Mechoulam *et al.*, 1970) its pharmacology was not investigated further. However, in view of the various promising actions of cannabidiol that, likewise, is not psychoactive, there has been a renewed interest in this minor cannabinoid.

Cannabigerol inhibits keratinocyte proliferation in a concentration-dependent manner (IC_{50} = 2.3 μM) (Wilkinson and Williamson, 2007). It activates TRPV1 receptors, but with a significantly lower potency than cannabidiol, which is also a more potent inhibitor of cancer-cell growth than CBG (Ligresti *et al.*, 2006).

Cannabigerol is a partial agonist at both the CB1 and CB2 cannabinoid receptors. Cannabigerol binds to CB1 (mouse brain membranes) with K_i = 439 nM and to CB2 (hCB2-CHO cells) with K_i = 337 nM (Pertwee, 2008b). It also displays significant potency as a 5-HT_{1A}-receptor antagonist (Gauson *et al.*, 2009) and is a potent alpha-2-adrenoreceptor partial agonist (Cascio *et al.*, 2009). There are currently no publications in animal models of disease; however a patent on such applications has been submitted (Pertwee, 2008b).

Endocannabinoids: anandamide and 2-arachidonoylglycerol

The discovery of anandamide and 2-arachidonoyl glycerol (2-AG), and the background to these projects have been reviewed (Hanuš, 2007, 2009a, 2009b; Mechoulam, 2007b). Anandamide has been shown to act via the cell-surface G protein-coupled receptors, CB1 and CB2, and the ion channel receptor, TRPV1. Recent publications bring evidence that additional targets are the peroxisome proliferator-activated receptors α and γ (PPARα and PPARγ). Thus, anandamide and possibly other endocannabinoids act via both cell surface and nuclear receptors (O'Sullivan and Kendall, 2009).

The CB2 receptor is involved in the pathogenesis of experimental encephalopathy in mice, caused by thioacetamide-induced acute liver failure. This is an animal model for hepatic encephalopathy, a neuropsychiatric syndrome. In encephalopathic mice there was a significant increase in brain levels of 2-AG, and systemic administration of 2-AG led to improvement of the neurological score, cognitive function and activity. These actions are mediated in part by adenosine monophosphate (AMP)-activated protein kinase. The effect of 2-AG on the neurological score could be fully eliminated by a CB2 antagonist. Interestingly, the best results were obtained by combining an inhibitor of CB1 activation with an exogenous CB2 agonist, indicating that activation of the two endocannabinoid receptors leads to opposite results (Avraham *et al.*, 2006; Dagon *et al.*, 2007; for a review see Magen *et al.*, 2008).

Additional endocannabinoids

Noladin ether, the ether analog of 2-AG, was identified in pig brain by our group (Hanuš *et al.*, 2001; see also Fezza *et al.*, 2002), but two research groups (Oka *et al.*, 2003; Richardson *et al.*, 2007) have not noted its presence in other tissues, and its existence as an endocannabinoid remains to be confirmed.

Tan *et al.* (2010) have identified a large number of endocannabinoid-like molecules, acyl amino acids, in the brain. The physiological function of most of these novel compounds is unknown.

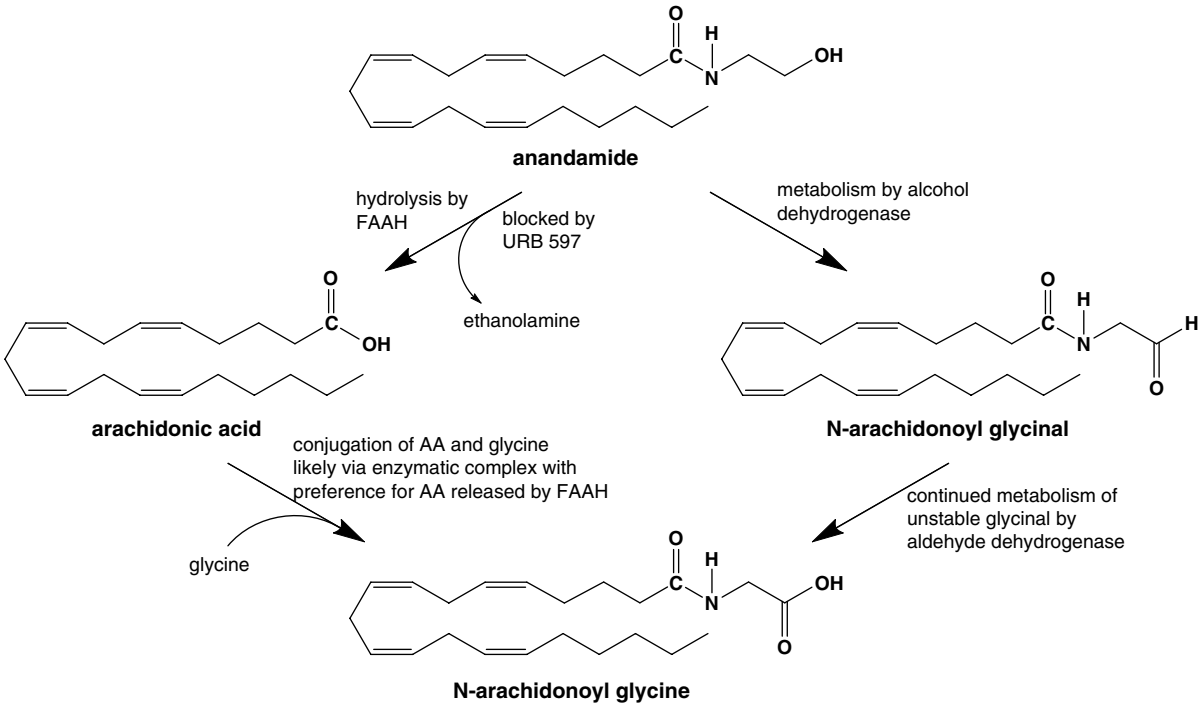

Figure 2.1. Biosynthetic pathways of N-arachidonoyl glycine.

Biosynthesis and inactivation of the endocannabinoids

The biosynthesis and degradation of endocannabinoids are of both basic and clinical interest and have recently been reviewed (Liu *et al.*, 2007; Hanuš, 2009b).

The human polymorphic cytochrome P450 2D6, which is present in high levels in the substantia nigra and pyramidal neurons of the cortex, plays an important role in the oxidation of endogenous substrates. Anandamide, which is one of its substrates, is metabolized to six oxygenated compounds (Snider *et al.*, 2008): 20-hydroxyeicosatetraenoic acid ethanolamide; 5,6-epoxyeicosatrienoic acid ethanolamide (5,6-EET-EA); 8,9-epoxyeicosatrienoic acid ethanolamide; 11,12-epoxyeicosatrienoic acid ethanolamide; and 14,15-epoxyeicosatrienoic acid ethanolamide. The anandamide-epoxygenated metabolite, 5,6-EET-EA, which is more stable than anandamide, is a potent and selective CB2 agonist (Snider *et al.*, 2009). It binds to the CB2 receptor with significantly higher affinity (K_i = 8.9 nM), and to the CB1 receptor with lower affinity (K_i = 3.2 μM) than anandamide. 5,6-Epoxyeicosatrienoic acid ethanolamide inhibits the forskolin-stimulated accumulation of cAMP in CHO cells, stably expressing the CB2 receptor (IC_{50} = 9.8 ± 1.3 nM).

Elucidation of the putative biological role of the oxygenated anandamide metabolites or prostaglandin-type metabolites is important for a full understanding of the role of anandamide in the body. However, data is still lacking as to whether these or related metabolites have physiological significance.

Bradshaw *et al.* (2009) have shown that anandamide acts as a biosynthetic precursor of the signaling lipid, N-arachidonoyl glycine. Indeed, some non-cannabinoid receptor effects of anandamide may be due to this lipid. Two biosynthetic pathways for this lipid have been put forward (see Figure 2.1).

Synthetic cannabinoids

Numerous new approaches toward cannabinoid ligands specific for the CB2 receptor have been reported (Huffman *et al.*, 2006; Han *et al.*, 2009). Several of these compounds have good affinity for the CB2 receptor and weak affinity for the CB1 receptor. Examples include: JWH-255 (24 nM vs. 4.3 μM); JWH-352 (31 nM vs. 1.5 μM); JWH-353 (47 nM vs. > 10 μM); and JWH-359 (13 nM vs. 2.9 μM).

Krishnamurthy *et al.* (2008) have reported the synthesis and characterization of new C1-substituted aryl analogs of Δ^8-THC. While some of these compounds are potent CB1 agonists, their specificity for this receptor was low. Thus, their most active compound, a 1′,1′,1′-dimethyl-2-thiophene derivative (K_i = 1.08 ± 0.04 nM for CB1 and 0.27 ± 0.01 nM for CB2), has high affinity at both receptors. Several compounds have in-vitro anti-glioma activities. No significant correlation between K_i and EC_{50} was found.

Several dimethylheptyl-Δ^8-THC derivatives and their 1-methoxy and 1-deoxy analogues have been prepared, and their affinities for the CB1 and CB2 receptor have been determined (Chen *et al.*, 2009). While the compounds in which the phenolic groups are not substituted have shown powerful binding to both receptors, the 1-methoxy-dimethylheptyl and the 1-deoxy-dimethylheptyl compounds have very low affinity to the CB1 receptor (> 10 μM), and modest affinity for the CB2 receptors.

Conclusions

Cannabinoid and endocannabinoid chemistry, biochemistry and pharmacology continue to be active fields of research. While advances in these areas have widened our understanding of numerous physiological processes and pathological states, there are as yet no new major cannabinoid therapeutic agents. As several companies are working on CB2 agonists, it is possible that we shall yet see cannabinoid-based drugs.

References

Adele, T., Stevenson, L.A., Wease, K.N., *et al.* (2005) Evidence that the plant cannabinoid Δ^9-tetrahydrocannabivarin is a cannabinoid CB1 and CB2 receptor antagonist. *Brit J Pharmacol,* **146**:917–26.

Ahmed, S.A., Ross, S.A., Slade, D., Radwan, M.M., Khan, I.A., ElSohly, M.A. (2008a) Structure determination and absolute configuration of cannabichromanone derivatives from high potency *Cannabis sativa*. *Tetrahedron Lett*, **49**:6050–3.

Ahmed, S. A., Ross, S.A., Slade, D., *et al.* (2008b) Cannabinoid ester constituents from high-potency *Cannabis sativa*. *J Nat Prod*, **71**:536–42. [Erratum] *J. Nat. Prod*, **71**:1119.

Ahrens, J., Demir, R., Leuwer, M., *et al.* (2009) The nonpsychotropic cannabinoid cannabidiol modulates and directly activates alpha-1 and alpha-1-beta glycine receptor function. *Pharmacology* **83**:217–22.

Appendino, G., Giana, A., Gibbons, S., *et al.* (2008) A polar cannabinoid from *Cannabis sativa* var. Carma. *Nat Prod Commun*, **3**:1977–80.

Avraham, Y., Israeli, E., Gabbay, E., *et al.* (2006) Cannabinoids affect neurological and cognitive function in thioacetamid-induced hepatic encephalopathy. *Neurobiol Disease* **21**:237–45.

Bradshaw, H.B., Rimmerman, N., Hu, S.S-J., *et al.* (2009) The endocannabinoid anandamide is a precursor for the signaling lipid N-arachidonoyl glycine by two distinct pathways. *BMC Biochemistry* **10**:1–11.

Cascio, G.M., Gauson, L.A., Stevenson, L.A., Ross, R.A., Pertwee, R.G. (2009) Cannabigerol behaves as a potent alpha-2-adrenoreceptor partial agonist. 19th Annual Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society, p.73.

Chen, J., Wiley, J.L., Martin, B.R., Huffman, J.W. (2009) Synthesis of geminally dimethyl substituted analogues of Δ^8-THC. 19th Annual Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society, p.2.

Dagon, Y., Avraham, Y., Ilan, R., Mechoulam, R., Berry, E.M. (2007) Cannabinoids ameliorate cerebral function following liver failure via AMP-activated protein kinase. *FASEB J.* **21**:2431–41.

ElSohly, M.A., Slade, D. (2005). Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sci*, **78**:539–48.

Fezza, F., Bisogno, T., Minassi, A., Appendino, G., Mechoulam, R., Di Marzo, V. (2002) Noladin ether, a putative novel endocannabinoid: inactivation mechanisms and a sensitive method for its quantification in rat tissues. *FEBS Lett,* **513**:294–8.

Gaoni, Y. and Mechoulam, R. (1964) Isolation, structure and partial synthesis of an active constituent of hashish. *J Am Chem Soc*, **86**:1646–7.

Gauson, L.A., Cascio, G.M., Ross, R.A., Pertwee, R.G. (2009) Cannabigerol displays significant potency as a 5-HT_{1A} receptor antagonist. 19th Annual Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society, p.25.

Guy, G. and Pertwee, R. (2006) Cannabinoids, in particular tetrahydrocannabivarin, as CB receptor antagonists, and therapeutic use. PCT Int. Appl., WO 2006054057 A2 20060526.

Han, S., Thatte, J., Jones, R.M. (2009) Recent advances in the discovery of CB2 selective agonists. *Ann Rep Med Chem*, **44**:227–46.

Hanuš, L.O. (2007) Discovery and isolation of anandamide and other endocannabinoids. *Chem Biodiv*, **4**:1828–41.

Hanuš, L.O. (2009a) Discovery and isolation of anandamide and other endocannabinoids. In Lambert, D. M. (ed) *Cannabinoids in Nature and Medicine*, Zurich: Verlag Helvetica Chimica Acta, Wiley VCH, pp. 251–66.

Hanuš, L.O. (2009b) Pharmacological and therapeutic secrets of plant and brain (Endo)Cannabinoids. *Med Res Rev*, **29**:213–71.

Hanuš L., Abu-Lafi S., Fride E., *et al.* (2001) 2-Arachidonoyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci. USA*, **98**:3662–5.

Huffman, J.W., Bushell, S.M., Joshi, S.N., Wiley, J.L., Martin, B.R. (2006) Enantioselective synthesis of 1-methoxy- and 1-deoxy-2'-methyl-Δ^8-tetrahydrocannbinols: New selective ligands for the CB2 receptor. *Bioorg Med Chem*, **14**:247–62.

Krishnamurthy, M., Gurley, S., Moore, B.M. (2008) Exploring the substituent effects on a novel series of C1'-dimethyl-aryl Δ^8-tetrahydrocannabinol analogs. *Bioorg Med Chem*, **16**:6489–500.

Ligresti, A., Moriello, A.S., Starowicz, K., *et al.* (2006) Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J Pharmacol Exp Ther,* **318**:1375–87.

Liu, J., Wang, L., Harvey-White, J., *et al.* (2007) Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacol*, **54**:1–7.

Magen, I., Avraham, Y., Berry E., Mechoulam, R. (2008) Endocannabinoids in liver disease and hepatic encephalopathy. *Curr Pharm Design*, **14**:2362–9.

Magen, I., Avraham, Y., Ackerman, Z., Vorobiev, L., Mechoulam, R., Berry. E.M. (2009) Cannabidiol ameliorates cognitive and motor impairement in mice with bile duct ligation. *J. Hepatology,* **51**:528–34.

Mato, S., Vidal, R., Castro, E., Diaz, A., Pazos, A., Valdizan, E.M. (2009) Chronic fluoxetine modulates CB1 receptor mediated inhibition of adenylyl cyclase in the rat prefrontal cortex through 5-HT1A receptor dependent mechanism. *Mol Pharmacol.* (Epub ahead of print).

McAllister, S.D., Christian, R.T., Horowitz, M.P., Garcia, A., Desprez, P.-Y. (2007) Cannabidiol as a novel inhibitor of Id-1 gene expression in aggressive breast cancer cells. *Mol Cancer Ther*, **6**:2921–7.

Mechoulam, R. (2005) Plant cannabinoids: a neglected pharmacological treasure trove. *Br J Pharmacol*, **146**:913–5.

Mechoulam, R. (2007b) Conversation with Raphael Mechoulam. *Addiction,* **102**:887–93.

Mechoulam, R., Shani, A., Edery, H., Grunfeld, Y. (1970) The chemical basis of hashish activity. *Science,* **169**:611–2.

Mechoulam, R., Peters, M., Murillo-Rodriguez, E., Hanuš, L.O. (2007a) Cannabidiol – Recent Advances. *Chem Biodiv*, **4**:1678–92.

Mechoulam, R., Peters, M., Murillo-Rodriguez, E., Hanuš, L.O. (2009) Cannabidiol – recent advances. pp.83–101. In Lambert, D. M. (ed) *Cannabinoids in Nature and Medicine*, Zurich: Verlag Helvetica Chimica Acta, Wiley VCH, pp. 83–101.

Oka, S., Tsuchie, A., Tokumura, A., *et al.* (2003) Ether-linked analogue of 2-arachidonoyl glycerol (noladin ether) was not detected in the brains of various mammalian species. *J Neurochem,* **85**:1374–81.

O'Sullivan, S.E. and Kendall, D.A. (2009) Cannabinoid activation of peroxisome proliferator-activated receptors: Potential for modulation of inflammatory disease. *Immunobiology* (Epub ahead of print).

Pertwee, R.G. (2008a) The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ^9-tetrahydrocannabinol, cannabidiol and Δ^9- tetrahydrocannabivarin. *Brit J Pharmacol*, **153**:199–215.

Pertwee, R.G. (2008b) Cannabigerol as CB1 and CB2 cannabinoid receptor agonist and therapeutic use. Patent Dec 31, GB 2450493 A.

Pertwee, R.G., Thomas, A., Stevenson, L.A., *et al.* (2007) The psychoactive plant cannabinoid, Δ^9-tetrahydrocannabinol, is antagonized by Δ^8- and Δ^9-tetrahydrocannabivarin in mice in vivo. *Brit J Pharmacol*, **150**:586–94.

Radwan, M.M., ElSohly, M.A., Slade, D., Ahmed, S.A., Khan, I.A., Ross, S.A. (2009) Biologically Active Cannabinoids from High-Potency *Cannabis sativa*. *J Nat Prod*, **72**:906–11.

Radwan, M.M., Ross, S.A., Slade, D., Ahmed, S.A., Zulfiqar, F., ElSohly, M.A. (2008) Isolation and characterization of new cannabis constituents from a high potency variety. *Planta Med*, **74**:267–72.

Richardson, D., Ortori, C.A., Chapman, V., Kendall, D.A., Barrett, D.A. (2007) Quantitative profiling of endocannabinoids and related compounds in rat brain using liquid chromatography-tandem electrospray ionization mass spectrometry. *Anal Biochem,* **360**:216–26.

Ross, S.A., ElSohly, M.A., Sultana, G.N.N., Mehmedic, Z., Hossain, C.F., Chandra, S. (2005) Flavonoid glycosides and cannabinoids from the pollen of *Cannabis sativa* L. *Phytochem Anal*, **16**:45–8.

Ross, S.A., Radwan, M.M., Ahmed, S.A., *et al.* (2009) Binding affinities of minor cannabis constituents to CB1 and CB2 receptors. 19th Annual Symposium on the Cannabinoids, Burlington, Vermont, International Cannabinoid Research Society, p.74.

Scuderi, C., De Filippis, D., Iuvone, T., Blasio, A., Steardo, A., Esposito, G. (2009) Cannabidiol in medicine: a review of its therapeutic potential in CNS disorders. *Phytother Res,* **23**:597–602.

Snider, N.T., Nast, J.A., Tesmer, L.A., Hollenberg, P.F. (2009) A cytochrome P450-derived epoxygenated metabolite of anandamide is a potent cannabinoid Receptor 2-selective agonist. *Mol Pharmacol*, **75**:965–72.

Snider, N.T., Sikora, M.J., Sridar, C., Feuerstein, T.J., Rae, J.M., Hollenberg, P.F. (2008) The endocannabinoid anandamide is a substrate for the human polymorphic cytochrome P450 2D6. *J Pharmacol Exp Ther*, **327**:538–45.

Tan, B., O'Dell, D.K., Yu, Y.W., *et al*. (2010) Identification of endogenous acyl amino acids based on a targeted lipidomics approach. *J Lipid Res,* **51**:112–9.

Wilkinson, J.D. and Williamson, E.M. (2007) Cannabinoids inhibit human keratinocyte proliferation through a non-CB1/CB2 mechanism and have a potential therapeutic value in the treatment of psoriasis. *J Dermatol Sci,* **45**:87–92.

Zanelati, T., Biojone, C., Moreira, F., Guimarães, F., and Joca, S. (2009) Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT receptors. *Br J Pharmacol* (Epub ahead of print).

Chapter 3

The function of the endocannabinoid system

Maria Grazia Cascio and Roger Pertwee

The plant *Cannabis sativa* has been widely used by humans over many centuries as a source of fiber, for medicinal purposes, for religious ceremonies and as a recreational drug. Currently almost 500 compounds have been identified in this plant (ElSohly and Slade, 2005). Among them are at least 70 phytocannabinoids, all of which are terpenophenolic compounds uniquely present in *Cannabis sativa*. Two phytocannabinoids that have attracted particular attention are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), the structures and stereochemistry of which were elucidated in the 1960s (Mechoulam and Shvo, 1963; Mechoulam and Gaoni, 1965; Pertwee, 2008). Δ^9-Tetrahydrocannabinol is considered to be the main psychotropic constituent of cannabis, whereas CBD lacks psychotropic activity but does possess anti-inflammatory and anti-psychotic properties (see Chapter 2).

Originally, because of its hydrophobic nature, it was suggested that the effects of THC were due to a non-specific perturbation of cell membranes. Subsequently, however, after the synthesis of the first THC enantiomers (Mechoulam *et al*., 1980, 1988), it was observed that the pharmacological actions of THC were stereoselective, raising the possibility that it might be targeting a specific receptor. Eventually a "cannabinoid receptor" was indeed discovered, opening up a new "era" in the field of cannabinoid research (Pertwee, 2006a). Although many of the effects of THC are cannabinoid receptor-mediated, evidence has emerged that at least some naturally occurring and synthetic cannabinoids can also target other receptors (Pertwee, 2010). These include the transient receptor potential (TRP) cation channel, TRPV1 (Zygmunt *et al*., 1999), nuclear peroxisome-proliferator-activated receptors (PPARs) (O'Sullivan, 2007), certain transmitter-gated channels and ion channels (Oz, 2006) and also several G-protein-coupled receptors, for example the orphan receptor, GPR55 (Ross, 2009). Review articles that provide more detailed information and list additional references have been cited throughout this chapter. The reader is also referred to Chapters 1 and 2 of this book.

Cannabinoid receptors

So far, two types of cannabinoid receptor, CB1 (Devane *et al*., 1988; Matsuda *et al*., 1990) and CB2 (Munro *et al*., 1993), have been identified. Both are 7-transmembrane receptors that signal through $G_{i/o}$ proteins to inhibit adenylate cyclase and activate mitogen-activated protein kinase (Howlett, 2002, 2005). Cannabinoid CB1 receptors, cloned in 1990, can also mediate inhibition of N-type and P/Q-type calcium currents, and activation of A-type and inwardly rectifying potassium currents. These receptors are mainly located in the terminals of central and peripheral neurons, where they mediate inhibition of ongoing release of various neurotransmitters that include acetylcholine, noradrenaline, dopamine, 5-hydroxytryptamine, γ-aminobutyric acid, glutamate, D-aspartate and cholecystokinin (Howlett, 2002; Pertwee and Ross, 2002). CB1 receptors have also been found at lower levels in testis, heart, vascular tissue and immune cells. Cannabinoid CB2 receptors, first cloned in 1993, were originally considered to be "peripheral receptors" because of their presence in immune cells and involvement in inflammatory reactions and immune responses. However, the expression of CB2 receptor mRNA and protein has now also been detected in some brainstem neurons (Van Sickle *et al*., 2005; Gong *et al*., 2006; Onaivi *et al*., 2006).

Endocannabinoids

Cannabinoid receptors can be activated not only by cannabis-derived and synthetic agonists but also by endogenous cannabinoids produced in mammalian tissues and usually referred to as "endocannabinoids" (Figure 3.1). The first endocannabinoid

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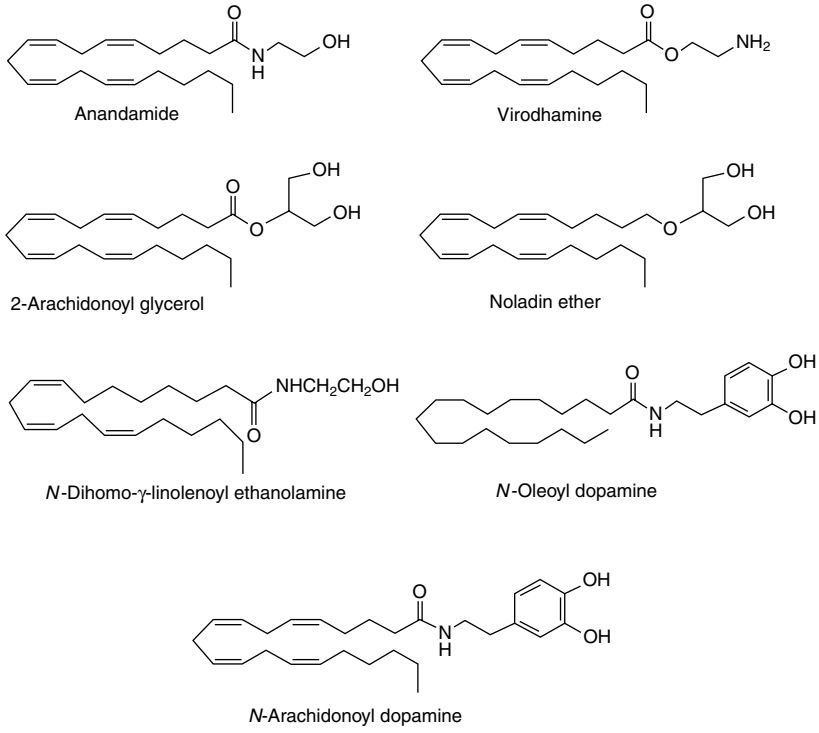

Figure 3.1. Chemical structures of some endocannabinoids.

was discovered in 1992 (Devane *et al*., 1992; Hanuš, 2007). This was the ethanolamide of arachidonic acid, *N*-arachidonoylethanolamine (anandamide; AEA), and is thought to be a partial CB1- and CB2-receptor agonist, as well as a TRPV1 receptor agonist (Devane *et al*., 1992; Smart *et al*., 2000; Al-Hayani *et al*., 2001; Di Marzo *et al*., 2001). Subsequently virodhamine, an endogenous molecule with the same molecular weight as anandamide, was discovered. In this molecule, arachidonic acid and ethanolamine are joined by an ester linkage, and not by an amide linkage as in anandamide (Porter *et al*., 2002). Virodhamine behaves as a CB2-receptor agonist and CB1-receptor partial agonist/antagonist. A second chemical class of endocannabinoid is represented by 2-arachidonoyl glycerol (2-AG). This is the arachidonate ester of glycerol, and activates CB1 and CB2 receptors with similar potency and efficacy (Mechoulam *et al*., 1995; Sugiura *et al*., 1995). There may also be a third ether-type class of endocannabinoid. Thus, there is some evidence that 2-arachidonoyl glyceryl ether (noladin ether, 2AGE) is an endogenous molecule that binds with relatively high affinity to CB1, and more weakly to CB2 (Hanuš *et al*., 2001). There is evidence too that *N*-arachidonoyl dopamine (NADA) is an endocannabinoid. Like AEA, it behaves as an agonist at both CB1 and TRPV1 receptors (Huang *et al*., 2002; De Petrocellis *et al*., 2004) and can antagonize the melastatin type-8 (TRPM8) cation channel (De Petrocellis *et al*., 2007). Other compounds that are thought be endocannabinoids include *N*-dihomo-γ-linolenoyl ethanolamine and *N*-oleoyl dopamine (OLDA) (Pertwee, 2005).

Biosynthesis of the endocannabinoids

Endocannabinoids are not stored in cells awaiting release, but are rather synthesized on demand in a Ca^{2+}-dependent manner in response to physiological or pathological stimuli (Di Marzo and Deutsch, 1998). Anandamide belongs to the large family of *N*-acylethanolamines (NAEs) and is generated by the hydrolysis of its precursor, *N*-acylphosphatidylethanolamine (NAPE) (Schmid *et al*., 1983, 1990, 1996; Hansen *et al*., 1998; Schmid and Berdyshev, 2002), a process that is catalyzed by the enzyme, NAPE-phospholipase D (NAPE-PLD) (Figure 3.2). Alternative biosynthetic pathways have also been proposed (Figure 3.2). For example, there is evidence that AEA is formed from *N*-acyl-lysophosphatidylethanolamine by a lysophospholipase-D-like enzyme (lysoPLD) (Sun *et al*., 2004). More recently, Simon and Cravatt (2006) reported the identification of an additional enzyme, alpha/beta/-

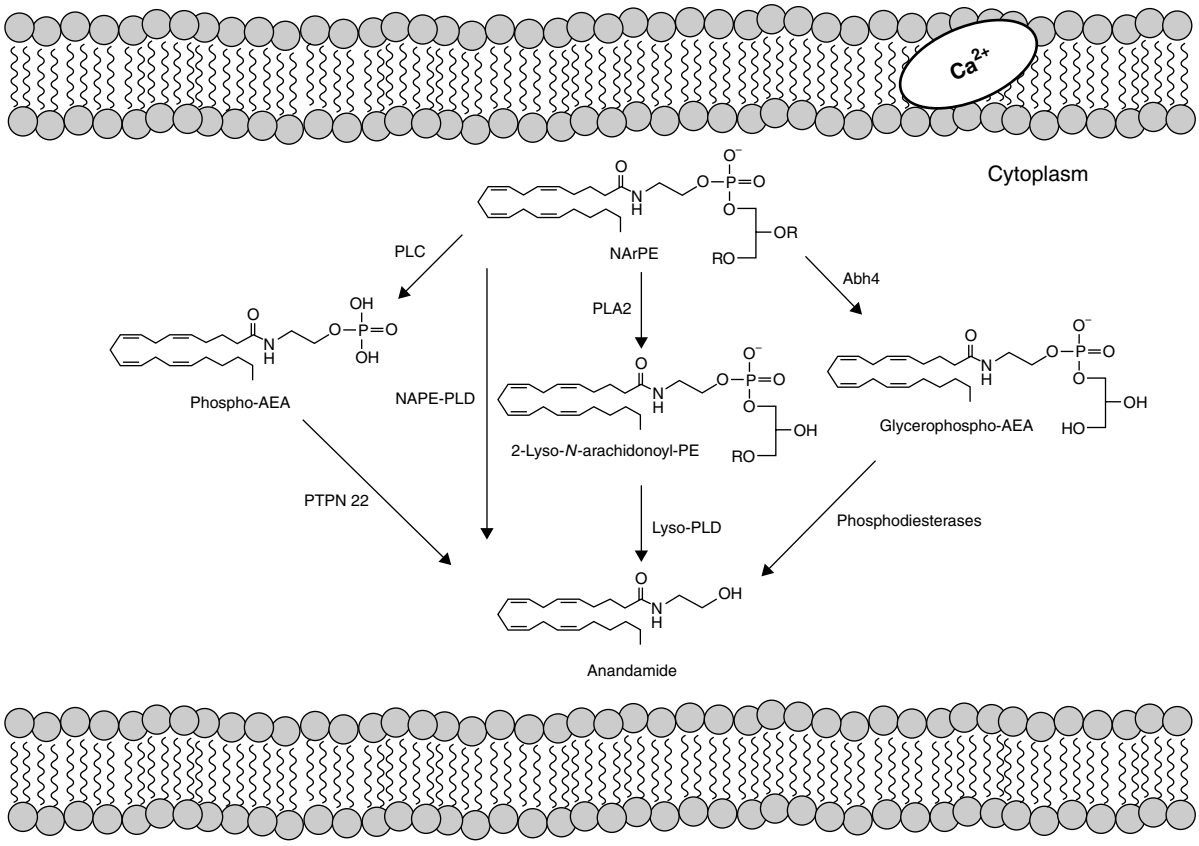

Figure 3.2. Schematic representation of anandamide biosynthesis routes. Abh4, alpha/beta-hydrolase 4; AEA, anandamide; NArPE, *N*-arachidonoylphosphatidylethanolamine; PLA_2, phospholipase A_2; PLC, phospholipase C; PE, phosphatidylethanolamine; PLD, phospholipase D; PTPN22, protein tyrosine phosphatase.

hydrolase 4 (Abh4), which can act on either NAPE (as shown in Figure 3.2) or lyso-NAPE to generate glycerophospho-arachidonoylethanolamide (GpAEA), that is subsequently converted to AEA in the presence of a phosphodiesterase. Finally, Liu and co-workers (2006) have obtained evidence for an alternative pathway in which NAPE is hydrolyzed to phosphoanandamide (pAEA) by phospholipase C (PLC), and pAEA dephosphorylated by phosphatases to AEA. As for 2-AG, many pathways have been proposed for its biosynthesis (Sugiura *et al*., 2002). However, the current view is that 2-AG is generated mainly through hydrolysis of 2-arachidonate-containing diacylglycerols (DAGs), reactions that are catalyzed by an *sn*-1 selective DAG lipase (Bisogno *et al*., 2003) (Figure 3.3).

Degradation of the endocannabinoids

After targeting their receptors, the endocannabinoids AEA and 2-AG are inactivated via a two-step process that begins with their transport from the extracellular to the intracellular space, and culminates in their intracellular degradation by hydrolysis or oxidation. So far, the mechanism responsible for endocannabinoid transport across the cell membrane is still unclear because, unlike several other proteins/enzymes that form part of the endocannabinoid system (ECS), the putative "*endocannabinoid membrane transporter*" (EMT) has yet to be cloned. Consequently, only indirect evidence for its existence has been reported in the literature to date. After their cellular uptake, AEA and 2-AG are metabolized by two different processes. The main process is the hydrolysis of AEA by fatty acid amide hydrolase (FAAH), and of 2-AG mainly by monoacylglycerol lipase (MGL), but also by FAAH. In addition to these two enzymes, an *N*-acylethanolamine-selective acid amidase (NAAA) (Ueda *et al*., 1999) and more recently, a second FAAH (Wei *et al*., 2006), as well as ABHD6 and ABHD12 (Blankman *et al*., 2007) have been reported to participate in the degradation of several

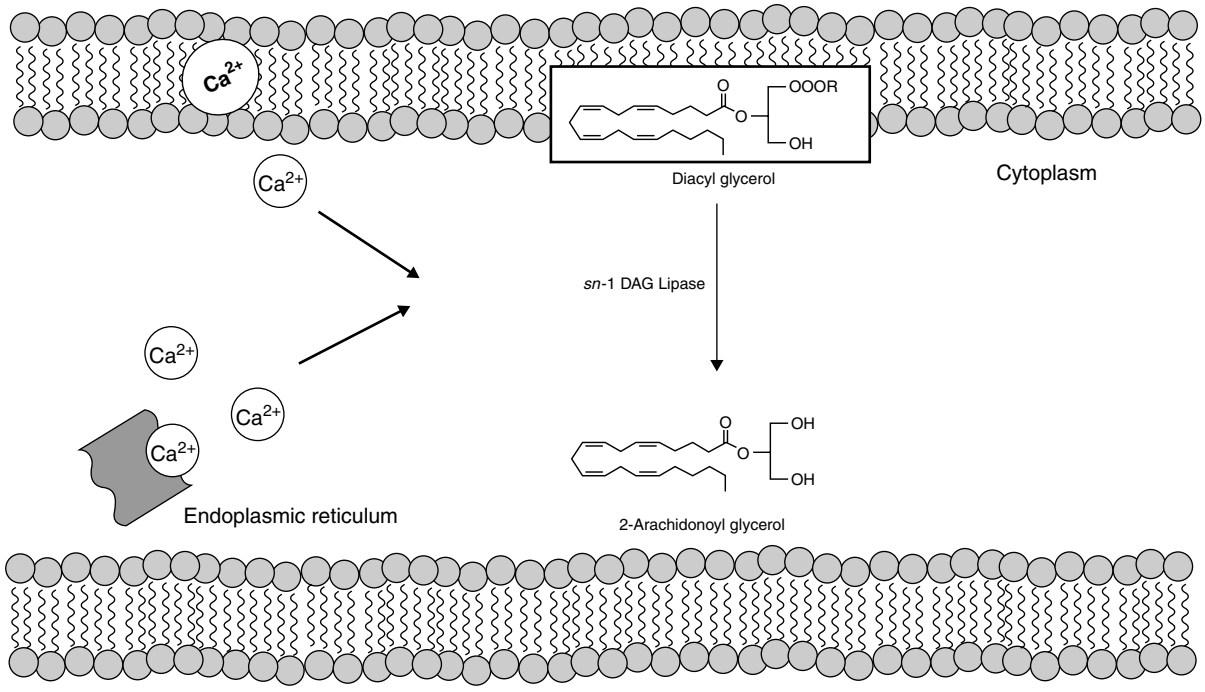

Figure 3.3. Schematic representation of the main 2-AG (2-arachidonoyl glycerol) biosynthesis route. DAG, diacylglycerol.

endocannabinoids. Both AEA and 2-AG can also be degraded by oxidation, catalyzed by cyclooxygenase-2 (COX-2) and the 12- and 15-lipoxygenases, 12-LOX and 15-LOX (Yates and Barker, 2009), to produce active prostaglandin ethanolamides (Yu *et al*., 1997) and glyceryl prostaglandins (Kozak *et al*., 2000), respectively.

The endocannabinoid system in health and disease

Some physiological functions

The physiological roles of the ECS are still a subject of intense research and speculation. However, there are already numerous data in the literature that provide strong support for the notion that the ECS plays a crucial role in the modulation of several other systems that range from the central and autonomic nervous systems to the endocrine system, the gastrointestinal tract and the reproductive, immune and cardiovascular systems (Di Marzo, 1998; Pertwee, 2005). Below, we highlight just three of the many physiological processes that are thought to have significant links with the ECS. The reader is also referred to Chapter 1 of this book.

1. *Retrograde signaling*. Endocannabinoids are known to mediate *retrograde signaling* at central synapses, a process in which stimulus-dependent synthesis of endocannabinoids in post-synaptic neurons leads to the activation of presynaptic CB1 receptors, and a subsequent inhibition of neurotransmitter release (Gerdeman, 2008). Presynaptic inhibition of transmitter release by endocannabinoids may give rise to two different forms of short-term synaptic plasticity. These are depolarization-induced suppression of inhibition (DSI), which involves GABAergic transmission, and depolarization-induced suppression of excitation (DSE), which involves glutamatergic transmission (Wilson and Nicoll, 2002; Diana and Marty, 2004). Endocannabinoid-induced DSI and DSE seem to play an important role in the coordination of neural networks within the hippocampus and cerebellum that are involved in physiological processes such as memory and motor coordination (Wilson and Nicoll, 2001; Wilson *et al*., 2001; Diana *et al*., 2002). As recently reviewed by Rodríguez de Fonseca *et al*. (2005), additional forms of synaptic transmission involve the induction of long-term synaptic plasticity: long-term potentiation (LTP) and long-term depression (LTD). Cannabinoid-receptor activation prevents the induction of LTP in

hippocampal synapses (Stella *et al*., 1997), and a facilitation of LTD in the striatum (Gerdeman *et al*., 2002) and the nucleus accumbens (Robbe *et al*., 2002). In the hippocampus, endocannabinoid messengers regulate a form of LTD that affects inhibitory GABAergic neurons (Chevaleyre and Castillo, 2003).

2. *Control of food intake*. That the ECS is involved in the regulation of food intake is supported by the following findings: (1) THC induces signs of hyperphagia by activating cannabinoid CB1 receptors (Williams *et al*., 1998; Williams and Kirkham, 2002); (2) low doses of AEA are able to increase food intake when administered either systemicaly (Williams and Kirkham, 1999; Hao *et al*., 2000) or into the ventromedial hypothalamus (Jamshidi and Taylor, 2001); (3) drugs that block cannabinoid CB1 receptors, such as SR141716, AM251 or AM1387, suppress food intake and disrupt food-reinforced behavior (Salamone *et al*., 2007); (4) food-deprived $CB1^{-/-}$ mice eat less than their wild-type littermates, and SR141716 does not affect the food intake of these animals but does reduce the food intake of wild-type mice to that of $CB1^{-/-}$ mice (Di Marzo *et al*., 2001; Wiley *et al*., 2005); (5) levels of endocannabinoids are elevated in leptin-deficient mice and rats, suggesting that endocannabinoids form part of the leptin-regulated neural circuitry that is involved in appetite regulation (Di Marzo *et al*., 2001).
3. *Control of the reproductive system*. As recently reviewed elsewhere, most human reproductive cells and tissues, including blastocytes, spermatozoa, uterus and testis, contain all components of the ECS (Battista *et al*., 2008), supporting the hypothesis that it plays a pivotal role in the regulation of both the female and male reproductive systems. In particular, it has been reported that endogenous levels of AEA are tightly regulated from the beginning of pregnancy, and that a dysregulation of AEA production seriously compromises pregnancy. Fatty acid amide hydrolase and COX-2, both enzymes involved in AEA degradation, help to keep AEA levels within the range needed to achieve a successful pregnancy (Wang *et al*., 2007). In males, *N*-acylethanolamines have been detected in human reproductive fluids (Schuel *et al*., 2002), in rodent testis (Cobellis *et al*., 2006), in Sertoli cells (Maccarrone *et al*., 2003) and in boar spermatozoa (Maccarrone *et al*., 2005). As for cannabinoid receptors, it seems that while CB1 receptors contribute to normal embryo development, and oviductal CB1 receptors mediate the timely transport of embryos from oviduct to uterus, CB2 receptors also play a crucial role in certain reproductive processes that include embryo development in females and spermatogenesis in males (Maccarrone, 2008).

Selected pathological functions

As discussed in greater detail elsewhere, the ECS has been reported to become upregulated in a wide range of disorders (Pertwee, 2005, 2006b, 2007). More specifically, in some disorders, such as multiple sclerosis, cancer, schizophrenia, post-traumatic stress disorders, certain types of pain, some intestinal and cardiovascular diseases, excitotoxicity and traumatic head injury, this upregulation may cause a reduction in the severity of symptoms, or a slowing of disease progression. There are also disorders, however, in which upregulation of the ECS contributes to the production or exacerbation of unwanted effects (Pertwee, 2005, 2006b). These disorders include obesity, impaired fertility, stroke, cystitis, ileitis and paralytic ileus. Current knowledge about the "autoprotective" and "autoimpairing" roles of the ECS in just a few of these pathological conditions is summarized below.

1. *Multiple sclerosis*. This is a disease of the central nervous system, in which the ability of neurons to conduct impulses becomes impaired through the loss of myelin, which normally forms the outer covering of many nerve fibers (Pertwee, 2007). As a consequence, people with multiple sclerosis (MS) show a variety of symptoms that can include tremor, spasticity and pain, as well as bladder and sexual dysfunction. Current treatment of MS involves the administration of anti-inflammatory, immunosuppressive and immunomodulatory drugs that, unfortunately, are frequently not particularly effective and can cause many side effects. It is noteworthy, therefore, that there is evidence that: (1) the ECS is activated in the central nervous system of MS patients; (2) endocannabinoids exert immunosuppressant and anti-inflammatory actions, and play a neuroprotective role in MS; and (3) augmenting levels of endocannabinoids by reducing their degradation and/or cellular uptake,

could constitute an important new strategy for treating this disorder (Hemmer *et al*., 2002; Baker *et al*., 2007; Pertwee, 2007). Thus, both FAAH inhibitors and inhibitors of endocannabinoid cellular uptake, for example AM374, AM404, VDM11, OMDM-1 and OMDM-2, show anti-spastic effects in a mouse model of MS (CREAE) (Baker *et al*., 2001; de Lago *et al*., 2004). The anti-spastic effects of AM374 were blocked by both SR141716 and the CB2-selective inverse agonist/antagonist, SR144528, supporting the hypothesis that cannabinoid CB1 and CB2 receptors mediate modulation of spasticity in MS. This hypothesis is also supported by the observation that $CB1^{-/-}$ CREAE mice show an earlier onset of spasticity and increased mortality compared with CB1 wild-type CREAE mice (Pryce *et al*., 2003). Exogenously administered cannabinoid-receptor agonists can also oppose spasticity in MS. Thus, the CB1/CB2-receptor agonists: AEA, 2-AG, *R*-(+)-WIN55 212 and THC; the selective CB1 receptor agonists: *R*-(+)-methanandamide and arachidonoyl-2-chloroethylamide (ACEA); and the selective CB2 receptor agonists: JWH-133 and JWH-015, have all been found to reduce spasticity, tremor and spasm in animal models of MS (Pertwee, 2005, 2007). There is also convincing evidence that cannabinoid receptor activation can ameliorate MS symptoms in patients (Pertwee, 2005). Indeed the Δ^9-THC- and CBD-containing medicine, Sativex, is now licensed in the UK and certain other European countries as an add-on treatment for symptom relief in patients with moderate to severe refractory spasticity caused by MS (Sastre-Garriga *et al*., 2011).

2. *Pain*. As outlined in Chapter 1 there is evidence that cannabinoid receptor agonists can reduce various kinds of pain, including acute, neuropathic, inflammatory, visceral and cancer pain, by acting on both CB1 and CB2 receptors that are located on pain pathways in the brain, spinal cord, peripheral sensory nerves and/or non-neuronal cells in the skin (Pertwee, 2001; 2005, 2009; Guindon and Hohmann, 2008). Evidence has also been obtained that certain kinds of pain, including inflammatory and neuropathic pain, trigger the release of endocannabinoids onto CB1 and CB2 receptors to induce signs of analgesia in animals, and that antinociception can be enhanced by compounds that inhibit the metabolism or cellular uptake of AEA or 2-AG. Thus, for example, two potent FAAH inhibitors, URB597 and OL135, have been reported to show high efficacy against signs of neuropathic pain, albeit not in all investigations, and also against signs of inflammatory pain (Jayamanne *et al*., 2006; Maione *et al*., 2007; Russo *et al*., 2007). There has also been a report that URB602, a monoacylglycerol lipase (MGL) inhibitor, elicited a dose-dependent, anti-edematous and antinociceptive effect in a murine model of inflammatory pain that was reversed exclusively by the CB2-receptor antagonist, SR144528 (Comelli *et al*., 2007). However, there has been another recent report that a novel MGL inhibitor, OMDM169, can reduce signs of formalin-induced inflammatory pain in a manner that seems to be both CB1- and CB2-receptor mediated (Bisogno *et al*., 2009). This effect was produced by doses of OMDM169 that elevated levels of 2-AG, but not of AEA. There has also been a report by Long and co-workers (2009) that another newly developed MGL inhibitor, JZL184, can induce an apparent cannabinoid CB1-receptor mediated inhibition of formalin-induced hyperalgesia. In addition, this compound produced some behavioral effects similar to those that can be produced by direct CB1-receptor agonists. Finally, since its two main constituents are THC and cannabidiol, it is noteworthy that Sativex is prescribed for the symptomatic relief of neuropathic pain in adults with multiple sclerosis (Perez and Ribera, 2008; Rahn and Hohmann, 2009) and as an adjunctive-analgesic treatment for adult patients with advanced cancer. Moreover, results from clinical trials suggest that nabilone, a synthetic cannabinoid receptor agonist, can relieve chronic neuropathic pain, fibromyalgia (diffuse musculoskeletal pain) and headache (Pinsger *et al*., 2006; Skrabek *et al*., 2008; Rahn and Hohmann, 2009).
3. *Anxiety and depression*. These are very common disorders world-wide, and represent a major cause of suicide. Importantly, therefore, evidence has emerged that the ECS plays a protective role in these disorders. Thus, cannabinoid receptors are present in the neuroanatomical structures and circuits implicated in depression are present in the neuroanatomical structures and circuits implicated in depression and anxiety, including

the prefrontal cortex, hippocampus, amygdala, hypothalamus and forebrain monoaminergic circuits (Herkenham *et al*., 1991). Moreover, it has also been found that: (1) $CB1^{-/-}$ mice display signs of increased anxiety in light-dark box, elevated plus-maze, and social interaction tests, and an increase in aggressive behavior in the resident-intruder test (Haller *et al*., 2002, 2004; Martin *et al*., 2002; Urigüen *et al*., 2004); (2) circulating levels of endocannabinoids decreased significantly in two different patient populations diagnosed with major depression (Hill *et al*., 2009); (3) CB1-receptor antagonists induce significant anxiogenic effects in animal models of anxiety (Navarro *et al*., 1997) as well as an increased incidence of depression and suicidality in obese patients (Nissen *et al*., 2008). In addition, there is evidence that anxiety can be reduced by increasing endogenous levels of endocannabinoids. Thus, mice and rats treated with a compound that inhibits either FAAH or endocannabinoid-cellular uptake display reduced anxiety-like behavior, suggesting that a facilitation of endocannabinoid tone in vivo could constitute a therapeutic strategy for the treatment of mood disorders (Bortolato *et al*., 2006; Rutkowska *et al*., 2006). Chapter 10 provides a detailed discussion of cannabis and depression.

4. *Parkinson's disease*. This is a chronic and progressive neurodegenerative disorder characterized by a severe loss of dopaminergic neurons in the substantia nigra pars reticulata (SNr), reduced dopamine levels and a loss of dopaminergic neurotransmission in the striatum, which interferes with motor function and coordination. The main symptoms of Parkinson's disease (PD) are resting tremor, muscular rigidity and bradykinesia/akinesia (Rodriguez-Oroz *et al*., 2009). In the past, the usual treatment for early PD has been levodopa, but long-term treatment with this drug causes unpleasant side effects in patients, such as disabling motor fluctuations and dyskinesia (Lang and Lozano, 1998; Ahlskog, 2001). Recently, new therapies have been developed that include the use of both monoamine oxidase B (MAO-B) inhibitors and anticholinergic agents (Horn and Stern, 2004). Importantly, there is evidence that overactivity of the ECS contributes to the production of bradykinesia in PD, raising the possibility that this major parkinsonian symptom could be ameliorated in the clinic with a CB1-receptor antagonist (Fernández-Ruiz, 2009). On the other hand, CB1 receptors seem to down-regulate/desensitize in the early presymptomatic phase of PD in a manner that may exacerbate the onset of neurodegeneration (Fernández-Ruiz, 2009). Consequently, it might well be possible to slow the development of PD with either a CB1 receptor agonist or a drug that can enhance endogenous levels of endocannabinoids within the basal ganglia. There is evidence too that underactivity of the ECS may contribute to the development of dyskinesia caused by long-term administration of levodopa to PD patients (Romero *et al*., 2000). Evidence has also recently emerged that combined blockade of CB1 receptors and activation of CB2 receptors may not only relieve some symptoms, but also slow disease progression in PD, raising the possibility that the phytocannabinoid, Δ^9-tetra hydrocannabinol, which is both a CB1 antagonist and a CB2 agonist, could be used to treat this disease (Garcia *et al*., 2011).
5. *Obesity*. A role for the ECS in obesity has been articulated in Chapter 1. That the ECS plays a significant role in endocrine and metabolic regulation and energy balance (Pagotto *et al*., 2006; Matias and Di Marzo, 2007; Cota, 2008; Di Marzo, 2008) is supported by the following observations: (1) CB1 receptor antagonists are significantly more efficacious in reducing caloric intake and body weight in rodents with diet-induced or genetic obesity than in their respective lean controls (Di Marzo *et al*., 2001; Ravinet Trillou *et al*., 2003; Vickers *et al*., 2003); (2) $CB1^{-/-}$ mice are resistant to diet-induced obesity (Ravinet Trillou *et al*., 2004; Osei-Hyiaman *et al*., 2005); and (3) both an upregulation of CB1 receptors and elevated endocannabinoid levels have been detected in the adipose tissue of obese compared with lean patients (Bensaid *et al*., 2003; Matias *et al*., 2006). Importantly, CB1 receptor antagonists show significant anti-obesity effects. More specifically, promising successes have been attained with rimonabant, which has been found to reduce food intake in both lean and obese rodents (Perez and Ribera, 2008) and to lower body weight both in experimental models of obesity and in clinical trials (Després, 2009).

Unfortunately, however, the use of rimonabant in the clinic has been suspended because of serious psychiatric side effects, particularly an increased incidence of depression and suicidality (Nissen *et al*., 2008). CB1 receptor antagonists other than rimonabant, including the cannabis-derived compound Δ^9-tetrahydrocannabivarin (see Chapter 2), have also been reported to induce hypophagic and weight-reducing effects (Di Marzo, 2008; Pertwee and Thomas, 2009; Riedel *et al*., 2009).

Conclusions

The discovery of the ECS has prompted a number of important advances in the field of cannabinoid research. As a result, it is now generally accepted that this system is a key player in several physiological processes and pathological conditions in both central and peripheral tissues. One challenge now is to develop new medicines from compounds that target cannabinoid receptors directly (Pertwee, 2009; Pertwee and Thomas, 2009), or that affect tissue levels of endocannabinoids at their receptors (Petrosino and Di Marzo, 2010) for the amelioration of a range of disorders.

References

Ahlskog, J.E. (2001) Parkinson's disease: medical and surgical treatment. *Neurol Clin,* **19**: 579–605.

Al-Hayani, A., Wease, K.N., Ross, R.A., *et al*. (2001) The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice. *Neuropharmacology,* **41**:1000–5.

Baker, D., Jackson, S.J. and Pryce, G. (2007) Cannabinoid control of neuroinflammation related to multiple sclerosis. *Brit J Pharmacol,* **152**:649–54.

Baker, D., Pryce, G., Croxford, J.L., *et al*. (2001) Endocannabinoids control spasticity in a multiple sclerosis model. *FASEB Journal,* **15**:300–2.

Battista, N., Pasquariello, N., Di Tommaso, M., *et al*. (2008) Interplay between endocannabinoids, steroids and cytokines in the control of human reproduction. *J Neuroendocrinol,* **20**:82–9.

Bensaid, M., Gary-Bobo, M., Esclangon, A., *et al*. (2003) The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol,* **63**:908–14.

Bisogno, T., Howell, F., Williams, G., *et al*. (2003) Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol,* **163**:463–8.

Bisogno, T., Ortar, G., Petrosino, S., *et al*. (2009) Development of a potent inhibitor of 2-arachidonoyl glycerol hydrolysis with antinociceptive activity in vivo. *Biochim Biophys Acta,* **1791**:53–60.

Blankman, J.L., Simon, G.M. and Cravatt, B.F. (2007) A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoyl glycerol. *Chemistry and Biology,* **14**:1347–56.

Bortolato, M., Campolongo, P., Mangieri, R.A., *et al*. (2006) Anxiolytic-like properties of the anandamide transport inhibitor AM404. *Neuropsychopharmacology,* **31**:2652–9.

Chevaleyre, V. and Castillo, P.E. (2003) Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron,* **38**:461–72.

Cobellis, G., Cacciola, G., Scarpa, D., *et al*. (2006) Endocannabinoid system in frog and rodent testis: type-1 cannabinoid receptor and fatty acid amide hydrolase activity in male germ cells. *Biol Reprod,* **75**:82–9.

Comelli, F., Giagnoni, G., Bettoni, I., *et al*. (2007) The inhibition of monoacylglycerol lipase by URB602 showed an anti-inflammatory and anti-nociceptive effect in a murine model of acute inflammation. *Brit J Pharmacol,* **152**:787–94.

Cota, D. (2008) Role of the endocannabinoid system in energy balance regulation and obesity. In *Obesity and Metabolism,* Korbonits, M. (ed), Basel: Karger, pp.135–45.

de Lago, E., Ligresti, A., Ortar, G., *et al*. (2004) In vivo pharmacological actions of two novel inhibitors of anandamide cellular uptake. *Eur J Pharmacol,* **484**:249–57.

De Petrocellis, L., Cascio, M.G. and Di Marzo, V. (2004) The endocannabinoid system: a general view and latest additions. *Brit J Pharmacol,* **141**:765–74.

De Petrocellis, L., Starowicz, K., Moriello Schiano, A., *et al*. (2007) Regulation of transient receptor potential channels of melastatin type 8 (TRPM8): effect of cAMP, cannabinoid CB1 receptors and endovanilloids. *Exp Cell Res,* **313**:1911–20.

Després, J.P. (2009) Pleiotropic effects of rimonabant: clinical implications. *Curr Pharm Des*, **15**:553–70.

Devane, W.A., Dysarz, F.A., Johnson, M.R., *et al*. (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol,* **34**:605–13.

Devane, W.A., Hanuš, L., Breuer, A., *et al*. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, **258**:1946–9.

Di Marzo, V. (1998) 'Endocannabinoids' and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance. *Biochim Biophys Acta,* **1392**:153–75.

Di Marzo, V. (2008) The endocannabinoid system in obesity and type 2 diabetes. *Diabetologia,* **51**:1356–67.

Di Marzo, V., Bisogno, T. and De Petrocellis, L. (2001) Anandamide: some like it hot. *Trends Pharmacol Sci,* **22**:346–9.

Di Marzo, V. and Deutsch, D.G. (1998) Biochemistry of the endogenous ligands of cannabinoid receptors. *Neurobiol Dis,* **5**:386–404.

Diana, M.A., Levenes, C., Mackie, K., *et al.* (2002) Short-term retrograde inhibition of GABAergic synaptic currents in rat Purkinje cells is mediated by endogenous cannabinoids. *J Neurosci,* **22**:200–8.

Diana, M.A. and Marty, A. (2004) Endocannabinoid-mediated short-term synaptic plasticity: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE). *Brit J Pharmacol,* **142**:9–19.

ElSohly, M.A. and Slade, D. (2005) Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sciences,* **78**:539–48.

Fernández-Ruiz, J. (2009) The endocannabinoid system as a target for the treatment of motor dysfunction. *Brit J Pharmacol*, **156**:1029–40.

Garcia, C., Paloma-Garo, C., Garcia-Arencibia, M., *et al.* (2011) Symptom-relieving and neuro protective, effects of the phytocannabinoid Δ^9 - THCV in animal models of Parkinson's Disease. *Br J Pharmacol*, **163**: 1495–1506.

Gerdeman, G.L. (2008) Endocannabinoids at the synapse: retrograde signaling and presynaptic plasticity in the brain. In Köfalvi, A. (ed). *Cannabinoids and the Brain*, New York: Springer Science+Business Media, pp.203–56.

Gerdeman, G.L., Ronesi, J. and Lovinger, D.M. (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci,* **5**:446–51.

Gong, J.-P., Onaivi, E.S., Ishiguro, H., *et al.* (2006) Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res,* **1071**:10–23.

Guindon, J. and Hohmann, A. G. (2008) Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. *Brit J Pharmacol,* **153**:319–34.

Haller, J., Bakos, N., Szirmay, M., *et al.* (2002) The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur J Neurosci,* **16**:1395–8.

Haller, J., Varga, B., Ledent, C., *et al.* (2004) CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. *Behav Pharmacol,* **15**:299–304.

Hansen, H.S., Lauritzen, L., Moesgaard, B., *et al.* (1998) Formation of *N*-acyl-phosphatidylethanolamines and *N*-acylethanolamines – proposed role in neurotoxicity. *Biochem Pharmacol*, **55**:719–25.

Hanuš, L., Abu-Lafi, S., Fride, E., *et al.* (2001) 2-arachidonoyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci USA*, **98**:3662–5.

Hanuš, L.O. (2007) Discovery and isolation of anandamide and other endocannabinoids. *Chem Biodivers*, **4**:1828–41.

Hao, S., Avraham, Y., Mechoulam, R., *et al.* (2000) Low dose anandamide affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice. *Eur J Pharmacol,* **392**:147–56.

Hemmer, B., Archelos, J.J. and Hartung, H.P. (2002) New concepts in the immunopathogenesis of multiple sclerosis. *Nat Rev Neurosci,* **3**:291–301.

Herkenham, M., Lynn, A.B., Johnson, M.R., *et al.* (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J Neurosci*, **11**:563–83.

Hill, M.N., Hillard, C.J., Bambico, F.R., *et al.* (2009) The therapeutic potential of the endocannabinoid system for the development of a novel class of antidepressants. *Trends Pharmacol Sci,* **30**:484–93.

Horn, S. and Stern, M.B. (2004) The comparative effects of medical therapies for Parkinson's disease. *Neurology,* **63**:S7 –S12.

Howlett, A.C. (2002) The cannabinoid receptors. *Prostag Oth Lipid M,* **68–9**:619–31.

Howlett, A.C. (2005) Cannabinoid receptor signaling. In Pertwee, R.G. (ed), *Cannabinoids* (*Handbook of Experimental Pharmacology*) **168**, Heidelberg: Springer-Verlag, pp.53–79.

Huang, S.M., Bisogno, T., Trevisani, M., *et al.* (2002). An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci USA,* **99**:8400–5.

Jamshidi, N. and Taylor, D.A. (2001) Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. *Brit J Pharmacol,* **134**:1151–4.

Jayamanne, A., Greenwood, R., Mitchell, V.A., *et al.* (2006) Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models. *Brit J Pharmacol,* **147**:281–8.

Kozak, K.R., Rowlinson, S.W. and Marnett, L.J. (2000) Oxygenation of the endocannabinoid,

2-arachidonoylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. *J Biol Chem,* **275**:33744–9.

Lang, A.E. and Lozano, A.M. (1998) Parkinson's disease. Second of two parts. *New Engl J Med,* **339**:1130–43.

Liu, J., Wang, L., Harvey-White, J., *et al.* (2006). A biosynthetic pathway for anandamide. *Proc Natl Acad Sci USA,* **103**:13345–50.

Long, J.Z., Nomura, D.K. and Cravatt, B.F. (2009) Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. *Chem Biol,* **16**:744–53.

Maccarrone, M. (2008) CB2 receptors in reproduction. *Brit J Pharmacol,* **153**:189–98.

Maccarrone, M., Barboni, B., Paradisi, A., *et al.* (2005) Characterization of the endocannabinoid system in boar spermatozoa and implications for sperm capacitation and acrosome reaction. *J Cell Sci,* **118**:4393–404.

Maccarrone, M., Cecconi, S., Rossi, G., *et al.* (2003). Anandamide activity and degradation are regulated by early postnatal aging and follicle-stimulating hormone in mouse sertoli cells. *Endocrinology,* **144**:20–28.

Maione, S., De Petrocellis, L., de Novellis, V., *et al.* (2007) Analgesic actions of *N*-arachidonoyl-serotonin, a fatty acid amide hydrolase inhibitor with antagonistic activity at vanilloid TRPV1 receptors. *Brit J Pharmacol*, **150**:766–81.

Martin, M., Ledent, C., Parmentier, M., *et al.* (2002) Involvement of CB1 cannabinoid receptors in emotional behavior. *Psychopharmacology,* **159**:379–87.

Matias, I. and Di Marzo, V. (2007) Endocannabinoids and the control of energy balance. *Trends Endocrin Met,* **18**:27–37.

Matias, I., Gonthier, M.P., Orlando, P., *et al.* (2006) Regulation, function, and dysregulation of endocannabinoids in models of adipose and β-pancreatic cells and in obesity and hyperglycemia. *J Clin Endocr Metab,* **91**:3171–80.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., *et al.* (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature,* **346**:561–4.

Mechoulam, R., Ben-Shabat, S., Hanuš, L., *et al.* (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol*, **50**:83–90.

Mechoulam, R., Feigenbaum, J.J., Lander, N., *et al.* (1988) Enantiomeric cannabinoids – stereospecificity of psychotropic activity. *Experientia,* **44**:762–4.

Mechoulam, R. and Gaoni, Y. (1965) A total synthesis of *dl*-Δ^1-tetrahydrocannabinol, the active constituent of hashish. *J Am Chem Soc*, **87**:3273–5.

Mechoulam, R., Lander, N., Varkony, T.H., *et al.* (1980) Stereochemical requirements for cannabinoid activity. *J Med Chem,* **23**:1068–72.

Mechoulam, R. and Shvo, Y. (1963) The structure of cannabidiol. *Tetrahedron,* **19**:2073–8.

Munro, S., Thomas, K.L. and Abu-Shaar, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature,* **365**:61–5.

Navarro, M., Hernández, E., Muñoz, R.M., *et al.* (1997) Acute administration of the CB1 cannabinoid receptor antagonist SR141716A induces anxiety-like responses in the rat. *Neuroreport*, **8**:491–6.

Nissen, S.E., Nicholls, S.J., Wolski, K., *et al.* (2008) Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease. The STRADIVARIUS randomized controlled trial. *J Am Med Assoc,* **299**:1547–60.

Onaivi, E.S., Ishiguro, H., Gong, J.P., *et al.* (2006) Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann NY Acad Sci,* **1074**:514–36.

Osei-Hyiaman, D., DePetrillo, M., Pacher, P., *et al.* (2005) Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest,* **115**:1298–305.

O'Sullivan, S.E. (2007) Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Brit J Pharmacol*, **152**:576–82.

Oz, M. (2006) Receptor-independent actions of cannabinoids on cell membranes: focus on endocannabinoids. *Pharmacol Therapeut,* **111**:114–44.

Pagotto, U., Marsicano, G., Cota, D., *et al.* (2006) The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev,* **27**:73–100.

Perez, J. and Ribera, M.V. (2008) Managing neuropathic pain with Sativex®: a review of its pros and cons. *Expert Opin Pharmacotherapy*, **9**:1189–95.

Pertwee, R.G. (2001) Cannabinoid receptors and pain. *Prog Neurobiol,* **63**:569–611.

Pertwee, R.G. (2005) The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. *The AAPS Journal,* **7**:E625–E654.

Pertwee, R.G. (2006a) Cannabinoid pharmacology: the first 66 years. *Brit J Pharmacol,* **147**:S163–S171.

Pertwee, R.G. (2006b) The pharmacology of cannabinoid receptors and their ligands: an overview. *Int J Obes,* **30**:S13–S18.

Pertwee, R.G. (2007) Cannabinoids and multiple sclerosis. *Mol Neurobiol,* **36**:45–59.

Pertwee, R.G. (2008) The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids:

Δ^9-tetrahydrocannabinol, cannabidiol and Δ^9-tetrahydrocannabivarin. *Brit J Pharmacol,* **153**:199–215.

Pertwee, R.G. (2009) Emerging strategies for exploiting cannabinoid receptor agonists as medicines. *Brit J Pharmacol,* **156**:397–411.

Pertwee, R.G. (2010) Receptors targeted by synthetic cannabinoid receptor agonists and antagonists. *Curr Med Chem*, **17**:1360–81.

Pertwee, R.G. and Ross, R.A. (2002) Cannabinoid receptors and their ligands. *Prostag Leukotr Ess,* **66**:101–21.

Pertwee, R.G. and Thomas, A. (2009) Therapeutic applications for agents that act at CB1 and CB2 receptors. In Reggio, P.H. (ed), *The Cannabinoid Receptors*, New York: Humana Press, pp. 361–92.

Petrosino, S. and Di Marzo, V. (2010) FAAH and MAGL inhibitors: therapeutic opportunities from regulating endocannabinoid levels. *Current Opinion in Investigational Drugs,* **11**:51–62.

Pinsger, M., Schimetta, W., Volc, D., *et al.* (2006) Benefits of an add-on treatment with the synthetic cannabinomimetic nabilone on patients with chronic pain – a randomized controlled trial. *Wien Klin Wochenschr,* **118**: 327–35.

Porter, A.C., Sauer, J.-M., Knierman, M.D., *et al.* (2002) Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther,* **301**:1020–4.

Pryce, G., Ahmed, Z., Hankey, D.J.R., *et al.* (2003) Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. *Brain,* **126**:2191–202.

Rahn, E.J. and Hohmann, A.G. (2009) Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. *Neurotherapeutics,* **6**:713–37.

Ravinet Trillou, C., Arnone, M., Delgorge, C., *et al.* (2003) Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol-Reg I,* **284**:R345–R353.

Ravinet Trillou, C., Delgorge, C., Menet, C., *et al.* (2004) CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. *Int Jour Obes,* **28**:640–8.

Riedel, G., Fadda, P., McKillop-Smith, S., *et al.* (2009) Synthetic and plant-derived cannabinoid receptor antagonists show hypophagic properties in fasted and non-fasted mice. *Brit J Pharmacol,* **156**:1154–66.

Robbe, D., Kopf, M., Remaury, A., *et al.* (2002) Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *P Natl Acad Sci USA,* **99**:8384–8.

Rodríguez de Fonseca, F., Del Arco, I., Bermudez-Silva, F.J., *et al.* (2005) The endocannabinoid system: physiology and pharmacology. *Alcohol Alcoholism*, **40**:2–14.

Rodriguez-Oroz, M.C., Jahanshahi, M., Krack, P., *et al.* (2009) Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. *The Lancet Neurology,* **8**:1128–39.

Romero, J., Berrendero, F., Pérez-Rosado, A., *et al.* (2000) Unilateral 6-hydroxydopamine lesions of nigrostriatal dopaminergic neurons increased CB1 receptor mRNA levels in the caudate-putamen. *Life Sci*, **66**:485–94.

Ross, R.A. (2009) The enigmatic pharmacology of GPR55. *Trends Pharmacol Sci,* **30**:156–63.

Russo, R., LoVerme, J., La Rana, G., *et al.* (2007) Synergistic antinociception by the cannabinoid receptor agonist anandamide and the PPAR-α receptor agonist GW7647. *Eur J Pharmacol*, **566**:117–9.

Rutkowska, M., Jamontt, J. and Gliniak, H. (2006) Effects of cannabinoids on the anxiety-like response in mice. *Pharmacol Rep,* **58**:200–6.

Salamone, J.D., McLaughlin, P.J., Sink, K., *et al.* (2007) Cannabinoid CB1 receptor inverse agonists and neutral antagonists: effects on food intake, food-reinforced behavior and food aversions. *Physiol Behav,* **91**:383–8.

Sastre-Garriga, J., Vila, C., Clissold, S., *et al.* (2011)THC and CBD oromucosal spray (Sativex®) in the management of spasicity associated with multiple sclerosis. *Expert Rev Neurother*, **11**: 627–637.

Schmid, H.H., Schmid, P.C. and Natarajan, V. (1990) *N*-acylated glycerophopholipids and their derivatives. *Prog Lipid Res*, **29**:1–43.

Schmid, H.H.O. and Berdyshev, E.V. (2002) Cannabinoid receptor-inactive *N*-acylethanolamines and other fatty acid amides: metabolism and function. *Prostag Leukotr Ess*, **66**:363–76.

Schmid, H.H., Schmid, P.C. and Natarajan, V. (1996) The *N*-acylation-phosphodiesterase pathway and cell signalling. *Chem Phys Lipids*, **80**:133–42.

Schmid, P.C., Reddy, P.V., Natarajan, V., *et al.* (1983) Metabolism of N-acylethanolamine phospholipids by a mammalian phosphodiesterase of the phospholipase-D type. *The J Biol Chem,* **258**:9302–6.

Schuel, H., Burkman, L.J., Lippes, J., *et al.* (2002) *N*-Acylethanolamines in human reproductive fluids. *Chem Phys Lipids*, **121**:211–27.

Simon, G.M. and Cravatt, B.F. (2006) Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for α/β-hydrolase 4 in this pathway. *J Biol Chem,* **281**:26465–72.

Skrabek, R.Q., Gallmova, L., Ethans, K., *et al.* (2008) Nabilone for the treatment of pain in fibromyalgia. *The Journal of Pain,* **9**:164–73.

Smart, D., Gunthorpe, M.J., Jerman, J.C., *et al.* (2000) The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Brit J Pharmacol,* **129**:227–30.

Stella, N., Schweitzer, P. and Piomelli, D. (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature,* **388**:773–8.

Sugiura, T., Kobayashi, Y., Oka, S., *et al.* (2002) Biosynthesis and degradation of anandamide and 2- arachidonoyl glycerol and their possible physiological significance. *Prostag Leukotr Ess,* **66**:173–92.

Sugiura, T., Kondo, S., Sukagawa, A., *et al.* (1995) 2-Arachidonoyl glycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Co,* **215**:89–97.

Sun, Y.X., Tsuboi, K., Okamoto, Y., *et al.* (2004) Biosynthesis of anandamide and *N*-palmitoylethanolamine by sequential actions of phospholipase A_2 and lysophospholipase D. *Biochem J*, **380**:749–56.

Ueda, N., Yamanaka, K., Terasawa, Y., *et al.* (1999) An acid amidase hydrolyzing anandamide as an endogenous ligand for cannabinoid receptors. *FEBS Letters,* **454**:267–70.

Urigüen, L., Pérez-Rial, S., Ledent, C., *et al.* (2004) Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. *Neuropharmacology*, **46**:966–73.

Van Sickle, M.D., Duncan, M., Kingsley, P.J., *et al.* (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science,* **310**:329–32.

Vickers, S.P., Webster, L.J., Wyatt, A., *et al.* (2003) Preferential effects of the cannabinoid CB1, receptor antagonist, SR 141716, on food intake and body weight gain of obese (*fa/fa*) compared to lean Zucker rats. *Psychopharmacology*, **167**:103–11.

Wang, H.B., Xie, H.R., Sun, X.F., *et al.* (2007) Differential regulation of endocannabinoid synthesis and degradation in the uterus during embryo implantation. *Prostag Oth Lipid M,* **83**:62–74.

Wei, B.Q.Q., Mikkelsen, T.S., McKinney, M.K., *et al.* (2006) A second fatty acid amide hydrolase with variable distribution among placental mammals. *J Biol Chem,* **281**:36569–78

Wiley, J.L., Burston, J.J., Leggett, D.C., *et al.* (2005) CB1 cannabinoid receptor-mediated modulation of food intake in mice. *Brit J Pharmacol,* **145**:293–300.

Williams, C.M. and Kirkham, T.C. (1999) Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. *Psychopharmacology,* **143**:315–7.

Williams, C.M. and Kirkham, T.C. (2002) Reversal of Δ^9-THC hyperphagia by SR141716 and naloxone but not dexfenfluramine. *Pharmacol Biochem Be*, **71**:341–348.

Williams, C.M., Rogers, P.J. and Kirkham, T.C. (1998) Hyperphagia in pre-fed rats following oral Δ^9-THC. *Physiol Behav*, **65**:343–6.

Wilson, R.I., Kunos, G. and Nicoll, R.A. (2001) Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron,* **31**:453–62.

Wilson, R.I. and Nicoll, R.A. (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature,* **410**:588–92.

Wilson, R.I. and Nicoll, R.A. (2002) Neuroscience – endocannabinoid signaling in the brain. *Science,* **296**:678–82.

Yates, M.L. and Barker, E.L. (2009) Inactivation and biotransformation of the endogenous cannabinoids anandamide and 2-arachidonoyl glycerol. *Mol Pharmacol,* **76**:11–17.

Yu, M., Ives, D. and Ramesha, C.S. (1997) Synthesis of prostaglandin E_2 ethanolamide from anandamide by cyclooxygenase-2. *J Biol Chem,* **272**:21181–6.

Zygmunt, P.M., Petersson, J., Andersson, D.A., *et al.* (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature,* **400**:452–7.

Chapter 4

Is cannabis becoming more potent?

Desmond Slade, Zlatko Mehmedic, Suman Chandra and Mahmoud ElSohly

The crux of the potency debate is the directly opposing views of pro- and anti-cannabis proponents: those for legalization of cannabis or, at a minimum, for the medicinal use of cannabis, reject the notion of an increase in cannabis potency; and those for total prohibition of the drug use the supposed increased potency as an argument for their viewpoint, extrapolating that an increase in potency must mean an increase in adverse health, mental and socio-economical effects. The conundrum this creates is that the politicization of this important question by both groups completely obscures and ignores the available scientific data, as well as the discussion about the additional scientific studies that are needed to fully address the issue. For a detailed discussion of these issues, the reader is referred to Chapter 5 of this book.

The potency debate should, therefore, not be seen as a simple question of increased potency, but rather as a comprehensive study into the characteristics of the available cannabis preparations, its health and cultural effects on users short- and long-term, especially adolescents and young adults, and the most rational and cost-effective way to legislate cannabis. The aim of this chapter is not to answer all of these complex questions, but rather to further the potency debate by exclusively focusing on the available data for the Δ^9-tetrahydrocannabinol (THC) content of different cannabis preparations. Solving this piece of the puzzle will hopefully elucidate the remainder of the questions so that science, and not politics, will resolve the potency debate.

What is cannabis?

Cannabis is the drug prepared from the dicotyledonous, herbaceous, dioecious, annual herb *Cannabis sativa* L. and its variants (family: Cannabaceae), which uniquely has the terpenophenolic cannabinoids as active ingredients (ElSohly and Slade, 2005), accumulating mainly in the glandular trichomes of the plant (De Meijer *et al*., 2003).

Cannabis is derived from *Cannabis sativa* pistillate inflorescence and usually refers to the herbal form, i.e., fresh or dried leaves and flowering tops (buds) without stalks, roots and seeds. The buds or leaves of pollinated female plants, typically outdoor grown, are dried to prepare marijuana (marihuana). This form is known as herbal cannabis in the United Kingdom. Sinsemilla comprises the buds of unfertilized female plants, usually grown indoors using specialized equipment. This form is known as skunk in the United Kingdom and as nederwiet in The Netherlands. In some cases, herbal cannabis is used as a general term to describe marijuana and sinsemilla. Ditchweed is fiber-type feral cannabis found in the midwestern region of the United States. The resin secreted from the glandular trichomes located around the buds of female plants can be compressed to prepare cannabis resin (United States and United Kingdom: hashish or hash), varying in color from black to golden brown depending upon purity and method of production. Solvent extraction or distillation of herbal cannabis or cannabis resin produces a dark green or black tar-like oily mixture known as cannabis oil (hash oil) (Stambouli *et al*., 2005).

The THC content decreases in the various parts of the plant as follows: bracts > flowers > leaves > smaller stems > larger stems > seeds (via external contamination) (King, 1997; McLaren *et al*., 2008; Potter *et al*., 2008).

Potency of cannabis

The psychoactive ingredients of cannabis are the terpenophenolic cannabinoids. The main psychoactive cannabinoid is THC (Mackie *et al*., 2007a, 2007b); however, other cannabinoids have also demonstrated

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pharmacological activities, as discussed elsewhere in this book. The living plant biosynthesizes these compounds in their acidic form, but the labile carboxyl group is lost as carbon dioxide to yield neutral cannabinoids under the influence of light, heat or as the harvested plant material ages. Growing, harvesting, processing, storage and use also produce breakdown products of cannabinoids, such as cannabinol (CBN) formed in aged cannabis via oxidative degradation of THC. Quantification of the total cannabinoid content present in fresh- or dried-plant material must therefore allow for analysis of the acidic and neutral cannabinoids as well as their degradation products; however, cannabis potency specifically refers to the THC content. Depending on the analytical method, THC content should be the sum of the free and the acidic forms of THC, e.g., HPLC analysis, or if the analytical method includes in-situ decarboxylation of THC, e.g., underivatized gas chromatography-flame ionization detection (GC-FID) or GC-mass spectrometry (MS) analysis, the total THC content is obtained directly. Complete decarboxylation of the acidic cannabinoids is important to ensure accurate results (De Backer *et al*., 2009).

Cannabis phenotypes

Cannabis is divided mainly into three phenotypes: phenotype I (drug-type), with THC > 0.5% and cannabidiol (CBD) < 0.5%; phenotype II (intermediate-type), with CBD as the major cannabinoid but with THC also present at various concentrations; and phenotype III (fiber-type or hemp), with especially low THC content. Hemp usually contains non-psychoactive cannabinoids as major constituents, e.g. CBD or cannabigerol (CBG) (De Backer *et al*., 2009; Galal *et al*., 2009). Although environmental factors play a role in the amount of cannabinoids present in different parts of the plant at different growth stages (Bócsa *et al*., 1997), the distribution of CBD:THC ratios in most populations are under genetic control (De Meijer *et al*., 2003).

A number of indexes are used to classify cannabis samples: [THC + CBN]/CBD > 1 indicates drug-type, while a ratio < 1 indicates non-drug or fiber-type (index I) (Lopes de Oliveira *et al*., 2008); THC > CBD indicates drug-type, while THC < 1% and CBD > THC indicates fiber-type (index II) (Ross *et al*., 2000); and THC/CBD or CBN/CBD > 1 indicates drug-type, while THC/CBD and CBN/CBD < 1 indicates fiber-type (index III) (Stefanidou *et al*., 1998).

United States

A small study (n = 36) reporting the potency of cannabis products seized in central Florida (June 2002) found a mean THC content of 6.2 ± 2.7% (range: 1.41–12.62%, 95% confidence interval [CI] range: 5.28, 7.13%) (Newell, 2003). Potency data in this chapter is always presented as mean ± standard deviation (SD), range (minimum to maximum) and 95% CI range, unless otherwise specified. The products had a normal distribution (p = 0.524), with THC frequency distribution of 36.1%, 55.6% and 8.3% (THC < 5%, THC 5–10% and THC > 10%, respectively). The report does not describe the products, but it can be assumed that it was herbal cannabis (marijuana and sinsemilla) based on the mean potency and range. Even though this is a relatively small study, it does exemplify the need for fully describing the cannabis samples under investigation to ensure that the data can be used to form a comprehensive picture of the potency trends of different cannabis preparations.

The National Institute on Drug Abuse (NIDA) and the Drug Enforcement Administration (DEA) established a cannabis research program in the early 1970s. The program performs a variety of cannabis research activities, including the Potency Monitoring (PM) program, which provides analytical potency data on confiscated cannabis and cannabis preparations. The PM program is administered by the NCNPR, University of Mississippi (ElSohly *et al*., 1984, 1985, 2000; Mehmedic *et al*., 2010). Cannabis seizures are classified as cannabis, hashish or hash oil. Cannabis, received as raw plant material, is further categorized as marijuana, sinsemilla and ditchweed. Hashish is the resinous parts of the buds, mixed with some plant particles and shaped into a variety of forms. Hash oil is a liquid or semi-solid-concentrated extract or distillate of cannabis or hashish.

During the past 35 years (1975–2009), 69 987 cannabis seizures have been analyzed at the PM laboratory. Cannabis, i.e. marijuana (79.8%), sinsemilla (13.9%) and ditchweed (3.8%), represents the overwhelming majority of the confiscations (97.5%), while the hashish- (1.9%) and hash oil- (0.7%) combined contribution has declined from 42.9% in 1975 to 1.1% in 2009. Marijuana typically represents at least 50% of the seizures. Sinsemilla seizures have gradually increased since the early 1990s, with a sharp increase from 2002 onwards (Figure 4.1).

The yearly mean THC content for the different types of cannabis seizures (Figures 4.2 and 4.3) shows

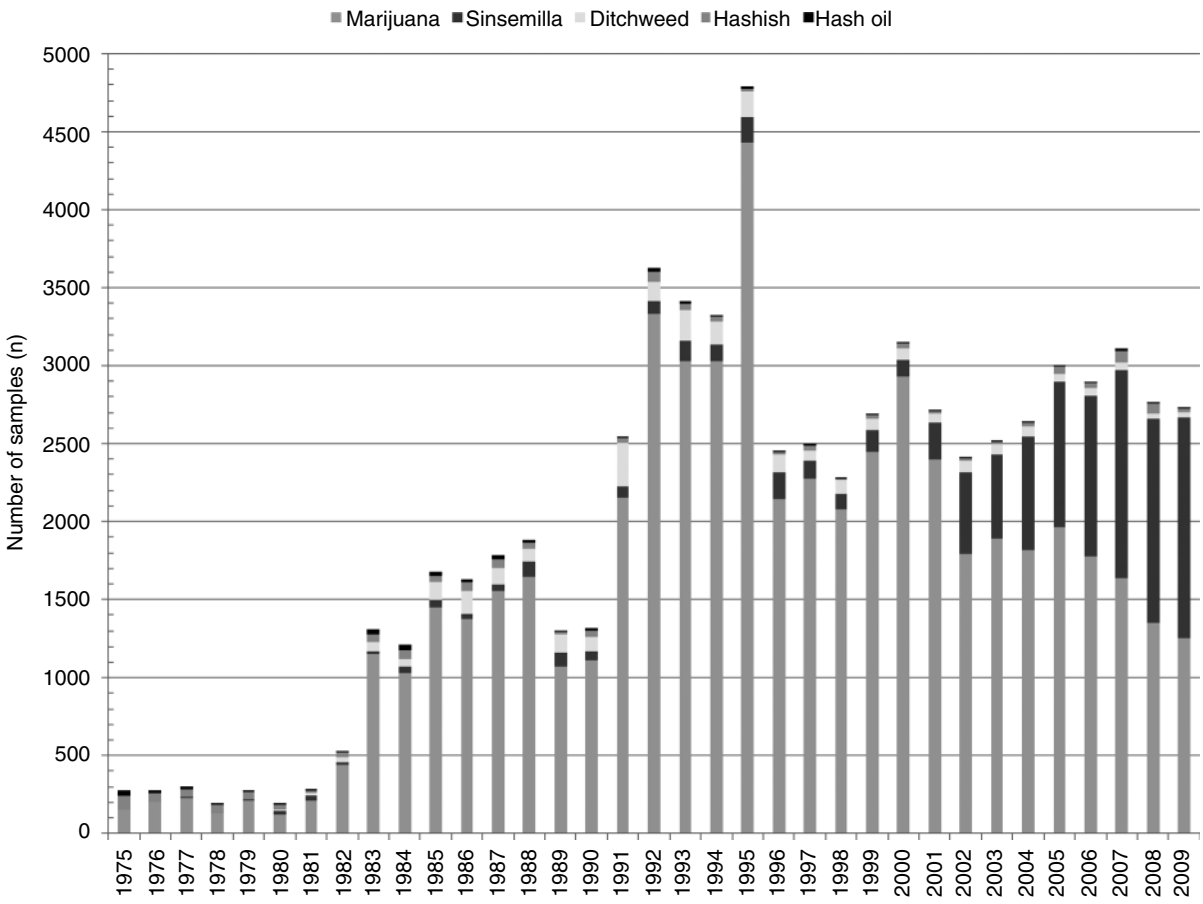

Figure 4.1. Number of cannabis seizures analyzed by type and year in the United States (1975–2009). See also color plate section.

large variation within categories and over time, with only ditchweed being relatively constant (0.37 ± 0.28%, 0.00–2.40%, 0.36, 0.38%). The marijuana mean THC content increased from 0.7 ± 0.9% (0.03–5.32%, 0.60, 0.88%) in 1975 to 5.1 ± 3.5% (0.00–27.13%, 4.91, 5.30%) in 2009, indicating a steady increase in marijuana potency over the past 35 years. The maximum yearly mean THC content was achieved in 2007 (6.1 ± 3.7%, 0.14–37.20%, 5.92, 6.29%). The data for 2008 (6.0 ± 3.9%, 0.02–26.02%, 5.76, 6.18%) was almost identical to 2007; however, 2009 did show a slight decrease in mean yearly potency for marijuana. The data for subsequent years will indicate if this is the start of a downtrend or just a statistical artifact.

The sinsemilla mean THC content increased from 4.1 ± 1.8% (1.45–6.77%, 2.85, 5.27%) in 1977 to 10.8 ± 6.0% (0.03–31.84%, 10.46, 11.09%) in 2009 (Figure 4.2). The maximum yearly mean THC content was achieved in 1999 (13.4 ± 4.7%, 2.03–27.08%, 12.59, 14.18%). The sinsemilla yearly mean potencies can be divided into two significantly different periods: 1977–1995 (7.6 ± 4.1%, 0.10–24.71%, 7.34, 7.83%) and 1996–2009 (11.3 ± 6.1%, 0.03–33.12%, 11.16, 11.42%) (one-way analysis of variance [ANOVA] [$\alpha = 0.05$]: $p < 0.001$). The 48.9% increase in THC content from 1977–1995 to 1996–2009 can probably be ascribed to indoor growing and improvements in cultivation techniques. The sinsemilla mean yearly potencies seemed to stabilize between 2002–2009.

One-way ANOVA ($\alpha = 0.05$) of the marijuana and sinsemilla mean potencies per year indicated, as expected, that these two categories are significantly different ($p < 0.001$).

The combined potencies for marijuana, sinsemilla and ditchweed, i.e. the seizures classified as cannabis, have often been used as an indication of herbal cannabis potency trends (Figure 4.2). The mean cannabis potency per year closely matches the marijuana

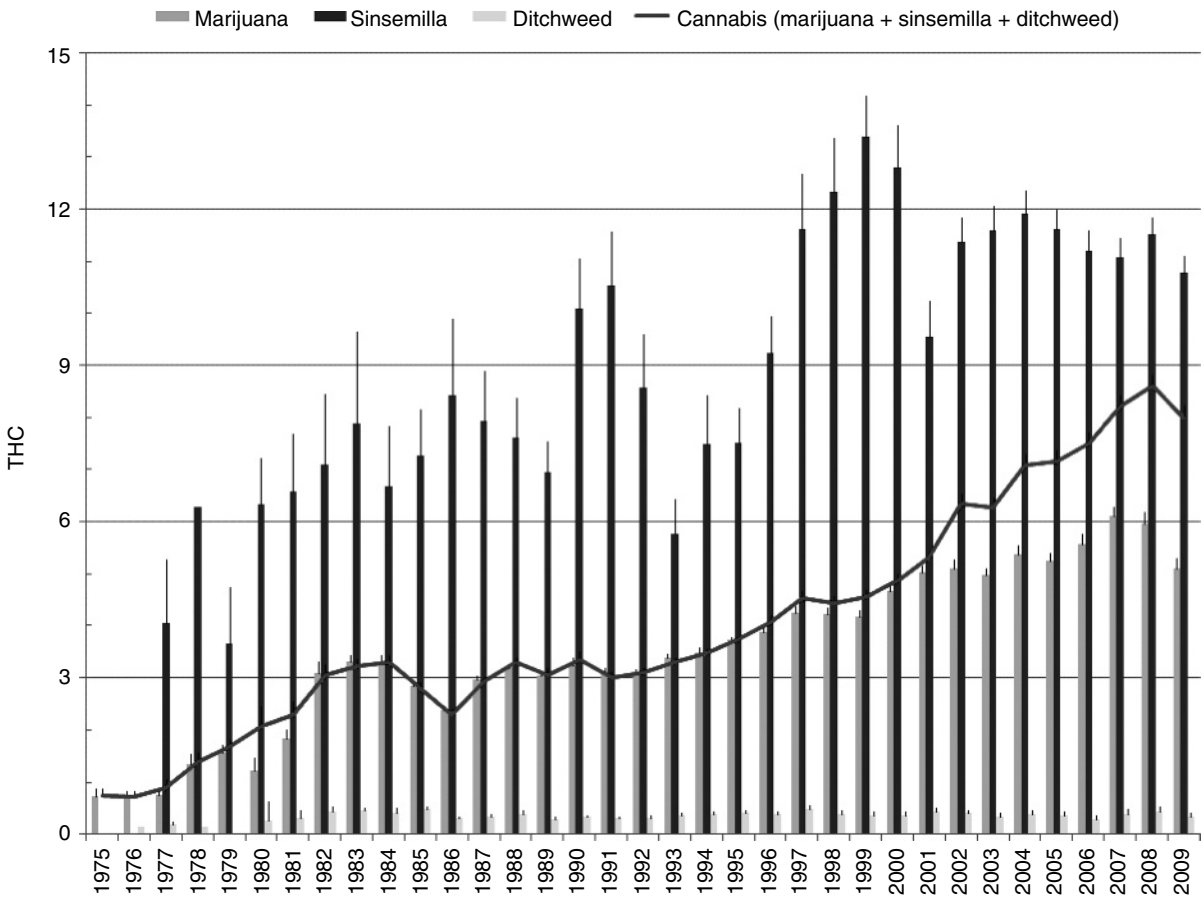

Figure 4.2. Mean THC content (%) with 95% confidence intervals for marijuana, sinsemilla and ditchweed seizures in the United States (1975–2009). THC, Δ^9-tetrahydrocannabinol. See also color plate section.

potencies between 1975–2000 (one-way ANOVA [$\alpha = 0.05$]: $p = 0.756$), while between 2001–2009 the values started to diverge (one-way ANOVA [$\alpha = 0.05$]: $p < 0.001$), with the cannabis potencies being significantly higher than the marijuana potencies. This can be ascribed to the influence of the increased number of sinsemilla seizures since 2001 (Figure 4.1).

Hashish- and hash oil-THC potencies showed the most variability over the 35-year period (7.8 ± 11.5%, 0.01–66.33%, 7.18, 8.43% and 16.2 ± 12.3%, 0.00–81.70%, 15.09, 17.35%, respectively) (Figure 4.3). The hashish mean potency for 2000–2009 (18.8 ± 17.2%, 0.03–66.33%, 16.97, 20.68%) was significantly higher than 1975–1999 (4.1 ± 4.7%, 0.01–52.87%, 3.76, 4.35%) (one-way ANOVA [$\alpha = 0.05$]: $p < 0.001$). This can possibly be attributed to the influence of sinsemilla on the illicit market since 2001 (Figure 4.1). The hash oil mean potency for 2000–2009 (18.0 ± 20.8%, 0.00–81.70%, 13.20, 22.84%) was only slightly higher than 1975–1999 (15.9 ± 9.8%, 0.01–47.01%, 14.88, 16.86%) (one-way ANOVA [$\alpha = 0.05$]: $p = 0.188$).

The change in cannabis potency over the past 40 years has been the subject of much debate and controversy. In an effort to clarify this issue, the influence of outliers on the overall mean THC content was examined. Outliers are determined based on the standard normal cumulative distribution of the absolute value of the z-score expressed as a percentage, known as the p-value:

$$p - \text{value} = NORMSDIST\left(\left|\frac{x-\bar{x}}{\bar{x}}\right|\right)\text{'}100$$

$$\begin{cases} < 5.0\% \rightarrow \text{OUTLIER} \\ - \\ - \\ \geq 5.0\% \rightarrow \text{NORMAL} \end{cases}$$

x = cannabis preparation THC potency, $\bar{x}$ = mean THC potency for specific year. A p-value < 5.0% indicates an

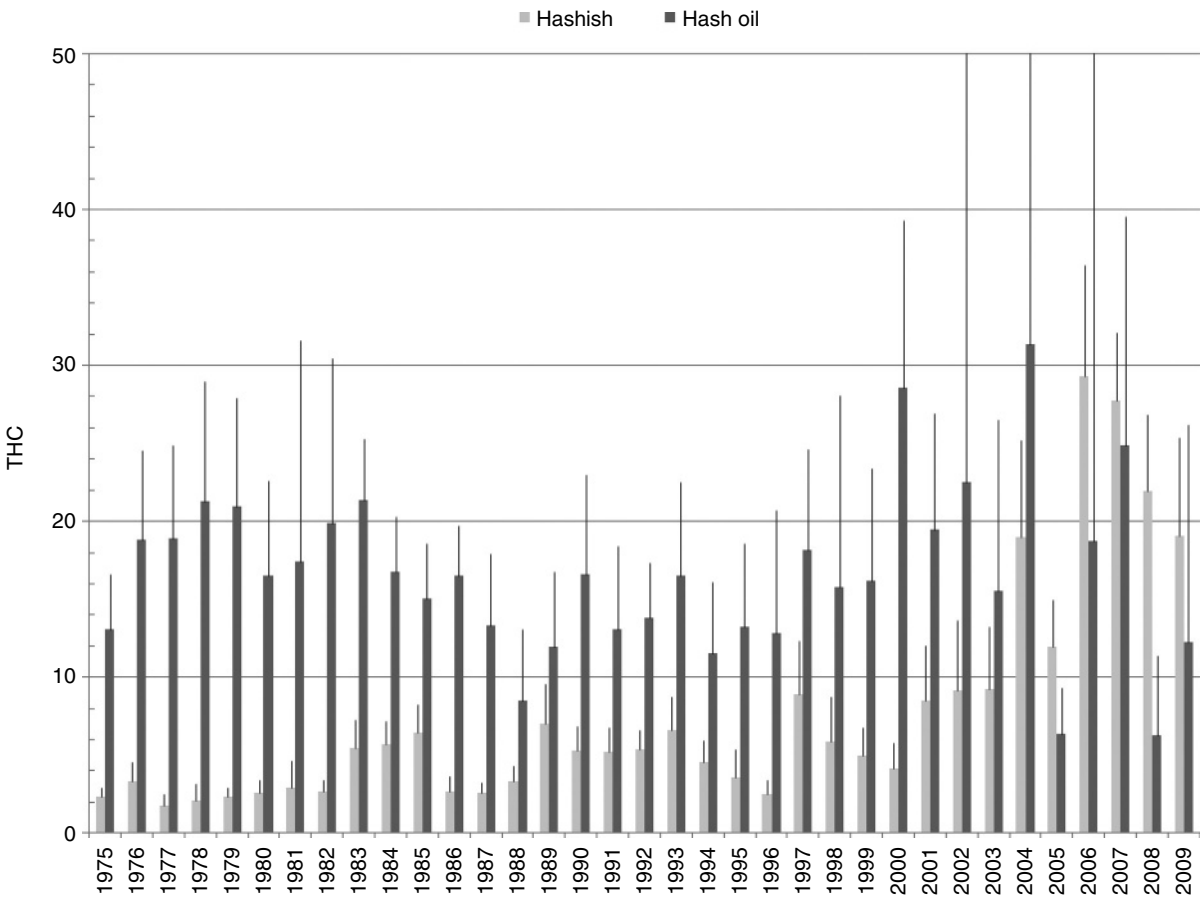

Figure 4.3. Mean THC content (%) with 95% confidence intervals for hashish and hash oil seizures in the United States (1975–2009). THC, Δ^9-tetrahydrocannabinol. See also color plate section.

outlier, while a p-value ≥ 5.0% indicates a normal THC potency value.

For example, the marijuana mean THC content for 2009 is 5.1% ($\bar{x}$) and a single marijuana seizure analyzed for 12.28% THC (x). The absolute value of the z-score is therefore 2.38, and the standard normal cumulative distribution of 2.38, calculated with the Microsoft Excel NORMSDIST function, expressed as a percentage is 0.86%, which is < 5.0%. This specific seizure was therefore classified as a positively skewed outlier.

Using this criterion, 1528 seizures were identified as outliers, representing 2.2% of the total. Marijuana (88.3%), sinsemilla (1.0%) and ditchweed (4.8%) comprised the majority of the outliers (hashish- and hash oil-combined: 5.9%) (1975–2009). Between 1975 and 2001, marijuana and sinsemilla contributed 74.0% and 0.5%, respectively, of the total outliers; between 2002 and 2009 these values changed to 14.3% and 0.5%, respectively. All the outliers were seizures with potencies higher than the mean potency, i.e. the distribution of THC content was positively skewed. It is therefore important that the potential effect of the outliers is examined to determine whether the apparent trend of increasing potency for marijuana and sinsemilla is real or simply a statistical artifact.

Comparison of the mean potency of marijuana, calculated for marijuana versus marijuana with outliers excluded, indicates that the mean THC content decreases for each year when the outliers are excluded. However, the general pattern of increasing potency of marijuana since 1975 appears to exist even when outliers are excluded. One-way ANOVA (α = 0.05) comparison of the marijuana mean potencies per year for marijuana and marijuana with outliers excluded, indicates that the two data sets are not significantly different (p = 0.568). The overall mean for

marijuana for 1975–2009 decreased from 4.0 ± 2.9% to 3.8 ± 2.5% after removal of the outliers, representing a 5.5% decrease. The positively skewed marijuana outlier potencies (n = 3378, 13.0 ± 4.1%, 1.94–37.20%, 12.77, 13.22%) were comparable to those of all sinsemilla samples for 1975–2009 (n = 4753, 10.9 ± 6.0%, 0.031–32.12%, 10.23, 11.01%).

Owing to the greater variability found in the potency of sinsemilla, fewer cases were excluded as outliers, and thus there was little effect on the mean potency for each of the years reported.

Analysis of the frequency distribution for each category gives an indication of how the dispersion of low (THC < 5%), medium (THC 5–10%) and high (THC > 10%) potency seizures have changed since 1975. For marijuana, the prevalence of low-potency seizures has steadily decreased from about 100% in 1975 to about 50% in 2009, with a concurrent increase in medium- and high-potency seizures. Although the picture for sinsemilla is more complex, it is clear that high potency seizures are much more ubiquitous during the second half of the study, contributing 50% or more of sinsemilla from 1997 onwards. About half of the high potency sinsemilla seizures since 1997 have a THC content > 15%.

State-eradication program seizures represent only cannabis and cannabis preparations know to originate from within the United States, and are therefore labeled as domestic seizures. Domestic seizures include plants grown indoors or outdoors at different stages of maturity. All DEA seizures are of final products produced from mature plant material with unknown origin.

The number of domestic seizures represents approximately one-third of all confiscations, with the number of yearly DEA seizures consistently higher than domestic seizures (1975–2009). Marijuana represents 71.7% and 83.6% of domestic and DEA seizures, respectively; however, sinsemilla seizures have increased significantly since 2002 for both categories. Sinsemilla classified as domestic seizures increased from 8.1% (1975–2001) to 44.0% (2002–2009); sinsemilla classified as DEA seizures increased from 1.8% (1975–2001) to 32.1% (2002–2009).

Comparing the mean THC content for domestic and DEA seizures classified as marijuana showed that domestic seizure potencies were relatively constant and lower than DEA seizure potencies (Figure 4.4). Marijuana DEA seizure potencies increased from 0.6 ± 0.6% (0.03–4.26%, 0.49, 0.69%) in 1975 to 6.6 ± 3.1% (0.06–27.13%, 6.42, 6.85%) in 2009. One-way ANOVA ($\alpha = 0.05$) comparison of the marijuana mean potencies per year for domestic and DEA seizures indicates that the two data sets are significantly different ($p < 0.001$).

Comparing the mean THC content for domestic and DEA seizures classified as sinsemilla showed that the two seizure types had similar potency patterns up to 2000 (one-way ANOVA [$\alpha = 0.05$]: $p = 0.734$), while the potency patterns were significantly different between 2001–2009 (one-way ANOVA [$\alpha = 0.05$]: $p < 0.001$). Sinsemilla DEA seizures potencies increased from 4.1 ± 1.8% (1.45–6.77%, 2.85, 5.27%) in 1977 to 12.6 ± 5.4% (0.42–31.84%, 12.29, 13.00%) in 2009 (Figure 4.5).

The mean content of the minor cannabinoids cannabichromene (CBC), CBD, CBN, CBG and Δ^9-tetrahydrocannabivarin (THCV) were also monitored. Cannabidiol is the major cannabinoid found in ditchweed, and is present in elevated amounts in intermediate-type cannabis (moderate levels of both THC and CBD) used to make hashish. The cannabinoid content of hash oil shows that it was prepared from intermediate-type cannabis during 1975–1991, while drug-type cannabis (high THC and low CBD levels) was used between 1992–2009 (Bócsa *et al*., 1997; Galal *et al*., 2009). Cannabichromene and CBN are usually higher in hashish and hash oil compared with cannabis. Cannabigerol content is typically about 3–6% of the THC content; however, in ditchweed this ratio increases to more than 11%, even though this type of seizure has the lowest overall mean CBG content. This is because of the low THC content (0.4 ± 0.3%) of ditchweed. Δ^9-Tetrahydrocannabivarin is generally present at about 0.5–5.0% of the THC content, with the highest levels found in hashish and hash oil. Phenotypic index analysis of the cannabis data for 1975–2009 indicates that about 93.8% of the seizures are drug-type and about 6.2% are intermediate- or fiber-type.

Canada

In Canada, seized cannabis products are submitted for THC analysis for court purposes, but are not classified by type, e.g. marijuana, sinsemilla or hashish; however, the majority of products are comprised almost exclusively of sinsemilla. Before the early 1980s, THC levels in cannabis were generally below 1% in Canada, increasing to about 6% in the late 1990s (RCMP, 2002). Between 1989–2003, the frequency of cannabis products with THC < 5% declined from 60% to 18%, while products with THC > 10% increased from 12% to 54% (Viau *et al*., 2004; Leggett, 2006). The THC content of cannabis analyzed in 2004 was 9.8%, followed by 10.0%

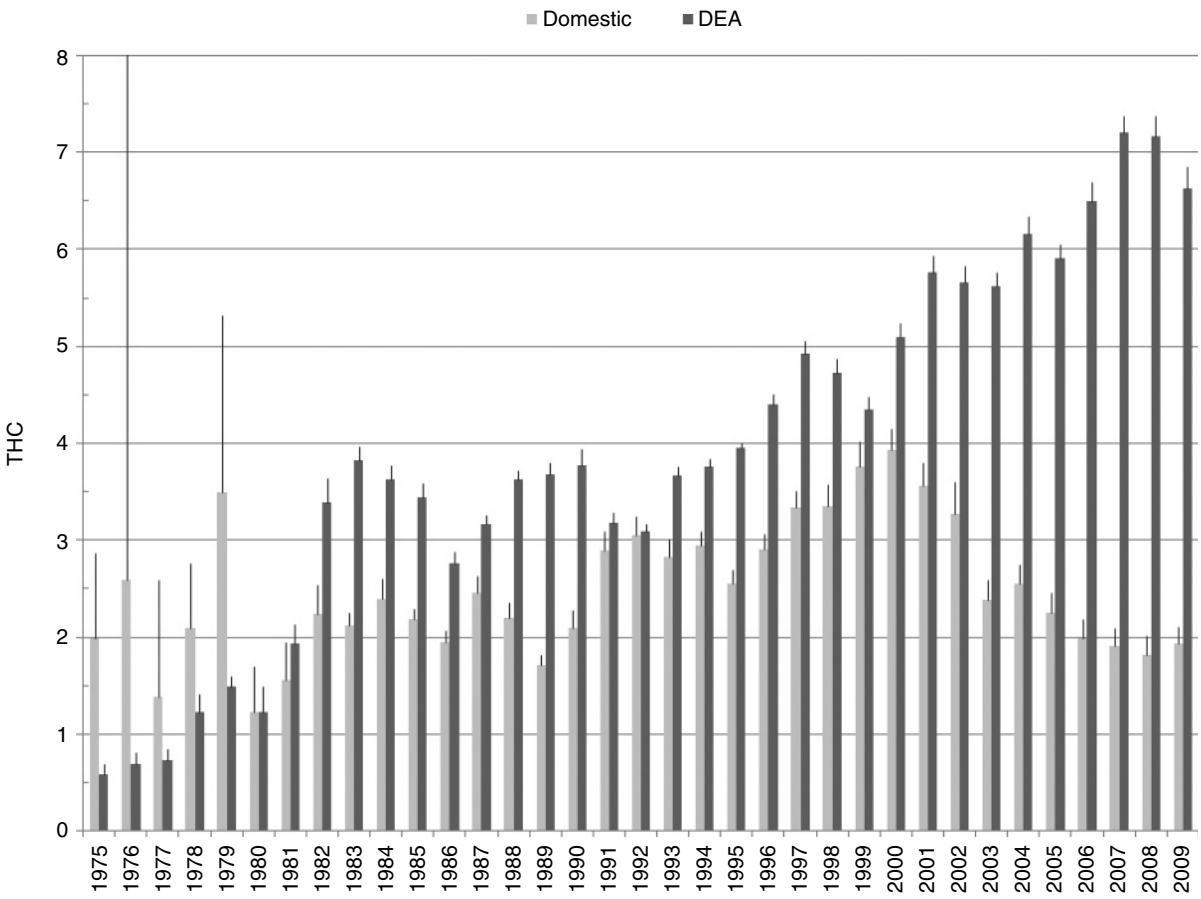

Figure 4.4. Mean THC content (%) with 95% confidence intervals for domestic and DEA marijuana seizures in the United States (1975–2009). DEA, Drug Enforcement Administration; THC, Δ^9-tetrahydrocannabinol. See also color plate section.

in 2005 and 10.3% in 2006 (United States – Canada Border, 2007).

The data on cannabis potency in Canada seems to indicate an upwards trend. The potency between 2004–2006 was about 10% and the frequency of products with THC > 10% in 2003 was more than 50%, which is consistent with a predominantly sinsemilla market.

The Netherlands

Data on cannabis potency is generally obtained from the analysis of law enforcement seizures. In The Netherlands, data have been derived from cannabis products purchased in coffee shops since 1999 (Korf, 2002; Pijlman *et al.*, 2005; Van Laar *et al.*, 2008). Products obtained in this way are generally of a better quality; however, it may not necessarily represent all cannabis consumed in The Netherlands (EMCDDA, 2009). Locally cultivated herbal cannabis (nederwiet) was selected based on the variety that was most popular. Imported marijuana consisted of fresh or dried leaves and buds, excluding stalk, roots and seeds. Nederhasj is cannabis resin prepared from locally cultivated herbal cannabis by sieving the resinous parts of the buds from other vegetable matter, while imported hashish is prepared from imported herbal cannabis. Preparations were purchased in January and September of each year.

The mean THC content of nederwiet purchased in January increased from 8.6 ± 2.8% in 2000 to a maximum of 20.4 ± 4.7% in 2004, followed by a decrease to 15.1 ± 3.7% in 2009 (Figure 4.6). The overall mean for the nederwiet samples purchased in January was 15.5 ± 3.4% (2000–2009). The mean THC content of imported marijuana purchased in January fluctuated between a minimum of 5.0 ± 2.8% (2000) and a maximum of 9.9 ± 3.6% (2009), with an overall mean of 6.5 ± 1.5% (2000–2009). The mean THC content of

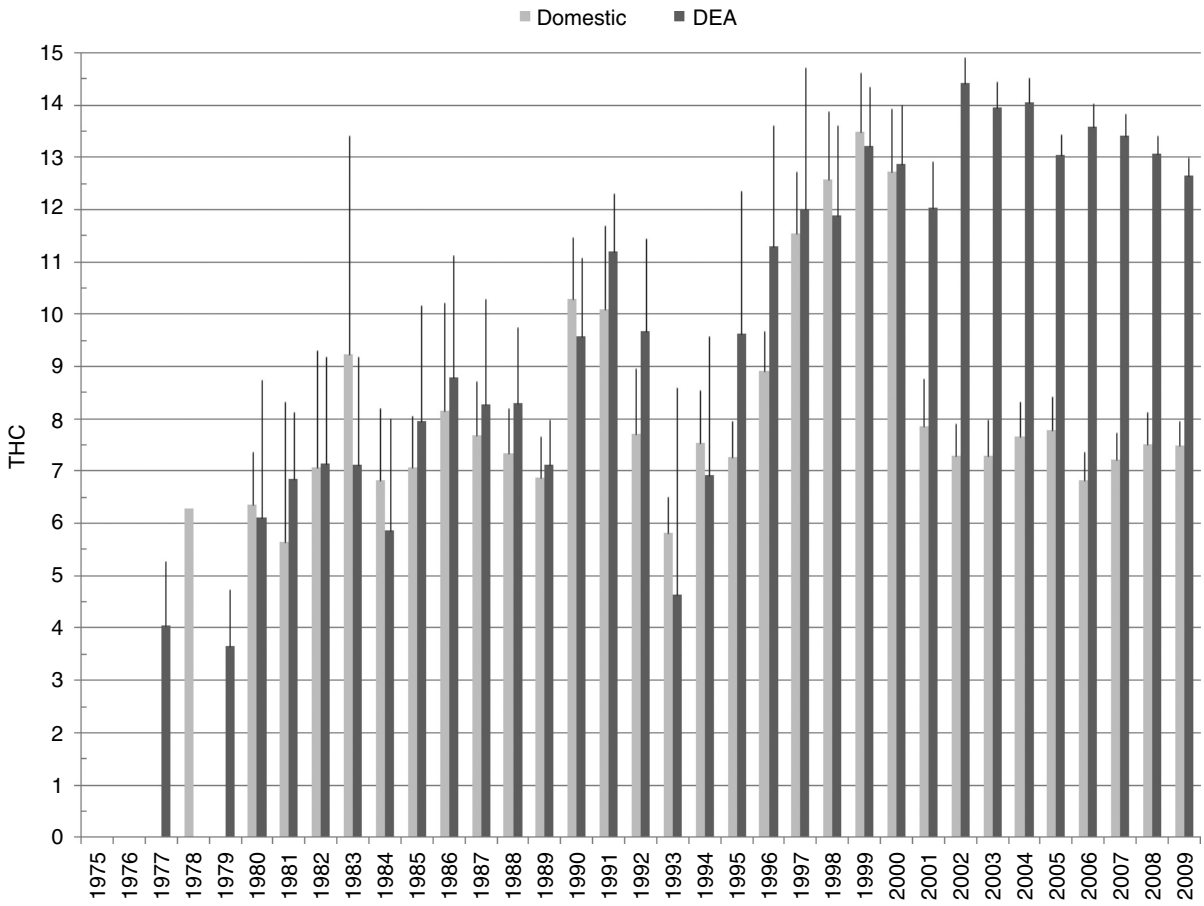

Figure 4.5. Mean THC content (%) with 95% confidence intervals for domestic and DEA sinsemilla seizures in the United States (1975–2009). DEA, Drug Enforcement Administration; THC, Δ^9-tetrahydrocannabinol. See also color plate section.

nederwiet was consistently higher than that of the imported marijuana. The THC data sets were both consistent with normal distributions (nederwiet – January: $p = 0.555$; imported marijuana – January: $p = 0.427$). The two data sets were analyzed using one-way ANOVA ($\alpha = 0.05$), revealing that they were significantly different ($p < 0.001$). The THC content of nederwiet samples purchased in January was consistently higher than the THC content of nederwiet samples purchased in September (Figure 4.6); however, the two data sets were not significantly different (one-way ANOVA [$\alpha = 0.05$]: $p = 0.174$). Cannabis sold in the summer usually includes a major portion that is grown outdoors, while cannabis sold in the winter is usually grown indoors. This could explain the seasonal variation in nederwiet potencies (Niesink *et al.*, 2002, 2003, 2006, 2007, 2008, 2009; Pijlman *et al.*, 2005). Although the yearly nederwiet (January and September) mean potencies peaked in 2004, in subsequent years the THC content seemed to stabilize, albeit at a slightly lower value. The same effect was observed for imported marijuana (Figure 4.6).

The mean THC content of nederhasj followed a similar pattern to that of nederwiet, increasing to a maximum in 2004 (39.3 ± 14.2%), followed by a relatively stable period between 2005 and 2009 (Figure 4.6). The overall mean for nederhasj was 30.0 ± 7.0% (2000–2009). The mean THC content of imported hashish ranged from 11.0 ± 4.4% (2000) to a maximum of 18.2 ± 5.8% (2004), with an overall mean of 15.8 ± 2.6% (2000–2009). The mean THC content of nederhasj was consistently higher than that of the imported hashish. The THC data sets were both consistent with normal distributions (nederhasj: $p = 0.733$; imported hashish: $p = 0.519$), and were significantly different (one-way ANOVA [$\alpha = 0.05$]: $p < 0.001$).

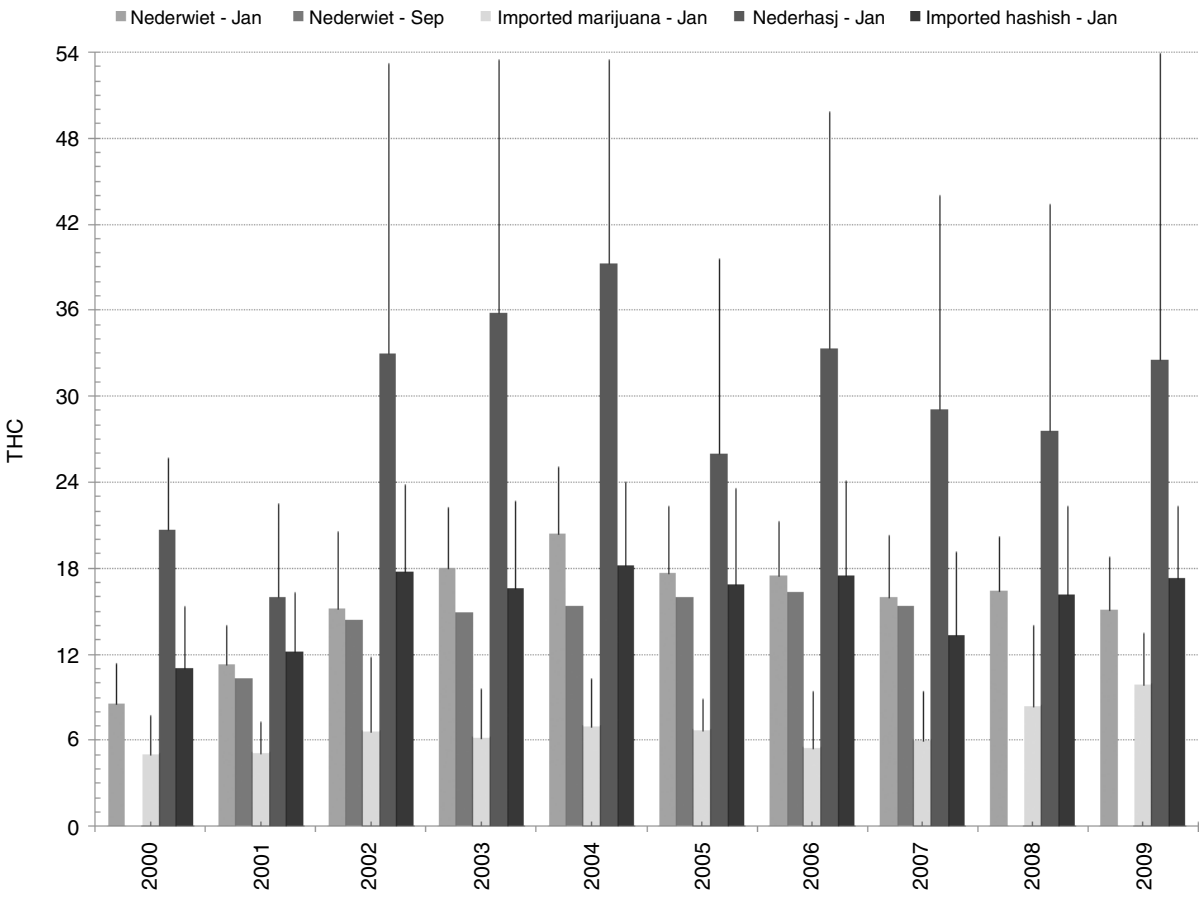

Figure 4.6. THC content (mean ± SD) (%) of cannabis products purchased in coffee shops in The Netherlands (2000–2009). THC, Δ^9-tetrahydrocannabinol. See also color plate section.

The twofold difference between locally produced and imported cannabis preparations purchased in the coffee shops are possibly due to improved cultivation and storage practices, genetic enhancement and cross breeding of varieties, and improvements in indoor hydroponic techniques employed in The Netherlands. Imported marijuana almost always contained seeds from fertilized female buds, while nederwiet is produced by using only unfertilized female buds high in THC. These advancements also ensure reproducibility of crops with high THC levels, resulting in a more constant final product.

United Kingdom

The THC content of seized illicit cannabis products in the United Kingdom has been reported since 1975 by different agencies. Between 1975–1989, the Laboratory of the Government Chemist (LGC) conducted a series of studies (Baker *et al*., 1980,1982a; Pitts *et al*., 1990), the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) collects data through the Reitox National Focal Points utilizing data provided by the Forensic Science Service (FSS) (Tables 4.1 and 4.2), and the FSS provides data to the Advisory Council on the Misuse of Drugs (ACMD) (ACMD, 2005, 2007; Eaton *et al*., 2005, 2006, 2007, 2008). Data provided to the ACMD by the FSS on the potency of imported herbal cannabis and cannabis resin relate only to samples sent to the FSS for evidential purposes and are therefore not necessarily representative of the actual street market.

A study on the THC content of fresh illicit cannabis products seized on entry into the United Kingdom was conducted by the LGC (1975–1989). The first publication (Baker *et al*., 1980) summarized several reports in the literature on the THC content of cannabis (pre-1975). The sample sizes were relatively small ($n \approx 32$ per year) and the data were not well described; however, it

Table 4.1. THC content (%) of herbal cannabis at retail level in Europe (1998–2007).

Country	Description	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Austria	cannabis leaves	—	—	—	—	—	4.0	4.8	5.6	7.2	6.7
Belgium	cannabis leaves [1]	—	—	10.4	6.0	6.0	13.8	13.3	—	6.7	—
Bulgaria	cannabis leaves	—	—	—	—	—	—	1 to 2	2.4	2.0	1.5
Czech Republic	cannabis leaves [2]	—	—	11.0	11.0	12.0	—	3.0	3.8	4.5	4.7
Czech Republic	other grass	—	—	—	1.6	2.7	—	—	—	—	—
Estonia	cannabis leaves	—	—	—	—	—	—	—	3.3	2.0	—
Finland	cannabis leaves	—	—	—	—	2.0	1.0	—	—	3.4	4.3
France	cannabis leaves	—	—	< 2.0	< 2.0	8.0	4.0–8.5	8.8	6.1–9.7	7.0–8.0	7.5
Germany [3]	cannabis leaves	—	6.0	6.4	8.6	8.4	—	10.8	9.0	7.8	—
Germany [3]	sinsemilla	—	—	—	—	—	—	—	—	—	10.0
Germany [3]	other grass	—	—	—	—	—	—	—	—	—	2.4
Hungary [4]	cannabis leaves [5]	—	—	—	—	1.1	1.2	1.7	1.7	1.8	1.2
Italy	cannabis leaves	8.3	16.9	6.3	5.8	5.5	8.8	5.8	—	—	—

Latvia	cannabis leaves	—	—	—	—	1.5	2.2	—	—	—	—
Luxembourg	cannabis leaves	—	—	—	—	8.0	—	—	—	—	10.21
Malta	cannabis leaves	—	—	—	—	—	7.0	4.7	8.5	5.5	—
Norway	cannabis leaves	—	—	—	—	8.0	4.0	—	—	—	3.0
Norway	sinsemilla	—	—	—	—	—	—	—	—	—	13.5
Poland	cannabis leaves	—	—	—	—	—	—	0.6	1.0	1.3	5.22
Portugal	cannabis leaves	1.6	—	—	5.2	3.1	1.4	3.2	3.0	6.3	3.9
Portugal	nederwiet	—	—	—	14.6	13.1	—	—	—	—	—
Slovakia [6]	cannabis leaves	—	—	—	—	—	3.8	2.6	6.1	6.4	4.9
The Netherlands[7,8]	cannabis leaves	—	7.5	10.1	14.6	—	7.0	6.7	6.7	5.5	6.0
The Netherlands [7]	nederwiet	—	8.6	11.3	15.2	—	20.3	17.7	—	17.5	16.0
The Netherlands [7]	other grass	—	5.0	5.1	6.6	—	—	—	—	—	—
United Kingdom [9]	cannabis leaves	7.9	9.5	12.0	9.5	10.8	10.7	12.7	13.5	11.3	—

[1] 2004: nederwiet. [2] 2000–2002: nederwiet. [3] 2004–2006: median. [4] 2007: free Δ^9-tetrahydrocannabinol (THC) only (without Δ^9-tetrahydrocannabinolic acid). [5] 2002: other grass. [6] 2004–2007: weighted mean. [7] 1999, 2000, 2001, 2003 and 2005 data refer to 1999/2000, 2000/2001, 2001/2002, 2003/2004 and 2004/2005, respectively. [8] Imported herbal cannabis. [9] England and Wales.

Table 4.2. THC content (%) of cannabis resin at retail level in Europe (1998–2007).

Country	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Austria	—	—	—	—	—	8.0	10.0	7.6	5.7	10.0
Belgium	—	—	7.1	13.6	9.7	15.4	14.6	—	8.0	—
Bulgaria	—	—	—	—	—	—	0.4–0.8	2.2	5.0	3.4
Czech Republic[1]	—	15.0	11.5	11.5	6.3	15.0	10.0	7.4	—	8.1
Estonia	—	—	—	—	—	—	—	4.4	3.7	—
France	—	—	7.5	7.5	8.0	9.0–10.0	10.7	8.8–10.0	9.0–9.5	10.0
Germany[2]	—	8.4	10.5	8.6	7.9	—	8.4	8.6	6.7	—
Hungary[3]	—	—	—	—	2.0	—	4.0	3.5	1.8	2.9
Italy	4.9	8.5	8.8	11.2	13.9	11.2	8.2	—	—	—
Latvia	—	—	—	—	4.5	3.8	—	—	—	—
Luxembourg	—	3.5	8.0	7.1	—	7.8	6.9	10.9	7.4	8.5
Malta	—	—	—	—	—	10.0	10.0	10.3	9.2	—
Norway	—	—	—	8.0	5.0	7.0	—	—	—	7.0
Poland	—	—	—	—	—	0.6	—	—	—	—
Portugal	4.3	3.7	2.2	5.5	2.6	7.1	7.1	5.4	5.8	6.6
Slovakia[4]	—	—	—	—	—	24.6	15.5	13.2	9.8	8.2
Slovenia	—	—	—	—	—	—	—	—	13.6	—
The Netherlands[5,6]	—	12.6	12.8	20.6	—	18.2	16.9	16.9	18.7	13.3
Turkey	—	—	—	—	—	—	2.5	—	—	—
United Kingdom[7]	7.3	2.6	18.1	7.4	2.0	9.8	3.4	5.3	3.3	—

[1] 2003: mean of minimum and maximum potencies. [2] 2004–2006: median. 2007: free Δ^9-tetrahydrocannabinol (THC) only (without Δ^9-tetrahydrocannabinolic acid).[4] 2004–2007: weighted mean. [5] 1999, 2000, 2001, 2003 and 2005 data refer to 1999/2000, 2000/2001, 2001/2002, 2003/2004 and 2004/2005, respectively. [6] Imported cannabis resin. [7] England and Wales.

was clear that the THC potencies of the analyzed samples varied considerably (sample range: 0.0–10.5%; mean range: 0.04–4.4%).

Physical and chemical data, as well as information from the drug dealer, were used to assign the country of origin for each cannabis preparation illegally brought into the United Kingdom (1975–1989). Although this data is limited in terms of the number of samples analyzed per year ($n \approx 45$), it does give an indication of the potencies of different cannabis products from various countries for this period. Inspection of the herbal cannabis data indicates that the THC content varied widely between countries and over time (sample range: 0.2–17.0%; mean range: 3.2–6.3%), with an overall potency of 4.2% (1975–1989). Between 1975 and 1981, Thailand and India frequently produced the highest THC-containing herbal cannabis, while between 1984 and 1989, the source of the most potent products shifted toward Jamaica and the United States. The mean potencies for 1975–1979, 1980–1985 and 1986–1989 were 3.6%, 5.0% and 4.3%, respectively, indicating a slight increase between the late 1970s and 1980s. As can be expected, cannabis resin and cannabis oil showed even wider sample potency ranges (0.5–26.0% and 2–70%, respectively), with overall potencies for the time-period of 8.4% and 24.5%, respectively. No discernable

pattern of increasing potency for cannabis resin or cannabis oil could be identified. India and Nepal produced especially high potency cannabis resin between 1975 and 1981. Morocco produced the majority of the high potency cannabis resin between 1984 and 1989. India and Pakistan were the origin of high potency cannabis oil between 1977 and 1981.

Seeds from illicitly imported cannabis of known origin were grown in greenhouses in South-East England during 1980 (Baker *et al*., 1982b). The imported cannabis as well as the cannabis cultivated from the seeds taken from these seized products were analyzed. The plant material was manicured by removing stems and stalks, but it is not clear whether only leaves or leaves and buds were analyzed. The relatively low potencies of the parent cannabis (sample range: 1.00–5.73%) seem to indicate that the leaves were analyzed. The cultivated cannabis (sample range: 0.12–7.16%; mean range: 0.84–4.38%) did not always show comparable potency compared with the parent cannabis, e.g. Thailand cannabis had a 338% increase, Morocco had a 101% increase and Zimbabwe had an 80% decrease in potency.

The cannabis potencies in the LGC (1975–1989) and EMCDDA (1998–2006) studies are given for herbal cannabis and cannabis resin, and are based on material seized by law enforcement agencies. No distinction is made between imported herbal cannabis and sinsemilla. The cannabis potencies in the ACMD study (1995–2007) are given for imported herbal cannabis, sinsemilla and cannabis resin; however, even though these reports do acknowledge the FSS as the source of the data, the cannabis resin potencies do not correlate with the data of the first two studies. The data from the ACMD study were used in two comprehensive studies on cannabis and cannabis potency in Europe (King *et al*., 2004; EMCDDA, 2009).

The herbal cannabis mean and mean-range THC content for 1975–1989 (LGC) and 1998–2006 (EMCDDA) was 4.2% and 3.2–6.3%, and 10.9% and 7.9–13.5%, respectively, while the herbal cannabis and sinsemilla values for 1995–2007 (ACMD) were 4.1% and 1.9–8.5%, and 10.9% and 5.8–13.7%, respectively. The similarity in mean and mean range for herbal cannabis in 1975–1989 (LGC) and 1995–2007 (ACMD) indicates that imported herbal cannabis has stayed relatively stable since the 1970s. The elevated values for herbal cannabis in 1998–2006 (EMCDDA) are probably due to the influence of sinsemilla being introduced into the market, as is evident from the comparable mean and mean-range values for sinsemilla in 1995–2007 (ACMD). Inspection of the sinsemilla data for 1995–2007 (normal distribution: $p = 0.369$) shows a steady increase from 1995 (5.8%) to 2000 (12.2%), followed by a stable period between 2000–2005 (12.2–13.7%), and a slight decrease in 2006–2007 (10.8–10.4%) (King *et al*., 2004; Leggett, 2006; EMCDDA, 2009).

Cannabis resin data shows a slight decrease in potency from 8.4% (mean range: 5.5–11.3%) in 1975–1989 (LGC) to 6.6% (mean range: 2.0–18.1%) in 1998–2006 (EMCDDA) or 4.4% (mean range: 1.6%–6.7%) in 1995–2007 (ACMD). The data reflects the cannabis resin potency of customs seizures, i.e. imported material. The majority of the cannabis resin is imported from North African countries such as Morocco, where cultivation and processing of cannabis has not changed appreciably for more than a generation, possibly explaining the relatively stable cannabis resin potencies since 1975.

The THC content of hash oil is typically in the range 25–45% in the United Kingdom, which is similar to the 1975–1989 (LGC) values (mean: 24.5%, mean range: 15.4–32.5%) (Baker *et al*., 1982a; King *et al*., 2004).

Illicit cannabis seized by police in five constabularies in England (2004–2005) consisted of indoor-grown sinsemilla (locally produced and imported), cannabis resin (mostly imported), outdoor-grown herbal cannabis (imported) and one sample of cannabis powder collected from a portable cannabis grinder (Potter *et al*., 2008). The mean THC content of the herbal cannabis ($n = 35$) (3.1%, 0.3–11.8%) and cannabis resin ($n = 169$) (3.7%, 0.4–10.8%) for 2004–2005 is slightly lower than the potencies obtained in the LGC, EMCDDA (Reitox) and ACMD (FSS) studies, while the sinsemilla-THC content ($n = 247$) (13.3%, 1.2–23.2%) was comparable to the values obtained in the ACMD (FSS) study, especially between 2000 and 2005. The majority of the herbal cannabis and cannabis resin had THC < 6% (90% and 80%, respectively); the sinsemilla displayed a wide range of potencies, with 92% having THC > 6%.

Police Forces in England and Wales seized cannabis from street-level users in early 2008 and submitted samples for analysis to the FSS and LGC Forensics. These seizures were separate from those sent to laboratories for evidentiary purposes (Hardwick and King, 2008). The cannabis was categorized as imported herbal cannabis ($n = 71$), sinsemilla ($n = 2281$) and cannabis resin ($n = 117$), with mean THC contents of 8.4% (0.3–22.0%), 16.2% (4.1–46.0%) and 5.9% (1.3–27.8%), respectively. The mean potencies for the different cannabis preparations in 2008 were higher than those in

the 2004–2005 study, and they were also higher than the ACMD (FSS) values for 1995–2007. The distribution of potencies in 2008 shifted toward the high potency end compared with those in 2004–2005, with only 36% and 68% of the herbal cannabis and cannabis resin samples having THC < 6%, respectively, while 98% of the sinsemilla samples had THC > 6%.

France

A study in France (1983, n = 37) assessed the chemical content and potency of cannabis resin obtained from habitual end-users. The mean THC content was reported as 8.8% (Le Vu *et al.*, 1983).

Two studies conducted in France covering 1993–2000 (n = 5152) (Mura *et al.*, 2001) and 2001–2004 (n = 3322) (Mura *et al.*, 2006) reported the THC content for herbal cannabis and cannabis resin products. The reports did not give mean THC data for the years covered, but analyzed the data by frequency of potency range. The data for the herbal cannabis products indicated that, while the prevalence of low- (THC < 5%) and medium-potency (THC 5–10%) products dominated the market (> 80% combined), the prevalence of high-potency (THC > 10%) products increased dramatically from 1993–1995 (0%) to 2001–2004 (18.6%). The picture for cannabis resin was even more striking, with an increase in the prevalence of high-potency (THC > 10%) products from 1% during 1993–1995 to 41% during 2003–2004. The mean THC content for cannabis resin increased from 4.4% during 1993–1995 to 8.6% during 2001–2004.

A subsequent French study in 2004, covering the regions of Bordeaux, Dijon, Lille and Martinique, analyzed herbal cannabis and cannabis resin products (Bello *et al.*, 2005). The herbal cannabis (n = 145) had an overall mean THC content of 8.8% (0.3–23.8%), with 62% of the samples having THC > 10%, while the cannabis resin (n = 96) had an overall mean THC content of 10.7% (1.1–26.1%), with 54% of the samples having THC > 10%.

The herbal-cannabis (Table 4.1) and cannabis-resin (Table 4.2) data for France (2000–2007) indicates relatively constant trends for both products, with mean and mean-range THC content for herbal cannabis (leaves) and cannabis resin of 6.2% and 2.0–9.7%, and 9.0% and 7.5–10.7%, respectively (King *et al.*, 2004). Herbal cannabis and cannabis resin data for France (1998–2003) is also reported in an EMCDDA monograph (EMCDDA, 2009) based on French police and customs data. The herbal cannabis mean THC content varied between 4.2–6.7%, and the cannabis resin varied between 9.0–10.5% for police and customs data combined. No discernable pattern over time could be observed for either herbal or resin data.

Digesting the potency data for France based on the above studies is rather complex. The cannabis resin potencies seem to have stabilized around 9–10%, while the herbal cannabis potencies varied between 4–9%, with an apparent increase in the availability of high potency products.

Greece

Customs and Police authorities in two districts of Greece, namely Ipiros (n = 18) and Lakonia (n = 18), seized and analyzed illicit herbal cannabis during 1996 (Stefanidou *et al.*, 1998). The upper part of the main stem of each flowering plant was dried and, after removing seeds and stems, the dried leaves were ground to a powder. Both THC data sets were consistent with normal distributions (Ipiros: p = 0.511; Lakonia: p = 0.355), and were not significantly different (one-way ANOVA [α = 0.05]: p = 0.145). One-way ANOVA (α = 0.05) revealed that the data sets for CBD (p = 0.376) and CBN (p = 0.064) were also not significantly different vis-à-vis the two regions. This suggests that there is no major variation in the cannabinoid content between the two districts. The THC mean potency for the 36 samples was 1.7 ± 1.3% (0.08–4.41%, 1.25, 2.12%) (CBD: 0.5 ± 1.1%, 0.002–5.98%, 0.10, 0.81%; CBN: 0.4 ± 0.4%, 0.005–1.61%, 0.27, 0.55%). The low-THC and high-CBD potencies of some of these seizures indicate that they might be intermediate or fiber phenotypes.

Italy

An Italian study analyzed cannabis products that were seized between 1997–2004 in Modena county (Licata *et al.*, 2005). The products were classified as marijuana (n = 947) or hashish (n = 4280), with each group subdivided into loose, kilo-brick, buds and domestic, or sticks and bars, respectively. The THC content of marijuana increased from 2.5% in 1997 to 15% in 2004, while hashish increased from 4.5% to 15.3% over the same period. Analyses of the frequency distribution based on THC content showed that between 1997–2000, the majority of the products had THC < 8% (marijuana range: 2.5–7%; hashish range: 4.5–6%), while between 2001–2004 the majority of the products

had THC > 8% (marijuana range: 10.7–15%; hashish range: 9.8–15.3%). During 2001–2004 there was a 79.3% increase compared with 1997–2000 in the percentage of marijuana products classified as buds, possibly explaining the concurrent increase in overall potency for marijuana.

The data on herbal cannabis (Table 4.1) and cannabis resin (Table 4.2) for Italy as provided by EMCDDA/Reitox (1998–2004) indicates relatively constant trends for both products. The mean and mean-range THC content for herbal cannabis and cannabis resin were 8.2% and 5.5–16.9%, and 9.5% and 4.9–13.9%, respectively. The herbal cannabis mean THC content for 1999 of 16.9% indicates that it could possibly be nederwiet. If this value is removed from the list, the mean and range for herbal cannabis is 6.7% and 5.5–8.8%, respectively (King *et al*., 2004).

Although the values in the two studies are consistent as far as potency range is concerned, the former study does suggest an increase in potency for both herbal cannabis and cannabis resin, while the EMCDDA/Reitox study suggests a more constant potency trend for both preparation types.

Brazil

The cannabinoid content of cannabis products (marijuana: $n = 52$; hashish: $n = 3$) seized in São Paulo, Brazil, were measured and reported (2006–2007). The marijuana was described as raw-plant material, and the hashish as resin of the plant shaped into a dark green ball (Lopes de Oliveira *et al*., 2008). Again, detailed information on the proper classification of the marijuana products was lacking. The marijuana had a mean THC content of 2.5 ± 1.9% (0.08–5.51%, 1.94, 2.99%), with only six seizures having THC > 5%. The hashish had a mean THC content of 3.5 ± 0.5% (3.15–4.02%, 2.28, 4.66%).

Colombia

A seizure of Colombian cannabis destined for North America (1977) consisted of 174 large bales of marijuana (34–45 kg each), 66 smaller bales of marijuana (14 kg each), 19 balls of hashish and hash oil (2.8 l in total) (Tucker and Graham, 1981). The bales were labeled with red, yellow and green crosses to differentiate between red, gold and green marijuana, respectively. The marijuana appeared to consist mainly of dried fruiting buds. The THC content of the red, gold and green marijuana was 5.0%, 3.6% and 3.0%, respectively. The hashish- and hash oil-THC content was 10.7% and 28.2%, respectively. Cannabidiol was undetectable in all the analyzed samples.

In a recent study, samples of illicit cannabis crops in four different geographical regions of Colombia, namely Llanos Orientales (n = 13), Cauca (n = 13), Santa Marta (n = 13) and Eje Cafetero (n = 13), were collected and analyzed (Florian *et al*., 2009). The samples were dried and manicured by removing stems, flowers and seeds, and the resulting cannabis leaf analyzed by GC-MS and GC-FID. Llanos Orientales (15.7 ± 2.9%, 13.37, 17.63%) and Cauca (11.0 ± 6.7%, 6.72, 15.48%) produced high-potency cannabis, while Santa Marta (2.8 ± 1.7%, 1.89, 4.00%) and Eje Cafetero (1.9 ± 1.3%, 1.11, 3.17%) produced much lower potencies. The high potencies from the former regions indicate that these samples possibly included buds. The Eje Cafetero region had especially low CBD content compared with the elevated levels of the other regions (0.02% versus 1.86%, 2.52% and 1.86%). The disparities in cannabinoid content between the regions could conceivably be explained by climate and cultivation variations.

The Llanos Orientales and Cauca regions produced herbal cannabis potencies two to three times higher than the 1977 seizure; however, the Santa Marta and Eje Cafetero regions produced much lower potencies. The data from these two studies are insufficient to hypothesize about potency trends in Colombia, except to say that high potency cannabis products (THC > 10%) are available on the illicit market.

Australia

The THC content of cannabis products produced in Australia is not routinely tested; however, seized cannabis is occasionally tested, and small independent research studies intermittently examine cannabis THC content. A number of small studies in 1970–1971 (n = 7) (Cartwright and Mather, 1972), 1996 (n = 168) (Hall and Swift, 2000), 1997 (n = 12) (Gowing *et al*., 2000) and 2002 (n = 20) (Leach and Deseo, 2002; Copeland *et al*., 2006) reported THC content.

A 1970–1971 study examined Australian grown cannabis, including six cultivated whole herbs and one feral female flowering-buds plant, by TLC and GC analyses. The whole-herb THC range was 0.04–0.92%, while the buds had a THC content of 1.1%. A collection of 168 seizures in 1996 (March to May) by Western Australian police yielded a mean THC content of 3.8%, while the subset of only buds (n = 59) yielded a mean

of 6.4%. A small study in 1997 of seizures of leaves (n = 6) and buds (n = 6) indicated a THC content of 3.4 ± 3.9% (0.6–13.0%, 0.97, 5.86%), with the majority being between 0.6–2.5%. The hydroponically grown cannabis was on average about six times more potent than the regular samples. A 2002 study examined cannabis from a controlled experimental crop (n = 10) and from the illicit market (n = 10) in New South Wales, South Australia and Queensland, revealing THC content of 1.4 ± 1.5% (0.10–5.05%, 0.59, 2.14%) and 5.0 ± 6.7% (0.51–22.25%, 1.61, 8.31%), respectively. The majority of the experimental and illicit cannabis contained THC below 2% and 5%, respectively. One-way ANOVA (α = 0.05) revealed that the controlled experimental crop and illicit market samples were not significantly different (p = 0.174). The data for Australia, although extremely limited in scope, indicates an increase in potency from about 1% in the 1970s to about 5% in 2002.

New Zealand

The potency of cannabis products seized and eradicated in New Zealand from 1976–1996 was studied and reported (Poulsen and Sutherland, 2000). Imported herbal cannabis (female buds) (n = 21) between 1976–1982 had mean THC content of 3.8 ± 1.9% (0.5–7.5%, 2.9, 4.7%); locally grown herbal cannabis (female buds) (n = 432) between 1979–1996 had mean THC content of 2.9 ± 0.6% (0.2–9.7%, 2.8, 2.9%). As can be expected, locally grown herbal cannabis (n = 613) between 1978–1996 consisting mainly of leaf material displayed significantly lower potencies (1.0 ± 0.3%, 0.1–4.2%, 1.0–1.1%) than the female buds (one-way ANOVA [α = 0.05]: $p < 0.001$). Neither the buds nor the leaves showed any indication of increased potencies over the period of the study.

Analysis of locally produced cannabis resin (n = 268) between 1978–1989 showed high variability between samples in a specific year, e.g. the range for 1985 was 0.2–19%, and between years, with an overall mean of 6.4 ± 1.9% (0.1–21%, 6.2, 6.6%). The resin did not show any indication of increased potencies over the period of the study. Comparison of imported (1975–1989, n = 106) and locally (1983–1995, n = 605) produced cannabis oil showed that the former had a higher overall mean potency and more variability (26.7 ± 14.0% [3.1–66%, 24.0, 29.4%] and 13.5 ± 3.9% [0.1–67%, 13.2, 13.8%], respectively). The overall combined potency for imported and locally produced cannabis oil peaked between 1984–1986, followed by a decrease in average potency in the subsequent years as imported oil was not available. The mean for the combined imported and locally produced cannabis oil potency for 1975–1986 and 1987–1995 was 24.5 ± 8.4% and 12.7 ± 1.6%, respectively, representing a twofold decrease. The highly variable oil did not show any indication of increased potencies over the period of the study.

Morocco

A study conducted in 2004 on cannabis resin (n = 30) seized in Morocco revealed a mean THC content of 6.0 ± 4.0% (0.4–16.0%, 4.5, 7.5%) (Stambouli *et al.*, 2005).

The THC content of cannabis (fresh male and female plants [n = 180], dried female plants [n = 52] and female-powdered plants [n = 13]) obtained from three provinces of Northern Morocco (2004), namely Al Hoceima, Chefchaouen and Larache, was determined. Al Hoceima and Chefchaouen have long traditions of cannabis cultivation, while cannabis cultivation has only recently started in Larache. Together these three regions accounted for more than 80% of the country's cannabis production in 2004 (Stambouli *et al.*, 2005). Analysis of green, growing plants indicated that female (0.5%, 0.1–2.2%, 0.4, 0.6%) and male (0.4%, 0.1–1.5%, 0.1, 0.7%) plants had similar THC potencies, and that there was not a significant difference between the leaves, buds, and leaves and buds-combined potencies compared across gender and within gender. Female dried plants (leaves and buds combined) showed significantly higher potencies (2.1%, 0.2–7.5%, 1.6, 2.5%) than the green, growing plants, as well as distinction between dried leaves (1.2%, 0.2–2.6%, 1.0, 1.5%) and dried buds (2.9%, 1.0–7.5%, 2.3, 3.8%). The female-powdered plants had the highest potencies (8.3%, 5.5–11.3%, 7.1, 9.4%). This study shows the variability between different growing stages, leaves and buds, and cannabis preparations with regard to THC content.

Cannabidiol content and its role in the effects of cannabis

The endogenous cannabinoid system acts via neuromodulatory action, generally inhibiting the release of other neurotransmitters. Cannabidiol, the main non-psychotropic constituent of cannabis, does not bind to the cannabinoid receptors, probably exerting its effects through novel cannabinoid receptors mediating non-CB1/CB2 receptor effects (Galal *et al.*, 2009). Cannabidiol can antagonize cannabinoid-receptor agonists such as THC.

The ratio of CBD to THC in the plant, which is genetically determined, is therefore significant in terms of psychoactivity, as changes in the ratio could modify the effects of cannabis. This is especially important for sinsemilla due to its high-THC and low-CBD content, and its steadily increasing market share.

Analysis of the CBD:THC ratios for marijuana and sinsemilla in the United States indicates that during 1981–1996 the ratios were both about 9.5%, whereas between 1997–2009 the ratio for marijuana increased to 14.3% and the ratio for sinsemilla decreased to 5.6%. This represents an increase of 50.4% for marijuana and a decrease of 46.8% for sinsemilla. One-way ANOVA ($\alpha = 0.05$) of the marijuana and sinsemilla CBD:THC ratios for 1981–1996 and 1997–2009 indicated that these two periods are significantly different ($p < 0.021$).

Improving the data

Classification of cannabis and cannabis preparations are of utmost importance to allow comparison of data across time and geographical location. This should include a description of the part of the plant used and classification by product, i.e. herbal cannabis, cannabis resin and cannabis oil. The nomenclature for herbal cannabis includes numerous terms that are location specific, such as skunk, tops, nederwiet, buds, seeded cannabis, marijuana and sinsemilla. Herbal cannabis comprising the dried and crushed flower-heads and surrounding leaves should be labeled marijuana, while the unfertilized buds of female plants should be labeled sinsemilla. Attention should also be given, if possible, to determining whether the cannabis is locally produced or imported. Cultivation techniques must also be considered. Cannabis is a versatile plant that grows in a variety of climates, with the amount and quality of resin produced depending on humidity, temperature, light and soil acidity and alkalinity. Outdoor-produced herbal cannabis usually has substantial variation in potency, while optimized indoor cultivation of female plants, often employing hydroponics, yields cannabis of a consistently higher potency. Potency data is most often reported as the mean THC content for a specified time period, usually per year. Unfortunately, additional data such as sampling method, sample size, mode, median, potency range, confidence interval, standard deviation and analysis of outlier samples is usually not included.

Adequate sampling is extremely important to ensure that the potency of the actual THC-containing parts of the plant is measured. This should include careful manicuring, i.e. removal of stalks, seeds and leaves (in the case of sinsemilla) and homogenization of the material. The actual analysis of the THC content can also be problematic. Issues such as extraction efficiency, conversion efficiency of cannabinoid acids to their neutral forms, THC stability, accuracy and precision should always be considered when performing potency analyses. Control measures must be included in any laboratory standard operating procedures to ensure accuracy and precision of the analyses. The use of THC as a reference standard can be problematic because of stability issues and accuracy of the labeled amount of THC present in commercially available standards.

Conclusions

The data discussed in this chapter does suggest that the cannabis products available on the illicit market and in coffee shops in The Netherlands are more potent today than before the turn of the millennium. Similar work on cannabis potency often uses past erroneous claims of 10–30 times-increased THC content as justification for proclaiming that cannabis has not become more potent. That is to say, just because cannabis potency has not increased 10–30 times does not necessarily mean that a more modest increase of, for example 2–3 times, is not possible. The issue is also considerably more complex than merely analysis of potency trends. The cannabis market has changed significantly over especially the last decade, with growers becoming much more sophisticated and focusing on high-potency sinsemilla. This was seen in a number of studies where the frequency of high-potency (THC > 10%) products has increased dramatically.

In conclusion, it is clear that high-potency cannabis products are freely available on the international drug markets, and that cannabis products have at least a twofold increased THC content compared with pre-2000 products. It does seem that the potencies have stabilized over the past five years; however, the variability of the cannabis plant does result in extremely high-potency (THC > 25%) products being consumed by end-users. These factors certainly warrant investigation into the effects of the availability of high-potency products on cannabis users.

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References

ACMD (2005) *Further Consideration of the Classification of Cannabis Under the Misuse of Drugs Act 1971*. London, England: Home Office.

ACMD (2007) *Cannabis: Classification and Public Health*. London, England: Home Office. Mathre, pp.35–55. Jefferson, NC: McFarland and Company.

Baker, P.B., Bagon, K.R., Gough, T.A. (1980) Variation in the THC content in illicitly imported cannabis products. *B Narcotics*, **32**:47–54.

Baker, P.B., Gough, T.A., Johncock, S.I.M., Taylor, B.J., Wyles, L.T. (1982a) Variation in the THC content in illicitly imported cannabis products – Part II. *B Narcotics*, **34**:101–8.

Baker, P.B., Gough, T.A., Taylor, B.J. (1982b) The physical and chemical features of cannabis plants grown in the United Kingdom of Great Britain and Northern Ireland from seeds of known origin. *B Narcotics*, **34**:27–36.

Bello, P.Y., Giraudon, I., Parent, M.-J., *et al*. (2005) Composition et caractéristiques de cannabis collectés auprès d'usagers dans quatre sites en France, 2004. *Bulletin Épidémiologique Hebdomadaire*, **20**:91–2.

Bócsa, I., Máthé, P., Hangyel, L. (1997) Effect of nitrogen on tetrahydrocannabinol (THC) content in hemp (Cannabis sativa L.) leaves at different positions. *J Int Hemp Assoc*, **4**:80–1.

Cartwright, L.G., Mather, L.E. (1972) Investigations of some samples of Australian grown cannabis. *Aust J Pharm Sci*, **1**:49–51.

Copeland, J., Gerber, S., Swift, W. (2006) *Evidence-Based Answers to Cannabis Questions: A Review of the Literature*. Canberra, Australia: Australian National Council on Drugs.

De Backer, B., Debrus, B., Lebrun, P., *et al*. (2009) Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material. *J Chromatogr, B: Anal Tech Biomed Life Sci*, **877**:4115–24.

De Meijer, E.P.M., Bagatta, M., Carboni, A., Crucitti, P., Moliterni, V.M.C., Ranalli, P., Mandolino, G. (2003) The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics*, **163**:335–46.

Eaton, G., Davies, C., English, L., Lodwick, A., Bellis, M.A., McVeigh, J. (2007) *United Kingdom Drug Situation: Annual Report to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)*. London, England: United Kingdom Focal Point on Drugs, Department of Health.

Eaton, G., Davies, C., English, L., Lodwick, A., McVeigh, J., Bellis, M.A. (2008) *United Kingdom Drug Situation: Annual Report to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)*. London, England: United Kingdom Focal Point on Drugs, Department of Health.

Eaton, G., Lodwick, A., Bellis, M.A., McVeigh, J. (2006) *United Kingdom Drug Situation: Annual Report to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)*. London, England: United Kingdom Focal Point on Drugs, Department of Health.

Eaton, G., Morleo, M., Lodwick, A., Bellis, M. A., McVeigh, J. (2005) *United Kingdom Drug Situation: Annual Report to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)*. London, England: United Kingdom Focal Point on Drugs, Department of Health.

ElSohly, M.A., Holley, J.H., Lewis, G.S., Russell, M.H., Turner, C.E. (1984) Constituents of *Cannabis sativa* L. XXIV. The potency of confiscated marijuana, hashish, and hash oil over a ten-year period. *Journal of Forensic Sci*, **29**:500–14.

ElSohly, M.A., Holley, J.H., Turner, C.E. (1985) Constituents of *Cannabis sativa* L. XXVI. The Δ^9-tetrahydrocannabinol content of confiscated marijuana, 1974–1983. In *Marijuana '84: Proceedings of the Oxford Symposium on Cannabis*, eds. D. J. Harvey, W. Paton, G. G. Nahas, pp.37–42. England: IRL Press.

ElSohly, M.A., Ross, S.A., Mehmedic, Z., Arafat, R., Yi, B., Banahan, B.F. (2000) Potency trends of Δ^9-THC and other cannabinoids in confiscated marijuana from 1980–1997. *J Forensic Sci*, **45**:24–30.

ElSohly, M.A., Slade, D. (2005) Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sci*, **78**:539–48.

EMCDDA. (2009) *A Cannabis Reader: Global Issues and Local Experiences. Monograph Series 8 – Volume 1*. Lisbon, Portugal: Office for Official Publications of the European Communities.

Florian, N.M., Parada, F., Garzón, W.F. (2009) Study of cannabinoid content in marihuana samples (*Cannabis sativa* L.) cultivated in several regions of Colombia. *Vitae*, **16**: 237–44.

Galal, A.M., Slade, D., Gul, W., El-Alfy, A.T., Ferreira, D., ElSohly, M.A. (2009) Naturally occurring and related synthetic cannabinoids and their potential therapeutic applications. *Recent Patents on CNS Drug Discovery*, **4**:112–36.

Gowing, L.R., Ali, R.L., White, J.M. (2000) *Respiratory Harms of Smoked Cannabis*. Adelaide, Australia: Drug and Alcohol Services Council.

Hall, W., Swift, W. (2000) The THC content of cannabis in Australia: evidence and implications. *Aust NZ J Publ Heal*, **24**:503–8.

Hardwick, S., King, L. (2008) *Home Office Cannabis Potency Study 2008*. St Albans, United Kingdom: Home Office Scientific Development Branch.

King, L.A. (1997) Drug content of powders and other illicit preparations in the UK. *Forensic Sci Int*, **85**:135–47.

King, L.A., Carpentier, C., Griffiths, P. (2004) *An Overview of Cannabis Potency in Europe*. EMCDDA Insights 6. Lisbon, Portugal: Office for Official Publications of the European Communities.

Korf, D.J. (2002) Dutch coffee shops and trends in cannabis use. *Addict Behav*, **27**:851–66.

Le Vu, S., Aquatias, S., Bonnet, N., Debrus, M., Fournier, G., Beauverie, P. (1983) Chemical content of street cannabis. *Presse Med*, **35**:755–8.

Leach, D., Deseo, M. (2002) *Cannabinoid Levels in Varieties of Cannabis*. Southern Cross University, East Lismore, Australia: Australian Phytochemicals Limited.

Leggett, T. (2006) A review of the world cannabis situation. *Bulletin on Narcotics*, **58**:1–155.

Licata, M., Verri, P., Beduschi, G. (2005) Δ^9-THC content in illicit cannabis products over the period 1997–2004 (first four months). *Ann Ist Super Sanità*, **41**:483–5.

Lopes de Oliveira, G., Voloch, M. H., Sztulman, G. B., Negrini Neto, O., Yonamine, M. (2008) Cannabinoid contents in cannabis products seized in São Paulo, Brazil, 2006–2007. *Forensic Tox*, **26**:31–5.

Mackie, K. (2007a) From active ingredients to the discovery of the targets: the cannabinoid receptors. *Chem Biodivers*, **4**:1693–706.

Mackie, K. (2007b). Understanding cannabinoid psychoactivity with mouse genetic models. *PLoS Biology*, **5**:2106–8.

McLaren, J., Swift, W., Dillon, P., Allsop, S. (2008) Cannabis potency and contamination: a review of the literature. *Addiction*, **103**:1100–9.

Mehmedic, Z., Chandra, S., Slade, D., *et al.* (2010) Potency trends of Δ^9-THC and other cannabinoids in confiscated cannabis preparations from 1993–2008. *J Forensic Sci*, **55**:1209–17.

Mura, P., Brunet, B., Dujourdy, L., *et al.* (2006) Cannabis d'hier et cannabis d'aujourd'hui. Augmentation des teneurs en THC de 1993 à 2004 en France. *Annales de Toxicologie Analytique*, **18**:3–6.

Mura, P., Perrin, M., Chabrillat, M., *et al.* (2001) L'augmentation des teneurs en delta-9 tétrahydrocannabinol dans les produits à base de cannabis en France: mythe ou réalité? *Ann Tox Anal*, **13**: 75–9.

Newell, C.J. (2003) Potency of cannabis seized in central Florida during June 2002. *Microgram Journal*, **1**:37–9.

Niesink, R.J.M., Pijlman, F.T.A., Rigter, S., Hoek, J., Mostert, L. (2002) *THC concentrations in wiet, nederwiet en hasj in Nederlandse coffeeshops (2001–2002)*. Utrecht, The Netherlands: Trimbos Institute, Netherlands Institute of Mental Health and Addiction.

Niesink, R.J.M., Pijlman, F.T.A., Rigter, S., Hoek, J., Mostert, L. (2003) *THC concentrations in wiet, nederwiet en hasj in Nederlandse coffeeshops (2002–2003)*. Utrecht, The Netherlands: Trimbos Institute, Netherlands Institute of Mental Health and Addiction.

Niesink, R.J.M., Rigter, S., Hoek, J., Den Boer, N. (2009) *THC concentrations in wiet, nederwiet en hasj in Nederlandse coffeeshops (2008–2009)*. Utrecht, The Netherlands: Trimbos Institute, Netherlands Institute of Mental Health and Addiction.

Niesink, R.J.M., Rigter, S., Hoek, J., Goldschmidt, H. (2006) *THC concentrations in wiet, nederwiet en hasj in Nederlandse coffeeshops (2005–2006)*. Utrecht, The Netherlands: Trimbos Institute, Netherlands Institute of Mental Health and Addiction.

Niesink, R.J.M., Rigter, S., Hoek, J., Goldschmidt, H. (2007) *THC concentrations in wiet, nederwiet en hasj in Nederlandse coffeeshops (2006–2007)*. Utrecht, The Netherlands: Trimbos Institute, Netherlands Institute of Mental Health and Addiction.

Niesink, R.J.M., Rigter, S., Hoek, J., Goldschmidt, H. (2008) *THC concentrations in wiet, nederwiet en hasj in Nederlandse coffeeshops (2007–2008)*. Utrecht, The Netherlands: Trimbos Institute, Netherlands Institute of Mental Health and Addiction.

Pijlman, F.T.A., Rigter, S.M., Hoek, J., Goldschmidt, H.M.J., Niesink, R.J.M. (2005) Strong increase in total delta-THC in cannabis preparations sold in Dutch coffee shops. *Addict Biol*, **10**:171–80.

Pitts, J.E., O'Neil, P.J., Leggo, K.P. (1990) Variation in the THC content of illicitly imported cannabis products – 1984–1989. *J Pharm Pharmacol*, **42**:817–20.

Potter, D.J., Clark, P., Brown, M.B. (2008) Potency of Δ^9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. *J Forensic Sci*, **53**:90–4.

Poulsen, H.A., Sutherland, G.J. (2000) The potency of cannabis in New Zealand from 1976 to 1996. Science and Justice: *J For Sci Soc*, **40**:171–6.

RCMP. (2002) *Marihuana Cultivation in Canada: Evolution and Current Trends*. Royal Canadian Mounted Police, Canada: Criminal Intelligence Directorate.

Ross, S.A., Mehmedic, Z., Murphy, T.P., ElSohly, M.A. (2000) GC-MS analysis of the total Δ^9-THC content of both drug- and fiber-type cannabis seeds. *J Anal Toxicol*, **24**:715–7.

Stambouli, H., El Bouri, A., Bellimam, M. A., Bouayoun, T., El Karni, N. (2005) Cultivation of *Cannabis sativa* L. in Northern Morocco. *B Narcotics*, **57**:79–118.

Stefanidou, M., Dona, A., Athanaselis, S., Papoutsis, I., Koutselinis, A. (1998) The cannabinoid content of marijuana samples seized in Greece and its forensic application. *Forensic Sci Int*, **95**:153–62.

Tucker, R.B., Graham, B.F. (1981) Cannabinoid content of Colombian cannabis. *Can Soc Forensic Sci J*, **14**: 41–5.

United States – Canada Border. (2007) Drug Threat Assessment 2007.

Van Laar, M., Cruts, G., Van Gageldonk, A., *et al*. (2008) The Netherlands Drug Situation 2007: Report to the EMCDDA by the Reitox National Focal Point. Utrecht, The Netherlands: Trimbos Institute, Netherlands Institute of Mental Health and Addiction.

Viau, R., Marro, L., Walker, M. (2004) *Retrospective Examination of the THC Levels of Marihuana Seized in Canada*. Vienna, Austria: Second Technical Conference on Drug Control Research.

Chapter 5

What are the policy implications of the evidence on cannabis and psychosis?

Wayne Hall and Louisa Degenhardt

In this chapter we explore the policy implications of the evidence on cannabis and psychosis for mental health services, health education about the risks of cannabis use, and public policy toward recreational cannabis use. We consider: (1) what the relationship should be between observational evidence and public health policy using comparative analyses of similar evidence on the harmful effects of alcohol, tobacco and amphetamine use; (2) arguments made on the grounds of public health prudence for discouraging cannabis use by young people; and (3) recommendations for policies that may reduce cannabis use among patients in mental-health services and the general population via health education and public policies toward cannabis.

We conclude that the observational evidence, as well as the biological plausibility of the hypothesis that cannabis is a contributory cause of psychosis, are at least as strong as the evidence for causal relationships between heavy alcohol and amphetamine use and psychosis. On the grounds of public-health prudence, there is also a good case for discouraging cannabis use among adolescents and young adults. Uncertainty remains about the best ways to do so, and who should be the targets of campaigns to reduce cannabis use. We should: discourage cannabis use among young adults seeking treatment in mental health services; inform young people about the probable mental health risks of cannabis use, especially of early and frequent use of cannabis, after conducting research to identify credible and persuasive ways of doing so; exercise caution in liberalizing cannabis laws in ways that may increase young people's access to cannabis, decrease their age of first use or increase their frequency of cannabis use; and consider the feasibility of reducing the availability of high-potency cannabis products under prohibition, by trialing and evaluating the efficacy of graduated penalties for producers and suppliers of higher Δ^9-tetrahydrocannabinol (THC) content cannabis products.

The interpretation of the policy implications of the evidence on cannabis use and psychosis has often been refracted through the pre-existing policy commitments of protagonists in the debate about whether cannabis use by adults should continue to attract criminal penalties. Those who favor a continuation of criminal penalties often invoke the evidence for a causal role of cannabis in psychosis to justify their stance (e.g. de Irala *et al*., 2005), while those who favor more liberal policies toward cannabis have generally been very sceptical about the evidence (e.g. Zimmer and Morgan, 1997; Grotenhermen, 2004; Mirken and Earleywine, 2005; Macleod and Hickman, 2010).

We attempt to avoid premature closure on the policy implications of the evidence by proceeding as follows. First, we ask "is the available evidence sufficient to warrant the conclusion that cannabis is a contributory cause of psychosis in young adults?" We argue that it is more likely than not that cannabis is a contributory cause of psychosis. We also believe that even those who remain sceptical should support health education that alerts young people to the possibility of these effects. Second, we ask what policies should be adopted in order to reduce the psychotogenic effects of cannabis use? We consider these policies under three broad headings that are organized in increasing order of their contentiousness, and the scope of those who would be affected by the policy. We begin by asking: how should we respond to young people with psychoses who use cannabis? We then pose the question what should we tell young people about the mental health risks of cannabis use? How can this be done in a way that is most likely to dissuade them from using cannabis? Finally, we address the most contentious issue that is at the forefront of the cannabis policy debate in many countries: what are the implications of the evidence on cannabis and psychosis for legal policies adopted toward cannabis use by young adults? Should jurisdictions that still impose

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criminal penalties for cannabis use reject proposals to liberalize these policies by imposing civil rather than criminal penalties for cannabis use, or legalizing the production, sale and use of cannabis? Should jurisdictions that have liberalized penalties for cannabis use consider re-imposing criminal sanctions?

Making causal inferences from observational data

As detailed elsewhere in this book (Chapters 12 and 15 and see Arseneault *et al*., 2004; Semple *et al*., 2005; Degenhardt and Hall, 2006; Fergusson *et al*., 2006b), there is observational evidence from large longitudinal studies in a number of different countries that young adults who are regular cannabis users are at increased risk of developing psychosis. These studies have found that: (1) cannabis users report more psychotic symptoms, and have higher rates of diagnosed psychosis, than individuals who have not used cannabis; (2) the risk of psychosis and psychotic symptoms increases with the frequency of use, and is greater if people begin using cannabis at an earlier age; (3) persons with a personal or family history of psychotic symptoms appear to be more likely to develop psychotic symptoms if they use cannabis; and (4) these relationships usually persist after controlling for a range of potentially confounding variables, such as personal characteristics, other types of drug use and a family history of psychiatric disorders.

The major uncertainty about the evidence arises from the fact that individuals are not randomly assigned to use cannabis or not, so we cannot be certain that the baseline risk of psychosis in those who did and did not use cannabis was the same (Fergusson *et al*., 2006b; Macleod *et al*., 2004). The two most plausible alternative explanations of the association between cannabis use and psychosis are: (1) that psychosis is caused by uncontrolled confounding e.g. the use of other drugs, such as psychostimulants and alcohol, both of which are more likely to be used by regular cannabis users, or a genetic vulnerability to develop a psychosis that also increases the risk of using cannabis (Weiser and Noy, 2005); and (2) cannabis use is an early symptom of emerging psychosis.

The epidemiological studies to date have attempted to address these forms of confounding by measuring and statistically adjusting for other drug use, personal characteristics that predict psychosis risk and a personal history of psychotic symptoms (e.g. van Os *et al*., 2002; Zammit *et al*., 2002, Fergusson *et al*., 2003, 2005; Henquet *et al*., 2004). The logic of this approach has been that the relationship between cannabis use and psychosis is unlikely to be because of confounding if the association persists after controlling for these variables. The number of confounding variables that have been assessed has varied between studies, as have the specific variables that have been statistically controlled. One recent study used fixed effects regression to control for *unmeasured* confounders (Fergusson *et al*., 2005).

We agree with the authors of a systematic review (Moore *et al*., 2007) that it is unlikely that confounding explains the association between regular and early cannabis use, and psychotic symptoms and psychosis. The studies that have controlled for personal characteristics and other drug use have still found a relationship. Those who continue to assert the possibility of uncontrolled confounding should identify *plausible* confounding variables (Cook and Campbell, 1979) that have not been adequately controlled in studies to date so that they can be controlled in future studies.

We also give weight to evidence supporting the biological plausibility of a causal relationship between cannabis use and psychosis. First, the principal psychoactive ingredient of cannabis – THC – acts upon CB1 cannabinoid receptors in the brain (Chapter 1 and see Hall *et al*., 2001), and the cannabinoid system, in turn, interacts with dopaminergic neurotransmission, which has been implicated in the production of psychotic symptoms (Hall *et al*., 2001). Second, there is direct evidence that the cannabinoid system may be disturbed in patients with schizophrenia and related psychotic disorders (Chapters 16 and 17 and see Fritzsche, 2001; Glass, 2001; Skosnik *et al*., 2001). Third, D'Souza and colleagues have shown in a double-blind provocation study that intravenous THC increases positive and negative psychotic symptoms in a dose-dependent way in both patients with schizophrenia and healthy volunteers with no history of psychosis (Chapter 18 and see D'Souza *et al*., 2004).

We think it more likely than not that cannabis use precipitates schizophrenia in persons who are vulnerable because of a personal or family history of schizophrenia (Hall *et al*., 2004). This hypothesis is consistent with the stress-diathesis model of schizophrenia (Gottesman, 1991; Bromet *et al*., 1995) and with the following facts: the overall relative risk (RR) of developing schizophrenia if cannabis use is relatively modest (RR = 2–3); the incidence of treated schizophrenia has

not substantially increased during the 1970s and 1980s (Der *et al.*, 1990) when there were substantial increases in cannabis use among young adults in Australia and North America (Donnelly and Hall, 1994), and among persons with psychosis who have used cannabis. The onset of these disorders is, on average, an earlier age than those who have not (Arendt *et al.*, 2005; Barnes *et al.*, 2006).

A comparative evaluation of the evidence

It is useful to compare the strength of the evidence on cannabis and psychosis with that of observational evidence on relationships between adverse health effects and other types of drug use. Such comparisons facilitate more consistent, even-handed appraisals of the comparative strengths and weakness of evidence on the adverse effects of different drugs (Hall, 1999).

Some commentators (de Irala *et al.*, 2005) have drawn analogies to the debate in the early 1960s (see Talley *et al.*, 2004; Parascandola, 2005) about whether the epidemiological evidence on associations between cigarette smoking and lung cancer was sufficient to warrant public health campaigns to discourage smoking. The eventual emergence of a consensus that cigarette smoking was a cause of lung cancer, heart disease and chronic obstructive pulmonary disease depended on observational evidence from large cohort studies, in the absence of pathophysiological explanations of how cigarette smoking caused any of these diseases (Royal College of Physicians, 1962; US Surgeon General's Advisory Committee on Smoking and Health, 1964). Some commentators argue that the evidence for the psychotogenicity of cannabis also depends upon observational evidence from cohort studies (de Irala *et al.*, 2005). A major difference, however, is that the association was far stronger for cigarette smoking and lung cancer (RR = 12 for a 20 cigarettes a day smoker) than it is for cannabis and psychosis (RR ~ 2 to 3).

A more relevant comparison can be made with the evidence on the psychotogenicity of alcohol and amphetamines. The evidence that heavy alcohol use causes psychosis is much weaker than the evidence for the psychotogenicity of cannabis. It largely consists of case series of *delirium tremens* in severely alcohol-dependent people undergoing alcohol withdrawal (Greenberg and Lee, 2001) and one very old experimental study that deliberately induced *delirium tremens* in drinkers by the abrupt cessation of alcohol, after several weeks of sustained heavy drinking, in a hospital ward (Isbell *et al.*, 1955). There are also case series of psychotic disorders ("alcoholic hallucinosis") that reportedly occur in heavy consumers of alcohol, but the status of this diagnostic entity and the role of alcohol in producing these disorders, is uncertain (Lishman, 1987; Greenberg and Lee, 2001).

The evidence that heavy amphetamine use can induce psychosis is much stronger than that for alcohol. The initial observations were derived from 200 case studies of heavy-amphetamine users who developed paranoid psychoses after sustained periods of heavy-amphetamine use and whose disorders remitted after a period of abstinence from amphetamine lasting from several days to a week (Connell, 1958). This case-series evidence was later supported by the experimental reproduction of psychoses in small numbers of amphetamine users (Bell, 1973) and normal volunteers (Angrist *et al.*, 1974). These studies (which would nowadays be considered unethical) involved administering chronic high doses of amphetamine to drug users in treatment (Bell, 1973) or to medical students (Curran *et al.*, 2004). More recently, observational studies have reported associations between the frequency of amphetamine injection, and the frequency and severity of psychotic symptoms among amphetamine users (Hall and Hando, 1993). The hypothesis that the relationship is causal is supported by animal evidence that amphetamine and cocaine produce major effects on dopaminergic neurotransmission (Curran *et al.*, 2004).

Methamphetamine is a much more potent psychotogenic agent than cannabis, as is evidenced by the relative frequency of hospitalizations for cannabis- and amphetamine-related psychotic disorders. In Australia, the prevalence of amphetamine use is around one-fifth that of cannabis use but there are more hospital stays for psychotic disorders attributed to methamphetamine use than there are for cannabis use (Degenhardt *et al.*, 2007a). The differences are even more marked when the number of users of cannabis and amphetamines are taken into account. A recent study estimated that the rates of psychotic disorders were between 2.5 and 11 times greater for amphetamine than cannabis users, depending on age group (Degenhardt *et al.*, 2007a).

To summarize, the evidence that cannabis use is a contributory cause of psychosis is not as strong as that for cigarette smoking and lung cancer because the association is much weaker (RR of 2–3 vs. 12 or greater), but it is arguably stronger than the observational evidence

for the psychotogenicity of alcohol. The epidemiological evidence for a causal role for cannabis is also arguably more extensive than that for a relationship between amphetamine use and psychosis. The magnitude of the relationship between drug use and psychosis is weaker for cannabis (2- to 3-fold) than it is for amphetamine (11-fold) (Degenhardt and Hall, 2001).

A public health case for prudence

How strong does the evidence for a causal relationship between cannabis and psychosis need to be before we are justified in taking action? If the standard of proof required for action was "beyond reasonable doubt," as is demanded in criminal cases, then we would find it difficult to take *any* public health policy decisions on any issue. If, however, we are prepared to act on the "balance of probabilities" (more likely than not), then some policy action is warranted (Hall, 1999). The latter standard of proof is the one used in judging whether adverse reactions are attributable to pharmaceutical drugs. In other words, if we had similar evidence of an association between use of a pharmaceutical drug and psychosis, the drug would either be withdrawn from the market, or it would only be prescribed with clear warnings about the risk to patients and prescribers. This has been the response to what some have argued is weak(er) evidence of an increased suicide risk after the initiation of selective serotonin reuptake inhibitor antidepressants (Klein, 2006).

There are, of course, important differences between the way that we regulate pharmaceuticals and recreational drugs. We generally (and we would argue appropriately) err in the direction of prudence when responding to evidence of harm caused by therapeutic drugs. We are less concerned as a society about voluntarily assumed risks from using alcohol and tobacco; we generally allow adults to decide whether to take these risks or not, while prohibiting the use of these drugs by minors. We discuss below how we think the evidence on cannabis and psychosis should affect the debate about the legal prohibition on cannabis use by adults.

In the case of uncertainty about a causal relationship, we need to consider the likely costs and benefits of different policy actions. For example, the decision to advise parents to avoid putting infants to sleep in the prone position was advocated as a way of reducing sudden infant death syndrome (SIDS) on the grounds that: this sleeping position was a strong risk factor for SIDS; the proposed behavior carried few risks; and if the relationship was *not* causal, parents and infants would not be greatly inconvenienced. A substantial reduction in SIDS deaths in countries that implemented this policy provided convincing evidence for a causal relationship between sleeping position and SIDS in the absence of any detailed understanding of the causal mechanism for the relationship (Dwyer *et al*., 1995).

The same sort of prudential reasoning would support efforts to discourage young people from using cannabis, or at least to delay their use until early adulthood (de Irala *et al*., 2005). Assuming for the moment that we know how to do this, the public-health gain if the relationship is truly causal (perhaps a 10% reduction in schizophrenia incidence), would arguably offset the foregone pleasure among those young people who either did not use cannabis, or delayed use until young adulthood. In principle, a reduction in cannabis use among incident cases of psychosis would also provide evidence for the effectiveness of this policy, but it may be difficult to detect any such reduction in incidence for the same reasons that it has been difficult to assess whether the increased use of cannabis among Australian youth has increased the incidence of schizophrenia (Degenhardt *et al*., 2003).

The case for discouraging adolescent cannabis use is strengthened by evidence that cannabis use by young people is also associated with other adverse effects on psychosocial adjustment in young adulthood (Hall, 2006; Hall *et al*., 2008, Hall and Degenhardt, 2009). These include: the development of cannabis dependence (Anthony, 2006; Hall *et al*., 2008); poor educational outcomes (Lynskey and Hall, 2000); an increased risk of using other illicit drugs (Hall and Lynskey, 2005; Fergusson *et al*., 2006a); a possible increased risk of depression (See Chapter 10 and see Patton *et al*., 2002); and lower quality of life and poorer social relations in early adulthood (Patton *et al*., 2007; Fergusson and Boden, 2008). These more prevalent adverse outcomes have been overshadowed in the public debate about cannabis use by the association with psychosis. There are similar debates about the causal interpretation of these associations (Macleod *et al*., 2004), but the fact that cannabis use is associated with multiple indicators of poor psychosocial outcomes in young adults strengthens the case for discouraging its use by adolescents. There is still room for debate about the best policies for achieving this goal.

Responding to cannabis use among people with psychosis

The implications of the evidence are probably least controversial for mental health services that treat young people with psychoses, among whom there are high rates of regular cannabis use (Green *et al*., 2005). Even if we believe that the relationship between cannabis use and psychosis is not causal, there is still reasonable evidence that people with psychoses who are regular cannabis users have more positive symptoms, more frequent relapses and require more hospitalization (Linszen *et al*., 1994; Grech *et al*., 2005; Degenhardt *et al*., 2007b). It is therefore wise to encourage young people with psychotic symptoms who are using cannabis to either stop, or reduce the frequency of their psychoactive drug use.

If we were able to reduce cannabis use among patients with schizophrenia, then we could potentially discover whether the course of their disorders improved and their risk of relapse was reduced. As outlined in Chapter 21, there are major challenges with this strategy, including finding ways of persuading persons with schizophrenia to stop doing something that they enjoy, and helping those who want to stop using cannabis but find it difficult to do so. Recent evaluations (see Roffman and Stephens, 2006) of psychological interventions for cannabis dependence in persons without psychoses achieve only modest rates of abstinence at the end of treatment (20–40%), and there are substantial rates of relapse thereafter. Nonetheless, treatment still substantially reduces cannabis use and problems, even among those who do not succeed in quitting, much like the outcome of treatment for alcohol and other drug dependence (Budney and Moore, 2002).

Many persons with schizophrenia have characteristics that predict a poor outcome from treatment for cannabis dependence, namely they lack social support, they may be cognitively impaired, are often unemployed and may not adhere to treatment involving antipsychotics (Mueser *et al*., 1992; Kavanagh, 1995). There are very few controlled outcome studies of drug dependence treatment in schizophrenia (Lehman *et al*., 1993). A recent Cochrane review identified 25 randomized controlled trials but found "no compelling evidence to support any one psychosocial treatment over another to reduce substance use (or improve mental state) by people with serious mental illnesses" (Cleary *et al*., 2008). Major methodological problems with these studies prevented any pooled estimate. A research priority should be the development of more effective psychological and pharmacological methods for treating cannabis dependence (Ujike and Morita, 2004).

Informing young people about the mental health risks of cannabis use

A major public health educational challenge will be finding effective and persuasive ways of explaining the psychotogenic and other risks of cannabis use to young people. In addition to a possible increased risk of psychosis, young people also need to be informed about the risks of developing dependence, impairing their educational attainment and possibly increasing their risk of depression (Patton *et al*., 2002; Hall, 2006).

Providing credible advice to young people on the mental health risks of cannabis use is complicated by the polarized views on the health risks of cannabis expressed in the larger cannabis policy debate. As argued above, community debates about whether cannabis use should continue to be a criminal offence increases the prominence of conflicting views about the evidence on psychosis. This difference of opinion probably increases scepticism among young people, by raising doubts about which information they should heed (and probably makes it easier for some young people to downplay the evidence of harm in order to justify their continued cannabis use).

We need to be realistic about the practical impacts of educational messages (White and Pitts, 1998; Caulkins *et al*., 2004). Reviews of the evidence on the effectiveness of school-based drug education have suggested that statistically significant reductions in cannabis use may be observed in well-conducted programs, but the effects are small (Gorman, 1995; White and Pitts, 1998; Tobler *et al*., 1999). The primary effect is on knowledge rather than behavior change (White and Pitts, 1998), and the latter is more likely to occur among less-frequent users (Gorman, 1995) rather than the heavier users who are at greater risk of psychotic symptoms and other adverse effects. It has been argued that there is more to be gained from programs aimed at smaller targeted groups identified as being at higher risk (Gorman, 1996).

There are also intergenerational differences of opinion about the risks of cannabis use (Hall and Nelson, 1996), and there may well be different views about how information should be presented to discourage its use. The political imperative has been to express community

concern via high profile mass media campaigns. This is done, at times, by educators who are seemingly indifferent to how the communications will be perceived by young people.

Mass-media and school-based campaigns must also deal with scepticism among youth about health advice given by adults. Adolescents are exquisitely sensitive to what they see as parental double standards in disapproving of cannabis use while approving of alcohol use. They are also alert to what they see as dishonest information about cannabis because of a past history of scare campaigns about cannabis. These responses make for a sceptical audience among young people about information on the mental health risks of cannabis.

Given this, it is foolhardy for us to suggest simple solutions to the question of what advice should be given to young people. The nature and delivery of the advice will need to differ for different groups facing different levels of risk (Toumbourou *et al*., 2005). The best way to deliver the advice will depend upon good social-marketing research on the pre-existing views of young people (Grier and Bryant, 2005).

We believe that education about the risks of cannabis use should be part of general health education about drug use and mental health (McBride, 2003). This should explain the mental-health risks of regular intoxication with alcohol and cannabis, and define the known high-risk groups such as those with a family history of psychosis and those who have had bad experiences with cannabis and alcohol. Such education needs to be directed not only at cannabis users but also at their peers, to increase the likelihood that young people can encourage peers who are adversely affected by cannabis use to cease using or seek help earlier than might otherwise be the case.

A major challenge will be framing the magnitude of the risk of psychosis from cannabis use. If cannabis use increases the incidence of psychosis among those who use it regularly, then the risk for regular cannabis users increases from around 7 in 1000 (Saha *et al*., 2005) to 14 in 1000, arguably still a low rate. If this risk is multiplicative with family history, then in persons with an affected first-degree relative, the risk could increase from 1 in 10 among those who do not use cannabis to as high as 1 in 5 or 1 in 3 among those who use cannabis. The consequences for those individuals who develop the disorder are serious. The temptation for parents and health educators is to play up the risk, arguing that everyone is at risk because it is difficult to predict which young people are most vulnerable. We think this a doubtful strategy that may undermine the credibility of the message by being seen to exaggerate the risk.

Policies toward recreational cannabis use

A major obstacle to a more considered cannabis policy is the implicit assumption that if we accept that cannabis is a contributory cause of psychosis then it follows that we should continue to prohibit cannabis use and probably increase the rigor with which the criminal penalties for use are enforced (e.g. Cresswell, 2005). The following statement illustrates a common inferential slippage between evidence that cannabis causes psychosis and support for cannabis prohibition:

> " … the appropriate question is: in light of all we know, should we recommend cannabis use to our youth and just wait and see until more evidence arises? Or is it wise to prohibit its use?" (de Irala *et al*., 2005, p.358)

This framing of the relationship between evidence and policy is an understandable response to the common simplification of the policy debate in the popular media in many developed countries, namely, that we should either legalize cannabis because its use is harmless, or we should continue to prohibit it because it harms some users (Hall, 1997; Hall and Pacula, 2003). If this is seen as the policy choice we face, then it is understandable why those who defend prohibition routinely invoke the evidence on psychosis. It is also understandable why the advocates of more liberal cannabis policies attack the same evidence because it undermines the simplest and most compelling argument for cannabis law reform, namely, the claim that cannabis use is harmless.

As is argued in more detail elsewhere (Hall and Pacula, 2003), it does not follow that cannabis use should be prohibited simply because it causes harm to some users. If it did we would be morally obliged to prohibit alcohol and tobacco use, not to mention motor cars and motorbikes. Those who advocate for cannabis prohibition need to provide additional arguments that criminal penalties are the best way to discourage use and reduce the harms that cannabis use causes. As a society, we also need to consider the social costs of using the criminal law to deter people from using cannabis. In order to decide whether the costs of prohibition are worth bearing in the interests of discouraging young people from using cannabis, we need information about both the harms caused by cannabis

and the social consequences of its prohibition (Hall and Pacula, 2003; Pollack and Reuter, 2007).

The evidence on cannabis and psychosis is clearly relevant in this societal decision, because psychosis is serious, and substantially and negatively affects life chances for the young people affected by it (Hall *et al.*, 2001). Nonetheless, we do not think that this health effect can or should be the sole basis for a social policy toward cannabis because if evidence of psychotogenicity was a sufficient warrant for prohibition, then we would be obliged to prohibit alcohol, which is also a probable contributory cause of psychosis.

Among the arguments offered for recriminalizing cannabis are the following: it would simplify cannabis laws and make it easier to educate the community by sending a simple, strong message of disapproval about cannabis use; and criminal penalties for use may deter more young people from using cannabis (e.g. Cresswell, 2005).

On the other side of the debate are a number of counter-arguments. First, there is no evidence that substituting civil for criminal penalties for cannabis use has affected rates of use in jurisdictions that have done so. For example, rates of cannabis use increased by the same amount in all Australian states during the early 1990s, despite differences in nominal legal penalties (Donnelly *et al.*, 1999; Williams, 2004). This mirrors experience in the USA in the 1980s (Single, 1989) and in The Netherlands in the early 1970s (MacCoun and Reuter, 2001). Moreover, rates of cannabis use have declined in Australia since 1998 (AIHW, 2007) and at much the same rate in all states and territories regardless of penalties for cannabis use.

Second, there are also concerns about the adverse social consequences of recriminalizing cannabis use. Reintroducing criminal penalties for an offence that is committed by around 10% of adults in many developed countries in any year will mean either that the law is not enforced, or that it is selectively enforced against social minorities and disadvantaged groups in the community (Hall and Pacula, 2003; Room, 2008).

Third, the debate about criminal penalties for cannabis use runs the risk of heightening the polarization of opinion about the risks of cannabis. It may also distract us from considering policy options that enjoy much wider public support, namely, more effective health education of young people about the mental health risks of cannabis use (Murray *et al.*, 2007).

Why prohibition is not enough

It seems likely that governments in most developed countries will continue to prohibit cannabis use by adults, regardless of whether they do so by imposing criminal or civil penalties for use. It is also clear that prohibiting cannabis has not been enough to prevent the occurrence of cannabis-induced psychoses among young people, although it can be argued that the problem may have been worse in the absence of prohibition (Hall and Pacula, 2003), nor has prohibition prevented a probable increase in the average THC content of cannabis products over recent decades (ElSohly, 2008; McLaren *et al.*, 2008; see also Chapter 4), a development that some fear has increased the risk of psychosis among users (Murray *et al.*, 2007).

We also need to consider two possible ways in which prohibition may have unwittingly made this outcome more likely, namely, creating incentives for black-market producers to maximize the average potency of their cannabis products (Miron, 1998), and in the absence of any regulation of an illicit market, allowing the most vulnerable young people to have access to more potent forms of cannabis from an early age (Room *et al.*, 2008). For example, enforcement of the prohibition against cannabis cultivation by disrupting outdoor-cannabis plantations may have driven cannabis cultivation indoors, and created incentives for illegal producers to maximize their profit and reduce their risk of detection, by breeding and cultivating smaller numbers of cannabis plants under indoor-growing conditions that maximize their THC content (UNODC, 2006). The shift to indoor-hydroponic cultivation has been one of the most generalized changes to cannabis production in a number of countries including Canada, The Netherlands and Australia (UNODC, 2008).

If our aim in imposing criminal penalties on cannabis use is to reduce the aggregate social harm that its use causes, then our policies should take more account of the potency of cannabis products that are readily available to young people. Tetrahydrocannabinol content cannot be regulated under prohibition in the way that it could if there were a legal market, but we could experiment with policies that take account of THC content in imposing legal sanctions upon persons who are arrested for growing and selling cannabis. This might, for example, mean imposing higher financial penalties and/or custodial sentences on persons who produce and distribute higher-potency cannabis seeds, plants and products. It may also mean reducing the severity of

penalties for people caught growing small numbers of plants of modest potency for their own use. The main uncertainties with this proposal are the feasibility and cost of enforcing such laws that would require assays of the THC content of confiscated cannabis. If such a policy was feasible, a useful by-product would be the collection of data on the THC content of black-market cannabis products that could be used in health education for users (Hall and Swift, 2000).

Conclusions

The observational evidence for a causal relationship between cannabis use and psychosis is at least as strong as observational evidence on associations between heavy alcohol and amphetamine use and psychotic symptoms. A causal relationship is also biologically plausible because the cannabinoids interact with dopaminergic neurotransmission, the cannabinoid system may be disturbed in psychosis, and high doses of THC provoke psychotic symptoms in people without psychosis.

The clearest policy implication of this evidence is that we should discourage cannabis use among the clients of mental health services (i.e. those who have already developed mental health problems). This could be done by screening all new patients with psychotic symptoms and advising those who use cannabis to stop, or at the very least, to reduce their use. More research is needed on how best to persuade them to stop, and better ways of assisting those who would like to stop but find it difficult to do so.

There is arguably an ethical imperative to inform young people of the probable mental health risks of cannabis use. On the grounds of prudence, young people should be discouraged from early and frequent use of cannabis, as they are for alcohol. The challenge will be finding credible and persuasive ways of doing so. This task may be complicated by the community debate about cannabis policy, with people's positions on this policy issue affecting their appraisals of the evidence. Political imperatives to express community concern via mass-media campaigns may work against effective education if it also unwittingly amplifies the sceptical views about the evidence expressed by critics of current policy. The tobacco industry's success in undermining tobacco control policies suggests that raising doubts about the quality of the evidence for harmful effects of cannabis is an effective way of reassuring users to continue using the drug (Glantz *et al*., 1996).

We should avoid making the common assumption that if the relationship between cannabis use and psychosis is causal, then we should continue to prohibit cannabis use, and reverse the liberalization of penalties for cannabis use that has occurred in some countries. As we have argued, accepting a causal relationship removes the strongest case for liberalization – the complete absence of any harmful effects on users. Given the seriousness of psychotic disorders for the life chances of young people who are affected by them, this evidence increases the case for caution in liberalizing cannabis laws in ways that may increase young people's access to cannabis, decrease their age of first use, or increase their frequency of cannabis use. But the effect of the law on young people is not the only outcome we should consider in framing cannabis policies. A considered decision about a policy toward cannabis requires an analysis of the harms caused by current policy, as much as the harms caused by cannabis use.

Even if we believe that some form of prohibition is the most prudent policy toward cannabis, it has clearly not been enough to prevent many young people from using high potency cannabis products at an early age in most developed societies. More needs to be done to reduce the availability of high-potency cannabis products to young people. This includes trialling the feasibility and efficacy of graduated penalties for producers and suppliers of higher THC content cannabis products.

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References

AIHW (2007). *Statistics on drug use in Australia, 2006*. Canberra: Australian Institute of Health and Welfare.

Angrist, B., Sathananthan, G., Wilk, S. and Gershon, S. (1974) Amphetamine psychosis: behavioral and biochemical aspects. *J Psychiatr Res*, **11**:13–23.

Anthony, J.C. (2006) The epidemiology of cannabis dependence. In *Cannabis dependence: its nature, consequences and treatment*, ed. R. A. Roffman and R. S. Stephens, pp. 58–105. Cambridge, UK: Cambridge University Press.

Arendt, M., Rosenberg, R., Foldager, L., Perto, G. and Munk-Jorgensen, P. (2005) Cannabis-induced psychosis and subsequent schizophrenia-spectrum disorders: follow-up study of 535 incident cases. *Br J Psychiatry*, **187**:510–15.

Arseneault, L., Cannon, M., Witton, J. and Murray, R.M. (2004) Causal association between cannabis and psychosis: examination of the evidence. *Br J Psychiatry*, **184**:110–17.

Barnes, T.R., Mutsatsa, S.H., Hutton, S.B., Watt, H.C. and Joyce, E.M. (2006) Comorbid substance use and age at onset of schizophrenia. *Br J Psychiatry*, **188**:237–42.

Bell, D.S. (1973) The experimental reproduction of amphetamine psychosis. *Arch Gen Psychiatry*, **29**:35–40.

Bromet, E.J., Dew, M.A. and Eaton, W. (1995) Epidemiology of psychosis with special reference to schzophrenia. In *Textbook of psychiatric epidemiology*, ed. M. T. Tsuang, M. Tohen and G. E. P. Zahner, pp.283–300. New York: Wiley-Liss.

Budney, A.J. and Moore, B.A. (2002) Development and consequences of cannabis dependence. *J Clin Pharmacol*, **42**:28S–33S.

Caulkins, J.P., Pacula, R.L., Paddock, S. and Chiesa, J. (2004) What we can – and cannot – expect from school-based drug prevention. *Drug Alcohol Rev*, **23**:79–87.

Cleary, M., Hunt, G., Matheson, S., Siegfried, N. and Walter, G. (2008) Psychosocial interventions for people with both severe mental illness and substance misuse. *Cochrane Database Syst Rev*, **1**, CD001088.

Connell, P.H. (1958) *Amphetamine psychosis*. London: published for the Institute of Psychiatry by Chapman and Hall.

Cook, T.D. and Campbell, D.T. (1979) *Quasi-experimentation: design and analysis issues for field settings*. Chicago: Rand McNally.

Cresswell, A. (2005) 'Weak drug laws linked to madness', *The Australian*, October 29.

Curran, C., Byrappa, N. and McBride, A. (2004) Stimulant psychosis: systematic review. *Brit J Psychiatry*, **185**:196–204.

D'Souza, D.C., Cho, H.S., Perry, E. and Krystal, J.H. (2004) Cannabinoid 'model' psychosis, dopamine-cannabinoid interactions and implications for schizophrenia. In *Marijuana and madness*, ed. D. J. Castle and R. M. Murray, pp. 142–165. Cambridge, UK: Cambridge University Press.

de Irala, J., Ruiz-Canela, M. and Martinez-Gonzalez, M. (2005) Causal relationship between cannabis use and psychotic symptoms or depression. Should we wait and see? A public health perspective. *Med Sci Monit*, **11**:RA355–8.

Degenhardt, L. and Hall, W.D. (2001) The association between psychosis and problematical drug use among Australian adults: findings from the National Survey of Mental Health and Well-being. *Psychol Med*, **31**:659–68.

Degenhardt, L., Hall, W.D. and Lynskey, M.T. (2003) Testing hypotheses about the relationship between cannabis use and psychosis. *Drug Alcohol Depend*, **71**:37–48.

Degenhardt, L. and Hall, W.D. (2006) Is cannabis a contributory cause of psychosis? *Can J Psychiatry*, **51**:556–65.

Degenhardt, L., Roxburgh, A. and McKetin, R. (2007a) Hospital separations for cannabis- and methamphetamine-related psychotic episodes in Australia. *Med J Aust*, **186**:342–5.

Degenhardt, L., Tennant, C., Gilmour, S., Schofield, D., Nash, L., Hall, W., *et al.* (2007b) The temporal dynamics of relationships between cannabis, psychosis and depression among young adults with psychotic disorders: findings from a 10-month prospective study. *Psychol Med*, **37**:927–34.

Der, G., Gupta, S. and Murray, R.M. (1990) Is schizophrenia disappearing? *Lancet*, **335**:513–16.

Donnelly, N. and Hall, W.D. (1994) *Patterns of cannabis use in Australia*. National Drug Strategy Monograph no. 27. Canberra: Australian Government Publishing Service.

Donnelly, N., Hall, W.D. and Christie, P. (1999) *Effects of the Cannabis Expiation Notice Scheme on levels and patterns of cannabis use in South Australia: evidence from the National Drug Strategy Household Surveys 1985–1995*. Canberra: Australian Government Publishing Service.

Dwyer, T., Ponsonby, A.L., Blizzard, L., Newman, N.M. and Cochrane, J.A. (1995) The contribution of changes in the prevalence of prone sleeping position to the decline in sudden infant death syndrome in Tasmania. *JAMA*, **273**:783–9.

ElSohly, M.A. (2008) *Quarterly report: December 16, 2007 thru March 15, 2008*. Potency Monitoring Project Report no. 100. University, MS: National Center for Natural Products Research, University of Mississippi.

Fergusson, D.M., Horwood, L.J. and Swain-Campbell, N.R. (2003) Cannabis dependence and psychotic symptoms in young people. *Psychol Med*, **33**:15–21.

Fergusson, D.M., Horwood, L.J. and Ridder, E.M. (2005) Tests of causal linkages between cannabis use and psychotic symptoms. *Addiction*, **100**:354–66.

Fergusson, D.M., Boden, J.M. and Horwood, L.J. (2006a) Cannabis use and other illicit drug use: testing the cannabis gateway hypothesis. *Addiction*, **101**:556–69.

Fergusson, D.M., Poulton, R., Smith, P.F. and Boden, J.M. (2006b) Cannabis and psychosis. *BMJ*, **332**:172–5.

Fergusson, D.M. and Boden, J.M. (2008) Cannabis use and later life outcomes. *Addiction*, **103**:969–76; discussion 977–8.

Fritzsche, M. (2001) Are cannabinoid receptor knockout mice animal models for schizophrenia? *Med Hypotheses*, **56**:638–43.

Glantz, S. A., Slade, J., Bero, L. A., Hanauer, P. and Barnes, D. E. (eds) (1996) *The cigarette papers*. Berkeley: University of California Press.

Glass, M. (2001) The role of cannabinoids in neurodegenerative diseases. *Prog Neuropsychopharmacol Biol Psychiatry*, **25**:743–65.

Gorman, D.M. (1995) On the difference between statistical and practical significance in school-based drug abuse prevention. *Drugs Educ Prev Policy*, **2**:275–83.

Gorman, D.M. (1996) Etiological theories and the primary prevention of drug use. *J Drug Issues*, **26**:505–20.

Gottesman, I.I. (1991) *Schizophrenia genesis: the origins of madness*. New York: W. H. Freeman.

Grech, A., Van Os, J., Jones, P.B., Lewis, S.W. and Murray, R.M. (2005) Cannabis use and outcome of recent onset psychosis. *Eur Psychiat*, **20**:349–53.

Green, B., Young, R. and Kavanagh, D. (2005) Cannabis use and misuse prevalence among people with psychosis. *Brit J Psychiatry*, **187**:306–13.

Greenberg, D.M. and Lee, J.W. (2001) Psychotic manifestations of alcoholism. *Curr Psychiatry Rep*, **3**:314–18.

Grier, S. and Bryant, C.A. (2005) Social marketing in public health. *Ann Rev Public Health*, **26**:319–39.

Grotenhermen, F. (2004) How to prevent cannabis-induced psychological distress … in politicians. *Lancet*, **363**:1568–9.

Hall, W.D. and Hando, J. (1993) Patterns of illicit psychostimulant use in Australia. In *Psychostimulant use in Australia*, ed. D. Burrows, B. A. Flaherty and M. MacAvoy. Canberra: Australian Government Publishing Service.

Hall, W.D. and Nelson, J. (1996) Correlates of the perceived health risks of marijuana use among Australian adults. *Drug Alcohol Rev*, **15**:137–43.

Hall, W.D. (1997) The recent Australian debate about the prohibition on cannabis use. *Addiction*, **92**:1109–15.

Hall, W.D. (1999) Assessing the health and psychological effects of cannabis use. In *The health effects of cannabis*, ed. H. Kalant, W. Corrigall, W. D. Hall and R. Smart, pp.1–17. Toronto: Centre for Addiction and Mental Health.

Hall, W.D. and Swift, W. (2000) The THC content of cannabis in Australia: evidence and implications. *Aust NZ J Publ Heal*, **24**:503–8.

Hall, W.D., Degenhardt, L. and Lynskey, M.T. (2001) *The health and psychological effects of cannabis use*. National Drug Strategy Monograph no. 44. Canberra: Commonwealth Department of Health and Aged Care.

Hall, W.D. and Pacula, R.L. (2003) *Cannabis use and dependence: public health and public policy*. Cambridge, UK: Cambridge University Press.

Hall, W.D., Degenhardt, L. and Teesson, M. (2004) Cannabis use and psychotic disorders: an update. *Drug Alcohol Rev*, **23**:433–43.

Hall, W.D. and Lynskey, M.T. (2005) Is cannabis a gateway drug? Testing hypotheses about the relationship between cannabis use and the use of other illicit drugs. *Drug Alcohol Rev*, **24**:39–48.

Hall, W.D. (2006). The mental health risks of adolescent cannabis use. *PLoS Med*, **3**:159–62.

Hall, W.D., Degenhardt, L. and Patton, G.C. (2008) Cannabis abuse and dependence. In *Adolescent addiction: epidemiology, treatment and assessment*, ed. C. A. Essau, pp. 117–148. London: Academic Press.

Hall, W.D. and Degenhardt, L. (2009) The adverse health effects of nonmedical cannabis use. *Lancet*, **374**:1383–91.

Henquet, C., Krabbendam, L., Spauwen, J., Kaplan, C., Lieb, R., Wittchen, H.U., *et al*. (2004) Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *BMJ*, **330**:11.

Isbell, H., Fraser, H.F., Wikler, A., Belleville, R.E. and Eisenman, A.J. (1955) An experimental study of the etiology of rum fits and delirium tremens. *Q J Stud Alcohol*, **16**:1–33.

Kavanagh, D.J. (1995) An intervention for substance abuse in schizophrenia. *Behav Change*, **12**:20–30.

Klein, D.F. (2006) The flawed basis for FDA post-marketing safety decisions: the example of anti-depressants and children. *Neuropsychopharmacology*, **31**:689–99.

Lehman, A.F., Herron, J.D., Schwartz, R.P. and Myers, C.P. (1993) Rehabilitation for adults with severe mental illness and substance use disorders: a clinical trial. *J Nerv Ment Dis*, **181**:86–90.

Linszen, D.H., Dingemans, P.M. and Lenior, M.E. (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. *Arch Gen Psychiatry*, **51**:273–9.

Lishman, W.A. (1987) *Organic psychiatry: the psychological consequences of cerebral disorder*, 2nd edn. Oxford: Blackwell Scientific.

Lynskey, M.T. and Hall, W.D. (2000) The effects of adolescent cannabis use on educational attainment: a review. *Addiction*, **96**:433–43.

MacCoun, R. and Reuter, P. (2001) *Drug war heresies: learning from other vices, times and places*. Cambridge, UK: Cambridge University Press.

Macleod, J., Oakes, R., Copello, *et al*. (2004) Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. *Lancet*, **363**:1579–88.

Macleod, J. and Hickman, M. (2010) How ideology shapes the evidence and the policy: what do we know

about cannabis and what should we do? *Addiction*, **105**:1326–30.

McBride, N. (2003) A systematic review of school drug education. *Health Edu. Res*, **18**:729–42.

McLaren, J., Swift, W., Dillon, P. and Allsop, S. (2008) Cannabis potency and contamination: a review of the literature. *Addiction*, **103**:1100–9.

Mirken, B. and Earleywine, M. (2005) The cannabis and psychosis connection questioned: a comment on Fergusson *et al*. 2005. *Addiction*, **100**:714–5.

Miron, J.A. (1998) An economic analysis of alcohol prohibition. *J Drug Issues*, **28**:741–62.

Moore, T.H., Zammit, S., Lingford-Hughes, A., *et al*. (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, **370**:319–28.

Mueser, K.T., Bellack, A.S. and Blanchard, J.J. (1992) Comorbidity of schizophrenia and substance abuse: implications for treatment. *J Consult Clin Psychol*, **60**:845–56.

Murray, R.M., Morrison, P.D., Henquet, C. and Di Forti, M. (2007) Cannabis, the mind and society: the hash realities. *Nat Rev Neurosci*, **8**:885–95.

Parascandola, M. (2005) Lessons from the history of tobacco harm reduction: the National Cancer Institute's Smoking and Health Program and the "less hazardous cigarette". *Nicotine Tob Res*, **7**:779–89.

Patton, G.C., Coffey, C., Carlin, J.B., Degenhardt, L., Lynskey, M.T. and Hall, W.D. (2002) Cannabis use and mental health in young people: cohort study. *BMJ*, **325**:1195–8.

Patton, G.C., Coffey, C., Lynskey, M.T., Reid, S., Hemphill, S., Carlin, J.B., *et al*. (2007) Trajectories of adolescent alcohol and cannabis use into young adulthood. *Addiction*, **102**:607–15.

Pollack, H.A. and Reuter, P. (2007) The implications of recent findings on the link between cannabis and psychosis. *Addiction*, **102**:173–6.

Roffman, R.A. and Stephens, R.S. (eds) (2006). *Cannabis dependence: its nature, consequences and treatment*. Cambridge, UK: Cambridge University Press.

Room, R., Fischer, B., Hall, W.D., Lenton, S. and Reuter, P. (2008) *Cannabis policy: moving beyond stalemate. The Global Cannabis Commission Report*. Oxford, UK: Beckley Foundation.

Royal College of Physicians (1962). *Smoking and health*. London: Pitman.

Saha, S., Chant, D., Welham, J. and McGrath, J. (2005) A systematic review of the prevalence of schizophrenia. *PLoS Med*, **2**:e141.

Semple, D.M., McIntosh, A.M. and Lawrie, S.M. (2005) Cannabis as a risk factor for psychosis: systematic review. *J Psychopharmacol*, **19**:187–94.

Single, E.W. (1989) The impact of marijuana decriminalization: an update. *J Publ Heal Policy*, **9**:456–66.

Skosnik, P.D., Spatz-Glenn, L. and Park, S. (2001). Cannabis use is associated with schizotypy and attentional dysinhibition. *Schizophr Res*, **48**:83–92.

Talley, C., Kushner, H.I. and Sterk, C.E. (2004) Lung cancer, chronic disease epidemiology, and medicine, 1948–1964. *J Hist Med Allied Sci*, **59**:329–74.

Tobler, N.S., Lessard, T., Marshall, D., Ochshorn, P. and Roona, M. (1999) Effectiveness of school-based drug prevention programs for marijuana use. *Sch Psychol Int*, **20**:105–37.

Toumbourou, J., Williams, J. and Patton, G. (2005) What do we know about preventing drug-related harm through social developmental intervention with children and young people? In *Preventing harmful substance use: the evidence base for policy and practice*, ed. T. Stockwell, P. Gruenwald, J. Toumbourou and W. Loxley, pp. 87–100. Chichester, UK: John Wiley.

US Surgeon General's Advisory Committee on Smoking and Health (1964), *Smoking and health: report of the Advisory Committee to the Surgeon General of the Public Health Service*. Washington, DC: US Department of Health, Education and Welfare, Public Health Service.

Ujike, H. and Morita, Y. (2004) New perspectives in the studies on endocannabinoid and cannabis: Cannabinoid receptors and schizophrenia. *J Pharmacol Sci*, **96**:376–81.

UNODC (2006) *World drug report 2006. Volume 1: analysis*. Vienna: United Nations Office on Drugs and Crime.

UNODC (2008) *World drug report 2008. Volume 1: analysis*. Vienna: United Nations Office on Drugs and Crime.

van Os, J., Bak, M., Hanssen, M., Bijl, R.V., de Graaf, R. and Verdoux, H. (2002) Cannabis use and psychosis: a longitudinal population-based study. *Am J Epidemiol*, **156**:319–27.

Weiser, M. and Noy, S. (2005) Interpreting the association between cannabis use and increased risk for schizophrenia. *Dialogues Clin Neurosci*, **7**:81–5.

White, D. and Pitts, M. (1998) Educating young people about drugs: a systematic review. *Addiction*, **93**:1475–87.

Williams, J. (2004) The effects of price and policy on marijuana use: what can be learned from the Australian experience? *Health Econ*, **13**:123–37.

Zammit, S., Allebeck, P., Andréasson, S., Lundberg, I. and Lewis, G. (2002) Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. *BMJ*, **325**:1199–201.

Zimmer, L. and Morgan, J.P. (1997) *Marijuana myths, marijuana facts: a review of the scientific evidence*. New York: The Lindesmith Center.

Chapter 6

Cannabis, endocannabinoids and neurodevelopment

Ismael Galve-Roperh

Recent advances from neurobiological studies have provided crucial insights into the cellular and molecular mechanisms involved in neural development, and support the notion that the endocannabinoid (eCB) system (ECS) constitutes a novel extracellular signaling system involved in the regulation of nervous system formation (Harkany *et al*., 2007). Research on the developmental role of the ECS is expected to provide the opportunity for a rational understanding of the impact of cannabinoid exposure during nervous-system generation and maturation. As discussed elsewhere in this book (Chapters 1 and 3), the ECS is a bioactive-lipid-signaling system that, by engaging the G-protein-coupled CB1 receptor, exerts a well-known neuromodulatory role (Hashimotodani *et al*., 2007; Heifets and Castillo, 2009). In addition, eCBs via CB1 or the so-called "peripheral" CB2 receptors exert a variety of regulatory actions on neuronal- and glial-cell generation, neuronal survival and glial activation (Galve-Roperh *et al*., 2007; Arevalo-Martin *et al*., 2008). The ECS elements (receptors, endogenous ligands and metabolizing enzymes) are expressed and function during neural-cell development, and their participation in neural-cell generation and survival have led to the hypothesis that neurodevelopmental alterations mediated by CB1 receptors may underlie the cognitive and emotional consequences occurring during adulthood in the offspring of animals exposed to chronic cannabinoid administration (Schneider, 2009). The impact of the developmental exposure to *Cannabis sativa*-derived cannabinoids and their implications in human neuropsychiatric disorders (control of emotions, cognition or psychosis) has been widely studied, and is reviewed in companion chapters of this book and elsewhere (D'Souza *et al*., 2009; Jutras-Aswad *et al*., 2009).

CB1 receptors are widely expressed in the adult brain and actively control different synapses and areas by regulating neuronal activity in a highly context-dependent manner (Hashimotodani *et al*., 2007; Heifets and Castillo, 2009). Thus, cannabinoids can influence various synapses e.g. acting on inhibitory gamma aminobutyric acid (GABA) communication, but also on excitatory glutamatergic neuronal transmission. In addition, CB1 receptors crosstalk and modulate other neurotransmitter systems, including those involved in dopaminergic, opioid, cholinergic and serotoninergic neuronal communication. Cannabinoid administration during development therefore can interfere with normal glutamatergic and GABAergic neuronal activity (Mereu *et al*., 2003; Antonelli *et al*., 2005; Castaldo *et al*., 2007). CB1 receptors are highly expressed by inhibitory GABAergic neurons in different brain areas (cortex, hippocampus, striatum, cerebellum), but CB1 receptors are also present in adult glutamatergic axon terminals in many locations (Katona *et al*., 2006; Kawamura *et al*., 2006), where they exert a crucial role that efficiently controls excessive excitatory neuronal activity (Marsicano *et al*., 2003; Monory *et al*., 2006). The neuromodulatory role of the ECS thus provides a variety of potential mechanisms of action that can be responsible for the neurochemical alterations induced by prenatal cannabinoid exposure. During nervous-system development exogenous or endogenous cannabinoids via CB1-receptor-mediated neuromodulation would influence neuronal communication and developing circuits, and thus may exert relevant consequences on adult neuronal function (Jutras-Aswad *et al*., 2009). Typically, dysfunction of the ECS is crucial in seizure onset and epileptogenesis, as a consequence of unbalanced excitatory and inhibitory neurotransmission (Katona and Freund, 2008; Lutz and Monory, 2008)(Figure 6.1).

Besides acute or long-lasting neuromodulatory regulation, cannabinoid exposure or altered eCB function during neurodevelopment can interfere with

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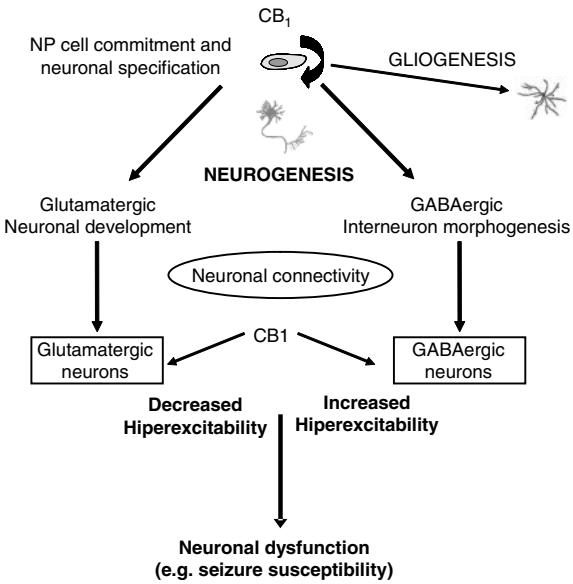

Figure 6.1. General perspective of the regulatory actions of the endocannabinoid system in nervous-system development and patterning. Endogenous cannabinoids via CB1 receptors modulate proper neurodevelopment by acting at early stages in neural progenitors (NP) and differentiating neuronal cells. During cortical development the endocannainoid system is crucially involved in excitatory neuronal commitment and interneuron migration and morphogenesis (see text for details). In addition, the endocannainoid system is involved in astrogliogenesis, oligodendrocyte maturation and neural-cell survival. In the mature nervous system, CB1 receptors exert a neuromodulatory role in excitatory and inhibitory neurons, and thus excessive or defective cannabinoid function play a crucial action in the regulation of neuronal activity as exemplified by their influence in the regulation of seizure onset.

proper neuronal generation owing to the recently described ability of CB receptors to act as neurogenic signaling cues (Galve-Roperh *et al*., 2007; Harkany *et al*., 2007). In the following sections the developmental actions of cannabinoids, with particular emphasis on their role in cortical development, is addressed. I summarize the expression pattern of the ECS during nervous system formation, followed by an analysis of the regulatory role of eCBs and their receptors in neural progenitor/stem (NP) cell proliferation, neurogenesis and commitment. The signaling mechanisms by which CB receptors modulate neural-cell fate are still poorly understood, but CB1-receptor regulation of different proliferative and prosurvival pathways has already been described. In addition, cannabinoids are known to modulate the transcriptional regulatory mechanisms involved in neurogenesis. Finally, how the developmental alterations regulated by the ECS may influence adult neuronal dysfunction and neuropsychiatric disorders, is briefly discussed.

The endocannabinoid system during neuronal development

Neurobiological substrate of action

The impact of prenatal cannabinoid exposure on brain development and interest in understanding its interference with normal adult neurological function has fostered the characterization of the ECS during nervous-system development. CB1 and CB2 receptors, endogenous ligands (2-arachidonoyl glycerol [2-AG] and anandamide, N-arachidonoylethanolamine [AEA]) and other ECS elements (synthesizing and degrading enzymes) are expressed since early blastocyst stages (Sun and Dey, 2008), in neuroectoderm-derived neural tissues (Harkany *et al*., 2007), and reach a major expression in the adult brain (Freund *et al*., 2003). Indeed, the CB1 receptor is the most abundant G-protein-coupled receptor in the adult nervous system (Freund *et al*., 2003; Heifets and Castillo, 2009). It is important to note that CB-receptor expression and eCB ligands are present at very early stages of development in which neuronal activity and communication is still absent or under maturation (Harkany *et al*., 2007). Therefore, in addition to their neuromodulatory role, eCBs via CB receptors contribute to the regulation of neurodevelopment most likely through their ability to control neural cell-fate-signaling pathways (Guzman, 2003; Mackie, 2006; Galve-Roperh *et al*., 2008).

Levels of the eCBs AEA and 2-AG are tightly regulated during embryo development. Initially, low AEA levels are required for proper embryo implantation, and AEA increases from mid-gestation until adult levels are reached (Berrendero *et al*., 1999). Thus, deletion of fatty acid amide hydrolase (FAAH), which yields high AEA levels, and Δ^9-tetrahydrocannabinol (THC) administration, constrains embryo preimplantation and induce aberrant expression of genes crucial in embryonic stem-cell renewal and proliferation (Nanog, Oct3/4 and Cdx2)(Wang *et al*., 2006). 2-Arachidonoyl glycerol, the other major eCB, is also present at embryonic stages and its levels evolve with a characteristic peak after birth (Berrendero *et al*., 1999).

The presence of CB receptors in embryonic stem cells (Jiang *et al*., 2007) and early blastocyst stages (Sun and Dey, 2008) is followed by its re-expression during neural development and gradually increases along neuronal differentiation until the final pattern of the adult brain is acquired (Freund *et al*., 2003; Harkany *et al*., 2007). CB1 receptors are particularly abundant

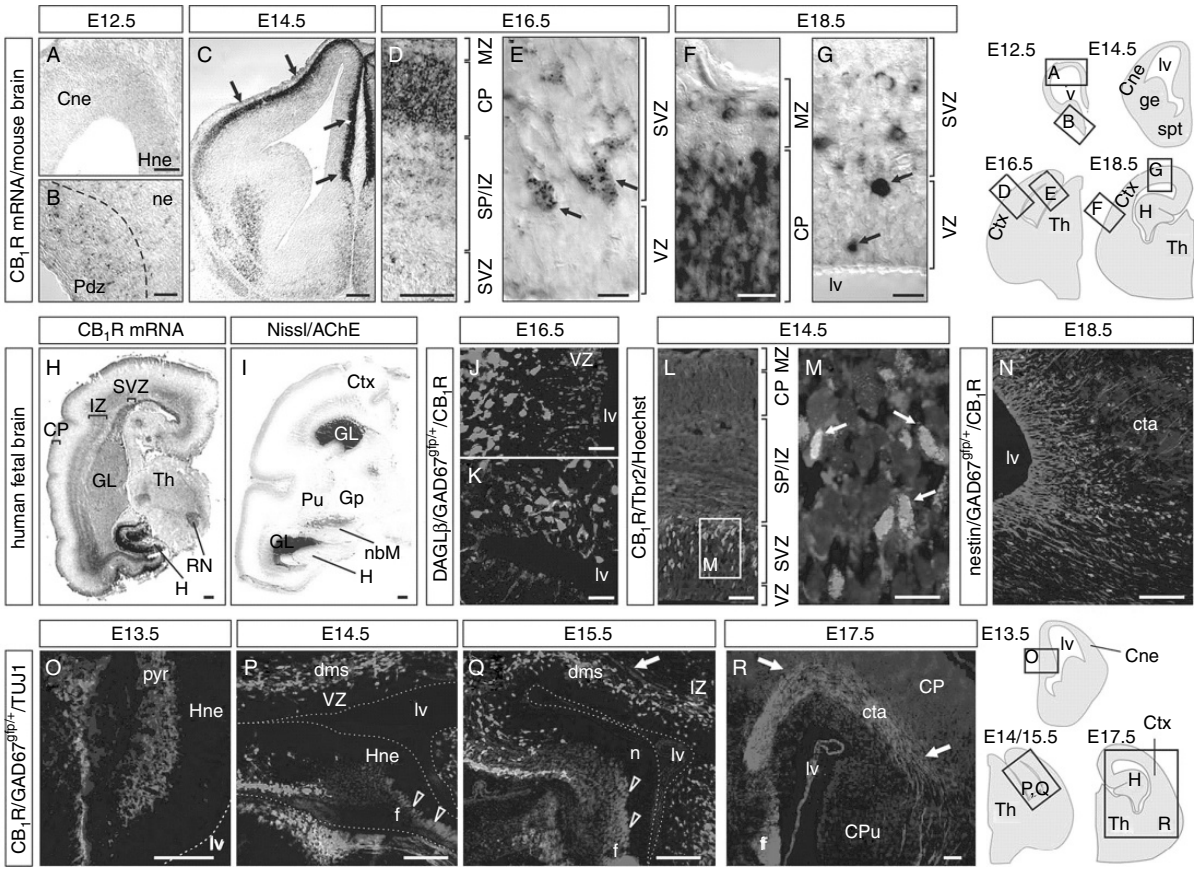

Figure 6.2. CB1 receptor expression in the developing neocortex. A–G show in-situ hybridization of CB1 mRNA at the indicated developmental stages. C, D and F show the presence of CB1 transcripts in pyramidal neurons, and E and G in VZ/SVZ neural progenitors. L–N reveal CB1 receptor expression in Tbr-2-positive intermediate progenitors. O–R, CB1 receptors are enriched in projecting axons of cortical neurons. Arrows indicate corticothalamic axons and arrowheads indicate axons commited to the fimbria. See original article for further details. Reproduced with permission from Mulder *et al.*, 2008. Copyright 2008 National Academy of Sciences, USA. See also color plate section.

in white matter areas of the embryonic brain, and their levels progressively increase from early prenatal stages to adulthood in grey matter areas (Berrendero *et al.*, 1998). These observations are in line with the putative role of the eCB system in the regulation of axonal elongation and fasciculation. During neocortical generation, higher CB1-receptor expression is observed in the intermediate zone and developing cortical plate, where postmitotic neurons are located and express early neuronal markers such as class III beta-tubulin (Tuj1)(Mulder *et al.*, 2008; Vitalis *et al.*, 2008)(Figure 6.2).

CB1 receptors are also present in pioneer neurons that populate the marginal zone in the dorsal cortex. In particular, Cajal-Retzius cells that express reelin are CB1 positive (Vitalis *et al.*, 2008; Morozov *et al.*, 2009). Reelin is well known for its role as instructive signaling cue that among other actions promote radial migration of differentiating neurons. Later, CB1 receptors are heterogeneously distributed through cortical layers and hippocampus, in either excitatory glutamatergic neurons, as identified by vGlut-1 expression, and cholecystokinin (CCK)-expressing GABAergic interneurons (Katona *et al.*, 2006; Monory *et al.*, 2006; Lafourcade *et al.*, 2007). CB1+CCK+ interneurons derived from the ganglionic eminences follow complex tangential migratory routes from the ventral telencephalon and reach the developing cortex, hippocampus and amygdala (Morozov and Freund, 2003; Berghuis *et al.*, 2005; Bodor *et al.*, 2005; Morozov *et al.*, 2009). This dual pattern of expression and functionality of the ECS in both excitatory and inhibitory neuronal lineages during development is reproduced in the adult brain, in which CB1 receptors are functional in

cortical excitatory projecting neurons and inhibitory GABAergic interneurons (Hashimotodani *et al*., 2007; Heifets and Castillo, 2009). Recently CB1-receptor expression, first in white matter and later in postnatal grey matter, has been shown to participate in whisker barrel map development of the rat somatosensory cortex (Li *et al*., 2009). These findings suggest a developmental role of CB1 at the systems level and postnatal plasticity of the whisker map (see Figure 6.2).

In addition to fully and partially differentiated neuronal and glial cells, NPs (multipotent self-renewing cells with the ability to differentiate into the neuronal and glial-cell lineages: astrocytes and oligodendrocytes), also express a functional ECS. Endocannabinoids present in the neurogenic niche of the developing cortex, the subventricular and ventricular zone (SVZ/VZ) are active engaging CB1 receptors on NPs identified by the expression of the neuroepithelial marker nestin and the transcription factor Sox2 (Aguado *et al*., 2006; Mulder *et al*., 2008). Intermediate progenitor cells, characterized by the expression of the transcription factor Eomes/Tbr2, that contribute to the generation of pyramidal cells in all layers of the cerebral cortex (Kowalczyk *et al*., 2009), are also regulated by CB1 receptors. CB1 receptors are present in actively dividing cells identified by 5-bromo-2′-deoxyuridine labeling, the expression of endogenous cell-cycle markers (Ki-67, phosphorylated-histone 3) and phosphorylation of vimentin (a marker of radial progenitor cell division) (Aguado *et al*., 2005; Jiang *et al*., 2005; Mulder *et al*., 2008). In the developing chick, however, embryonic CB1-receptor expression follows neuronal differentiation and, at least in the spinal cord, might be restricted to postmitotic neurons (Begbie *et al*., 2004; Watson *et al*., 2008).

The expression of the eCB signalling-system elements in undifferentiated cells in vivo, has been expanded to ex-vivo and in-vitro studies. Using the neurosphere model, in which NPs are grown in non-adherent conditions, CB1 receptors and eCBs have been shown to be active regulators of the neurogenic niche. Thus, neurospheres from different stages of embryonic and postnatal development express CB1 receptors, the AEA-degrading FAAH enzyme, and elevation of intracellular calcium concentration increase eCB production (Aguado *et al*., 2005). N-Arachidonoylethanolamine and 2-AG can act therefore in an autocrine or paracrine manner on NPs or surrounding neighbor cells. Two isoforms (α and β) of the diacylglycerol lipase (DAGL) enzyme, responsible for 2-AG synthesis, have been cloned (Bisogno *et al*., 2003). Diacylglycerol lipase α is present in ependymal cells of the adult subventricular zone, that act as neural-stem cells, and mediates the generation of 2-AG involved in the regulation of neurogenesis (Goncalves *et al*., 2008). Likewise, high-expression levels of DAGLα in the NSC line Cor-1, rapidly decrease along their differentiation into GABAergic neuronal cells (Walker *et al*., 2010). The analysis and characterization of the DAGL locus has identified the minimal core promoter sequence and the involvement of the transcriptional regulator specificity protein Sp1, in DAGLα expression.

The expression and functionality of the ECS has also been characterized in the developing human brain (Mato *et al*., 2003; Wang *et al*., 2003, Zurolo *et al*., 2010). In human-fetal brain, in-situ hybridization and binding assays show evidence of a heterogeneous pattern of CB1-receptor expression with preferential limbic expression and high levels throughout the cerebral cortex, hippocampus, caudate nucleus, putamen and cerebellum. In the second trimester intense labeling for CB1 receptors is evident in the hippocampal CA region (Wang *et al*., 2003). High densities of CB1 receptors are detected during prenatal development in fiber-enriched areas, that later in the adult brain are practically devoid of these receptors (Mato *et al*., 2003). Overall, the early expression pattern and functionality of CB1 receptors during nervous system development, together with their transient and atypical localization in prenatal stages, suggest a specific role of the ECS in human brain development, with potential implications in neuropsychiatric disorders (Galve-Roperh *et al*., 2009; Jutras-Aswad *et al*., 2009).

The endocannabinoid system in neural progenitor/stem cell proliferation

Multiple lines of evidences show that the ECS regulates the functionality of neural progenitor cell populations during development and in adult neurogenic areas (Galve-Roperh *et al*., 2007). The finding that NPs from adult neurogenic brain areas are also regulated by CB1 receptor expression and agonists (Jin *et al*., 2004; Aguado *et al*., 2005; Jiang *et al*., 2005) reveals that the role of eCBs as developmental signaling cues is conserved in the mature nervous system (Galve-Roperh *et al*., 2007). One of the most commonly described actions of CB receptors in undifferentiated cells is their ability to regulate cell proliferation and survival. Neurosphere cultures of embryonic cortical NPs

derived from knockout mice show that the absence of CB1 and CB2 receptors reduces cell proliferation and affects their ability to self-renew (Aguado *et al*., 2005; Palazuelos *et al*., 2006). Accordingly, pharmacological regulation with selective CB1 and CB2 cannabinoid receptor agonists or antagonists exerts a positive and negative action, respectively, on NP cell division (Aguado *et al*., 2005; Jiang *et al*., 2005; Palazuelos *et al*., 2006; Goncalves *et al*., 2008; Trazzi *et al*., 2010). In vivo, CB1 receptor loss of function induces alterations of cortical and hippocampal development (Aguado *et al*., 2005; Berghuis *et al*., 2005) and, whereas CB1-null mice have reduced cortical progenitor proliferation, in FAAH-deficient mice the opposite is observed (Aguado *et al*., 2005; Mulder *et al*., 2008). Abnormal cortical development in CB1-deficient mice is characterized by defective SVZ/VZ pyramidal progenitor proliferation and radial migration, deficits in axonal navigation and aberrant corticofugal projections (Mulder *et al*., 2008). The role of the ECS in the regulation of pyramidal neural progenitor cell expansion during cortical development is also reproduced in cortical brain slice cultures, in which pharmacological regulation of CB1 receptors or genetic manipulation of the endogenous cannabinoid tone disrupts proper pyramidal neuronal generation and specification (Mulder *et al*., 2008). Neural progenitor proliferation from other brain areas such as the cerebellum is also dependent on CB1-receptor activation (Trazzi *et al*., 2010).

The physiopathological relevance of cannabinoid-mediated NP cell proliferation is highlighted by their ability to prime and mobilize progenitor cells under circumstances in which a neurogenic response is initiated (Danzer, 2008). In this context, the absence of CB1 or CB2 receptors is associated with a defective response to excitotoxicity-induced response of hippocampal subgranular zone progenitor cells (Palazuelos *et al*., 2006; Aguado *et al*., 2007). CB1 receptor activation in the adult brain has been shown to increase hippocampal progenitor proliferation and neurogenesis, which was associated to an anxyolitic/antidepressive cannabinoid action (Jiang *et al*., 2005). In agreement, it has recently been shown that cannabinoid regulation of amigdala cell proliferation may impact in a sex-dimorphic manner in social rat behaviour (Krebs-Kraft *et al*., 2010). Likewise, aging-associated CA1 and CA3 neuronal loss and cognitive impairment are exacerbated in CB1-deficient mice (Bilkei-Gorzo *et al*., 2005). Thus, aging-associated decline of hippocampal and olfactory-bulb neurogenesis can be partially reverted by the mixed CB1/CB2 agonist WIN55 212–2 and the selective CB2 agonist JWH-133 (Goncalves *et al*., 2008; Marchalant *et al*., 2009b).

An emerging paradigm from cannabinoid research is that the ECS constitutes an allostatic signaling system that contributes to cellular plasticity responses in adaptation to stress-induced alterations (Patel and Hillard, 2008). Stress insults induce an inhibitory effect on neurogenesis that can be partially reverted by engaging the ECS (Hill *et al*., 2006). In addition, eCB signaling via CB1 receptors are required for the proneurogenic actions of voluntary exercise- and environmental enrichment-induced hippocampal neurogenesis (Hill *et al*., 2009; Wolf *et al*., 2010).

It is important to note that the ability of CB receptors to regulate stem/progenitor cell proliferation is not restricted to the neuroectodermal lineage, and before neuralization the ECS is also active. In-vitro studies with mouse embryonic stem cells have revealed that embryoid body generation (aggregates derived from embryonic stem cells) occurs in parallel with the induction of CB1/CB2 receptor expression and eCB production (Jiang *et al*., 2007). Δ^9-Tetrahydrocannabinol-induced CB-receptor activation increases embryoid-body formation, possibly by promoting cell survival, and promotes differentiated-embryoid body chemotaxis (Jiang *et al*., 2007). More recently, DNA microarray-based comparison of gene expression patterns of human embryonic and induced-pluripotent stem cells suggests that CB1-receptor mediated G_i-signaling participates in human stem cell function (Nakamura *et al*., 2009).

Cannabinoid-induced alterations of brain patterning

Cannabinoid administration studies during nervous-system development have provided diverse examples on the role of the ECS in the appropriate generation of neurons, astrocytes and oligodendrocytes. Here is summarized some of the findings in which the alterations of brain patterning by pharmacological regulation of CB receptors have been investigated at the cellular and molecular level, and these are correlated with the observations derived from the study of the ECS.

Blockade of CB1 receptors by SR1417116 (rimonabant) administration in utero at early embryonic stage (E14) has been shown to induce aberrant subcortical axon projections (Mulder *et al*., 2008). These alterations are likely due to rimonabant-mediated CB1-receptor blockade, and the interference with eCB actions in VZ/SVZ cortical progenitor proliferation, radial migration

and axonal navigation. Thus eCBs are proposed to act as regulators of the "handshake" interaction between corticothalamic and thalamocortical axons (Wu *et al*., 2010). Whether reduced, excessive or misspecification of pyramidal neurogenesis is at the origin of alterations of glutamatergic signaling in the offspring of cannabinoid-treated animals remains to be investigated. In addition, CB1-receptor activity governs proper interneuron placement and integration during corticogenesis, and in coordination with TrkB receptor-dependent signaling pathways regulates interneuron subtype-selective migration and specification. CB1 receptors enriched in filopodial tips and axonal growth cones, regulate neurite branching, elongation and dendrite arborization (Berghuis *et al*., 2005; Berghuis *et al*., 2007). The participation of CB1 receptors in interneuron morphogenesis and migration is reflected by an increased density of CCK-expressing interneurons in the hippocampus in vivo after prenatal chronic THC administration (Berghuis *et al*., 2005). The absence of CB1 receptors results in impaired target selection of cortical GABAergic interneurons, and navigation of axonal projection neurons, which indicates that eCBs may act as axon guidance cues and synaptogenic regulatory factors in vivo.

Pharmacological regulation of the ECS induces a variety of neurochemical alterations including markers of neuronal- and glial-cell populations (e.g. glutamate decarboxylase, tyrosine hydroxylase, vGlut, EAAC1). Prenatal, but not postnatal, WIN55 212–2 administration induces alterations in cortical cerebellar neuronal activity with increased glutamate decarboxylase and GABA immunoreactivities (Benagiano *et al*., 2007). In addition, postnatal administration of THC and CB1 synthetic agonists alters the expression of proteins involved in neuronal migration such as the L1 protein and polysialic acid-neural cell adhesion molecule (Gomez *et al*., 2007; Mackowiak *et al*., 2007). Pharmacological manipulation of eCB signaling in other models has been shown to induce very early actions on neural development. In chicks, chronic administration of the soluble THC analogue 0–2545 severely disrupts neural development by impairing brain, somite, spinal cord primordia and heart development (Psychoyos *et al*., 2008). Neural plate and initial steps of telencephalon formation are also affected.

CB1 receptors are expressed in the peripheral nervous system of whole mount chicken embryos from stage 19, reach the dorsal root and sympathetic ganglia, and, by stage 25 are coincident with a more advanced differentiation state; CB1 expression is widespread (Watson *et al*., 2008). Expression of DAGLα and β in the white matter of the spinal cord mediates the synthesis of 2-AG that, via CB1 receptors, influences neuronal axonal growth and pathfinding. Thus, morpholino-mediated CB1 knockdown during early neural zebrafish development and CB1-receptor antagonism interfere with correct axonal fasciculation and growth of mesenchephalic trigeminal neurons (Watson *et al*., 2008). The regulatory role of CB receptors during spinal cord development remains active later in the adult nervous system and responds to pathological insults including demyelination, trauma or neuroinflammation, thereby contributing to neuronal and oligodendrocyte survival, as well as myelination (Garcia-Ovejero *et al*., 2009). In addition, cannabinoids, by regulating microglial activity via CB2 receptors, play a protective role in neuroinflammatory-induced neuronal loss (Stella, 2009). Inhibition of non-autonomous neuronal cell death by CB2 receptor activity in neurodegenerative disorder models, e.g. multiple sclerosis and Huntington's disease (Maresz *et al*., 2007; Palazuelos *et al*., 2009), may also be accompanied by increased neurogenesis, which is usually impaired in a neuroinflammatory context. Therefore, modulation of the state of microglial activation and inflammation by CB2 receptors attenuates aging-associated decline of neurogenesis, and palliates its inhibition occurring in a viral encephalitis model (Goncalves *et al*., 2008; Solbrig and Hermanowicz, 2008; Marchalant *et al*., 2009a).

Cannabinoid signaling during neural development

Studies of the mechanisms of cannabinoid action during neural development are still in their infancy, but indicate that eCBs, by activating CB receptors, constitute a novel family of extracellular signaling cues that participate and orchestrate embryonic and postnatal nervous system development (Galve-Roperh *et al*., 2007; Harkany *et al*., 2007). Current understanding of the signal transduction mechanisms regulated by CB1 receptors in NPs is summarized in Figure 6.3. CB1-receptor signal transduction pathways can be mediated by direct or indirect coupling to heterotrimeric G-proteins, and in addition CB1 receptors can crosstalk with other membrane receptor signaling pathways, in particular growth factor and neurotrophin receptors. Ultimately, CB1 receptors will regulate protein kinase cascades and the transcriptional factor networks that

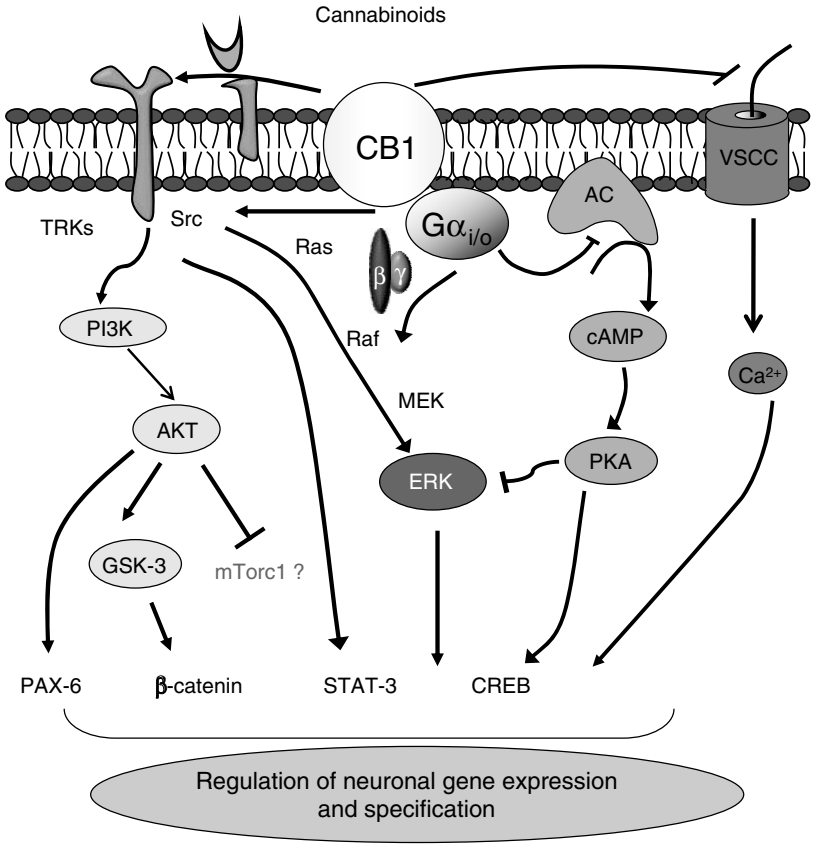

Figure 6.3. CB1 cannabinoid receptor signaling and regulation of neural-cell fate. CB1 receptors are coupled to $G_{i/o}$ proteins, thereby mediating the inhibition of adenylyl cyclase (AC) and protein kinase A (PKA). CB1 receptor coupling to $G_{i/o}$ signaling is also associated with activation of the extracellular-signal-regulated kinase (ERK) pathway via different mechanisms (see text for details). Direct activation of the PI3K/AKT and ERK pathways by CB1 receptors may converge and synergize with their regulation by other membrane receptors, such as growth-factor receptors with tyrosine kinase activity (RTK). CB1 receptor-induced activation of RTKs can occur by promoting the processing of membrane-bound growth-factor inactive precursors to yield active growth factors, or by activating intracellular Src family protein kinases. Activation of CB1 receptors ultimately controls different transcriptional regulators including CREB, STAT-3, PAX-6 and β-catenin. CB1 receptors are also coupled to the regulation of ion channels e.g. voltage-sensitive calcium channels (VSCC), and may also affect the mammalian target of rapamycin complex 1 (mTORC1, shown in grey).

are responsible for gene expression regulation involved in NP proliferation, specification and survival.

CB1 cannabinoid receptor crosstalk with extracellular signaling pathways

CB1 receptors have been show to crosstalk with growth factor and neurotrophin signaling at different levels. CB1-receptor expression is associated to changes in growth-factor expression, and CB1 activation can regulate tyrosine kinase growth-factor receptors by transactivation mechanisms. In the adult nervous system CB1-receptor expression is involved in the regulation of the neurotrophin brain-derived neurotrophic factor (BDNF) levels, thus CB1-deficient mice have reduced hippocampal BDNF levels under basal circumstances, which may explain some of the neuronal plasticity and emotional alterations shown in those animals (Marsicano *et al.*, 2002; Aso *et al.*, 2008; Bergami *et al.*, 2008). Growth-factor levels are also regulated by cannabinoid signaling under different neurodegenerative paradigms such as hippocampal and striatal excitotoxicity in which BDNF, fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) are sensitive to CB1 expression (Marsicano *et al.*, 2003; Aguado *et al.*, 2007; De March *et al.*, 2008). Reciprocally, FGF receptors promote axonal growth and guidance via DAGL activation and 2-AG generation (Williams *et al.*, 2003).

Transactivation of growth-factor receptors with tyrosine kinase activity (EGF receptor, Trk B receptor)

constitutes an additional mechanism for CB1-receptor-induced signal transduction. CB1-receptor-induced transactivation can be mediated by inducing growth factor or cytokine (TNFα) production by their processing and shedding from inactive membrane-bound precursors (Hart *et al*., 2004; Rubio-Araiz *et al*., 2008). Moreover, transactivation can occur via intracellular protein kinases of the Src family (Berghuis *et al*., 2005; He *et al*., 2005).

CB1 receptor activation can also lead to the regulation of small G-proteins and subsequent regulation of cytoskeleton and microtubule dynamics, which may be responsible for cannabinoid actions on neuritogenesis and synaptogenesis. Activation of CB1 receptors can induce either neurite outgrowth or retraction (Zhou and Song, 2001; Ishii and Chun, 2002; Rueda *et al*., 2002; He *et al*., 2005; Jordan *et al*., 2005). CB1 receptors are enriched in the axonal growth cones of GABAergic interneurons at late gestation and, when activated, they induce a chemorepulsive collapse of axonal growth cones by activating RhoA (Ishii and Chun, 2002; Berghuis *et al*., 2007). Nerve growth factor-induced neurite outgrowth of PC12 cells is inhibited by CB1 receptor modulation of Trk A/Rap1/B-Raf-mediated sustained ERK activation (Rueda *et al*., 2002). Likewise, CB-1 receptor-mediated axonal growth cone collapse in retina development occurs by interfering with Deleted in colorectal cancer receptor membrane trafficking (Argaw *et al*., 2011). On the contrary, CB1 receptor-induced neurite outgrowth in Neuro2A neuroblastoma cells occurs via Rap1, Src and the signal transducer and activator of transcription 3 (STAT-3)(He *et al*., 2005; Jordan *et al*., 2005). A synergistic effect of CB1 activation and IL6-R signaling in CREB and STAT3 activation enforces neurite outgrowth (Zorina *et al*., 2010). Glutamatergic synapse establishment is also regulated by CB1 receptors. In particular, inhibition of 2-AG synthesis in pyramidal cells reduced vGlut1 expression and altered the expression of the glutamatergic synapse markers SNAP25 and synaptophysin (Mulder *et al*., 2008). Other studies based on the use of cannabinoid agonists have shown either positive or inhibitory actions on hippocampal synapse formation or loss (Kim and Thayer, 2001; Kim *et al*., 2008).

CB1 cannabinoid receptor intracellular signaling mechanisms

CB1 receptor signal transduction is coupled to different proliferative and survival pathways that can contribute to the regulation of nervous system development and neurogenesis (Galve-Roperh *et al*., 2008). Besides regulation of cyclic adenosine monophosphate (AMP) levels, perhaps the most recognized downstream target of CB1 receptor activation is the regulation of mitogen-activated protein kinases, with particular relevance of the ERK pathway, and regulation of the PI3K/AKT axis (Figure 6.2). CB1 receptors via canonical $G_{i/o}$-mediated inhibition of adenylyl cyclases decrease cAMP levels and this can contribute to de-inhibition of the ERK pathway by protein kinase A (Davis *et al*., 2003). Moreover, $G_{i/o}$ proteins, by activating Rap1, leads to the activation of a signaling network that includes the small GTPases Ral and Rac, the cytosolic tyrosine kinase Src, and c-Jun N-terminal kinases (Rueda *et al*., 2002; He *et al*., 2005). In addition, βγ subunits liberated upon CB1 receptor activation stimulate the ERK pathway in a PI3K-dependent manner (Galve-Roperh *et al*., 2002). In cerebellar granular progenitor cells, CB1 receptor coupling to the PI3K/AKT pathway is ensued by the activation of the GSK-3β/β-catenin pathway (Trazzi *et al*., 2010). CB1 receptor activity therefore increases β-catenin nuclear localization and the activation of LEF/TCF transcription factor induces proliferation, thereby modulating cell-cycle regulatory genes including cyclin D1.

CB1 receptor activation can regulate more than 20 transcription factors that are part of the gene expression signatures involved in NP maintenance, neuronal commitment and maturation. CB1 signaling converges onto the activation of STAT3, a transcription factor responsible for gene expression regulation that is involved in cannabinoid-induced neurite outgrowth (He *et al*., 2005). In neuroblastoma cells, CB1 receptor-induced STAT3 activation relies on PI3K-dependent activation of the transcription factor Pax6 (Bromberg *et al*., 2008), a paired box family member essential for the generation of the glutamatergic neuronal lineage and cortical neurogenesis (Osumi *et al*., 2008). In addition, CB1 receptor prevents the inhibitory effect of BRCA (breast resistance cancer associated) on neuritogenesis. During cortical development and pyramidal neurogenesis, CB1 receptors are also able to modulate Pax6 and Tbr-2 transcriptional activity in VZ/SVZ progenitors (Diaz-Alonso, J., Aguado, T., Galve-Roperh, I., unpublished). Noteworthy, chronic administration of a THC analogue severely disrupts chick neural development, and this was associated to gene expression changes of critical neurogenic transcription factors including Krox20, Otx2, Pax6 and Sox2 (Psychoyos *et al*., 2008). The involvement of CB1

receptors not only in the regulation of embryonic neuronal specification, but also in postnatal astrogliogenesis, oligodendrocyte survival and myelination, suggests that CB1 receptor signaling is likely to be transduced also by, as yet, unknown pro-gliogenic transcription factors (Guillemot *et al*., 2006). STAT3 regulation by CB1 receptors is a likely candidate in the regulation of astrogliogenesis (Fukuda *et al*., 2007). Thus, CB1 receptors exert a dual role as either pro-neurogenic factors in some cases, or by favoring gliogenesis in others, indicating that differences in the intrinsic progenitor features or in the surrounding niche may be responsible for alternative CB1 receptor neural outcomes.

Recent findings suggest that CB1 signaling may involve the regulation of mammalian target of rapamycin complex 1 (mTORC1), a serine/threonine protein kinase that regulates cell growth, proliferation and survival. CB1 receptor stimulation in hippocampal GABAergic neurons activates mTORC1 and downstream p70S6K in pyramidal neurons, that, by controlling protein synthesis, is responsible for the amnesic effects of THC administration (Puighermanal *et al*., 2009). Therefore, CB1 receptor-induced mTORC1 and protein synthesis regulation can explain some long-term cannabinoid actions on neuronal plasticity and cognition. The role of CB1 receptors in mTORC1 signaling during brain development remains unknown, and different outcomes are possible according to the precise cellular context. Whereas in neuronal cells mTORC1 is activated by CB1 receptors (Puighermanal *et al*., 2009), in transformed glioma cells, cannabinoids, via tribbles homolog 3-dependent inhibition of the AKT/mTORC1 axis, can switch an autophagy program that results in cell death by apoptosis (Carracedo *et al*., 2006; Salazar *et al*., 2009).

The developmental role of the endocannabinoid system: implications in dysfunction

Neurodevelopmental disorders can originate by subtle or severe alterations of various neurogenic processes, including neuronal generation, migration, maturation and connectivity that may be later responsible for adult brain dysfunction (Danzer, 2008; Pang *et al*., 2008). Malformations of cortical development constitute an important example of how embryonic alterations affect adult neurological function (Pang *et al*., 2008). The variety of neuronal processes affected by CB1 cannabinoid receptor signaling makes it difficult to ascribe the consequences of cannabinoid exposure during gestation to a unique mechanism of action. The existence of eCB signaling alterations induced by genetic polymorphism of some of its elements (Norrod and Puffenbarger, 2007) are expected to induce subtle changes during development, e.g. eCB tone dosage, hyper- or hypofunctionality of neurotransmitter signaling, etc. that, however, can have a significant impact and may interfere with proper brain function (Heng *et al*., 2007; Ramocki and Zoghbi, 2008). In agreement, it has recently been reported that progressive demyelinating peripheral polyneuropathy (PHARC) may be associated to polymorphisms in the 2-AG degrading enzyme ABDH 12 (Fiskerstrand T., *et al*., 2010). Mutations in genes involved in the regulation of neurogenesis can interfere with progenitor cell proliferation, aberrant migration or neuronal fate commitment resulting in architecture disruption such as microcephaly, cortical dysplasia or periventricular heterotopias (Pang *et al*., 2008). Developmental alterations are frequently associated to alterations in neuronal excitability and status epilepticus progression, in addition to influence behavioral and emotional responses (Danzer, 2008).

However, the mechanisms by which these early alterations influence cognitive or emotional brain function remain elusive and difficult to extrapolate from animal models to human disorders. For instance, loss of function of doublecortin, a marker of migrating neuroblasts, has a profound impact on excitatory neuronal migration, induces discrete lamination defects and has a profound impact on neuronal excitability (Nosten-Bertrand *et al*., 2008). CB1-deficient mice at early development possess altered cortical projecting neurons due to defective VZ/SVZ pyramidal progenitor cell proliferation, alterations in radial migration and axonal pathfinding, and thus aberrant corticothalamic projections (Mulder *et al*., 2008; Wu *et al*., 2010). Complementary gain of function studies by in-utero electroporation and enforced FAAH expression indicates that depletion of the eCB tone mimics the CB1-deficient phenotype. These results suggest that exacerbated excitotoxicity in CB1-deficient mice (Marsicano *et al*., 2003) may be due, at least in part, to defective excitatory cortical neuronal migration. Expression of CB1 receptors in developing human brain with cortical development – associated

intractable epilepsy points towards this direction (Zurolo *et al*., 2010). The influence of CB1 signaling in the specification of the different cortical neuron subtypes remains to be elucidated, as it is still unknown what is the specific action of CB1 receptors in cortical layer generation. However, considering the heterogeneous CB1 receptor expression in the adult cortex and enrichment in particular cortical layers (Freund *et al*., 2003) it is tempting to speculate that the CB1 receptors are likely to influence in a selective manner the formation of upper versus deeper cortical layers. The possibility of manipulating and counteracting developmental neuronal migration deficits and associated epilepsy (Manent *et al*., 2009) is a proof of concept supporting that palliation of some consequences of neurodevelopmental alterations might be effectively achieved by therapeutic strategies in the future (Ehninger *et al*., 2008).

Epileptogenesis

Impaired GABAergic activity is a natural candidate mechanism of origin for generalized epilepsies (Rakhade and Jensen, 2009). Considering the involvement of the ECS in inhibitory neuronal development and morphogenesis (Berghuis *et al*., 2005; Morozov *et al*., 2009), it is likely that these developmental alterations can be responsible for changes in susceptibility to epileptogenesis. Disruption of cortical interneuron development is known to exert GABA cell type-specific deficits, epilepsy and behavioral dysfunction (Powell *et al*., 2003; Cobos *et al*., 2005). Thus, the decrease in the number of interneurons observed in Dlx1-deficient mice, a homeodomain transcription factor essential during embryonic development for the production of forebrain GABAergic interneurons, is associated with a reduction of GABA-mediated inhibitory postsynaptic currents, electrographic seizures and cortical dysrhythmia in vivo (Cobos *et al*., 2005). Chronic cannabinoid administration induces alterations of CCK+ interneurons density in the hippocampus and cortex (Berghuis *et al*., 2005; Morozov *et al*., 2009) and, even considering the correct integration and normal function of these additional inhibitory interneurons, it is likely that unbalanced inhibitory or excitatory activity influences the development of status epilepticus and excitotoxicity susceptibility.

Alternatively, the crucial role of CB1 receptors in excitatory neuronal generation implies that defective CB1 signaling during embryonic development would result in unbalanced excitatory input due to aberrant pyramidal cell progenitor specification and migration (Mulder *et al*., 2008). Unfortunately, the influence of cannabinoid-mediated regulation of neurogenesis in status epilepticus development and epileptogenesis has not yet been described. At mature stages, excitotoxicity and seizure episodes induce hippocampal neurogenesis (Parent and Murphy, 2008), and newly born neurons influence seizure development due to aberrant granule cell dispersion, mossy fiber sprouting, dendritic remodeling and alterations in excitability threshold (Jakubs *et al*., 2006; Overstreet-Wadiche *et al*., 2006). In CB1-deficient mice the excitotoxicity-induced neurogenic response is abolished (Aguado *et al*., 2007), although the consequences on status epilepticus progression is unknown.

Since early postnatal stages, CB1 receptor activation participates in the homeostatic control of synaptic transmission and network signaling. Once neuronal activity is established, CB1-mediated neuromodulation in differentiated cells will constitute the major mechanism for unbalanced neuronal activity through the disruption of excitatory and inhibitory activity (Hashimotodani *et al*., 2007; Heifets and Castillo, 2009). CB1 activation by retrograde eCB messengers are key regulators of rapid synaptic plasticity, both of inhibitory synapses (depolarization-induced suppression of inhibition and long-term depression of inhibitory transmission) and excitatory synapses (depolarization-induced suppression of excitation and long-term depression of excitatory transmission) (Hashimotodani *et al*., 2007; Heifets and Castillo, 2009)(see Chapters 1 and 3). Thus, CB1 blockade induces epileptic discharges that have been attributed to the absence of depolarization-induced suppression of GABA postsynaptic currents (Bernard *et al*., 2005). CB1 receptors are involved in limbic hyperexcitability and fever-induced seizures through the potentiation of depolarization-induced suppression of inhibition in CCK+ interneurons (Chen *et al*., 2003; Chen *et al*., 2007). In addition, CB1 receptors expressed solely in excitatory hippocampal vGlut1 neurons can allow protection from kainic acid-induced seizures (Marsicano *et al*., 2003; Monory *et al*., 2006). It is important to note that within the first days of development GABA is excitatory instead of inhibitory, therefore CB1 receptor activation and subsequent inhibition of GABA release would result in different outcomes depending on the developmental stage in which the ECS function is altered.

Adult psychiatric disorders

Developmental studies point to a possible role of – often subtle – alterations occurring in embryonic and postnatal nervous system generation and maturation in the pathogenesis of psychiatric disorders (Danzer, 2008; Pang *et al*., 2008; Ramocki and Zoghbi, 2008). Thus, it is plausible that either excessive or defective eCB signaling during brain development may have a significant influence in adult nervous system function and homeostasis (Galve-Roperh *et al*., 2009; Jutras-Aswad *et al*., 2009). Alterations of cannabinoid signaling that influence human emotion-, threat- and reward-related brain function may occur at different levels. On the one hand, CB1 receptor gene (*CNR1*) polymorphisms may reduce or enhance G-protein-mediated signaling and have already been associated with major depression, schizophrenia and drug addiction problems (Ponce *et al*., 2003; Martinez-Gras *et al*., 2006). Surprisingly, recent findings also suggest to CB2 cannabinoid receptor gene (*CNR2*) polymorphisms being associated with depressive syndromes and schizophrenia (Onaivi *et al*., 2008). On the other hand, mutations of eCB metabolizing enzymes, including FAAH and MAG lipases, may result in less active degrading enzymes that would increase the endogenous CB tone and signaling. FAAH polymorphisms have been associated with drug abuse behaviors (Sipe *et al*., 2002; Hariri *et al*., 2009). The available experimental information regarding altered eCB signaling during brain development is still scarce. According to the temporal dynamics in which aberrant CB1 signaling would be active, different actions on neuronal (Berghuis *et al*., 2007; Mulder *et al*., 2008) and glial cell populations (Molina-Holgado *et al*., 2002; Aguado *et al*., 2006; Arevalo-Martin *et al*., 2007) may be predicted, although their influence in the normal adult brain when exposed to pathological insults or stressors remains to be elucidated. The ECS can be influenced as well by prenatal exposure to plant derived cannabinoids, or by incidental contact with drugs targeting CB receptors either directly (e.g. rimonabant) or indirectly (antidepressants). The neurobiological consequences of plant-derived cannabinoid intake on pre- and postnatal stages have been recently reviewed from the perspective of animal models and humans (Jutras-Aswad *et al*., 2009; Schneider, 2009), and indicate that the brain burst period is of special susceptibility.

Changes in the appropriate number, specification or migration of projection neurons and interneurons will initiate neuronal transmission variations that will in turn be followed by a more generalized disruption of neurochemical alterations. The glutamatergic neuronal dysfunction hypothesis of schizophrenia suggests that malfunction of the developmental role of CB1 receptors in pyramidal neurogenesis may contribute to the pathogenesis of psychoses or schizophrenia symptoms (Paz *et al*., 2008). Malfunction of the ECS may be one of the causes underlying neuronal dysfunction, but CB receptors and eCB-metabolizing enzymes are also likely to adapt to aberrant neuronal homeostasis as an attempt to counteract the changes of neuronal transmission (Eggan *et al*., 2008). It remains unknown whether the ECS adaptations exert positive effects to cope with those alterations or may worsen the pathological processes.

Conclusions

Recent findings have demonstrated that endocannabinoids and CB1 receptors are crucial regulators of neurogenic processes including neural progenitor cell proliferation and survival, neuronal specification, migration, synapse establishment and the correct connectivity of newly formed cells. Thus, the ECS may be considered as a novel regulatory signaling system of neurogenesis and nervous system maturation. These studies provide the opportunity for a better understanding of the mechanism of action of aberrant cannabinoid function during nervous system formation and maturation. Developmental cannabinoid exposure alters neurochemical homeostasis, impacts brain plasticity and deregulates neuronal activity. In addition, some cannabinoid-induced alterations of adult neuronal function and behavior may be a consequence of the ability of cannabinergic drugs to interfere with the developmental role of the ECS. Future studies of CB1 cannabinoid receptor signal transduction mechanisms and eCB actions in neurogenic and specification processes should provide useful crucial information to understand the potential association between altered function of the ECS during brain development and neurological and psychiatric dysfunction in adulthood.

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References

Aguado, T., Monory, K., Palazuelos, J., *et al.* (2005) The endocannabinoid system drives neural progenitor proliferation. *FASEB J*, **19**:1704–6.

Aguado, T., Palazuelos, J., Monory, K., *et al.* (2006) The endocannabinoid system promotes astroglial differentiation by acting on neural progenitor cells. *J Neurosci*, **26**:1551–61.

Aguado, T., Romero, E., Monory, K., *et al.* (2007) The CB1 cannabinoid receptor mediates excitotoxicity-induced neural progenitor proliferation and neurogenesis. *J Biol Chem*, **282**:23892–8.

Antonelli, T., Tomasini, M.C., Tattoli, M., *et al.* (2005) Prenatal exposure to the CB1 receptor agonist WIN 55,212–2 causes learning disruption associated with impaired cortical NMDA receptor function and emotional reactivity changes in rat offspring. *Cereb Cortex*, **15**:2013–20.

Arevalo-Martin, A., Garcia-Ovejero, D., Gomez, O., *et al.* (2008) CB2 cannabinoid receptors as an emerging target for demyelinating diseases: from neuroimmune interactions to cell replacement strategies. *Brit J Pharmacol*, **153**:216–25.

Arevalo-Martin, A., Garcia-Ovejero, D., Rubio-Araiz, A., *et al.* (2007) Cannabinoids modulate Olig2 and polysialylated neural cell adhesion molecule expression in the subventricular zone of post-natal rats through cannabinoid receptor 1 and cannabinoid receptor 2. *Eur J Neurosci*, **26**:1548–59.

Argaw, A., Duff, G., Zabouri, N., *et al.* (2011) Concerted action of CB1 cannabinoid receptor and deleted in colorectal cancer in axon guidance. *J. Neurosci*, **31**:1489–99.

Aso, E., Ozaita, A., Valdizan, E.M., *et al.* (2008) BDNF impairment in the hippocampus is related to enhanced despair behavior in CB1 knockout mice. *J Neurochem*, **105**:565–72.

Begbie, J., Doherty, P. and Graham, A. (2004) Cannabinoid receptor, CB1, expression follows neuronal differentiation in the early chick embryo. *J Anat*, **205**:213–8.

Benagiano, V., Lorusso, L., Flace, P., *et al.* (2007) Effects of prenatal exposure to the CB-1 receptor agonist WIN 55212–2 or CO on the GABAergic neuronal systems of rat cerebellar cortex. *Neuroscience*, **149**:592–601.

Bergami, M., Rimondini, R., Santi, S., Blum, R., Gotz, M. and Canossa, M. (2008) Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. *Proc Natl Acad Sci U S A*, **105**:15570–5.

Berghuis, P., Dobszay, M.B., Wang, X., *et al.* (2005) Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor. *Proc Natl Acad Sci USA*, **102**:19115–20.

Berghuis, P., Rajnicek, A.M., Morozov, Y.M., *et al.* (2007) Hardwiring the brain: endocannabinoids shape neuronal connectivity. *Science*, **316**:1212–6.

Bernard, C., Milh, M., Morozov, Y.M., Ben-Ari, Y., Freund, T.F. and Gozlan, H. (2005) Altering cannabinoid signaling during development disrupts neuronal activity. *Proc Natl Acad Sci USA*, **102**:9388–93.

Berrendero, F., GarciaGil, L., Hernandez, M.L., *et al.* (1998) Localization of mRNA expression and activation of signal transduction mechanisms for cannabinoid receptor in rat brain during fetal development. *Development*, **125**:3179–88.

Berrendero, F., Sepe, N., Ramos, J.A., Di Marzo, V. and Fernandez-Ruiz, J.J. (1999) Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestation and early postnatal period. *Synapse*, **33**:181–91.

Bilkei-Gorzo, A., Racz, I., Valverde, O., *et al.* (2005) Early age-related cognitive impairment in mice lacking cannabinoid CB1 receptors. *Proc Natl Acad Sci USA*, **102**:15670–5.

Bisogno, T., Howell, F., Williams, G., *et al.* (2003) Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol*, **163**:463–8.

Bodor, A.L., Katona, I., Nyiri, G., *et al.* (2005) Endocannabinoid signaling in rat somatosensory cortex: laminar differences and involvement of specific interneuron types. *J Neurosci*, **25**:6845–56.

Bromberg, K.D., Ma'ayan, A., Neves, S.R. and Iyengar, R. (2008) Design logic of a cannabinoid receptor signaling network that triggers neurite outgrowth. *Science*, **320**:903–9.

Carracedo, A., Lorente, M., Egia, A., *et al.* (2006) The stress-regulated protein p8 mediates cannabinoid-induced apoptosis of tumor cells. *Cancer Cell*, **9**:301–12.

Castaldo, P., Magi, S., Gaetani, S., *et al.* (2007) Prenatal exposure to the cannabinoid receptor agonist WIN 55,212–2 increases glutamate uptake through overexpression of GLT1 and EAAC1 glutamate transporter subtypes in rat frontal cerebral cortex. *Neuropharmacology*, **53**:369–378.

Cobos, I., Calcagnotto, M.E., Vilaythong, A.J., *et al.* (2005) Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. *Nat Neurosci*, **8**:1059–68.

Chen, K., Neu, A., Howard, A.L., *et al.* (2007) Prevention of plasticity of endocannabinoid signaling inhibits

persistent limbic hyperexcitability caused by developmental seizures. *J Neurosci*, **27**:46–58.

Chen, K., Ratzliff, A., Hilgenberg, L., *et al.* (2003) Long-term plasticity of endocannabinoid signaling induced by developmental febrile seizures. *Neuron*, **39**, 599–611.

D'Souza, D.C., Sewell, R.A. and Ranganathan, M. (2009) Cannabis and psychosis/schizophrenia: human studies. *Eur Arch Psychiatry Clin Neurosci*, **259**:413–31.

Danzer, S.C. (2008) Postnatal and adult neurogenesis in the development of human disease. *Neuroscientist*, **14**:446–58.

Davis, M.I., Ronesi, J. and Lovinger, D.M. (2003) A predominant role for inhibition of the adenylate cyclase/protein kinase A pathway in ERK activation by cannabinoid receptor 1 in N1E-115 neuroblastoma cells. *J Biol Chem*, **278**:48973–80.

De March, Z., Zuccato, C., Giampa, C., *et al.* (2008) Cortical expression of brain derived neurotrophic factor and type-1 cannabinoid receptor after striatal excitotoxic lesions. *Neuroscience*, **152**:734–40.

Eggan, S.M., Hashimoto, T. and Lewis, D.A. (2008) Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. *Arch Gen Psychiatry*, **65**:772–84.

Ehninger, D., Li, W., Fox, K., Stryker, M.P. and Silva, A.J. (2008) Reversing neurodevelopmental disorders in adults. *Neuron*, **60**:950–60.

Fiskerstrand, T., H'mida-Ben Brahim, D., Johansson, S., *et al.* (2010) Mutations in ABHD12 cause the neurodegenerative disease PHARC: An inborn error of endocannabinoid metabolism. *Am J Hum Genet*, **87**:410–17.

Freund, T.F., Katona, I. and Piomelli, D. (2003) Role of Endogenous Cannabinoids in Synaptic Signaling. *Physiol Rev*, **83**:1017–66.

Fukuda, S., Abematsu, M., Mori, H., *et al.* (2007) Potentiation of astrogliogenesis by STAT3-mediated activation of bone morphogenetic protein-Smad signaling in neural stem cells. *Mol Cell Biol*, **27**:4931–7.

Galve-Roperh, I., Aguado, T., Palazuelos, J. and Guzman, M. (2007) The endocannabinoid system and neurogenesis in health and disease. *Neuroscientist*, **13**, 109–14.

Galve-Roperh, I., Aguado, T., Palazuelos, J. and Guzman, M. (2008) Mechanisms of control of neuron survival by the endocannabinoid system. *Curr Pharm Des*, **14**:2279–88.

Galve-Roperh, I., Palazuelos, J., Aguado, T. and Guzman, M. (2009) The endocannabinoid system and the regulation of neural development: potential implications in psychiatric disorders. *Eur Arch Psychiatry Clin Neurosci*, **259**:371–82.

Galve-Roperh, I., Rueda, D., Gomez Del Pulgar, T., Velasco, G. and Guzman, M. (2002) Mechanism of Extracellular Signal-Regulated Kinase Activation by the CB(1) Cannabinoid Receptor. *Mol Pharmacol*, **62**:1385–92.

Garcia-Ovejero, D., Arevalo-Martin, A., Petrosino, S., *et al.* (2009) The endocannabinoid system is modulated in response to spinal cord injury in rats. *Neurobiol Dis*, **33**:57–71.

Gomez, M., Hernandez, M. and Fernandez-Ruiz, J. (2007) The activation of cannabinoid receptors during early postnatal development reduces the expression of cell adhesion molecule L1 in the rat brain. *Brain Res*, **1145**:48–55.

Goncalves, M.B., Suetterlin, P., Yip, P., *et al.* (2008) A diacylglycerol lipase-CB2 cannabinoid pathway regulates adult subventricular zone neurogenesis in an age-dependent manner. *Mol Cell Neurosci*, **38**:526–36.

Guillemot, F., Molnar, Z., Tarabykin, V. and Stoykova, A. (2006) Molecular mechanisms of cortical differentiation. *Eur J Neurosci*, **23**:857–68.

Guzman, M. (2003) Neurons on cannabinoids: dead or alive? *Br J Pharmacol*, **140**:439–40.

Hariri, A.R., Gorka, A., Hyde, L.W., *et al.* (2009) Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. *Biol Psychiatry*, **66**:9–16.

Harkany, T., Guzman, M., Galve-Roperh, I., Berghuis, P., Devi, L.A. and Mackie, K. (2007) The emerging functions of endocannabinoid signaling during CNS development. *Trends Pharmacol Sci*, **28**:83–92.

Hart, S., Fischer, O.M. and Ullrich, A. (2004) Cannabinoids induce cancer cell proliferation via tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor. *Cancer Res*, **64**:1943–50.

Hashimotodani, Y., Ohno-Shosaku, T. and Kano, M. (2007) Endocannabinoids and synaptic function in the CNS. *Neuroscientist*, **13**:127–37.

He, J.C., Gomes, I., Nguyen, T., *et al.* (2005a) The G alpha (o/i)-coupled cannabinoid receptor-mediated neurite outgrowth involves Rap regulation of Src and Stat3. *J Biol Chem*, **280**: 33426–34.

Heifets, B.D. and Castillo, P.E. (2009) Endocannabinoid signaling and long-term synaptic plasticity. *Annu Rev Physiol*, **71**:283–306.

Heng, J.I., Moonen, G. and Nguyen, L. (2007) Neurotransmitters regulate cell migration in the telencephalon. *Eur J Neurosci*, **26**:537–46.

Hill, M.N., Kambo, J.S., Sun, J.C., Gorzalka, B.B. and Galea, L.A. (2006) Endocannabinoids modulate stress-induced suppression of hippocampal cell proliferation and activation of defensive behaviors. *Eur J Neurosci*, **24**:1845–9.

Hill, M.N., Titterness, A.K., Morrish, A.C., *et al.* (2009) Endogenous cannabinoid signaling is required for

voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. *Hippocampus,* **20**:513–23.

Ishii, I. and Chun, J. (2002) Anandamide-induced neuroblastoma cell rounding via the CB1 cannabinoid receptors. *Neuroreport*, **13**:593–6.

Jakubs, K., Nanobashvili, A., Bonde, S., *et al.* (2006) Environment matters: synaptic properties of neurons born in the epileptic adult brain develop to reduce excitability. *Neuron*, **52**:1047–59.

Jiang, S., Fu, Y., Williams, J., *et al.* (2007) Expression and function of cannabinoid receptors CB1 and CB2 and their cognate cannabinoid ligands in murine embryonic stem cells. *PLoS ONE*, **2**:e641.

Jiang, W., Zhang, Y., Xiao, L., *et al.* (2005) Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. *J Clin Invest*, **115**:3104–16.

Jin, K., Xie, L., Kim, S.H., *et al.* (2004) Defective Adult Neurogenesis in CB1 Cannabinoid Receptor Knockout Mice. *Mol Pharmacol*, **66**:204–8.

Jordan, J.D., He, J.C., Eungdamrong, N.J., *et al.* (2005) Cannabinoid receptor-induced neurite outgrowth is mediated by Rap1 activation through G(alpha)o/i-triggered proteasomal degradation of Rap1GAPII. *J Biol Chem*, **280**:11413–21.

Jutras-Aswad, D., DiNieri, J.A., Harkany, T. and Hurd, Y.L. (2009) Neurobiological consequences of maternal cannabis on human fetal development and its neuropsychiatric outcome. *Eur Arch Psychiatry Clin Neurosci*, **259**:395–412.

Katona, I. and Freund, T.F. (2008) Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med*, **14**:923–30.

Katona, I., Urban, G.M., Wallace, M., *et al.* (2006) Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci*, **26**:5628–37.

Kawamura, Y., Fukaya, M., Maejima, T., *et al.* (2006) The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J Neurosci*, **26**:2991–3001.

Kim, D. and Thayer, S.A. (2001) Cannabinoids inhibit the formation of new synapses between hippocampal neurons in culture. *J Neurosci*, **21**:RC146.

Kim, H.J., Waataja, J.J. and Thayer, S.A. (2008) Cannabinoids inhibit network-driven synapse loss between hippocampal neurons in culture. *J Pharmacol Exp Ther*, **325**:850–8.

Kowalczyk, T., Pontious, A., Englund, C., *et al.* (2009) Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. *Cereb Cortex*, **19**:2439–50.

Krebs-Kraft, D.L., Hill, M.N., Hillard, C.J., *et al.* (2010) Sex difference in cell proliferation in developing rat amygdala mediated by endocannabinoids has implications for soical behavior. *Proc Natl Acad Sci U S A*, **107**:20535–40.

Lafourcade, M., Elezgarai, I., Mato, S., Bakiri, Y., Grandes, P. and Manzoni, O.J. (2007) Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex. *PLoS ONE*, **2**:e709.

Li, L., Bender, K.J., Drew, P.J., Jadhav, S.P., Sylwestrak, E. and Feldman, D.E. (2009) Endocannabinoid signaling is required for development and critical period plasticity of the whisker map in somatosensory cortex. *Neuron*, **64**:537–49.

Lutz, B. and Monory, K. (2008) Soothing the seizures of children. *Nat Med*, **14**:721–2.

Mackie, K. (2006) Cannabinoid receptors as therapeutic targets. *Annu Rev Pharmacol Toxicol*, **46**:101–22.

Mackowiak, M., Chocyk, A., Markowicz-Kula, K. and Wedzony, K. (2007) Acute activation of CB1 cannabinoid receptors transiently decreases PSA-NCAM expression in the dentate gyrus of the rat hippocampus. *Brain Res*, **1148**:43–52.

Manent, J.B., Wang, Y., Chang, Y., Paramasivam, M. and LoTurco, J.J. (2009) Dcx reexpression reduces subcortical band heterotopia and seizure threshold in an animal model of neuronal migration disorder. *Nat Med*, **15**:84–90.

Marchalant, Y., Brothers, H.M., Norman, G.J., Karelina, K., DeVries, A.C. and Wenk, G.L. (2009a) Cannabinoids attenuate the effects of aging upon neuroinflammation and neurogenesis. *Neurobiol Dis*, **34**:300–7.

Marchalant, Y., Brothers, H.M. and Wenk, G.L. (2009b) Cannabinoid agonist WIN-55,212–2 partially restores neurogenesis in the aged rat brain. *Mol Psychiatry*, **14**:1068–9.

Maresz, K., Pryce, G., Ponomarev, E.D., *et al.* (2007) Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB(1) on neurons and CB(2) on autoreactive T cells. *Nat Med*, **13**:492–7.

Marsicano, G., Goodenough, S., Monory, K., *et al.* (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*, **302**:84–8.

Marsicano, G., Wotjak, C.T., Azad, S.C., *et al.* (2002) The endogenous cannabinoid system controls extinction of aversive memories. *Nature*, **418**:530–4.

Martinez-Gras, I., Hoenicka, J., Ponce, G., *et al.* (2006) (AAT)n repeat in the cannabinoid receptor gene, CNR1: association with schizophrenia in a Spanish population. Eur *Arch Psychiatry Clin Neurosci*, **256**:437–41.

Mato, S., Del Olmo, E. and Pazos, A. (2003) Ontogenetic development of cannabinoid receptor expression and

signal transduction functionality in the human brain. *Eur J Neurosci*, **17**:1747–54.

Mereu, G., Fa, M., Ferraro, L., *et al.* (2003) Prenatal exposure to a cannabinoid agonist produces memory deficits linked to dysfunction in hippocampal long-term potentiation and glutamate release. *Proc Natl Acad Sci USA*, **100**:4915–20.

Molina-Holgado, E., Vela, J.M., Arevalo-Martin, A., *et al.* (2002) Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *J Neurosci*, **22**:9742–53.

Monory, K., Massa, F., Egertova, M., *et al.* (2006) The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron*, **51**:455–66.

Morozov, Y.M. and Freund, T.F. (2003) Post-natal development of type 1 cannabinoid receptor immunoreactivity in the rat hippocampus. *Eur J Neurosci*, **18**:1213–22.

Morozov, Y.M., Torii, M. and Rakic, P. (2009) Origin, early commitment, migratory routes, and destination of cannabinoid type 1 receptor-containing interneurons. *Cereb Cortex*, **19 Suppl** 1:i78–89.

Mulder, J., Aguado, T., Keimpema, E., *et al.* (2008) Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. *Proc Natl Acad Sci USA*, **105**:8760–5.

Nakamura, K., Salomonis, N., Tomoda, K., Yamanaka, S. and Conklin, B.R. (2009) G(i)-coupled GPCR signaling controls the formation and organization of human pluripotent colonies. *PLoS One*, **4**:e7780.

Norrod, A.G. and Puffenbarger, R.A. (2007) Genetic polymorphisms of the endocannabinoid system. *Chem Biodivers*, **4**:1926–32.

Nosten-Bertrand, M., Kappeler, C., Dinocourt, C., *et al.* (2008) Epilepsy in Dcx knockout mice associated with discrete lamination defects and enhanced excitability in the hippocampus. *PLoS One*, **3**:e2473.

Onaivi, E.S., Ishiguro, H., Gong, J.P., *et al.* (2008) Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. *PLoS ONE*, **3**:e1640.

Osumi, N., Shinohara, H., Numayama-Tsuruta, K. and Maekawa, M. (2008) Concise review: Pax6 transcription factor contributes to both embryonic and adult neurogenesis as a multifunctional regulator. *Stem Cells*, **26**:1663–72.

Overstreet-Wadiche, L.S., Bromberg, D.A., Bensen, A.L. and Westbrook, G.L. (2006) Seizures accelerate functional integration of adult-generated granule cells. *J Neurosci*, **26**, 4095–103.

Palazuelos, J., Aguado, T., Egia, A., Mechoulam, R., Guzman, M. and Galve-Roperh, I. (2006) Non-psychoactive CB2 cannabinoid agonists stimulate neural progenitor proliferation. *FASEB J*, **20**:2405–407.

Palazuelos, J., Aguado, T., Pazos, M.R., *et al.* (2009) Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain*, **132**:3152–64.

Pang, T., Atefy, R. and Sheen, V. (2008) Malformations of cortical development. *Neurologist*, **14**:181–91.

Parent, J.M. and Murphy, G.G. (2008) Mechanisms and functional significance of aberrant seizure-induced hippocampal neurogenesis. *Epilepsia*, **49 Suppl** 5:19–25.

Patel, S. and Hillard, C.J. (2008) Adaptations in endocannabinoid signaling in response to repeated homotypic stress: a novel mechanism for stress habituation. *Eur J Neurosci*, **27**:2821–9.

Paz, R.D., Tardito, S., Atzori, M. and Tseng, K.Y. (2008) Glutamatergic dysfunction in schizophrenia: from basic neuroscience to clinical psychopharmacology. *Eur Neuropsychopharmacol*, **18**:773–86.

Ponce, G., Hoenicka, J., Rubio, G., *et al.* (2003) Association between cannabinoid receptor gene (CNR1) and childhood attention deficit/hyperactivity disorder in Spanish male alcoholic patients. *Mol Psychiatry*, **8**:466–7.

Powell, E.M., Campbell, D.B., Stanwood, G.D., Davis, C., Noebels, J.L. and Levitt, P. (2003) Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction. *J Neurosci*, **23**:622–31.

Psychoyos, D., Hungund, B., Cooper, T. and Finnell, R.H. (2008) A cannabinoid analogue of Delta9-tetrahydrocannabinol disrupts neural development in chick. *Birth Defects Res B Dev Reprod Toxicol*, **83**:477–88.

Puighermanal, E., Marsicano, G., Busquets-Garcia, A., Lutz, B., Maldonado, R. and Ozaita, A. (2009) Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nat Neurosci*, **12**:1152–8.

Rakhade, S.N. and Jensen, F.E. (2009) Epileptogenesis in the immature brain: emerging mechanisms. *Nat Rev Neurol*, **5**:380–91.

Ramocki, M.B. and Zoghbi, H.Y. (2008) Failure of neuronal homeostasis results in common neuropsychiatric phenotypes. *Nature*, **455**:912–18.

Rubio-Araiz, A., Arevalo-Martin, A., Gomez-Torres, O., *et al.* (2008) The endocannabinoid system modulates a transient TNF pathway that induces neural stem cell proliferation. *Mol Cell Neurosci*, **38**:374–80.

Rueda, D., Navarro, B., Martinez-Serrano, A., Guzman, M. and Galve-Roperh, I. (2002) The endocannabinoid anandamide inhibits neuronal progenitor cell

differentiation through attenuation of the Rap1/B-Raf/ERK pathway. *J Biol Chem*, **277**:46645–50.

Salazar, M., Carracedo, A., Salanueva, I.J., *et al.* (2009) Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. *J Clin Invest*, **119**:1359–72.

Schneider, M. (2009) Cannabis use in pregnancy and early life and its consequences: animal models. *Eur Arch Psychiatry Clin Neurosci*, **259**:383–93.

Sipe, J.C., Chiang, K., Gerber, A.L., Beutler, E. and Cravatt, B.F. (2002) A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc Natl Acad Sci USA*, **99**:8394–9.

Solbrig, M.V. and Hermanowicz, N. (2008) Cannabinoid rescue of striatal progenitor cells in chronic Borna disease viral encephalitis in rats. *J Neurovirol*, **14**:252–60.

Stella, N. (2009) Endocannabinoid signaling in microglial cells. *Neuropharmacology*, **56 Suppl** 1:244–53.

Sun, X. and Dey, S.K. (2008) Aspects of endocannabinoid signaling in periimplantation biology. *Mol Cell Endocrinol*, **286**:S3–11.

Trazzi, S., Steger, M., Mitrugno, V.M., Bartesaghi, R. and Ciani, E. (2010) CB1 cannabinoid receptors increase neuronal precursor proliferation through AKT/glycogen synthase kinase-3beta/beta-catenin signaling. *J Biol Chem*, **285**:10098–109.

Vitalis, T., Laine, J., Simon, A., Roland, A., Leterrier, C. and Lenkei, Z. (2008) The type 1 cannabinoid receptor is highly expressed in embryonic cortical projection neurons and negatively regulates neurite growth in vitro. *Eur J Neurosci*, **28**:1705–18.

Walker, D.J., Suetterlin, P., Reisenberg, M., Williams, G. and Doherty, P. (2010) Down-regulation of diacylglycerol lipase-alpha during neural stem cell differentiation: identification of elements that regulate transcription. *J Neurosci Res*, **88**:735–45.

Wang, H., Xie, H., Guo, Y., *et al.* (2006) Fatty acid amide hydrolase deficiency limits early pregnancy events. *J Clin Invest*, **116**:2122–31.

Wang, X., Dow-Edwards, D., Keller, E. and Hurd, Y.L. (2003) Preferential limbic expression of the cannabinoid receptor mRNA in the human fetal brain. *Neuroscience*, **118**:681–94.

Watson, S., Chambers, D., Hobbs, C., Doherty, P. and Graham, A. (2008) The endocannabinoid receptor, CB1, is required for normal axonal growth and fasciculation. *Mol Cell Neurosci*, **38**:89–97.

Williams, E.J., Walsh, F.S. and Doherty, P. (2003) The FGF receptor uses the endocannabinoid signaling system to couple to an axonal growth response. *J Cell Biol*, **160**:481–6.

Wolf, S.A., Bick-Sander, A., Fabel, K., *et al.* (2010) Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. *Cell Commun Signal*, **8**:12.

Wu, C.S., Zhu, J., Wager-Miller, J., *et al.* (2010) Requirement of cannabinoid CB(1) receptors in cortical pyramidal neurons for appropriate development of corticothalamic and thalamocortical projections. *Eur J Neurosci*, **32**:693–706.

Zhou, D. and Song, Z.H. (2001) CB1 cannabinoid receptor-mediated neurite remodeling in mouse neuroblastoma N1E-115 cells. *J Neurosci Res*, **65**:346–53.

Zorina, Y., Iyengar, R. and Bromberg, K.D. (2010) Cannabinoid 1 receptor and interleukin-6 receptor together induce integration of protein kinase and transcription factor signaling to trigger neurite outgrowth. *J Biol Chem*, **285**:1358–70.

Zurolo, E., Iyer, A.M., Spliet, W.G., *et al.* (2010) CB1 and CB2 cannabinoid receptor expression during development and in epileptogenic developmental pathologies. *Neuroscience*, **170**:28–41.

Chapter 7

The impact of pubertal exposure to cannabis on the brain: a focus on animal studies

Miriam Schneider

The use of psychoactive preparations of *Cannabis sativa* is highly prevalent worldwide, especially among teenagers. In recent years growing evidence from animal research and also human studies has indicated the existence of particular vulnerable developmental periods, mainly puberty and mid-adolescence, during which exposure to cannabis and cannabinoids might lead to deleterious consequences in later life (Arseneault *et al*., 2004; Hall, 2006; for review see Schneider, 2008). In the debate about possible long-term consequences of cannabis use and abuse the age of onset of cannabis consumption has therefore gained increasing attention. Although the association between early cannabis use and subsequent problems may be due, in part, to common risk factors, it nevertheless remains important to monitor the age of initial cannabis use. It has been shown in the last decades that the use of cannabis has increased in young people, and the age of first use has declined, with most consumers starting cannabis use in their mid-to-late teens (Monshouwer *et al*., 2005; EMCDDA, 2006; Hall, 2006). Therefore, those who might be at the highest risk for adverse consequences of cannabis exposure tragically represent the major consumer group of cannabis derivatives.

Human studies, in particular those using retrospective evaluations, have some limitations because of the vast heterogeneity of cannabis use (different consumption patterns, cannabis products and cannabinoid concentrations), and also owing to possible problems in monitoring and confirming self-reported drug use, as well as concurrent use of other drugs. Hence, research with laboratory animals offers an important link to gaining further knowledge about specific effects of cannabinoids during postnatal development and underlying neurobiological mechanisms mediating this heightened susceptibility. Although, developmental cannabinoid exposure in animals might not be able to completely capture the entire situation and predict the exact risk of cannabis use in teenagers, the information obtained in valid animal models is still crucial and necessary for a better understanding of the underlying neurobiological mechanisms and possible deleterious consequences. This chapter therefore surveys possible lasting consequences of cannabinoid exposure during crucial periods of pubertal and adolescent maturation reported from animal research.

Timing and neurobiological characteristics of puberty

Puberty and adolescence are important developmental periods during which an individual matures from a biologically non-reproductive, infertile child into an adult. The term "puberty" (latin *pubertas* = (sexual) maturity), which has to be clearly distinguished from "adolescence" (latin *adolescere* = to grow up), refers exactly to the time period during which sexual maturation is achieved and is initiated by an increased secretion of gonadotropin-releasing hormone (GnRH), resulting in gonadal maturation and steroid hormone secretion. Although puberty and adolescence are overlapping time periods, with puberty being a part of adolescence, the terms cannot be used interchangeably. In contrast, adolescence refers to the gradual period of behavioral and cognitive transition from childhood to adulthood, and the boundaries of this period are less precisely defined (Schneider, 2008). However, gonadal alterations in puberty and adolescent behavioral maturations are intimately linked in timing through multiple and complex interactions between the nervous system and gonadal steroid hormones, which are involved in the maturation of reproductive and social behaviors (e.g. sexual salience of sensory stimuli and sexual motivation) (Sisk and Foster, 2004).

Numerous neurodevelopmental alterations take place during puberty, including maturational processes

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in cortical (mainly the medial prefrontal cortex [mPFC]) and limbic regions, which are characterized by both progressive and regressive changes, e.g. myelination and synaptic pruning (Spear, 2000; De Bellis *et al*., 2001; Powell, 2006). Typically, an overproduction of axons and synapses can be found during early puberty, followed by rapid pruning during later puberty, indicating that connections and communication between subcortical and cortical regions are in a highly transitional state during adolescence and, in particular, during puberty. Furthermore, maturation of neurotransmitter systems, such as the glutamatergic, the dopaminergic and also the endocannabinoid system, occur during adolescence, with developmental peaks in receptor overexpression often seen concomitant with the onset of puberty (Rodriguez de Fonseca *et al*., 1993; Andersen *et al*., 2000; Spear, 2000).

Some of these neurodevelopmental changes during puberty have been linked directly to the presence of steroid hormones. It has been shown, for example, that gonadal hormone modulation of cell numbers and cell group volume is a potential mechanism for the active maintenance of sexual dimorphisms during adolescent development in rats (Ahmed *et al*., 2008). Furthermore, ovarian hormone-modulated cell death during puberty appears to be responsible for the postnatal emergence of sex differences in volume of the rat primary visual cortex (Nunez *et al*., 2002). Finally, there is evidence that testicular hormones influence changes in white matter volume during adolescent brain development. The increase in white matter volume during adolescence in humans (Paus *et al*., 1999; Lenroot and Giedd, 2006) has recently been linked in males to testosterone levels and androgen receptor activity (Perrin *et al*., 2008). Notably, testosterone appears to influence white matter volume by increasing axonal caliber and not myelination. Additionally, it has been shown in the Syrian hamster that patterns of synaptic connectivity change across adolescence within the medial amygdala, concomitant with the pubertal rise in gonadal hormones (Zehr *et al*., 2006).

The timing of puberty is quite easy to determine in rodents, since external physical signs exist that indicate the onset of this specific developmental period. Puberty is reached from around postnatal day (pd) 28 and extends to ~ pd 40 in female rats (onset indicated by vaginal opening), and from pd 40 to pd 60 in males (onset indicated by complete balanopreputial separation) (Schneider, 2008) (Figure 7.1). It is therefore of great importance for animal research to factor in gender differences in the timing of puberty and adolescence in female and male animals (with females maturing earlier than males), in particular for studies comparing the sexes.

In contrast to puberty, the exact timing of adolescence is rather difficult to define in laboratory animals. Per definition, adolescence covers the complete time span from childhood to adulthood, including the pubertal period. Therefore, adolescence begins in the juvenile period directly after weaning (around pd 22), extending to sexual maturity by the end of puberty (females ~ pd 40; males ~ pd 60), and continuing into early adulthood (females ~ pd 50/60; males ~ pd 70/80) (Figure 7.1).

Clarification of the exact timing of these developmental periods is of great importance if animal research is expected to give indications about crucial time windows for cannabinoid exposure. If animal studies are aiming to translate the findings from cannabis exposure in laboratory rats toward possible risks of cannabis use in humans, the specific age of exposure has to be considered very carefully. As mentioned above, cannabis use is normally initiated during puberty or mid-adolescence (EMCDDA, 2006) and, therefore, cannabinoid exposure during early adolescence, before puberty onset, might not provide a good model of the timing of cannabis use during the teenage years in humans. Furthermore, since the most important adolescent behavioral changes and many important neuronal maturational processes are closely linked to pubertal development (Sisk and Foster, 2004), and since major developmental alterations in the endocannabinoid system are taking place during pubertal development (see below), the more exact time window of puberty might be the best choice for the evaluation of possible lasting consequences of cannabis exposure in the laboratory rat (Schneider, 2008).

Postnatal development of the endocannabinoid system

Endocannabinoids and their cannabinoid receptors, CB1 and CB2, are present from the early stages of gestation and play a number of vital roles for the developing organism (for review see Galve-Roperh *et al*., 2009 and Chapter 6). However, only a few studies have so far investigated the maturational processes occurring in the endocannabinoid system during further postnatal development, including adolescence and puberty. A thorough study by Rodriguez de Fonseca

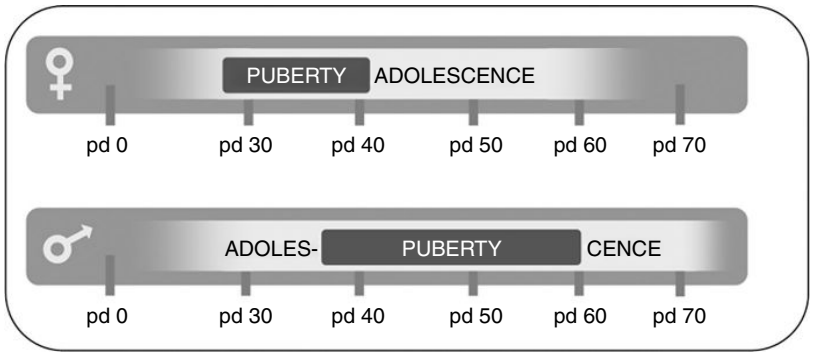

Figure 7.1. Timing of puberty and adolescence in female and male rats. The pubertal period in female rats (~postnatal day (pd) 28 to 40) is determined by vaginal opening and first estrus. Balano preputial separation indicates pubertal onset in male rats (around pd 40), and sexual maturity is indicated by the presence of mature spermatozoa in the vas deferens, which is achieved around pd 60. In both male and female rats, adolescence begins during the juvenile period and reaches into early adulthood, thereby including the pubertal period.

and coworkers demonstrated a sex-dependent progressive increase in CB1 receptor radioligand binding in rats in the limbic system, mesencephalon and striatum. Binding increased gradually starting from pd 10, and reached maximum values around pd 30 in females and pd 40 in males, respectively (Rodriguez de Fonseca *et al*., 1993). Thereafter CB1 binding seems to decrease during the pubertal period until it reaches adult values (measured on pd 70). Interestingly, the peak of CB1 receptor binding coincides in male and female animals, with the age of approximate onset of puberty.

A similar postnatal increase in CB1 receptor binding has been reported in other studies in rats for the striatum, cerebellum and the cortex (Belue *et al*., 1995) as well as for the whole brain (McLaughlin *et al*., 1994). Additionally, Berrendero *et al*. detected an increase in anandamide levels during the early postnatal period (Berrendero *et al*., 1999). However, in all these studies animals were investigated only up to pd 21 and again in early adulthood, omitting the pubertal developmental period. Interestingly, CB1 mRNA expression is present in the whole brain as early as pd 3 (McLaughlin *et al*., 1994) and does not differ from later postnatal (only tested up to pd 21) or adult expression levels (pd 60), as has been shown for CB1 binding. The authors suggested that the observed progressive increase in CB1 binding might reflect the final manifestation of complete functional maturity of the receptors. These interpretations were supported by a study investigating the development of behavioral responses to exogenous and endogenous cannabinoids in mice from pd 6 up to the age of weaning (pd 20) and in adulthood (Fride and Mechoulam, 1996). A significant behavioral response (locomotor activity and analgesia) as seen in adult mice to either the endocannabinoid anandamide (AEA) or Δ^9-tetrahydrocannabinol (THC) was not observed in juvenile mice at any postnatal age tested. Unfortunately, neither of these studies included the pubertal period in their investigations.

In addition to the progressive increase in CB1 binding during development, it has been shown that hypothalamic levels of AEA display a peak immediately before the onset of puberty in female rats, indicating a possible involvement of the endocannabinoid system in the timing of puberty (Wenger *et al*., 2002). Accordingly, chronic THC treatment delays the onset of puberty in female rats by two days (Wenger *et al*., 1988) and it has been shown in vitro that CB1 and CB2 receptors are expressed on GnRH neurons, and that these neurons are able to produce and release endocannabinoids (AEA and 2-arachidonoyl glycerol) (Gammon *et al*., 2005).

In summary, the activity of the endocannabinoid system, including receptors and endogenous ligands, seems to be highest around puberty onset, indicating a high vulnerability of this specific developmental period for the consequences of exposure to exogenous cannabinoids (Schneider, 2008).

Consequences of pubertal cannabinoid exposure

The maturational processes occurring during puberty and adolescence are necessary for the occurrence of adult behavioral performance, but simultaneously render the organism vulnerable to perturbations during this crucial developmental time span (Chambers *et al*., 2003). Regarding the various developmental processes

in the endocannabinoid and related neurotransmitter systems during puberty, it is not surprising that immature individuals seem to be particularly susceptible to the exposure of exogenous cannabinoids. The following sections will give an overview of these possible adverse consequences of cannabis exposure during adolescence and pubertal maturation in rats.

Studies using animal models to investigate possible long-term cannabinoid effects are of major importance for a better understanding of the crucial developmental time windows, and also for the underlying neurobiological mechanisms, since animal research offers the possibility to examine possible links between histological or biochemical alterations directly with behavioral abnormalities. Animal models of psychiatric disorders are normally based on the concept of homology of brain structures and the equivalence of their function in animals and humans (Koch, 2002). This is especially true for the endocannabinoid system, with CB1 and CB2 receptors and the endogenous ligands being detectable in a similar neuroanatomical distribution in all vertebrates; also basic elements of the endocannabinoid system have even been found in some invertebrates (Elphick and Egertova, 2001). However, one should be always be careful when translating findings from animal studies to humans. It is, for example, not possible to relate directly the dosage of cannabinoid injections administered to rodents, to human cannabis ingestions. This is not only because of the different routes of administration, but also to the fact that rodents have a much higher metabolism than humans and, therefore, rodents have to be exposed to higher cannabinoid concentrations than humans to obtain similar effects. Hence, in our studies with laboratory rats we use moderate cannabinoid doses that affect cognitive processing but do not alter basic locomotor activity (e.g. Schneider and Koch, 2002; Schneider and Koch, 2003).

Neurobiological consequences

So far, only few animal studies have investigated possible lasting neurobiological perturbations after cannabinoid exposure during adolescence and puberty. A lasting decrease in CB1 receptor density and functionality was shown in the nucleus accumbens (NAC), amygdala and ventral tegmental area (VTA) in adult female rats that had been treated with THC during puberty (pd 35–45). Additionally, different persistent alterations in the endogenous opioid system were detected. Chronic THC treatment during early puberty in male rats (pd 28–49) increased preproenkephalin mRNA expression in the NAC shell. Moreover, mu-opioid receptor GTP-coupling was found to be potentiated in mesolimbic and nigrostriatal brainstem regions in THC-pretreated animals (Ellgren *et al*., 2007).

Beside direct alterations in the endocannabinoid or other neurotransmitter systems, adolescent/pubertal cannabinoid exposure has been reported to affect cortical and limbic systems in particular. Chronic treatment with the synthetic cannabinoid agonist CP55 940 (CP) during puberty in female rats (pd 28–38) induced changes in brain glucose metabolism, indicating a hyperactivation of the frontal cortex and a hypoactivation of the amygdalo-enthorinal area (Higuera-Matas *et al*., 2008). In addition, pubertal treatment with the cannabinoid receptor agonist WIN55 212-2 (WIN) revealed persistent changes in neuronal activity assessed by c-Fos protein quantification in several brain regions (e.g. NAC, striatum and hippocampus) (Wegener and Koch, 2009). Pubertal cannabinoid exposure in female rats decreased cAMP response element-binding protein (CREB) activity in the hippocampus and PFC and increased activity in the NAC (Rubino *et al*., 2008). A significant decrease was also found in the astroglial marker glial fibrillar acid protein (GFAP) and in pre- and postsynaptic protein expression (VAMP2, PSD95) (Rubino *et al*., 2009b).

Furthermore, a recent proteomic analysis revealed differences between adolescent and adult cannabinoid pre-exposure on protein expression in the hippocampus (Quinn *et al*., 2008). The analysis uncovered a much higher number of proteins, mainly involved in regulating oxidative stress/mitochondrial functioning and cytoarchitecture, that were altered after adolescent THC treatment, than after adult treatment. Some specific alterations were also reported for the PFC. A significant decrease in presynaptic (synaptophysin) and postsynaptic (PSD95) proteins was found in the PFC of THC-pretreated female rats (pd 35–45), with no alterations in the hippocampus (Rubino *et al*., 2009a). Finally, proteomic analysis of the synapses in the PFC revealed the presence of less-active synapses. In particular, mitochondrial proteins (e.g., cytochrome b–c1 complex subunit 1 and subunit 2, ATP synthase alpha and beta subunits) were all found to be less abundant, thus suggesting a reduction in mitochondria co-isolated within synaptosomes.

So, taken together, some lasting and region-specific neurobiological alterations have been reported after chronic adolescent and pubertal cannabinoid

treatment, mainly altering the expression of various proteins in the NAC, hippocampus and PFC. However, these findings are so far very heterogeneous and restricted, for example regarding alterations in the endocannabinoid system and other neurotransmitter systems, as only few studies have investigated such long-term changes. In addition, the detailed relevance of the differences detected in expression of various proteins on behavioral performance needs to be examined; this will require further research.

Effects on cognition

The first studies investigating differences in residual effects on cognitive ability in rats after cannabinoid exposure during different developmental periods were performed by Stiglick and Kalant. They demonstrated that chronic exposure of immature animals to THC caused more irreversible residual effects on cognitive performance (Stiglick and Kalant, 1982a; Stiglick and Kalant, 1982b) than chronic treatment of mature rats (Stiglick and Kalant, 1985). However, treatment periods in this study were relatively long (3 to 6 months) so it remained difficult to isolate the specific vulnerable period. We could demonstrate that chronic treatment with the synthetic cannabinoid receptor agonist WIN throughout the period of pubertal development in male rats (pd 40–65) leads to long-lasting behavioral disturbances in adulthood. A comparable treatment in adult (> pd 70) and prepubertal (pd 15–40) rats induced no, or only minor, lasting impairments on behavioral performance respectively, identifying puberty as the most vulnerable period for the adverse effects of exogenous cannabinoids (Schneider and Koch, 2003; Schneider *et al*., 2005; for review see Schneider, 2008).

Pubertal WIN-treated rats showed persistent alterations in sensorimotor gating, object recognition memory, progressive ratio (PR) performance, social behavior and wake-sleep rhythm (Schneider and Koch, 2003; Schneider and Koch, 2005; Schneider *et al*., 2008). These findings were confirmed by two studies showing that chronic treatment with the cannabinoid receptor agonist CP during puberty in female rats (pd 30–50) (O'Shea *et al*., 2006) and during early puberty in males (pd 34–55) (Quinn *et al*., 2008) persistently and specifically affected object-recognition memory. We also demonstrated recently that acute WIN administration affects object- and social-recognition memory in pubertal and adult rats, although the decrease in short-term memory performance was more pronounced after pubertal WIN administration (Schneider *et al*., 2008).

It has also been shown that acute THC treatment impaired both spatial and non-spatial learning in the water maze more powerfully in male juvenile rats (pd 30) than in adults (pd 65–70), whereas no residual alterations were seen in this study after chronic treatment from pd 30 to 50 (Cha *et al*., 2006). However, treatment in these male rats started 10 days before puberty onset and ended about 10 days before sexual maturity was reached and might therefore not have been sufficient to observe persistent effects. In addition, THC treatment in pubertal female rats (pd 35–45) was found to decrease performance in the radial maze in adulthood, but no effects were detected on aversive memory (Rubino *et al*., 2009a). Interestingly, the same group reporting adolescent specific residual cannabinoid effects on object recognition (O'Shea *et al*., 2004; Quinn *et al*., 2008) failed in an additional study to confirm these findings (O'Shea *et al*., 2006). That study even reported similar residual alterations in object-recognition memory, irrespective of age when chronic treatment occurred (pd 4–24, pd 30–50 and pd 56–76), and it is the only study showing persistent effects after adult cannabinoid exposure. However, this supposed "adult" treatment was started in immature males at an age of 56 days before sexual maturity was reached in rats. Therefore, animals during this late pubertal period might still be susceptible to cannabinoid exposure.

In summary, findings from animal studies indicate puberty/mid-adolescence as a highly susceptible time window for possible residual, but also acute, aversive effects on cognitive processing of cannabinoid exposure.

Implications for neuropsychiatric disorders

Global evidence indicates that cannabis use/abuse acts as a risk factor for the emergence of schizophrenia, especially among early-onset cannabis users (Arseneault *et al*., 2004; Caspi *et al*., 2005; see Chapter 5). Similar indications were observed in animal studies, where chronic pubertal, but not adult, cannabinoid treatment resulted in lasting behavioral deficits, resembling at least some aspects of schizophrenia. Cannabinoid exposure during pubertal development induced working memory deficits (Schneider and Koch, 2003; O'Shea *et al*., 2004; Quinn *et al*., 2008), impaired sensorimotor gating and led to abnormal social behavior and anhedonia in adulthood (Schneider and Koch, 2003; 2005; Schneider *et al*., 2008); these are all among the symptoms of schizophrenia. Some of these behavioral deficits were even more pronounced if the animals were

rendered more vulnerable to the effects of the pubertal cannabinoid administration by neonatal lesion of the mPFC on pd 7 (Schneider and Koch, 2005, 2007), indicating that susceptible individuals show a higher risk for adverse consequences after cannabis exposure. Furthermore, the deficit observed in sensorimotor gating after pubertal WIN treatment was completely rescued by an acute injection of the typical antipsychotic drug haloperidol, indicating changes in the dopaminergic system (Schneider and Koch, 2003).

There is also increasing evidence that regular, and in particular heavy cannabis use, might be linked to depression, anxiety and other mood related disorders (e.g. Patton *et al*., 2002; Rey *et al*., 2002; Degenhardt *et al*., 2003; Poulin *et al*., 2005; see Chapter 10). Similar to the risk for schizophrenia, this association has been reported to be mainly linked to an early onset of problematic cannabis use in young people (Degenhardt *et al*., 2003).

Data from animal studies on emotional behavior and anxiety are partially conflicting, depending strongly on the age when cannabinoid exposure took place and on the behavioral tests applied. In pubertal rats (pd 40) acute CP injections induced hyperreactivity and anxiogenic responses (vocalizations) (Romero *et al*., 2002). In addition, we found in a previous study that chronic WIN treatment in pubertal (pd 40–65) rats after neonatal mPFC lesion alters the pattern of social-play behavior in adult animals in a way that could be interpreted as increased anxiety (Schneider and Koch, 2005). Additionally, chronic juvenile (pd 15–40) and pubertal WIN treatment reduced the time spent in the center of an open field and the number of rearings in adulthood, indicating reduced exploratory and increased anxiety-related behavior (Schneider *et al*., 2005; Schneider and Koch, 2005). Consistent with these findings it was shown that chronic cannabinoid treatment during puberty in female rats (pd 30–50) resulted in decreased social interaction (O'Shea *et al*., 2004). Chronic THC treatment (pd 35–45) in female rats did not affect anxiety-related behavior, but induced a "behavioral despair" response in the forced-swim test and reduced sucrose preference (Rubino *et al*., 2008). However, chronic CP injections during pre-/early puberty in male rats (pd 35–45), as well as pubertal WIN treatment, were found to decrease anxiety-related behavior in the elevated-plus maze in adult animals (Biscaia *et al*., 2003; Wegener and Koch, 2009).

In conclusion, data from animal research indicate that cannabinoid exposure specifically during puberty or mid-adolescence might contribute to the occurence of behavioral deficits that reflect some aspects of psychotic symptomatology and lead to alterations in emotional behavior. However, much more research is needed on this topic to clarify the detailed influence of cannabis on emotionality and to examine further the impact of possible pre-existing vulnerability factors on cannabis exposure.

Cannabis dependence and gateway effects

A very delicate and controversial issue is whether cannabis might act as a gateway drug, and subsequently lead to increased intake of other illicit drugs (e.g. Fergusson and Horwood, 2000; Fergusson *et al*., 2006; Lynskey *et al*., 2006; Schneider, 2008). Thus, whether or not there is a causal relationship between cannabis use in humans and a progression to other illicit drug use is still heavily debated (for detailed discussion see: Kandel *et al*., 2006; MacCoun, 2006), and clarification of this contentious issue definitely requires further research. In this context experimental animal models provide the opportunity to evaluate directly the relationship between prior cannabis exposure and further response to other drugs of abuse. Evidence from animal studies indicates that cannabinoids might induce lasting neuronal modulations that could alter the perception and/or reinforcing values of other drugs of abuse, independent of genetic, social or cultural factors.

Pistis *et al*. (2004) demonstrated that subchronic WIN treatment induces long-lasting tolerance to acute administration of cannabinoids in VTA dopaminergic neurons. When the cannabinoid treatment took place between pd 35–42, tolerance was not restricted to cannabinoids as observed in adult rats, but cross-tolerance developed to morphine, amphetamine and cocaine. The mechanisms underlying the observed cross-tolerance are not yet known in detail. Notably, CB1, mu and dopamine D2 receptors share similar inhibitory G-protein systems and effectors, and subchronic CB1 stimulation might therefore dysregulate common signaling cascades (Pistis *et al*., 2004). In addition, CP treatment (pd 35–45) was found to induce higher morphine self-administration rates under a fixed ratio, but not under a progressive ratio schedule in male rats, whereas no such effects were detected in females (Biscaia *et al*., 2008). A similar CP treatment in prepubertal male and pubertal female rats (pd 28–38) detected subtle and sex-specific alterations in cocaine self-administration (Higuera-Matas *et al*., 2008).

During the acquisition phase, female CP-treated rats showed a higher rate of cocaine self-administration as compared with vehicle-treated controls. Furthermore, a recent study demonstrated that chronic THC administration during early puberty of male rats (pd 28–49) enhanced heroin self-administration in adulthood (Ellgren *et al*., 2007). A previous study by the same group failed to show an association between juvenile THC pretreatment in males (pd 28–32) and a later response to amphetamine (Ellgren *et al*., 2004). These findings confirm the assumption that a narrow time window around puberty, as described before, represents the most vulnerable developmental period toward lasting consequences of cannabis exposure.

Taken together, these studies indicate that cannabis exposure during pubertal development might have a priming effect on the brain, and render cannabis users more susceptible to the effects of other illicit drugs. Although strong evidence suggests that cannabinoids induce neurobiological alterations in common reward pathways during this crucial period, these findings do not completely exclude the possibility that other factors such as genetic predispositions, social structures and environment might influence these neurodevelopmental cannabinoid effects and either enhance or attenuate further progression into illicit drug use.

Conclusions

The public debate about cannabis in politics and the media is often confined to two very extreme and conflicting opinions: either to legalize cannabis because its use is thought to be harmless and useful for therapeutic purposes; or strict prohibition of its use because cannabis is considered extremely harmful (see Chapter 5). This rather emotional quarrel often prevents the very necessary objective discussion of the potential specific risks of cannabis exposure for young consumers during the teenage years. Data from animal studies and human studies are indicating young cannabis users might be extremely susceptible to residual cognitive impairments, have a higher risk for neuropsychiatric disorders, in particular when genetic or environmental predispositions exist, and might be more susceptible to further drug use and cannabis dependence. The neurobiological mechanisms mediating the specific vulnerability of teenage brains to cannabinoid exposure are not yet known in detail and the association between early cannabis use and subsequent problems may be owing partially to common risk factors. Nevertheless, data from animal research reviewed in the present chapter all point out clearly that the age at which an individual is exposed to cannabinoids has a major impact on the subsequent effects of this drug. In particular the period of pubertal development, during which the endocannabinoid system appears to be overactive, seems to represent the period most susceptible to possible lasting negative cannabinoid effects.

Taken together, these findings suggest that teeagers, in particular during the susceptible period around pubertal development, represent a highly vulnerable consumer group for cannabis preparations and seem to be at a higher risk of suffering from adverse consequences of cannabinoid exposure than adults. Hence, there is an urgent need for long-term follow-up studies and further animal research to shed light on the neurobiological mechanisms, specific consequences and possible additional risk factors for deleterious cannabinoid effects during puberty.

References

Ahmed, E.I., Zehr, J.L., Schulz, K.M., Lorenz, B.H., DonCarlos, L.L. and Sisk, C.L. (2008) Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions. *Nat Neurosci,* **11**:995–7.

Andersen, S.L., Thompson, A.T., Rutstein, M., Hostetter, J.C. and Teicher, M.H. (2000) Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse,* **37**:167–9.

Arseneault, L., Cannon, M., Witton, J. and Murray, R.M. (2004) Causal association between cannabis and psychosis: examination of the evidence. *Br J Psychiatry,* **184**:110–7.

Belue, R.C., Howlett, A.C., Westlake, T.M. and Hutchings, D.E. (1995) The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. *Neurotoxicol Teratol,* **17**:25–30.

Berrendero, F., Sepe, N., Ramos, J.A., Di Marzo, V. and Fernandez-Ruiz, J.J. (1999) Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestation and early postnatal period. *Synapse,* **33**:181–91.

Biscaia, M., Fernandez, B., Higuera-Matas, A., *et al*. (2008) Sex-dependent effects of periadolescent exposure to the cannabinoid agonist CP-55,940 on morphine self-administration behavior and the endogenous opioid system. *Neuropharmacology,* **54**:863–73.

Biscaia, M., Marin, S., Fernandez, B., *et al*. (2003) Chronic treatment with CP 55,940 during the periadolescent period differentially affects the behavioral responses of male and female rats in adulthood. *Psychopharmacology,* **170**:301–8.

Caspi, A., Moffitt, T.E., Cannon, M., *et al*. (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by functional polymorphism in the catechol-O-methyltransferase gen: longitudinal evidence of a gene x environment interaction. *Biol Psychiatry,* **57**:1117–27.

Cha, Y.M., White, A.M., Kuhn, C.M., Wilson, W.A. and Swartzwelder, H.S. (2006) Differential effects of delta9-THC on learning in adolescent and adult rats. *Pharmacol Biochem Behav,* **83**:448–55.

Chambers, R.A., Taylor, J.R. and Potenza, M.N. (2003) Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. *Am J Psychiatry,* **160**:1041–52.

De Bellis, M.D., Keshavan, M.S., Beers, S.R., *et al*. (2001) Sex differences in brain maturation during childhood and adolescence. *Cereb Cortex,* **11**:552–7.

Degenhardt, L., Hall, W. and Lynskey, M. (2003) Exploring the association between cannabis use and depression. *Addiction,* **98**:1493–504.

Ellgren, M., Hurd, Y.L. and Franck, J. (2004) Amphetamine effects on dopamine levels and behavior following cannabinoid exposure during adolescence. *Eur J Pharmacol,* **497**:205–13.

Ellgren, M., Spano, S.M. and Hurd, Y.L. (2007) Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. *Neuropsychopharmacology,* **32**:607–15.

Elphick, M.R. and Egertova, M. (2001) The neurobiology and evolution of cannabinoid signalling. *Philos Trans R Soc Lond B Biol Sci*, **356**:381–408.

EMCDDA (2006) Report on european drug use. Internet Communication: http://ar2006.emcdda.europa.eu/en/home-en.html?CFID=6556355andCFTOKEN=775dc8d09f40f3c7-C10A6570–94C2–5A43-C5A9EED9672B4893andjsessionid=2e30aa2470a451721240.

Fergusson, D.M., Boden, J.M. and Horwood, L.J. (2006) Cannabis use and other illicit drug use: testing the cannabis gateway hypothesis. *Addiction,* **101**:556–69.

Fergusson, D.M. and Horwood, L.J. (2000) Does cannabis use encourage other forms of illicit drug use? *Addiction,* **95**:505–20.

Fride, E. and Mechoulam, R. (1996) Ontogenetic development of the response to anandamide and delta 9-tetrahydrocannabinol in mice. *Brain Res Dev Brain Res,* **95**:131–4.

Galve-Roperh, I., Palazuelos, J., Aguado, T. and Guzman, M. (2009) The endocannabinoid system and the regulation of neural development: potential implications in psychiatric disorders. *Eur Arch Psychiatry Clin Neurosci,* **259**:371–82.

Gammon, C.M., Freeman, G.M., Jr., Xie, W., Petersen, S.L. and Wetsel, W.C. (2005) Regulation of gonadotropin-releasing hormone secretion by cannabinoids. *Endocrinology* **146**:4491–9.

Hall, W. (2006) The mental health risks of adolescent cannabis use. *PLoS Me.* **3**:e39.

Higuera-Matas, A., Soto-Montenegro, M.L., del Olmo, N., *et al*. (2008) Augmented acquisition of cocaine self-administration and altered brain glucose metabolism in adult female but not male rats exposed to a cannabinoid agonist during adolescence. *Neuropsychopharmacology,* **33**:806–13.

Kandel, D.B., Yamaguchi, K. and Klein, L.C. (2006) Testing the Gateway Hypothesis. *Addiction,* **101**:470–2.

Koch, M. (2002) Animal models in schizophrenia research. *Neuroforum,* **8**:236–44.

Lenroot, R.K. and Giedd, J.N. (2006) Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. *Neurosci Biobehav Rev,* **30**:718–29.

Lynskey, M.T., Vink, J.M. and Boomsma, D.I. (2006) Early onset cannabis use and progression to other drug use in a sample of Dutch twins. *Behav Genet,* **36**:195–200.

MacCoun, R.J. (2006) Competing accounts of the gateway effect: the field thins, but still no clear winner. *Addiction,* **101**:473–4.

McLaughlin, C.R., Martin, B.R., Compton, D.R. and Abood, M.E. (1994) Cannabinoid receptors in developing rats: detection of mRNA and receptor binding. *Drug Alcohol Depend,* **36**:27–31.

Monshouwer, K., Smit, F., de Graaf, R., van Os, J. and Vollebergh, W. (2005) First cannabis use: does onset shift to younger ages? Findings from 1988 to 2003 from the Dutch National School Survey on Substance Use. *Addiction,* **100**:963–70.

Nunez, J.L., Sodhi, J. and Juraska, J.M. (2002) Ovarian hormones after postnatal day 20 reduce neuron number in the rat primary visual cortex. *J Neurobiol,* **52**:312–21.

O'Shea, M., McGregor, I.S. and Mallet, P.E. (2006) Repeated cannabinoid exposure during perinatal, adolescent or early adult ages produces similar longlasting deficits in object recognition and reduced social interaction in rats. *J Psychopharmacol,* **20**:611–21.

O'Shea, M., Singh, M.E., McGregor, I.S. and Mallet, P.E. (2004) Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. *J Psychopharmacol*, **18**:502–8.

Patton, G.C., Coffey, C., Carlin, J.B., Degenhardt, L., Lynskey, M. and Hall, W. (2002) Cannabis use and mental health in young people: cohort study. *BMJ,* **325**:1195–8.

Paus, T., Zijdenbos, A., Worsley, K., *et al*. (1999) Structural maturation of neural pathways in children and adolescents: in vivo study. *Science,* **283**:1908–11.

Perrin, J.S., Herve, P.Y., Leonard, G., *et al.* (2008) Growth of white matter in the adolescent brain: role of testosterone and androgen receptor. *J Neurosci*, **28**:9519–24.

Pistis, M., Perra, S., Pillolla, G., Melis, M., Muntoni, A.L. and Gessa, G.L. (2004) Adolescent exposure to cannabinoids induces long-lasting changes in the response to drugs of abuse of rat midbrain dopamine neurons. *Biol Psychiatry,* **56**:86–94.

Poulin, C., Hand, D., Boudreau, B. and Santor, D. (2005) Gender differences in the association between substance use and elevated depressive symptoms in a general adolescent population. *Addiction,* **100**:525–35.

Powell, K. (2006) Neurodevelopment: how does the teenage brain work? *Nature,* **442**:865–7.

Quinn, H.R., Matsumoto, I., Callaghan, P.D., *et al.* (2008) Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology,* **33**:1113–26.

Rey, J.M., Sawyer, M.G., Raphael, B., Patton, G.C. and Lynskey, M. (2002) Mental health of teenagers who use cannabis. Results of an Australian survey. *Br J Psychiatry,* **180**:216–21.

Rodriguez de Fonseca, F., Ramos, J.A., Bonnin, A. and Fernandez-Ruiz, J.J. (1993) Presence of cannabinoid binding sites in the brain from early postnatal ages. *Neuroreport,* **4**:135–8.

Romero, E.M., Fernandez, B., Sagredo, O., *et al.* (2002) Antinociceptive, behavioral and neuroendocrine effects of CP 55,940 in young rats. *Brain Res Dev Brain Res,* **136**:85–92.

Rubino, T., Realini, N., Braida, D., *et al.* (2009a) The depressive phenotype induced in adult female rats by adolescent exposure to THC is associated with cognitive impairment and altered neuroplasticity in the prefrontal cortex. *Neurotox Res,* **15**:291–302.

Rubino, T., Realini, N., Braida, D., *et al.* (2009b) Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus,* **19**:763–72.

Rubino, T., Vigano', D., Realini, N., *et al.* (2008) Chronic delta(9)-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. *Neuropsychopharmacology,* **33**:2760–71.

Schneider, M. (2008) Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addiction Biol,* **13**:253–63.

Schneider, M., Drews, E. and Koch, M. (2005) Behavioral effects in adult rats of chronic prepubertal treatment with the cannabinoid receptor agonist WIN 55,212–2. *Behav Pharmacol,* **16**:447–54.

Schneider, M. and Koch, M. (2002) The cannabinoid agonist WIN 55,212–2 reduces sensorimotor gating and recognition memory in rats. *Behav Pharmacol,* **13**:29–37.

Schneider, M. and Koch, M. (2003) Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology,* **28**:1760–9.

Schneider, M. and Koch, M. (2005) Deficient social and play behavior in juvenile and adult rats after neonatal cortical lesion: effects of chronic pubertal cannabinoid treatment. *Neuropsychopharmacology,* **30**:944–57.

Schneider, M. and Koch, M. (2007) The effect of chronic peripubertal cannabinoid treatment on deficient object recognition memory in rats after neonatal mPFC lesion. *Eur Neuropsychopharmacol*, **17**:180–6.

Schneider, M., Schomig, E. and Leweke, F.M. (2008) Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addict Biol,* **13**:345–57.

Sisk, C.L. and Foster, D.L. (2004) The neural basis of puberty and adolescence. *Nat Neurosci*, **7**:1040–7.

Spear, L.P. (2000) The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev,* **24**:417–63.

Stiglick, A. and Kalant, H. (1982a) Learning impairment in the radial-arm maze following prolonged cannabis treatment in rats. *Psychopharmacology,* **77**:117–23.

Stiglick, A. and Kalant, H. (1982b) Residual effects of prolonged cannabis administration on exploration and DRL performance in rats. *Psychopharmacology,* **77**:124–8.

Stiglick, A. and Kalant, H. (1985) Residual effects of chronic cannabis treatment on behavior in mature rats. *Psychopharmacology,* **85**:436–9

Wegener, N. and Koch, M. (2009) Behavioral disturbances and altered Fos protein expression in adult rats after chronic pubertal cannabinoid treatment. *Brain Res*, **1253**:81–91.

Wenger, T., Croix, D. and Tramu, G. (1988) The effect of chronic prepubertal administration of marihuana (delta-9-tetrahydrocannabinol) on the onset of puberty and the postpubertal reproductive functions in female rats. *Biol Reprod,* **39**:540–5.

Wenger, T., Gerendai, I., Fezza, F., *et al.* (2002) The hypothalamic levels of the endocannabinoid, anandamide, peak immediately before the onset of puberty in female rats. *Life Sci,* **70**:1407–14.

Zehr, J.L., Todd, B.J., Schulz, K.M., McCarthy, M.M. and Sisk, C.L. (2006) Dendritic pruning of the medial amygdala during pubertal development of the male Syrian hamster. *J Neurobiol,* **66**:578–90.

Chapter 8

Cannabis and cognition: short- and long-term effects

Nadia Solowij and Nicole Pesa

Twenty years ago cannabis was generally perceived to be a benign drug with few significant adverse effects. As outlined elsewhere in this book, evidence has since mounted in the scientific literature for a range of harms associated with the use of cannabis, including the development of dependence and health-related harms (see also Hall and Solowij, 1998; Hall and Degenhardt, 2009). As the overall theme of this book indicates, an association between cannabis use and the development of psychotic symptoms or overt psychosis has grown to be recognized as a significant potential harm, and investigating the mechanisms by which cannabis may trigger psychosis is a priority. This includes understanding the effects of cannabis on brain structure, biology and function. We recently highlighted a similarity between the cognitive impairment that has been reported in cannabis users and the deficits observed in schizophrenia (Solowij and Michie, 2007), suggesting common underlying neuropathology. Few would argue that cognition is not impaired to some degree during acute intoxication with cannabis. That impaired cognition persists beyond the period of acute intoxication is more contentious. Despite objective appraisals of the literature in interpreting the evidence, it is inevitable that researchers will be influenced by the weight of their own data in formulating scientific opinion. Accordingly, and on the basis of the accumulating evidence, this review will come to some rather different conclusions from those made in the first edition of this book (Pope and Yurgelun-Todd, 2004).

The goal of this chapter is to update our knowledge of the short- and long-term effects of cannabis on cognition based on integrating evidence from the most recent literature on this topic. We acknowledge the weight of evidence from our own studies that must inevitably guide us to the conclusions that we draw, while also aiming objectively to assess the evidence from multiple sources. We consider evidence from preclinical research, studies of acute administration of cannabinoids to humans, studies of long-term or heavy cannabis users tested in the unintoxicated state, including adults and adolescents and patients with schizophrenia, and we evaluate the evidence for recovery of function after prolonged abstinence.

Animal studies

A wealth of preclinical research shows an unequivocal role for the endogenous cannabinoid system in attention, memory, inhibitory control and multiple other cognitive processes, and that these are impaired following both acute and chronic cannabinoid administration (Egerton *et al*., 2006; Solowij and Michie, 2007; Pattij *et al*., 2008; Solowij and Battisti, 2008). Even a single administration of an ultra-low dose of Δ^9-tetrahydrocannabinol (THC) (0.001–0.002 mg/kg) has been shown to result in long-term cognitive impairments in mice (3 weeks to 4 months post-injection) (Tselnicker *et al*., 2007; Amal *et al*., 2010).

Recent animal research supports the notion that the developing brain is more susceptible to the acute and chronic effects of exogenous cannabinoids, particularly the hippocampus. As outlined in Chapter 7, evidence is building from studies in which animals have been exposed to cannabinoids prenatally or during the pubertal/adolescent period, with greater immediate adverse effects on cognition and behavior observed in comparison to animals exposed during adulthood, as well as such effects persisting into adulthood with no further cannabinoid exposure (Kang-Park *et al*., 2007; Schneider, 2008; Realini *et al*., 2009; Rubino *et al*., 2009).

A recent study reported that the endocannabinoid system is significantly altered by exposure to THC during early, middle and late adolescence in rats (Ellgren *et al*., 2008). The normal proportional ratio of

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anandamide and 2-arachidonoyl glycerol (2-AG) in the prefrontal cortex (PFC) and the nucleus accumbens was reversed by exposure to THC, and anandamide levels were increased in the nucleus accumbens. These dynamic changes in the mesocorticolimbic endocannibinoid (eCB) system (ECS) were induced by intermittent exposure to THC, which emulates the pattern of use among teenagers.

Short-term effects in humans

Numerous studies have examined the acute effects of cannabis on human cognition. That cannabis induces perceptual distortions and impairs memory and concentration during acute intoxication is generally well accepted. However, a recent systematic review of the literature to 2007 identified considerable inconsistency across findings. Zuurman *et al.* (2009) examined the effects of acute administration of cannabis or THC to healthy volunteers from 165 studies utilizing 318 measures with the goal of identifying specific biomarkers of cannabis intoxication and central nervous system effects, and considering dose of THC administered. While functional impairment was observed across an extensive range of measures, few met the criteria for biomarkers in terms of consistency of effect. This may have been somewhat obscured by variability across multiple factors in the studies reviewed, including the nature of the subjects (varying in degree of experience with cannabis and hence tolerance) and the wide range of test measures. The most reliable biomarkers were increased heart rate and subjective effects. Dose-related decrements were observed in some domains (e.g. auditory/verbal delayed recall and recognition), less clear effects in others (e.g. immediate recall) and reverse effects (decreased decrements with higher doses) in yet others (e.g. working memory, digit-symbol substitution, focused selective attention, visuomotor control). Inhibition, reasoning/association and reaction time, while impaired, showed no consistent dose–response effect. Biphasic effects of lower versus higher doses were also observed and the authors highlighted that the pattern of effects supported a relaxing, sedating and reduced attention effect of THC at lower doses, and greater stimulatory and aggressive effects at higher doses. They further commented on the potential for additional reliable biomarkers within the domains of memory and motor functions if the wide range of tests and measures were standardized.

Despite a degree of inconsistency and complexity associated with biphasic dose effects and tolerance, cannabis has been shown in many studies to acutely impair attention, learning, short-term memory, working memory, executive function, abstract ability and decision making (Hall and Solowij, 1998; Solowij, 1998; Iversen, 2003; Fletcher and Honey, 2006; Ranganathan and D'Souza, 2006; Solowij and Michie, 2007; Hall and Degenhardt, 2009; Zuurman *et al.*, 2009; Sewell *et al.*, 2010; Solowij and Pesa, 2010). A revival of interest in examining the acute effects of cannabinoids on cognition in humans has been evident in recent years, with greater application of prospective, double-blind, placebo-controlled, cross-over designs, and with particular interest in understanding the psychotomimetic effects of cannabis. Here we summarize key findings, focusing predominantly on these most recent studies.

There have been growing concerns regarding the increasing potency of cannabis preparations (see Chapter 4). Many studies of acute administration have demonstrated dose–response effects whereby the greater the dose of THC, the greater the impairment. One recent study examined a range of doses of THC relevant to designer-grade cannabis in common use in Europe and the UK (eg. sinsemilla, nederweed), administered to regular but not daily users in the form of joints mixed with tobacco. There were linear decrements with increasing dose in reaction time and errors in attention, and short-term memory tasks and impaired motor control (Hunault *et al.*, 2009).

A range of attentional processes is impaired by cannabis acutely. Impaired performance on sustained attention (eg. on continuous performance tasks), selective, focused and divided attention tasks, as well as in preattentive sensory memory have been demonstrated after acute administration of cannabis or THC to humans (Ilan *et al.*, 2004; O'Leary *et al.*, 2007; Hunault *et al.*, 2009; Ramaekers *et al.*, 2009). Accuracy, increased error rates and slowed reaction times were shown in some studies to be dose-related. Ramaekers and colleagues (2009) found impaired performance on a divided attention task following high-dose (500 μg/kg) THC only in occasional, but not heavy users, suggesting tolerance. In contrast, both occasional and heavy users exhibited inhibitory control deficits in a "Stop Signal" task. Altered inhibitory processing is evident following acute intoxication, in particular through impulsive responding (Hart *et al.*, 2001; McDonald *et al.*, 2003). Imaging studies have found that THC-attenuated activation in

the right inferior frontal and anterior cingulate cortex (ACC) (Borgwardt *et al*., 2008) and opposing effects of THC and cannabidiol (CBD) in the hippocampus were found during a "Go/NoGo" task (Bhattacharyya *et al*., 2009). A study of decision making, as assessed by the Iowa Gambling Task, found no disruption to risky behavior, only a slowing of performance in daily cannabis users during acute intoxication (Vadhan *et al*., 2007), while another found increased risky decision making and altered sensitivity to consequences after a higher dose of THC was given to occasional users (Lane *et al*., 2005b).

D'Souza and colleagues (2004) conducted a rigorous investigation of the effects of intravenous THC administered to healthy volunteers who had experience with cannabis use, but who were not heavy users. Δ^9-Tetrahydrocannabinol induced transient positive and negative schizophrenia-like symptoms and impaired working memory, verbal memory, distractibility and verbal fluency. Similarly, Morrison *et al*. (2009) reported induction of positive psychotic symptoms and deficits in verbal episodic memory and executive function following administration of intravenous THC. Deficits in verbal learning and memory are perhaps the most robust impairments associated with acute cannabis use (Curran *et al*., 2002; D'Souza *et al*., 2004; Ilan *et al*., 2004; Morrison *et al*., 2009), with evidence of impaired immediate and delayed free recall of information, and difficulties in manipulating the contents of working memory, along with failure to use semantic processing and organization to optimize episodic memory encoding and impaired retrieval performance (Fletcher and Honey, 2006; Ranganathan and D'Souza, 2006).

Bhattacharyya and colleagues have reported a series of neuroimaging studies of the effects of orally administered THC or CBD (Bhattacharyya *et al*., 2009a; 2009b). They found that the effects of cannabis on verbal learning were mediated through its influence on left temporal activity (particularly parahippocampal), with modulation also of medial PFC and ACC activity during encoding or retrieval of information. Δ^9-THC and CBD showed opposing effects in the striatum during verbal recall. These studies also elucidated the neural basis of the anxiogenic or anxiolytic effects of THC and CBD, respectively, as pertinent to understanding the propensity for cannabis to induce psychotic symptoms. Other recent neuroimaging studies of acute administration of cannabinoids have been reviewed by Martin-Santos *et al.* (2010) (see also Chapter 14).

Working memory is disrupted by acute cannabis use, with impaired performance, electroencephalogram (EEG) and event-related potential (ERP) measures (Ilan *et al*., 2004; D'Souza *et al*., 2004; Lane *et al*., 2005a). Regular but infrequent cannabis users showed dose-dependently impaired performance (greater errors) on a Sternberg memory task following acute administration of THC (O'Leary *et al*., 2007), and these have been associated with reduced frontal-midline EEG theta power (Bocker *et al*., 2007). Acute effects of cannabinoids on electrophysiology have also been demonstrated in infrequent cannabis users for the mismatch negativity (MMN) component of the ERP (MMN being an index of preattentive sensory memory) (Juckel *et al*., 2007) and the P300 component (an index of the allocation of attentional resources and updating of memory traces) (Roser *et al*., 2008).

Thus, further evidence has accumulated for a disruption of attention, memory and inhibitory control following acute administration of cannabis to humans, with some elucidation of the neural substrates of these effects, including evidence of differential effects of different cannabinoids (such as THC and CBD). It appears also that the response to acute cannabinoid administration is mediated by cannabis-use history and the development of tolerance to the acute effects in some cognitive tasks. However, more research is required to determine systematically the parameters of cannabis use that lead to the development of tolerance, the doses that may or may not elicit impaired performance in regular users and the cognitive tasks that are amenable to tolerance. For example, Boucher and colleagues (2009) showed that impairments in spatial working memory in rats are resistant to tolerance after extended administration of THC. We also do not know whether, or how, regular users may develop compensatory strategies during acute intoxication to facilitate performance that might otherwise be impaired. For example, in a risky decision-making task, Rogers *et al*. (2007) showed a reduction of risky behavior following low-dose sublingual administration of THC to healthy young adults (not regular cannabis users), with an adoption of more cautious cognitive strategies to compensate for the perceived disruption of effective decision making by cannabis. Thus regular users, due to their greater experience with cannabis, might be more likely to develop alternate compensatory strategies.

Long-term effects

Studies of long-term and heavy cannabis users have continued to investigate residual or persistent effects of cannabis on cognitive function. Most studies have assessed cannabis users within 12–48 hours of last use of cannabis and cognitive impairment during this phase informs the functioning of regular users in the course of their daily lives when not acutely intoxicated. An increasing number of studies are applying longer periods of abstention, from 1 week through to 1 month or more. Some years ago, we postulated that the consequences of cannabis use may differ across the lifespan, with greater psychosocial, educational, maturational and mental health issues for adolescents and young adults, and cognitive deficits manifesting only after years of heavy cannabis use (Solowij and Grenyer, 2002). However, much evidence has now emerged for cognitive deficits to exist in younger cannabis users and interest has focused on the impact of cannabis use on the adolescent brain (see Chapter 7). Accordingly, we have structured this section to consider studies of adult cannabis users separately to those of cannabis-using adolescents and young adults who commenced cannabis use during early adolescence. We also consider briefly the growing literature on cognitive functioning in patients with schizophrenia who also use cannabis.

Adult Studies

Attention

Sustained attention, most often measured by continuous performance tasks (CPTs), is inconsistently impaired in chronic cannabis users (Pope *et al*., 2001; Indlekofer *et al*., 2009). However, even in the absence of overt performance deficits, lower glucose metabolism in orbitofrontal, temporal, hippocampal and parahippocampal regions has been observed during CPT performance in regular cannabis users (Voytek *et al*., 2005). Tonic alertness was impaired in moderate users (Indlekofer *et al*., 2009). A study of preattentive prepulse inhibition (PPI) attributed poor performance by chronic cannabis users to deficits in sustained attention, which were associated with greater frequency cannabis use (Scholes *et al*., 2009).

Selective and divided attention deficits in chronic cannabis users have been shown to be related to frequency and duration of long-term use, with only partial recovery after a mean of two years of abstinence (Solowij, 1998). But even relatively light use (once a week) was related to some attentional dysfunction in young adults (Skosnik *et al*., 2001). The evidence suggests differential deficits associated with frequency versus duration of cannabis use, reflecting shorter- versus longer-lasting effects.

Inhibition

Impaired inhibitory processing, assessed through behavioral tasks such as the "Stroop, Go/NoGo" and a variety of decision-making and gambling tasks, is also impaired in long-term cannabis users (Bolla *et al*., 2002; Solowij *et al*., 2002; Solowij and Michie, 2007; Hester *et al*., 2009). Such tasks require the selection of an appropriate response while simultaneously inhibiting the inappropriate response. It has been suggested that the eCB system may modulate dopaminergic PFC and accumbal activity and contribute to inappropriate incentive salience to irrelevant stimuli; this may underlie attentional and inhibitory processing and decision-making deficits (Melis *et al*., 2004; Solowij and Michie, 2007; Pattij *et al*., 2008). Imaging studies show altered dorsolateral prefrontal cortical (DLPFC) and ACC activation during the interference condition of the Stroop task, despite reasonable task performance, in current cannabis users (Gruber and Yurgelun-Todd, 2005) and 1-month abstinent users (Eldreth *et al*., 2004). Performance on the Stroop task is inconsistently impaired in chronic cannabis users, but poorer performance has been associated with duration and dose, possibly interacting with low IQ and with altered electrophysiology (Bolla *et al*., 2002; Solowij *et al*., 2002; Battisti *et al*., 2010a). In chronic adult users with adequate inhibitory control performance, commission errors increased and a diminished capacity for behavior monitoring and error-awareness was associated with hypoactivity in the ACC and right insula (Hester *et al*., 2009).

Working memory and other executive functions

Working memory is the temporary encoding and manipulation of information that is a core component of executive functions of cognition. The involvement of the endogenous cannabinoid system in working memory has been well documented (Solowij and Michie, 2007; Pattij *et al*., 2008). A range of executive function tasks have been found to be impaired in both acute and chronic cannabis use (e.g. verbal fluency, Wisconsin Card Sorting Task, Ravens Progressive Matrices, Tower of London) (see Solowij and Michie,

2007) but few studies have addressed working memory directly in cannabis users and this is an area that is receiving increasing interest. We have shown that chronic cannabis users are impaired on several measures from the Cambridge Neuropsychological Test Automated Battery (CANTAB), including Rapid Visual Information Processing, Pattern Recognition Memory, Spatial Recognition Memory, Spatial Span, Spatial Working Memory and Visuospatial Paired Associate Learning (Solowij *et al.*, 2008). Abstinent cannabis users showed no performance deficits, but did demonstrate altered parietal brain activation in a Sternberg working memory task (Jager *et al.*, 2006). Further neuroimaging studies indicate that cannabis users recruit additional brain regions in a compensatory manner in order to achieve adequate performance on working memory tasks (Kanayama *et al.*, 2004; Martin-Santos *et al.*, 2010).

In a recent study of verbal fluency, visual memory and short- and long-interval prospective memory thought to rely on executive functions, McHale and colleagues (2008) found that young adult cannabis users with recent use (past week) showed impaired memory function and generated fewer words than those abstinent longer than a week; both groups generated fewer words than non-user controls. The authors showed that these deficits were specific to cannabis use despite the fact that cannabis is often mixed with tobacco, as the deficits were not apparent in a tobacco-user control group. They suggested some recovery of cognitive ability with abstinence, but this may have been confounded by frequency of use as the "abstinent" group comprised twice-weekly users, whereas the "recent-use" group smoked five to six times per week.

Verbal memory and other memory processes

Verbal memory is consistently impaired in chronic cannabis users, with impaired performance on word list learning tasks (e.g. Rey Auditory Verbal Learning Task [RAVLT], the California Verbal Learning Task [CVLT] and Buschke's Selective Reminding Task). These studies have been extensively reviewed elsewhere, together with some early neuroimaging studies of verbal memory in cannabis users (Solowij and Michie, 2007; Solowij and Battisti, 2008). Overall, the evidence suggests that long-term or heavy cannabis users show impaired encoding, storage, manipulation and retrieval mechanisms. Users learn fewer words across trials and recall fewer words, particularly after interference or delay. Several studies have shown that these deficits are variously attributed to duration of cannabis use (Solowij *et al.*, 2002; Messinis *et al.*, 2006), frequency of use (Pope *et al.*, 2001) or cumulative dosage effects (Bolla *et al.*, 2002).

Recent neuroimaging studies have sought to elucidate the acute effects of THC and other cannabinoids (e.g. CBD) on neural substrates subserving verbal memory, as discussed above (Bhattacharyya *et al.*, 2009; Martin-Santos *et al.*, 2010), or attempted to relate brain structural changes in cannabis users to verbal memory deficits. For example, Yücel *et al.* (2008) found significantly reduced hippocampal volumes in long-term heavy cannabis users, who were also significantly impaired on the RAVLT, but memory performance was unrelated to hippocampal volumes. Such complex verbal learning tasks likely involve functional connectivity across a wide range of brain regions, and impaired performance is likely to be associated more with the functional activation of those regions, rather than their structure. A recent electrophysiological study in chronic users found poor word recall and alteration of the ERP-subsequent memory effect during encoding, a component thought to originate in the hippocampal region; this alteration was associated with a longer duration and an earlier onset of cannabis use (Battisti *et al.*, 2010b).

More specific hippocampal-dependent tasks, such as pictorial-associative memory tasks, have also been investigated in 1-week abstinent cannabis users (Jager *et al.*, 2007; Luijten *et al.*, 2007). Task performance did not differ between moderately using young adults and non-user controls, but recall accuracy decreased as a function of exposure to cannabis and decreased activation was observed in users in bilateral parahippocampal regions and in the right DLPFC during learning (Jager *et al.*, 2007). A study of hippocampal-dependent face-name learning in young adult frequent users found impaired learning, short- and long-term memory and hypoactivation of frontal and temporal regions, with concomitant hyperactivation of parahippocampal regions during learning, reflective of both functional deficits and compensatory processes (Nestor *et al.*, 2008). Similarly, Becker *et al.* (2010) found greater activation of the left parahippocampal gyrus during encoding in a face-profession associative-learning task in high- compared with low-frequency users; however, there were no apparent effects associated with duration of use, or age of onset of use. This too was interpreted as functional compensation to maintain performance.

Other cognitive functions

Indlekofer *et al.* (2009), in a population-based study of moderate cannabis users, found deficits in prose recall (logical memory test) in association with lifetime cannabis use, and significantly increased self-reported cognitive failures (of memory, attention, perception and motor function) with more extensive cannabis use. Time estimation has been found to be altered during both acute intoxication and in some studies of chronic users (Solowij and Michie, 2007; Pattij *et al.*, 2008). Typically, time is underestimated – the subjective experience is of time passing more slowly. Time estimation is thought to involve the cerebellum and chronic cannabis users have been shown to be impaired in a classical delayed eye-blink conditioning task that reflects cerebellar functional integrity (cerebellar-dependent associative learning) (Skosnik *et al.*, 2008). Recent data suggest cerebellar structural alterations in chronic cannabis users (Solowij *et al.*, 2011b).

Since cannabis alters mood during acute intoxication, interest has grown in exploring emotion and affect processing in chronic users. Gruber and colleagues (2009) examined regional brain activation to masked affective stimuli in heavy cannabis users and found altered frontal and limbic activity, with decreased activation of ACC and amygdala regions compared with controls, as well as differential effects for masked happy versus angry faces. Three studies of acute cannabis administration also found modulation of amygdala activity during processing of fearful faces, with opposing effects of THC and CBD (Phan *et al.*, 2008; Bhattacharyya *et al.*, 2009b; Fusar-Poli *et al.*, 2009). We reported significantly reduced amygdala volumes in long-term heavy cannabis users (Yücel *et al.*, 2008) but it is not yet known whether this is associated with emotional or affect processing deficits. Other recent neuroimaging research has examined reward processing mechanisms in chronic users, showing increased cerebellar and ventrostriatal activation during reward anticipation; the latter was correlated with the duration of cannabis use and lifetime dose of exposure (Nestor *et al.*, 2010).

Adolescent and early-onset young-adult studies

Adolescence is the prime period for initiation of cannabis use and a significant proportion of adolescents use cannabis regularly. Adolescence is also a period of significant neural development, with resculpting of the brain in terms of pruning, apoptosis and myelination (Schneider, 2008). This suggests that substance use during this crucial neurodevelopmental period may impact brain maturation and plasticity, and an increasing body of evidence from animal research indicates that the adolescent brain is more vulnerable to some of the adverse effects of cannabis (Schneider, 2008; see also Chapter 7).

Two recent reviews have examined the literature on cognitive functioning specifically in adolescent cannabis users (Schweinsburg *et al.*, 2008a; Jacobus *et al.*, 2009). The primary findings from these reviews indicated evidence for impaired attention, processing speed, learning and memory, functional and subtle structural brain alterations and sleep disturbances in adolescents who use cannabis heavily. Further, it was suggested that cognitive deficits may persist for longer in adolescent users (6 weeks to 3 months) than has been shown in adult users, and particularly so in the domains of learning, memory and working memory. Here we highlight some of the recent findings in adolescent users.

Attention

Jacobsen and colleagues (2004) found that adolescent cannabis users made significantly more errors on a CPT task than non-using controls. Increased errors trended toward an association with greater exposure to cannabis. Early onset of cannabis use (i.e. before age 15 or 16 years) was found to be a strong predictor of attentional deficits during adulthood (Ehrenreich *et al.*, 1999; Novaes *et al.*, 2008). P300 amplitude, thought to reflect the allocation of attentional resources, has been found to be reduced in early-onset users (Kempel *et al.*, 2003).

Inhibition

Poor performance on the Stroop task has been associated with early-onset cannabis use (Novaes *et al.*, 2008; Battisti *et al.*, 2010a). In a "Go/NoGo task," adolescent cannabis users' performance was adequate following 1 month abstinence, but altered activation was observed in frontal and parietal brain regions, with users requiring increased neural effort during the inhibition condition to maintain performance levels (Tapert *et al.*, 2007). We found that adolescent cannabis use, but not alcohol use, was associated with increased risky and impulsive decision making, with users adopting strategies with higher levels of uncertainty and not utilising information effectively; also performance

was related to an earlier onset of regular cannabis use (Solowij *et al*., in press).

Working memory and executive functions

Executive functions have been shown to be impaired in early-onset cannabis users (Pope *et al*., 2003), and adolescent users show a range of attention, working memory and executive function deficits on the CANTAB (Harvey *et al*., 2007). Performance on an n-back auditory working-memory task was shown to be impaired, as memory load increased in abstinent adolescent cannabis users, with some evidence of altered regional brain activation emerging during nicotine withdrawal (Jacobsen *et al*., 2007). Abstinent adolescent male cannabis users showed overactivity in prefrontal regions but no performance deficits in a Sternberg working memory task, and no alterations in an associative memory task (Jager *et al*., 2010). The authors suggested that their results supported the vulnerability of the developing frontal lobes to early-onset cannabis use. Two neuroimaging studies reported functional brain activation abnormalities in 28-day abstinent adolescents in a spatial working memory task and provide further evidence in these young users of the application of alternate strategies, and recruitment of additional brain regions in a compensatory manner in order to achieve adequate performance (Padula *et al*., 2007; Schweinsburg *et al*., 2008b).

Verbal memory and other memory processes

Verbal memory was found to be impaired in adolescent cannabis users (Harvey *et al*., 2007) and minimum 23-day abstinent adolescents, and associated with lifetime episodes of use (Medina *et al*., 2007). We have recently reported impaired verbal learning and memory in adolescent cannabis users compared with matched adolescent alcohol users and non-user controls and this was shown to increase with duration, quantity, frequency and age of onset of cannabis use and was unrelated to alcohol use (Solowij *et al*., 2011a). Importantly, an effect of earlier age of onset of cannabis use was retained after controlling for the extent of exposure to cannabis. This young sample had only moderate exposure to cannabis over 2–3 years, yet showed impairment relative to their age-matched counterparts similar to that seen in adults with greater than 20 years of heavy use; we previously reported no such impairment in heavy adult users with 10 years use (Solowij *et al*., 2002). These robust findings indicate that cannabis adversely affects the developing brain and reinforce concerns regarding the impact of early exposure and the greater vulnerability of the adolescent brain.

Prospective memory has also been demonstrated to be impaired in adolescent and young adult users (Bartholomew *et al*., 2008; McHale *et al*., 2008). Altered electrophysiology during encoding of words was associated with an earlier onset of use in an adult sample (Battisti *et al*., 2010b). In contrast to decreased parahippocampal and DLPFC activation during learning in adult cannabis users, a study of adolescents found increased activation in the fusiform/parahippocampal area, inferior frontal gyrus, DLPFC, superior parietal cortex and the ACC (Luijten *et al*., 2007) suggestive of increased neural effort.

Recovery of function with abstinence

While an increasing number of studies have now assessed adult and adolescent cannabis users following abstinence of several weeks, very few have been specifically designed to determine whether functioning recovers. The study frequently cited as being definitive was that by Pope and colleagues (2001) that showed impaired memory function at baseline and after 7 days abstinence, but an apparent full recovery after 28 days abstinence (Pope *et al*., 2001). Delayed recall was still impaired relative to controls in analyses that did not adjust for verbal IQ differences, and in a reanalysis of their data, these authors found that those participants with an earlier onset of cannabis use were less likely to show full recovery (Pope *et al*., 2002). The participants in Pope *et al*.'s (2001) study remained in the general community for the course of the study, with abstinence monitored by the provision of urine samples. In another study using a similar verbal learning and memory test, participants were not assessed at baseline, but were admitted to an inpatient unit for supervised abstinence of 28 days before neuropsychological assessment (Bolla *et al*., 2002). This study found that memory deficits persisted and were dose-related, and similar decrements were also observed on tests of executive function, psychomotor speed and manual dexterity. Our own data from cannabis users engaged in a 4-month treatment program not aimed at abstinence (Solowij *et al*., 2002) suggest partial recovery with cessation or reduction of use (unpublished data). Adolescent cannabis users were also shown to be impaired in memory, attention, psychomotor speed and planning ability after 23 or more days of urine monitored abstinence; poor performance

was shown to be a function of lifetime episodes of cannabis use after controlling for lifetime alcohol use (Medina *et al*., 2007). These findings suggest that cognitive deficits may indeed persist for a significant period beyond last use of cannabis and it is not known how long it may take before deficits recover and whether this may differ between adult and adolescent users.

A subset of participants from Pope *et al.*'s (2001) study were also found to show diminished activation in motor cortical circuits (Pillay *et al*., 2008) and persistent alterations of cerebral blood flow in the temporal lobe and cerebellum after 28 days abstinence (Sneider *et al*., 2008). Altered flow in frontal regions was apparent after 7 days abstinence but not 28 days, suggesting gradual normalization of neural activity in some regions but not others. Other neuroimaging studies have also reported functional activation differences in cannabis users after 7 days (Jager *et al*., 2006; 2007), 25 days (Eldreth *et al*., 2004; Bolla *et al*., 2005) or > 2 months abstinence (Chang *et al*., 2006). We found impaired electrophysiological measures of selective attention in users who had been abstinent for an average of 2 years (Solowij, 1998). Clearly, further research is required to clarify the extent and time course of recovery of function after cessation of cannabis use.

Patients with schizophrenia

As discussed elsewhere in this book (Chapters 19, 20) cannabis exerts greater adverse effects on cognition when administered to patients with schizophrenia than it does in healthy individuals (D'Souza *et al*., 2008). This section will briefly review the evidence from the growing body of studies that have examined long-term effects on cognition in patients with schizophrenia who also use cannabis.

Since long-term or heavy cannabis use generally impairs cognition in otherwise healthy users, it might be expected that people with schizophrenia who are already cognitively impaired may be even more vulnerable to the adverse effects of cannabis on cognition. Surprisingly, the evidence to date has suggested the reverse (Potvin *et al*., 2008). Løberg and Hugdahl (2009) reviewed 23 recent studies that included a range of samples with psychosis and substance use (primarily cannabis) and found that 14 of these reported better cognition in the cannabis-using patient groups than in their non-using counterparts. More recently, we conducted a meta-analysis of 10 studies of cognition comprising 572 patients with established schizophrenia with and without cannabis use and found that patients with a history of cannabis use had superior neuropsychological functioning (Yücel *et al*., 2010). However, we observed that these findings were driven more so by those studies that included patients with any history of cannabis use, than by studies of patients with current or recent use. We also reported data from a first-episode sample and found that, relative to healthy controls, patients who used cannabis showed only selective neuropsychological impairment, while those without cannabis use had generalized deficits. Previous conjecturing of better premorbid functioning in cannabis-using patients has not been borne out in most studies that have considered this, but current explanations for better functioning in cannabis-using patients suggest that these findings may be driven by a subgroup of neurocognitively less-impaired patients who only developed psychosis after a relatively early initiation into cannabis use. Thus cannabis may cause a transient cognitive breakdown associated with the development of psychosis among less cognitively vulnerable individuals who might, in the absence of cannabis use, never have developed schizophrenia (Løberg and Hugdahl, 2009; Schnell *et al*., 2009; Yücel *et al*., 2010).

Not all studies report better cognition in clinical samples. For example, Ringen and colleagues (2009) found some evidence of better cognition in cannabis-using patients with bipolar disorder, but significantly worse cognition in cannabis-using patients with schizophrenia, with relatively low-level cannabis use. Our own data in a small sample of chronic schizophrenia patients with extensive cannabis use (22 years, near daily, 5 joints/day) found little difference in neuropsychological functioning compared with non-using counterparts, although performance on some measures appeared to worsen with the extent of exposure to cannabis (Grenyer *et al*., 2010). However, the patients with extensive cannabis-use histories showed significant alterations in cerebellar white matter (Solowij *et al*., 2010b) and in hippocampal shape (Solowij *et al*., 2010). Clearly, the impact of cannabis use on brain function and structure in schizophrenia warrants further investigation.

Conclusions

A range of cognitive functions, encompassing attentional, memory, executive and inhibitory processes, are impaired during both the acute intoxication period and following long-term use of cannabis. There has been some elucidation of the neural substrates underlying

these cognitive impairments. Cannabis users, and in particular regular users, may employ compensatory strategies to aid performance or require increased neural effort to maintain performance on certain tasks that may otherwise have been impaired. Cannabis-use history and the development of tolerance may mediate these effects.

Cognitive dysfunction in long-term or heavy cannabis users has been shown to increase as a function of frequency, duration, dose and age of onset of cannabis use. Recent interest has been directed toward cannabis use during adolescence, and evidence from animal and human studies suggests that the adolescent brain is more susceptible to the adverse effects of cannabis. Adolescent cannabis users show similar deficits to those observed in adult users, but greater cognitive impairment is evident the earlier that cannabis use commences. Cognitive dysfunction in long-term users tends to persist for at least one month following the cessation of cannabis use, and may persist for longer in adolescents, but the literature in both populations regarding extent of persistence is not definitive, although it is likely that deficits recover following prolonged abstinence.

Similarities between cognitive deficits in cannabis users and in people with schizophrenia, together with an overlap in brain morphological changes observed in each population (see Chapter 10), suggest that further research into the cognitive effects of cannabis may inform the mechanisms by which cannabis triggers symptoms of psychosis. The endogenous cannabinoid system modulates cognition and is altered in schizophrenia. Individual differences and variability in response to cannabis, during both acute intoxication and in the long-term, dictates a need to understand the mechanisms that constitute increased risk or susceptibility to both the adverse effects of cannabis on cognition and the development of psychosis. Further attention should be given to genetic variation, neurodevelopmental processes, and to the differential opposing or interactive effects of cannabinoids. When humans consume cannabis, they expose themselves not only to THC but also CBD and multiple other compounds that may exacerbate or diminish the effects of THC on the brain (See Chapters 1, 2).

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References

Amal, H., Fridman-Rozevich, L., Senn, R., *et al.* (2010) Long-term consequences of a single treatment of mice with an ultra-low dose of Δ9-tetrahydrocannabinol (THC). *Behav Brain Res*, **206**:245–53.

Bartholomew, J., Holroyd, S. and Heffernan, T.M. (2008) Does cannabis use affect prospective memory in teenagers and young adults? *Eur Psych,* **23**:S305.

Battisti, R.A., Roodenrys, S., Johnstone, S., *et al.* (2010a) Chronic cannabis users show altered neurophysiological functioning on Stroop task conflict resolution. *Psychopharmacology*.

Battisti, R.A., Roodenrys, S., Johnstone, S.J., *et al.* (2010b) Chronic use of cannabis and poor neural efficiency in verbal memory ability. *Psychopharmacology*, **209**:319–40.

Becker, B., Wagner, D., Gouzoulis-Mayfrank, E., *et al.* (2010) Altered parahippocampal functioning in cannabis users is related to the frequency of use. *Psychopharmacology*, **209**:361–74.

Bhattacharyya, S., Fusar-Poli, P., Borgwardt, S., *et al.* (2009a) Modulation of mediotemporal and ventrostriatal function in humans by Δ^9-tetrahydrocannabinol: A neural basis for the effects of cannabis sativa on learning and psychosis. *Arch Gen Psy*, **66**:442–51.

Bhattacharyya, S., Morrison, P., Fusar-Poli, P., *et al.* (2009b) Opposite effects of Δ^9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacol*, **35**:764–74.

Bocker, K.B.E., Gerritsen, J., Kruidenier, M., *et al.* (2007) Smoking high potency marijuana: theta power correlates with memory search. *Eur Neuropsychopharmacology*, **17**, S553.

Bolla, K.I., Brown, K., Eldreth, D., *et al.* (2002) Dose-related neurocognitive effects of marijuana use. *Neurology,* **59**:1337–43.

Bolla, K.I., Eldreth, D.A., Matochik, J.A., *et al.* (2005) Neural substrates of faulty decision-making in abstinent marijuana users. *Neuroimage,* **26**:480–92.

Borgwardt, S.J., Allen, P., Bhattacharyya, S., *et al.* (2008) Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. *Biol Psychiatry,* **64**:966–73.

Boucher, A.A., Vivier, L., Metna-Laurent, M., *et al.* (2009). Chronic treatment with Delta(9)-tetrahydrocannabinol impairs spatial memory and reduces zif268 expression in the mouse forebrain. *Behav Pharmacol*, **20**:45–55.

Chang, L., Yakupov, R., Cloak, C., *et al.* (2006). Marijuana use is associated with a reorganized visual-attention network and cerebellar hypoactivation. *Brain,* **129**:1096–112.

Curran, H.V., Brignell, C., Fletcher, S., *et al.* (2002). Cognitive and subjective dose-response effects of acute oral Δ9-tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology*, **164**:61–70.

D'Souza, D.C., Abi-Saab, W.M., Madonick, S., *et al.* (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biol Psychiatry*, **57**:594–608.

D'Souza, D.C., Perry, E., MacDougall, L., *et al.* (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: Implications for psychosis. *Neuropsychopharmacology*, **29**:1558–72.

Egerton, A., Allison, C., Brett, R.R., *et al.* (2006) Cannabinoids and prefrontal cortical function: Insights from preclinical studies. *Neurosci Biobehav Rev,* **30**:680–95.

Ehrenreich, H., Rinn, T., Kunert, H.J., *et al.* (1999) Specific attentional dysfunction in adults following early start of cannabis use. *Psychopharmacology,* **142**:295–301.

Eldreth, D.A., Matochik, J.A., Cadet, J.L., *et al.* (2004) Abnormal brain activity in prefrontal regions in abstinent marijuana users. *Neuroimage,* **23**:914–20.

Ellgren, M., Artmann, A., Tkalych, O., *et al.* (2008) Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence: THC effects. *European Neuropsychopharmacol*, **18**:826–34.

Fletcher, P.C. and Honey, G.D. (2006) Schizophrenia, ketamine and cannabis: Evidence of overlapping memory deficits. *Trends Cog Sci,* **10**:167–74.

Fusar-Poli, P., Crippa, J.A., Bhattacharyya, S., *et al.* (2009) Distinct effects of (delta)9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Arch Gen Psych,* **66**:95–105.

Grenyer, B., Respondek, C., Gordon, S., *et al.* (2010) Cognitive, executive and social functioning in schizophrenia with and without comorbid cannabis use. *Schizophrenia Res*, **117**:425.

Gruber, S.A., Rogowska, J. and Yurgelun-Todd, D.A. (2009) Altered affective response in marijuana smokers: An fMRI study. *Drug Alcohol Depend,* **105**:139–53.

Gruber, S. and Yurgelun-Todd, D. (2005) Neuroimaging of marijuana smokers during inhibitory processing: a pilot investigation. *Brain Res Cogni Brain Res*, **23**:107–18.

Hall, W. and Degenhardt, L. (2009) Adverse health effects of non-medical cannabis use. *Lancet,* **374**:1383–91.

Hall, W. and Solowij, N. (1998) Adverse effects of cannabis. *Lancet,* **352**:1611–16.

Hart, C.L., van Gorp, W., Haney, M., *et al.* (2001) Effects of acute smoked marijuana on complex cognitive performance. *Neuropsychopharmacology,* **25**:757–65.

Harvey, M.A., Sellman, J.D., Porter, R.J., *et al.* (2007) The relationship between non-acute adolescent cannabis use and cognition. *Drug Alcohol Rev,* **26**:309–19.

Hester, R., Nestor, L. and Garavan, H. (2009) Impaired error awareness and anterior cingulate cortex hypoactivity in chronic cannabis users. *Neuropsychopharmacology,* **34**:2450–8.

Hunault, C.C., Mensinga, T.T., Böcker, K.B.E., *et al.* (2009) Cognitive and psychomotor effects in males after smoking a combination of tobacco and cannabis containing up to 69 mg delta-9-tetrahydrocannabinol (THC). *Psychopharmacology*, **204**:85–94.

Ilan, A.B., Smith, M.E. and Gevins, A. (2004) Effects of marijuana on neuropsychological signals of working and episodic memory. *Psychopharmacology,* **176**:214–22.

Indlekofer, F., Piechatzek, M., Daamen, M., *et al.* (2009) Reduced memory and attention performance in a population-based sample of young adults with a moderate lifetime use of cannabis, ecstasy and alcohol. *J Psychopharmacology,* **23**:495–509.

Iversen, L. (2003) Cannabis and the brain. *Brain,* **126**:1252–70.

Jacobsen, L.K., Mencl, W.E., Westerveld, M., *et al.* (2004) Impact of cannabis use on brain function in adolescents. *Ann NY Acad Sci,* **1021**:384–90.

Jacobsen, L.K., Pugh, K.R., Constable, R.T., *et al.* (2007) Functional correlates of verbal memory deficits emerging during nicotine withdrawal in abstinent adolescent cannabis users. *Biol Psychiatry,* **61**:31–40.

Jacobus, J., Bava, S., Cohen-Zion, M., *et al.* (2009) Functional consequences of marijuana use in adolescents. *Pharmacol Biochem Behav*, **92**:559–65.

Jager, G., Block, R.I., Luijten, M., *et al.* (2010) Cannabis use and memory brain function in adolescent boys: A cross-sectional multicenter functional magnetic resonance imaging study. *J Am Acad Child Adolesc Psychiatry,* **49**:561–72.

Jager, G., Kahn, R.S., Van den Brink, W., *et al.* (2006) Long-term effects of frequent cannabis use on working memory and attention: an fMRI study. *Psychopharmacology (Berl),* **185**:358–68.

Jager, G., Van Hell, H.H., De Win, M.M.L., *et al.* (2007) Effects of frequent cannabis use on hippocampal activity during an associative memory task. *Eur Neuropsychopharmacol,* **17**:289–97.

Juckel, G., Roser, P., Nadulskic, T., *et al.* (2007) Acute effects of Δ9-tetrahydrocannabinol and standardized cannabis extract on the auditory evoked mismatch negativity. *Schizophr Res*, **97**:109–17.

Kanayama, G., Rogowska, J., Pope, H.G., *et al.* (2004) Spatial working memory in heavy cannabis users: a functional magnetic resonance imaging study. *Psychopharmacology,* **176**:239–47.

Kang-Park, M., Wilson, W.A., Kuhn, C.M., *et al.* (2007) Differential sensitivity of GABAA receptor-mediated IPSCs to cannabinoids in hippocampal slices from adolescent and adult rats. *J Neurophysiol,* **98**:1223–30.

Kempel, P., Lampe, K., Parnefjord, R., *et al.* (2003) Auditory-evoked potentials and selective attention: different ways of information processing in cannabis users and controls. *Neuropsychobiology,* **48**:95–101.

Lane, S.D., Cherek, D.R., Lieving, L.M., *et al.* (2005a) Marijuana effects on human forgetting functions. *J Exp Anal Behav,* **83**:67–83.

Lane, S.D., Cherek, D.R., Tcheremissine, O.V., *et al.* (2005b) Acute marijuana effects on human risk taking. *Neuropsychopharmacology*, **30**:800–9.

Løberg, E.M. and Hugdahl, K. (2009) Cannabis use and cognition in schizophrenia. *Front Hum Neurosci*, **3**:53.

Luijten, M., Jager, G., Block, R.I., *et al.* (2007) Effects of cannabis use on associative memory in adolescents, and the role of personality measures: an fMRI study. *Eur Neuropsychopharmacol*, **17**:S552–S553.

Martin-Santos, R., Fagundo, A.B., Crippa, J.A., *et al.* (2010) Neuroimaging in cannabis use: a systematic review of the literature. *Psychol Med*, **40**:383–98.

McDonald, J., Schleifer, L., Richards, J.B., *et al.* (2003) Effects of THC on behavioral measures of impulsivity in humans. *Neuropsychopharmacology,* **28**:1356–65.

McHale, S. and Hunt, N. (2008) Executive function deficits in short-term abstinent cannabis users. *Human Psychopharmacol,* **23**:409–15.

Medina, K.L., Hanson, K.L., Schweinsburg, A.D., *et al.* (2007) Neuropsychological functioning in adolescent marijuana users: Subtle deficits detectable after a month of abstinence. *J Int Neuropsychol Soc,* **13**:807–20.

Melis, M., Perra, S., Muntoni, A.L., *et al.* (2004) Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. *J Neurosci*, **24**:10707–15.

Messinis, L., Kypriandou, A., Malefaki, S., *et al.* (2006) Neuropsychological deficits in long-term frequent cannabis users. *Neurology,* **66**:737–9.

Morrison, P.D., Zois, V., McKeown, D.A., *et al.* (2009) The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychol Med*, **39**:1607–16.

Nestor, L., Hester, R. and Garavan, H. (2010) Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. *Neuroimage,* **49**:1133–43.

Nestor, L., Roberts, G., Garavan, H., *et al.* (2008) Deficits in learning and memory: parahippocampal hyperactivity and frontocortical hypoactivity in cannabis users. *Neuroimage,* **40**:1328–39.

Novaes, M.A.F.P., Guindalini, C., Almeida, P., *et al.* (2008) Cannabis use before age 15 years is associated with poorer attention and executive function. *Biol Psychiatry,* **63**:18S–19S.

O'Leary, D.S., Block, R.I., Koeppel, J.A., *et al.* (2007) Effects of smoking marijuana on focal attention and brain blood flow. *Human Psychopharmacol*, **22**:135–48.

Padula, C.B., Schweinsburg, A.D. and Tapert, S.F. (2007) Spatial working memory performance and fMRI activation interaction in abstinent adolescent marijuana users. *Psychol Addict Behav*, **21**:478 – 87.

Pattij, T., Wiskerke, J. and Schoffelmeer, A.N. (2008) Cannabinoid modulation of executive functions. *Eur J Pharmacol*, **585**:458–63.

Phan, K.L., Angstadt, M., Golden, J., *et al.* (2008) Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. *J Neurosci,* **28**:2313–9.

Pillay, S.S., Rogowska, J., Kanayama, G., *et al.* (2008) Cannabis and motor function: fMRI changes following 28 days of discontinuation. *Exp Clin Psychopharmacol,* **16**:22–32.

Pope, H.G., Gruber, A.J., Hudson, J.I., *et al.* (2003) Early-onset cannabis use and cognitive deficits: What is the nature of the association? *Drug Alcohol Depend,* **69**:303–10.

Pope, H.G., Gruber, A.J., Hudson, J.I., *et al.* (2001) Neuropsychological performance in long-term cannabis users. *Arch Gen Psychiatry*, **58**:909–15.

Pope, H.G., Gruber, A.J., Hudson, J.I., *et al.* (2002) Cognitive measures in long-term cannabis users. *J Clin Pharmacol*, **42**:41S–47S.

Pope, H.G. and Yurgelun-Todd, D. (2004) Residual cognitive effects of long-term cannabis use. In Castle, D.J. and Murray, R.M. (Eds.), *Marijuana and Madness*, 1st ed. (pp. 198–210). Cambridge: Cambridge University Press.

Potvin, S., Joyal, C.C., Pelletier, J., *et al.* (2008) Contradictory cognitive capacities among substance-abusing patients with schizophrenia: a meta-analysis. *Schizophr Res,* **100**:242–51.

Ramaekers, J.G., Kauert, G., Theunissen, E.L., *et al.* (2009). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *J Psychopharmacol,* **23**:266–77.

Ranganathan, M. and D'Souza, D.C. (2006) The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology*, **188**:425–44.

Realini, N., Rubino, T. and Parolaro, D. (2009) Neurobiological alterations at adult age triggered by adolescent exposure to cannabinoids. *Pharmacol Res,* **60**:132–8.

Ringen, P.A., Vaskinn, A., Sundet, K., *et al.* (2009) Opposite relationships between cannabis use and neurocognitive functioning in bipolar disorder and schizophrenia. *Psychological Med,* **6**:1–11.

Rogers, R.D., Wakeley, J., Robson, P.J., *et al*. (2007) Reinforcement processing in the risky decision-making of young healthy adults. *Neuropsychopharmacology,* **32**:417–28.

Roser, P., Juckel, G., Rentzsch, J., *et al*. (2008) Effects of acute oral Δ^9-tetrahydrocannabinol and standardized cannabis extract on the auditory P300 event-related potential in healthy volunteers. *Eur Neuropsychopharmacology*, **18**:569–77.

Rubino, T., Realini, N., Braida, D., *et al.* (2009) Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus,* **19**:763–72.

Schneider, M. (2008) Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addict Biol,* **13**:253–63.

Schnell, T., Koethe, D., Daumann, J., *et al*. (2009) The role of cannabis in cognitive functioning of patients with schizophrenia. *Psychopharmacology (Berl),* **205**: 45–52.

Scholes, K.E. and Martin-Iverson, M.T. (2009) Alterations to pre-pulse inhibition (PPI) in chronic cannabis users are secondary to sustained attention deficits. *Psychopharmacology,* **207**:469–84.

Schweinsburg, A.D., Brown, S.A. and Tapert, S.F. (2008a) The influence of marijuana use on neurocognitive functioning in adolescents. *Curr Drug Abuse Rev*, **1**:99–111.

Schweinsburg, A.D., Nagel, B.J., Schweinsburg, B.C., *et al.* (2008b) Abstinent adolescent marijuana users show altered fMRI response during spatial working memory. *Psychiatry Res,* **163**:40–51.

Sewell, R.A., Skosnik, P.D., Garcia-Sosa, I., *et al*. (2010) Behavioral, cognitive and psychophysiological effects of cannabinoids: relevance to psychosis and schizophrenia. *Rev Bras Psiquiatr*, **32**:S15–S30.

Skosnik, P.D., Edwards, C.R., O'Donnell, B.F., *et al*. (2008) Cannabis use disrupts eyeblink conditioning: Evidence for cannabinoid modulation of cerebellar-dependent learning. *Neuropsychopharmacology*, **33**:1432–40.

Skosnik, P.D., Spatz-Glenn, L. and Park, S. (2001) Cannabis use is associated with schizotypy and attentional disinhibition. *Schizophr Res,* **48**:83–92.

Sneider, J.T., Pope Jr, H.G., Silveri, M.M., *et al*. (2008) Differences in regional blood volume during a 28-day period of abstinence in chronic cannabis smokers. *Eur Neuropsychopharmacol,* **18**, 612–19.

Solowij, N. (1998). *Cannabis and cognitive functioning*. Cambridge: Cambridge University Press.

Solowij, N. and Battisti, R. (2008) The chronic effects of cannabis on memory in humans: a review. *Curr Drug Abuse Rev,* **1**:81–98.

Solowij, N. and Grenyer, B. (2002) Are the adverse consequences of cannabis use age-dependent? *Addiction*, **97**:1083–6.

Solowij, N., Jones, K.A., Rozman, M.E., *et al*. (2011) Verbal learning and memory in adolescent cannabis users, alcohol users and non-users. *Psychopharmacology*, **216**:131–144.

Solowij, N., Jones, K.A., Rozman, M.E., *et al*. (2011) Reflection impulsivity in adolescent cannabis users. *Psychopharmacology*, **24**(3):A63.

Solowij, N. and Michie, P. (2007). Cannabis and cognitive dysfunction: Parallels with endophenotypes of Schizophrenia? *J Psychiatry Neurosci*, **32**:30–52.

Solowij, N., and Pesa, N. (2010) Cognitive abnormalities and cannabis use. *Rev Bras Psiquiatr,* **32**:S31–S40.

Solowij, N., Respondek, C., Battisti, R., *et al*. (2008). Visuospatial memory deficits in long term heavy cannabis users: relation to psychotic symptoms and regional brain volumes. *Int J Neuropsychopharmacol*, **11**: 242.

Solowij, N., Stephens, R.S., Roffman, R.A., *et al*. (2002). Cognitive functioning of long-term heavy cannabis users seeking treatment. *J Am Med Assoc,* **287**:1123–31.

Solowij, N., Walterfang, M., Respondek, C., *et al*. (2010). Alterations of hippocampal shape in cannabis users with and without schizophrenia. *Schizophr Res*, **117**:426.

Solowij, N., Yücel, M., Respondek, C., *et al*. (2011b) Cerebellar white matter changes in cannabis users with and without schizophrenia. *Psychol Med*. DOI: 10.1017/S003329171100050X.

Tapert, S.F., Schweinsburg, A.D., Drummond, S.P.A., *et al*. (2007) Functional MRI of inhibitory processing in abstinent adolescent marijuana users. *Psychopharmacology,* **194**:173–83.

Tselnicker, I., Keren, O., Hefetz, A., *et al*. (2007) A single low dose of tetrahydrocannabinol induces long-term cognitive deficits. *Neuroscience Letters,* **411**:108–11.

Vadhan, N.P., Hart, C.L., van Gorp, W.G., *et al*. (2007) Acute effects of smoked marijuana on decision making, as assessed by a modified gambling task, in experienced marijuana users. *J Clin Exp Neuropsychol,* **29**:357–64.

Voytek, B., Berman, S.M., Hassid, B.D., *et al*. (2005) Differences in regional brain metabolism associated with marijuana abuse in methamphetamine abusers. *Synapse,* **57**:113–15.

Yücel, M., Bora, E., Lubman, D.I., *et al*. (2010) The impact of cannabis use on cognitive functioning in patients with schizophrenia: A meta-analysis of existing findings and new data in a first-episode sample. *Schizophrenia Bulletin*, DOI:10.1093/schbul/sbq079.

Yücel, M., Solowij, N., Respondek, C., *et al*. (2008) Regional brain abnormalities associated with long-term heavy cannabis use. *Arch Gen Psychiatry*, **65**:694–701.

Zuurman, L., Ippel, A.E., Moin, E., *et al*. (2009) Biomarkers for the effects of cannabis and THC in healthy volunteers. *Brit J Clin Pharmacol*, **67**:5–21.

Chapter 9

Does cannabis cause lasting brain damage?

Nadia Solowij, Murat Yücel, Valentina Lorenzetti and Dan Lubman

Until recently, it was possible to state with some confidence that there was no evidence of cannabis-related brain damage in humans. There was some support from the animal literature, but few human studies had been conducted where the findings could not be explained by methodological or other confounding factors. Recent evidence for gross morphological, connectivity and microstructural changes has now emerged that warrants further consideration. If cannabis were found to alter the structural integrity of the brain, then this may assist us to understand the mechanisms by which cannabis triggers psychotic symptoms or overt psychosis in vulnerable individuals.

Evidence from animal studies

Cannabinoids, either endogenous or exogenous, possess both neuroprotective and neurotoxic properties (Sarne and Mechoulam, 2005; Kano *et al*., 2009). Cannabinoid-receptor activation induces morphological changes to neurons, such as inhibition of new synapse formation (Kano *et al*., 2009), and at crucial neurodevelopmental stages (prenatal and adolescent), exposure to cannabinoids impacts on neural cell survival and maturation (Chapters 6, 7) (Downer and Campbell, 2010). The role of different cannabinoids in controlling neural-cell survival or death is a complex issue that is influenced by the dose, duration of exposure and route of administration, but also the neural-cell type and its stage of differentiation (Downer and Campbell, 2010). Contradictory hypotheses circulate regarding the doses of Δ^9-tetrahydrocannabinol (THC) that may be neurotoxic or neuroprotective. Some suggest that single high doses of THC are neuroprotective within a limited timeframe, but that low doses are neurotoxic and, with chronic exposure, induce neuronal death (Sarne and Keren, 2004; Tselnicker *et al*, 2007; Sarne and Mechoulam, 2005). However, large doses of THC applied directly to cultured hippocampal neurons, and both high and low doses to cultured cortical neurons, have been shown to cause cell death or significant neurotoxic changes (eg. shrinkage of cell bodies and DNA-strand breaks) characteristic of neuronal apoptosis (Chan *et al*., 1998; Campbell, 2001; Downer *et al*., 2001). Indeed, even a single administration of an ultra-low dose of THC (0.001–0.002 mg/kg) has been shown to result in long-term cognitive impairment (in spatial learning, strategy and working memory) in mice. These defecits persisted for at least 5 months post-injection and were associated with activation of extracellular-regulated kinase (ERK) in the cerebellum and hippocampus (Tselnicker *et al*., 2007; Amal *et al*., 2010). The authors suggested that low THC concentration is the main determinant of long-lasting neuronal effects following chronic exposure to cannabinoids, due to their slow clearance and accumulation (Amal *et al*., 2010).

A study of cannabinoid application in vitro showed that THC appears to accumulate primarily in neurons and that transformation to its metabolite, THC-COOH, depends on the presence of glia (Monnet-Tschudi *et al*., 2008). The authors suggested that the adverse effects of cannabinoids on the brain may occur through a combination of pathways involving cannabinoid receptor activation, accumulation of cannabinoids and their metabolites and upregulation of neuroinflammatory cytokines. Given the dependence on glia for metabolism of THC, if white-matter aberrations develop in cannabis users (as discussed further below), more THC could potentially accumulate in neurons, causing toxicity.

Studies of chronic cannabinoid administration to animals have demonstrated cognitive impairment associated with specific neurochemical, transmission and cell firing alterations, particularly in the hippocampus, but also the prefrontal cortex (PFC), similar to impairment

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induced by lesions or transient inactivation (Egerton *et al*., 2006). Chronic administration of THC to rats and primates has been shown to result in dose-dependent neurotoxic changes in brain regions that are rich in cannabinoid receptors. Specifically, THC-induced neurotoxic effects are prominent within the hippocampus (Heath *et al*., 1980; Scallet *et al*., 1987; Landfield *et al*., 1988; Chan *et al*., 1998; Lawston *et al*., 2000), amygdala (Heath *et al*., 1980), septum (Harper *et al*., 1977; Myers and Heath., 1979) and cerebral cortex (Harper *et al*., 1977; Downer *et al*., 2001). These neurotoxic effects include shrinkage of neural cell nuclei and bodies (Heath *et al*., 1980; Scallet *et al*., 1987) and reductions in pyramidal cell density (Lawston *et al*., 2000), dendritic length (Landfield *et al*., 1988) and number of synapses (Heath *et al*., 1980). Some of these studies have emulated chronic-use patterns seen in humans: for example, in the study by Landfield and colleagues (1988) THC was administered to rats five times a week for 8 months, representing approximately 30% of the rats' lifespan.

Cannabis use in humans typically commences during adolescence and young adulthood, a crucial period of neurodevelopment (see Chapter 7). Neuromaturational changes primarily occur within PFC and limbic circuits, and include progressive and regressive changes such as myelination and synaptic pruning, neurogenesis and apoptosis, axonal growth and sprouting, dendritic arborization and retraction, synaptogenesis and synapse elimination, alongside the maturation of multiple neurotransmitter systems (Schneider, 2008; Realini *et al*., 2009). The endocannabinoid (eCB) system (ECS) is crucially involved in these developmental processes (Harkany *et al*., 2008; Schneider, 2008; Realini *et al*., 2009) that are thought to be essential for the acquisition of adult cognition, decision-making and social behaviors. As such, exposure to THC during adolescence may perturb neurodevelopmental processes with potential long lasting consequences.

As discussed in detail in Chapter 7, a number of studies have examined the impact of THC administration during adolescence on the adult brain. For instance, Rubino and colleagues (2009a) administered THC twice daily to adolescent rats for 10 days and then left them undisturbed until adulthood, at which point they assessed learning and memory capacities, as well as their underlying neural substrates. Deficits in spatial memory were evident in the pretreated rats and were accompanied by, and correlated with, significantly lower total dendritic length and number, reduced spine density and decreases in astroglial markers, protein expression and N-methyl-D-aspartate receptor levels within the hippocampus. Thus, adolescent exposure to THC resulted in long-lasting alterations to the structural and functional plasticity of both neurons and glia, with a reduction in synaptic contacts and/or less efficient synaptic connections throughout the hippocampus. In other studies, these authors found significant gender-related THC neurotoxic effects, demonstrating that CB1 receptor density and G-protein coupling were significantly reduced in the amygdala, ventral tegmental area and nucleus accumbens in female rats, but only in the amygdala and hippocampus of male rats, accompanied by different behavioral profiles (Rubino *et al*., 2008). Further, spatial working memory impairment was similar between genders but was underpinned by hippocampal alterations in males, in contrast to PFC alterations in females (Rubino *et al*., 2009b). This work supports a growing literature demonstrating sex differences in adulthood in animals chronically administered THC during adolescence, as well as alterations within circuits underlying emotional processing (Realini *et al*., 2009).

There is also growing evidence of differential responses to cannabis during adolescence compared with adulthood. Quinn *et al*. (2008) found that repeated exposure to THC was less behaviorally aversive for adolescent compared with adult rats, but caused greater persistent memory deficits and hippocampal structural and functional alterations. Altered protein expression in the hippocampus was observed in both adult and adolescent rats, but adolescent rats showed a greater number of altered proteins related to oxidative stress, mitochondrial and metabolic function, cell proliferation and repair, and cytoskeletal structure and signaling. Further recent research on neurotransmitter system functionality and cannabinoid receptor changes following chronic exposure to cannabinoids suggests that the adolescent brain does not compensate for the biological changes in response to cannabis exposure in the same way as the adult brain (Dalton and Zavitsanou, 2010; Zavitsanou *et al*., 2010). The ECS also appears to be altered by exposure to THC during early, middle and late adolescence (Ellgren *et al*., 2008). In a rat study, intermittent exposure to THC (a pattern of use common among teenagers) was found to reverse the normal proportional ratio of eCBs (anandamide and 2-arachidonoyl glycerol [2-AG]) in the PFC and nucleus accumbens. These studies support the notion that THC effects on neural integrity may depend on different developmental stages of exposure.

Thus, evidence from preclinical research has identified neurotoxic, morphological and microstructural alterations to the brain in vitro and, when animals are acutely or chronically exposed to cannabinoids, at doses relevant to human use. With discrepant results concerning the neurotoxicity of low and high doses, and accumulation of cannabinoids, further research must reconcile dose-effects in vitro versus in vivo, and consider the various cannabinoids that human users expose themselves to. Some of these have been shown to have differential properties and opposing effects in humans (eg. THC versus cannabidiol; see Chapter 1). As such, animal research could examine each of these in isolation and in combination, and further elucidate their impact on the developing brain.

Evidence from human studies

Adult chronic cannabis users

Findings of persistent alteration of brain function or cognitive impairment in human cannabis users (as reviewed in Chapter 8), together with the animal work discussed above, support the notion that long-term cannabis use may result in morphological alterations of brain structures that subserve attention, learning, memory, executive functions and emotional processes (such as the prefrontal and temporal cortices). To date, findings from structural neuroimaging studies of long-term cannabis users have been contradictory, with evidence for both the presence and absence of morphological changes in specific brain regions (DeLisi, 2008; Solowij *et al*., 2009; Lorenzetti *et al*., 2010; Martin-Santos *et al*., 2010). However, a number of variables, such as demographic, clinical, genetic and drug-use factors are likely to mediate the relationship between cannabis use and brain structural alterations.

A recent review (Lorenzetti *et al*., 2010) identified only 13 structural neuroimaging studies where the primary substance used was cannabis and major psychopathologies were excluded. The main imaging modality utilized was magnetic resonance imaging (MRI) (eight studies), with three studies employing computed tomography (CT) and two early studies using pneumo-encephalography and echo-encephalography, respectively. The MRI studies used either a region-of-interest approach (six studies) or voxel-based morphometry (VBM; two studies). No significant differences were found in any of the studies on global measures of brain volume. More specific regional brain analyses demonstrated evidence of structural brain abnormalities, but these were not consistent across studies.

Six studies reported specific regional structural alterations in regular cannabis users (Campbell *et al*., 1971; Block *et al*., 2000; Wilson *et al*., 2000; Matochik *et al*., 2005; Medina *et al*., 2007b; Yücel *et al*., 2008), while the remaining seven studies found no significant volumetric differences between users and controls (Stefanis, 1976; Co *et al*., 1977; Kuehnle *et al*., 1977; Hannerz and Hindmarsh, 1983; Jager *et al*., 2007; Medina *et al*., 2007a; Tzilos *et al*., 2005). Alterations in hippocampal or parahippocampal volumes were the most consistently reported findings, but the nature of the findings were still mixed. Hippocampal volumes in cannabis users were found to be smaller (Matochik *et al*., 2005; Yücel *et al*., 2008), larger (Medina *et al*., 2007b), or no different to controls (Block *et al*., 2000; Wilson *et al*., 2000; Medina *et al*., 2007a). Of three studies that examined parahippocampal volume, two reported no change (Jager *et al*., 2007; Tzilos *et al*., 2005), while one found an alteration in grey and white matter composition (Matochik *et al*., 2005). Two studies examined amygdala volumes, with one reporting reduced volume (Yücel *et al*., 2008) and the other no change (Wilson *et al*., 2000). Finally, there were a number of brain regions that were investigated only within a single study, with significant between-group differences found for the precentral gyrus, thalamus, parietal lobule, fusiform gyrus, lentiform nucleus and pons (Matochik *et al*., 2005), but not for the cerebellum (Block *et al*., 2000). While few studies have specifically examined white-matter volume, we recently identified significant cerebellar white matter reduction in adult long-term very heavy cannabis users (Solowij *et al*., 2011).

Dose and duration of cannabis exposure may differentiate between those studies that did or did not find volumetric differences between users and controls. For example, in our study (Yücel *et al*., 2008) the cannabis users had a similar exposure to that of Landfield *et al*.'s (1988) rodent study (cited above). Both of these studies found significant dose-related reductions in hippocampal volume. The cannabis users within our study had the most extensive exposure to cannabis of all the studies of human cannabis users (near daily use for a mean 19.7 years, range 10–32 years), and the most striking findings. We reported a 12% reduction bilaterally in hippocampal volumes, as well as an approximate 7% reduction in bilateral amygdala volumes (Yücel *et al*., 2008), and a 24% reduction in cerebellar white matter (Solowij *et al*., 2011). The reduction of left hippocampal

volume was of a similar magnitude to that observed in schizophrenia, was dose-related and was associated with subclinical psychotic symptoms, even though our sample was carefully screened for DSM-IV psychotic disorders.

One other study with a similar mean duration of use (mean 22.6 years, range 12–33 years) to the sample in our study, reported no brain alterations, but the minimum duration of *daily* use in that sample was only one year (Tzilos *et al*., 2005). In contrast, the minimum duration of *near daily* use in our study was 10 years. A further key difference between the Tzilos *et al.* (2005) study and ours was in the estimated episodes of use, and hence the cumulative dose of exposure to cannabis. Tzilos *et al*.'s sample reported an average of 20 100 lifetime episodes of use. Our sample had an average 62 000 estimated episodes of use over the lifetime. Thus, despite a similar mean duration of use, our cannabis users used more than three times as much cannabis, which may be the crucial factor in explaining our finding of a dose–response relationship between hippocampal volume and cumulative cannabis use. In addition, Tzilos *et al.* (2005) acquired their images at a lower field strength and with a coarser spatial resolution (1.5 T with 3-mm-thick slices vs. 3 T with 1-mm-thick slices in our study), an important consideration given the small size and boundary definition of the brain structures investigated. Moreover, the region of interest measured in their study was less specific to the hippocampus relative to ours because they also included the parahippocampal gyrus (ours was restricted to the hippocampus itself using well-defined boundaries).

A general trend for an inverse relationship between indices of cannabis use and hippocampal or parahippocampal volume appears to exist in other studies. Aside from our own study, samples with greater cannabis exposure demonstrated reductions in hippocampal or parahippocampal volumes (Matochik *et al*., 2005), whereas samples with a lower quantity or frequency of cannabis use exhibited no change (Block *et al*., 2000; Wilson *et al*., 2000; Jager *et al*., 2007; Medina *et al*., 2007a; Tzilos *et al*., 2005), or even volumetric increases (Medina *et al*. 2007b). Studies of heavy cannabis users (Matochik *et al*., 2005; Yücel *et al*., 2008) were more likely to detect regional abnormalities than those of lighter cannabis users. Greater brain alterations with an earlier age of onset of cannabis use have been reported in some studies (Wilson *et al*., 2000), but not others (Matochik *et al*., 2005; Tzilos *et al*., 2005), but this aspect of human cannabis use remains underinvestigated.

Several recent studies have examined the integrity of white matter fiber tracts in cannabis users using diffusion tensor imaging (DTI), including studies of adolescent users (reported below). A pilot study in ten heavy cannabis users demonstrated trends toward both increased mean diffusivity and lower fractional anisotropy in the anterior cingulate cortex (Gruber and Yurgelun-Todd, 2005). Another study of heavy users found significantly increased mean diffusivity in the anterior region of the corpus callosum, where white matter passes between the prefrontal lobes (Arnone *et al*., 2008). The data suggest impaired structural integrity of the corpus callosum fiber tracts with prolonged cannabis exposure, particularly as the authors reported an association with duration of cannabis use within the sample. White matter tractography investigations in cannabis users are only at a preliminary stage of investigation and hold much promise for the future.

A post-mortem study of cannabinoid receptor density and integrity in human brains found that the receptor becomes hypofunctional with chronic cannabis use (Villares, 2007). Downregulation was observed in the hippocampus, basal ganglia and mesencephalon of chronic users, and reduced binding levels were accompanied by parallel decreases in mRNA levels. These findings suggest that the primary effect of chronic exposure was on the CB1 receptor gene rather than on the receptor protein. Evidence of diminished neuronal and axonal integrity in the dorsolateral prefrontal cortex has been indicated by magnetic resonance spectroscopic markers of metabolism (NAA/tCr ratio) (Hermann *et al*., 2007). Dose-related changes in this study were also found in the anterior cingulate and putamen/globus pallidum, but not in the hippocampus. Acute and chronic exposure to cannabis in humans has also been associated with reduced serum concentrations of neurotrophins, including nerve growth factor (Angelucci *et al*., 2008) and brain derived neurotrophic factor (BDNF) (D'Souza *et al*., 2009).

Thus, there is growing evidence for alterations to the structural integrity of the brain as a result of chronic cannabis exposure in adult users. This includes gross structural anatomical studies of long term and heavy users, as well as more refined studies of white matter and connectivity, and neurotoxic markers in vivo.

Adolescent and young-adult cannabis users

An increasing number of studies have investigated brain morphology in adolescent cannabis users or in adults who started using cannabis at a young age. A

study of adult users reported that early onset cannabis users (before age 17 years) had smaller whole brain volumes, lower percent cortical grey matter, higher percent white matter and increased cerebral blood flow compared with later onset users (Wilson *et al*., 2000).

The two studies by Medina and colleagues discussed above were of adolescents, one reporting larger hippocampal volumes in users (Medina *et al*., 2007b), while the other found no volumetric differences from controls (Medina *et al*., 2007a). Medina *et al*. (2007a) also found an association between whole brain white matter volume and depressive symptoms in young adult cannabis users. While a DTI study of young adults who had at least one year of daily or several times/week cannabis during adolescence, found no evidence of pathological white matter integrity differences between users and controls, it identified several regions of apparently greater integrity among users (DeLisi *et al*., 2006). However, a solid body of evidence for pathology in white matter tracts within the corpus callosum and various fronto-temporal, occipito-frontal and posterior connections that develop during adolescence, has come from other recent DTI studies of young adult (Arnone *et al*., 2008; Allin *et al*., 2009) and adolescent (Ashtari *et al*., 2009; Bava *et al*., 2009; Yücel *et al*., 2011) cannabis users, as well as adolescents with substance use disorders (primarily cannabis) (Thatcher *et al*., 2010). Abnormalities in this latter study were greater in females than in males. The results from these studies overall suggest that cannabis use, particularly during adolescence, may affect the trajectory of normal brain maturation resulting in white matter aberrations, which may underlie compromised cognitive processing and may even underpin the propensity for cannabis to cause psychosis (Allin *et al*., 2009; Solowij *et al*., 2011).

Interestingly, Jacobus *et al*. (2009) reported greater white matter integrity alterations in several brain regions in adolescent binge drinkers than in adolescent heavy cannabis users who were also binge drinkers. The latter group differed from controls in three regions, while alcohol only users differed in eight regions. The data suggest subtle white-matter-tissue microstructural abnormalities reflecting poor tract coherence and organization, but not tissue loss or demyelination, and imply interactive effects of cannabis and alcohol or a possible neuroprotective role of cannabis in binge drinking. In a further investigation of cognitive function in relation to white matter integrity, these same authors found that reduced white matter integrity in temporal regions in cannabis and alcohol using adolescents was associated with poor performance in attention, working memory and speed of processing tasks (Bava *et al*., 2010). Higher integrity of white matter fiber tracts in the cannabis users relative to controls (interpreted as a neurodevelopmental compensatory mechanism in response to exposure to cannabis) was associated with better performance, except for in one anterior region where higher integrity was associated with poorer contextual verbal memory performance. The interactive effects of cannabis and alcohol should be further investigated, particularly as they are frequently used together by adolescents.

Altered cortical gyrification in the frontal lobe and abnormal age-related changes to gyrification and cortical thickness have also recently been reported in adolescent and young adult users (Mata *et al*., 2010). Cannabis users showed bilaterally decreased concavity of the sulci (i.e. greater flattening) in frontal, temporal and parietal lobes, and thinner sulci in the right frontal lobe, in the absence of global brain structural differences between users and controls. Abnormal cortical gyrification may reflect abnormal neurodevelopment or neurodegeneration. A lack of normal association between these measures and increasing age in the cannabis users, together with a lack of observed associations with specific cannabis-use parameters led the authors to speculate that cannabis use during adolescence or young adulthood might prematurely alter cortical gyrification toward patterns usually seen at a later age.

Further specific investigations of brain structure and function are clearly warranted in adolescent cannabis-using samples to verify whether cannabis has specific and/or more detrimental effects than in adult users; whether there are age-of-onset-dependent and gender effects; and whether there is a progression of brain morphological abnormalities with continued cannabis use, or reversal with abstinence.

Patients with psychosis

Since brain structural changes are evident in patients with schizophrenia, and there is mounting evidence for similar changes in association with heavy cannabis use, it is possible that cannabis may exert greater adverse effects on brain morphology when the brain is already compromised. Indeed, this is most likely to occur in brain regions known to be altered in both heavy cannabis users and patients with schizophrenia (e.g., hippocampus). In line with this, a number of recent studies have investigated brain morphology in patients with

schizophrenia or early psychosis and comorbid cannabis use.

No differences in brain structure between patients with established schizophrenia who did and did not use cannabis were reported by Cahn *et al.* (2004), while Potvin *et al.* (2007) found increased striatal grey matter densities in schizophrenia patients with comorbid substance-use disorders (primarily cannabis). In first-episode psychosis patients who use cannabis, decreased grey matter volumes of the anterior cingulate (Szeszko *et al.*, 2007), right posterior cingulate cortex and left hippocampus (Bangalore *et al.*, 2008) were reported relative to their non-using counterparts and to healthy controls. Trends toward smaller left and right cerebellar volumes were also apparent (Bangalore *et al.*, 2008). Rais and colleagues (2008) reported greater lateral and third ventricle enlargements and more pronounced total cerebral grey matter volume reduction over 5 years in first-episode schizophrenia patients who used cannabis compared with those who did not, as well as in comparison with healthy controls (2.67% and 5.09% reduction, respectively). The results were suggested to explain some of the detrimental effects of cannabis use in patients with schizophrenia. We have recently reported hippocampal shape alteration (Solowij *et al.*, 2010) and an almost 30% loss of cerebellar white matter relative to healthy controls in patients with schizophrenia and extensive cannabis use histories (Solowij *et al.*, 2011). Finally, Dekker *et al.* (2010) found that the age of onset of cannabis use (before age 15 years versus age 17 years or later) had no bearing on white matter integrity of the corpus callosum in a sample of young adults with recent onset schizophrenia, while cannabis-naïve patients showed greater abnormalities. These results support the notion that cannabis-using patients may represent a group who developed psychosis in part at least as a consequence of their cannabis use (Dekker *et al.*, 2010; Yücel *et al.*, 2010). Clearly, the impact of cannabis use on brain function and structure in schizophrenia also warrants further investigation.

What might be the implications of structural brain changes in cannabis users?

It is often assumed that alterations in the morphology of the brain may underlie impaired cognition and may indicate neural substrates of risk for the development of psychosis, but there are limited data to support these notions. The interrelationships between cognition and psychopathology, and indeed between brain structure and function, are complex.

Few structural brain imaging studies of cannabis users have specifically examined the relationship between brain volumes and cognitive performance measures and most of those that did found no associations (Tzilos *et al.*, 2005; Jager *et al.*, 2007; Medina *et al.*, 2007b; Yücel *et al.*, 2008) or isolated relationships (Medina *et al.*, 2007a; Solowij *et al.*, 2008). An exception to this was the finding of poor white-matter structural integrity being related to poorer cognitive performance in cannabis and alcohol-using adolescents (Bava *et al.*, 2010). The lack of association in most studies might be interpreted as aberrant associations between brain structure and function, as discussed elsewhere (Solowij *et al.*, 2009).

The growing literature reporting an association between cannabis use and the development of psychopathology, including both psychotic and depressive symptoms, has searched for mediators of risk such as genes (eg. *COMT*; Caspi *et al.*, 2005), but associations between the development of psychotic or depressive symptoms and brain changes in cannabis users have not been rigorously investigated. We reported an association between smaller left hippocampal volume in cannabis users and subclinical positive psychotic symptoms as measured by the Scale for the Assessment of Positive Symptoms (SAPS) (Yücel *et al.*, 2008). Positive symptom scores were also correlated with cumulative cannabis exposure. The cannabis users in our sample were carefully screened for DSM-IV Psychotic Disorders, had never been diagnosed with Major Depressive Disorder and had never sought treatment for any psychological disorders. Yet the majority of the sample endorsed beliefs (scores on the SAPS ranged from Questionable to Mild) concerning ideas of persecution, reference, mind reading, sin and/or guilt, while some displayed bizarre clothing/appearance or reported bizarre social/sexual behavior. Smaller left hippocampal volume was also significantly correlated with higher scores on the paranoid subscale of the Brief Symptom Inventory (BSI) (unpublished data). Negative symptoms were elevated in the cannabis users but were unrelated to hippocampal volumes. Depressive symptoms, which were also elevated, did not correlate with volumetric measures of any brain region, and the relationship between left hippocampal volume and cumulative exposure to cannabis remained significant

after controlling for depressive symptoms. An association between depression and hippocampal volume is seen in the more persistent forms of Major Depressive Disorder (eg. MacQueen *et al*., 2005; Lorenzetti *et al*., 2009), which does not apply to our sample. One other study has reported an association between overall brain white-matter volume and depressive symptoms in adolescent/young adult cannabis users without diagnosable mood disorders (Medina *et al*., 2007b).

Subclinical positive psychotic symptom scores in the heavy cannabis users of our sample correlated with spatial span errors, but no other associations between cognitive measures and symptoms were observed (Solowij *et al*., 2008). Skosnik and colleagues (2001, 2006, 2008) have found associations between cognitive (eg. poor negative priming) and psychophysiological measures (e.g. P300 to affective stimuli; 20 Hz neural synchrony), and higher scores on the Schizotypal Personality Questionnaire, on which cannabis users generally obtained high positive-syndrome scores.

Acute administration of THC to healthy volunteers and patients with schizophrenia induces both cognitive impairment and transient positive and negative symptoms (Chapter 18; D'Souza *et al*., 2004, 2005; Koethe *et al*., 2006), and sensitivity to the psychosis-inducing *and* cognitive-impairing effects of cannabis may be genetically mediated (Chapter 12, Henquet *et al*., 2006). In patients with schizophrenia, associations between positive psychotic symptoms and memory deficits, and volumetric measures of the hippocampus, the superior temporal gyrus, and the temporal lobe in general, have been demonstrated, as well as between negative symptoms, executive function and prefrontal cortical measures (Antonova *et al*., 2005; Gur *et al*., 2007; Nestor *et al*., 2007; Hurlemann *et al*., 2008) and in particular, in relation to white matter structural integrity (Szeszko *et al*., 2008). This suggests that further investigation of brain structural changes in cannabis users, in relation to symptoms and cognition, is warranted.

A crucial question is the extent to which interrelated structural-functional aberrations involving the hippocampus, prefrontal regions or indeed other brain structures in cannabis users, might reflect a vulnerability to schizophrenia. Our own findings suggest that long-term exposure to cannabis constitutes a vulnerability to psychopathology by disrupting the structural integrity of brain regions that are also involved in psychotic (and affective) disorders. We propose that long-term heavy cannabis use leads to structural brain changes and associated deleterious functional (cognitive and mental health) sequelae that resemble aspects of schizophrenia. These changes may occur not only in individuals who are vulnerable to the development of such disorders, but also in nonvulnerable individuals if cannabis is used heavily for prolonged periods or commences during crucial neurodevelopment periods such as early adolescence.

Conclusions

Strong evidence for cumulative, sometimes dose-related, neuronal damage or microstructural alterations following chronic exposure to cannabinoids (largely THC) comes from the animal literature. While previous research failed to identify structural brain abnormalities in human cannabis users, more recent studies using high-resolution imaging techniques, combined with more robust delineations of specific brain regions in very heavy cannabis users, have revealed evidence of dose-related alterations, mostly in the hippocampal and parahippocampal regions. Our own findings of significant hippocampal and amygdala volume loss in cannabis users suggest potential toxicity due to cumulative exposure to large doses of cannabis over many years. However, the structural neuroimaging studies of cannabis users have so far focused on a narrow range of brain regions. Cannabis use, particularly during early adolescence, may affect the morphology of other cortical (e.g. PFC) and subcortical (e.g. striatum) brain areas, where cannabinoid receptors are heavily concentrated. Hippocampal changes accord with hippocampal functional alterations in functional imaging studies, which together with evidence of aberrations from spectroscopic and DTI studies, implicate the PFC. Evidence for damage to white-matter integrity in cannabis users implicates neural circuitry across multiple regions. Differences in the methods of measurement used and the brain regions investigated and small sample sizes of varying age and exposure to cannabis, may have contributed to the heterogeneity of findings across human studies overall.

While the evidence is only beginning to accumulate from a small number of studies that have used rigorous methods to investigate structural brain alterations in cannabis users, it seems that long-term heavy cannabis use can result in brain pathophysiological and functional changes that resemble aspects of schizophrenia. The data suggest that such alterations are likely to occur when cannabis is used very heavily over a prolonged period and typically involve medial

temporal lobe structures. The cumulative evidence for neurocognitive dysfunction similar to that seen in schizophrenia and the development of subclinical psychotic symptoms in cannabis users, combines with the limited data from structural neuroimaging studies to support our proposition that chronic cannabis use may result in schizophrenia-like changes in brain structure and function. This is further supported by evidence that long-term exposure to cannabis may result in lasting dysfunction of the endogenous cannabinoid system, as well as alterations in the functionality of a number of neurotransmitter systems – changes that resemble schizophrenia-like conditions in the brain (see Solowij *et al*., 2009). There may be multiple moderators or mediators of adverse sequelae from long-term heavy cannabis use, including genetic variation, gender, environmental factors and early neurodevelopmental insults and stress, that interact with cumulative exposure to high-dose cannabis use to produce schizophrenia-like sequelae. A crucial factor is in determining the parameters of cannabis use that lead to these structural and functional alterations in individuals who are, compared with those who are not, at high risk for the development of neuropsychiatric disorders, at various neurodevelopmental periods, and identifying the protective mechanisms that prevent the onset of such potentially devastating disorders.

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References

Allin, M., Khan, O., Walshe, M., *et al.* (2009) Cannabis smoking and white matter in healthy volunteers. *Schizophr Bull*, **35**:201.

Amal, H., Fridman-Rozevich, L., Senn, R., *et al.* (2010) Long-term consequences of a single treatment of mice with an ultra-low dose of Δ^9-tetrahydrocannabinol (THC). *Behav Brain Res*, **206**:245–53.

Antonova, E., Kumari, V., Morris, R., *et al.* (2005) The relationship of structural alterations to cognitive deficits in schizophrenia: a voxel-based morphometry study. *Biol Psychiatry*, **58**:457–67.

Angelucci, F., Ricci, V., Spalletta, G., *et al.* (2008) Reduced serum concentrations of nerve growth factor, but not brain-derived neurotrophic factor, in chronic cannabis abusers. *Eur Neuropsychopharmacol*, **18**:882–7.

Arnone, D., Barrick, T.R., Chengappa, S., *et al.* (2008) Corpus callosum damage in heavy marijuana use: Preliminary evidence from diffusion tensor tractography and tract-based spatial statistics. *Neuroimage*, **41**:1067–74.

Ashtari, M., Cervellione, K., Cottone, J., *et al.* (2009) Diffusion abnormalities in adolescents and young adults with a history of heavy cannabis use. *J Psychiatr Res*, **43**:189–204.

Bangalore, S.S., Prasad, K.M., Montrose, D.M., *et al.* (2008) Cannabis use and brain structural alterations in first episode schizophrenia – a region of interest, voxel based morphometric study. *Schizophr Res*, **99**:1–6.

Bava, S., Frank, L.R., McQueeny, T., *et al.* (2009) Altered white matter microstructure in adolescent substance users. *Psychiatry Res*, **173**:228–37.

Bava, S., Jacobus, J., Mahmood, O., *et al.* (2010) Neurocognitive correlates of white matter quality in adolescent substance users. *Brain Cogn*, **72**:347–54.

Block, R.I., O'Leary, D.S., Ehrhardt, J.C., *et al.* (2000) Effects of frequent marijuana use on brain tissue volume and composition. *Neuroreport*, **11**:491–6.

Cahn, W., Hulshoff Pol, H.E., Caspers, E., *et al.* (2004) Cannabis and brain morphology in recent-onset schizophrenia. *Schizophr Res*, **67**:305–7.

Campbell, A.M.G., Evans, M., Thomson, J.L.G., *et al.* (1971) Cerebral atrophy in young cannabis smokers. *Lancet*, **298**:1219–24.

Campbell, V.A. (2001) Tetrahydrocannabinol-induced apoptosis of cultured cortical neurones is associated with cytochrome c release and caspase-3 activation. *Neuropharmacology*, **40**:702–9.

Caspi, A., Moffitt, T.E., Cannon, M., *et al.* (2005) Moderation of the effect of adolescent onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene x environment interaction. *Biol Psychiatry*, **57**:1117–27.

Chan, G.C., Hinds, T.R., Impey, S., *et al.* (1998) Hippocampal neurotoxicity of Delta9-tetrahydrocannabinol. *J Neurosci*, **18**:5322–32.

Co, B.T., Goodwin, D.W., Gado, M., *et al.* (1977) Absence of cerebral atrophy in chronic cannabis users: Evaluation by computerized transaxial tomography. *J Am Med Assoc*, **237**:1229–30.

Dalton, V.S. and Zavitsanou, K. (2010) Cannabinoid effects on CB1 receptor density in the adolescent brain: an autoradiographic study using the synthetic cannabinoid HU210. *Synapse*, DOI:10.1002/Syn.20801.

Dekker, N., Schmitz, N., Peters, B.D., *et al.* (2010) Cannabis use and callosal white matter structure and integrity in recent-onset schizophrenia. *Psychiatry Res*, **181**:51–6.

DeLisi, L.E. (2008) The effect of cannabis on the brain: can it cause brain anomalies that lead to increased risk for schizophrenia? *Curr Opin Psychiatry*, **21**:140–50.

DeLisi, L.E., Bertisch, H.C., Szulc, K.U., *et al.* (2006) A preliminary DTI study showing no brain structural change associated with adolescent cannabis use. *Harm Reduct J*, **3**:17.

Downer, E., Boland, B., Fogarty, M., *et al.* (2001) Delta 9-tetrahydrocannabinol induces the apoptotic pathway in cultured cortical neurones via activation of the CB1 receptor. *Neuroreport*, **12**:3973–8.

Downer, E.J. and Campbell, V.A. (2010) Phytocannabinoids, CNS cells and development: A dead issue? *Drug Alcohol Rev*, **29**:91–8.

D'Souza, D.C., Abi-Saab, W.M., Madonick, S., *et al.* (2005) Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biol Psychiatry*, **57**:594–608.

D'Souza, D.C., Perry, E., MacDougall, L., *et al.* (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology*, **29**:1558–72.

D'Souza, D.C., Pittman, B., Perry, E., *et al.* (2009) Preliminary evidence of cannabinoid effects on brain-derived neurotrophic factor (BDNF) levels in humans. *Psychopharmacology*, **202**:569–78.

Egerton, A., Allison, C., Brett, R.R., *et al.* (2006) Cannabinoids and prefrontal cortical function: Insights from preclinical studies. *Neurosci Biobehav Rev,* **30**:680–95.

Ellgren, M., Artmann, A., Tkalych, O., *et al.* (2008) Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence: THC effects. *Eur Neuropsychopharmacol*, **18**:826–34.

Gruber, S.A. and Yurgelun-Todd, D.A. (2005) Neuroimaging of marijuana smokers during inhibitory processing: a pilot investigation. *Cogn Brain Res*, **23**:107–18.

Gur, R.E., Keshavan, M.S. and Lawrie, S.M. (2007) Deconstructing psychosis with human brain imaging. *Schizophr Bull*, **33**:921–31.

Hannerz, J. and Hindmarsh, T. (1983) Neurological and neuroradiological examination of chronic cannabis smokers. *Ann Neurol*, **13**:207–10.

Harkany, T., Keimpema, E., Barabás, K., *et al.* (2008) Endocannabinoid functions controlling neuronal specification during brain development. *Mol Cell Endocrinol*, **286**:S84–90.

Harper, J.W., Heath, R.G. and Myers, W.A. (1977) Effects of *Cannabis sativa* on ultrastructure of the synapse in monkey brain. *J Neurosci Res*, **3**:87–93.

Heath, R.G., Fitzjarrell, A.T., Fontana, C.J., *et al.* (1980) *Cannabis sativa*: effects on brain function and ultrastructure in rhesus monkeys. *Biol Psychiatry*, **15**:657–90.

Henquet, C., Rosa, A., Krabbendam, L., *et al.* (2006) An experimental study of catechol-O-methyltransferase Val158Met moderation of Δ-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology*, **31**:2748–57.

Hermann, D., Sartorius, A., Welzel, H., *et al.* (2007) Dorsolateral prefrontal cortex N-acetylaspartate/total creatine (NAA/tCr) loss in male recreational cannabis users. *Biol Psychiatry*, **61**:1281–89.

Hurlemann, R., Jessen, F., Wagner, M., *et al.* (2008) Interrelated neuropsychological and anatomical evidence of hippocampal pathology in the at-risk mental state. *Psychol Med*, **38**:843–51.

Jacobus, J., McQueeny, T., Bava, S., *et al.* (2009) White matter integrity in adolescents with histories of marijuana use and binge drinking. *Neurotoxicol Teratol*, **31**:349–55.

Jager, G., Van Hell, H.H., De Win, M.M., *et al.* (2007) Effects of frequent cannabis use on hippocampal activity during an associative memory task. *Eur Neuropsychopharmacol*, **17**:289–97.

Kano, M., Ohno-Shosaku, T., Hashimotodani Y., *et al.* (2009) Endocannabinoid-mediated control of synaptic transmission. *Physiology Rev*, **89**:309–80.

Koethe, D., Gerth, C.W., Neatby, M.A., *et al.* (2006) Disturbances of visual information processing in early states of psychosis and experimental delta-9-tetrahydrocannabinol altered states of consciousness. *Schizophr Res*, **88**:142–50.

Kuehnle, J., Mendelson, J.H., Davis, K.R., *et al.* (1977) Computed tomographic examination of heavy marijuana smokers. *J Am Med Assoc*, **237**:1231–2.

Landfield, P.W., Cadwallader, L.B., Vinsant, S. (1988) Quantitative changes in hippocampal structure following long-term exposure to delta 9-tetrahydrocannabinol: possible mediation by glucocorticoid systems. *Brain Res*, **443**:47–62.

Lawston, J., Borella, A., Robinson, J.K., *et al.* (2000) Changes in hippocampal morphology following chronic

treatment with the synthetic cannabinoid WIN 55,212–2. *Brain Res*, **877**:407–10.

Lorenzetti, V., Allen, N.B., Fornito, A., *et al.* (2009) Structural brain abnormalities in major depressive disorder: A selective review of recent MRI studies. *J Affect Disord*, **117**:1–17.

Lorenzetti, V., Lubman, D.I., Whittle, S., *et al.* (2010) Structural MRI findings in cannabis users: what do we know? *Subst Use Misuse*, **45**: 1787–808.

MacQueen, G.M., Campbell, S., McEwen, B.S., *et al.* (2005) Course of illness, hippocampal function, and hippocampal volume in major depression. *Proc Nat Acad Sci USA*, **100**:1387–92.

Martin-Santos, R., Fagundo, A.B., Crippa, J.A., *et al.* (2010) Neuroimaging in cannabis use: a systematic review of the literature. *Psychol Medi*, **40**:383–98.

Mata, I., Perez-Iglesias, R., Roiz-Santiañez, R., *et al.* (2010) Gyrification brain abnormalities associated with adolescence and early-adulthood cannabis use. *Brain Res*, **1317**:297–304.

Matochik, J.A., Eldreth, D.A., Cadet, D.A., *et al.* (2005) Altered brain tissue composition in heavy marijuana users. *Drug Alcohol Depend*, **77**:23–30.

Medina, K.L., Nagel, B.J., Park, A., *et al.* (2007a) Depressive symptoms in adolescents: associations with white matter volume and marijuana use. *J Child Psychol Psychiatry*, **48**, 592–600.

Medina, K.L., Schweinsburg, A.D., Cohen-Zion, M., *et al.* (2007b) Effects of alcohol and combined marijuana and alcohol use during adolescence on hippocampal volume and asymmetry. *Neurotoxicol Teratol*, **29**:141–52.

Monnet-Tschudi, F., Hazekamp, A., Perret, N., *et al.* (2008) Delta-9-tetrahydrocannabinol accumulation, metabolism and cell-type-specific adverse effects in aggregating brain cell cultures. *Toxicol Appl Pharmacol*, **228**:8–16.

Myers, W.A. and Heath, R.G. (1979) *Cannabis sativa*: ultrastructural changes in organelles of neurons in brain septal region of monkeys. *J Neurosci Res*, **4**:9–17.

Nestor, P.G., Kubicki, M., Kuroki, N., *et al.* (2007) Episodic memory and neuroimaging of hippocampus and fornix in chronic schizophrenia. *Psychiatry Res*, **155**:2128.

Potvin, S., Mancini-Marie, A., Fahim, C., *et al.* (2007) Increased striatal gray matter densities in patients with schizophrenia and substance use disorder: a voxel-based morphometry study. *Psychiatry Res*, **154**:275–9.

Quinn, H.R., Matsumoto, I., Callaghan, P.D., *et al.* (2008). Adolescent rats find repeated Δ^9-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology*, **33**:1113–26.

Rais, M., Cahn, W., Van Haren, N., *et al.* (2008) Excessive brain volume loss over time in cannabis-using first-episode schizophrenia patients. *Am J Psychiatry*, **165**:490–6.

Realini, N., Rubino, T. and Parolaro, D. (2009) Neurobiological alterations at adult age triggered by adolescent exposure to cannabinoids. *Pharmacol Res*, **60**, 132–8.

Rubino, T., Realini, N., Braida, D, *et al.* (2009a) Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus*, **19**:763–72.

Rubino, T., Realini, N., Braida, D., *et al.* (2009b) The depressive phenotype induced in adult female rats by adolescent exposure to THC is associated with cognitive impairment and altered neuroplasticity in the prefrontal cortex. *Neurotox Res*, **15**:567–607.

Rubino, T., Viganò, D., Realini, N., *et al.* (2008) Chronic delta(9)-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. *Neuropsychopharmacology*, **33**:2760–71.

Sarne, Y. and Keren, O. (2004) Are cannabinoid drugs neurotoxic or neuroprotective? *Med Hypotheses*, **63**:187–92.

Sarne, Y. and Mechoulam, R. (2005) Cannabinoids: between neuroprotection and neurotoxicity. *Curr Drug Targets CNS Neurol Disord*, **4**:677–84.

Scallet, A.C., Uemura, E., Andrews, A., *et al.* (1987) Morphometric studies of the rat hippocampus following chronic delta-9-tetrahydrocannabinol (THC). *Brain Res*, **436**:193–8.

Schneider, M. (2008) Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addict Biol*, **13**:253–63.

Skosnik, P.D., Grishnan, G.P. and Aydt, E.E. (2006) Psychophysiological evidence of altered neural synchronization in cannabis use: relationship to schizotypy. *Am J Psychiatry*, **163**:1798–805.

Skosnik, P.D., Park, S., Dobbs, L., *et al.* (2008) Affect processing and positive syndrome schizotypy in cannabis users. *Psychiatry Res*, **157**:279–82.

Skosnik, P.D., Spatz-Glenn, L. and Park, S. (2001) Cannabis use is associated with schizotypy and attentional disinhibition. *Schizophr Res*, **48**:83–92.

Solowij, N., Yücel, M., Lorenzetti, V., *et al.* (2009) Structural brain alterations in cannabis users: Association with cognitive deficits and psychiatric symptoms. In Ritsner, M.S. (Ed) *The Handbook of Neuropsychiatric Biomarkers, Endophenotypes, and Genes. Vol. II Neuroanatomical and Neuroimaging Endophenotypes and Biomarkers*, (pp. 215–225). Dordrecht: Springer.

Solowij, N., Respondek, C., Battisti, R., *et al.* (2008) Visuospatial memory deficits in long term heavy cannabis users: relation to psychotic symptoms and regional brain volumes. *Int J Neuropsychopharmacol*, **11**:242.

Solowij, N., Walterfang, M., Respondek, C., *et al.* (2010) Alterations of hippocampal shape in cannabis users with and without schizophrenia. *Schizophr Res*, **117**:426.

Solowij, N., Yücel, M., Respondek C., *et al.* (2011) Cerebellar white matter changes in cannabis users with and without schizophrenia. *Psychol Med*. DOI: 10.1017/S003329171100050X.

Stefanis, C. (1976) Biological aspects of cannabis use. In Petersen, R.C., ed. *The International Challenge of Drug Abuse*, 149–178. Rockville, MD: National Institute on Drug Abuse.

Szeszko, P.R., Robinson, D.G., Sevy, S., *et al.* (2007) Anterior cingulate grey-matter deficits and cannabis use in first-episode schizophrenia. *Brit J Psychiatry*, **190**:230–6.

Szeszko, P.R., Robinson, D.G., Ashtari, M., *et al.* (2008) Clinical and neuropsychological correlates of white matter abnormalities in recent onset schizophrenia. *Neuropsychopharmacology*, **33**:976–84.

Thatcher, D.L., Pajtek, S., Chung, T., *et al.* (2010) Gender differences in the relationship between white matter organization and adolescent substance use disorders. *Drug Alcohol Depend*, **110**:55–61.

Tselnicker, I., Keren, O., Hefetz, A., *et al.* (2007) A single low dose of tetrahydrocannabinol induces long-term cognitive deficits. *Neurosci Lett,* **411**:108–11.

Tzilos, G.K., Cintron, C.B., Wood, J.B., *et al.* (2005) Lack of hippocampal volume change in long-term heavy cannabis users. *Am J Addict*, **14**:64–72.

Villares, J. (2007) Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. *Neuroscience*, **145**:323–34.

Wilson, W., Mathew, R., Turkington, T., *et al.* (2000) Brain morphological changes and early marijuana use: a magnetic resonance and positron emission tomography study. *J Addict Dis*, **19**:1–22.

Yücel, M., Bora, E., Lubman, D.I., *et al.* (2010) The impact of cannabis use on cognitive functioning in patients with schizophrenia: A meta-analysis of existing findings and new data in a first-episode sample. *Schizophr Bull*, DOI:10.1093/schbul/sbq079.

Yücel, M., Solowij, N., Respondek, C., *et al.* (2008) Regional brain abnormalities associated with long-term heavy cannabis use. *Arch Gen Psychiatry*, **65**:694–701.

Yücel, M., Zalesky, A., Takagi, M.J., *et al.* (2010) Age-related white-matter abnormalities in long-term inhalant misusing adolescents: a diffusion MRI comparison with cannabis users and drug-naive controls. *J Psychiatry Neurosci*, **35**:409–12.

Zavitsanou, K., Wang, H., Dalton, V.S., *et al.* (2010) Cannabinoid administration increases 5HT1 receptor binding and mRNA expression in the hippocampus of adult but not adolescent rats. *Neuroscience*, **169**:315–24.

Chapter

10

The association between cannabis use and depression: a review of the evidence

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The association between cannabis and depression has received less attention than the links between cannabis use and psychosis (Degenhardt *et al*., 2003; Degenhardt and Hall, 2006; Di Forti *et al*., 2007). Over past decades, however, rising rates of cannabis use (Donnelly and Hall, 1994; Hall *et al*., 1999; Degenhardt *et al*., 2000; Johns, 2001), depression (Andrews *et al*., 1998; Cicchetti and Toth, 1998) and suicide (Diekstra *et al*., 1995; Lynskey *et al*., 2000) among young adults have increased public concern about the possible role of drug use, including cannabis, in depression and other non-psychotic mental disorders. There has also been increasing advocacy for interventions to prevent and treat problematic cannabis use and depressed mood among young people. This chapter evaluates the nature of the relationship between cannabis use and depression by addressing the following questions:

1. Is there evidence of an association between cannabis use and depression?
2. If there is, what are the potential explanations for the association?
3. What evidence is needed to test these different explanations?
4. What are the public health implications of the evidence to date?

Comorbidity between cannabis use and depression

Within psychiatry, comorbidity is commonly used to refer to the overlap of two or more psychiatric disorders (Boyd *et al*., 1984). However, as shall become apparent in the following review, much of the research examining associations between cannabis use and depression has studied relatively infrequent, low-level cannabis use.

Clinical samples

Case histories reporting associations between cannabis use and mood have long been reported in clinical literature (Pond, 1948; Ablon and Goodwin, 1974). Some cases report persons who develop manic symptoms after using cannabis (Stoll *et al*., 1991; Bowers, 1998); others have reported cannabis being used as an antidepressant (Zelwer, 1994), while others have reported persons with mania or bipolar disorder using cannabis to *moderate* their manic symptoms (Grinspoon and Bakalar, 1998). The reader is referred to Chapter 11 for a more detailed discussion of cannabis and bipolar disorder.

There is little literature on cannabis use in clinical populations with affective disorders. In one study of depressed outpatients, a history of substance-use disorders was associated with a greater number of depressive episodes (Alpert *et al*., 1994) but there was no difference in the age of onset or in severity of depression at assessment (Alpert *et al*., 1994). Studies of patients with bipolar disorder (Estroff *et al*., 1985; Miller *et al*., 1989; Brady *et al*., 1991; Marken *et al*., 1992; Mueser *et al*., 1992; Sonne *et al*., 1994) have found rates of problematic cannabis use between 3% (Sonne *et al*., 1994) and 19% (Marken *et al*., 1992). Among samples of heroin-dependent persons in methadone treatment, daily cannabis users reported the highest rates of depression (compared with occasional and non-users of cannabis) (Bell *et al*., 1995; Best *et al*., 1999).

Among people presenting for treatment of cannabis use, there are elevated rates of depressive symptoms (White *et al*., 2004; Diamond *et al*., 2006; Konings and

Marijuana and Madness, Second Edition, ed. David Castle, Robin M. Murray and Deepak Cyril D'Souza. Published by Cambridge University Press.

Maharajh, 2006). Among adolescents receiving outpatient treatment for problem cannabis use for example, rates of depression range from 24 to 50% (Kaminer *et al.*, 2008). Some studies have suggested cannabis-dependent people with comorbid depression have poorer outcomes when treated for their cannabis, compared with those who do not have depression (White *et al.*, 2004).

Representative samples of the general population

Clinical samples are ill-suited to examining the question of whether comorbidity exists between cannabis use and depression as it is not possible to distinguish between "artefactual" comorbidity and "true" comorbidity (Caron and Rutter, 1991). *Artefactual* comorbidity arises because of the ways in which participants are selected, or the behavior is conceptualized, measured and classified. *True* comorbidity refers to the actual co-occurrence of two separate conditions at a rate higher than expected by chance.

There are a number of reasons, related to sampling biases, which make artefactual comorbidity more likely in research in clinical populations. The first is Berkson's bias (Berkson, 1946): persons who have two disorders at a given point in time are more likely to receive treatment because there are two separate disorders for which they might seek help (Roberts *et al.*, 1978). The second reason is clinical bias (Galbaud Du Fort *et al.*, 1993): persons who have two disorders may be more likely to seek treatment *because* they have two disorders (Galbaud Du Fort *et al.*, 1993). Third, referral biases may exist, whereby persons are referred for treatment because of other background factors, such as having a family history of psychopathology, which makes it more likely that they will have a number of different mental health problems (Caron and Rutter, 1991).

In order to minimize the effects of sampling and selection biases it is best to study the patterns of association between cannabis use and depression in representative samples of the general population (Berkson, 1946; Caron and Rutter, 1991; Galbaud Du Fort *et al.*, 1993). A number of large-scale surveys have examined associations between substance-use disorders (including cannabis) and other mental disorders in the US and other developed countries.

Cross-sectional studies

Data from one of the major US epidemiological surveys, the National Comorbidity Survey (NCS), have been analyzed to explore the association between cannabis use and major depressive episodes (Chen *et al.*, 2002). The more often cannabis had been used, the higher the risk of having experienced a major depressive episode. Persons with a lifetime DSM-III-R diagnosis of cannabis dependence were 3.4 times more likely to have a diagnosis of major depression, and 9.5% of those who had experienced a major depressive episode met criteria for cannabis dependence, compared with 4% of those who had not (Chen *et al.*, 2002).

Grant and colleagues found that persons in the US meeting criteria for DSM-IV cannabis abuse or dependence within the prior year were 6.4 times more likely to meet criteria for DSM-IV major depressive disorder than those without cannabis abuse (29% and 14%, compared with 3% in the whole sample) (Grant, 1995). Similar but weaker associations were found in the Canadian CAMH Monitor Survey conducted from 2001–2006: daily cannabis users were about twice as likely to meet criteria for anxiety and mood disorders than non-users (Cheung *et al.*, 2010).

Degenhardt and colleagues examined the relationship between different levels of cannabis use (no use, use, abuse or dependence) and depression in the Australian National Survey of Mental Health and Wellbeing (Degenhardt *et al.*, 2001). Cannabis users were between two to three times more likely to meet criteria for a mood disorder than non-users and the prevalence of these disorders increased from 6% among non-users to 14% among those who met criteria for cannabis dependence. Similarly, research on drug use and mental disorders in a representative sample of Australians aged 13–17 years found that those who had used cannabis were three times more likely than those who had not, to meet criteria for depression (Rey *et al.*, 2002).

Prospective cohort studies

One recent study examined this issue using multistage samples of adults surveyed in 17 countries across the globe (De Graaf *et al.*, 2010). Specifically, the analyses examined the association between early-onset (age < 17 years) cannabis use with later-onset (age ≥ 17 years) risk of depression, using data on 85 088 participants collected using structured diagnostic interviews, from 17 countries of the World Health Organization World Mental Health Survey Initiative (2001–2005). The overall association was moderate (relative risk [RR] 1.5, 95% confidence intervals [CI]: 1.4, 1.7, after adjusting for age and sex), with no sex differences; stronger associations were found in older age groups. The association

remained after controlling for other mental disorders apart from childhood conduct disorder (which abolished the association). Interestingly, country-level analyses showed that the association was found in only 5 of the 17 countries (Germany, Ukraine, Nigeria, South Africa, New Zealand). Although this study looked at the order of onset of cannabis use and depressive episodes and, therefore, attempted to address temporality of the associations, the weaknesses of this study are, of course, that participants were being asked to recall events that, for many, occurred quite some time previously. Furthermore, there was no capacity to examine different levels of cannabis use since frequency was not measured in the survey.

A range of prospective cohort studies have been able to examine the temporality of the association and consider frequency of cannabis use, while measuring both cannabis use and depression much more recently. Fergusson and colleagues examined the association between cannabis use and major depression using data from a birth cohort of 1265 children born in mid-1977 in Christchurch, New Zealand (Fergusson and Horwood, 1997; Fergusson *et al*., 2002). They found that adolescents who had used cannabis ten or more times by the age of 15–16 years were more likely to meet criteria for a mood disorder at that age: 11% of those who had never used cannabis compared with 18% of those who had used cannabis one to nine times, and 36% of those who had used it ten or more times (Fergusson and Horwood, 1997). At age 20–21 years, 30% of those who were using cannabis at least weekly met criteria for depression, compared with 15% of those who were not using cannabis at that age (Fergusson *et al*., 2002).

Similarly, the Zurich cohort study of young people (assembled when they were 20 years of age) found that by age 30 years, those who met criteria for depression over the period of the study were 2.3 times more likely to report weekly cannabis use during this time (Angst, 1996).

A study by Patton and colleagues, using a representative cohort of young adults (aged 20–21 years) in Australia, found that 68% of females who reported daily cannabis use in the prior year were depressed – an odds of 8.6 compared with non-users (Patton *et al*., 2002). No other level of cannabis use was associated with an increased risk of depression. Among males there was *no* association between cannabis use in the prior year and depression (Patton *et al*., 2002).

In one cohort of American adolescents, those who had experimented with cannabis, reported *better* social adjustment than those who had never used cannabis and those who were heavy cannabis users (Shedler and Block, 1990). This U-shaped curve needs to be considered within its social and historical context. Because this cohort had very high rates of cannabis use, the authors suggested that those who had never tried cannabis had poorer social adjustment, more anxiety and emotional constriction than those who experimented (Shedler and Block, 1990).

Other longitudinal studies have reported conflicting results. Brook and colleagues (Brook *et al*., 1998) found no relationship between cannabis use and DSM-III-R depressive disorders over 10 years of follow-up from adolescence to young adulthood. By contrast, a study of students aged 12–14 years found that those reporting lifetime cannabis use had higher depression scores, and 42% met criteria for DSM-IV major depression at some point in their lives (Kelder *et al*., 2001). Cohort studies examining adolescent cannabis use and young adult depression, conducted in Ontario (Georgiades and Boyle, 2007), have also failed to find an association.

Does cannabis use increase the risk of suicide?

Some have suggested that cannabis use may be a contributory cause of suicidal behaviors (Holden and Pakula, 2001; Johns, 2001; Maharajh and Konings, 2005). This causal hypothesis might explain apparent parallel increases in cannabis use (Degenhardt *et al*., 2000) and suicide among young males (Diekstra *et al*., 1995; Lynskey *et al*., 2000).

However, few studies have examined this issue. One study of Italian university students found no association between frequency of cannabis use and suicidal ideation (Innamorati *et al*., 2008). A systematic review described four studies of the association between cannabis use and suicide (two cohort and one case–control) (Calabria *et al*., 2010). One study found that after adjustment for background factors, neither DSM-III-R cannabis abuse nor dependence was associated with medically serious suicide attempts, defined as requiring hospitalization for more than 24 hours and fulfilling one of three treatment options (specialized unit treatment, surgery under general anesthesia or other medical treatment as specified in the article) (Beautrais *et al*., 1999).

A number of studies have found associations between cannabis use and suicide, but the quality of

control for confounding variables has varied widely. Ever-use of cannabis was found to be associated with an increased risk of completed suicide in one study (Kung *et al.*, 2003). In a school sample, early-onset cannabis use marginally increased the risk of a suicide attempt (Wilcox and Anthony, 2004) but potential confounding variables that are strongly related to suicide were not controlled for (namely depression and alcohol use). Fergusson and colleagues found significant associations between annual cannabis use and suicidal ideation, when attempting to control for fixed- and time-dynamic confounding factors (Fergusson *et al.*, 2002).

Data from a population-based sample of 2033 Norwegians followed up over a 13-year period from adolescence to young adulthood, found that there were no associations between early onset cannabis use and suicidal ideation or suicide attempts in young adulthood (Pedersen, 2008). There were cross-sectional associations in young adulthood.

Summary

There is increasing evidence that regular cannabis use and depression occur together more often than we might expect by chance. Systematic reviews have been conducted on these longitudinal studies, including some meta-analyses. The consensus is that the evidence linking cannabis use with later depression is less consistent and provides less convincing evidence for a causal role than for psychotic symptoms and disorders (Arseneault *et al.*, 2004; Moore *et al.*, 2007; McLaren *et al.*, 2008; and see Chapter 15). However, there are enormous methodological variations in age, sampling and level of exposure to cannabis (see later discussion). Recent reviews have concluded that there is, as yet, insufficient evidence to make inferences about whether there is a causal relationship between cannabis use and suicide (Hall and Degenhardt, 2009; Calabria *et al.*, 2010).

What explains the association between cannabis use and depression?

There are a number of reasons why cannabis use and depression might be associated. The most commonly studied classes of explanation for comorbidity can be broadly classified into three categories (Caron and Rutter, 1991; Klein and Riso, 1994; Kessler, 1995; Neale and Kendler, 1995): (1) cannabis use may be a contributory cause of depression (e.g. cannabis use precipitates depression); (2) depression may be a contributory cause of cannabis use (e.g. if a depressed person uses cannabis to improve their mood); and (3) there is no direct relationship between the two with the association explained by shared risk factors that increase the risk of both disorders.

Cannabis use causes depression

Heavy cannabis use could precipitate depression in at least two ways: (1) cannabis intoxication could produce depression indirectly by impairing psychological adjustment; or (2) large doses of the active ingredient of cannabis, Δ^9-tetrahydrocannabinol (THC) could affect serotonin and other neurotransmitters in ways that produce depressive symptoms.

Popular concerns about the effects of cannabis use on depression often implicitly assume the second of these hypotheses. Nonetheless, this is not the only potential mechanism for a causal link between cannabis use and depression. Cannabis use may lead to a cascade of life events such as early school leaving, early unplanned parenthood and reduced earning capacity that predispose to developing depression in young adulthood.

Evidence in support of either forms of this hypothesis would include evidence from controlled studies: (1) that cannabis or THC worsens or does not improve mood; (2) that persons who use cannabis in adolescence are more likely to develop depression during early adulthood; (3) that persons who are depressed at baseline are *no* more likely to become cannabis users during a follow-up period; and (4) that associations between cannabis use and depression are not explained by potentially confounding variables.

Depression causes cannabis use

Most advocates of the notion that depression leads to cannabis use invoke the self-medication hypothesis: that persons who are depressed use cannabis to improve their depressive mood (Mueser *et al.*, 1998). The self-medication hypothesis would be supported by evidence from controlled studies that: (1) cannabis or THC improve mood; (2) persons who are depressed at baseline are more likely to begin, continue or increase their cannabis use during follow-up; (3) persons who are cannabis users at baseline are no more likely to become depressed during follow-up; and (4) the associations in (2) are not explained by confounding variables.

Research on self-reported reasons for substance use has provided some support for this idea (Warner *et al*., 1994). Nonetheless, it can be argued that alleviating dysphoria is simply one among many factors – such as poor social skills, poor social functioning and peer group influences – that increase the likelihood of developing both substance use and mental disorders (Mueser *et al*., 1998).

A recent study of cannabis-dependent people examined the reasons for, and reactions to, cannabis among those with lifetime depression, with those who had no such history (Arendt *et al*., 2007). Participants who had experienced lifetime depression reported using cannabis for the same reasons as those without such a history. People with a history of depression were more likely to report experiencing depression, sadness, anxiety and paranoia when under the effects of cannabis, and less likely to report euphoria, than those without a history of depression (Arendt *et al*., 2007).

Common factors increase the risk of both depression and cannabis use

The association between cannabis use and depression may arise because the same factors that predispose people to use cannabis also increase their risk of depression (Caron and Rutter, 1991; Kessler, 1995; Mueser *et al*., 1998). These common factors might include biological, personality, social and environmental factors, or a combination of all of these.

This is a plausible hypothesis because there is a wealth of evidence that mental and substance-use disorders share common risk factors. For example, social disadvantage is more common among persons who are problem substance users (Institute of Medicine, 1996) and persons who meet criteria for depressive disorders (Weissman *et al*., 1991; Kessler *et al*., 1994; Blazer, 1995). There are also higher rates of separation and divorce, and lower rates of being married or in defacto relationships among persons with mental and substance use disorders (Jablensky *et al*., 1991; Weissman *et al*., 1991; Kessler *et al*., 1994; Blazer, 1995). Other factors that have been associated with both cannabis-use disorders and depression include parental psychiatric illness and family dysfunction (Rutter, 1987; Velez *et al*., 1989; Fergusson *et al*., 1990; Fergusson *et al*., 1994).

If common risk factors explain the association between cannabis use and depression then they would no longer be associated after these risk factors were taken into account. This explanation would be supported by evidence from controlled studies that: (1) the administration of cannabis or THC does not affect mood; (2) there is no temporal relationship between cannabis use and depressed mood (i.e. that cannabis use does not predict depression at a later point in time, and vice versa); and (3) the association between cannabis use and depression in epidemiological studies did not persist after statistical control for "confounding" or "common" risk factors.

A review of relevant evidence

Studies of the effects of cannabis use upon mood: "biological plausibility"

In surveys, cannabis users often report increased well-being, euphoria and contentment after using cannabis (Hall *et al*., 2001; Hall and Degenhardt, 2009), but controlled studies have been inconsistent. One study found that cannabis had no effect upon mood in experienced cannabis users, while significantly worsening mood in inexperienced users (Mathew *et al*., 1989). Controlled studies of persons with depression have found that THC significantly increased dysphoria (Pond, 1948; Ablon and Goodwin, 1974), while another found that THC did not improve depressed mood in a small sample of severely depressed inpatients (Kotin *et al*., 1973). In contrast, a more recent prospective study of a cohort of people with psychotic disorders found that there was no relationship between the number of days cannabis was used in 1 month and the level of depressive symptomatology in the following month (or vice versa) (Degenhardt *et al*., 2007).

More recent animal research examining the effect of cannabinoid agonists and endocannabinoid enhancers has suggested that they have an *antidepressant* effect (Ashton *et al*., 2005; Vinod and Hungund, 2006; Leweke and Koethe, 2008; Rodriguez Bambico *et al*., 2009). Specifically, cannabinoid agonists and endocannabinoid enhancers increase serotonin and noradrenergic activity, and the CB1 antagonist, rimonabant, *increases* the risk of depression and suicidality. These findings support the hypothesis that the endocannabinoid system is related to mood (Bambico and Gobbi, 2008).

Recent reviews have suggested that there may be specific points during the lifespan, in particular during adolescence (puberty) – when changes in endocannabinoid activity (caused by THC) might have more long-lasting effects in brain functions and behavior (Sundram, 2006; Schneider, 2008; Malone *et al*., 2010).

Such a possibility would be consistent with the increasing evidence that the associations between cannabis use and depression are stronger when cannabis use begins during adolescence.

Cross-sectional surveys of the general population

Cross-sectional surveys can employ multivariate statistical analysis to examine whether common factors explain any of the observed association between cannabis use and depression. In the Australian National Survey of Mental Health and Wellbeing, for example, the relationship between cannabis use and depression among adults did not remain significant in multiple regression analyses that adjusted for potential confounders (Degenhardt *et al*., 2001). Specifically, the relationship disappeared after controlling for alcohol, tobacco and other drug use, and for neuroticism. This suggests that the association arose because cannabis users were more likely to: meet criteria for an alcohol use disorder; to smoke tobacco regularly; to use other drug types; and to have higher neuroticism scores.

In the Australian child and adolescent survey, the increased risks of depression among lifetime cannabis users remained significant after statistical adjustment for confounders, but the risk was reduced to two and the lower limit of the 95% CI was close to one (Rey *et al*., 2002). Among those who had used cannabis ten or more times in the previous month, this association was stronger, with a threefold increase in risk of depression (Rey *et al*., 2001).

In a sample of adult males, a weak association between early initiation of cannabis use and depression was not significant, after controlling for educational attainment, marital status, alcohol and tobacco use (Green and Ritter, 2000). Similarly, other research has found that associations between cannabis use and depression were no longer significant after accounting for demographic factors and other drug use (Rowe *et al*., 1995). Finally, as noted above, analyses of data from the World Mental Health Survey found that an association between early-onset cannabis use and later depression did not remain after adjusting for childhood conduct problems (De Graaf *et al*., 2010).

The use of longitudinal research to examine questions about causality

Longitudinal studies provide better information on the nature of relationships between cannabis use and depression (Caron and Rutter, 1991; Merikangas and Angst, 1995). Evidence from such studies is reviewed in the following two sections: first, whether depression at one point in time predicts later cannabis use; and second, whether cannabis use at one point in time predicts later depression. In each case, the "common cause" hypothesis is examined by multivariate adjustment for confounders.

Does cannabis use predict later depression?

Among the earliest work is that of Kandel and colleagues, who followed up a cohort of adolescents in New York State (Kandel *et al*., 1986). They found that cannabis use at age 15–16 years was *not* associated with depressive symptoms at age 24–25 years. However, greater involvement with cannabis was associated with a lower degree of life satisfaction and a higher chance of consulting a mental health professional or being hospitalized for a psychiatric disorder (Kandel, 1984). A study of a birth cohort from Dunedin, New Zealand found that cannabis use by age 15 years was *not* associated with an increased risk of a mental disorder (depression, anxiety disorders, substance dependence or antisocial personality disorder) at age 18 years (McGee *et al*., 2000).

The most comprehensive examination of the "common cause" hypothesis has been conducted by Fergusson and colleagues, who controlled for a wide range of possible confounding variables collected on a cohort followed from birth to young adulthood (Fergusson and Horwood, 2001). In an early report, the use of cannabis ten or more times by age 15–16 years was *not* associated with either major depression or suicide attempts at age 16–18 years, after controlling for the effects of confounding individual, familial, peer and sociodemographic variables (Fergusson and Horwood, 1997).

Fergusson and colleagues re-examined the association between cannabis use during adolescence and depression, suicidal ideation and suicide attempts at age of 21 years (Fergusson *et al*., 2002). They explored the effects of heavier patterns of cannabis use than in their earlier study. At age 20–21 years, 30% of those using cannabis weekly or more often met criteria for depression, compared with 15% of those who did not use cannabis at that age. A fixed effects regression that adjusted for sociodemographic and individual factors, adverse life events, peer affiliation, school and home leaving age, and alcohol dependence substantially reduced the association; however significant associations remained between cannabis use during adolescence and depression, suicidal ideation and suicide attempts in the same

year. After adjustment, weekly or more frequent cannabis use in a given year was associated with a 1.7 times greater risk of depression in the same year. For suicidal ideation and suicide attempts, there was an interaction between cannabis use and age: the association between weekly cannabis use in a given year and suicidal ideation/attempts in the same year was highest among those aged 14–15 years. This association declined as the cohort aged, so that by 20–21 years there was no significant association between these outcomes and weekly cannabis use (Fergusson *et al.*, 2002).

Similar findings were obtained from an Australian cohort of adolescents who were followed up into young adulthood. This study examined the link between early onset *regular* cannabis use and early adulthood depression (Patton *et al.*, 2002). It found that among *females only*, weekly cannabis use in adolescence predicted a twofold increase in rates of depression at 20–21 years, while daily use predicted a fourfold increased risk. This was after adjusting for confounding factors including sociodemographic variables, alcohol use, gender and antisocial behavior. Similarly, analyses of data from a birth cohort in Queensland found that early-onset, regular cannabis use was associated with anxiety and depression in young adulthood, an association that persisted after adjustment for a range of covariates (Hayatbakhsh *et al.*, 2007).

One study used both individual and group approaches to analyze data from a longitudinal cohort across adolescence (Fleming *et al.*, 2008). Multiple-group latent-growth curve models were used to assess relationships during adolescence and how this differed by sex, using data from annual interviews from around 13–17 years of age. Depressive symptoms and drug use in early adolescence were positively associated with all types of drug use for girls; but only with cannabis use among boys. Increases in depressive symptoms across adolescence were positively associated with all types of drug use (Fleming *et al.*, 2008).

One study failed to find any such associations. A potentially important feature of this sample was that the period of follow-up was during adulthood only (Harder *et al.*, 2006). Slightly elevated odds of depression among cannabis users were no longer significant after adjustment for covariates (using propensity score weights). There was a failure to find an association in analyses that examined only heavy cannabis users, stratifying for sex or age; and using a 4-year lag between cannabis use and depression. The authors concluded that after adjusting for differences in baseline risk factors, past-year cannabis use did not predict depression (Harder *et al.*, 2006).

Two other prospective studies examining the relationship between cannabis use and depression in adulthood *did* find predictive associations (Bovasso, 2001; van Laar *et al.*, 2007). One study used data from a follow-up of the Baltimore site of the Epidemiologic Catchment Area (ECA), in which a subsample of 1920 people were re-assessed 14–16 years later (Bovasso, 2001). Those who reported cannabis use and at least one symptom of cannabis abuse/dependence at baseline were 4.5 times more likely to report depressive symptoms, and 4.6 times more likely to report suicidal ideation during follow-up than those who were "non-abusers." This relationship remained after adjusting for baseline depressive symptoms and demographic variables (Bovasso, 2001). Approximately 4% of those who reported depressive symptoms during the follow-up period met criteria for cannabis abuse at baseline, compared with 1% of those who did not report depressive symptoms.

The other adult prospective study used data from the Netherlands Mental Health Survey and Incidence Study (NEMESIS) (van Laar *et al.*, 2007). A total of 3881 people without mood disorders at baseline were included, and cannabis use was measured at baseline. After adjustment for confounders, cannabis use at baseline predicted a modest increase in incident depression (odds ratio [OR] = 1.62; 95% CI: 1.06, 2.48), with a stronger effect for bipolar disorder (OR = 5.0; 95% CI: 1.8, 13.8). Risk appeared to be elevated primarily among heavy users.

Does depression predict later cannabis use?

A number of longitudinal studies of representative samples of children and adolescents, or birth cohorts, have examined the association between depression and later cannabis use. In general, with a few exceptions these studies have failed to find a significant association (Wittchen *et al.*, 2007; Marmorstein *et al.*, 2010).

Paton and colleagues found no significant relationship between depressive mood and cannabis use either cross-sectionally or prospectively (over 6 months of follow-up) in a cohort of adolescents (16–17 years) from New York State (Paton *et al.*, 1977). However, they found that depressed mood was related to the *onset* of cannabis use among those who had not used it previously (Paton *et al.*, 1977). In a later analysis, Kandel and colleagues found that depression at age 16–17 years was not associated with higher rates of cannabis use at age

24–25 years (Kandel and Davies, 1986). Indeed, males with depression at the first assessment were *less* likely to have used cannabis than those without a history of depression. Later analyses of this cohort revealed that at age 34–35 years, depression at age 15–16 years was *not* associated with either early-onset or current-heavy cannabis use (Kandel and Chen, 2000).

A study of a cohort of African American students followed from grade 6 to grade 10 found that depression in 6th grade was not associated with subsequent cannabis use (Miller-Johnson *et al*., 1998). Similarly, a study of a cohort of Dutch children found that depression did not predict later substance dependence (including cannabis) (Hofstra *et al*., 2002). The Dunedin, New Zealand, birth-cohort study analyzed the relationships between depression at age 15 years and alcohol or cannabis dependence at age 21 years in females (Bardone *et al*., 1998). There was no significant association between the early onset depression and later cannabis dependence, with or without statistically controlling for covariates.

A longitudinal study of children with prepubertal major depression found that there was no significant association with drug abuse or dependence by the time they were in their mid to late 20s (Weissman *et al*., 1999). The same results were found by Brook and colleagues when they analyzed the association between adolescent depression and later cannabis use (ranging from "light" to "heavy"), after controlling for age and gender (Brook *et al*., 1998). Patton and colleagues analyzed the strength of association between depression at ages 14–18 years, and use of cannabis either weekly or daily at age 20–21 years (Patton *et al*., 2002). There was no significant relationship between adolescent depression and weekly or daily cannabis use in young adulthood, after adjusting for sociodemographic variables, alcohol use, gender, adolescent cannabis use and antisocial behavior. The Bovasso study, cited above, also found that among those who did not meet criteria for cannabis abuse at baseline, depressive symptoms at baseline did not predict an increased risk of cannabis abuse during follow-up (Bovasso, 2001).

One published study reported contradictory results. A 10-year prospective study of participants followed up from 14–17 years, to ages 24–27 years, found that in both cross-sectional and prospective analyses, mood disorders *were* associated with later cannabis use and cannabis use disorders, even after controlling for other mental disorders (Wittchen *et al*., 2007).

In summary, the results of longitudinal studies on this issue have not been consistent, but the majority have found that *regular* early-onset cannabis use is associated with an increased risk of later depression. A recent systematic review of longitudinal population-based studies found inconsistent evidence of an association between any level of cannabis use and later depression (Moore *et al*., 2007); a meta-analysis of those studies that measured more frequent or problematic cannabis use (Brook *et al*., 1998; Newcomb *et al*., 1999; Fergusson *et al*., 2002) found an increased risk of depression among regular or problem cannabis users (pooled OR = 1.5; 95% CI: 1.2, 1.9) (Moore *et al*., 2007).

Potential age and sex effects

Some studies have investigated potential differences in associations between males and females. As mentioned earlier, the study by Patton *et al*. found that associations with depression at age 20–21 years were demonstrable only among young women (Patton *et al*., 2002).

Not all studies have found sex differences. A cross-sectional study of adolescent students in Canada found alcohol and tobacco use to be associated with elevated depression in females, but not males; cannabis use was an independent predictor of depressive symptoms in *both* males and females (Poulin *et al*., 2005). In a cohort study of youths from the USA, repeated assessments from 1985 (at age 6 years) through 2002 (at age 21 years) were made for 1494 participants and the risk of depression in young adulthood estimated (Harder *et al*., 2008); differences between individuals with and without problematic cannabis use were controlled using propensity score techniques. The risk of young adult depression for adolescents with problematic cannabis use did not differ from non-users, regardless of sex, with the authors concluding there was no evidence of an association in this population (Harder *et al*., 2008).

It is also possible that effects might be age-specific. As noted earlier, there is increasing evidence from prospective cohort studies that it is early-onset regular cannabis use that is the strongest predictor of depression in early adulthood. Later-onset cannabis use does not appear to be as strongly linked with the development of depression.

Summary

Cross-sectional and longitudinal studies have provided mixed evidence on the association between cannabis use and depression. Cross-sectional studies have suggested that the relationship can be explained

by other factors, such as the use of other drugs. Longitudinal studies have suggested that the "self-medication" hypothesis does not fit the pattern of cannabis use over time *among cohorts of adolescents and young adults*. There is more evidence that regular cannabis use increases the risk of depression during follow-up, and this relationship is partly, but possibly *not completely*, explained by confounding variables. Relationships appear to be stronger for early-onset cannabis use, and sex differences in effects have been reported, with some studies suggesting that the relationship is stronger for females.

The Bovasso study (Bovasso, 2001) allows an estimation of the population-attributable risk for this association, *if it is causal*. Approximately 67% of those with cannabis abuse but no depressive symptoms at baseline developed depression after 14–16 years of follow-up, compared with 31% of those without cannabis abuse. The number of persons who met criteria for cannabis abuse at baseline without also reporting depressive symptoms was extremely small (only 15 out of 849 who did not report depressive symptoms at baseline).

This suggests that at most 0.6% of the sample may have developed depressive symptoms over 14–16 years *possibly* as a result of using cannabis. These figures are likely to be overestimates of the effect of problematic cannabis use as they assume a strong causal relationship when a variety of potentially confounding factors were not assessed in the study. Given current rates of cannabis use, assuming that the link is causal, then 1.9% of the depressive symptoms that developed over 15 years could be attributed to cannabis abuse. Thus, in a population in which problematic cannabis use is uncommon (as is still the case in most developed countries), regular cannabis use explains only a modest proportion of depression in the population. These estimates of attributable risk need to be improved upon in future longitudinal studies.

Implications for future research

Our review of the literature has identified a number of limitations in the available research on cannabis use and depression. In the following section, we outline these limitations, and suggest ways in which future research might overcome them.

Measurement of cannabis use

The measurement of cannabis use in published research includes the following limitations. First, some epidemiological studies have grouped cannabis with other drugs (Anthony and Helzer, 1991; Kessler *et al*., 1996), so it is not clear what specific contribution has been made by cannabis use. Second, some studies have grouped cannabis abuse and dependence into "use disorders" (Anthony and Helzer, 1991), although some epidemiological research has examined cannabis abuse and dependence separately for comorbidity with major depression (Grant, 1995; Degenhardt *et al*., 2001). Third, some studies have examined only cannabis *use* without distinguishing between increasing levels of such use (Abel, 1971; Gale and Guenther, 1971; Shedler and Block, 1990; Zablocki *et al*., 1991; Gruber *et al*., 1997; Milich *et al*., 2000).

It is also important to consider the *level* of cannabis use. Most often studies have examined patterns of comorbidity between the problem of regular cannabis use and other mental health problems. This is most likely because: (1) it is at higher levels of use such that we might expect to see associations with other problems; and (2) the clinical concept of comorbidity emphasizes the co-occurrence of *disorders*.

In support of this distinction between low-level or lifetime use and regular/problematic use, studies reporting relatively low levels of cannabis use have usually failed to find a significant relationship with depression. It is a reasonable hypothesis that: (1) low-level cannabis use is *not* associated with a *significant* increase in the risk of depression; (2) it is only when persons are using cannabis heavily (perhaps weekly or more often) that the risk of depression is increased. Future work needs to test these hypotheses directly.

Measurement of depression

Our review has been complicated by the fact that a variety of different ways have been used to measure depression. Some studies have assessed major depression as defined by the American Psychiatric Association's classification system for mental disorders, the DSM (Degenhardt *et al*., 2001; Fergusson *et al*., 2002; Marmorstein *et al*., 2010). Others have used measures of "depressive symptoms" (Kandel and Davies, 1986; Kandel *et al*., 1986; Bovasso, 2001), continuous measures of depression (Troisi *et al*., 1998; Milich *et al*., 2000), or cut-off scores on continuous depression scales (Patton *et al*., 2002). It is likely that some of the discrepant findings might have reflected differential sensitivity of these differences in measurement.

Study designs

Well-designed surveys of the general population have indicated that heavy cannabis use and depression occur at a level greater than chance, but these studies are not well-suited to testing causal hypotheses. Longitudinal studies (Fergusson and Horwood, 2001) and genetically informative research designs (Neale and Kendler, 1995; Rutter *et al*., 2001) are better suited to the task of testing causal hypotheses.

Longitudinal studies

To date most longitudinal studies have used adolescent or young adult samples. From a public health perspective, this group is important because of the high rates of incident cannabis use and depression. In this age group there is emerging evidence that frequent cannabis use predicts depression.

This relationship may change across the lifespan. It is likely that many frequent users will reduce or cease using when the detrimental consequences of use, such as depression, become evident. Whether risk of depression resolves with cessation of use is an important and, as yet, unanswered question that would illuminate the relationship, as it has done with the analogous issue of the relationship between alcohol disorders and depression.

The use of genetically informative designs to examine causality

Standard genetic modelling of twin data has found moderate to high heritability of both cannabis use/dependence and liability to depression. Specifically, estimates of the heritability of cannabis dependence have ranged from 45% to 62% (Kendler and Prescott, 1998; Kendler *et al*., 2000; Lynskey *et al*., 2002) and a meta-analysis of twin studies has suggested that 37% of the liability to major depression is owing to heritable factors (Sullivan *et al*., 2000).

One study suggested that the association between major depression and cannabis dependence may be explained by a high degree of overlap in the genetic factors predisposing to each disorder (Fu *et al*., 2002). Specifically, the shared genetic risk between cannabis dependence and depression was largely explained by genetic effects on ASPD, which in turn was associated with an increased risk of both disorders.

Another US study involved a cross-sectional survey of a general population sample of 277 adult-twin pairs who were discordant for lifetime cannabis dependence ($n = 277$ pairs) and for early cannabis use (before age 17 years, $n = 311$) (Lynskey *et al*., 2004). Twins who were cannabis dependent had higher risks of suicidal ideation and suicide attempt than their non-cannabis-dependent co-twin. Cannabis dependence was associated with an elevated risk of major depression in dizygotic but not in monozygotic twins. Those who initiated cannabis use before age 17 years had elevated rates of subsequent suicide attempt, but not of major depression or suicidal ideation. The authors concluded that the comorbidity between cannabis dependence and depression largely reflected shared genetic and environmental vulnerabilities whereas associations between cannabis dependence and suicidal behaviors could not be explained in that way.

Further, genetically informative research strategies may make substantial contributions to our understanding of the relationship between cannabis use and depression. Potential designs include further twin studies, reared together or apart, adoption studies and studies of the children of twins and other extended family designs.

Conclusions

Surveys of representative samples of the general population have found that rates of depression are often elevated in those who use cannabis frequently or who are cannabis dependent. The extent of this comorbidity exceeds levels we would expect to see by chance. There does not appear to be an increased risk of depression associated with infrequent cannabis use.

The reasons for this comorbidity are uncertain. Research does not support the self-medication hypothesis. It is too early to rule out shared risk factors since not all cross-sectional studies have adequately controlled for confounding variables, the results of cohort studies to date have been mixed, and one twin study has suggested that shared genetic risk may explain the association.

There appears to be a modest association between early-onset regular or problem cannabis use and later depression. There are at least two broad classes of explanation: the first of these is biological, in which cannabis use causes changes in neurotransmitter systems that make depressed mood more likely. There is limited research evidence to support this possibility, though there is increasing interest in the potential impact of cannabis use in adolescence given that the

significant maturational change in the endocannabinoid system that occurs in this period.

There is better evidence for a causal explanation in which the effects of regular or problematic cannabis use on depression are socially mediated. Regular and early-onset cannabis use are associated with reduced educational attainment (Lynskey and Hall, 2000), and unemployment and crime (Fergusson and Horwood, 1997; Lynskey and Hall, 2000), all factors that may lead to increased risks of later mental health problems.

There is a need for longitudinal and twin studies that assess the relationship between cannabis use, depression and confounding factors. There is a need for more work to examine relationships in older adult samples, since few studies have extended into middle or late adulthood.

If we assume that cannabis use and depression are causally related, the proportion of depression that is attributable to cannabis use is modest. On the basis of current patterns of cannabis use in the general population (in which very few people use cannabis regularly), regular cannabis use explains only a small proportion (for example, perhaps 2% in the USA) of depression in the population.

References

Abel, E. (1971) Changes in anxiety feelings following marihuana smoking. *Brit J Addict,* **66**:185–7.

Ablon, S.L. and Goodwin, F.K. (1974) High frequency of dysphoric reactions to tetrahydrocannabinol among depressed patients. *Am J Psychiatry,* **131**:448–53.

Alpert, J., Maddocks, A., Rosenbaum, J.F. and Fava, M. (1994). Childhood psychopathology retrospectively assessed among adults with early onset depression. *J Affect Disord,* **31**:165–71.

Andrews, G., Mathers, C. and Sanderson, K. (1998) The burden of disease. *Med J Aust,* **169**:156–8.

Angst, J. (1996) Comorbidity of mood disorders: A longitudinal prospective study. *Brit J Psychiatry,* **168**(Suppl 30): 31–7.

Anthony, J.C. and Helzer, J. (1991) Syndromes of drug abuse and dependence. *Psychiatric Disorders in America*. L. N. Robins and D. A. Regier. New York, The Free Press: 116–54.

Arendt, M., Rosenberg, R., Fjordback, L. *et al.* (2007) Testing the self-medication hypothesis of depression and aggression in cannabis-dependent subjects. *Psychol Med,* **37**:935–45.

Arseneault, L., Cannon, M., Whitten, J. and Murray, R.M. (2004) Causal association between cannabis and psychosis: examination of the evidence. *Brit J Psychiatry,* **184**:110–7.

Ashton, C.H., Moore, P.B., Gallagher, P. and Young, A.H. (2005) Cannabinoids in bipolar affective disorder: A review and discussion of their therapeutic potential. *J Psychopharmacol,* **19**:293–300.

Bambico, F.R. and Gobbi, G. (2008) The cannabinoid CB1 receptor and the endocannabinoid anandamide: Possible antidepressant targets. *Expert Opin Ther Targets,* **12**:1347–66.

Bardone, A., Moffitt, T., Caspi, A., Dickson, N., Stanton, W.R. and Silva, P.A. (1998) Adult physical health outcomes of adolescent girls with conduct disorder, depression, and anxiety. *J Am Acad Child Adolesc Psychiatry,* **37**:594–601.

Beautrais, A.L., Joyce, P.R. and Mulder, R.T. (1999) Cannabis abuse and serious suicide attempts. *Addiction,* **94**:1155–64.

Bell, J., Ward, J., Mattick, R., Hay, A., Chan, J. and Hall, W.D. (1995) *An Evaluation of Private Methadone Clinics*. Canberra, Australian Government Publishing Service.

Berkson, J. (1946) Limitations of the application of fourfold table analysis to hospital data. *Biometrics Bull,* **2**:47–53.

Best, D., Gossop, M., Greenwood, J., Marsden, J., Lehman, P. and Strang, J. (1999) Cannabis use in relation to illicit drug use and health problems among opiate users in treatment. *Drug Alcohol Rev,* **18**:31–8.

Blazer, D. (1995) Mood disorders: Epidemiology. *Comprehensive Textbook of Psychiatry*. H. Kaplan and B. Sadock. Baltimore, MA, Williams and Wilkins. **1**:1079–89.

Bovasso, G. (2001) Cannabis abuse as risk factor for depressive symptoms. *Am J Psychiatry,* **158**:2033–7.

Bowers, M. (1998) Family history and early psychotogenic response to marijuana. *J Clin Psychiatry,* **59**:198–9.

Boyd, J.H., Burke, J.D., Gruenberg, E., *et al.* (1984) Exclusion criteria of DSM-III: A study of co-occurrence of hierarchy-free syndromes. *Arch Gen Psychiatry,* **41**:983–9.

Brady, K.T., Casto, S., Lydiard, R.B., Malcolm, R. and Arana, G. (1991) Substance abuse in an inpatient psychiatric sample. *Am J Drug Alcohol Abuse,* **17**:389–97.

Brook, J.S., Cohen, P. and Brook, D.W. (1998) Longitudinal study of co-occurring psychiatric disorders and substance use. *J Am Acad Child Adolesc Psychiatry,* **37**: 322–30.

Calabria, B., Degenhardt, L., Hall, W. and Lynskey, M. (2010) Does cannabis use increase the risk of death? Systematic review of epidemiological evidence on adverse effects of cannabis use. *Drug Alcohol Rev,* **29**:318–30.

Caron, C. and Rutter, M. (1991) Comorbidity in child psychopathology: Concepts, issues and

research strategies. *J Child Psychol Psychiatry,* **32**:1063–80.

Chen, C.-Y., Wagner, F. and Anthony, J.C. (2002) Marijuana use and the risk of major depressive episode: Epidemiological evidence from the United States National Comorbidity Survey. *Soc Psychiatry Psychiatr Epidemiol,* **37**:199–206.

Cheung, J., Mann, R., Ialomiteanu, A., *et al.* (2010) Anxiety and Mood Disorders and Cannabis Use. *Am J Drug Alcohol Abuse,* **36**:118–22.

Cicchetti, D. and Toth, S. (1998) The development of depression in children and adolescents. *Am Psychologist* **53**:221–41.

De Graaf, R., Radovanovic, M., van Laar, M., *et al.* (2010) Early cannabis use and estimated risk of later onset of depression spells: epidemiologic evidence from the population-based World Health Organization World Mental Health Survey Initiative. *Am J Epidemiol* **172**:149–59.

Degenhardt, L., Hall, W. and Lynskey, M. (2001) The relationship between cannabis use, depression and anxiety among Australian adults: Findings from the National Survey of Mental Health and Well-Being. *Soc Psychiatry Psychiatr Epidemiology* **36**:219–27.

Degenhardt, L., Hall, W. and Lynskey, M. (2003) Exploring the association between cannabis use and depression. *Addiction,* **98**:1493–504.

Degenhardt, L. and Hall, W.D. (2006) Is cannabis a contributory cause of psychosis? *Can J Psychiatry,* **51**:556–65.

Degenhardt, L., Lynskey, M. and Hall, W. (2000) Cohort trends in the age of initiation of drug use in Australia. *Aust NZ J Pub Health,* **24**:421–6.

Degenhardt, L., Tennant, C., Gilmour, St., *et al.* (2007). *The Temporal Dynamics of Relationships Between Cannabis, Psychosis and Depression Among Young Adults with Psychotic Disorders: Findings From a 10-month Prospective Study. (31 refs.)*. Sydney, University of New South Wales National Drug and Alcohol Research Centre.

Di Forti, M., Morrison, P.D., Butt, A. and Murray, R.M. (2007) Cannabis use and psychiatric and cogitive [sic] disorders: the chicken or the egg? *Curr Opin Psychiatry,* **20**:228–34.

Diamond, G., Panichelli-Mindel, S.M., *et al.* (2006) Psychiatric syndromes in adolescents with marijuana abuse and dependency in outpatient treatment. *J Child Adolesc Subst Abuse,* **15**:37–54.

Diekstra, R., Kienhorst, C., Shera, D., Dennis, M., Tims, F. and Ungemack, J. (1995) Suicide and suicidal behavior among adolescents. *Psychosocial Disorders Among Young People: Time Trends and Their Causes*. M. Rutter and D. J. Smith. Chichester, John Wiley and Sons: 686–761.

Donnelly, N. and Hall, W. (1994) Patterns of cannabis use in Australia. NCADA Monograph Sries No. 27. Canberra, Australian Government Publishing Service: p.106.

Estroff, T., Dackis, C., Gold, M. and Pottash, A. (1985) Drug abuse and bipolar disorders. *Int J Psychiatry Med,* **15**:37–40.

Fergusson, D., Horwood, J., and Lawton, J. (1990) Vulnerability to childhood problems and family social background. *J Child Psychol Psychiatry,* **31**:1145–60.

Fergusson, D., Horwood, L. and Lynskey, M. (1994) Parental separation, adolescent psychopathology and problem behaviors. *J Am Acad Child Adolesc Psychiatry,* **33**:1122–31.

Fergusson, D., Horwood, L. and Swain-Campbell, N. (2002) Cannabis use and psychosocial adjustment in adolescence and young adulthood. *Addiction,* **97**:1123–35.

Fergusson, D.M. and Horwood, L.J. (1997) Early onset cannabis use and psychosocial adjustment in young adults. *Addiction,* **92**:279–96.

Fergusson, D.M. and Horwood, L.J. (2001) The Christchurch Health and Development Study: review of findings on child and adolescent mental health. *Austr NZ J Psychiatry,* **35**:287–96.

Fleming, C.B., Mason, W.A., Mazza, J.J., Fleming A., Abbott, R.D. and Catalano, R.F. (2008) Latent growth modeling of the relationship between depressive symptoms and substance use during adolescence. *Psychol Addict Behav,* **22**:186–97.

Fu, Q., Heath, A., Bucholz, K.K. *et al.* (2002) Shared genetic risk of major depression, alcohol dependence, and marijuana dependence: contribution of antisocial personality disorder in men. *Arch Gen Psychiatry,* **59**:1125–32.

Galbaud Du Fort, G., Newman, S. and Bland, R.C. (1993) Psychiatric comorbidity and treatment seeking: Sources of selection bias in the study of clinical populations. *J Nerv Ment Dis,* **181**:464–74.

Gale, E.N. and Guenther, G. (1971). Motivational factors associated with the use of cannabis (marihuana). *Brit J Addict,* **66**:188–94.

Georgiades, K. and Boyle, M.H. (2007) Adolescent tobacco and cannabis use: Young adult outcomes from the Ontario Child Health Study. *J Child Psychol Psychiatry,* **48**:724–31.

Grant, B.F. (1995) Comorbidity between DSM-IV drug use disorders and major depression: results of a national survey of adults. *J Subst Abuse,* **7**:481–97.

Green, B.E. and Ritter, C. (2000) Marijuana use and depression. *J Health Soc Behav,* **41**:40–9.

Grinspoon, L. and Bakalar, J.B. (1998) The use of cannabis as a mood stabilizer in bipolar disorder: Anecdotal

evidence and the need for clinical research. *J Psychoactive Drugs,* **30**:171–7.

Gruber, A.J., Pope, H.G. and Oliva, P. (1997) Very long-term users of marijuana in the United States: A pilot study. *Subst Use Misuse,* **32**:249–24.

Hall, W. and Degenhardt, L. (2009) The adverse health and psychological effects of non-medical cannabis use. *Lancet,* **374**:1383–91.

Hall, W., Degenhardt, L. and Lynskey, M. (2001) The health and psychological consequences of cannabis use. Canberra, Australian Publishing Service.

Hall, W., Johnston, L. and Donnelly, N. (1999) Epidemiology of cannabis use and its consequences. *The Health Effects of Cannabis*. H. Kalant, W. Corrigall, W. Hall and R. Smart. Toronto: Canada, Centre for Addiction and Mental Health: 71–125.

Harder, V.S., Morral, A.R. and Arkes, J. (2006) Marijuana use and depression among adults : testing for causal associations. *Addiction,* **101**:1463–72.

Harder, V.S., Stuart, E.A. and Anthony, J.C. (2008) Adolescent cannabis problems and young adult depression: male-female stratified propensity score analyses. *Am J Epidemiology,* **168**:592–601.

Hayatbakhsh, M.R., Najman, J.M., Jamrozik, K., Mamun, A.A., Alati, R. and Bor, W. (2007) Cannabis and anxiety and depression in young adults : a large prospective study. *J Am Acad Child Adolesc Psychiatry,* **46**:408–17.

Hofstra, M., van der Ende, J. and Verhulst, F.C. (2002) Child and adolescent problems predict DSM-IV disorders in adulthood: A 14-year follow-up of a Dutch epidemiological sample. *J Am Acad Child Adolesc Psychiatry,* **41**:182–9.

Holden, R. and Pakula, I. (2001) Marijuana, stress and suicide: A neuroimmunological explanation. *Austr NZ J Psychiatry,* **32**:465–6.

Innamorati, M., Pompili, M., Ferrari, V., *et al*. (2008) Cannabis use and the risk behavior syndrome in Italian university students: Are they related to suicide risk? *Psycholog Rep,* **102**:577–94.

Institute of Medicine (1996) *Pathways of Addiction*. Washington, National Academy Press.

Jablensky, A., Sartorius, N., Ernberg, G., *et al*. (1991) Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization Ten-Country Study. *Psychological Medicins Supplement No. 20*.

Jian, W., Zhang, Y., Xiao, L., *et al*. (2005) Cannabinoid may make new brain cells grow, reduce depression in rats. *Alcoholism and Drug Abuse Weekly,* **7**:7.

Johns, A. (2001) Psychiatric effects of cannabis. *Brit J Psychiatry,* **178**:116–22.

Kaminer, Y., Connor, D.F. and Curry, J.F. (2008) Treatment of comorbid adolescent cannabis use and major depressive disorder. *Psychiatry,* **5**:34–9.

Kandel, D. and Chen, K. (2000) Types of marijuana users by longitudinal course. *J Stud Alcohol,* **61**:367–78.

Kandel, D. and Davies, M. (1986) Adult sequelae of adolescent depressive symptoms. *Arch Gen Psychiatry,* **43**:255–62.

Kandel, D.B. (1984) Marijuana users in young adulthood. *Arch Gen Psychiatry,* **41**:200–9.

Kandel, D.B., Davies, M., Karus, D. and Yamaguchi, K. (1986) The consequences in young adulthood of adolescent drug involvement. *Arch Gen Psychiatry,* **43**:746–54.

Kelder, S., Murray, N.G., Orpinas, P., *et al*. (2001) Depression and substance use among minority middle-school students. *Am J Pub Health,* **91**:761–6.

Kendler, K., Karkowski, L., Neale, M. and Prescott, C. (2000) Illicit psychoactive substance use, heavy use, abuse and dependence in a US population-based sample of male twins. *Arch Gen Psychiatry,* **57**:261–9.

Kendler, K.S. and Prescott, C.A. (1998) Cannabis use, abuse, and dependence in a population-based sample of female twins. *Am J Psychiatry,* **155**:1016–22.

Kessler, R.C. (1995) Epidemiology of psychiatric comorbidity. *Textbook in Psychiatric Epidemiology*. M.T. Tsuang, M. Tohen and G.E.P. Zahner. New York, Wiley and Sons: 179–97.

Kessler, R.C., McGonagle, K.A., Zhao, S., *et al*. (1994) Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry,* **51**:8–19.

Kessler, R.C., Nelson, C.B., McGonagle, K.A., *et al*. (1996) The epidemiology of co-occurring addictive and mental disorders: implications for prevention and service utilization. *Am J Orthopsychiatry,* **66**:17–31.

Klein, D. and Riso, L. (1994) Psychiatric disorders: Problems of boundaries and comorbidity. *Basic Issues in Psychopathology*. G. Costello. New York, Guilford: 19–66.

Konings, M. and Maharajh, H.D. (2006) Cannabis use and mood disorders: Patterns of clinical presentations among adolescents in a developing country. *International Journal of Adolescent Medicine and Health* **18**(2): 221–233.

Kotin, J., Post, R.M. and Goodwin, F.K. (1973) Δ9-Tetra-hydrocannabinol in depressed outpatients. *Arch Gen Psychiatry,* **28**:345–8.

Kung, H.-C., Pearson, J.L. and Liu, X. (2003) Risk factors for male and female suicide decedents ages 15–64 in the United States. Results from the 1993 National Mortality Followback Survey. *Soc Psychiatry Psychiatr Epidemiol,* **38**:419–26.

Leweke, F.M. and Koethe, D. (2008) Cannabis and psychiatric disorders : it is not only addiction. [Review

For Special Issue On Cannabinoids]. *Addict Biol,* **13**:264–75.

Lynskey, M., Degenhardt, L. and Hall, W.D. (2000) Cohort trends in youth suicide in Australia 1964 to 1997. *Austr NZ J Psychiatry,* **34**:408–12.

Lynskey, M. and Hall, W. (2000) The effects of adolescent cannabis use on educational attainment: A review. *Addiction,* **95**:1621–30.

Lynskey, M.T., Glowinski, A.L., Todorov, A.A., *et al*. (2004) Major depressive disorder, suicidal ideation, and suicide attempt in twins discordant for cannabis dependence and early-onset cannabis use. *Arch Gen Psychiatry,* **61**:1026–32.

Lynskey, M.T., Heath, A.C. and Nelson, E.C. (2002) Genetic and environmental contributions to cannabis dependence in a national young adult twin sample. *Psycholog Med,* **32**:195–207.

Maharajh, H.D. and Konings, M. (2005) Suicidal behavior and cannabis-related disorders among adolescents. *Suicidal Behavior in Adolescence: An International Perspective*. London, England: Freund Publishing House: 119–29.

Malone, D.T., Hill, M.N. and Rubino, T. (2010) Adolescent cannabis use and psychosis: epidemiology and neurodevelopmental models. *Brit J Pharmacol,* **160**:511–22.

Marken, P., Stanislav, S., Lacombe, S., Pierce, C., Hornstra, R. and Sommi, R.W. (1992) Profile of a sample of subjects admitted to an acute care psychiatric facility with manic symptoms. *Psychopharmacol Bull,* **28**:201–5.

Marmorstein, N., Iacono, W. and Malone, S.M. (2010) Longitudinal associations between depression and substance dependence from adolescence through early adulthood. *Drug Alcohol Depend,* **107**:154–60.

Mathew, R., Wilson, W. and Tant, S.R. (1989) Acute changes in cerebral blood flow associated with marijuana smoking. *Acta Psychiatr Scand,* **79**:118–28.

McGee, R., Williams, S., Poulton, R. and Moffit, T. (2000) A longitudinal study of cannabis use and mental health from adolescence to early adulthood. *Addiction,* **95**:491–503.

McLaren, J., Lemon, J., Robins, L. and Mattick, R.P. (2008) *Cannabis and Mental Health*. Canberra, ACT, Australian Government Department of Health and Ageing.

Merikangas, K. and Angst, J. (1995) The challenge of depressive disorders in adolescence. *Psychosocial Disturbances in Young People*. M. Rutter. Cambridge, Cambridge University Press.

Milich, R., Lynam, D., Zimmerman, R., *et al*. (2000) Differences in young adult psychopathology among drug abstainers, experimenters, and frequent users. *J Subst Abuse,* **11**:69–88.

Miller-Johnson, S., Lochman, J., Coie, J.D., Terry, R. and Hyman, C. (1998) Comorbidity of conduct and depressive problems at sixth grade: Substance use outcomes across adolescence. *J Abnorm Child Psychol,* **26**:221–32.

Miller, F., Busch, F. and Tanenbaum, J.H. (1989). Drug abuse in schizophrenia and bipolar disorder. *Am J Drug Alcoh Abuse*, **15**:291–5.

Moore, T.H., Zammit, S., Lingford-Hughes, A., *et al*. (2007) Cannabis use and risk of psychotic or affective mental heath outcomes: A systematic review. *Lancet,* **370**:319–28.

Mueser, K., Yarnold, P.R. and Bellack, A.S. (1992) Diagnostic and demographic correlates of substance abuse in schizophrenia and major affective disorder. *Acta Psychiatr Scan,* **85**:48–55.

Mueser, K.T., Drake, R.E. and Wallach, M.A. (1998) Dual diagnosis: A review of etiological theories. *Addict Behav,* **23**:717–34.

Neale, M.C. and Kendler, K.S. (1995) Models of comorbidity for multifactorial disorders. *Am J Human Gen,* **57**:935–53.

Newcomb, M.D., Vargas-Carmona, J. and Galaif, E.R. (1999). Drug problems and psychological distress among a community sample of adults: predictors, consequences, or confound? *J Comm Psychol* **27**(4): 405–429.

Paton, S., Kessler, R. and Kandel, D. (1977) Depressive mood and adolescent illicit drug use: a longitudinal analysis. *J Gen Psychol,* **131**:267–89.

Patton, G., Coffey, C., Carlin, J.B., Degenhardt, L., Lynskey, M. and Hall, W. (2002) Cannabis use and mental health in young people: cohort study. *BMJ,* **325**:1195.

Pedersen, W. (2008) Does cannabis use lead to depression and suicidal behaviors? A population-based longitudinal study. *Acta Psychiatrica Scandinavica* **118**:395–403.

Pond, D. (1948) Psychological effects in depressive patients of the marijuana homologue, synhexyl. *J Neurol Neurosurg Psychiatry* **11**:271–9.

Poulin, C., Hand, D., Boudreau, B. and Santor, D. (2005) Gender differences in the association between substance use and elevated depressive symptoms in a general adolescent population. *Addiction,* **100**:525–35.

Rey, J., Sawyer, M., Clark, J.J. and Baghurst, P.A. (2001) Depression among Australian adolescents. *Med J Aus,* **175**:19–23.

Rey, J., Sawyer, M., Raphael, B., Patton, G.C. and Lynskey, M. (2002) Mental health of teenagers who use cannabis: Results of an Australian survey. *Brit J Psychiatry,* **180**:216–21.

Roberts, R.S., Spitzer, W.O., Delmore, T. and Sackett, D.L. (1978) An empirical demonstration of Berkson's bias. *J Chron Dis,* **31**:119–28.

Rodriguez Bambico, F., Duranti, A., Tontini, A., Tarzia, G. and Gobbie, G. (2009) Endocannabinoids in the treatment of mood disorders: Evidence from animal models. *Curr Pharm Des,* **15**:1623–46.

Rowe, M., Fleming, M., Michael, F., *et al.* (1995) Correlates of depression in primary care. *J Fam Pract,* **41**:551–8.

Rutter, M. (1987) Parental mental disorder as a psychiatric risk factor. *Psychiatric Update: American Psychiatric Association: Annual Review*. R. Hales and A. Frances. Washington, American Psychiatric Press. **6**:647–63.

Rutter, M., Pickles, A., Murray, R. and Eaves, L. (2001) Testing hypotheses on specific environmental causal effects on behavior. *Psychol Bull,* **127**:291–324.

Schneider, M. (2008) Review for special issue on cannabinoids: Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addict Biol,* **13**:253–63.

Shedler, J. and Block, J. (1990) Adolescent drug use and psychological health: A longitudinal inquiry. *Am Psychologist,* **45**:612–30.

Sonne, S., Brady, K. and Morton, W.A. (1994) Substance abuse and bipolar affective disorder. *J Nerv Ment Dis,* **182**:349–52.

Stoll, A., Cole, J. and Lukas, S.E. (1991) A case of mania as a result of fluoxetine-marijuana interaction. *J Clin Psychiatry,* **52** 280–1.

Sullivan, P.F., Neale, M.C. and Kendler, K.S. (2000) Genetic epidemiology of major depression: Review and meta-analysis. *Am J Psychiatry,* **157**:1552–62.

Sundram, S. (2006) Cannabis and neurodevelopment: Implications for psychiatric disorder. *Human Psychopharmacol,* **21**:245–54.

Troisi, A., Pasini, A., Saracco, M. and Spalletta, G. (1998) Psychiatric symptoms in male cannabis users not using other illicit drugs. *Addiction,* **93**:487–92.

van Laar, M., van Dorsselaer, S., Monshouwer, K and de Graaf, R. (2007) Does cannabis use predict the first incidence of mood and anxiety disorders in the adult population? *Addiction,* **102**:1251–60.

Velez, C., Johnson, J. and Cohen, P. (1989) A longitudinal analysis of selected risk factors for childhood psychopathology. *J Am Acad Child Adoles Psychiatry,* **28**:861–4.

Vinod, K.Y. and Hungund, B.L. (2006) Role of the endocannabinoid system in depression and suicide. *Trends Pharmacol Sci,* **27**:539–45.

Warner, R., Taylor, D., Wright, J., *et al.* (1994) Substance use among the mentally ill: prevalence, reasons for use and effects on illness. *Am J Orthopsychiatry*, **74**:30–9.

Weissman, M., Livingston Bruce, M., Leaf, P.J., *et al.* (1991) Affective disorders. *Psychiatric Disorders in America*. L. Robins and D. Regier. New York, MacMillan: 53–80.

Weissman, M.M., Wolk, S., Wickramaratne, P., *et al.* (1999) Children with prepubertal-onset major depressive disorder and anxiety grown up. *Arch Gen Psychiatry,* **56**:794–801.

White, A.M., Jordan, J.D., Schroeder, K.M., *et al.* (2004) Predictors of relapse during treatment and treatment completion among marijuana-dependent adolescents in an intensive outpatient substance abuse program. *Substance Abuse,* **25**:53–9.

Wilcox, H. C. and Anthony, J.C. (2004) The development of suicide ideation and attempts: an epidemiologic study of first graders followed into young adulthood. *Drug Alcohol Depend,* **76** (Suppl):S53–S67.

Wittchen, H.-U., Frohlick, C., Behrendt, S., *et al.* (2007) Cannabis use and cannabis use disorders and their relationship to mental disorders : a 10-year prospective-longitudinal community study in adolescents. *Drug Alcohol Depend,* **88** (Suppl 1):S60–S70.

Zablocki, B., Aidala, A., Hansell, S. and White, H.R. (1991) Marijuana use, introspectiveness, and mental health. *J Health Soc Behav,* **32**:65–79.

Zelwer, A. (1994) Depression and marijuana. *Aus NZ Psychiatry,* **28**:528.

Chapter

11

Cannabis, cannabinoids and bipolar disorder

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As detailed elsewhere in this book, the associations between cannabis use and mental illnesses are well established, especially psychotic illnesses. However, there is a paucity of research into the associations between cannabis use and bipolar affective disorder, in particular with respect to prevalence, causality, treatment and outcomes. This chapter reviews the literature on cannabis and bipolar disorder, identifies gaps in current knowledge, and outlines areas for future study.

Comorbidity between cannabis use and bipolar disorder

Much of the published research on comorbidity between bipolar disorder and cannabis abuse is taken from studies investigating the more general concept of substance use or abuse, rather than specifically looking at cannabis abuse. In terms of population-based studies, the US-based Epidemiological Catchment Area (ECA) Study found the lifetime prevalence of drug abuse or dependence in patients with bipolar I to be 40.7%, compared with 6.2% in general population (Regier *et al*., 1990). Many other studies have replicated the findings of higher prevalence of drug abuse or dependence in bipolar patients.

Sherwood-Brown *et al*. (2001) reviewed studies of the prevalence of substance abuse in bipolar disorder. They point out the limitations of various methodologies; inpatient studies may introduce bias by including subjects with more severe illness, and lifetime rates do not measure frequency of current abuse, which may be contributing to illness and indicate the need for treatment. Lifetime rates of cannabis abuse were reported as between 30 and 64% in the various studies reviewed. One such study of 392 patients hospitalized for manic or mixed episodes of bipolar reported current cannabis abuse as 22% and lifetime abuse rates of 36% (Cassidy *et al*., 2001); there were no significant differences in these rates between patients with mixed or manic episodes. Males were significantly more likely to have reported a history of cannabis abuse. Like many studies examining substance abuse, this one was limited by the retrospective nature of the data gathering and the dependence on patient self-report, which may have underestimated the true rates of cannabis use.

In a recent clinical sample, substance use in general, and cannabis use in particular, were more frequent in patients with schizophrenia compared with a much smaller bipolar comparison group (De Hert *et al*., 2010). Patients were diagnosed by their treating psychiatrist and not by a structured diagnostic interview, and only bipolar patients with current psychotic symptoms were included. Cannabis use was associated with an earlier age of first hospitalization in both schizophrenic and bipolar patients when compared with non-users from the same groups. Interestingly, cannabis use had a significantly greater effect in lowering the age of first hospitalization, with bipolar cannabis users being hospitalized 9 years earlier than non-users, as compared with an average of 1.5 years earlier in the schizophrenia group.

In a further population-based study on a subset of patients drawn from a large genomic sample of individuals with bipolar disorder (n = 471), the bipolar patients were 6.8 times more likely than controls (n = 1761) to report a lifetime history of cannabis use; 29.4% met DSM-IV criteria for either cannabis abuse or dependence. Bipolar patients with cannabis-use disorder had significantly greater self-reported disability attributable to bipolar disorder, and were more likely to have attempted suicide or experienced a mixed episode (Agrawal *et al*., 2011). This study had methodological limitations, not the least being that there were no data on cannabis use disorders in the control subjects.

Marijuana and Madness, Second Edition, ed. David Castle, Robin M. Murray and Deepak Cyril D'Souza. Published by Cambridge University Press.

Given such high rates of comorbidity between substance abuse and bipolar disorder, diagnostic dilemmas can arise regarding whether the mental illness is primary (i.e. not substance-induced) or secondary to the substance use. Levin and Hennessy (2004) offer useful guidelines to discerning these, including close attention to the temporality of the substance use and onset, as well as resolution of mood symptoms, and use of validated screening instruments such as the Psychiatric Research Interview for Substance and Mental Disorders (PRISM).

Explaining the association between cannabis and bipolar disorder

From the foregoing, it is clear that there is an association between cannabis use and bipolar disorder. However, it remains unclear what factors underpin this association, and especially whether cannabis could be considered a causal factor in bipolar disorder. While it seems that cannabis use usually precedes the onset of bipolar disorder (at least manic symptoms), temporality is not the only criterion to establish causality and it is also likely that in at least some cases depressive symptoms antedate the manic ones and lead to cannabis use (see also Chapter 10). Other hypotheses for the association include bipolar disorder being a risk factor for cannabis use, or that patients use cannabis as self-medication for their bipolar symptoms. The kindling hypothesis has been much explored for the associations between other substances and bipolar disorder (see Tohen *et al*., 1998), but this has not to our knowledge been investigated specifically for cannabis.

Regarding the self-medication hypothesis, which posits that patients use substances to try and alleviate distressing symptoms, studies of cannabis and bipolar disorder are equivocal. Levin and Hennessy (2004) reviewed this literature and found that whereas some studies reported that patients self-medicate to try and counteract symptoms, others report patients using substances to accentuate their symptoms, for example a manic patient using amphetamines. One of the difficulties with this literature is disentangling the effects of cannabis from the other substances often also being used.

Baethge *et al*. (2008) performed a prospective study of 166 first-episode bipolar patients and looked at the temporal relationships between substance use and affective morbidity (see Table 11.1). They divided the longitudinal course of each subject's illness into three-month periods, and scored for the presence of affective symptoms and substance abuse in each period. Dose–response relationships were disregarded: the researchers chose to analyze both substance use and symptoms simply as either present or absent. They found that manic and hypomanic morbidity was significantly associated with cannabis use in the preceding or same period; the reverse situation of mood symptoms preceding substance use was not found to be significantly associated. The authors suggested that this represents "selective association" of cannabis use with manic symptoms, and that the temporal associations found in the study gives strength to the argument that cannabis has a causal relationship with manic symptoms in people with bipolar disorder.

To explore whether cannabis has a potential role in actually causing bipolar disorder, a general population cohort study design is informative. Van Laar *et al*. (2007) performed such a study, investigating whether cannabis use is associated with an increased risk of bipolar disorder in people with no pre-existing history thereof. They employed a prospective dataset of 3881 people who had no lifetime history of mood disorders. Cannabis exposure was defined as use more than five times at baseline; any subjects who had used less than five times were classed as non-users. They created a scale for frequency of use during the subject's period of heaviest use (no use; 1–3 days per month; 1–4 days per week; almost daily). Confounders adjusted for included demographic characteristics, socioeconomic status and degree of urbanicity, neuroticism, family psychiatric history, experiences of childhood trauma, alcohol and other substance use disorders, and lifetime mood, anxiety and psychotic disorders. After adjustment for these confounders, they found that any use of cannabis at baseline was significantly associated (odds ratio [OR] = 4.98; 95% confidence intervals [CI]: 1.80, 13.81) with an increased risk of a first diagnosis of bipolar disorder during the three year follow-up period. The results, when analyzed for frequency of cannabis use, were not statistically significant, although there was a trend toward a dose–response relationship. These authors do concede that there were some significant limitations to their study, including reliance on self-report of cannabis use. Also, the average age (39 years) of study participants is higher than the average age of the onset of mood disorders in the sample and is certainly later than the mean age at onset of bipolar disorder. This is likely due to the investigators excluding anyone with a lifetime diagnosis of a mood disorder

Table 11.1 Selected prospective studies of interactions between cannabis and bipolar disorder.

Study	*n*	Population	Findings
Jarvis *et al.*, 2008	14	adolescent inpatients	Greater structural brain abnormalities on MRI in patients with comorbid cannabis use.
Baethge *et al.*, 2008	166	adult inpatients	Significant temporal association of cannabis use preceding mania.
Henquet *et al.*, 2006	4815	general population	Cannabis use at baseline increased risk for manic symptoms during follow-up.
Van Laar *et al.*, 2007	3881	general population	Cannabis use at baseline increased risk fivefold for first diagnosis of bipolar disorder.
Strakowski *et al.*, 2007	144	adult inpatients	Non-significant association between cannabis use and total time of affective episode and rapid cycling.
Rottanburg, 1982	20	adult inpatients	High urinary drug screen levels of cannabis was associated with manic-like syndrome and rapid recovery.
Van Rossum *et al.*, 2009	3459	adult in- and outpatients	Cannabis use associated with greater illness severity, particularly with mania and psychosis and poorer treatment compliance. Quality of life measures showed lower life satisfaction, and lower likelihood of being in a relationship.
Baethge *et al.*, 2005	112	adult inpatients	Patients with cannabis dependence spent more time in manic episode.

at baseline; the incident cases of bipolar disorder were therefore people who developed their illness later than usual and thus these results may not be generalizable to disorders with onset earlier in life.

Henquet *et al.* (2006) explored whether cannabis is a risk factor for the development of manic symptoms. They used a prospective general population cohort of 4815 people, who were followed up over three years to examine whether cannabis use at baseline increased the risk of developing manic symptoms, and whether the association between cannabis and manic symptoms was independent of the development of psychotic symptoms. The investigators also sought to explore the "self-medication hypothesis"; that is, whether baseline mania was predictive of later cannabis use. Cannabis use at baseline significantly increased the risk of the development of manic-like symptoms during the follow-up period. This effect was found to be independent of psychotic symptoms. The association was robust even after controlling for many other demographic and illness variables, including other substance use and depressive symptoms. The presence of manic symptoms at baseline was not significantly associated with the commencement of cannabis use during the follow-up period; reverse causality did not therefore account for the association. There were some limitations to this study, including the broad definition of manic-like symptoms in people in the general community, rather than patients with diagnosed DSM-IV bipolar disorder; the relationship between this syndrome of subthreshold mania and bipolar disorder needs further clarification. This study suggests that cannabis use can lead to non-psychotic manic symptoms, and increases the risk of onset of manic symptoms in the general population, independent of psychosis.

Bertolín-Guillén *et al.* (2008) described a case that is suggestive of cannabis-induced mania. The patient abruptly developed clear-cut manic symptoms – rated at 36 on the Young Mania Rating Scale at admission – after several days of heavy cannabis use. They hypothesized that this could be explained by the acute induction model, in which the acute effects of cannabis are additive, interacting with the patient's underlying genetic vulnerability to develop mania. This is in line with previous findings by Rottanburg *et al.* (1982) (see later for further discussion), who reported more pronounced manic symptoms in cannabis positive patients suffering acute psychosis.

Levin and Hennessy's review (2004) addresses the hypothesis of bipolar disorder as the cause of substance abuse. As bipolar disorder's symptoms include such behaviors as excessive involvement in pleasurable

activities, impulsivity and impaired judgment, it is reasonable to suppose that those symptoms may lead to patients abusing substances such as cannabis. This is supported by several studies that have found that a proportion of patients do commence their substance abuse after the onset of bipolar disorder. None of the studies reviewed, however, were specific to cannabis abuse; these relationships warrant further investigation.

Mood effects of cannabis in established bipolar disorder

Numerous studies have investigated the effects of substance abuse on the course of bipolar disorder, but there is limited evidence specific to cannabis abuse. Levin and Hennessy's review (2004) found several studies where substance abuse in general was associated with earlier age of onset of bipolar disorder, and with more severe subtypes including dysphoric mania and rapid cycling. There have also been associations found with higher rates of hospitalization and slower remission from mania (Goldberg *et al*., 1999), as well as more incidents of medication non-adherence and higher rates of suicide attempts.

A preliminary report from Strakowski *et al*. (2000) of the first 50 inpatients with bipolar disorder recruited to their larger study (see later), suggested an association between cannabis abuse and the manic phase of bipolar illness; alcohol abuse was associated with the depressive phase. This is consonant with the findings of Baethge *et al*. (2005), who examined substance abuse in first-episode bipolar patients and found that those with cannabis dependence spent more time manic, whereas alcohol-dependent subjects spent more time depressed; however, their results were not statistically significant and many of the subjects were abusing both drugs.

Turning specifically to cannabis use, Strakowski *et al*. (2007) examined the effects of cannabis use on the course of the bipolar disorder in patients diagnosed with first-episode mania. This study included data obtained from the earlier observational study quoted above, raising the question of whether it was specifically designed to investigate the associations between cannabis use and mania, or was simply a further analysis of extant data. In the 2007 analysis, the investigators hypothesized that the sequence of onset of cannabis abuse and bipolar disorder would differentially affect the outcome; namely, that in patients where the onset of bipolar disorder occurred after the onset of cannabis abuse, the course of both disorders would be better than when bipolar disorder preceded cannabis use. They also anticipated that cannabis abuse would be more strongly associated with mania rather than depression. First-episode patients were chosen to lessen the confounding effects of long-term disease progression and the effects of chronicity. They were followed up for a minimum of 4 months; some were followed up for 5 years. Patients were classified into three groups: those with no cannabis use (n = 75), those who developed bipolar disorder before or within a year of cannabis use disorder ("bipolar first": n = 36) and those who had a cannabis-use disorder before the development of bipolar disorder ("cannabis first": n = 33). The "cannabis first" group recovered more rapidly and had higher overall rates of recovery; female gender and earlier age of onset of bipolar disorder were significantly associated with slower recovery. The "bipolar first" group spent a significantly longer time in a mixed or manic phase than the other groups. Recovery from cannabis-use disorder was found to be significantly more common in the "cannabis first" group than in the "bipolar first" group. While the initial hypothesis – that patients who develop a bipolar illness after using cannabis will have a better course of both diagnoses compared with those who commence using cannabis after their bipolar diagnosis – was confirmed using survival analyses, these results lost statistical significance when confounders were included in the model. However, the results were still important in showing that the use of cannabis can worsen or complicate the course of bipolar illness.

There were some limitations to this study. First, the use of previous data suggests that the study was not primarily designed to answer the question at hand. Subsequent subgroup analyses involved much smaller samples, limiting statistical power. The researchers note that while the study demonstrated associations between bipolar and cannabis use, they were unable to determine causality. Furthermore, interpretation of the results was complicated by the use of other substances of abuse, particularly alcohol. Other methodological problems include self-report measures of substance use and medication adherence, use of patients from a single site also may limit generalizability to other populations. More worryingly, group assignments may have been influenced by difficulties distinguishing cannabis abuse from occasional use, which would have minimized any differences found between groups in the analysis. Overall, the study was negative in that there were no statistically significant relationships found,

despite strong associations between cannabis use and rapid cycling and total time in affective episodes.

Other published data include case reports, such as one by El-Mallakh and Brown (2007), who reported on a case of one bipolar patient who kept daily mood charts during a 2-year period off marijuana (while in prison and on probation) followed by a 2-year period of heavy daily cannabis use after release. There were no changes in the patients' medication during this period. There was a statistically significant higher total number of days of hypomania during the period of cannabis use, and significantly fewer days of depression. The authors conclude that there is an overall "hypomanic promoting" effect of cannabis.

An earlier study by Rottanburg *et al.* (1982) compared 20 inpatients with a diagnosis of "psychosis" who tested positive on urine screens for cannabis, with cannabis-negative controls; other drugs of abuse were excluded through toxicology. Patients who had positive cannabis tests showed significantly more hypomanic symptoms and agitation, and fewer auditory hallucinations, negative-type symptoms and thought disorder. Furthermore, the patients in the cannabis group showed a much greater improvement within 1 week, suggesting that cannabis may induce a psychosis with prominent hypomanic features. There were several methodological issues: this study had many drop outs, and it was not mentioned how these patients were accounted for in the statistical analysis; also, the controls were only matched for age and diagnosis. Nevertheless, it remains an interesting study in its demonstration of cannabis causing a manic-like syndrome with few negative symptoms or hallucinations, and with rapid recovery.

Clinical and psychosocial outcomes in bipolar patients who use cannabis

Van Rossum *et al.* (2009) performed an observational study of bipolar patients as part of the EMBLEM trial (European Mania in Bipolar Evaluation of Medication) study, which examined 3459 in- and outpatients to assess how cannabis use impacts both clinical and psychosocial outcomes. Cannabis use was assessed over a 15-month period and judged based on patient self-report as well as by the investigators. Clinical measures included severity of psychopathology (assessed using the Clinical Global Impressions scales), medication adherence and use of alcohol and other substances. Other outcomes included independent living, work impairment, relationships, social activities, life satisfaction and number of dependents. Cannabis use was significantly associated with higher scores of overall illness, mania scores and hallucinations/delusions; there was no association with depression scores. Cannabis use was also associated with poorer treatment adherence; however, the significance did not persist once other substance use was included in the regression model. Cannabis use was significantly associated with alcohol and other substance use/abuse/dependence. Turning to psychosocial outcomes, patients with cannabis use were significantly less likely to have a confiding relationship, and were significantly less satisfied with their life overall. Interestingly, cannabis use was significantly associated with engaging in more social activities although the association did not persist when mediator variables were included in the model. The observational design did not allow determination of the degree of causality that underpinned the significant associations they found; cannabis use may be either the cause or the result of poorer clinical outcomes, or there may be other factors such as genes or demographics that may increase the vulnerability for both. However, the results are certainly suggestive of a negative association between cannabis use and treatment outcome.

Cognitive effects of cannabis in bipolar disorder

Cahill *et al.* (2006) reviewed the interactions between chronic cannabis use and cognitive compromise in bipolar disorder. They highlighted the inherent difficulties in researching this area including small sample sizes, differences in clinical presentation or phases of illness, and the confounding effects of medication. Baseline neuropsychological deficits have been found in people with bipolar disorder; these include executive functioning deficits, attentional difficulties and deficits in memory and verbal learning. There are also neuropsychological deficits associated with substance abuse in general, and chronic cannabis use in particular, notably in executive functioning and impulse control (see Chapter 8). When looking at cognitive functioning in cannabis users with bipolar disorder, the deficits seem to lie mostly in the domain of executive dysfunction. Cahill *et al.* (2006) suggest that these findings in bipolar disorder represent difficulties with encoding, concentration and memory, and have some overlap with cannabis use where the primary deficits are in decision making, response inhibition, cognitive flexibility and abstraction, in addition

to impaired memory and learning abilities. Extending these findings to functional outcomes, the authors expected to find poorer functioning in patients with bipolar disorder and comorbid cannabis abuse, but few studies have investigated this issue. Further studies are warranted to elucidate the associations between the neuropsychological deficits and integrate the findings into functional and clinical outcomes.

The brain in bipolar patients who abuse cannabis

Jarvis *et al.* (2008) sought to clarify the neurophysiological correlates between cannabis and bipolar disorder. They performed MRI scans on 14 adolescents with bipolar disorder, half of whom had comorbid cannabis-use disorder either before the scan, or within two years following the scan. There was evidence of greater structural abnormalities in adolescents who had comorbid cannabis-use disorder, and the investigators speculated that this might reflect underlying neuroanatomic differences given the subjects' limited exposure to cannabis before the scans. However, the small sample size and methodological issues – including not reporting on the statistical significance of their result – limit the conclusions that can be drawn from this study. Further exploration of brain structure and function in people with cannabis and bipolar disorder are needed.

The endocannabinoid system and bipolar disorder

In trying to understand the interaction between cannabis and bipolar disorder, attention has been given to the endocannabinoid system (see also Chapter 3). For example, Koethe *et al.* (2007) found no evidence of increased or decreased density of CB1 receptor immunopositive cells in the anterior cingulate in a group of patients with bipolar disorder. However, exposure to first-generation antipsychotics (FGA) was associated with significantly reduced numerical density of CB1 receptor immunoreactive glial cells in the bipolar patients (Koethe *et al.*, 2007). In a recent further study, post mortem tissue of human hippocampus was investigated regarding expression of cannabinoid CB1 receptors. There were no significant differences in the numerical density of immunoreactive neurons or glial cells in bipolar disorder compared with healthy controls, depressive disorder or schizophrenia (data not published).

Regarding levels of cannabinoid-receptor ligands such as anandamide (AEA) in bipolar disorder, no systematic investigations have been published. In the study of Giuffrida *et al.*, the elevation of AEA in cerebrospinal fluid was apparently restricted to schizophrenia and was not observed in patients affected by dementia or affective disorders. This study investigated only 22 patients with affective disorder, consisting of depressive and bipolar disorder; therefore definitive conclusions cannot be drawn at present (Giuffrida *et al.*, 2004).

Exploration of cannabinoid genes in the mood disorders is of interest given, for example, that the CB1 receptor rs1049353 single nucleotide polymorphism (1359 G/A) could affect mRNA stability or translation, which might result in an alteration of CB1 receptor function; and the rs324420 SNP (cDNA 385C to A) of the fatty acid amide hydrolase (FAAH) gene has been shown to reduce the activity of the FAAH enzyme. Monteleone *et al.* (2010) found that the CB1 receptor rs1049353 (1359 G/A) SNP was significantly associated with major depression, but not with bipolar disorder; there was a trend for an association of the FAAH rs324420 SNP with both diseases. These findings, although preliminary, point to a potential role for the endocannabinoid in both major depression and bipolar disorder, and might give a perspective in the development of exogenous cannabinoid agonists like FAAH inhibitors with potential antidepressant effects.

Treatment

The neuropharmacological properties of cannabinoids have not been studied systematically in bipolar disorder. It has been suggested that the cannabinoids Δ^9-tetrahydrocannabinol (THC) and cannabidiol have multimodal action, including sedative, hypnotic, anxiolytic, antidepressant, antipsychotic and anticonvulsant effects (Ashton *et al.*, 2005; Leweke and Koethe, 2008) (see Chapters 1 and 2). This has led to the hypothesis that cannabidiol may offer therapeutic potential in bipolar patients because of its anticonvulsant effects and neuroprotection against glutamate toxicity, suggesting it may have mood-stabilizing effects similar to some other anticonvulsants (Ashton *et al.*, 2005; Leweke and Koethe, 2008). Indeed, there is a great deal of anecdotal evidence from patients who claim that smoking cannabis can provide symptomatic relief from depressive as well as manic symptoms. Ashton *et al.* (2005) have detailed the mechanisms of the

endocannabinoid system that have been implicated in brain reward pathways, pain, sleep, mood and anxiety. The authors acknowledged the many difficulties with studying the cannabinoids, including issues with doses, mode of administration and tolerance. They called for a placebo-controlled trial of cannabinoids as adjunctive treatment in patients with bipolar disorder. There are obvious potential drawbacks to such a study, including potential exacerbation or precipitation of manic or psychotic symptoms, cognitive problems, induction of cytochrome P450 enzymes and issues with tolerance and dependence. Nonetheless, such a study may be warranted to investigate the potential of adjunctive treatment, with cannabinoids in a select group of closely monitored, treatment-resistant patients.

At the time of writing, there is one placebo-controlled trial ongoing, employing a within subject, crossover experimental intervention using a standardized plant extract of cannabis containing a 1:1 ratio of THC and cannabidiol to investigate the impact on mood symptoms in bipolar patients unresponsive to standard treatment (http://clinicaltrials.gov/ct2/show/NCT00397605). This is an ambitious study, and the mixture of THC and cannabidiol may obscure potential effects of one or the other of these compounds, and the long overlapping effects of these compounds are likely to jeopardize the trial in its crossover design.

Conclusions

There is no doubt a great deal of comorbidity between bipolar disorder and cannabis use. There is also a growing body of evidence regarding the deleterious effects of cannabis use in patients with bipolar disorder. Cannabis use has been associated with higher risks of inducing mania or manic symptoms, and there are studies suggesting that the onset of bipolar illness may be earlier in patients who use cannabis. Furthermore, there is good evidence that cannabis use can influence the course and treatment of bipolar illness, and lead to poorer clinical and psychosocial outcomes. Preliminary studies suggest that bipolar patients who use cannabis may have greater cognitive deficits, in particular executive dysfunction; there is also a suggestion there may be underlying neuroanatomical differences, although this evidence is meagre.

Studies on the role of cannabis use or an involvement of the endocannabinoid system in bipolar disorders are sparse, but suggest that the endocannabinoid system might be involved in pathophysiological mechanisms of bipolar disorders. This is an area for further research.

The majority of existing studies are complicated by methodological limitations, and there is a clear indication for well-designed basic, epidemiologic and clinical studies to further clarify the relationship between bipolar disorder and cannabis use, and guide future treatment and research.

References

Agrawal, A., Nurnberger, J.I., Jr. and Lynskey, M.T. (2011) Cannabis involvement in individuals with bipolar disorder. *Psychiatry Res*, **185**:459–61.

Ashton, C.H., Moore, P.B., Gallagher, P. and Young, A.H. (2005) Cannabinoids in bipolar affective disorder: a review and discussion of their therapeutic potential. *J Psychopharmacol*, **19**:293–300.

Baethge, C., Baldessarini, R.J., Khalsa, H.M., Hennen, J., Salvatore, P. and Tohen, M. (2005) Substance abuse in first-episode bipolar I disorder: indications for early intervention. *Am J Psychiatry*, **162**:1008–10.

Baethge, C., Hennen, J., Khalsa, H.M., Salvatore, P., Tohen, M. and Baldessarini, R.J. (2008) Sequencing of substance use and affective morbidity in 166 first-episode bipolar I disorder patients. *Bipolar Disord*, **10**:738–41.

Bertolín-Guillén, J.M., Lopez-Arquero, F.J. and Martinez-Franco, C. (2008) Cannabis-induced mania? *J Subs Use*, **13**:139–41.

Cahill, C.M., Malhi, G.S., Ivanovski, B., Lagopoulos, J. and Cohen, M. (2006) Cognitive compromise in bipolar disorder with chronic cannabis use: cause or consequence? *Expert Rev Neurother*, **6**:591–8.

Cassidy, F., Ahearn, E.P. and Carroll, B.J. (2001) Substance abuse in bipolar disorder. *Bipolar Disord*, **3**:181–8.

De Hert, M., Wampers, M., Jendricko, T., *et al.* (2010) Effects of cannabis use on age at onset in schizophrenia and bipolar disorder. *Schizophr Res*. doi:10.1016/j.schres.2010.07.003.

El-Mallakh, R.S. and Brown, C. (2007) The effect of extreme marijuana use on the long-term course of bipolar I illness: a single case study. *J Psychoactive Drugs*, **39**:201–2.

Giuffrida, A., Leweke, F.M., Gerth, C.W., *et al.* (2004) Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology*, **29**:2108–14.

Goldberg, J.F., Garno, J.L., Leon, A.C., Kocsis, J.H. and Portera, L. (1999) A history of substance abuse complicates remission from acute mania in bipolar disorder. *J Clin Psychiatry*, **60**:733–40.

Henquet, C., Krabbendam, L., de Graaf, R., ten Have, M. and van Os, J. (2006) Cannabis use and expression of mania in the general population. *J Affect Disord*, **95**:103–10.

Jarvis, K., DelBello, M.P., Mills, N., Elman, I., Strakowski, S.M. and Adler, C.M. (2008) Neuroanatomic comparison of bipolar adolescents with and without cannabis use disorders. *J Child Adolesc Psychopharmacol*, **18**:557–63.

Koethe, D., Llenos, I.C., Dulay, J.R., *et al.* (2007) Expression of CB1 cannabinoid receptor in the Anterior Cingulate Cortex in Schizophrenia, Bipolar Disorder, and Major Depression. *J Neural Transmission*, **114**:1055–63.

Levin, F.R. and Hennessy, G. (2004) Bipolar disorder and substance abuse. *Biol Psychiatry*, **56**:738–48.

Leweke, F.M. and Koethe, D. (2008) Cannabis and psychiatric disorders: it is not only addiction. *Addict Biol*, **13**:264–75.

Monteleone, P., Bifulco, M., Maina, G., *et al.* (2010) Investigation of CNR1 and FAAH endocannabinoid gene polymorphisms in bipolar disorder and major depression. *Pharmacological Research*, **61**:400–4.

Regier, D.A., Farmer, M.E., Rae, D.S., *et al.* (1990) Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. *JAMA*, **264**:2511–8.

Rottanburg, D., Robins, A.H., Ben-Arie, O., Teggin, A. and Elk, R. (1982) Cannabis-associated psychosis with hypomanic features. *Lancet*, **2**:1364–6.

Sherwood Brown, E., Suppes, T., Adinoff, B. and Rajan Thomas, N. (2001) Drug abuse and bipolar disorder: comorbidity or misdiagnosis? *J Affect Disord*, **65**:105–15.

Strakowski, S.M., DelBello, M.P., Fleck, D.E., *et al.* (2007) Effects of co-occurring cannabis use disorders on the course of bipolar disorder after a first hospitalization for mania. *Arch Gen Psychiatry*, **64**:57–64.

Strakowski, S.M., DelBello, M.P., Fleck, D.E. and Arndt, S. (2000) The impact of substance abuse on the course of bipolar disorder. *Biol Psychiatry*, **48**:477–85.

Tohen, M., Greenfield, S.F., Weiss, R.D., Zarate, C.A., Jr. and Vagge, L.M. (1998) The effect of comorbid substance use disorders on the course of bipolar disorder: a review. *Harv Rev Psychiatry*, **6**:133–41.

van Laar, M., van Dorsselaer, S., Monshouwer, K. and de Graaf, R. (2007) Does cannabis use predict the first incidence of mood and anxiety disorders in the adult population? *Addiction*, **102**:1251–60.

van Rossum, I., Boomsma, M., Tenback, D., Reed, C. and van Os, J. (2009) Does cannabis use affect treatment outcome in bipolar disorder? A longitudinal analysis. *J Nerv Ment Dis*, **197**:35–40.

Chapter

12

Which cannabis users develop psychosis?

Marta Di Forti, Cécile Henquet, Hélène Verdoux, Sir Robin M. Murray and Jim van Os

Numerous epidemiological studies have described a significant association between cannabis use and onset of psychosis, though all have also shown that only a minority of cannabis users develop such disorders (Murray *et al*., 2007). Nevertheless, since the global number of people who used cannabis at least once in 2007 was between 143 and 190 million (World Drug Report, 2009), this psychotic minority represents a large number of people. Indeed, the proportion of schizophrenia attributed to cannabis use in different countries has been found to be between 8% and 15% (Henquet *et al*., 2005b; Moore *et al*., 2007), indicating considerable potential for preventive measures. It is therefore important to understand which factors determine an individual's vulnerability to: (1) start using cannabis; and (2) to experience the long-term negative effects of cannabis exposure on psychosis outcome.

Cannabis and sub-clinical expression of psychosis

Cannabis use not only predicts onset of psychotic disorder, but is also associated with subthreshold expression of psychosis, either in the form of schizotypy (Williams *et al*., 1996; Barkus and Lewis, 2008; Esterberg *et al*., 2009) or subclinical psychotic experiences (van Os *et al*., 2002; Verdoux *et al*., 2003b; Henquet *et al*., 2005). Verdoux *et al*. (2003a), for example, explored the pattern of associations between cannabis use and dimensions of psychosis in a sample of female undergraduates. Dimensions of psychosis were measured by the Community Assessment of Psychic Experiences (CAPE), a 42-item self-report questionnaire developed to measure the positive, negative and depressive symptom dimensions of psychosis in the general population (Konings *et al*., 2006). Significant associations were found between cannabis use and positive and negative symptom dimension scores, such that increased levels of cannabis use were associated with higher positive and negative symptom dimension scores. No such association was found however between cannabis use and the depression dimension score (Verdoux *et al*., 2003).

There has been much debate as to whether the association between cannabis and subclinical expression of psychosis is causal, or whether psychotic experiences may prompt cannabis use in individuals at genetic risk for psychosis as a means of self-medication. Given that there is evidence that subclinical expression of psychosis may in part have a genetic origin, the self-medication hypothesis has been interpreted in terms of gene-environment correlation (Henquet *et al*., 2008), referring to genetic control of cannabis use. Kendler *et al*. (2008), examining monozygotic (MZ) and dizygotic (DZ) twins, have shown that the impact of genes and environment on cannabis use varies according to stages and characteristics of use. For instance, initiation and early patterns of cannabis use are more influenced by environmental factors, whereas cannabis abuse and dependence are more genetically mediated (Kendler *et al*., 2008).

Moreover, Ferdinand and colleagues (2005) showed that psychotic symptoms in cannabis-naïve children and adolescents (4–16 years) predicted later onset of cannabis use (after 14 years). Henquet *et al*. (2005), however, found no evidence in a German cohort study that baseline psychotic symptoms predicted the onset of cannabis use 3.5 years later. Also, the Christchurch Health and Development Study in which cannabis use and psychotic symptoms were examined at ages 18, 21 and 25 years, showed that the data were more compatible with a causal rather than a self-medication explanation (Fergusson *et al*., 2003; Fergusson *et al*., 2005). Veling and colleagues (2008) investigated cannabis use and psychosis liability associations in a case–control design, including first-episode schizophrenia patients,

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unaffected siblings of cases and controls. In this study, cannabis use was associated with schizophrenia as an outcome, but there was no evidence for gene-environment correlation, as siblings of cases (at increased genetic risk) did not have higher rates of cannabis use than controls (Veling *et al*., 2008). Similarly, a recent large sib-pair study, including patients with a psychotic disorder, their siblings and community controls, found no evidence for genetic confounding (G.R.O.U.P., 2011). Thus, CAPE positive dimension scores in the patients did not predict cannabis use in the siblings, nor did CAPE positive dimension scores in the siblings predict cannabis use in the patients.

Thus, although there is some modest evidence that self-medication may play a role, the association between cannabis and psychosis cannot be reduced largely or entirely to gene-environment correlation. Several studies adjusted statistically for self-medication effects and still found an effect of cannabis use on psychosis outcome. Van Os *et al.* (2002), for example, excluded individuals with psychotic symptoms at baseline and nonetheless found an effect of baseline cannabis on psychosis outcome at 3-year follow-up. Other studies used the method of statistical adjustment and similarly found that the effect of cannabis on psychosis outcome remained significant after controlling for pre-existing psychotic symptoms (Arseneault *et al*., 2002; Henquet *et al*., 2005).

Gene-environment interaction

Psychosis proneness

Clearly, individuals differ greatly in their sensitivity to the psychosis-inducing effects of cannabis. Instead of adjusting for pre-existing psychotic symptoms, Henquet and colleagues applied a model of interaction in which psychosis liability, measured psychometrically by questionnaire, was studied for its potential synergistic effects on the psychosis-inducing effects of cannabis in adolescents from the general population (Henquet *et al*., 2005); the effect of baseline cannabis use on the psychosis outcome at 4-year follow-up was much stronger in those individuals who had higher levels of psychosis proneness. Barkus and Lewis (2008) also investigated psychosis proneness and acute reactions to cannabis use in university students by means of the Cannabis Experiences Questionnaire (CEQ) and the Schizotypal Personality Questionnaire (SPQ) (Raine, 1991). High-psychosis proneness scores in this study were associated with higher levels of pleasurable experiences, psychosis-like experiences and cannabis after-effects. Acute effects of cannabis on psychotic symptoms were also investigated by Mason *et al.* (2009) using a naturalistic design. Level of psychosis proneness was determined using the Psychotomimetic States Inventory (PSI) (Mason *et al*., 2009). The results showed that highly psychosis prone individuals were more sensitive to experiencing enhanced psychotomimetic states following acute cannabis use than individuals with average levels of psychosis proneness.

Applying a momentary assessment technique (the Experience Sampling Method [ESM]), Verdoux and colleagues (2003a) investigated the acute effects of cannabis on psychotic experiences in the flow of daily life, and compared these between students with high and average psychosis proneness (defined by the CAPE questionnaire and the MINI-International Neuropsychiatric Interview criteria). They also found that in daily life the acute effects of cannabis were moderated by an individual's level of psychosis liability, in that those with high psychosis vulnerability reported more intense increases in psychosis-like symptoms. Individuals with low CAPE scores, on the other hand, were more likely to interpret the social context as more friendly when under the influence of cannabis (Verdoux *et al*., 2003a).

Familial liability

From the aforementioned studies, it is clear that psychometrically defined psychosis liability moderates both the acute and the long-term effects of cannabis. Whether psychometric psychosis liability reflects a familial or genetic liability, however, remains a matter of debate. The proposition that subclinical psychotic symptoms may have a familial origin comes from studies showing that first-degree relatives of patients with schizophrenia display higher levels of subclinical symptoms than individuals from the general population (Appels *et al*., 2004). In addition, there is research to show that in samples that were not selected specifically to investigate psychotic disorders, the positive dimensions of subclinical psychosis cluster in families (Hanssen *et al*., 2006). Twin studies similarly show that genetic factors play a significant role in the manifestation of subclinical psychotic symptoms (MacDonald *et al*., 2001).

Recently, the Genetic Risk and Outcome in Psychosis (GROUP) study (G.R.O.U.P., 2011), investigated the association between familial liability for

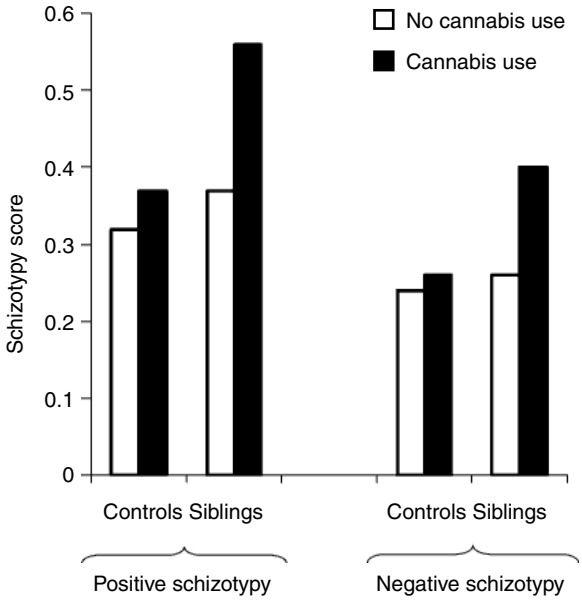

Figure 12.1. Positive and negative "Structured Interview for Schizotypy – Revised" schizotypy scores in controls and healthy siblings of patients with psychotic disorder as a function of cannabis use assessed by urinalysis. Siblings displayed more than 15 times greater sensitivity to positive schizotypy associated with current cannabis use than did controls. Similar results were found with respect to the negative dimension of schizotypy.

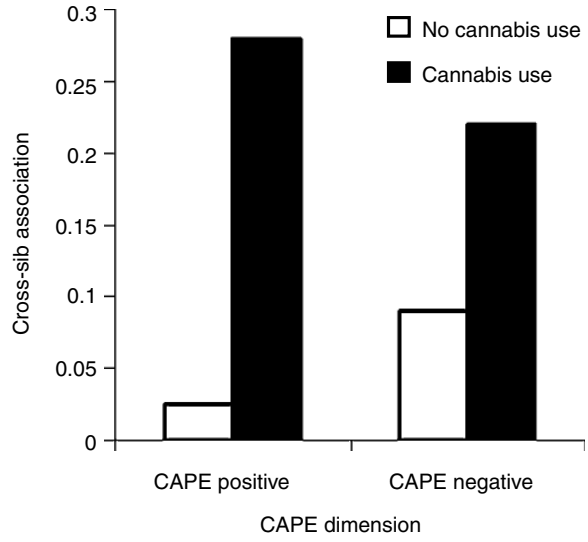

Figure 12.2. Patient-healthy sibling cross-sib associations in Community Assessment of Psychic Experiences (CAPE) positive and CAPE negative symptoms as a function of cannabis use in the healthy sibling, assessed by urinalysis. Siblings exposed to cannabis resembled their patient relative nearly 10 times more closely in the positive psychotic dimension of the CAPE compared with non-exposed siblings. No such effects of cannabis were found for the CAPE negative domain.

psychosis and sensitivity to cannabis, using patient-sibling and sibling-control pairs analyses. This study, focussing on gene-environment interactions relevant to psychotic disorders (G.R.O.U.P., 2011), included patients with a psychotic disorder (n = 1120), their siblings (n = 1057) and community controls (n = 509). First, associations between current cannabis use (by urinalysis) and schizotypy (measured with the Schizotypy Interview Schedule – Revised, SIS-R) were calculated in unaffected siblings versus healthy controls; siblings displayed more than 15 times greater sensitivity to positive schizotypy associated with current cannabis use than controls. Similar results were found with respect to the negative dimension of schizotypy (Figure 12.1). Furthermore, positive and negative psychotic experiences (measured with the CAPE) were assessed in patient-sibling pairs, comparing siblings with and without lifetime exposure to cannabis. Siblings exposed to cannabis resembled their patient relatives nearly ten times more closely in the positive psychotic dimension of the CAPE than non-exposed siblings (Figure 12.2). No such effects of cannabis were found for the CAPE negative domain (G.R.O.U.P., 2011).

Can we measure genetic vulnerability directly?

In the Dunedin study, Caspi and colleagues (2005) highlighted the importance of individual genetic vulnerability when they reported an interaction between cannabis use and variation in the gene that encodes catecholamine-O-methyl transferase (COMT). Catecholamine-O-methyl transferase is the key enzyme involved in the pre-frontal cortex metabolism of dopamine released into synapses, and contains a G to A missense mutation that generates a valine (Val) to methionine (Met) substitution at codon 158 (*Val*^{158}*Met*), producing less enzymatic activity and slower break down of dopamine. In the Dunedin cohort, adolescents who used cannabis had a significantly greater increase in the risk of subsequent schizophreniform disorder if they carried the *Val/Val* genotype compared with a lesser increase if they carried the *Val/Met* genotype and no increase for the *Met/Met* genotype. Interestingly, however, there was no evidence for gene environment correlation, as individuals with the *Val/Val* genotype were not more prone to start using cannabis at a young age, than individuals with the *Val/Met* or *Met/Met* genotype.

The interaction between cannabis use and the *COMT Val^{158}Met* polymorphism gene was later tested experimentally by Henquet *et al.* (2009b) who gave 300 μg of Δ^9-tetrahydrocannabinol (THC) per kg of body weight, or a placebo to patients with psychotic disorders, relatives of patients with a psychotic disorder and healthy controls. Those with the homozygous Val genotype were more likely to develop THC-induced psychotic symptoms, but this was conditional on prior evidence of psychometric psychosis liability. These findings were replicated using the experience sampling method to collect data on cannabis use and the occurrence of psychotic symptoms in daily life (Henquet *et al.*, 2009b). The frequency of hallucinatory experiences following cannabis use was significantly increased only in those individuals who were carriers of the Val allele and had high levels of psychometric psychosis liability. Once again, these data suggested that *COMT Val^{158}Met* genotype might moderate the association between cannabis use and psychotic symptoms in psychosis-prone people. In this study, carriers of the Val allele similarly were not more likely to use cannabis more frequently than individuals with the Met allele.

In contrast, a study of patients with a psychotic disorder (Zammit *et al.*, 2007) found no evidence for a differential effect of cannabis use on psychosis risk according to variation in *COMT Val^{158}Met*. Unfortunately, in this study, information on whether cannabis was used or not was obtained retrospectively from hospital records, which reflects a relatively unreliable source of information for drug use of individuals. In short, there is intriguing evidence suggesting an interaction between cannabis consumption and the *COMT Val^{158}Met* genotype in provoking psychosis. However, the hypothesis remains to be adequately confirmed or refuted, and, of course, individual response to cannabis use is likely to be moderated by a number of genes rather a single polymorphism.

Indeed, recent evidence suggests that variation within the *AKT1* gene may also be involved in differential sensitivity to cannabis. Using data from the previously described G.R.O.U.P. study, van Winkel and G.R.O.U.P. (2011) applied a family-based design to investigate genetic moderation of selected schizophrenia-candidate single-nucleotide polymorphisms (SNPs) on cannabis-induced psychosis. First, an at-risk paradigm (including 740 unaffected siblings) was used to examine 152 SNPs in 42 a-priori candidate genes. Associations between recent cannabis use (by urinary analyses) and positive schizotypy (assessed by the Structured Interview for Schizotypy – revised [SIS-R]) and genetic moderation thereof, were investigated. Sixteen SNPs in 12 genes showed significant interaction, of which three survived correction for multiple testing; these were situated in *AKT1* and *LRRTM1*. Follow-up replication analyses of these three SNPs furthermore showed a significant interaction between *AKT1* (rs2494732) and cannabis-lifetime use, using a case only, a case-sib and a case–control design. This study is the first to show that *AKT1* may moderate both the short-term and the longer-term effects on psychosis expression associated with cannabis use. Interestingly, acute administration of THC in mice has been found to activate *AKT1* in vivo in several brain areas, including the striatum. Decreased *AKT1* levels have been observed in post-mortem prefrontal cortex of patients with schizophrenia. The authors suggest that the interaction between cannabis exposure and variation within the *AKT1* gene may be explained by a mechanism of cannabinoid-regulated *AKT1-GSK3* signaling downstream of the dopamine D2 receptor (Van Winkel and G.R.O.U.P., 2011). This study failed to find an interaction between cannabis and COMT.

Early environmental exposure apart from genetic factors, interplay between environmental factors may also determine an individual's sensitivity to cannabis (see Figure 12.3). First, additivity of environmental risks for psychosis (i.e. urbanicity, trauma and cannabis) was shown in two independent cohort studies (Cougnard *et al.*, 2007). Recently, however, more-than-additive effects of the combination of childhood trauma and cannabis use became apparent, as three separate population-based studies have now shown that early traumatic experiences moderate the psychosis-inducing effects of cannabis (Konings *et al.*, submitted; Houston *et al.*, 2008; Harley *et al.*, 2010). Similarly, an interaction between urbanicity and cannabis exposure has been shown in a longitudinal population-based study, indicating that the effects of cannabis use may be particularly detrimental for adolescents who are growing up in an urban environment (Henquet *et al.*, 2009a). These findings suggest that cannabis on the one hand and trauma and urbanicity on the other do not index the same environmental influence but may impact on related biological pathways. There is emerging evidence that childhood trauma and urban environment may index aspects of social adversity associated with chronic experience of marginalization, subordination or exclusion, also referred to as social defeat (Selten and Cantor-Graae, 2005). The

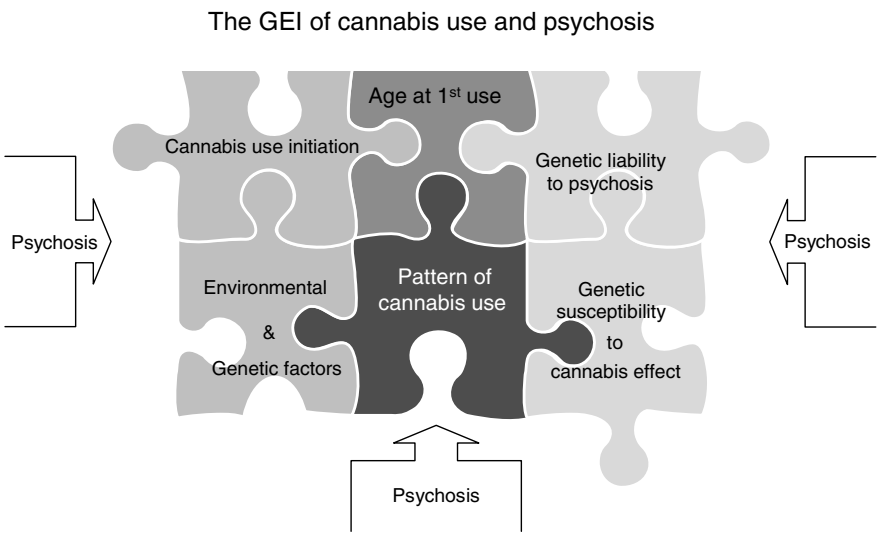

Figure 12.3. Illustration of all the different factors contributing to the GEI (Gene Environment Interaction) puzzle of cannabis use and psychosis.

finding that the psychosis-inducing effects of cannabis are moderated by trauma and urbanicity, therefore suggests that cross-sensitization between cannabis and stress may play a role in shaping risk of psychotic symptoms (Henquet *et al*., 2008). The underlying biological mechanisms, however, remain unclear.

"Tell me your pattern of use and I shall predict your risk": fiction or science?

The main psychoactive ingredient of cannabis is THC, which is present in the different types of cannabis at different levels of concentration (see Chapter 4). For example, marijuana and resin have traditionally contained about 4% THC compared with the concentration of THC in sensimilla (or skunk) that has recently reached between 16 and 22% in Holland and England (Potter *et al*., 2008). Although a clear dose–response association has consistently been shown between cannabis exposure and psychosis risk, few of the existing studies have collected detailed data on the pattern of cannabis use or its potency. There is increasing evidence, however, that different strains of cannabis may have differential impact on mental health risk (Smith, 2005), depending not only upon the concentration of THC but also of cannabidiol (CBD, another cannabis compound). This has the ability to reduce the anxiety and psychotomimetic symptoms as well as cognitive impairments induced by THC (Leweke *et al*., 2000 and see Chapter 3). In addition, a preliminary report claims that CBD may even hold antipsychotic properties (Leweke *et al*., 2007).

An interesting study by Morgan and Curran measured cannabinoid traces in the hair of three groups of normal volunteers, and found that those with "THC only" had higher level of schizophrenia-like symptoms than the "THC plus CBD" and "no cannabinoids at all" groups (Morgan and Curran, 2008).

Furthermore, a recent study by Di Forti *et al*. (2009) collected detailed data on patterns of cannabis use (ever used, age at first use, duration and frequency of use) and potency from a sample of 280 first-episode psychosis patients and 174 healthy controls. The first-episode psychosis patients were almost twice as likely to have used cannabis for more than 5 years, compared with controls. When potential confounders (age, gender, ethnicity, use of stimulants, level of education achieved and employment status) were controlled for, this difference was only slightly reduced, albeit no longer statistically significant. Furthermore, first-episode psychosis patients were over six times more likely than the control group to use cannabis every day, even after adjusting for the above potential confounders. Most interestingly, patients with a first episode of psychosis were almost seven times more likely to have used high-potency cannabis preparations, such as skunk, than controls (adjusted odds ratio = 6.8, 95% confidence intervals: 2.6, 25.4), who seemed to prefer the much less potent variety. Overall, these findings suggest that the potency and frequency of cannabis use may play a crucial role in further increasing the risk of psychosis. This has potentially important public health implications given how the availability of skunk in a number of countries is increasing (see also

Chapter 5). Indeed, Di Forti *et al*. (2009) calculated from the study described above a population attributable fraction of 27% for skunk use, which indicates the proportion of first-episode psychosis cases that could be prevented in South-East London were the use of skunk to be abolished (unpublished data). In theory, it is possible that those subjects who later develop a psychotic disorder, choose high-potency cannabis to self-medicate their pre-existing symptoms, for example in the prodrome. It remains paradoxical why anyone at genetic risk for psychosis, who is already experiencing prodomal and/or psychotic symptoms, should choose to use a potent type of cannabis such as skunk, the high level of THC of which is likely to exacerbate their symptoms, rather than use resin (hash), which contains the potentially ameliorating CBD, as well as a low level of THC (Miettunen *et al*., 2008).

Given that different types of cannabis clearly affect mental health differentially, more research is needed to understand how genetic liability may increase sensitivity to, or preference for, the specific constituents of cannabis. Furthermore, recent data on the differential effects of different cannabis components, stress the importance of taking into account differences in potency of cannabis when studying the acute and long-term effects of cannabis use.

References

Appels, M.C., Sitskoorn, M.M., Vollema, M.G. and Kahn, R.S. (2004) Elevated levels of schizotypal features in parents of patients with a family history of schizophrenia spectrum disorders. *Schizophr Bull,* **30**:781–90.

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A. and Moffitt, T.E. (2002) Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *BMJ,* **325**:1212–3.

Barkus, E. and Lewis, S. (2008) Schizotypy and psychosis-like experiences from recreational cannabis in a non-clinical sample. *Psychol Med,* **38**:1267–76.

Caspi, A., Moffitt, T.E., Cannon, M., *et al*. (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry,* **57**:1117–27.

Cougnard, A., Marcelis, M., Myin-Germeys, I., *et al*. (2007) Does normal developmental expression of psychosis combine with environmental risk to cause persistence of psychosis? A psychosis proneness-persistence model. *Psychol Med,* **37**:513–27.

Di Forti, M., Morgan, C., Dazzan, P., *et al*. (2009) High-potency cannabis and the risk of psychosis. *Br J Psychiatry,* **195**:488–91.

Esterberg, M.L., Goulding, S.M., McClure-Tone, E.B. and Compton, M.T. (2009) Schizotypy and nicotine, alcohol, and cannabis use in a non-psychiatric sample. *Addict Behav,* **34**:374–9.

Ferdinand, R.F., Sondeijker, F., van der Ende, J., Selten, J.P., Huizink, A. and Verhulst, F.C. (2005) Cannabis use predicts future psychotic symptoms, and vice versa. *Addiction,* **100**:612–8.

Fergusson, D.M., Horwood, L.J. and Ridder, E.M. (2005) Tests of causal linkages between cannabis use and psychotic symptoms. *Addiction,* **100**:354–66.

Fergusson, D.M., Horwood, L.J. and Swain-Campbell, N.R. (2003) Cannabis dependence and psychotic symptoms in young people. *Psychol Med,* **33**:15–21.

G.R.O.U.P. (2011) Evidence that familial liability for psychosis is expressed as differential sensitivity to cannabis: an analysis of patient-sibling and sibling-control pairs. *Arch Gen Psychiatry*, **68**:138–47.

Hanssen, M., Krabbendam, L., Vollema M., Delespaul, P. and van Os, J. (2006) Evidence for instrument and family-specific variation of subclinical psychosis dimensions in the general population. *J Abnorm Psychol,* **115**:5–14.

Harley, M., Kelleher, I., Clark, M. *et al*. (2010) Cannabis use and childhood trauma interact additively to increase the risk of psychotic symptoms in adolescence. *Psychol Med,* **40**:1627–34.

Henquet, C., Di Forti, M., Morrison, P., Kuepper, R. and Murray, R.M. (2008) Gene-environment interplay between cannabis and psychosis. *Schizophr Bull,* **34**:1111–21.

Henquet, C., Krabbendam, L., Spauwen, J., *et al*. (2005) Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *BMJ,* **330**:11–15.

Henquet, C., Kuepper, R. Konings, M., Lieb, R., Wittchen, H.-U. and van Os, J. (2009a) Do cannabis and urbanicity interact in causing psychosis? *Schizophr Bull,* **35**:87.

Henquet, C., Murray, R., Linszen, D. and van Os, J. (2005b) The environment and schizophrenia: the role of cannabis use. *Schizophr Bull,* **31**:608–12.

Henquet, C., Rosa, A., Delespaul, P., *et al*. (2009b) COMT ValMet moderation of cannabis-induced psychosis: a momentary assessment study of 'switching on' hallucinations in the flow of daily life. *Acta Psychiatr Scand,* **119**:156–60.

Houston, J.E., Murphy, J., Adamson, G., Stringer, M. and Shevlin, M. (2008) Childhood sexual abuse, early cannabis use, and psychosis: testing an interaction

model based on the National Comorbidity Survey. *Schizophr Bull,* **34**:580–5.

Kendler, K.S., Schmitt, E., Aggen, S.H. and Prescott, C.A. (2008) Genetic and environmental influences on alcohol, caffeine, cannabis, and nicotine use from early adolescence to middle adulthood. *Arch Gen Psychiatry,* **65**:674–82.

Konings, M., Bak, M., Hanssen, M., van Os., J. and Krabbendam, L. (2006) Validity and reliability of the CAPE: a self-report instrument for the measurement of psychotic experiences in the general population. *Acta Psychiatr Scand,* **114**:55–61.

Konings, M.B., Bakoula, C., *et al.* (2011) Evidence from two prospective population-based studies that cannabis interacts with childhood trauma in causing psychosis. *Submitted Manuscript*.

Leweke, F.M., Gerth, C.W., Koethe, D., Nolden, B.M. and Klosterkotter, J. (2007) Efficacy and tolerability of quetiapine in hospitalised schizophrenic patients during the acute treatment phase: a PMS study. *Fortschr Neurol Psychiatr,* **75**:211–9.

Leweke, F.M., Schneider, U., Radwan, M., Schmidt, E. and Emrich, H.M. (2000) Different effects of nabilone and cannabidiol on binocular depth inversion in Man. *Pharmacol Biochem Behav,* **66**:175–81.

MacDonald, A.W., 3rd, Pogue-Geile, M. F., Debski, T.T. and Manuck, S. (2001) Genetic and environmental influences on schizotypy: a community-based twin study. *Schizophr Bull,* **27**:47–58.

Mason, O., Morgan, C.J., Dhiman, S.K., *et al.* (2009) Acute cannabis use causes increased psychotomimetic experiences in individuals prone to psychosis. *Psychol Med.* **39**:951–6.

Miettunen, J., Tormanen, S., Murray, G.K., *et al.* (2008) Association of cannabis use with prodromal symptoms of psychosis in adolescence. *Br J Psychiatry,* **192**:470–1.

Moore, T.H., Zammit, S., Lingford-Hughes, A., *et al.* (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet,* **370**:319–28.

Morgan, C.J. and Curran, H.V. (2008) Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *Br J Psychiatry,* **192**:306–7.

Murray, R.M., Morrison, P.D., Henquet, C. and Di Forti, M. (2007) Cannabis, the mind and society: the hash realities. *Nat Rev Neurosci,* **8**:885–95.

Potter, D.J., Clark, P. and Brown, M.B. (2008) Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. *J Forensic Sci,* **53**:90–4.

Raine, A. (1991) The SPQ: a scale for the assessment of schizotypal personality based on DSM-III-R criteria. *Schizophr Bull,* **17**:555–64.

Selten, J.P. and Cantor-Graae, E., (2005) Social defeat: risk factor for schizophrenia? *Br J Psychiatry,* **187**:101–2.

Smith, N. (2005) High potency cannabis: the forgotten variable. *Addiction,* **100**:1558–60; author reply 1560–1.

van Os, J., Bak, M., Hanssen, M., Bijil, R.V., de Graaf, R. and Verdoux, H. (2002) Cannabis use and psychosis: a longitudinal population-based study. *Am J Epidemiol,* **156**:319–27.

van Winkel, R. and G.R.O.U.P. (2011) Family-based Analysis of Genetic Variation Underlying Psychosis-Inducing Effects of Cannabis: Sibling Analysis and Proband Follow-up. *Arch Gen Psychiatry*, **68**: 148–57.

Veling, W., Mackenbach, J.P., van Os, J. and Hoek, H.W. (2008) Cannabis use and genetic predisposition for schizophrenia: a case-control study. *Psychol Med,* **38**:1251–6.

Verdoux, H., Gindre, C., Sobara, F., Tournier, M. and Swendsen, J.D. (2003a) Effects of cannabis and psychosis vulnerability in daily life: an experience sampling test study. *Psychol Med,* **33**:23–32.

Verdoux, H., Sorbara, F., Gindre, C., Swendsen, J.D. and van Os., J. (2003b) Cannabis use and dimensions of psychosis in a nonclinical population of female subjects. *Schizophr Res,* **59**:77–84.

Williams, J. H., Wellman, N.A. and Rawlins, J.N.P. (1996) Cannabis use correlates with schizotypy in healthy people. *Addiction,* **91**:869–77.

Zammit, S., Spurlock, G., Williams, H., *et al.* (2007) Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. *Br J Psychiatry,* **191**:402–7.

United Nation Office on Drugs and Crime. UNODC World Drug Report 2009. http://www.unodc.org/unodc/en/data-and-analysis/WDR-2009.html

Chapter

13

Cannabinoids and the cerebellum: a potential role in the development of psychosis

Patrick D. Skosnik

The cerebellum: a brain within a brain

Situated just posterior to the cerebrum (Latin for "big brain") lies the "little brain," otherwise known as the cerebellum. A remarkable structure, with vast connections via the pons and thalamus to numerous areas of cerebral cortex, the cerebellum contains more than half of all the neurons in the entire human brain (~50–85 billion) (Lange, 1975; Zagon *et al*., 1977; Azevedo *et al*., 2009). For decades, the cerebellum has been conceptualized as a structure devoted primarily to motor function, particularly the acquisition and maintenance of fine motor control, gait and posture. Indeed, empirical support for this view has been substantial (Ito, 2002; Apps and Garwicz, 2005; Ioffe *et al*., 2007; Morton and Bastian, 2007).

Aside from the sheer number of neuronal cells making up this relatively small structure (the cerebellum represents only 10% of total brain volume), another noteworthy characteristic is the functional organization of its information processing units, or microcomplexes (Ito, 1984; Braitenberg, 2002; Apps and Garwicz, 2005). Similar to the cerebrum, the cerebellum contains its own cortex, which is organized into three distinctive structural and functional layers: the molecular, Purkinje and granular layers (Figure 13.1). As first summarized by Eccles *et al*. (1967), five major cerebellar cortical cell types exist: stellate, basket and Golgi cells are inhibitory interneurons, granule cells are excitatory interneurons and Purkinje cells are inhibitory projection neurons that synapse onto cells in the deep cerebellar nuclei (DCN) (Figure 13.1). Thus, Purkinje cells, which release gamma-aminobutyric acid (GABA), are the sole output relays from the cerebellar cortex. Excitatory inputs to the cerebellar cortex arise from climbing fibers (inferior olive axons) and mossy fibers (pons and spinal cord afferents). These inputs to the cerebellar cortex are essentially a "side-loop," as collaterals from climbing and mossy fibers also provide input to the DCN (Hansel *et al*., 2001).

What is particularly striking from an information processing perspective is the idiosyncratic organization of these cortical afferents. Each Purkinje cell receives input from upwards of 200 000 granule-cell parallel fibers, while only a single climbing fiber synapses onto each Purkinje cell. This strong climbing-fiber input, when temporally paired with concurrent parallel-fiber activity, is thought to represent an "error" or "teaching" signal during the process of motor learning (see below). Such convergent inputs provide a mechanism for unique forms of coincidence detection and synaptic plasticity, which has inspired the notion of the cerebellum as a "neuronal learning machine" (Eccles *et al*., 1967; Raymond *et al*., 1996). Thus, as elegantly affirmed by Ramnani (2006), the combination of extremely large cell number, extensive interconnectedness and crystalline microstructure provides the mechanistic potential for extremely powerful neural processing.

Basic cerebellar structure and function

While a detailed examination of cerebellar gross anatomy is beyond the scope of this chapter, a brief exposition of its major features is included here (for a comprehensive treatment of cerebellar anatomy see Angevine, 1961; Larsell and Jansen, 1970; Schmahmann, 2000).

The major subdivisions of the cerebellar cortex include the vestibulocerebellum, spinocerebellum and the cerebrocerebellum. As the nomenclature indicates, each of these divisions includes reciprocal connections with brainstem vestibular nuclei, spinal cord and cerebral cortex, respectively. The cerebellum can also be generally divided into three major lobes in the rostral to caudal dimension, the most rostral of which is

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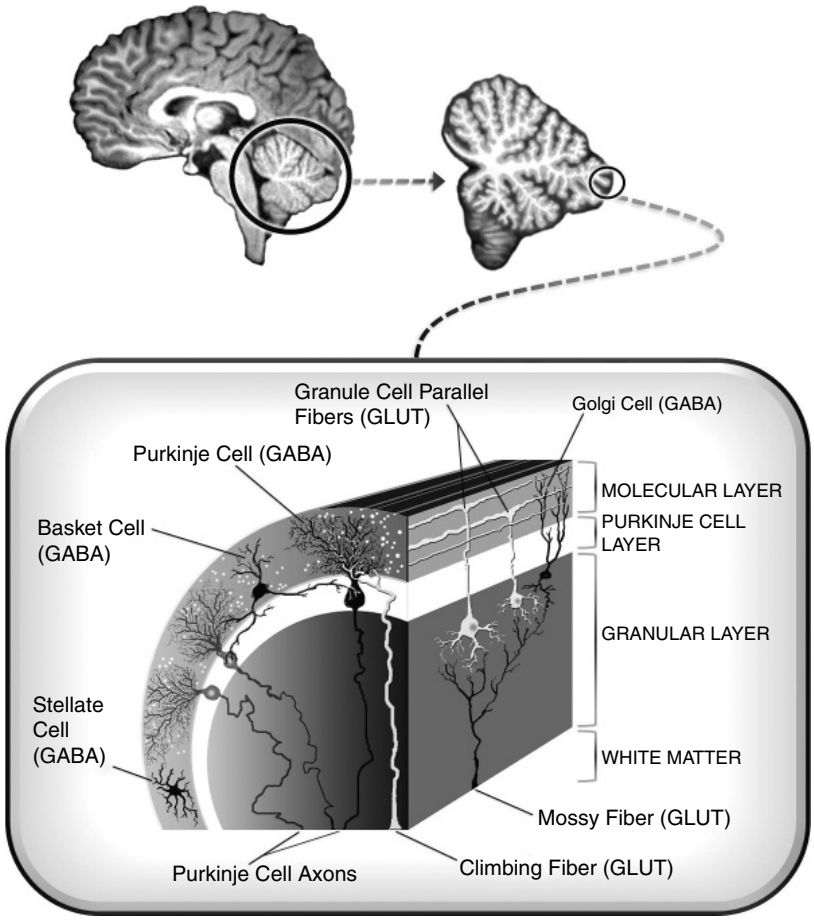

Figure 13.1. Intrinsic circuitry of the cerebellar cortex, showing the five major cell types. In terms of input, the climbing fibers make direct excitatory contact with the Purkinje cells. Mossy fibers terminate in the granular layer and make excitatory synaptic contacts with granule and Golgi cells. The granule cells then send their ascending axons to the molecular layer, which bifurcate, thus forming the parallel fibers. The parallel fibers extend for several millimeters, and make synaptic contacts with Purkinje, stellate and basket cells. Purkinje cells represent the sole output from the cerebellar cortex and, when active, provide inhibitory input to the deep cerebellar nuclei. Not shown are the less-well-understood brush and Lugaro cells, as well as the aminergic afferent inputs (Dino *et al.*, 1999; Laine and Axelrad, 2002; Schweighofer *et al.*, 2004). DCN, deep cerebellar nuclei; GLUT, glutamate-releasing cells; GABA, gamma-aminobutyric acid-releasing cells.

the anterior lobe, and consists of Larsell's lobules I-V (Larsell and Jansen, 1967, 1970). The posterior lobe is separated from the anterior lobe by the primary fissure, and comprises Larsell's lobules VI-IX (and crus I and II), while Larsell's lobule X represents the flocculonodular lobe (Schmahmann, 2000).

Phylogenetically, the oldest portion of the cerebellar cortex is the vestibulocerebellum. This substructure consists of the most caudal cerebellar lobules (the flocculus and nodulus), and is concerned primarily with equilibrium and balance. The spinocerebellum, which exhibits a loosely somatotopic organization (Manni and Petrosini, 2004), resides in the median and paramedian portions of the cerebellar cortex and includes the central vermis. It is principally devoted to motor functions including gait and the coordination of distal limbs. Lastly, the cerebrocerebellum, which represents the most lateral portions of the cerebellar cortex, is the largest division of cortex in primates and humans. Highly interconnected to the cerebrum, this division of the cerebellum is essential for complex movement, speech and possibly cognition.

The cerebellar peduncles are large axon bundles that interconnect the cerebellum with other areas of the nervous system. Afferents to the cerebellum arise primarily from the pons, inferior olive, spinal cord and vestibular nuclei. Importantly, cells at the base of the pons (i.e. the pontine nuclei) represent the largest source of input to the cerebellum, and relay information from the frontal and parietal cortices. Cerebellar efferents arise from the DCN (dentate, interposed and fastigial nuclei), and project to the descending motor tracts, thalamus and cerebral cortex.

Cerebellar intrinsic circuitry and information processing

As alluded to previously, the intrinsic circuitry of the cerebellar cortex is highly uniform, indicating

similar modes of information processing throughout this structure. While different areas of the cerebellum ultimately project to various targets, comparable computations and synaptic plasticity may take place within each microcomplex. Thus, as asserted by Boyden *et al.* (2004), studying cerebellar dynamics in the context of known cerebellar-dependent tasks, such as the vestibuloocular reflex and/or classical eyeblink conditioning (see below), could elucidate general principles of cerebellar function that may be relevant to other domains such as affect or cognition (Boyden *et al.*, 2004).

While several models of cerebellar-dependent information processing have been proposed (Ito, 2005; Ramnani, 2006; Dean *et al.*, 2010), most have been influenced by the original Marr-Albus-Ito scheme (Marr, 1969; Albus, 1971; Ito, 1982). This model was inspired by the unique functional organization of inputs to cerebellar Purkinje cells, the synaptic arrangement of which is well suited for sensory-motor integration and neural representations of learning and memory (i.e. alterations in parallel fiber-Purkinje cell synaptic strength). Briefly, taking a cerebral-cortical motor command as an example, an "efference copy" of a frontal-parietal generated movement is sent to the cerebellum via relays in the pons. Thus, the cerebellum is recruited to compare the "planned" movement with the actual "performed" motor output (via proprioceptive feedback from the spinal cord and vestibular nuclei). A representation of the planned movement is relayed from the pons to the granule cells (via mossy fibers), thus enabling the large numbers of parallel fibers to perform pattern separation (Boyden, *et al.*, 2004). It is thought that climbing fibers (from the inferior olive) provide the "error" or "teaching" signal based upon proprioceptive feedback. During ongoing movement, the mossy and climbing fibers provide excitatory input to the DCN, while simultaneously sending collaterals to the cerebellar-cortical Purkinje cells (which inhibit the DCN). If a movement error occurs, the climbing fibers become active, and the Purkinje cells will receive nearly simultaneous input from both parallel and climbing fiber afferents. Counterintuitively, these concurrent excitatory inputs induce long-term depression (LTD) of the parallel fiber-Purkinje cell synapse, which disinhibits the DCN (since Purkinje cells are GABAergic). In this way, the DCN is able to send error-related information out of the cerebellum and back to the upper motor neurons of the prefrontal cortex, thus allowing for movement correction. While this description is highly simplified, and leaves out other crucial elements such as the role of interneurons and long-term potentiation (LTP) of DCN and parallel fiber-Purkinje cell synapses (Hansel *et al.*, 2001; Zhu *et al.*, 2007; Hong and Optican, 2008; Bender *et al.*, 2009; Wang *et al.*, 2009), the key point is that Purkinje cells are well adapted to integrate behaviorally-relevant spatio-temporal patterns of neural activity and adjust their synaptic strengths accordingly. Thus, the cerebellum may represent a general pattern classification device, coincidence detector or encoder of internal models of thought and action, which performs learning-related computations across different tasks, motor effectors and sensory modalities with high temporal precision (Keele and Ivry, 1990; Boyden *et al.*, 2004; Ito, 2008).

The role of the endocannabinoid system in cerebellar neural plasticity

It is now well established that the cerebellum contains one of the highest densities of CB1 receptors in the mammalian brain (Herkenham *et al.*, 1990; Pertwee 1997; Tsou *et al.*, 1998a; Ong and Mackie, 1999; and see Chapter 1). A recent CB1 receptor-binding study carried out in both primates and humans provides further support for this notion, as levels of CB1 receptor immunoreactivity in the cerebellum were second only to the basal ganglia (globus pallidus and substantia nigra) (Eggan and Lewis, 2007). The highest levels of the receptor are found in the cerebellar molecular layer, with more moderate concentrations found in the granular layer (Pettit *et al.*, 1998; Eggan and Lewis, 2007; Suarez *et al.*, 2008). As schematically illustrated in Figure 13.2, CB1 receptors primarily reside on glutamatergic granule cell axons (i.e. parallel fibers) (Breivogel *et al.*, 2004; Daniel *et al.*, 2004; Kawamura *et al.*, 2006), and GABAergic stellate and basket cell axon terminals (Diana *et al.*, 2002; Ashton *et al.*, 2004). Limited evidence also suggests CB1 receptor localization on climbing fibers (Safo *et al.*, 2006; Suarez *et al.*, 2008). Concerning endogenous cannabinoid transmitters, Purkinje cells appear to be the only cerebellar neuron type to synthesize, release and metabolize endocannabinoids (Egertova *et al.*, 1998; Tsou *et al.*, 1998a,1998b; Lee *et al.*, 2000).

Parallel fibers, climbing fibers, stellate cells and basket cells all have synaptic contacts with Purkinje cells in the cerebellar cortex. It thus appears that the primary mode of action of endocannabinoids in the cerebellum is self-regulation by Purkinje cells via retrograde

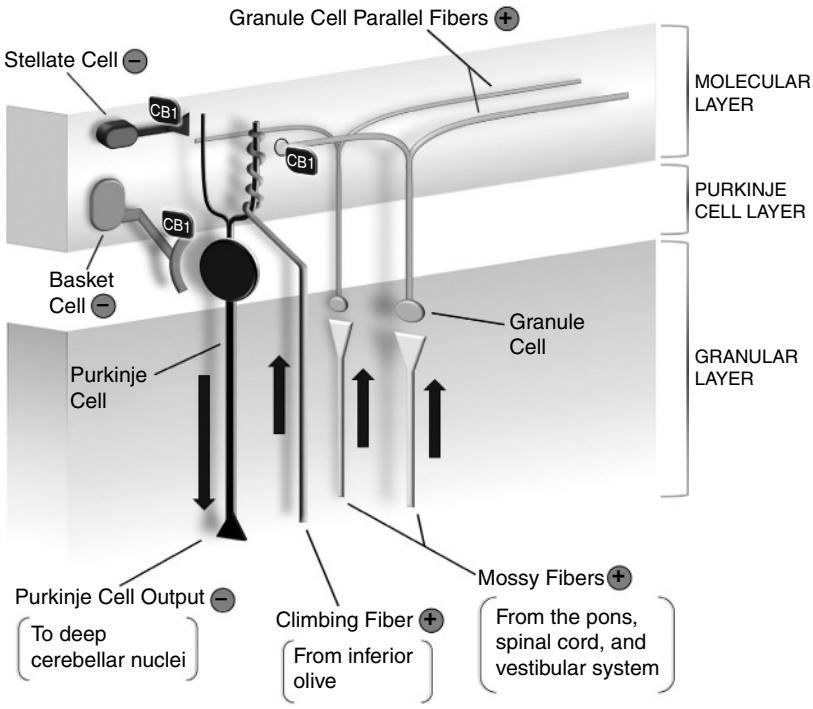

Figure 13.2. Illustration of the known locations of CB1 receptors in the cerebellar cortex. CB1 receptors primarily occupy glutamatergic granule cell axons (i.e. parallel fibers), and GABAergic stellate and basket cell axon terminals. Purkinje cells synthesize, release and metabolize endocannabinoids, but do not express CB1 receptors.

signaling back to the presynaptic terminals that innervate them (Takahashi and Linden, 2000; Tanimura *et al.*, 2009). This retrograde signaling system by Purkinje cells to inhibitory GABAergic basket and stellate cells has been termed depolarization-induced suppression of inhibition (DSI) (Kreitzer and Regehr, 2001a), or in the case of Purkinje cell to excitatory, glutamatergic granule-cell communication, depolarization-induced suppression of excitation (DSE) (Kreitzer and Regehr, 2001b; Howlett *et al.*, 2004). In addition to this short-term form of synaptic modulation, it also appears that endocannabinoids play a key role in LTD at parallel fiber-Purkinje cell synapses, thus implicating the cannabinoid system in cerebellar-dependent motor learning and synaptic plasticity (Levenes *et al.*, 1998; Safo and Regehr, 2005; Qiu and Knopfel, 2009). It thus appears that Purkinje cells are well adapted as "coincidence detectors," and release endocannabinoids during temporally paired parallel and climbing-fiber excitatory inputs (Brenowitz and Regehr, 2005; Rancz and Hausser, 2006). For a full review of the role of retrograde endocannabinoids in cerebellar synaptic plasticity, please see Safo *et al.* (2006).

In sum, an abundance of evidence suggests that the endocannabinoid system is integrally involved in mediating both short- and long-term plasticity of Purkinje cells, which are the sole output neurons of the cerebellar cortex. Thus, CB1 receptor signaling via associated endocannabinoids (e.g. 2-arachidonoyl glycerol) (Szabo *et al.*, 2006) likely modulates cerebellar-dependent processes related to motor coordination, associative learning and neural timing. Indeed, CB1 receptor-knockout mice have been reported to display various symptoms related to altered cerebellar function, including changes in locomotor activity (Zimmer *et al.*, 1999) and disrupted motor learning (Kishimoto and Kano, 2006). It has also been shown that exogenous cannabinoids disrupt LTD in cerebellar Purkinje cells (Levenes *et al.*, 1998). Furthermore, intracerebellar Δ^9-tetrahydrocannibinol (THC) and synthetic cannabinoid-agonist administration produces disruptions in motor coordination in a dose-dependent manner, an effect that is mediated by CB1 receptors (Dar, 2000; DeSanty and Dar, 2001).

The effect of cannabis intake on cerebellar brain activity

Over the past two decades, the majority of studies assessing the effects of exogenous cannabinoids on human brain function have focused on higher sensory, attentional and memory processes (Patrick *et al.*,

1995; Patrick *et al*., 1997; Skosnik *et al*., 2001; Iversen 2003; D'Souza *et al*., 2004; Jager *et al*., 2006; Ramaekers *et al*., 2006; Ranganathan and D'Souza, 2006; Skosnik *et al*., 2006a; Skosnik *et al*., 2006b; D'Souza *et al*., 2008; Jager and Ramsey, 2008; Jager *et al*., 2007; Ramaekers *et al*., 2009). Given the known role of endocannabinoids in cerebellar synaptic plasticity described above, paradigms that examine cerebellar function should be particularly useful indices of the neural effects of exogenous cannabinoids. Several lines of work have therefore sought to assess the effect of cannabinoids on cerebellar function in humans utilizing neuroimaging methodologies.

For example, a positron emission tomography (PET) study by O'Leary *et al*. (2003) demonstrated that cannabis-induced alterations in a self-paced timing task were related to increased activity in the medial cerebellar cortex (O'Leary *et al*., 2003). Several other studies have also shown increased regional cortical blood flow (rCBF) in the cerebellum as a result of cannabinoid administration (Volkow *et al*., 1996; Mathew *et al*., 1998; Block *et al*., 2000a; Mathew *et al*., 2002). Interestingly, cerebellar metabolism during acute cannabis administration has been shown to correlate with subjective ratings of intoxication (Volkow *et al*., 1996). Conversely, several studies have also shown that chronic cannabis users assessed during periods of abstinence consistently demonstrate hypoactive cerebellar activity, which could be interpreted as neuroadaptive endocannabinoid down-regulation (Volkow *et al*., 1996; Block *et al*., 2000a; Chang *et al*., 2006). Taken together, these studies indicate that both acute and chronic cannabinoids are associated with alterations in cerebellar metabolism.

Cannabis effects on cerebellar-mediated behavior

In terms of behavioral outcomes, several studies have shown that cannabinoid administration in humans is associated with alterations in temporal processing and psychomotor performance, both of which are at least partly cerebellar-mediated (O'Leary *et al*., 2003; Huestegge *et al*., 2009; Hunault *et al*., 2009; Ramaekers *et al*., 2009; Roser *et al*., 2009). For example, altered time perception (in the seconds to minutes range) has traditionally been one of the most frequently reported subjective experiences associated with acute cannabis intoxication (Clark *et al*., 1970; Melges *et al*., 1970a, 1970b; Tinklenberg *et al*., 1972; Vachon *et al*., 1974; Tinklenberg *et al*., 1976; Hicks *et al*., 1984). Interestingly, several studies have also noted time-estimation deficits in patients with schizophrenia (Densen, 1977; Wahl and Sieg, 1980; Rammsayer, 1990; Volz *et al*., 2001; Penney *et al*., 2005; Lee *et al*., 2009). More recent studies have confirmed that direct cannabinoid administration induces temporal disintegration, which is typically exhibited in the form of increased internal-clock speeds (the experience of time passing more slowly) (Mathew *et al*., 1998; O'Leary *et al*., 2003; Solowij, 1998). Furthermore, disturbance in temporal processing under the influence of THC has been related to its effects on the cerebellum, as subjects who showed a decrease in cerebellar CBF while administered THC also had significant alterations in time estimation (Mathew *et al*., 1998). Such alterations in time perception may not just occur acutely, as it has been shown that disturbed time sense persists in chronic cannabis users after periods of abstinence (Solowij *et al*., 2002).

Cannabinoids and cerebellar-dependent delay eyeblink conditioning

While the above described data examining cannabinoid mediation of time estimation and psychomotor ability are intriguing, it should be noted that the majority of these tasks are not primarily cerebellar-dependent, and likely recruit a network of brain areas such as the motor cortex and basal ganglia (Bengtsson *et al*., 2005). This caveat thus serves to obfuscate specific interpretations regarding cannabinoid effects on cerebellar function. One paradigm that has been shown to be mediated almost completely by the cerebellum is classical delay eyeblink conditioning (dEBC) (Steinmetz, 2000; Christian and Thompson, 2003; Gerwig *et al*., 2007; Thompson and Steinmetz, 2009). Delay eyeblink conditioning is an associative motor-learning task that involves the paired presentation of a neutral conditioned stimulus (CS), such as a tone, followed by an unconditioned stimulus (US), such as an ocular air puff (Figure 13.3, top). The US air puff evokes a reflexive eyeblink, the unconditioned response (UR), and after repeated CS-US paired presentations, a conditioned response (CR), a blink of the eye, forms subsequent to CS presentation. Given enough CS-US pairings, the learned CR becomes optimally timed such that the protective eyelid closure occurs just before the onset of the US (Steinmetz, 2000). As mentioned above, over three

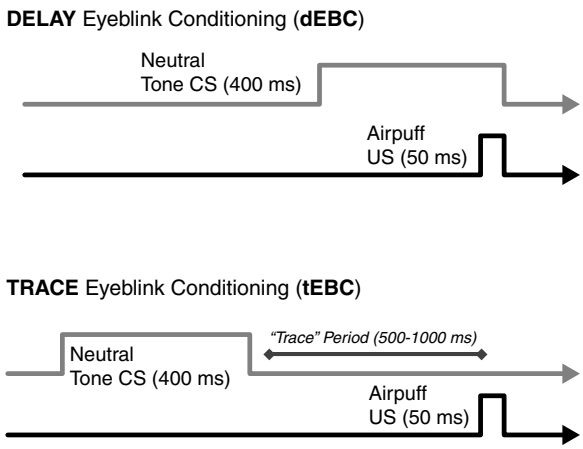

Figure 13.3. Cerebellar-dependent delay eyeblink conditioning (dEBC; top) versus forebrain-dependent trace eyeblink conditioning (tEBC; bottom). The standard dEBC paradigm utilizes a coterminating conditioned and unconditioned stimulus (CS and US, respectively). Optimal conditioning in humans is typically achieved with a 400 ms CS tone and a 50 ms air puff US. In the tEBC procedure, a stimulus-free gap (typically 500–1000 ms) is inserted between the CS and US. Thus, a forebrain-dependent, declarative memory "trace" is needed to store the temporal relationship and allow for acquisition of the conditioned response (CR).

decades of lesion, neural-unit recording and reversible inactivation studies have provided compeling evidence that regions of the DCN (interpositus nucleus) and anterior cerebellar cortex constitute the memory trace for dEBC. The role of the cerebellum in this learned response has been further confirmed pharmacologically (Vogel *et al*., 2009), through metabolic mapping in animals (Plakke *et al*., 2007) and with functional neuroimaging in humans (Logan and Grafton, 1995; Dimitrova *et al*., 2002; Cheng *et al*., 2008).

It is now well established that in dEBC, CS information is conveyed to the cerebellum via mossy fibers, while the US is transmitted via climbing fibers (Steinmetz, 2000). Interestingly, it has been shown that concurrent parallel-fiber-climbing fiber activation of Purkinje cells (which is analogous to convergent CS-US input) leads to short-term depression of the parallel-fiber synapse, an effect that is mediated by endocannabinoids (Brenowitz and Regehr, 2005). Thus, cannabinoids may be integrally involved in forms of synaptic plasticity crucial in the acquisition of CRs during dEBC.

In the first-ever study of dEBC in the context of cannabinoids in humans, Skosnik *et al*. (2008) demonstrated robust deficits in the acquisition of CRs and alterations in CR timing in chronic users of cannabis (Skosnik *et al*., 2008). In a follow-up study, it was demonstrated that heavy cannabis use was not associated with deficits in trace eyeblink conditioning (tEBC; Figure 13.3, bottom), a variation of the task that is thought to be dependent on forebrain structures such as the hippocampus (Edwards *et al*., 2008; Woodruff-Pak and Disterhoft, 2008). Additional evidence for the role of cannabinoids in dEBC came from a study of CB1 receptor-knockout animals, in which mice lacking the CB1 receptor were shown to exhibit severely disrupted dEBC in an animal analogue of the task (Kishimoto and Kano, 2006). Wild-type mice administered the potent CB1 receptor antagonist SR141716A (rimonabant), also showed impaired dEBC, ruling out the possibility that the learning deficits observed in knockout animals were due to ancillary neurodevelopmental changes. This pattern of decreased learning in CB1 receptor-deficient animals is nearly identical to data from Skosnik *et al*. showing disrupted dEBC in chronic cannabis users (Skosnik *et al*., 2008). Interestingly, the CB1 receptor knockouts exhibited normal forebrain-dependent tEBC, which was also the case in the follow-up study assessing trace conditioning in heavy users of cannabis (Edwards *et al*., 2008).

In sum, the data from human cannabis users in delay and trace EBC mirrors the CB1 receptor-knockout mice results, and suggest that cannabinoid system disturbances are specifically associated with cerebellar-dependent learning (Kishimoto and Kano, 2006; Edwards and Skosnik, 2009). Changes in CB1 receptors (either by chronic cannabis use or genetic deletion) may alter learning in the cerebellum by disrupting short and long-term plasticity at parallel fiber-Purkinje cell synapses (DSE and/or LTD, respectively), which could lead to increased inhibition of the DCN (thus preventing CR acquisition). At the same time, alterations in CB1 receptors could disrupt the normal operation of cerebellar-cortical inhibitory interneurons. In the case of stellate and basket cells, these receptor changes could disturb the process of DSI and LTD in inhibitory interneurons, which could lead to altered timing of Purkinje cell firing (Mittmann and Hausser, 2007), or dysregulated lateral inhibition, thus changing the spatial distribution of Purkinje cell activity (Kreitzer *et al*., 2002).

The cerebellum and schizophrenia

Compared with areas of the cerebrum such as the prefrontal, temporal and parietal cortices, the contribution

of the cerebellum to schizophrenia pathogenesis has received relatively little attention, most likely owing to the common notion of the cerebellum as a simple motor structure. Nevertheless, abnormalities in cerebellar structure in schizophrenia have been noted as far back as half a century ago (Roizin *et al.*, 1959; Heath *et al.*, 1979). Snider was one of the first to postulate that pathology of the cerebellum could contribute to schizophrenia symptomatology (Snider, 1982). This proposition has been reinvigorated in recent years by Schmahmann (1991), with his assertion of a "dysmetria-of-thought hypothesis," and further expounded by Andreasen and colleagues (1998), with the notion of "cognitive dysmetria" (Schmahmann, 1991; Andreasen *et al.*, 1998; Andreasen, 1999; Andreasen *et al.*, 1999). These ideas emphasize the loss of synchrony and the coordinated sequence of thoughts and actions in schizophrenia, which could be modulated by neurodevelopmental disruptions of cortical-cerebellar-thalamic-cortical circuits. In other words, just as the cerebellum is thought to coordinate the accurate sequence of complex motor commands, it may perform similar computations in the perceptual, cognitive and affective domains. Indeed, as reviewed by Strick *et al.* (2009), recent neuroanatomical and functional neuroimaging studies strongly indicate that the cerebellum is involved in a wide array of non-motor functions including attention, executive control, language, working memory, learning, pain, affect and even addiction (Strick *et al.*, 2009). In support of this hypothesis, and germane to schizophrenia, Schmahmann and Sherman (1998), in their characterization of individuals with cerebellar lesions, have described a distinct cerebellar cognitive-affective syndrome that shares many of the core symptoms of schizophrenia (Schmahmann and Sherman, 1998; Schmahmann, 2004). Thus, as stated by Schmahmann and Sherman, this schizophrenia-like syndrome is characterized by "impairments of executive function often with perseveration, distractibility or inattention; visual-spatial disorganization and impaired visual-spatial memory; personality change with blunting of affect or disinhibited and inappropriate behavior; and difficulties with language."

Several contemporary reviews have cogently summarized the growing body of evidence linking schizophrenia with cerebellar abnormalities (Andreasen and Pierson, 2008; Picard *et al.*, 2008). Picard *et al.* (2008) comprehensively reviewed the evidence for cerebellar-related deficits in schizophrenia in the context of a number of outcomes including hallucinations, formal-thought disorder, affect, neurological soft signs, classic cerebellar symptoms, language and cognition. While the majority of studies implicated the cerebellum at some level (via correlations with either cerebellar volume or activity), the most unequivocal data arose from behavioral experiments assessing classic cerebellar symptoms in schizophrenia such as posture, equilibrium, intentional tremor, dysdiadochokinesis, dysarthria and the vestibular-ocular reflex (Picard *et al.*, 2008). Schizophrenia-related alterations in the vestibular-ocular reflex are particularly striking, as this a highly cerebellar-dependent process and is also abnormal in cannabis users (Yee *et al.*, 1987; Warren and Ross, 1998; Huestegge *et al.*, 2009). Thus, while modest evidence of behavior-related cerebellar aberrations seems to exist in schizophrenia, data supporting a relationship between the cerebellum and the core symptoms of psychosis remain equivocal.

In addition to alterations in cerebellar-mediated behavioral outcomes, numerous studies have shown that patients with schizophrenia exhibit abnormalities in cerebellar structure and metabolism (for review, see Andreasen and Pierson, 2008). For example, a large number of studies examining cerebellar structure in schizophrenia (utilizing neuroimaging) have demonstrated alterations in cerebellar size in patients with schizophrenia (Weinberger *et al.*, 1979; Jacobsen *et al.*, 1997; Levitt *et al.*, 1999; Nopoulos *et al.*, 1999; Loeber *et al.*, 2001; Okugawa *et al.*, 2002; Szeszko *et al.*, 2003; James *et al.*, 2004; Edwards *et al.*, 2008). In addition, neuropathological studies of the cerebellum have shown decreased size and density of cerebellar-cortical Purkinje cells in schizophrenia patients (Reyes and Gordon, 1981; Tran *et al.*, 1998; Maloku *et al.*, 2010). Concerning neural activity in the cerebellum, a series of experiments using PET have shown that schizophrenia patients exhibit decreased cerebellar blood flow during varied behavioral tasks including those involving attention, memory, social cognition and affect (Andreasen *et al.*, 1996; Andreasen *et al.*, 1997; Crespo-Facorro *et al.*, 2001a; Crespo-Facorro *et al.*, 2001b; Paradiso *et al.*, 2003). Lastly, dysfunctional or delayed infant neuromotor development has been shown to be a risk factor for the genesis of schizophrenia (Walker *et al.*, 1996; Fish and Kendler, 2005; Murray *et al.*, 2006; Ridler *et al.*, 2006). Taken together, a large assortment of convergent evidence has continued to accrue implicating the cerebellum in the pathogenesis of schizophrenia.

Schizophrenia and cerebellar-dependent delay eyeblink conditioning

Given the known dependence of dEBC on the cerebellum (see above), a number of groups have attempted to utilize this paradigm to assay cerebellar function in patients with schizophrenia. As reviewed by Lubow, the results to date have been somewhat mixed (Lubow, 2009). Several early studies have shown increased learning (larger percentage of CRs) or no differences in schizophrenia patients compared with controls (Taylor and Spence, 1954; O'Connor and Rawnsley, 1959; Spain 1966; Sears *et al*., 2000; Stevens *et al*., 2002). Marenco *et al*. (2003) found no CR acquisition differences in schizophrenia during dEBC, but did report more adaptive CR latencies in the patient group (Marenco *et al*., 2003). Conversely, Hofer *et al*. (2001) and Brown *et al*. (2005) showed that schizophrenia subjects exhibited decreased conditioning during dEBC compared with controls (Hofer *et al*., 2001; Brown *et al*., 2005). More recently, Edwards *et al*. also demonstrated that schizophrenia patients had CR acquisition deficits compared with matched controls, and that the schizophrenia group had decreased anterior cerebellar volumes as determined by structural MRI (Edwards *et al*., 2008).

In terms of the inconsistent eyeblink-conditioning results just described, it should be noted that most of the previous assessments of dEBC in schizophrenia have utilized either small samples or unequal sample sizes between the control and schizophrenia groups. A recent study by Bolbecker *et al*. (2009) attempted to address this issue with larger samples, and examined 62 individuals with schizophrenia, and 62 age-matched non-psychiatric comparison subjects. They demonstrated that individuals with schizophrenia exhibited robust deficits in conditioned responding, along with concomitant earlier (less adaptively timed) CR latencies (Bolbecker *et al*., 2009). Further, a detailed analysis of UR amplitudes, bad trials and spontaneous blink rates was undertaken, which was not considered in most previous studies. To an extent, therefore, these more recent data help clarify the dEBC picture in the context of schizophrenia, and provide a stronger argument for cerebellar anomalies in psychosis. It is therefore reasonable to postulate that the discrepant results of earlier studies were due to confounding factors, which could be related to a number of variables including sample size, differences in schizophrenia subtype, dEBC methodology and medication status. It should also be mentioned that most studies of schizophrenia tend to utilize older populations, which further complicates interpretation, as it is well known that aging is associated with dEBC deficits (Woodruff-Pak *et al*., 2010). Future studies attempting to assess cerebellar function in schizophrenia using the dEBC paradigm should take these confounding variables into consideration.

The dénouement: could cerebellar cannabinoid dysfunction be psychotogenic?

Heretofore, a parallel picture of cannabinoid modulation of cerebellar function and anomalies of the cerebellum in schizophrenia has been described. The strong relationship between cannabinoids and psychosis has already been summarized in recent reviews (Moore *et al*., 2007; D'Souza *et al*., 2009) and expanded upon in the current volume. The remaining question concerns the mechanism whereby cannabinoids, whether endogenously in schizophrenia (Leweke *et al*., 1999, 2007; Eggan *et al*., 2008), or exogenously with acute and chronic cannabis use (D'Souza *et al*., 2004; Skosnik *et al*., 2006a), contribute to psychotogenesis. To date, no studies have directly assessed the interaction between the cerebellum and cannabinoids in the context of schizophrenia spectrum disorders. Therefore, the following section will postulate several mechanisms through which alterations in the endocannabinoid system could influence the genesis of psychosis via cerebellar mechanisms.

One potential mechanism relates to the known developmental trajectory of the cerebellum in humans. Along with the prefrontal cortex, the cerebellum is one of the last brain structures to complete neural development, which can extend well into adolescence and early adulthood (Giedd *et al*., 1999; Tiemeier *et al*., 2010). This heterochronicity with respect to the rest of brain has several implications concerning the role of the cerebellum in psychotogenesis. First, the time course of cerebellar maturation corresponds with the typical age of onset of schizophrenia. Second, it has recently been discovered that the endocannabinoid system plays a key role in neural development, including processes such as neurogenesis, neural specification, neural maturation, neuronal migration, axonal

elongation and glia formation (Harkany *et al.*, 2008 and see Chapter 6). It is therefore tenable that genetic or environmental perturbations in the endocannabinoid system could disrupt the development of cortical-cerebellar-thalamic-cortical circuits, thus leading to the expression of psychosis-related behaviors at the culmination of ontogeny. This emerging role of cannabinoids in development, and the particular vulnerability of the cerebellum in adolescence, could partially explain the fact that early-onset cannabis use is a risk factor, and perhaps, component cause of schizophrenia (Caspi *et al.*, 2005; Sundram, 2006; Moore *et al.*, 2007; Harley *et al.*, 2009).

Concerning the specific mechanism whereby cannabinoid alterations in the cerebellum could contribute to the core symptoms of schizophrenia, one possibility relates to the putative role of the cerebellum in modulating temporal processing. As aptly affirmed by Meck (1996), "time perception is a guiding force in the behavior of all organisms." In other words, whether it is predicting a schedule of reinforcement, coordinating motor commands, sequencing cognitive operations or maintaining the cadence of internal and external speech, accurate time estimation in the millisecond-to-second range is central to the adaptive behavior of almost all animals (Grondin, 2010; Meck, 1996, 2005). Pertinent to the current postulate, the cerebellum was put forth as a candidate for the internal neural clock nearly fifty years ago (Braitenberg, 1967). Since then, data from a host of time-estimation and time-reproduction studies suggest that the cerebellum, possibly in concert with the basal ganglia (Wild-Wall *et al.*, 2008; Jin *et al.*, 2009), represents the neural substrate of time (Pellionisz and Llinas, 1982; Keele and Ivry, 1990; Salman, 2002; Lewis and Miall, 2003; Spencer and Ivry, 2005; Fierro *et al.*, 2007; Koch *et al.*, 2007; Lee *et al.*, 2007; Yamazaki and Tanaka, 2007; Bueti *et al.*, 2008; Oliveri *et al.*, 2009); for exceptions see Harrington *et al.* (2004) and Bengtsson *et al.* (2005). Such a role is structurally valid, as the unique organization of the cerebellar cortex, with its microcomplex arrangement of orthogonal parallel fibers intersecting Purkinje cell dendrites, could provide a putative mechanism whereby a series of Purkinje cells might encode parallel-fiber conduction times, thus hinting at a possible neural correlate of clock function and the coding of time (Heck and Sultan, 2002).

As mentioned earlier in this chapter, evidence suggests that both schizophrenia (Densen, 1977; Wahl and Sieg, 1980; Rammsayer, 1990; Volz *et al.*, 2001; Penney *et al.*, 2005; Lee *et al.*, 2009) and acute cannabis intoxication (Clark *et al.*, 1970; Melges *et al.*, 1970a, 1970b; Tinklenberg *et al.*, 1972; Vachon *et al.*, 1974; Tinklenberg *et al.*, 1976; Hicks *et al.*, 1984) share a profound disintegration in temporal processing and alterations in the subjective sense of time. Consequently, it appears tenable that a breakdown in endocannabinoid function in the cerebellum (e.g. altered DSE, DSI and/or LTD) could lead to inaccurate neural timing, which could induce positive symptoms by desynchronizing widespread cerebral-cortical processes such as sensory-motor integration, affect, task-shifting and inner speech. Thus, cannabinoids in the cerebellum could provide a mechanism for the dysmetria of thought in schizophrenia proposed by both Schmahmann and Andreasen (Schmahmann, 1991; Andreasen *et al.*, 1998; Andreasen, 1999; Andreasen *et al.*, 1999). Such deficits in temporal processing and time perception might be common to altered states of consciousness in general (Ludwig, 1966), and a disruption in cerebellar representations of spatial-temporal reality might be one powerful mechanism linking cannabinoids and psychosis (Fritzsche, 2001, 2002).

Conclusions

To recapitulate, the current chapter has attempted to synthesize known literatures regarding the effect of cannabinoids on cerebellar function and the role of the cerebellum in schizophrenia, with the implication that endocannabinoids may contribute to psychosis via cerebellar mechanisms. Toward this end, studies were reviewed that have examined CB1 receptor binding, synaptic plasticity (i.e. DSE, DSI and LTD) and cerebellar-mediated behavior, all of which point to the crucial role of cannabinoids in cerebellar dynamics. In parallel, the emerging role of the cerebellum in non-motor functions, and experiments demonstrating altered cerebellar structure and function in schizophrenia were summarized, which further implicates this structure in psychosis. These converging lines of evidence thus provide the impetus for future studies to explicitly examine CB1 receptors and associated endocannabinoid transmitters in relation to the cerebellum in schizophrenia. Such work may go far in clarifying the long-recognized relationship between cannabis, cannabinoids and the form of madness we call schizophrenia.

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References

Albus, J.S. (1971) A theory of cerebellar function. *Mathematical Biosciences,* **10**:25–61.

Andreasen, N.C. (1999) A unitary model of schizophrenia: Bleuler's "fragmented phrene" as schizencephaly. *Arch Gen Psychiatry,* **56**:781–7.

Andreasen, N.C., Nopoulos, P., O'Leary, D.S., Miller, D.D., Wassink, T. and Flaum, M. (1999) Defining the phenotype of schizophrenia: cognitive dysmetria and its neural mechanisms. *Biol Psychiatry,* **46**:908–20.

Andreasen, N.C., O'Leary, D.S., Cizadlo, T., *et al.* (1996) Schizophrenia and cognitive dysmetria: a positron-emission tomography study of dysfunctional prefrontal-thalamic-cerebellar circuitry. *Proc Natl Acad Sci U S A*, **93**:9985–90.

Andreasen, N.C., O'Leary, D.S., Flaum, M., *et al.* (1997) Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. *Lancet*, **349**:1730–4.

Andreasen, N.C., Paradiso, S. and O'Leary, D.S. (1998) "Cognitive dysmetria" as an integrative theory of schizophrenia: a dysfunction in cortical-subcortical-cerebellar circuitry? *Schizophr Bull*, **24**:203–18.

Andreasen, N.C. and Pierson, R. (2008) The role of the cerebellum in schizophrenia. *Biol Psychiatry,* **64**:81–8.

Angevine, J.B. (1961) *The human cerebellum; an atlas of gross topography in serial sections* (1st ed.). Boston: Little.

Apps, R. and Garwicz, M. (2005) Anatomical and physiological foundations of cerebellar information processing. *Nat Rev Neurosci,* **6**:297–311.

Ashton, J.C., Appleton, I., Darlington, C.L. and Smith, P.F. (2004) Immunohistochemical localization of cannabinoid CB1 receptor in inhibitory interneurons in the cerebellum. *Cerebellum,* **3**:222–6.

Azevedo, F.A., Carvalho, L.R., Grinberg, L.T., *et al.* (2009) Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol,* **513**:532–41.

Bender, V.A., Pugh, J.R. and Jahr, C.E. (2009) Presynaptically expressed long-term potentiation increases multivesicular release at parallel fiber synapses. *J Neurosci,* **29**:10974–8.

Bengtsson, S.L., Ehrsson, H.H., Forssberg, H. and Ullen, F. (2005) Effector-independent voluntary timing: behavioral and neuroimaging evidence. *Eur J Neurosci,* **22**:3255–65.

Block, R.I., O'Leary, D.S., Ehrhardt, J.C., *et al.* (2000a) Effects of frequent marijuana use on brain tissue volume and composition. *Neuroreport*, **11**:491–6.

Block, R.I., O'Leary, D.S., Hichwa, R.D., *et al.* (2000b) Cerebellar hypoactivity in frequent marijuana users. *Neuroreport*, **11**:749–53.

Bolbecker, A.R., Mehta, C.S., Edwards, C.R., Steinmetz, J.E., O'Donnell, B.F. and Hetrick, W.P. (2009) Eye-blink conditioning deficits indicate temporal processing abnormalities in schizophrenia. *Schizophr Res*, **111**:182–91.

Boyden, E.S., Katoh, A. and Raymond, J.L. (2004) Cerebellum-dependent learning: the role of multiple plasticity mechanisms. *Annu Rev Neurosci,* **27**:581–609.

Braitenberg, V. (1967) Is the cerebellar cortex a biological clock in the millisecond range? *Prog Brain Res,* **25**:334–46.

Braitenberg, V. (2002) In defense of the cerebellum. *Ann N Y Acad Sci,* **978**:175–83.

Breivogel, C.S., Walker, J.M., Huang, S.M., Roy, M.B. and Childers, S.R. (2004) Cannabinoid signaling in rat cerebellar granule cells: G-protein activation, inhibition of glutamate release and endogenous cannabinoids. *Neuropharmacology,* **47**:81–91.

Brenowitz, S.D. and Regehr, W.G. (2005) Associative short-term synaptic plasticity mediated by endocannabinoids. *Neuron,* **45**:419–31.

Brown, S.M., Kieffaber, P.D., Carroll, C.A., *et al.* (2005) Eyeblink conditioning deficits indicate timing and cerebellar abnormalities in schizophrenia. *Brain Cogn,* **58**:94–108.

Bueti, D., Walsh, V., Frith, C. and Rees, G. (2008) Different brain circuits underlie motor and perceptual representations of temporal intervals. *J Cogn Neurosci,* **20**:204–14.

Caspi, A., Moffitt, T.E., Cannon, M., *et al.* (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry,* **57**:1117–27.

Chang, L., Yakupov, R., Cloak, C. and Ernst, T. (2006) Marijuana use is associated with a reorganized visual-attention network and cerebellar hypoactivation. *Brain,* **129**:1096–112.

Cheng, D.T., Disterhoft, J.F., Power, J.M., Ellis, D.A. and Desmond, J.E. (2008) Neural substrates underlying human delay and trace eyeblink conditioning. *Proc Natl Acad Sci USA,* **105**:8108–13.

Christian, K.M. and Thompson, R.F. (2003) Neural substrates of eyeblink conditioning: acquisition and retention. *Learn Mem,* **10**:427–55.

Clark, L.D., Hughes, R. and Nakashima, E.N. (1970) Behavioral effects of marihuana. Experimental studies. *Arch Gen Psychiatry,* **23**:193–8.

Crespo-Facorro, B., Paradiso, S., Andreasen, N.C., *et al.* (2001a) Neural mechanisms of anhedonia in schizophrenia: a PET study of response to unpleasant and pleasant odors. *JAMA,* **286**:427–35.

Crespo-Facorro, B., Wiser, A.K., Andreasen, N.C., *et al.* (2001b) Neural basis of novel and well-learned recognition memory in schizophrenia: a positron emission tomography study. *Hum Brain Mapp,* **12**:219–31.

D'Souza, D.C., Perry, E., MacDougall, L., *et al.* (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology*, **29**:1558–72.

D'Souza, D.C., Ranganathan, M., Braley, G., *et al.* (2008) Blunted psychotomimetic and amnestic effects of delta-9-tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology*, **33**: 2505–16.

D'Souza, D.C., Sewell, R.A. and Ranganathan, M. (2009) Cannabis and psychosis/schizophrenia: human studies. *Eur Arch Psychiatry Clin Neurosci*, **259**:413–31.

Daniel, H., Rancillac, A. and Crepel, F. (2004) Mechanisms underlying cannabinoid inhibition of presynaptic Ca2+ influx at parallel fiber synapses of the rat cerebellum. *J Physiol,* **557**:159–74.

Dar, M.S. (2000) Cerebellar CB(1) receptor mediation of Delta(9)-THC-induced motor incoordination and its potentiation by ethanol and modulation by the cerebellar adenosinergic A(1) receptor in the mouse. *Brain Res,* **864**:186–94.

Dean, P., Porrill, J., Ekerot, C.F. and Jorntell, H. (2010) The cerebellar microcircuit as an adaptive filter: experimental and computational evidence. *Nat Rev Neurosci,* **11**:30–43.

Densen, M.E. (1977) Time perception and schizophrenia. *Percept Mot Skills,* **44**:436–8.

DeSanty, K.P. and Dar, M.S. (2001) Cannabinoid-induced motor incoordination through the cerebellar CB(1) receptor in mice. *Pharmacol Biochem Behav,* **69**:251–9.

Diana, M.A., Levenes, C., Mackie, K. and Marty, A. (2002) Short-term retrograde inhibition of GABAergic synaptic currents in rat Purkinje cells is mediated by endogenous cannabinoids. *J Neurosci,* **22**:200–8.

Dimitrova, A., Weber, J., Maschke, M., *et al.* (2002) Eyeblink-related areas in human cerebellum as shown by fMRI. *Hum Brain Mapp,* **17**:100–15.

Dino, M.R., Willard, F.H. and Mugnaini, E. (1999) Distribution of unipolar brush cells and other calretinin immunoreactive components in the mammalian cerebellar cortex. *J Neurocytol,* **28**:99–123.

Eccles, J.C., Ito, M. and Szentágothai, J. (1967) *The cerebellum as a neuronal machine*. Berlin, New York [etc.]: Springer-Verlag.

Edwards, C.R., Newman, S., Bismark, A., *et al.* (2008) Cerebellum volume and eyeblink conditioning in schizophrenia. *Psychiatry Res,* **162**:185–94.

Edwards, C.R. and Skosnik, P. D. (2009) Cerebellar-dependent learning as a neurobehavioral index of the cannabinoid system. *Crit Rev Neurobiol,* **19**:29–57.

Edwards, C.R., Skosnik, P.D., Steinmetz, A.B., Vollmer, J.M., O'Donnell, B.F. and Hetrick, W.P. (2008) Assessment of forebrain-dependent trace eyeblink conditioning in chronic cannabis users. *Neurosci Lett*, **439**:264–8.

Egertova, M., Giang, D.K., Cravatt, B.F. and Elphick, M.R. (1998) A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. *Proc Biol Sci,* **265**:2081–5.

Eggan, S.M., Hashimoto, T. and Lewis, D.A. (2008) Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. *Arch Gen Psychiatry,* **65**:772–84.

Eggan, S.M. and Lewis, D.A. (2007) Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. *Cereb Cortex,* **17**:175–91.

Fierro, B., Palermo, A., Puma, A., *et al.* (2007) Role of the cerebellum in time perception: a TMS study in normal subjects. *J Neurol Sci,* **263**:107–12.

Fish, B. and Kendler, K. S. (2005) Abnormal infant neurodevelopment predicts schizophrenia spectrum disorders. *J Child Adolesc Psychopharmacol,* **15**:348–61.

Fritzsche, M. (2001) Are cannabinoid receptor knockout mice animal models for schizophrenia? *Med Hypotheses,* **56**:638–43.

Fritzsche, M. (2002) Impaired information processing triggers altered states of consciousness. *Med Hypotheses,* **58**:352–8.

Gerwig, M., Kolb, F.P. and Timmann, D. (2007) The involvement of the human cerebellum in eyeblink conditioning. *Cerebellum,* **6**:38–57.

Giedd, J.N., Blumenthal, J., Jeffries, N.O., *et al.* (1999) Brain development during childhood and adolescence: a longitudinal MRI study. *Nat Neurosci,* **2**:861–3.

Grondin, S. (2010) Timing and time perception: a review of recent behavioral and neuroscience findings and theoretical directions. *Atten Percept Psychophys,* **72**:561–82.

Hansel, C., Linden, D.J. and D'Angelo, E. (2001) Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. *Nat Neurosci*, **4**:467–75.

Harkany, T., Mackie, K. and Doherty, P. (2008) Wiring and firing neuronal networks: endocannabinoids take center stage. *Curr Opin Neurobiol,* **18**:338–45.

Harley, M., Kelleher, I., Clarke, M., *et al.* (2009) Cannabis use and childhood trauma interact additively to increase the risk of psychotic symptoms in adolescence. *Psychol Med*, **40**:1627–34.

Harrington, D.L., Lee, R.R., Boyd, L.A., Rapcsak, S.Z. and Knight, R.T. (2004) Does the representation of time depend on the cerebellum? Effect of cerebellar stroke. *Brain,* **127**:561–74.

Heath, R.G., Franklin, D.E. and Shraberg, D. (1979) Gross pathology of the cerebellum in patients diagnosed and treated as functional psychiatric disorders. *J Nerv Ment Dis,* **167**:585–92.

Heck, D. and Sultan, F. (2002) Cerebellar structure and function: making sense of parallel fibers. *Hum Mov Sci,* **21**:411–21.

Herkenham, M., Lynn, A.B., Little, M.D., *et al.* (1990) Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA,* **87**:1932–6.

Hicks, R.E., Gualtieri, C.T., Mayo, J.P., Jr. and Perez-Reyes, M. (1984) Cannabis, atropine, and temporal information processing. *Neuropsychobiology,* **12**:229–37.

Hofer, E., Doby, D., Anderer, P. and Dantendorfer, K. (2001) Impaired conditional discrimination learning in schizophrenia. *Schizophr Res,* **51**:127–36.

Hong, S. and Optican, L.M. (2008) Interaction between Purkinje cells and inhibitory interneurons may create adjustable output waveforms to generate timed cerebellar output. *PLoS One,* **3**:e2770.

Howlett, A.C., Breivogel, C.S., Childers, S.R., Deadwyler, S.A., Hampson, R.E. and Porrino, L.J. (2004) Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology,* **47** *Suppl* 1:345–58.

Huestegge, L., Radach, R. and Kunert, H.J. (2009) Long-term effects of cannabis on oculomotor function in humans. *J Psychopharmacol,* **23**:714–22.

Hunault, C.C., Mensinga, T.T., Bocker, K.B., *et al.* (2009) Cognitive and psychomotor effects in males after smoking a combination of tobacco and cannabis containing up to 69 mg delta-9-tetrahydrocannabinol (THC). *Psychopharmacology (Berl),* **204**:85–94.

Ioffe, M.E., Chernikova, L.A. and Ustinova, K.I. (2007) Role of cerebellum in learning postural tasks. *Cerebellum,* **6**:87–94.

Ito, M. (1982) Experimental verification of Marr-Albus' plasticity assumption for the cerebellum. *Acta Biol Acad Sci Hung,* **33**:189–99.

Ito, M. (1984) *The cerebellum and neural control*. New York: Raven Press.

Ito, M. (2002) Historical review of the significance of the cerebellum and the role of Purkinje cells in motor learning. *Ann N Y Acad Sci,* **978**:273–88.

Ito, M. (2005) Bases and implications of learning in the cerebellum – adaptive control and internal model mechanism. *Prog Brain Res,* **148**:95–109.

Ito, M. (2008) Control of mental activities by internal models in the cerebellum. *Nat Rev Neurosci,* **9**:304–13.

Iversen, L. (2003) Cannabis and the brain. *Brain,* **126**:1252–70.

Jacobsen, L.K., Giedd, J.N., Berquin, P.C., *et al.* (1997) Quantitative morphology of the cerebellum and fourth ventricle in childhood-onset schizophrenia. *Am J Psychiatry,* **154**:1663–9.

Jager, G., Kahn, R.S., Van Den Brink, W., Van Ree, J.M. and Ramsey, N.F. (2006) Long-term effects of frequent cannabis use on working memory and attention: an fMRI study. *Psychopharmacology (Berl),* **185**:358–368.

Jager, G. and Ramsey, N.F. (2008) Long-term consequences of adolescent cannabis exposure on the development of cognition, brain structure and function: an overview of animal and human research. *Curr Drug Abuse Rev,* **1**:114–23.

Jager, G., Van Hell, H.H., De Win, M.M., *et al.* (2007) Effects of frequent cannabis use on hippocampal activity during an associative memory task. *Eur Neuropsychopharmacol,* **17**:289–97.

James, A.C., James, S., Smith, D.M. and Javaloyes, A. (2004) Cerebellar, prefrontal cortex, and thalamic volumes over two time points in adolescent-onset schizophrenia. *Am J Psychiatry,* **161**:1023–9.

Jin, D.Z., Fujii, N. and Graybiel, A.M. (2009) Neural representation of time in cortico-basal ganglia circuits. *Proc Natl Acad Sci USA,* **106**:19156–61.

Kawamura, Y., Fukaya, M., Maejima, T., *et al.* (2006) The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J Neurosci,* **26**:2991–3001.

Keele, S.W. and Ivry, R. (1990) Does the cerebellum provide a common computation for diverse tasks? A timing hypothesis. *Ann NY Acad Sci,* **608**:179–207; discussion 207–11.

Kishimoto, Y. and Kano, M. (2006) Endogenous cannabinoid signaling through the CB1 receptor is essential for cerebellum-dependent discrete motor learning. *J Neurosci,* **26**:8829–37.

Koch, G., Oliveri, M., Torriero, S., Salerno, S., Lo Gerfo, E. and Caltagirone, C. (2007) Repetitive TMS of cerebellum interferes with millisecond time processing. *Exp Brain Res,* **179**:291–9.

Kreitzer, A.C., Carter, A.G. and Regehr, W.G. (2002). Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. *Neuron,* **34**:787–96.

Kreitzer, A.C. and Regehr, W.G. (2001a) Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. *J Neurosci,* **21**:RC174.

Kreitzer, A.C. and Regehr, W.G. (2001b) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron,* **29**:717–27.

Lange, W. (1975) Cell number and cell density in the cerebellar cortex of man and some other mammals. *Cell Tissue Res,* **157**:115–24.

Larsell, O. and Jansen, J. (1967) *The comparative anatomy and histology of the cerebellum*. Minneapolis: University of Minnesota Press.

Larsell, O. and Jansen, J. (1970) *The comparative anatomy and histology of the cerebellum from monotremes through apes*. Minneapolis: University of Minnesota Press.

Lee, K.H., Bhaker, R.S., Mysore, A., Parks, R.W., Birkett, P.B. and Woodruff, P.W. (2009) Time perception and its neuropsychological correlates in patients with schizophrenia and in healthy volunteers. *Psychiatry Res,* **166**:174–83.

Lee, K.H., Egleston, P.N., Brown, W.H., Gregory, A.N., Barker, A.T. and Woodruff, P.W. (2007) The role of the cerebellum in subsecond time perception: evidence from repetitive transcranial magnetic stimulation. *J Cogn Neurosci,* **19**:147–57.

Lee, M., Jo, Y., Chun, M., Chung, J., Kim, M. and Min, D. (2000) Immunohistochemical localization of phospholipase D1 in rat central nervous system. *Brain Res,* **864**:52–59.

Levenes, C., Daniel, H., Soubrie, P. and Crepel, F. (1998) Cannabinoids decrease excitatory synaptic transmission and impair long-term depression in rat cerebellar Purkinje cells. *J Physiol,* **510**:867–79.

Levitt, J.J., McCarley, R.W., Nestor, P.G., *et al.* (1999) Quantitative volumetric MRI study of the cerebellum and vermis in schizophrenia: clinical and cognitive correlates. *Am J Psychiatry,* **156**:1105–7.

Leweke, F.M., Giuffrida, A., Koethe, D., *et al.* (2007) Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophr Res,* **94**: 29–36.

Leweke, F.M., Giuffrida, A., Wurster, U., Emrich, H.M. and Piomelli, D. (1999) Elevated endogenous cannabinoids in schizophrenia. *Neuroreport,* **10L** 1665–9.

Lewis, P.A. and Miall, R.C. (2003) Brain activation patterns during measurement of sub- and supra-second intervals. *Neuropsychologia,* **41**: 1583–92.

Loeber, R.T., Cintron, C.M. and Yurgelun-Todd, D.A. (2001) Morphometry of individual cerebellar lobules in schizophrenia. *Am J Psychiatry,* **158**: 952–4.

Logan, C.G. and Grafton, S.T. (1995) Functional anatomy of human eyeblink conditioning determined with regional cerebral glucose metabolism and positron-emission tomography. *Proc Natl Acad Sci USA,* **92**: 7500–4.

Lubow, R.E. (2009) Classical eyeblink conditioning and schizophrenia: a short review. *Behav Brain Res,* **202**:1–4.

Ludwig, A.M. (1966) Altered states of consciousness. *Arch Gen Psychiatry,* **15**: 225–34.

Maloku, E., Covelo, I.R., Hanbauer, I., *et al.* (2010) Lower number of cerebellar Purkinje neurons in psychosis is associated with reduced reelin expression. *Proc Natl Acad Sci USA,* **107**:4407–11.

Manni, E. and Petrosini, L. (2004) A century of cerebellar somatotopy: a debated representation. *Nat Rev Neurosci,* **5**:241–9.

Marenco, S., Weinberger, D. R. and Schreurs, B. G. (2003). Single-cue delay and trace classical conditioning in schizophrenia. *Biol Psychiatry,* **53**:390–402.

Marr, D. (1969) A theory of cerebellar cortex. *J Physiol,* **202**:437–70.

Mathew, R.J., Wilson, W.H., Turkington, T.G. and Coleman, R.E. (1998) Cerebellar activity and disturbed time sense after THC. *Brain Res,* **797**:183–9.

Mathew, R.J., Wilson, W.H., Turkington, T.G., *et al.* (2002) Time course of tetrahydrocannabinol-induced changes in regional cerebral blood flow measured with positron emission tomography. *Psychiatry Res,* **116**:173–85.

Meck, W.H. (1996) Neuropharmacology of timing and time perception. *Brain Res Cogn Brain Res,* **3**:227–42.

Meck, W.H. (2005) Neuropsychology of timing and time perception. *Brain Cogn,* **58**:1–8.

Melges, F.T., Tinklenberg, J.R., Hollister, L.E. and Gillespie, H.K. (1970a) Marihuana and temporal disintegration. *Science,* **168**:1118–20.

Melges, F.T., Tinklenberg, J.R., Hollister, L.E. and Gillespie, H.K. (1970b) Temporal disintegration and depersonalization during marihuana intoxication. *Arch Gen Psychiatry,* **23**:204–10.

Mittmann, W. and Hausser, M. (2007) Linking synaptic plasticity and spike output at excitatory and inhibitory synapses onto cerebellar Purkinje cells. *J Neurosci,* **27**:5559–70.

Moore, T.H., Zammit, S., Lingford-Hughes, A., *et al.* (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet,* **370**:319–28.

Morton, S.M. and Bastian, A.J. (2007) Mechanisms of cerebellar gait ataxia. *Cerebellum,* **6**:79–86.

Murray, G.K., Veijola, J., Moilanen, K., *et al.* (2006) Infant motor development is associated with adult cognitive categorisation in a longitudinal birth cohort study. *J Child Psychol Psychiatry,* **47**:25–9.

Nopoulos, P.C., Ceilley, J.W., Gailis, E.A., and Andreasen, N.C. (1999) An MRI study of cerebellar vermis morphology in patients with schizophrenia: evidence in support of the cognitive dysmetria concept. *Biol Psychiatry,* **46**:703–11.

O'Connor, N. and Rawnsley, K. (1959) Two types of conditioning in psychotics and normals. *J Abnorm Psychol*, **58**:157–61.

O'Leary, D.S., Block, R.I., Turner, B.M., *et al.* (2003) Marijuana alters the human cerebellar clock. *Neuroreport*, **14**:1145–51.

Okugawa, G., Sedvall, G., Nordstrom, M., *et al.* (2002) Selective reduction of the posterior superior vermis in men with chronic schizophrenia. *Schizophr Res,* **55**:61–7.

Oliveri, M., Bonni, S., Turriziani, P., *et al.* (2009) Motor and linguistic linking of space and time in the cerebellum. *PLoS One,* **4**:e7933.

Ong, W.Y. and Mackie, K. (1999) A light and electron microscopic study of the CB1 cannabinoid receptor in primate brain. *Neuroscience,* **92**:1177–91.

Paradiso, S., Andreasen, N.C., Crespo-Facorro, B., *et al.* (2003) Emotions in unmedicated patients with schizophrenia during evaluation with positron emission tomography. *Am J Psychiatry,* **160**:1775–83.

Patrick, G., Straumanis, J.J., Struve, F.A., Fitz-Gerald, M.J. and Manno, J.E. (1997) Early and middle latency evoked potentials in medically and psychiatrically normal daily marihuana users: a paucity of significant findings. *Clin Electroencephalogr,* **28**:26–31.

Patrick, G., Straumanis, J.J., Struve, F.A., *et al.* (1995) Auditory and visual P300 event related potentials are not altered in medically and psychiatrically normal chronic marihuana users. *Life Sci,* **56**:2135–40.

Pellionisz, A. and Llinas, R. (1982) Space-time representation in the brain: the cerebellum as a predictive space-time metric tensor. *Neuroscience,* **7**:2949–70.

Penney, T.B., Meck, W.H., Roberts, S.A., Gibbon, J., and Erlenmeyer-Kimling, L. (2005) Interval-timing deficits in individuals at high risk for schizophrenia. *Brain Cogn,* **58**:109–18.

Pertwee, R.G. (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther,* **74**:129–80.

Pettit, D.A., Harrison, M.P., Olson, J.M., Spencer, R.F. and Cabral, G.A. (1998) Immunohistochemical localization of the neural cannabinoid receptor in rat brain. *J Neurosci Res,* **51**:391–402.

Picard, H., Amado, I., Mouchet-Mages, S., Olie, J.P. and Krebs, M.O. (2008) The role of the cerebellum in schizophrenia: an update of clinical, cognitive, and functional evidences. *Schizophr Bull,* **34**:155–72.

Plakke, B., Freeman, J.H. and Poremba, A. (2007) Metabolic mapping of the rat cerebellum during delay and trace eyeblink conditioning. *Neurobiol Learn Mem,* **88**:11–18.

Qiu, D.L. and Knopfel, T. (2009) Presynaptically expressed long-term depression at cerebellar parallel fiber synapses. *Pflugers Arch,* **457**:865–75.

Ramaekers, J.G., Kauert, G., Theunissen, E.L., Toennes, S.W. and Moeller, M.R. (2009) Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *J Psychopharmacol,* **23**:266–77.

Ramaekers, J.G., Kauert, G., van Ruitenbeek, P., Theunissen, E.L., Schneider, E. and Moeller, M.R. (2006) High-potency marijuana impairs executive function and inhibitory motor control. *Neuropsychopharmacology,* **31**:2296–303.

Rammsayer, T. (1990) Temporal discrimination in schizophrenic and affective disorders: evidence for a dopamine-dependent internal clock. *Int J Neurosci,* **53**:111–20.

Ramnani, N. (2006) The primate cortico-cerebellar system: anatomy and function. *Nat Rev Neurosci,* **7**:511–22.

Rancz, E.A. and Hausser, M. (2006) Dendritic calcium spikes are tunable triggers of cannabinoid release and short-term synaptic plasticity in cerebellar Purkinje neurons. *J Neurosci,* **26**:5428–5437.

Ranganathan, M. and D'Souza, D.C. (2006) The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl)*, **188**:425–44.

Raymond, J.L., Lisberger, S.G. and Mauk, M.D. (1996) The cerebellum: a neuronal learning machine? *Science,* **272**:1126–31.

Reyes, M.G. and Gordon, A. (1981) Cerebellar vermis in schizophrenia. *Lancet,* **2**:700–1.

Ridler, K., Veijola, J.M., Tanskanen, P., *et al.* (2006) Fronto-cerebellar systems are associated with infant motor and adult executive functions in healthy adults but not in schizophrenia. *Proc Natl Acad Sci USA,* **103**:15651–6.

Roizin, L., True, C. and Knight, M. (1959) Structural effects of tranquilizers. *Res Publ Assoc Res Nerv Ment Dis,* **37**:285–324.

Roser, P., Gallinat, J., Weinberg, G., Juckel, G., Gorynia, I. and Stadelmann, A.M. (2009) Psychomotor performance in relation to acute oral administration of Delta 9-tetrahydrocannabinol and standardized cannabis extract in healthy human subjects. *Eur Arch Psychiatry Clin Neurosci,* **259**:284–92.

Safo, P.K., Cravatt, B.F. and Regehr, W.G. (2006) Retrograde endocannabinoid signaling in the cerebellar cortex. *Cerebellum,* **5**:134–45.

Safo, P.K. and Regehr, W.G. (2005) Endocannabinoids control the induction of cerebellar LTD. *Neuron,* **48**:647–59.

Salman, M.S. (2002) The cerebellum: it's about time! But timing is not everything – new insights into the role of the cerebellum in timing motor and cognitive tasks. *J Child Neurol,* **17**:1–9.

Schmahmann, J.D. (1991) An emerging concept. The cerebellar contribution to higher function. *Arch Neurol,* **48**:1178–87.

Schmahmann, J.D. (2000) *MRI atlas of the human cerebellum*. San Diego: Academic Press.

Schmahmann, J.D. (2004) Disorders of the cerebellum: ataxia, dysmetria of thought, and the cerebellar cognitive affective syndrome. *J Neuropsychiatry Clin Neurosci,* **16**:367–78.

Schmahmann, J.D. and Sherman, J.C. (1998) The cerebellar cognitive affective syndrome. *Brain,* **121**:561–79.

Sears, L.L., Andreasen, N.C. and O'Leary, D.S. (2000) Cerebellar functional abnormalities in schizophrenia are suggested by classical eyeblink conditioning. *Biol Psychiatry*, **48**; 204–09.

Skosnik, P.D., Edwards, C.R., O'Donnell, B.F., Steffen, A., Steinmetz, J.E. and Hetrick, W.P. (2008) Cannabis use disrupts eyeblink conditioning: evidence for cannabinoid modulation of cerebellar-dependent learning. *Neuropsychopharmacology*, **33**:1432–40.

Skosnik, P.D., Krishnan, G.P., Aydt, E.E., Kuhlenshmidt, H.A. and O'Donnell, B.F. (2006a) Psychophysiological evidence of altered neural synchronization in cannabis use: relationship to schizotypy. *Am J Psychiatry*, **163**:1798–805.

Skosnik, P.D., Krishnan, G.P., Vohs, J.L. and O'Donnell, B.F. (2006b) The effect of cannabis use and gender on the visual steady state evoked potential. *Clin Neurophysiol*, **117**:144–56.

Skosnik, P.D., Spatz-Glenn, L. and Park, S. (2001) Cannabis use is associated with schizotypy and attentional disinhibition. *Schizophr Res,* **48**:83–92.

Snider, S.R. (1982) Cerebellar pathology in schizophrenia – cause or consequence? *Neurosci Biobehav Rev,* **6**:47–53.

Solowij, N. (1998) *Cannabis and cognitive functioning*. Cambridge; New York: Cambridge University Press.

Solowij, N., Stephens, R.S., Roffman, R.A., *et al*. (2002) Cognitive functioning of long-term heavy cannabis users seeking treatment. *JAMA,* **287**:1123–31.

Spain, B. (1966) Eyelid conditioning and arousal in schizophrenic and normal subjects. *J Abnorm Psychol,* **71**:260–6.

Spencer, R.M. and Ivry, R.B. (2005) Comparison of patients with Parkinson's disease or cerebellar lesions in the production of periodic movements involving event-based or emergent timing. *Brain Cogn,* **58**:84–93.

Steinmetz, J.E. (2000) Brain substrates of classical eyeblink conditioning: a highly localized but also distributed system. *Behav Brain Res,* **110**:13–24.

Stevens, A., Schwarz, J., Schwarz, B., Ruf, I., Kolter, T. and Czekalla, J. (2002) Implicit and explicit learning in schizophrenics treated with olanzapine and with classic neuroleptics. *Psychopharmacology (Berl),* **160**:299–306.

Strick, P.L., Dum, R.P. and Fiez, J.A. (2009) Cerebellum and nonmotor function. *Annu Rev Neurosci,* **32**:413–34.

Suarez, J., Bermudez-Silva, F.J., Mackie, K., *et al*. (2008) Immunohistochemical description of the endogenous cannabinoid system in the rat cerebellum and functionally related nuclei. *J Comp Neurol,* **509**:400–21.

Sundram, S. (2006) Cannabis and neurodevelopment: implications for psychiatric disorders. *Hum Psychopharmacol,* **21**:245–54.

Szabo, B., Urbanski, M.J., Bisogno, T., *et al*. (2006) Depolarization-induced retrograde synaptic inhibition in the mouse cerebellar cortex is mediated by 2-arachidonoyl glycerol. *J Physiol,* **577**:263–80.

Szeszko, P.R., Gunning-Dixon, F., Goldman, R.S., *et al*. (2003) Lack of normal association between cerebellar volume and neuropsychological functions in first-episode schizophrenia. *Am J Psychiatry,* **160**:1884–7.

Takahashi, K.A. and Linden, D.J. (2000) Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. *J Neurophysiol,* **83**:1167–80.

Tanimura, A., Kawata, S., Hashimoto, K. and Kano, M. (2009) Not glutamate but endocannabinoids mediate retrograde suppression of cerebellar parallel fiber to Purkinje cell synaptic transmission in young adult rodents. *Neuropharmacology,* **57**:157–63.

Taylor, J.A. and Spence, K.W. (1954) Conditioning level in the behavior disorders. *J Abnorm Psychol,* **49**:497–502.

Thompson, R.F. and Steinmetz, J.E. (2009) The role of the cerebellum in classical conditioning of discrete behavioral responses. *Neuroscience,* **162**:732–55.

Tiemeier, H., Lenroot, R.K., Greenstein, D.K., Tran, L., Pierson, R. and Giedd, J.N. (2010) Cerebellum development during childhood and adolescence: a

longitudinal morphometric MRI study. *Neuroimage,* **49**:63–70.

Tinklenberg, J.R., Kopell, B.S., Melges, F.T. and Hollister, L.E. (1972) Marihuana and alcohol, Time production and memory functions. *Arch Gen Psychiatry,* **27**:812–15.

Tinklenberg, J.R., Roth, W.T. and Kopell, B.S. (1976) Marijuana and ethanol: differential effects on time perception, heart rate, and subjective response. *Psychopharmacology (Berl),* **49**:275–9.

Tran, K.D., Smutzer, G. S., Doty, R. L. and Arnold, S. E. (1998). Reduced Purkinje cell size in the cerebellar vermis of elderly patients with schizophrenia. *Am J Psychiatry,* **155**(9), 1288–90.

Tsou, K., Brown, S., Sanudo-Pena, M.C., Mackie, K. and Walker, J.M. (1998a). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience,* **83**(2), 393–411.

Tsou, K., Nogueron, M.I., Muthian, S., *et al.* (1998b). Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. *Neurosci Lett,* **254**(3), 137–40.

Vachon, L., Sulkowski, A. and Rich, E. (1974) Marihuana effects on learning, attention and time estimation. *Psychopharmacologia,* **39**:1–11.

Vogel, R.W., Amundson, J.C., Lindquist, D.H. and Steinmetz, J.E. (2009) Eyeblink conditioning during an interstimulus interval switch in rabbits (*Oryctolagus cuniculus*) using picrotoxin to disrupt cerebellar cortical input to the interpositus nucleus. *Behav Neurosci,* **123**:62–74.

Volkow, N.D., Gillespie, H., Mullani, N., *et al.* (1996) Brain glucose metabolism in chronic marijuana users at baseline and during marijuana intoxication. *Psychiatry Res,* **67**:29–38.

Volz, H.P., Nenadic, I., Gaser, C., Rammsayer, T., Hager, F. and Sauer, H. (2001) Time estimation in schizophrenia: an fMRI study at adjusted levels of difficulty. *Neuroreport,* **12**:313–16.

Wahl, O.F. and Sieg, D. (1980) Time estimation among schizophrenics. *Percept Mot Skills,* **50**:535–41.

Walker, E.F., Lewine, R.R. and Neumann, C. (1996) Childhood behavioral characteristics and adult brain morphology in schizophrenia. *Schizophr Res,* **22**:93–101.

Wang, X., Chen, G., Gao, W. and Ebner, T. (2009) Long-term potentiation of the responses to parallel fiber stimulation in mouse cerebellar cortex in vivo. *Neuroscience,* **162**:713–22.

Warren, S. and Ross, R.G. (1998) Deficient cancellation of the vestibular ocular reflex in schizophrenia. *Schizophr Res,* **34**:187–93.

Weinberger, D.R., Torrey, E.F. and Wyatt, R.J. (1979) Cerebellar atrophy in chronic schizophrenia. *Lancet,* **1**:718–19.

Wild-Wall, N., Willemssen, R., Falkenstein, M. and Beste, C. (2008) Time estimation in healthy ageing and neurodegenerative basal ganglia disorders. *Neurosci Lett,* **442**:34–8.

Woodruff-Pak, D.S. and Disterhoft, J.F. (2008) Where is the trace in trace conditioning? *Trends Neurosci,* **31**:105–12.

Woodruff-Pak, D.S., Foy, M.R., Akopian, G.G., *et al.* (2010) Differential effects and rates of normal aging in cerebellum and hippocampus. *Proc Natl Acad Sci USA,* **107**:1624–9.

Yamazaki, T. and Tanaka, S. (2007) A spiking network model for passage-of-time representation in the cerebellum. *Eur J Neurosci,* **26**: 2279–92.

Yee, R.D., Baloh, R.W., Marder, S.R., Levy, D.L., Sakala, S.M. and Honrubia, V. (1987). Eye movements in schizophrenia. *Invest Ophthalmol Vis Sci,* **28**:366–74.

Zagon, I.S., McLaughlin, P.J. and Smith, S. (1977) Neural populations in the human cerebellum: estimations from isolated cell nuclei. *Brain Res,* **127**:279–82.

Zhu, L., Scelfo, B., Hartell, N.A., Strata, P. and Sacchetti, B. (2007) The effects of fear conditioning on cerebellar LTP and LTD. *Eur J Neurosci,* **26**:219–27.

Zimmer, A., Zimmer, A.M., Hohmann, A.G., Herkenham, M. and Bonner, T.I. (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci USA,* **96**:5780–5.

Chapter 14

The neural basis for the acute effects of cannabis on learning and psychosis

Sagnik Bhattacharyya and Philip McGuire

Accumulating epidemiological evidence (reviewed in Chapter 18 of this book and elsewhere [Moore *et al*., 2007]) suggests that regular cannabis use can increase the long-term risk of development of psychotic disorders like schizophrenia, and this risk may be particularly high in individuals who start smoking cannabis at a young age. Among the many environmental risk factors for schizophrenia (van Os and Kapur, 2009), the influence of regular frequent cannabis use on the risk of development of psychotic disorders like schizophrenia is particularly important, as it is the most widely used illicit drug in the world (Chawla and Pichon, 2006). Furthermore, the age of first cannabis use is currently decreasing (Hall and Degenhardt, 2007), to the extent that cannabis use is becoming more common than cigarette smoking among young people in some countries (Rey and Tennant, 2002).

Evidence has also accumulated regarding the occurrence of a wide range of psychotic symptoms acutely in the context of cannabis use (D'Souza, 2007) and following the experimental administration of its principal psychoactive ingredient, Δ^9-tetrahydrocannabinol (THC) (Isbell *et al*., 1967; Melges *et al*., 1974). Methodologically improved, placebo-controlled studies have demonstrated that the acute administration of THC can induce psychotic symptoms as measured using standardized rating scales in healthy volunteers (D'Souza *et al*., 2004; Morrison *et al*., 2009; Stokes *et al*., 2009; Bhattacharyya *et al*., 2009b) and can exacerbate them in patients with schizophrenia (D'Souza *et al*., 2005). However, while existing anecdotal, experimental and epidemiological studies constitute a very important guiding strand in the evidence supporting the role of cannabis use in the development of psychosis, obvious methodological limitations associated with large-scale epidemiological studies (Moore *et al*., 2007) that examine a relatively rare outcome, indicate the need for complementary strands of evidence.

Studies that provide mechanistic evidence delineating how the enhancement of the risk of psychosis by cannabis may be mediated at the neural level in humans are therefore important. But, robust evidence obtained under controlled experimental conditions regarding the longer-term mediation of the effects of cannabis at the neural level in humans are difficult to obtain for logistical and ethical reasons. Examination of the acute neural effects of cannabis and its principal ingredients in humans allows an alternative window into plausible mechanistic links between cannabis use and psychosis. This chapter will review currently available human evidence regarding the acute neural effects of cannabinoids. Particular emphasis will be put on the neural mechanisms that may underlie the acute effects of cannabis and its main psychoactive ingredients on learning, as impairment in learning and memory is one of the most prominent acute cognitive effects of cannabis and THC in healthy individuals (Ranganathan and D'Souza, 2006), and possibly the only cognitive domain that continues to be impaired in chronic users (Solowij *et al*., 2002) (also reviewed in Chapter 9). This is also crucial to understanding the link between cannabis and psychosis as verbal memory is one of the key neuropsychological impairments in schizophrenia (Reichenberg and Harvey, 2007).

Effects of THC during verbal learning

The main central cannabinoid (CB1) receptors have a high density in the medial temporal and prefrontal cortex (Elphick and Egertova, 2001), areas crucial to learning and memory (Wagner *et al*., 1998; Buckner and Wheeler, 2001), and THC affects medial temporal function in animals (Robbe *et al*., 2006; Puighermanal *et al*., 2009; Wise *et al*., 2009) and memory performance in animals and humans (D'Souza *et al*., 2004; Robbe *et al*., 2006; Puighermanal *et al*., 2009; Wise

Marijuana and Madness, Second Edition, ed. David Castle, Robin M. Murray and Deepak Cyril D'Souza. Published by Cambridge University Press.

et al., 2009). Therefore, one could hypothesize that it may influence learning and memory by modulating function in these regions.

We did just this (Bhattacharyya *et al*., 2009b), and employed functional MRI in conjunction with oral administration of the two major psychoactive cannabinoids, THC and cannabidiol (CBD), in a group of occasional cannabis users. Fifteen healthy, right-handed, English-speaking men who had been exposed to cannabis < 15 times in their life, but not in the previous month, and had minimal exposure to other illicit drugs, were scanned using functional MRI while performing a task which engaged verbal learning. The task comprised three sequentially presented conditions that involved visual presentation of stimuli: encoding, recall and baseline. During the encoding condition, subjects viewed a series of word-pairs. One word from each pair was then shown during the recall condition and subjects had to articulate the missing word. During the baseline condition, different words were presented in pairs, printed with identical or different fonts. This cycle was repeated four times with the same word pairs to facilitate learning and recall of associations over successive encoding or recall blocks respectively. Contrasting the active (encoding and recall) task conditions against the baseline condition allowed us to control for activation related to visually presented words.

Each subject was scanned on three separate occasions, with each session preceded by oral administration of either 10 mg THC, 600 mg CBD or placebo, in a double-blind, randomized, repeated-measures, within-subject design. There was a progressive improvement in word recall with repeated presentation of word pairs across encoding blocks for all the drug conditions, but no significant effect of drug on this repetition-related improvement. Under the placebo condition there was a linear reduction in the engagement of various areas including the parahippocampal cortex bilaterally during the repeated presentation of word pairs across successive encoding blocks, which was associated with a progressive improvement in recall score. Further, the decrement in parahippocampal response was directly correlated with recall score ($r = 0.502$, $p = 0.028$).

The linear decrement in the engagement of the medial temporal cortex and its relationship with the recall score observed during learning under placebo conditions is consistent with previous findings (Zeineh *et al*., 2003). Medial temporal activation has been related to the quantity of novel and successful mnemonic processing (Brewer *et al*., 1998; Wagner *et al*., 1998; Zeineh *et al*., 2003), and more specifically to relational memory binding (Hannula and Ranganath, 2008). Administration of THC augmented the parahippocampal activation such that the normal linear decrement in activation across successive encoding blocks and its relationship with the recall score was no longer evident (Figure 14.1). The augmentation of parahippocampal activation by THC and elimination of the relationship between medial temporal activation and recall score were consistent with evidence that THC impairs medial temporal function in animals (Robbe *et al*., 2006; Puighermanal *et al*., 2009) and memory performance in animals and humans (Ranganathan and D'Souza, 2006; Robbe *et al*., 2006; Puighermanal *et al*., 2009).

We interpreted these findings as reflective of increased demands on encoding under the influence of THC. Encoding-related parahippocampal activation is thought to serve the encoding of contextual information about stimuli that may be reactivated later to aid in recollection (Eichenbaum *et al*., 2007). Under the placebo condition, engagement of this region occurred most during the initial presentation of word pairs and progressively diminished during subsequent presentations, consistent with behavioral evidence from subsequent recall performance that most of the learning occurred during the first presentation of the encoding condition. The pattern of minimal change in the engagement of this region over the first three blocks of the encoding condition under the influence of THC suggests that it impairs the efficient encoding of contextual information in the parahippocampal cortex.

During repeated presentations of the recall condition under the influence of placebo, progressive improvement in recall score was associated with a linear reduction in activation in the dorsal anterior cingulate/medial prefrontal cortex bilaterally, and this decline in activation was correlated with the recall score ($r = 0.619$, $p = 0.007$). Δ^9-tetrahydrocannabinol augmented activation in these regions during recall, such that the linear decline in the left dorsal anterior cingulate/medial prefrontal cortical response across successive recall blocks seen under placebo conditions, and the correlation between this response and the progressive improvement in recall score, were abolished. Anterior cingulate/medial prefrontal activation in the context of recall has been related to retrieval monitoring and verification (Simons *et al*., 2005), suggesting that this effect of THC may be a correlate of increased demands on these processes in the presence of the drug.

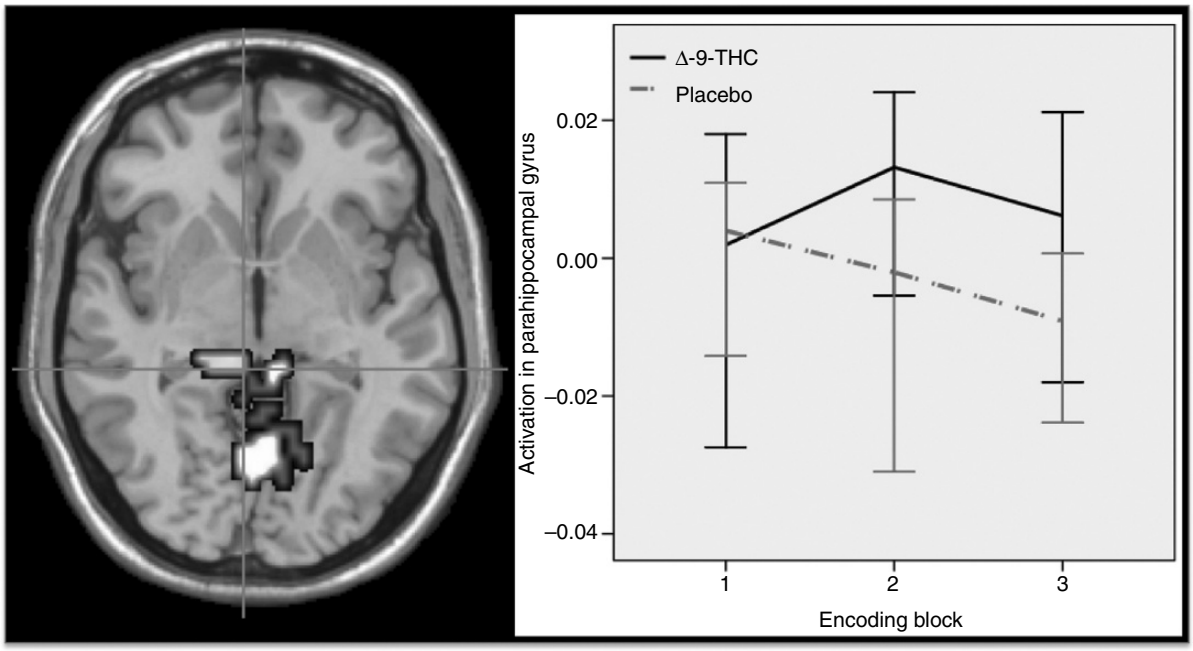

Figure 14.1. Effect of Δ^9-tetrahydrocannabinol (THC) on activation in the parahippocampal gyri (cross-hair in the transverse section of brain on the left of the panel) bilaterally extending to the midbrain and cerebellum during verbal learning. The left side of the brain is shown on the left side of the image. The plot on the right side of the panel shows the mean magnitude of activation (in aribitrary units; error bars show standard error of mean) in the parahippocampal gyral cluster on the left during each encoding block (x-axis) following administration of THC (solid line) and placebo (dashed line). See also color plate section.

We also found an attenuation of activation over successive presentations of the recall condition under the influence of THC in the striatum bilaterally and the left rostral anterior cingulate gyrus, where there was a non-significant increase under placebo conditions. These effects of THC in the ventral striatum (Figure 14.2) and the rostral anterior cingulate cortex were directly correlated with the severity of psychotic symptoms concurrently induced by it ($r = 0.568$, $p = 0.014$ and $r = 0.506$, $p = 0.027$, respectively). Thus in these brain regions, attenuation of activation by THC was greatest in those subjects who became most psychotic. The correlations between symptoms and brain activation were specific to the brain regions as well as to the symptoms, i.e. they were not evident in relation to anxiety, intoxication or sedation. The correlations between the psychotic symptoms induced by THC and its effects on functional activity in the striatum and anterior cingulate, both of which are rich in CB1 receptors (Elphick and Egertova, 2001) and dopaminergic innervation (Seamans and Yang, 2004), are consistent with evidence that THC modulates central dopamine transmission in animals (Chen *et al.*, 1990; French, 1997; Tanda *et al.*, 1997) and humans (Bossong *et al.*, 2009), and that acute psychotic symptoms in first-episode psychosis and schizophrenia are associated with increased striatal dopamine release (Guillin *et al.*, 2007). These regions have also been implicated in the pathophysiology of psychotic symptoms in schizophrenia (Allen *et al.*, 2007).

This effect of THC is possibly of more direct relevance to understanding the neurobiology of the relationship between cannabis use and psychosis. Although it has been hypothesized that the induction of psychotic symptoms by cannabis reflects a secondary effect of THC on dopamine release in the striatum (Murray *et al.*, 2007), and recent evidence obtained using positron emission tomography (PET) suggests that THC may acutely modulate dopamine release in this area (Bossong *et al.*, 2009), it was not clear until recently whether the effects of THC on the human striatum underlie the acute induction of psychotic symptoms by cannabis and THC. This study has provided the first human evidence to this effect.

The modulatory effects of THC on medial temporal, anterior cingulate/medial prefrontal and striatal

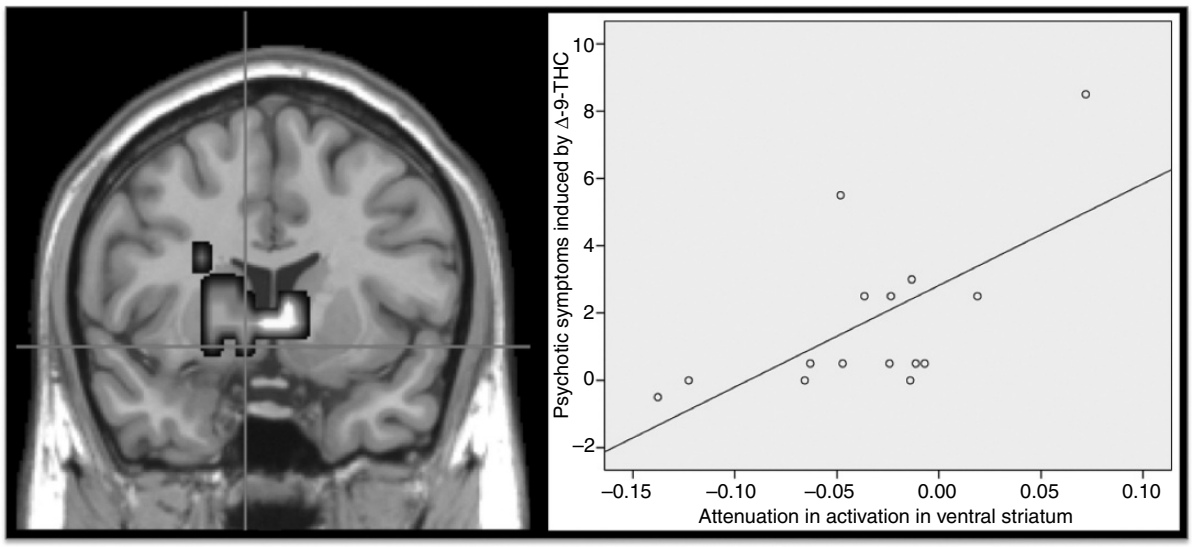

Figure 14.2. Effect of Δ^9-tetrahydrocannabinol (THC) on activation in the ventral striatum (cross-hair in the coronal section of brain on the left of the panel) during repeated recall trials. The plot on the right of the panel shows the correlation between attenuation of activation in the ventral striatum (in arbitrary units) caused by THC across repeated recall blocks and psychotic symptoms (y-axis) induced by it. See also color plate section.

activation during verbal learning were observed in the absence of differential task performance between the drug conditions. This was because our objective was not to examine the effects of THC on behavioral performance during a verbal learning task, which has already been examined in many previous studies (Ranganathan and D'Souza, 2006), but to assess the effects of THC on the neural underpinnings of verbal learning. So we employed a relatively easy task to ensure that performance across the drug conditions would be matched. This ensured that any differences in brain activation between the drug conditions could be interpreted without the confounding effect of differential task performance, thus allowing detection of drug effects at the neurophysiological level while the behavioral effects were matched.

None of the above effects of THC during learning were evident following the administration of CBD, consistent with evidence that CBD does not impair learning and memory (Fadda *et al*., 2004; Ilan *et al*., 2005), and suggesting that the effects of cannabis on memory and psychotic symptoms may be specifically related to its THC content. In a subsequent study, further evidence emerged that CBD may in fact have opposite neural effects to that of THC during a range of cognitive processes and also block the acute induction of psychotic symptoms under its influence (Bhattacharyya *et al*., 2010).

Other acute neural effects of cannabis relevant to psychosis

This section will summarize the evidence from studies that have examined the neural effects of cannabis and its main psychoactive ingredients during other cognitive tasks, as well as on resting state activity of the brain. Finally, the acute effects of THC on central dopaminergic transmission will be described. They are equally important as the previous evidence regarding the effects on the neural substrate for learning, as they help to understand the neural basis of the relationship between cannabis use and psychotic symptoms or disorder, because of the various converging domains of neurocognitive impairments in cannabis use and psychotic disorders like schizophrenia (Solowij and Michie, 2007 and see Chapters 8 and 9).

Effects on resting state activity/blood flow

Early studies investigated the acute effects of cannabis or THC on the brain in chronic or recreational cannabis users, employing various imaging techniques, ranging from single photon emission computed tomography (SPECT) (Mathew *et al*., 1989; Mathew *et al*., 1992; Mathew and Wilson, 1993) to "positron emission tomography" (PET) (Volkow *et al*., 1996; Mathew *et al*., 1997; Mathew *et al*., 1998; Mathew *et al*., 1999).

Relative to baseline, acute administration of pure THC, or cannabis extract rich in THC, causes an increase in resting global cerebral blood flow (CBF) as well as increased activity in the anterior cingulate, insula, prefrontal and orbitofrontal cortices, left temporal lobe and the cerebellum (Mathew *et al*., 1989; Mathew *et al*., 1992; Mathew *et al*., 1993; Volkow *et al*., 1996; Mathew *et al*., 1997; Mathew *et al*., 1998; Mathew *et al*., 1999; Mathew *et al*., 2002). The effect of administration of cannabis extract rich in THC on activity in the basal ganglia, thalamus, amygdala and hippocampus (Volkow *et al*., 1996; Mathew *et al*., 1997; Mathew *et al*., 1999) is less consistent. Results from these studies are difficult to compare and integrate because of differences in the severity and duration of cannabis use in the subjects recruited in the various studies, presence of psychiatric and drug misuse co-morbidities, as well as variation in the mode, dose and purity of cannabis administered, notwithstanding the different imaging modalities used. Other confounding factors such as tolerance, withdrawal and sensitization to repeated use further complicate the interpretation and generalization of these results. However, the evidence suggests that acute cannabis administration modulates brain function as measured using metabolic rate or blood flow in a wide network that includes the prefrontal cortex, limbic and paralimbic areas, basal ganglia and cerebellum, consistent with the distribution of the CB1 receptors in the brain (Elphick and Egertova, 2001).

Effects during cognitive activation tasks

The acute effects of cannabis on neural activity during cognitive processing paradigms apart from learning have been examined by few other studies. Employing a task that involved the inhibition of prepotent motor responses in conjunction with functional MRI, our group (Borgwardt *et al*., 2008) observed that acute administration of THC attenuated activation in the right inferior frontal and anterior cingulate gyrus, normally crucial for response inhibition (Aron *et al*., 2003; Sharp *et al*., 2010). An effect of THC on the neural substrate for response inhibition is consistent with the adverse effects of cannabis use on motor control (Hall and Solowij, 1998; Rogers and Robbins, 2001), inhibitory processing (Solowij and Michie, 2007), driving safety (Lamers and Ramaekers, 2001) and evidence that THC impairs performance on certain tasks that engage response inhibition (Ramaekers *et al*., 2006; Ramaekers *et al*., 2009). This is also consistent with impairments in response inhibition (Huddy *et al*., 2009) and altered activation of the inferior frontal gyrus during the same (Rubia *et al*., 2001) or related response inhibition paradigms (Kaladjian *et al*., 2007) in psychotic disorders like schizophrenia. Attenuation of inferior frontal and anterior cingulate activity during a response-inhibition task under the influence of THC may result in impairments in the inhibitory control of thoughts and emotions, as well as motor responses, and therefore contribute to the generation of paranoid beliefs under the influence of THC and cannabis, if these are derived from thoughts and feelings that are normally suppressed. This is of interest not just in the context of understanding how cannabis modulates inhibitory processes and symptoms in the brain, but also because neurophysiological studies (Freedman *et al*., 2000; Daskalakis *et al*., 2002) suggest an inhibitory deficit as a central pathophysiologic mechanism in psychotic disorders and as abnormal activation of the network underlying motor response inhibition is well documented in schizophrenia (Rubia *et al*., 2001; Kaladjian *et al*., 2007).

Acute modulation of attentional processing, commonly impaired in the context of cannabis use and in schizophrenia (Solowij and Michie, 2007) has also been examined employing PET (O'Leary *et al*., 2002; O'Leary *et al*., 2007). Increased regional CBF in the orbital and mesial frontal lobes, insula, temporal poles, anterior cingulate and cerebellum, and reductions in the auditory and visual cortices, and in an attentional network comprising the parietal lobe, frontal lobe and thalamus, were reported during an auditory attention (dichotic listening) task in recreational or occasional cannabis users relative to placebo.

Effects on central neurotransmission

As outlined in Chapters 1 and 3 of this volume, basic research suggests that cannabis and THC may modulate a number of neurotransmitters in the brain (Pertwee, 2008). Only a couple of studies have systematically examined this in humans (Bossong *et al*., 2009; Stokes *et al*., 2009). Both studies examined the effects of acute administration of THC on the release of dopamine in the striatum. However, the earliest evidence regarding this came from a case report demonstrating a reduction in [^{11}C]iodobenzamide binding, suggesting an increase in the levels of synaptic dopamine immediately following recreational cannabis use in a single volunteer with a history of schizophrenia (Voruganti

et al., 2001). Employing the inhalation route of administration, Bossong *et al.* (2009) demonstrated that an acute dose (8 mg) of THC resulted in a modest 3–4% reduction in the binding of [^{11}C]raclopride (suggestive of an increase in synaptic dopamine levels) in the ventral striatum and the precommissural dorsal putamen in healthy mild cannabis users ($n = 7$). However another study (Stokes *et al.*, 2009), employing the oral route of administration of a slightly larger dose of THC (10 mg) in a slightly larger sample ($n = 13$) failed to detect any significant effect on [^{11}C]raclopride binding.

The discrepancy between the evidence from basic research regarding the effects of THC on dopaminergic neurotransmission (French, 1997; Tanda *et al.*, 1997) and the equivocal results from the human studies may be because of several factors. First, the results of the study by Stokes *et al.* (2009) suggest that an oral route of administration may not be appropriate to examine the acute effects of THC on central dopamine levels because of the variability in bioavailability as well as slower rate of transfer to the brain of orally administered THC. The genetic make-up of volunteers taking part in such studies, especially with regard to genes for the catechol-O-methytransferase (COMT) enzyme or the dopamine transporter (DAT), may have a modulatory effect on the measurement of THC-induced dopamine release. Further, lifetime exposure to cannabis may also have an influence on these measures, being relatively higher in occasional use, as opposed to regular/heavy users.

Integration of the evidence

The various studies summarized here suggest that cannabis and THC may acutely modulate functional activity in a wide network of brain areas, including the medial temporal, prefrontal and paralimbic cortex and striatum, consistent with the widespread distribution of the CB1 receptors in the brain (Elphick and Egertova, 2001). Dysfunctions in the limbic, paralimbic and prefrontal cortices and striatum are well known in schizophrenia (Ross *et al.*, 2006), which is also characterized by positive (psychotic), negative and anxiety symptoms similar to that induced by THC (D'Souza *et al.*, 2005).

The precise neurochemical mechanisms underlying the acute effects of THC and cannabis are less clear. While animal studies clearly suggest that THC modulates dopaminergic transmission, the evidence from human PET studies is equivocal (Bossong *et al.*, 2009; Stokes *et al.*, 2009). As THC modulates a number of other neurotransmitters including glutamate and gamma-aminobutyric acid (GABA) in the brain, these effects may play a role in mediating its effects (Pertwee, 2008). The main molecular target of THC in the brain is the CB1 receptor, where it acts as a partial agonist (Pertwee, 2008). While this has not yet been examined directly in humans, it is likely that the acute neural, behavioral and symptomatic effects of THC seen in the studies reported here were a result of either a direct inhibitory effect of THC on GABAergic neurotransmission, or an indirect facilitatory effect on dopaminergic neurotransmission (Julian *et al.*, 2003; Kofalvi *et al.*, 2005; Pertwee, 2008). This has been hypothesized to be mediated through the inhibition of glutamatergic neurotransmission from the prefrontal cortex to the ventral striatal GABAergic interneurons, resulting in the disinhibition of midbrain dopaminergic neurons that project to the ventral striatum (Pertwee, 2008).

Alternatively, impairment of learning and memory by cannabis are predominantly a result of its effect on GABAergic signaling through the activation of CB1 receptors located on GABAergic interneurons in the medial temporal cortex (Puighermanal *et al.*, 2009). This has been shown to result in a disruption of the temporal coordination of principal cell assemblies (Robbe *et al.*, 2006). The neural effects of THC are thus a result of its effects on multiple neurotransmitter systems. This is not too dissimilar to current understandings regarding the involvement of multiple neurotransmitter systems in the pathophysiology of schizophrenia (Lisman *et al.*, 2008). Unraveling these precise mechanisms in humans appears increasingly likely with the refinement of neuroimaging methods and application of multimodal neuroimaging techniques. Such studies are particularly important as currently available evidence suggests a potential role for the acute cannabinoid challenge paradigm as a valid human psychopharmacological model for schizophrenia for use in early drug discovery research (Bhattacharyya *et al.*, 2009a).

Conclusions

While currently available evidence summarized here provides invaluable insight into how cannabis may impair learning and induce psychotic symptoms acutely, it does not shed light on the specific neural and genetic mechanisms underlying the wide variability in the individual response to cannabis.

Studies are underway that aim to provide mechanistic insights into the genetic modulation of the acute symptomatic, cognitive and neural effects of cannabis in humans. Further studies employing multimodal neuroimaging and molecular imaging techniques are also warranted to delineate the precise neurocognitive and neurochemical mechanisms that may mediate the longer-term effects of cannabis use on the risk of psychosis by examining enriched samples that may have a higher risk of development of psychosis. Examination of the precise neural mechanisms of the genetic moderation of this longer-term effect of cannabis use may also serve as an exemplar for future lines of research examining the neurobiological basis of other gene–environment interactions relevant to schizophrenia.

References

Allen, P., Aleman, A. and McGuire, P.K. (2007) Inner speech models of auditory verbal hallucinations: evidence from behavioral and neuroimaging studies. *Int Rev Psychiatry,* **19**:407–15.

Aron, A.R., Fletcher, P.C., Bullmore, E.T., Sahakian, B.J. and Robbins, T.W. (2003) Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nat Neurosci,* **6**:115–6.

Bhattacharyya, S., Crippa, J.A., Martin-Santos, R., Winton-Brown, T. and Fusar-Poli, P. (2009a) Imaging the neural effects of cannabinoids: current status and future opportunities for psychopharmacology. *Curr Pharm Des,* **15**:2603–14.

Bhattacharyya, S., Fusar-Poli, P., Borgwardt, S., *et al.* (2009b) Modulation of mediotemporal and ventrostriatal function in humans by Delta9-tetrahydrocannabinol: a neural basis for the effects of *Cannabis sativa* on learning and psychosis. *Arch Gen Psychiatry,* **66**:442–51.

Bhattacharyya, S., Morrison, P.D., Fusar-Poli, P., *et al.* (2010) Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology,* **35**:764–74.

Borgwardt, S.J., Allen, P., Bhattacharyya, S., *et al.* (2008) Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. *Biol Psychiatry,* **64**:966–73.

Bossong, M.G., van Berckel, B.N., Boellard, R., *et al.* (2009) Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology,* **34**:759–66.

Brewer, J.B., Zhao, Z., Desmond, J.E., Glover, G.H. and Gabrieli, J.D. (1998) Making memories: brain activity that predicts how well visual experience will be remembered. *Science,* **281**:1185–7.

Buckner, R.L. and Wheeler, M.E. (2001) The cognitive neuroscience of remembering. *Nat Rev Neurosci,* **2**:624–34.

Chawla, S. and Pichon, T.L. (2006) *2006 world drug report*, Vienna, Austria, United Nations Office on Drugs and Crime.

Chen, J., Paredes, W., Lowinson, J.H. and Gardner, E.L. (1990) Delta 9-tetrahydrocannabinol enhances presynaptic dopamine efflux in medial prefrontal cortex. *Eur J Pharmacol,* **190**:259–62.

D'Souza, D.C. (2007) Cannabinoids and psychosis. *Int Rev Neurobiol*, **78**:289–326.

D'Souza, D.C., Abi-Saab, W.M., Madonick, S., *et al.* (2005) Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biol Psychiatry*, **57**:594–608.

D'Souza, D.C., Perry, E., Macdougall, L., *et al.* (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology*, **29**:1558–72.

Daskalakis, Z.J., Christensen, B.K., Chen, R., Fitzgerald, P.B., Zipursky, R.B. and Kapur, S. (2002) Evidence for impaired cortical inhibition in schizophrenia using transcranial magnetic stimulation. *Arch Gen Psychiatry,* **59**:347–54.

Eichenbaum, H., Yonelinas, A.P. and Ranganath, C. (2007) The medial temporal lobe and recognition memory. *Annu Rev Neurosci,* **30**:123–52.

Elphick, M.R. and Egertova, M. (2001) The neurobiology and evolution of cannabinoid signalling. *Phil Trans R Soc Lond B,* **356**:381–408.

Fadda, P., Robinson, L., Fratta, W., Pertwee, R.G. and Riedel, G. (2004) Differential effects of THC- or CBD-rich cannabis extracts on working memory in rats. *Neuropharmacology,* **47**:1170–9.

Freedman, R., Adams, C.E., Adler, L.E., *et al.* (2000) Inhibitory neurophysiological deficit as a phenotype for genetic investigation of schizophrenia. *Am J Med Genet,* **97**:58–64.

French, E.D. (1997) delta9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. *Neurosci Lett,* **226**:159–62.

Guillin, O., Abi-Dargham, A. and Laruelle, M. (2007) Neurobiology of dopamine in schizophrenia. *Int Rev Neurobiol,* **78**:1–39.

Hall, W. and Degenhardt, L. (2007) Prevalence and correlates of cannabis use in developed and developing countries. *Curr Opin Psychiatry,* **20**:393–7.

Hall, W. and Solowij, N. (1998) Adverse effects of cannabis. *Lancet,* **352**:1611–6.

Hannula, D.E. and Ranganath, C. (2008) Medial temporal lobe activity predicts successful relational memory binding. *J Neurosci,* **28**:116–24.

Huddy, V.C., Aron, A.R., Harrison, M., Barnes, T.R., Robbins, T.W. and Joyce, E.M. (2009) Impaired conscious and preserved unconscious inhibitory processing in recent onset schizophrenia. *Psychol Med,* **39**:907–16.

Ilan, A.B., Gevins, A., Coleman, M., Elsohly, M.A. and De Wit, H. (2005) Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. *Behav Pharmacol,* **16**:487–96.

Isbell, H., Gorodetzsky, C.W., Jasinski, D., Claussen, U., von Spulak, F. and Korte, F. (1967) Effects of (–)delta-9-trans-tetrahydrocannabinol in man. *Psychopharmacologia,* **11**:184–8.

Julian, M.D., Martin, A.B., Cuellar, B., *et al.* (2003) Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. *Neuroscience,* **119**:309–18.

Kaladjian, A., Jeanningros, R., Azorin, J.M., Grimault, S., Anton, J.L. and Mazzola-Pomietto, P. (2007) Blunted activation in right ventrolateral prefrontal cortex during motor response inhibition in schizophrenia. *Schizophr Res,* **97**:184–93.

Kofalvi, A., Rodrigues, R.J., Ledent, C., *et al.* (2005) Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: a combined immunochemical and pharmacological analysis. *J Neurosci,* **25**:2874–84.

Lamers, C.T. and Ramaekers, J.G. (2001) Visual search and urban driving under the influence of marijuana and alcohol. *Hum Psychopharmacol,* **16**:393–401.

Lisman, J.E., Coyle, J.T., Green, R.W., *et al.* (2008) Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci,* **31**:234–42.

Mathew, R.J. and Wilson, W.H. (1993) Acute changes in cerebral blood flow after smoking marijuana. *Life Sci,* **52**:757–67.

Mathew, R.J., Wilson, W.H., Chiu, N.Y., Turkington, T.G., Degrado, T.R. and Coleman, R.E. (1999) Regional cerebral blood flow and depersonalization after tetrahydrocannabinol administration. *Acta Psychiatr Scand,* **100**:67–75.

Mathew, R.J., Wilson, W.H., Coleman, R.E., Turkington, T.G. and Degrado, T.R. (1997) Marijuana intoxication and brain activation in marijuana smokers. *Life Sci,* **60**:2075–89.

Mathew, R.J., Wilson, W.H., Humphreys, D., Lowe, J.V. and Weithe, K.E. (1993) Depersonalization after marijuana smoking. *Biol Psychiatry,* **33**:431–41.

Mathew, R.J., Wilson, W.H., Humphreys, D.F., Lowe, J.V. and Wiethe, K.E. (1992) Regional cerebral blood flow after marijuana smoking. *J Cereb Blood Flow Metab,* **12**:750–8.

Mathew, R.J., Wilson, W.H. and Tant, S.R. (1989) Acute changes in cerebral blood flow associated with marijuana smoking. *Acta Psychiatr Scand,* **79**:118–28.

Mathew, R.J., Wilson, W.H., Turkington, T.G. and Coleman, R.E. (1998) Cerebellar activity and disturbed time sense after THC. *Brain Res,* **797**:183–9.

Mathew, R.J., Wilson, W.H., Turkington, T.G., *et al.* (2002) Time course of tetrahydrocannabinol-induced changes in regional cerebral blood flow measured with positron emission tomography. *Psychiatry Res,* **116**:173–85.

Melges, F.T., Tinklenberg, J.R., Deardorff, C.M., Davies, N.H., Anderson, R.E. and Owen, C.A. (1974) Temporal disorganization and delusional-like ideation. Processes induced by hashish and alcohol. *Arch Gen Psychiatry,* **30**:855–61.

Moore, T.H., Zammit, S., Lingford-Hughes, A., *et al.* (2007) Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet,* **370**:319–28.

Morrison, P.D., Zois, V., McKeown, D.A., *et al.* (2009) The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychol Med,* **39**:1607–16.

Murray, R.M., Morrison, P.D., Henquet, C. and Di Forti, M. (2007) Cannabis, the mind and society: the hash realities. *Nat Rev Neurosci,* **8**:885–95.

O'Leary, D.S., Block, R.I., Koeppel, J.A., *et al.* (2002) Effects of smoking marijuana on brain perfusion and cognition. *Neuropsychopharmacology*, **26**:802–16.

O'Leary, D.S., Block, R.I., Koeppel, J.A., *et al.* (2007) Effects of smoking marijuana on focal attention and brain blood flow. *Hum Psychopharmacol*, **22**:135–48.

Pertwee, R.G. (2008) The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br J Pharmacol,* **153**:199–215.

Puighermanal, E., Marsicano, G., Busquets-Garcia, A., Lutz, B., Maldonado, R. and Ozaita, A. (2009) Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nat Neurosci,* **12**:1152–8.

Ramaekers, J.G., Kauert, G., Theunissen, E.L., Toennes, S.W. and Moeller, M.R. (2009) Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *J Psychopharmacol,* **23**:266–77.

Ramaekers, J.G., Kauert, G., van Ruitenbeek, P., Theunissen, E.L., Schneider, E. and Moeller, M.R. (2006) High-potency marijuana impairs executive function and

inhibitory motor control. *Neuropsychopharmacology,* **31**:2296–303.

Ranganathan, M. and D'Souza, D.C. (2006) The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl),* **188**:425–44.

Reichenberg, A. and Harvey, P.D. (2007) Neuropsychological impairments in schizophrenia: integration of performance-based and brain imaging findings. *Psychol Bull,* **133**:833–58.

Rey, J.M. and Tennant, C.C. (2002) Cannabis and mental health. *BMJ,* **325**:1183–4.

Robbe, D., Montgomery, S.M., Thome, A., Rueda-Orozco, P.E., McNaughton, B.L. and Buzsaki, G. (2006) Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nat Neurosci,* **9**:1526–33.

Rogers, R.D. and Robbins, T.W. (2001) Investigating the neurocognitive deficits associated with chronic drug misuse. *Curr Opin Neurobiol,* **11**:250–7.

Ross, C.A., Margolis, R.L., Reading, S.A., Pletnikov, M. and Coyle, J.T. (2006) Neurobiology of schizophrenia. *Neuron,* **52**:139–53.

Rubia, K., Russell, T., Bullmore, E.T., *et al.* (2001) An fMRI study of reduced left prefrontal activation in schizophrenia during normal inhibitory function. *Schizophr Res,* **52**:47–55.

Seamans, J.K. and Yang, C.R. (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol,* **74**:1–58.

Sharp, D.J., Bonnelle, V., De Boissezon, X., *et al.* (2010) Distinct frontal systems for response inhibition, attentional capture, and error processing. *Proc Natl Acad Sci USA*, **107**:6106–11.

Simons, J.S., Gilbert, S.J., Owen, A.M., Fletcher, P.C. and Burgess, P.W. (2005) Distinct roles for lateral and medial anterior prefrontal cortex in contextual recollection. *J Neurophysiol,* **94**:813–20.

Solowij, N. and Michie, P.T. (2007) Cannabis and cognitive dysfunction: parallels with endophenotypes of schizophrenia? *J Psychiatry Neurosci,* **32**:30–52.

Solowij, N., Stephens, R.S., Roffman, R.A., *et al.* (2002) Cognitive functioning of long-term heavy cannabis users seeking treatment. *JAMA,* **287**:1123–31.

Stokes, P.R., Mehta, M.A., Curran, H.V., Breen, G. and Grasby, P.M. (2009) Can recreational doses of THC produce significant dopamine release in the human striatum? *Neuroimage,* **48**:186–90.

Tanda, G., Pontieri, F.E. and Di Chiara, G. (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science,* **276**:2048–50.

van Os, J. and Kapur, S. (2009) Schizophrenia. *Lancet,* **374**:635–45.

Volkow, N.D., Gillespie, H., Mullani, N., *et al.* (1996) Brain glucose metabolism in chronic marijuana users at baseline and during marijuana intoxication. *Psychiatry Res,* **67**:29–38.

Voruganti, L.N., Slomka, P., Zabel, P., Mattar, A. and Awad, A.G. (2001) Cannabis induced dopamine release: an in-vivo SPECT study. *Psychiatry Res,* **107**:173–7.

Wagner, A.D., Schacter, D.L., Rotte, M., *et al.* (1998) Building memories: remembering and forgetting of verbal experiences as predicted by brain activity. *Science,* **281**:1188–91.

Wise, L.E., Thorpe, A.J. and Lichtman, A.H. (2009) Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol. *Neuropsychopharmacology,* **34**:2072–80.

Zeineh, M.M., Engel, S.A., Thompson, P.M. and Bookheimer, S.Y. (2003) Dynamics of the hippocampus during encoding and retrieval of face-name pairs. *Science,* **299**:577–80.

Chapter

15

Does cannabis use cause schizophrenia? The epidemiological evidence

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In this chapter we examine the strength of the epidemiological evidence to support a causal relationship between cannabis use and increased risk of schizophrenia and other psychotic disorders. We discuss the implications of this for individuals who use cannabis and for public health at a population level. The reader is also referred to Chapters 5 and 12 of this volume.

There is little dispute that cannabis use can cause short-lived and mild psychotic experiences (hallucinations, delusions and periods of thought disorder) directly following use. Such adverse effects are commonly reported in surveys of users and have support from experimental studies of the effects of Δ^9-tetrahydrocannabinol (THC) in humans, too (see Chapter 12). It is likely that the transient nature of such symptoms cause minimal functional impairment in most individuals. Of much greater concern, however, is whether cannabis use increases the risk of more severe and prolonged psychotic states that persist beyond the period reasonably attributable to direct biological effects of exogenous cannabinoids. Whether such a relationship exists is often disputed in the literature, but it is important to establish the truth given that these disorders lead to substantial distress to individuals and their families and to public burden from healthcare costs.

Association between cannabis use and psychosis

For multifactorial complex diseases, risk factors are rarely, if ever, necessary or sufficient to cause disease (Rothman and Greenland, 2005). Thus, even if use of cannabis does play a causal role in some cases of schizophrenia, it is clear that this disorder can occur without preceding use of cannabis, and use of cannabis can occur without subsequent development of schizophrenia. It is not possible to determine whether use of cannabis contributed to the development of schizophrenia in any one particular individual, as we cannot know whether the outcome for that individual would have been different had they not used this drug. At a population level, however, we can examine whether risk of developing schizophrenia is different among the subset of the population who use cannabis compared with the subset of those who have not used this drug. As we discuss later, there are a number of reasons why such an association might exist apart from that of causality, including chance, bias, confounding and reverse causation. Nevertheless, providing evidence of association is the first and necessary step toward determining whether a causal relationship between cannabis use and schizophrenia exists.

Case–control and cross-sectional studies

The proportion of individuals with schizophrenia reporting use of cannabis shows a wide variation across studies, probably reflecting different baseline frequency of cannabis use in different countries, secular trends and differences in methods and samples used. Nevertheless in most such studies the frequency of cannabis use in individuals with chronic psychotic disorders such as schizophrenia appears to be elevated when compared with individuals without such disorders. For example, a study in Scotland compared rates of substance misuse in patients with schizophrenia with rates in general population controls drawn from rural, suburban, and urban settings (McCreadie, 2002); 7% of patients reported problematic drug use (4% related specifically to cannabis use) compared with 2% of controls. In a case–control study in The Netherlands where cannabis use is legally and socially more acceptable, it was still more common in first-episode cases of schizophrenia (59%) compared with general hospital controls (21%) (Veling *et al.*, 2008). In South London, where more

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than half of both patients with first-episode psychosis and general population controls had smoked cannabis, patients were six times more likely than controls to smoke cannabis everyday (Di Forti *et al*., 2009).

One of the main limitations in case–control studies is the possibility of selection bias arising from biased selection of controls. This is less likely to occur in cross-sectional study designs. Cross-sectional data from a number of studies provide information as to whether rates of schizophrenia are higher among people who have used cannabis compared with those who have never used this drug. The US National Epidemiological Catchment Area (ECA) study, the Australian National Survey of Mental Health and Well-Being (NSMHWB) and the Netherlands Mental Health Survey and Incidence Study (NEMESIS) all reported that psychotic disorders including schizophrenia were more common in individuals who used cannabis or were dependent on this drug compared with non-users (Regier *et al*., 1990; Hall and Degenhardt, 2000; van Os *et al*., 2002), whereas in the Australian Mater-University Study of Pregnancy (Mater-University) birth cohort, duration of cannabis use was associated with non-affective psychoses (McGrath *et al*., 2010). In the UK National Psychiatric Morbidity Survey (NPMS) there was no difference in frequency of cannabis use between individuals with schizophrenia and those without (Farrell *et al*., 1998). However, the NPMS consisted of a general population household survey and a separate survey of individuals with schizophrenia who were long-term residents in psychiatric institutions. Long-term institutional residents are likely to include a rather select sub-group of individuals with schizophrenia, and the proportion of those with schizophrenia reporting cannabis use is almost certainly an under-estimate of the true frequency of cannabis use in all individuals with this disorder.

There is also evidence from cross-sectional surveys that prevalence of experiencing psychotic symptoms, rather than psychotic disorders, is also greater in individuals who have used cannabis compared with those who have not. These include studies from Finland (Miettunen *et al*., 2008), Greece (Stefanis *et al*., 2004), the Netherlands (Ferdinand *et al*., 2005), France (Verdoux *et al*., 2003), New Zealand (Thomas, 1996) and Australia (Hides *et al*., 2009), as well as additional data from the Mater-University cohort (McGrath *et al*., 2010), the NPMS (Johns *et al*., 2004) and NSMHWB surveys (Degenhardt and Hall, 2001; Scott *et al*., 2009).

In a number of these surveys no attempt was made to determine whether symptoms reported were due to cannabis intoxication. In the Mater-University study, the association between cannabis use and psychotic symptoms persisted after omitting individuals who had used cannabis in the month before assessing psychosis (McGrath *et al*., 2010). This suggests that the relationship between cannabis and psychosis is not simply due to intoxication, although it is not clear whether the psychotic symptoms occurred in the cannabis-free month before assessment. Problems in determining a causal relationship between cannabis and psychotic symptoms persisting beyond intoxication effects from case–control and cross-sectional designs also include those of reverse causation and recall bias. The former refers to the possibility that the association observed between cannabis use and schizophrenia is the result of individuals initiating or increasing cannabis use after, rather than before, the onset of their psychotic illness. This is a concern as it is possible that individuals use cannabis to reduce the anxiety associated with their psychotic experiences, and there is some evidence to support the existence of such a relationship (Ferdinand *et al*., 2005; McGrath *et al*., 2010).

Some case–control studies have attempted to overcome this problem by enquiring about the timing of first use of cannabis as well as onset of psychosis. For example, in a study of 232 first-episode cases of schizophrenia, pre-morbid use of cannabis and age of initiation of cannabis use, as well as age at onset of schizophrenia, were assessed retrospectively (Hambrecht and Hafner, 2000). Compared with age- and sex-matched controls, subjects with schizophrenia were twice as likely to report using cannabis. The majority of subjects with schizophrenia reported that they had started using cannabis before any positive symptoms of psychosis. However, the temporal relationship with prodromal symptoms was much less clear, with equal numbers of subjects reporting that their first use of cannabis preceeded, followed or occurred at approximately the same time as their illness began.

There are substantial problems in establishing the validity of time of first exposure to cannabis, in relation to time of illness onset, using retrospective data. Therefore, although case–control and cross-sectional studies show a strong association between cannabis use and psychotic disorders, in the absence of randomized controlled trials (RCTs), the most robust evidence concerning a possible causal role for cannabis use is likely

to come from longitudinal, epidemiological cohorts, where effects of recall and selection biases and reverse causation are minimized.

Cohort studies

There are limited data examining the relationship between cannabis use and psychotic outcomes in longitudinal studies. A systematic review published in 2007 identified only seven cohort studies with such data (Moore *et al*., 2007). Of these, only three studies examined psychotic disorders as outcomes; one of schizophrenia, one of schizophreniform disorder and one of psychotic symptoms adjudged to fulfil criteria for a needs-based diagnosis of psychotic disorder (summarized in Table 15.1). These three studies are described below.

The Swedish conscript cohort

The first longitudinal study to examine whether cannabis use is associated with subsequent risk of psychosis came from a cohort study of 50 087 Swedish men conscripted into the military in 1969 (Andreasson *et al*., 1987). At conscription (age 18 years) all men were interviewed by a psychologist and a psychiatrist to identify mental health problems at baseline, and were asked about cannabis use. Record-linkage with the National Hospital Discharge Register was used to identify all admissions to hospital with International Classification of Diseases (WHO, 1974) diagnoses of schizophrenia over a 15-year follow-up period. A dose–response relationship was observed between cannabis use at conscription and subsequent schizophrenia diagnosis.

A follow-up study of the same Swedish conscript cohort was conducted to address concerns raised about interpretation of results from this study, primarily that the association may have been confounded by other drug use or personality traits of the subjects, or that reverse causation may have occurred due to an unrecognized prodrome of schizophrenia at the time of conscription. Consistent with previous findings, self-reported "heavy cannabis users" (i.e. who had used cannabis more than 50 times) by the age of 18 years were 6.7 times more likely than non-users to be diagnosed with schizophrenia 27 years later (Zammit *et al*., 2002). The risk was attenuated by approximately 50%, but persisted after controlling for other drug use, other psychiatric diagnoses at conscription, disturbed behavior, IQ score and social traits, as well as other potential confounding factors (adjusted odds ration [OR] = 3.1, 95% confidence intervals [CI]: 1.7, 5.5).

The Dunedin birth cohort

The Dunedin Multidisciplinary Health and Development Study (Dunedin) has followed-up a general-population birth cohort of 1037 individuals born in Dunedin, New Zealand, in 1972–1973 (96% follow-up rate at age 26). Self-reports of cannabis use were obtained at ages 15 and 18 years, and information was also obtained on self-reported psychotic symptoms at age 11 years, before the onset of cannabis use. At age 26 years the cohort members were assessed again using a standardized psychiatric interview schedule that allowed the examination of psychotic symptoms and a DSM-IV (American Psychiatric Association, 1994) diagnosis of schizophreniform disorder as outcomes (Poulton *et al*., 2000).

Cannabis use by age 15 years was associated with an increased likelihood of meeting diagnostic criteria for schizophreniform disorder at age 26 years (Arseneault *et al*., 2002): 10.3% of the age 15-year cannabis users in this cohort were diagnosed with schizophreniform disorder at age 26 years, as opposed to 3% of the controls. After controlling for age 11-year psychotic symptoms, the risk for adult schizophreniform disorder remained elevated, though it was no longer statistically significant. Onset of cannabis use by age 18 years was not associated with an increased likelihood of developing schizophreniform disorder. However, there were only 25 subjects who met criteria for this disorder in the study and therefore statistical power for such sub-group analyses is likely to have been very limited. Subjects who had used cannabis either at age 15 or 18 years had higher rates of psychotic symptoms at age 26 years compared with non-users. These remained significant after controlling for other drug use and for psychotic symptoms predating the onset of cannabis use.

The Dutch NEMESIS sample

In the NEMESIS study, 4045 subjects without any symptoms of psychosis at baseline were administered follow-up assessments at 1 year and 3 years after baseline. For those subjects who reported psychotic symptoms, an additional clinical interview was conducted by an experienced psychiatrist or psychologist (at baseline and at 3-year follow-up), and an assessment made at follow-up for a need for clinical care. There was a dose–response relationship between cannabis use at

Table 15.1 Summary of longitudinal studies on cannabis use and psychotic disorders

Cohort label and author	Setting and sample size	Cannabis measure and age	Follow-up and attrition	Outcome measure (age)	Outcome, n (%)	Main results	Confounders adjusted for	Dose response effects
Dunedin	birth cohort, Dunedin, New Zealand ($n = 759$)	used cannabis > 3 times Ages 15 and 18 years	8–11 years attrition 4%	DIS (age 26 years)	(i) psychotic symptoms (≈ 25%)	*cannabis use by age 15 years†:* adjusted β = 6.6 (0.9), p < 0.001 *cannabis use age 15–18 years†:* adjusted β = 1.0 (0.4), p < 0.01	stratified analyses adjusted for sex, socio-economic status, other drug use, psychotic symptoms at age 11 years	not studied
					(ii) schizo-phreniform disorder 25 (3.3%)	*whole sample:** adjusted OR = 2.9 (1.2 to 7.0) *cannabis use by age 15 years†:* adjusted OR = 3.1 (0.7 to 13.3) *cannabis use age 15–18 years†:* adjusted OR = 1.4 (0.5 to 3.7)	whole sample analysis adjusted for confounders above and also IQ	

NEMESIS	adult population based cohort, Netherlands ($n = 4045$)	lifetime ever use and cumulative frequency from baseline to follow-up, summed as lowest, middle & highest levels	3 years attrition 30%	BPRS, also CIDI and SCID for "need for care"	(i) any psychotic symptoms (BPRS > 1) 38 (0.94%)	*ever use*: adjusted OR = 2.1 (0.8 to 5.7) cumulative frequency: adjusted OR = 1.7 (1.0 to 2.7)	age, sex, ethnicity, marital status, education, urbanicity, discrimination, and other drug use Subjects with any lifetime ever psychotic symptoms at baseline excluded	yes
				(age 21–67 years)	(ii) pathology level symptoms (BPRS > 4) 10 (0.25%)	*ever use*: adjusted OR = 16.9 (3.3 to 86.1) cumulative frequency: adjusted OR = 3.7 (2.0 to 7.0)		
		Age 18–64 years			(iii) "need for care" 7 (0.17%)	*ever use*: adjusted OR = 10.5 (1.8 to 63.2) cumulative frequency: adjusted OR = 3.5 (1.6 to 7.4)		

Table 15.1 (*cont.*)

Cohort label and author	Setting and sample size	Cannabis measure and age	Follow-up and attrition	Outcome measure (age)	Outcome, n (%)	Main results	Confounders adjusted for	Dose response effects
Swedish conscripts	Adult population based conscript cohort, Sweden (***n*** = 48 481)	Ever use Frequency: None, 1 time, 2–4, 5–10, 11–50, > 50 times Age 18–20 years	27 years No data on attrition available	ICD8/9 clinical diagnoses following inpatient admission (age 18–47 years)	schizophrenia / schizoaffective disorder 362 (0.7%)	ever use: adjusted HR = 1.5 (1.1 to 2.0) *frequency of use:* adjusted HR = 1.2 (1.1 to 1.3) *frequency stratified by age of first use*: first use age ≤ 15 adjusted HR = 1.2 (0.9 to 1.4) first use age > 15 adjusted HR = 1.2 (1.1 to 1.4)	other drug use, IQ, social personality traits, other diagnoses at conscription (excluded if psychotic at baseline), place of birth, childhood behavior, family history, alcohol use, family income, paternal occupation, tobacco use, paternal age	yes

* Additional data provided by study authors; †Results adjusting for other drug use not presented as uncertain validity (large increase in confidence intervals for schizophreniform disorder, indicating possible collinearity or problems related to small numbers). β, linear regression coefficient; BPRS, brief psychiatric rating scale; CIDI, composite international diagnostic interview; DIS, diagnostic interview schedule; HR, hazard ratio, 95% confidence intervals in parentheses; ICD, international classification of diseases; OR, odds ratio, 95% confidence intervals in parentheses.

baseline and development of a needs-based diagnosis of psychotic disorder at follow-up, both before (OR for trend across four categories of cannabis use = 4.0, 95% CI: 2.2, 7.1) and after (adjusted OR = 3.5, 95% CI: 1.6, 7.4) adjustment for other drug use, ethnic group, marital status, educational level, urbanicity and discrimination (van Os *et al*., 2002). There were, however, only seven people meeting criteria for this outcome. A similar dose–response relationship was also observed between cannabis use at baseline and risk of psychotic symptoms at follow-up, which again persisted after adjustment for confounders.

Other studies

The other four studies identified in the systematic review by Moore *et al*. (2007), as well as one other published since then, examined only the presence of psychotic experiences regardless of functional impairment or likelihood of clinical disorder. In the Christchurch Health and Development Study (CHDS) frequency of cannabis use was associated with development of psychotic experiences in a dose–response manner (Fergusson *et al*., 2005). This persisted after adjustment for a comprehensive range of potential confounding factors. In the Early Developmental stages of Psychopathology (EDSP) study, cannabis use was associated with presence of psychotic symptoms 4 years later, even after adjustment for age, sex, socio-economic status, urbanicity, childhood trauma, other substance use and psychotic symptoms at baseline (Henquet *et al*., 2005). In the ECA study, people who used cannabis on a daily basis were approximately twice as likely to report 1-year incident psychotic experiences as non-daily cannabis users after controlling for socio-demographic factors, social role and psychiatric conditions and other substance use (Tien and Anthony, 1990). In the NPMS there was no association observed between non-dependent cannabis use and 1-year incidence of psychotic symptoms (Wiles *et al*., 2006). An association between cannabis dependence and psychotic symptoms was present (OR = 3.4, 95% CI: 1.5, 7.7), but this association did not persist after adjusting for a wide range of potential confounders (adjusted OR = 1.5, 95% CI: 0.6, 3.9).

Finally, in the Zurich study, repeat measures of psychotic experiences over a 20-year period were used to identify clusters of symptoms over time (Rossler *et al*., 2007). Cannabis use at baseline was associated with a continuously high loading on a cluster labelled "schizophrenia nuclear symptoms" (although non-psychotic experiences also contributed to this measure).

A meta-analysis of the seven studies included in the systematic review was conducted based on the assumption that measures of psychosis could be considered to be on a continuum of symptoms from mild (self-report of psychotic symptoms) to severe (clinical diagnosis of schizophrenia) (Moore *et al*., 2007). On average, there was a 40% increase in risk (95% CI: 20%, 65%) of any psychotic outcome in those who had ever used cannabis compared with individuals who had never used this drug after adjustment (Figure 15.1). This effect was stronger for more regular or greater cumulative use of cannabis (adjusted OR = 2.1, 95% CI: 1.5, 2.8), and was also stronger when restricted to the three studies that reported results for psychotic disorders, where results are likely to be of greater clinical relevance (adjusted OR = 2.6, 95% CI: 1.1, 6.1).

The empirical evidence from these longitudinal studies is therefore very consistent. However, although such consistency is in keeping with a causal effect of cannabis on psychotic outcomes, a number of other, non-causal explanations need to be considered when interpreting these findings.

Over-estimation of a true causal relationship

There are three main reasons why these longitudinal studies may have over-estimated the true causal relationship between cannabis use and development of psychosis.

Confounding

The main non-causal explanation that is most likely to have led to studies over-estimating any true causal effects of cannabis on risk of psychosis is confounding. Individuals who use cannabis tend to differ from those who have never used this drug on a number of characteristics, some of which may have causal effects on psychosis. Examples of such potential confounders include use of psycho-stimulant drugs such as amphetamines, social personality traits or adverse childhood conditions. All seven longitudinal studies described above made some attempt to adjust for confounding to some degree, and original estimates of association were attenuated by approximately 50% on average. Nevertheless, associations persisted in six of these studies after adjustment, suggesting that not all of the association reported in each study was due to confounding.

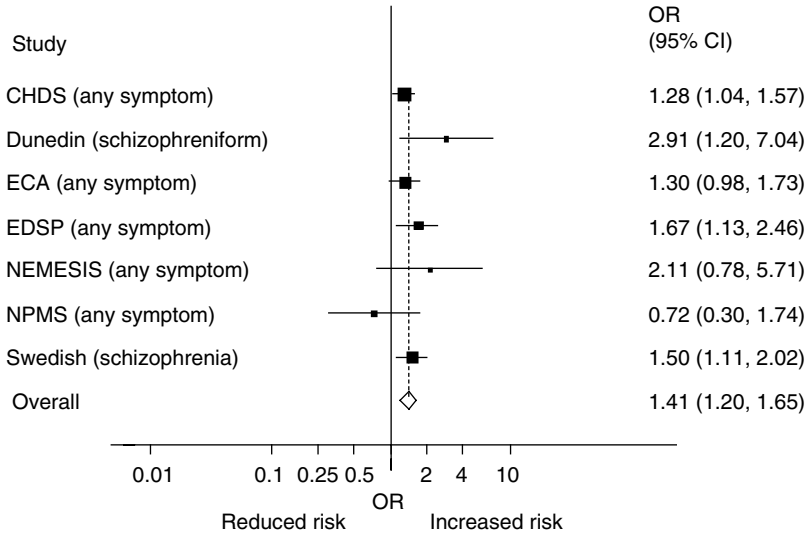

Figure 15.1. Forest plot showing adjusted odds ratio (OR) and 95% confidence intervals (CI) for any psychosis outcome according to ever use of cannabis in individual studies†. Taken from Moore *et al.* (2007). CHDS, Christchurch Health and Development Study; ECA, Epidemiological Catchment Area; EDSP, Early Developmental stages of Psychopathology; NEMESIS, Netherlands Mental Health Survey and Incidence Study; NPMS, National Psychiatric Morbidity Survey.

Additional data from the *CHDS* and *Dunedin* studies were kindly provided by the authors. Exposure studied in all studies was 'ever use' of cannabis, except for the *NPMS* study where the measure was 'ever use over the past 1-year only'

The degree of confounding apparent in these studies raises concerns that persisting associations might have been explained fully if more comprehensive measures of such confounders had been available. In a novel approach to account for residual confounding the authors from the Mater-University study examined sibling-pair data and found an association between the difference in cannabis-use duration within siblings of each sibling pair and the difference in psychotic symptom scores within siblings of each pair (McGrath *et al.*, 2010). This method minimizes effects of genetic and environmental characteristics shared between siblings that could confound the relationship between cannabis and psychosis, though it does not deal with non-shared confounding.

Selection bias

Although most studies made some attempt to examine psychotic phenomena that were not directly attributable to very recent use of cannabis, this is likely to have been difficult if not impossible to determine for subjects using cannabis on a frequent (especially daily) basis. It is possible therefore that the associations reported are, in part, owing to psychotic symptoms occurring solely during intoxication rather than effects persisting beyond those attributable to direct biological effects of exogenous cannabinoids. Intoxication effects are unlikely to have biased results substantially in the Swedish conscript cohort given the ICD criteria for diagnosing schizophrenia (Zammit *et al.*, 2002), and similarly for schizophreniform disorder in the Dunedin cohort where symptoms were also required to be present for more than one month (Arseneault *et al.*, 2002).

Reverse causation

As most of the studies excluded subjects with psychosis at baseline, or adjusted for psychotic symptoms at baseline, the observed associations are unlikely to reflect reverse causation. Although minimising reverse causation is an advantage of longitudinal studies, nevertheless the presence of undetected symptoms, or of an illness prodrome at baseline, was possible in all studies, though it seems very unlikely that this could account for the associations observed. To minimize the possibility of reverse causation in the Swedish conscript study, analyses were repeated on a subsample of individuals who only developed schizophrenia at least 5 years after conscription, as individuals with a prodrome at conscription were likely to have been diagnosed with schizophrenia within this 5-year period. The findings obtained in this sensitivity analysis were similar to the ones with the entire cohort (Zammit *et al.*, 2002).

Under-estimation of a true causal relationship

It is also possible that studies examining the longitudinal relationship between cannabis use and psychosis under-estimated the true causal relationship. There are two main reasons whereby this may have occurred.

Misclassification bias

Measurement of cannabis use is particularly difficult as there is almost certainly large variation in biologically available cannabinoid levels resulting from different sources of cannabis and from different intake practices, while self-reported frequency of use is also prone to error. Such misclassification, if random across the cohort (non-differential), almost always leads to underestimates of association. As none of the individuals in these studies had psychotic disorders at the time of cannabis-use assessment, it seems likely that misclassification would have been similar for those who subsequently went on to develop a psychotic illness compared with those who did not. If misclassification was differential though, it is possible that associations reported might be over-estimated. This would have occurred, for example, if individuals who went on to subsequently develop a psychotic illness were more candid or exaggerated their cannabis use to a greater degree compared with those who did not go on to develop such an illness. However, such a scenario seems rather unlikely and a more realistic expectation is that studies will have underestimated the true association between cannabis use and psychosis.

Attrition

Attrition in cohort studies is usually more likely in subjects who use drugs and also those who develop mental health problems (Fischer *et al.*, 2001; Allott *et al.*, 2006). Such a pattern of attrition would also lead to under-estimates of association. It is not clear to what extent such bias would affect any of the results, although modeling for attrition in two of these studies (CHDS [Fergusson *et al.*, 2005], NEMESIS [van Os *et al.*, 2002]) suggest it may have had little impact on the overall findings. Furthermore, attrition in the Dunedin cohort was very low (5%) and therefore unlikely to have substantially biased results.

Unfortunately we are not able to determine whether factors that might have led to over-estimating associations in these studies occurred to a greater or lesser degree than those leading to under-estimating associations. However, although attempts have been made to minimize confounding, selection bias and reverse causation, possible effects of misclassification in the data has not been examined, although this is likely to be important.

Identifying high-risk groups

There has been substantial interest in the media and in the scientific community regarding differential effects of cannabis use on risk of psychosis according to the presence or absence of other factors. Although identification of individuals likely to be at particularly high risk of developing a psychotic illness following use of cannabis seems sensible, the study of such environment-environment or gene-environment interactions is complex, and the extent to which studies of interaction will be useful for informing disease aetiology or prevention (Caspi and Moffitt, 2006) has been the subject of some debate (Zammit *et al.*, 2010). Here we summarize some of the literature in this field. The reader is also referred to Chapter 12 of this book.

Adolescent onset of cannabis use:

In the Dunedin study, a stronger effect of cannabis use on psychotic symptoms was reported for subjects who first used cannabis at age 15 years or before, as opposed to after this age (Arseneault *et al.*, 2002). First use of cannabis by age 15 years was associated with a 3.1-fold increase (95% CI: 0.7, 13.3) in risk of schizophreniform disorder, while first use by age 18 years was associated with a 1.4-fold increase in risk (95% CI: 0.5, 3.7). These results could reflect a sensitive period of risk, or could simply reflect a greater cumulative exposure to cannabis in those initiating use at a younger age. A cross-sectional analysis of a Greek birth cohort also reported a stronger association with psychotic symptoms for subjects with earlier first use of cannabis (Stefanis *et al.*, 2004). This was independent of a measure of frequency of cannabis use, but there was limited ability to account for cumulative exposure over time.

In the Swedish conscript study, where cumulative use of cannabis was accounted for more fully (although probably still inadequately), there was no evidence that the effect of cannabis on risk of schizophrenia was different in subjects who first used cannabis before, as opposed to after age 16 years (Zammit, 2004). However, age of first use was collected retrospectively in this study, and the youth of the cohort when conscripted limited the range at age of first use.

Studies in animal models lend some support to the presence of sensitive periods to cerebral insults during adolescent development, including effects of exposure to cannabis (Schneider and Koch, 2003; O'Shea *et al*., 2006). Such studies are discussed more fully in Casadio *et al.* (2011 and in Chapter 7 of this book).

Catechol-O-methyltransferase genotype

As described in Chapter 12, a paper from the Dunedin study reported a much stronger effect of cannabis on risk of schizophreniform disorder in subjects homozygous for the valine allele at Val^{158}Met within the catechol-O-methyltransferase (*COMT*) gene, than for subjects who were methionine homozygotes (Caspi *et al*., 2005). This putative interaction was observed only in subjects who first used cannabis before age 18 years, with no evidence of interaction in those who first used after this age. There have been no replications of this finding to date, although this relationship has not yet been examined in similar longitudinal samples. A finding of a greater psychotomimetic effect of cannabis use in valine homozygotes was reported in an experimental setting, but was again only observed in a subgroup of subjects, this time with evidence of pre-existing psychotic symptoms (Henquet *et al*., 2006). A case-only study of the relationship between *COMT* and cannabis use in schizophrenia failed to observe the expected association as reported in the Dunedin study (Zammit *et al*., 2007), although data on cannabis use was assessed retrospectively and therefore the timing of cannabis use in relation to onset of schizophrenia is of uncertain reliability.

At present, therefore, the evidence that the effect of cannabis varies substantially according to *COMT* genotype, although intriguing, is not sufficiently supported by current literature and awaits replication or refutation in independent samples.

Childhood trauma

Three studies have examined whether there is a synergistic effect of cannabis use and childhood trauma in increasing the risk of psychotic outcomes (Cougnard *et al*., 2007; Houston *et al*., 2008; Harley *et al*., 2009). All three reported that the presence of both childhood trauma and cannabis use significantly increased the absolute risk for psychotic outcomes beyond the risk posed by either risk factor alone. Some researchers argue that findings such as these can help us to elucidate the biological mechanisms underlying the aetiology of psychosis, and that they can also identify "at-risk" groups who could be targeted for selective interventions (Harley *et al*., 2009).

However, others argue that such interactive effects are what we would expect for joint exposure to any combination of risk factors in multi-factorial complex diseases (Greenland *et al*., 2008), and there is some empirical evidence to support this view (Zammit *et al*., 2010). If so, the implications of finding such interactions, both for understanding aetiology and for informing prevention, may be rather limited (Zammit *et al*., 2010).

Evidence in support of a causal relationship

A number of aspects of the findings reviewed in this chapter lend support to the view that cannabis use has a causal relationship in increasing risk of psychotic outcomes. First, results across studies have been reasonably consistent, with almost all the associations reported persisting (though substantially attenuated) after adjustment for confounding. Second, the studies that have examined a dose–response effect all find evidence to support such a relationship, whereby greater use of cannabis is associated with greater risk of psychosis in a dose-dependent manner. Third, experimental studies that demonstrate that administration of THC can result in acute but transient psychotic symptoms (D'Souza *et al*., 2004) indicates that this major psychoactive constituent of cannabis can produce biological effects that translate into the occurrence of psychotic experiences. The plausibility of effects of cannabis on longer-term psychotic outcomes, independent of intoxication effects, are supported by this, whether through long-term effects on exogenous cannabinoids exposure, or through a "kindling" effect of having previously experienced a psychotic state during intoxication. Fourth, the neurobiological sequelae of cannabis use, including modulation of dopaminergic, gamma-aminobutyric acid (GABA) and glutamatergic activity, are consistent with abnormalities described in subjects with psychotic disorders (Kuepper *et al*., 2010).

Finally, there is also indirect evidence from epidemiological studies that lends support to a causal relationship between cannabis and psychosis. For example, there is some evidence of specificity of exposure, in that the evidence of association between other drug use and

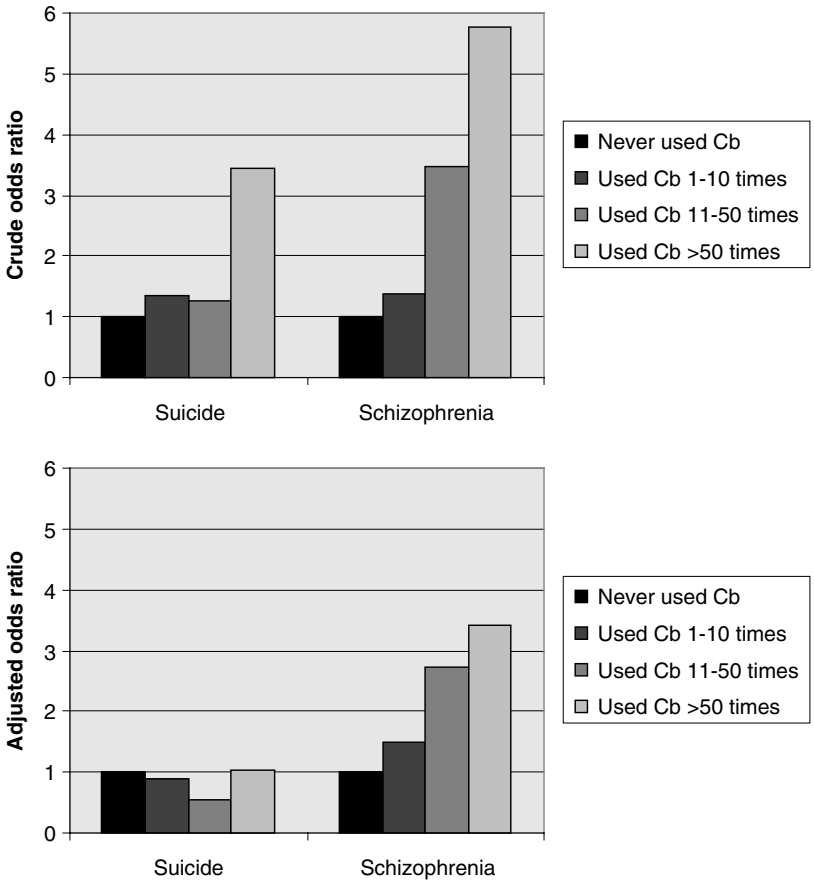

Figure 15.2. Crude and adjusted odds ratios for suicide and for schizophrenia in relation to frequency of cannabis use. Taken from Price *et al.* (2009). Cb, cannabis.

psychosis is substantially weaker than that for cannabis (Arseneault *et al.*, 2002; van Os *et al.*, 2002; Zammit *et al.*, 2002), although frequency of use of other drugs, for example amphetamines, is lower than that for cannabis, and power in these studies is therefore likely to be substantially less. There is also some evidence of specificity of outcome; in the Dunedin study the association between cannabis use and depression was much weaker compared with the association between cannabis use and psychosis (Arseneault *et al.*, 2002). This specificity of effect is perhaps best demonstrated by findings from the Swedish conscript cohort where an association between cannabis use and suicide showed a relationship that was similar to that between cannabis and schizophrenia in the crude analysis (Price *et al.*, 2009). However, after adjustment for the same set of confounders, the association with suicide was completely eliminated, whereas that for schizophrenia, though attenuated, remained strongly associated (Figure 15.2).

Evidence inconsistent with a causal relationship

One argument made against a causal relationship between cannabis and psychosis is that although the frequency of cannabis use has increased greatly over the past few decades, evidence of changes in the incidence of schizophrenia has been very inconsistent. Although a few studies have reported an increase in psychotic outcomes over recent decades (Boydell *et al.*, 2003; Ajdacic-Gross *et al.*, 2007), other studies have shown no change or even a decrease in incidence over time (Bresnahan *et al.*, 2003; Frisher *et al.*, 2009). There are a number of difficulties in interpreting results from such ecological studies however. First, studies often use first-admission data as a marker of incidence, though practice of admission to hospital, as well as criteria for diagnoses, have changed over time. Furthermore, projections based on assumptions of causal effects of cannabis on psychosis suggest that time lags, and

lack of reliable incidence data might mean observable changes in schizophrenia incidence are yet to become fully apparent (Hickman *et al.*, 2007). Finally, it is not possible to know whether the frequency of other risk factors for schizophrenia have reduced over this same time period, and that might therefore nullify the effects of increasing cannabis use over time. Findings from ecological studies therefore provide relatively weak arguments against a causal relationship between cannabis and schizophrenia in light of the other sources of evidence available.

Addressing the uncertainty

Uncertainty regarding causality is inevitable in observational studies. Randomized controlled trials that examine the effects of cannabis use on incidence of psychosis are not feasible, whereas trials of interventions to reduce cannabis use are unlikely to be sufficiently informative regarding changes in incidence of rare outcomes such as schizophrenia. Indeed, as incidence of psychotic disorders is so uncommon, a more fruitful approach for observational studies may be to study the longitudinal effects of cannabis on broader psychosis outcomes or endophenotypes of psychosis. To be of any value though, endophenotypes need to be strongly causally related to psychotic disorders, and although specific neurocognitive or neurophysiological deficits have been proposed as potential endophenotypes to study, it is unclear at present to what extent study of such endophenotypes has the potential to increase our understanding of schizophrenia aetiology (Walters and Owen, 2007).

Further epidemiological studies may help to resolve some of the issues discussed above regarding potential biases and identification of high-risk groups. They may also help by examining the effects of potency of cannabis on risk as well as by examining the effects of differing relative concentrations of THC and cannabidiol, a cannabinoid that appears to have some antagonistic properties to THC. Although very few such studies exist at present, preliminary evidence suggests that risk of psychosis is increased in those using higher potency forms of cannabis (Di Forti *et al.*, 2009) where the relative concentration of THC to cannabidiol is high (Morgan and Curran, 2008 and also see Chapter 12).

However, even if future studies are methodologically more rigorous, residual confounding will remain a limitation in any causal interpretations of association. Studies aimed at understanding the biological consequences of cannabinoids on neuronal structure and function are more likely to facilitate our understanding of the role of cannabis use in psychotic disorders.

Implications

If regular use of cannabis doubles the risk of developing schizophrenia, as suggested by some studies, the individual lifetime risk in such individuals would increase to about 1.5%. This is unlikely to deter many people from using cannabis, although the risk may be much greater for less severe psychotic outcomes, for people with a family history of psychosis or for those using high potency cannabis.

Issues regarding policy implications of the association between cannabis and psychosis are coherently discussed in a review (Hall and Degenhardt, 2006 and in Chapter 5 of this book) where the authors address the pertinent questions of how strong evidence needs to be before we should take action and what actions might minimize individual and societal harm from use of cannabis. From a clinical perspective it seems sensible for clinicians to educate patients about the potential adverse effects of cannabis, and particularly to target education at unaffected relatives of patients with psychoses if they are at an age where they may be using or potentially initiating use of this drug. Adequate provision of psychiatric services to assess cannabis use and to employ motivational interviewing or other strategies to encourage harm reduction are required.

From a public-health viewpoint the important question is how much the incidence of psychotic disorders would decrease if cannabis use decreased. Approximately 50% of adolescents and young adults in the UK report having used cannabis at least once (Singleton *et al.*, 2001). Even weak detrimental effects of cannabis on mental health outcomes could therefore potentially have a large impact at a population level. The population-attributable fraction calculated in the recent systematic review of cannabis and psychosis indicates that approximately 15% of cases of psychosis would not occur if cannabis did not exist (Moore *et al.*, 2007). These calculations rely heavily on the assumptions that the associations reported are accurate estimates of a truly causal effect of cannabis use on psychosis. Nevertheless they indicate that several hundred cases of schizophrenia might be prevented in the UK each year if cannabis use was eliminated (Nordentoft and Hjorthoj, 2007). Education

campaigns to inform individuals about the risks of cannabis are clearly warranted, but information on risk has all too often been mixed with debate about prohibition or legalization, and education has not been adequately targeted at the relevant at-risk groups (Hall and Degenhardt, 2006), for example young people at an age of initiating or experimenting with use of this drug. Honest communication of the risk of harm from using cannabis, and open discussion of the difficulties in interpreting results from epidemiological studies are essential to allow individuals to make informed choices about use of this drug. Specious debate as to whether evidence of adverse effects of cannabis supports the current legal status of this drug encourages polarized views about the safety of cannabis and distracts from the public health message that needs to be promoted.

The scale of the problem facing public health is reflected in a recent study that estimated the number of heavy cannabis users who would have to become abstinent from cannabis to prevent one new case of schizophrenia developing over a 1-year period in the UK (Hickman *et al*., 2009). This "number needed to prevent" (NNP) was estimated under the assumption that cannabis use is causally related to schizophrenia, with an increase in risk of this disorder in those who use cannabis as described in a recent systematic review. These estimations do not take into account the possible additional increase in risk related to use of higher-potency forms of cannabis over recent years (Di Forti *et al*., 2009). Nevertheless, for men, the NNP ranged from 2800 (90% CI: 2018, 4530) in those aged 20–24 years, to 4700 (90% CI: 3114, 8416) in those aged 35–39. The NNP for women were approximately double these. Note also that these assume that everyone who is a heavy user can become abstinent. In reality of course, interventions to reduce addiction are effective in probably less than 25% of individuals. If so, the "numbers needed to treat" (NNT) would have to be at least four times greater, highlighting the huge difficulties facing attempts to reduce the public health burden of psychosis in the population, even if modifiable risk factors such as cannabis are identified.

Conclusions

Despite the inevitable uncertainty inherent when relying upon observational studies rather than RCTs, we believe there is a strong body of evidence from epidemiological studies that use of cannabis increases risk of developing psychotic disorders, supported by findings in other research fields. Although attempts to reduce the public-health burden of psychosis in the population by reducing cannabis use are complex, studies showing association between cannabis use and psychosis have firmly captured scientific, public and media interest and, as a result, have alerted the public to the fact that cannabis use, and perhaps particularly early onset use, is associated with non-negligible (and possibly irreversible) adverse effects on mental health. This wider knowledge may have contributed to the fact that cannabis use has been declining in the UK and other major European Countries since 2004.

References

Ajdacic-Gross, V., Lauber, C., Warnke, I., Haker, H., Murray, R.M. and Rossler, W. (2007) Changing incidence of psychotic disorders among the young in Zurich. *Schizophr Res*, **95**:9–18.

Allott, K., Chanen, A. and Yuen, H.P. (2006) Attrition bias in longitudinal research involving adolescent psychiatric outpatients. *J Nerv Ment Dis*, **194**:958–61.

Andreasson, S., Allebeck, P., Engstrom, A. and Rydberg, U. (1987) Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet*, **2**:1483–6.

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A. and Moffitt, T.E. (2002) Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *BMJ*, **325**:1212–3.

Association, A.P. (1994) *Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Association Washington, DC.

Boydell, J., Van Os, J., Lambri, M., *et al*. (2003) Incidence of schizophrenia in south-east London between 1965 and 1997. *Br J Psychiatry*, **182**:45–9.

Bresnahan, J., Boydell, J., Murray, R. and Susser, E. (2003) Temporal variation in the incidence, course and outcome of schizophrenia. In *The Epidemiology of Schizophrenia* (ed. R. Murray, P.B. Jones, E. Susser, J. van Os and M. Cannon), pp. 34–48. Cambridge University Press: Cambridge.

Casadio, P., Fernandes, C., Di Forti, M. and Murray, R.M. (2011). Adolescent cannabis use: the risk for schizophrenia. *Neuroscience and Biobehavioral Reviews*, **35**:1779–87.

Caspi, A. and Moffitt, T.E. (2006) Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat Rev Neurosci,* **7**:583–90.

Caspi, A., Moffitt, T.E., Cannon, M., *et al*. (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism

in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry,* **57**:1117–27.

Cougnard, A., Marcelis, M., Myin-Germeys, I., *et al.* (2007) Does normal developmental expression of psychosis combine with environmental risk to cause persistence of psychosis? A psychosis proneness-persistence model. *Psychol Med,* **37**:513–27.

D'Souza, D.C., Perry, E., MacDougall, L., *et al.* (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology*, **29**:1558–72.

Degenhardt, L. and Hall, W. (2001) The association between psychosis and problematical drug use among Australian adults: findings from the National Survey of Mental Health and Well-Being. *Psychol Med,* **31**:659–68.

Di Forti, M., Morgan, C., Dazzan, P., *et al.* (2009) High-potency cannabis and the risk of psychosis. *Br J Psychiatry,* **195**:488–91.

Farrell, M., Howes, S., Taylor, C., *et al.* (1998) Substance misuse and psychiatric comorbidity: an overview of the OPCS National Psychiatric Morbidity Survey. *Addict Behav,* **23**:909–18.

Ferdinand, R.F., Sondeijker, F., van der Ende, J., *et al.* (2005) Cannabis use predicts future psychotic symptoms, and vice versa. *Addiction,* **100**:612–8.

Fergusson, D.M., Horwood, L.J. and Ridder, E.M. (2005) Tests of causal linkages between cannabis use and psychotic symptoms. *Addiction,* **100**:354–66.

Fischer, E.H., Dornelas, E.A. and Goethe, J.W. (2001) Characteristics of people lost to attrition in psychiatric follow-up studies. *J Nerv Ment Dis,* **189**:49–55.

Frisher, M., Crome, I., Martino, O. and Croft, P. (2009) Assessing the impact of cannabis use on trends in diagnosed schizophrenia in the United Kingdom from 1996 to 2005. *Schizophr Res,* **113**:123–8.

Greenland, S., Lash, T.L. and Rothman, K.J. (2008) Concepts of Interaction. In *Modern Epidemiology* (ed. K.J. Rothman, S. Greenland and T.L. Lash). Lippincott Williams and Wilkins: Philadelphia.

Hall, W. and Degenhardt, L. (2000) Cannabis use and psychosis: a review of clinical and epidemiological evidence. *Aust N Z J Psychiatry,* **34**:26–34.

Hall, W. and Degenhardt, L. (2006) What are the policy implications of the evidence on cannabis and psychosis? *Can J Psychiatry,* **51**:566–74.

Hambrecht, M. and Hafner, H. (2000) Cannabis, vulnerability, and the onset of schizophrenia: an epidemiological perspective. *Aust N Z J Psychiatry,* **34**:468–75.

Harley, M., Kelleher, I., Clarke, M., *et al.* (2009) Cannabis use and childhood trauma interact additively to increase the risk of psychotic symptoms in adolescence. *Psychol Med*, **40**:1627–34.

Henquet, C., Krabbendam, L., Spauwen, J., *et al.* (2005) Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *BMJ,* **330**:11.

Henquet, C., Rosa, A., Krabbendam, L., *et al.* (2006) An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology,* **31**:2748–57.

Hickman, M., Vickerman, P., Macleod, J., Kirkbride, J. and Jones, P.B. (2007) Cannabis and schizophrenia: model projections of the impact of the rise in cannabis use on historical and future trends in schizophrenia in England and Wales. *Addiction,* **102**:597–606.

Hickman, M., Vickerman, P., Macleod, J., *et al.* (2009) If cannabis caused schizophrenia – how many cannabis users may need to be prevented in order to prevent one case of schizophrenia? England and Wales calculations. *Addiction,* **104**:1856–61.

Hides, L., Lubman, D.I., Buckby, J., *et al.* (2009) The association between early cannabis use and psychotic-like experiences in a community adolescent sample. *Schizophr Res,* **112**:130–5.

Houston, J.E., Murphy, J., Adamson, G., Stringer, M. and Shevlin, M. (2008) Childhood sexual abuse, early cannabis use, and psychosis: testing an interaction model based on the National Comorbidity Survey. *Schizophr Bull,* **34**:580–5.

Johns, L.C., Cannon, M., Singleton, N., *et al.* (2004) Prevalence and correlates of self-reported psychotic symptoms in the British population. *Br J Psychiatry,* **185**:298–305.

Kuepper, R., Morrison, P., van Os, J., Murray, R., Kenis, G. and Henquet, C. (2010) Does dopamine mediate the psychosis-inducing effects of cannabis? A review and integration of findings. *Schizophrenia Research*, **121(1–3):107–17**.

McCreadie, R.G. (2002) Use of drugs, alcohol and tobacco by people with schizophrenia: case-control study. *Br J Psychiatry,* **181**:321–5.

McGrath, J., Welham, J., Scott, J., *et al.* (2010) Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults. *Arch Gen Psychiatry*, **67**:440–7.

Miettunen, J., Tormanen, S., Murray, G.K., *et al.* (2008) Association of cannabis use with prodromal symptoms of psychosis in adolescence. *Br J Psychiatry,* **192**:470–1.

Moore, T.H., Zammit, S., Lingford-Hughes, A., *et al.* (2007) Cannabis use and risk of psychotic or affective

mental health outcomes: a systematic review. *Lancet,* **370**:319–28.

Morgan, C.J. and Curran, H.V. (2008) Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *Br J Psychiatry,* **192**:306–7.

Nordentoft, M. and Hjorthoj, C. (2007) Cannabis use and risk of psychosis in later life. *Lancet,* **370**:293–4.

O'Shea, M., McGregor, I.S. and Mallet, P.E. (2006) Repeated cannabinoid exposure during perinatal, adolescent or early adult ages produces similar longlasting deficits in object recognition and reduced social interaction in rats. *J Psychopharmacol*, **20**:611–21.

Poulton, R., Caspi, A., Moffitt, T.E., Cannon, M., Murray, R. and Harrington, H. (2000) Children's self-reported psychotic symptoms and adult schizophreniform disorder: a 15-year longitudinal study. *Archives of General Psychiatry,* **57**:1053–8.

Price, C., Hemmingsson, T., Lewis, G., Zammit, S. and Allebeck, P. (2009) Cannabis and suicide: longitudinal study. *Br J Psychiatry,* **195**:492–7.

Regier, D.A., Farmer, M.E., Rae, D.S., *et al*. (1990) Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. *JAMA,* **264**:2511–8.

Rossler, W., Riecher-Rossler, A., Angst, J., *et al*. (2007) Psychotic experiences in the general population: a twenty-year prospective community study. *Schizophr Res,* **92**:1–14.

Rothman, K.J. and Greenland, S. (2005) Causation and causal inference in epidemiology. *Am J Public Health,* **95 Suppl** 1:S144–50.

Schneider, M. and Koch, M. (2003) Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology,* **28**:1760–9.

Scott, J., Martin, G., Bor, W., Sawyer, M., Clark, J. and McGrath, J. (2009) The prevalence and correlates of hallucinations in Australian adolescents: results from a national survey. *Schizophr Res,* **107**:179–85.

Singleton, N., Bumpstead, R., O'Brien, M., Lee, A. and Meltzer, H. (2001) *Psychiatric morbidity among adults living in private households, 2000*. Office for National Statistics, DOH: London.

Stefanis, N.C., Delespaul, P., Henquet, C., Bakoula, C., Stefanis, C.N. and Van Os, J. (2004) Early adolescent cannabis exposure and positive and negative dimensions of psychosis. *Addiction,* **99**:1333–41.

Thomas, H. (1996) A community survey of adverse effects of cannabis use. *Drug Alcohol Depend,* **42**:201–7.

Tien, A.Y. and Anthony, J.C. (1990) Epidemiological analysis of alcohol and drug use as risk factors for psychotic experiences. *J Nerv Ment Dis,* **178**:473–80.

van Os, J., Bak, M., Hanssen, M., Bijl, R.V., de Graaf, R. and Verdoux, H. (2002) Cannabis use and psychosis: a longitudinal population-based study. *Am J Epidemiol,* **156**:319–27.

Veling, W., Mackenbach, J.P., van Os, J. and Hoek, H.W. (2008) Cannabis use and genetic predisposition for schizophrenia: a case-control study. *Psychol Med,* **38**:1251–6.

Verdoux, H., Sorbara, F., Gindre, C., Swendsen, J.D. and van Os, J. (2003) Cannabis use and dimensions of psychosis in a nonclinical population of female subjects. *Schizophr Res*, **59**:77–84.

Walters, J.T. and Owen, M.J. (2007) Endophenotypes in psychiatric genetics. *Mol Psychiatry*, **12**:886–90.

WHO (1974) *Glossary of mental disorders and guide to their classification, for use in conjunction with the International Classification of Diseases*. World Health Organisation: Geneva.

Wiles, N.J., Zammit, S., Bebbington, P., Singleton, N., Meltzer, H. and Lewis, G. (2006) Self-reported psychotic symptoms in the general population: results from the longitudinal study of the British National Psychiatric Morbidity Survey. *Br J Psychiatry,* **188**:519–26.

Zammit, S. (2004) An investigation into the use of cannabis and tobacco as risk factors for schizophrenia. PhD. In *The Department of Psychological Medicine, School of Medicine*. Cardiff University: Cardiff.

Zammit, S., Allebeck, P., Andreasson, S., Lundberg, I. and Lewis, G. (2002) Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. *BMJ,* **325**:1199.

Zammit, S., Lewis, G., Dalman, C. and Allebeck, P. (2010) Examining interactions between risk factors for schizophrenia. *Br J Psychiatry*, **197**: 207–11.

Zammit, S., Spurlock, G., Williams, H., *et al.* (2007) Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. *Br J Psychiatry,* **191**:402–7.

Zammit, S., Wiles, N. and Lewis, G. (2010). The study of gene-environment interactions in psychiatry: limited gains at a substantial cost? *Psychological Medicine,* **40**:711–16.

Chapter

16

Postmortem studies of the brain cannabinoid system in schizophrenia

Suresh Sundram, Brian Dean and David Copolov

As outlined elsewhere in this book epidemiological, clinical and genetic approaches have linked cannabis to schizophrenia from aetiological, contributory and exacerbating perspectives. These studies implicate cannabis use as deleterious in schizophrenia either in increasing its incidence, precipitating illness onset or worsening its outcomes. This suggests that inhaled or ingested components of cannabis may interact directly with biological systems relevant to schizophrenia. Much work has focussed on the main psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (THC), which appears to induce psychotic symptoms in both healthy controls and those with schizophrenia (D'Souza *et al*., 2005). However, there are at least 60 other bioactive components that could mediate the effects of cannabis in humans (Mechoulam and Hanus, 2000; and see Chapters 2 and 3). Hence, one explanation for the association of cannabis with schizophrenia is that the human endocannabinoid (eCB) system (ECS) may be disrupted in vulnerable individuals, predisposing them to the risk of developing, precipitating or exacerbating schizophrenia when exposed to cannabis.

The human ECS is detailed elsewhere (see Chapter 3) and consists of a number of eCBs, their synthetic, degradative and transport pathways and the receptors to which they bind, principally the cannabinoid CB1 and CB2 receptors. The two major endocannabinoids are anandamide and 2-arachidonoyl glycerol (2-AG), with the latter predominating in the human central nervous system (CNS) (Piomelli, 2003). In addition to the CB1 and CB2 receptors, the endocannabinoids also activate other receptors in the CNS, including vanilloid (VR1), peroxisome proliferator-activated receptors, orphan G-protein coupled receptors (e.g. GPR55) and transmitter-gated ion channels (Pertwee, 2010). Although it is possible that any component of the ECS may be altered in individuals vulnerable to developing schizophrenia, methodological constraints have restricted investigation to a number of key elements.

One methodological approach is to use post-mortem human brain tissue from people with schizophrenia and matched controls that did not have schizophrenia. This allows direct examination of some elements of the ECS in brain regions plausibly implicated in schizophrenia. This direct advantage is nevertheless compromised by a number of potentially confounding variables such as mode of death, agonal state, post-mortem interval (PMI), symptom state at time of death and medication and substance exposure, of which only some may be partially controlled. Moreover, post-mortem investigation is best restricted to those components least subject to autolytic change such as membrane bound receptors. For these reasons studies examining the ECS in schizophrenia using post-mortem human brain tissue have focussed upon the CB1 receptor given its high prevalence and central role in mediating eCB function in the human brain.

The cannabinoid CB1 receptor in the brain

The CB1 receptor was the first component of the human endogenous cannabinoid system to be identified (Herkenham *et al*., 1990). Its gene in humans is located on region q14-q15 of chromosome 6 (Hoehe *et al*., 1991) and encodes for a 472-amino acid protein (Matsuda *et al*., 1990). The CB1 receptor has seven trans-membrane spanning domains and interacts with guanine nucleotide binding proteins (G-proteins) as part of its signal transduction mechanism, placing it within the superfamily of G-protein coupled receptors (GPCRs). There is a posttranscriptional splice variant of the CB1 receptor, provisionally termed the CB1A receptor, which contains 411 amino acids (Shire *et al*., 1995). This splice variant does not appear to differ

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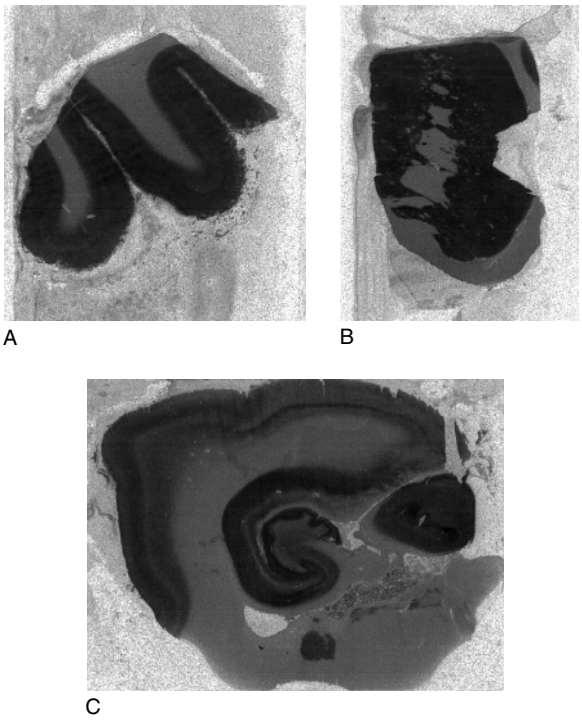

Figure 16.1. Representative autoradiograms showing the pattern of distribution of the cannabinoid CB1 receptor in: (A) the dorsolateral prefrontal cortex; (B) caudate putamen; and (C) hippocampus and surrounding entorhinal cortex from postmortem human brain, as demonstrated by the total binding of the tritium-labelled cannabinoid CB1 receptor antagonist, [^{3}H]CP55 940.

functionally from the CB1 receptor and is less than 20% of the total CB1 receptor pool as determined by reverse transcriptase PCR (Matsuda, 1997).

The distribution of the CB1 receptor has been mapped in human brain (Figure 16.1) (Herkenham *et al*., 1990; Westlake *et al*., 1994; Glass *et al*., 1997; and see Chapter 1). There is a very high density of CB1 receptor in the globus pallidus, substantia nigra pars reticulata, subiculum, Ammon's horn and the molecular layers of the dentate gyrus in the hippocampus and cerebellum, with a dense but lower level of binding in the neocortex, the remainder of the hippocampus, entorhinal cortex, amygdaloid complex and striatum. Neocortical binding is laminated, with highest levels in laminae I, V and VI, a thin dense band in IV(b) and low binding in II, III and IV(a and c). The regional density of cortical CB1 receptor also varies, with the densest binding being in the association areas of the frontal, temporal and limbic lobes and lowest densities in the primary motor and sensory cortices. Thalamic CB1 receptor binding anatomically corresponds to cortical binding, with moderate binding in the mediodorsal and anterior complex nuclei that connect to cortical associational areas, and very low levels in the geniculate bodies, ventral posterior and ventrolateral nuclei that connect to the primary sensory and motor cortices. The hypothalamus, nucleus solitarius and central grey substance exhibit moderate levels of CB1 receptor binding whereas there are minimal levels in the brainstem and area postrema.

In areas of very dense CB1 receptor binding, levels are of the same order of magnitude as that of striatal dopamine, cortical benzodiazepine and whole brain glutamate receptor densities (Herkenham *et al*., 1990). These comparisons, however, need to be viewed in light of the physiological activity of the cannabinoid agonist, R-(+)-WIN55 212 in CB1 receptor-knockout mice (Di Marzo *et al*., 2000; Breivogel *et al*., 2001). This has raised the possibility of non-CB1 cannabinoid receptors in the CNS that, although estimated to be small (Elphick and Egertova, 2001), may confound estimates of CB1 receptor density. Low levels of the cannabinoid CB2 receptor have also been identified in the CNS, specifically in microglia and brain stem neurons (Nunez *et al*., 2004; Van Sickle *et al*., 2005) where they may play a role in CNS inflammatory processes (Benito *et al*., 2008).

The distribution of mRNA for the CB1 receptor follows a pattern of distribution closely paralleling that of CB1 receptor binding (Mailleux *et al*., 1992; Westlake *et al*., 1994). The localization of the mRNA in the cortex is densest in laminae I and II and in the deep laminae IV, V and VI with variation between cortical regions. However, both in the hippocampus and cerebral cortex, the mRNA is extremely dense in some neurons surrounded by low to moderate densities in the majority of cells. This contrasts with other regions, for example the cerebellum, where mRNA distribution is relatively uniform across neurons. Equivalent levels of mRNA and binding are not maintained in the molecular layer of the hippocampal dentate gyrus, globus pallidus, substantia nigra and entopeduncular nucleus, where binding is high with minimal levels of mRNA, and conversely in the dentate hilus and medial habenula with high mRNA signal and low binding levels. These differences between mRNA and binding levels may indicate gene transcription of the CB1 receptor in a cell body remote from the receptor's terminal axonal location.

Relative to the density of the mRNA for the CB1 receptor, the mRNA for the CB1A receptor shows a

variable pattern of brain regional densities (between 1 and 20% of the CB1 receptor) (Shire *et al*., 1995). The physiological significance of this variable difference between the distributions of the mRNA for the CB1 and CB1A receptors remains to be determined (Matsuda, 1997; Elphick and Egertova, 2001).

The functional properties of the CB1 receptor are detailed in Chapters 1 and 3 but may be summarized as being located predominantly presynaptically, where they are proposed to inhibit synaptic neurotransmitter release (Schlicker and Kathmann, 2001).

Post-mortem human brain studies

Post-mortem human brain studies allow clear regional localization of changes in stable components of the endogenous cannabinoid system. There are three methods that have been used to date to quantify changes in the CB1 receptor in schizophrenia: in-situ radioligand binding and quantitative autoradiography; in-situ hybridization; and immunohistochemistry. Irrespective of the method used, the changes in CB1 receptor density reported in schizophrenia have been in the order of 10–20%, which contrasts sharply with the dramatic loss of CB1 receptor binding in the substantia nigra and globus pallidus seen in Huntington's disease, consistent with loss of striatal gamma-aminobutyric acid (GABA) projection neurons (Glass *et al*., 1993; Glass *et al*., 2000). The absence in schizophrenia of such an unequivocal change has resulted in studies reporting discordant findings owing in part to different methodologies and which require detailed interpretation.

In-situ radioligand binding and quantitative autoradiography studies

To date there have been four published studies using in-situ radioligand binding and quantitative autoradiography to measure CB1 receptor density in post-mortem human brain tissue from people with schizophrenia. The first such study (Dean *et al*., 2001) compared binding of the tritium-labelled CB1 receptor agonist [^{3}H]CP55 940 in the dorsolateral prefrontal cortex (DLPFC), Brodmann's area 9, caudate-putamen (C-P) and hippocampal formation of post-mortem tissue obtained from 14 subjects with schizophrenia and 14 non-psychiatric controls. Some subjects from both groups had consumed cannabis before death, allowing a comparison between recent cannabis users and those who had been abstinent. The methodology used was previously shown to provide a good measure of the density of the CB1 receptor (Herkenham *et al*., 1990) and the concentration of [^{3}H]CP55 940 was likely to provide single-point saturation.

When all subjects with schizophrenia were compared with all control subjects, the mean CB1 receptor density was increased by approximately 19% only in the DLPFC ($p < 0.05$). There were no significant differences between the groups in receptor density in the C-P or hippocampal formation. In subjects who had recently consumed cannabis (as determined by GC/MS of post-mortem plasma), there was a 23% increase in CB1 receptor density in the C-P compared with non-users, independent of schizophrenia ($p < 0.05$); in this comparison there were no significant differences in the DLPFC nor, again, in the hippocampus. The differences in the DLPFC between control and schizophrenia subjects and in the C-P between users and non-users could not be accounted for by post-mortem interval (PMI), brain pH, age or gender. There were also no significant correlations between [^{3}H]CP55 940 binding and duration of illness or final recorded antipsychotic drug dose in those with schizophrenia or with plasma THC levels in the cannabis users.

Another study also using in-situ binding with (3H) CP55 940 and quantitative autoradiography demonstrated increased CB1 recepter density in schizophrenia in the posterior cingulate cortex (PCC). (Newell *et al*., 2006). The study comprised post-mortem human brain tissue from 16 male subjects, eight controls and eight with schizophrenia matched for age and PMI, none of whom had used cannabis in the time preceding death. The pattern of [^{3}H]CP55 940 binding in the PCC showed a laminar distribution with highest binding in superficial layers compared with deep layers. From adjacent Nissl stained sections the authors concluded the superficial binding was in layers I and II and the deep binding in layers III-VI. The CB1 receptor density was increased by 25% only in the superficial laminar and was not different in the lower laminar compared with the control group. The increased binding was not accounted for by suicide or final recorded antipsychotic drug dose.

Immunohistochemical studies (Tsou *et al*., 1998) have identified densely staining cells in the superficial layers of the PCC that in rat (Tsou *et al*., 1999) and primate (Ong and Mackie, 1999) may be on GABAergic or glutamatergic neurons. Newell *et al*. (2007) had previously found strikingly increased $GABA_A$ receptor

binding in the superficial and deep layers of the PCC from 13 of the 16 subjects used in the CB1 receptor study. Moreover, there was a non-significant positive correlation (rho = 0.49) between CB1 receptor and $GABA_A$ receptor binding in the superficial layers of the PCC in these subjects. Given that CB1 receptor activation is proposed to decrease pre-synaptic neurotransmitter release, the increase in CB1 receptor density may be an attempt to compensate for increased signaling through the $GABA_A$ receptor; alternatively it may be owing to increased numbers of cells expressing these receptors.

An alternative radioligand to [^{3}H]CP55 940 is the CB1 receptor antagonist [^{3}H]SR141716A, which overcomes the limitation of agonist-induced affinity changes in the receptor and also does not bind the cannabinoid CB2 recptor. [^{3}H]SR141716A was used by Zavitsanou and colleagues (2004) to measure CB1 receptor density in the anterior cingulate cortex (ACC) in schizophrenia (n = 10) compared with controls (n = 9). This study found that [^{3}H]SR141716A binding was homogenous across all cortical layers, a finding different from the other studies detailed above. This study also found CB1 receptor density was increased in schizophrenia in the ACC (by 64%) and this was not associated with PMI. Moreover, like Dean *et al.* (2001), Zavitsanou *et al.* (2004) also noted no difference in CB1 receptor density in cortex between cannabis users and non-users and no relationship with antipsychotic drug dose. Intriguingly they did find that cannabis users with schizophrenia treated with clozapine had the highest CB1 receptor density, suggesting a possible specific clozapine-ECS interaction. In adjacent tissue sections from the same subjects, glutamate AMPA and NMDA receptor densities were also increased in schizophrenia whereas serotonin 5HT2 receptor density was decreased (Zavitsanou and Huang, 2002; Zavitsanou *et al.*, 2002). In the ACC, CB1 receptors are located on both pyramidal and non-pyramidal cells (Mailleux *et al.*, 1992; Ong and Mackie, 1999). Therefore, the changes in receptor densities may again indicate a functional interaction between eCB and other neurotransmitter systems or changes in cell numbers expressing these receptors.

In contrast to the above studies, one quantitative autoradiography study used both CB1 receptor radioligands, [^{3}H]CP55 940 and [^{3}H]SR141716A (Deng *et al.*, 2007), and found differences in receptor density in schizophrenia in the superior temporal gyrus (STG; Brodmann's Area 22). This suggests that changes in CB1 receptor density in schizophrenia may be brain-region specific. The study used tissue from 16 subjects, eight with schizophrenia and eight controls matched for age, sex (all male) and PMI. [^{3}H]CP55 940 showed a trilaminar pattern of binding with the upper band corresponding to cortical layers I and II, the middle band to layers III and IV and the deepest band corresponding to layers V and VI with the middle band having a lower level of binding compared with the other two. There were no significant differences between schizophrenia and control subjects in [^{3}H]CP55 940 binding in any of the bands. [^{3}H]SR141716A demonstrated a homogenous pattern of binding through the STG and, again, no difference between schizophrenia and control subjects was demonstrated. Recent cannabis use did not alter binding significantly in the schizophrenia group and there was no effect of antipsychotic drug treatment.

Fifteen of the sixteen subjects in the study of Deng *et al.* (2007) had also been included in the study of Newell *et al.* (2006) where there was an increase in CB1 receptor density in the ACC, supporting regional specificity.

The use of the two radioligands also demonstrated that the amount of CB1 receptor protein measured was different between the two compounds. The [^{3}H]CP55 940 assay showed levels of at least 97 fmol/mg tissue equivalents (TE) compared to [^{3}H]SR141716A levels of approximately 35 fmol/mg TE. This discrepancy may be due to suboptimal assay conditions that may not have resulted in binding saturation [^{3}H]CP55 940 binding to other receptors, or [^{3}H]CP55 940 inducing affinity change in the CB1 receptor.

In-situ hybridization and immunohistochemical studies

The first study to use immunohistochemistry to measure the CB1 receptor in schizophrenia measured protein in the ACC in tissue from the Stanley Neuropathology Consortium Collection (Koethe *et al.*, 2007). This allowed comparison between schizophrenia, bipolar disorder, major depression and non-psychiatrically ill controls (n = 15 in each group), although subjects were not matched on PMI, substance abuse or suicide. This study did not find any change in the number of CB1 receptor-immunopositive cells in the ACC in schizophrenia, although they did report a small decrease in the number of immunopositive glial cells in major depression compared with control subjects. There were also no changes in cell numbers

or apparent neuron density in any of the diagnostic groups compared with the control group.

The contrast with the striking increase in CB1 receptor density in the ACC as measured by quantitative autoradiography (Zavitsanou *et al.*, 2004) is not readily explicable. Two obvious factors are the methodological difference and the heterogeneity of the ACC (Vogt *et al.*, 1987). Immunohistochemistry can cover only small regions and the Koethe *et al.* (2007) study may have selected a sub-region of the ACC unaffected in schizophrenia.

A study with a larger sample ($n = 77$) used immunohistochemistry and in-situ hybridization to measure the protein and mRNA levels of the CB1, dopamine D2 and adenosine A2A receptors in the DLPFC in people with schizophrenia ($n = 31$); non-schizophrenia suicide ($n = 13$); and non-suicide, non-psychiatrically ill controls ($n = 33$) (Uriguen *et al.*, 2009). Recent cannabis use was an exclusion criterion. Interpretation of the findings of this study is complicated by 27 of the cases of schizophrenia having died by suicide; nevertheless this is partially addressed by including a sample of people with other psychiatric disorders who died by suicide.

There were no differences between groups in protein levels for the dopamine D2 or adenosine A2A receptor. The antibody used for immunodetection of the dopamine D2 receptor recognized the class of D2-like receptors and therefore measured the dopamine D3 and D4 receptors as well. There was also no difference overall in CB1 receptor protein levels between the groups. However, when the schizophrenia group were divided into those who had received antipsychotic drug treatment in the period immediately preceding death and those who were antipsychotic-free (as determined by post-mortem toxicological assay), differences were noted. The antipsychotic drug-treated group ($n = 11$) had a 29% decrease in CB1 receptor protein density, whereas the antipsychotic drug-free and non-schizophrenia suicide groups did not differ significantly from control subjects. The decrease was not attributable to suicide or a decrease in total protein (as measured by beta-actin). The study found no differences in the mRNA levels between schizophrenia and the other groups in the DLPFC for the CB1, dopamine D2 or adenosine A2A receptors.

The finding of a decrease in CB1 receptor protein density without change in mRNA suggests that there is a down-regulation in the receptor post-transcription attributable to antipsychotic drug treatment or some associated variable. Alternatively, CB1 receptor-producing cells in the DLPFC may be unaffected and only the CB1 receptors of cells that are transcribed outside the DLPFC and transported to axon terminals within the DLPFC are affected, resulting in lower CB1 receptor protein without detectable change in mRNA. The possible effect of antipsychotic drug treatment on the CB1 receptor is discussed below.

Two studies from the University of Pittsburgh using in-situ hybridization, radioimmunochemistry and immunohistochemistry have observed decreased levels of CB1 receptor mRNA and protein level in schizophrenia (Eggan *et al.*, 2008, 2010). The first study compared 23 pairs of schizophrenia and control subjects matched on age, sex and PMI using tissue from the DLPFC (Brodmann's Area 9) (Eggan *et al.*, 2008). The mRNA expression pattern was banded and confined to neurons with greatest density in layer II and the superficial part of layer III, intermediate in layers IV, V and VI, lowest in the deep part of layer III and absent in layer I; this was similar in both groups. The subjects with schizophrenia had a mean overall decrease of 14.8% of CB1 receptor mRNA compared with controls; this applied to each band.

The radioimmunoreactivity of CB1 receptor protein also showed a banded pattern with greatest density in layer IV, next highest in layers II and III, then layer VI and lowest in layer V. There is no comment on layer I binding that appears of a density between layer III and VI. Using a pairwise comparison a significant overall decrease of 11.6% was noted in the schizophrenia subjects that was not significant in a whole-group unpaired comparison. Rather surprisingly given the discordant banding patterns observed, a significant correlation (rho = 0.67; $p < 0.001$) was noted between the pairwise change in CB1 receptor mRNA and protein levels. Using standard immunohistochemistry on 12 of the 23 subject pairs, an overall significant decrease of 13.9% was observed in the subjects with schizophrenia that also was noted in deep portion of layer III, layer IV and VI (on both pairwise and unpaired comparisons).

This research group had previously described in the same region in the same subjects significantly decreased mRNA levels of glutamic acid decarboxylase of molecular weight 67 kDa (GAD67) and cholecystokinin (CCK) (Hashimoto *et al.*, 2008). These two levels were highly correlated, suggesting that it was a decrease of mRNA in the CCK-expressing GABA interneurons. CB1 receptors are expressed on CCK-positive GABA interneurons (Eggan and Lewis, 2007) and this study

found a significant positive correlation in decreases in the mRNA levels for CB1 receptor and GAD67 and CCK within each pair. The proposed function of the CB1 receptor to inhibit neurotransmitter release and its localization on CCK-positive GABA interneurons led the authors to conclude that decreased CB1 receptor mRNA was a compensatory response to decreased GAD67 mRNA causing reduced GABA release.

There was no effect on any of the CB1 receptor levels by sex, death by suicide, antidepressant exposure, sodium valproate (antipsychotic) or benzodiazepine medication use, or any substance abuse or dependence (including cannabis).

A subsequent study by the same group examined another DLPFC region (Brodmann's Area 46) in the same subjects and in tissue from a different cohort of subjects with schizophrenia (Eggan *et al*., 2010). CB1 receptor immunoreactivity was decreased by 19% in the same 12 pairs of subjects that had a 13.9% decrease in Brodmann's Area 9, and there was a significant correlation (rho = 0.73; $p < 0.001$) between the two regions. In the new cohort, CB1 receptor immunoreactivity was decreased by 20% compared with control subjects and by 23% compared with subjects with major depression. The pattern of binding in this area was similar to that observed in Brodmann's Area 9, with the subjects with schizophrenia from the new cohort having lower levels of CB1 receptor immunoreactivity in layers I-IV but not V or VI. The differences observed were not a function of sex, death by suicide, antidepressant exposure, benzodiazepine or antipsychotic drug use, substance use disorder diagnosis or history of cannabis use (Eggan *et al*., 2010).

In summary, the majority of studies demonstrate changes in CB1 receptor protein density in some brain regions in post-mortem tissue from subjects with schizophrenia. However, some brain regions such as the superior temporal gyrus, hippocampus and caudate-putamen showed no difference, while other brain regions such as the anterior cingulate gyrus and DLPFC have exhibited apparently contradictory findings of increased, unchanged or decreased CB1 receptor density. A smaller number of studies have measured mRNA, with the majority demonstrating unchanged levels and one study a decrease.

Interpretation

A number of factors need to be considered in interpreting these studies; the first being the small sample sizes and the small reported changes, generally in the range of 20%. Based on number of subjects with schizophrenia (ranging from 7 to 31) with these reductions in CB1 receptor density the applicability of the findings to the pathology of schizophrenia remain preliminary.

A possible important factor is the role of long term antipsychotic medication in influencing CB1 receptor levels. One study (Uriguen *et al*., 2009) reported a decrease in CB1 receptor immunoreactivity in the DLPFC only in subjects with schizophrenia treated with antipsychotic medication. However, the antipsychotic drug-free group were determined by having no detectable drug in post-mortem plasma, and may well have received long-term antipsychotic treatment up until a short time before death. Therefore, the increase in CB1 receptor density may more reflect an antipsychotic-withdrawal state than an antipsychotic-free condition. Also, no study demonstrated any correlation between final recorded antipsychotic dose and CB1 receptor density, but this is a poor correlate of the possible effect of chronic antipsychotic treatment on CB1 receptor density. To better address this issue requires animal treatment studies of chronic antipsychotic treatment and measurement of brain levels of the CB1 receptor. In-situ radioligand binding and quantitative autoradiography studies in rats did not show changes in CB1 receptor binding in the cerebral cortex, caudate-putamen or hippocampus (Sundram *et al*., 2005; Wiley *et al*., 2008). Small-scale monkey-treatment studies have also not reported changes in CB1 receptor protein or mRNA levels in the frontal cortex (Eggan *et al*., 2008, 2010). Therefore, from these limited data it would seem unlikely that antipsychotic treatment exerts a major effect on CB1 receptor levels in the frontal cortex of rats or monkeys; however, subtle effects or withdrawal effects of antipsychotics in humans cannot be definitively excluded.

It is possible that exogenous cannabinoids may influence CB1 receptor levels in schizophrenia, especially given the high rate of cannabis use in this group. The reported studies controlled for this variable by excluding subjects with recent cannabis use or demonstrating no effect on binding in cortical regions when comparing users and non-users. One study (Dean *et al*., 2001) did show an effect of cannabis consumption in the caudate-putamen, but not hippocampus or DLPFC. A subsequent study (Dean *et al*., 2003), using tissue from the same control and schizophrenia subjects, examined levels of the dopamine transporter (DAT) and tyrosine hydroxylase (TH) in the caudate-putamen in subjects who were: (1) non-cannabis users

($n = 19$); and (2) cannabis users ($n = 9$) at time of death. The mean DAT level, as measured by [^{3}H]mazindol binding, was significantly decreased (by 19%; $p = 0.01$) in non-using subjects with schizophrenia ($n = 9$) compared with the non-using controls ($n = 10$). The difference was not apparent between the two cannabis using groups, nor were these groups significantly different from the non-using control group. Tyrosine hydroxylase levels did not vary across any of the groups (Dean *et al*., 2003). Therefore, there appear to be functional effects of cannabis on CB1 receptor density and downstream consequences in schizophrenia that are region specific. The regional differences are consistent with animal studies in rodents and monkeys that show variable effects dependent on cannabinoid type, dose, duration and brain region examined. Hence, cannabis use or withdrawal remains a possible factor in explaining some of the variance between studies in schizophrenia. It is also possible that other exogenous agents such as alcohol, caffeine or nicotine may influence CB1 receptor levels (Basavarajappa and Hungund, 2002; Marco *et al*., 2007) and this may have contributed to within- and between-study differences.

The changes in CB1 receptor binding observed in schizophrenia are modest compared with those described in Huntington's disease (97.5% decrease in the substantia nigra pars reticulata) (Glass *et al*., 1993). However, the loss in Huntington's disease is due to the specific degeneration of striatonigral terminals (Glass *et al*., 1993) with no analogous pathology identified in schizophrenia (Harrison and Weinberger, 2005). In contrast, CB1 receptor binding decreases seen in Alzheimer's disease (37–45% in the hippocampus and 49% in the caudate) did not correlate with neuropathology but did correlate with age and were seen in other cortical disorders (Westlake *et al*., 1994). In the studies that reported increased CB1 receptor density in schizophrenia, it would seem unlikely the changes were a non-specific marker of cortical neurodegeneration.

Conclusions

It is premature to speculate on the possible functional sequelae of CB1 receptor changes in the frontal cortical regions in schizophrenia given the discordant findings. It is possible to recognize that changes are small and region specific, in particular in the DLPFC and ACC, and the possible influence of confounding variables such as chronic antipsychotic treatment and withdrawal, chronic cannabis use and withdrawal and nicotine, alcohol or caffeine use have not been definitively excluded.

Nevertheless, there is some speculative correlational data to support functional effects of CB1 receptor changes in schizophrenia (Dean *et al*., 2003; Eggan *et al*., 2008). The CB1 receptor is located predominantly if not exclusively on presynaptic neurons (Egertova and Elphick, 2000), particularly axons (Eggan and Lewis, 2007). When stimulated by retrograde diffusion of anandamide (Wilson and Nicoll, 2001) or 2-arachidonoyl glycerol (Kim and Alger, 2004; Makara *et al*., 2005) the CB1 receptor inhibits neurotransmitter release through inhibition of voltage dependent Ca^{2+} channels (Hoffman and Lupica, 2000) and possibly other mechanisms (for review see Schlicker and Kathmann, 2001). Therefore, a decrease in CB1 receptor density will result in an increase in the release of the co-located neurotransmitter and vice versa. Hence, the changes in CB1 receptor in schizophrenia need to be definitively localized and quantified before speculating as to the possible implications for the pathology of schizophrenia.

References

Basavarajappa, B.S. and Hungund, B.L. (2002) Neuromodulatory role of the endocannabinoid signaling system in alcoholism: an overview. *Prostaglandins Leukot Essent Fatty Acids*, **66**:287–99.

Benito, C., Tolon, R.M., Pazos, M.R., Nunez, E., Castillo, A.I. and Romero, J. (2008) Cannabinoid CB2 receptors in human brain inflammation. *Br J Pharmacol*, **153**:277–85.

Breivogel, C.S., Griffin, G., Di, M.V. and Martin, B.R. (2001) Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol Pharmacol*, **60**:155–63.

D'Souza, D.C., Abi-Saab, W.M., Madonick, S., *et al*. (2005) Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biol Psychiatry*, **57**:594–608.

Dean, B., Bradbury, R. and Copolov, D.L. (2003) Cannabis-sensitive dopaminergic markers in postmortem central nervous system: changes in schizophrenia. *Biol Psychiatry*, **53**:585–92.

Dean, B., Sundram, S., Bradbury, R., Scarr, E. and Copolov, D. (2001) Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience*, **103**:9–15.

Deng, C., Han, M. and Huang, X.F. (2007) No changes in densities of cannabinoid receptors in the superior temporal gyrus in schizophrenia. *Neurosci Bull*, **23**:341–7.

Di Marzo, V., Breivogel, C.S., Tao, Q., *et al.* (2000) Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. *J Neurochem*, **75**:2434–44.

Egertova, M. and Elphick, M.R. (2000) Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. *J Comp Neurol*, **422**:159–71.

Eggan, S.M., Hashimoto, T. and Lewis, D.A. (2008) Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. *Arch Gen Psychiatry*, **65**:772–84.

Eggan, S.M. and Lewis, D.A. (2007) Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. *Cereb Cortex*, **17**:175–91.

Eggan, S.M., Stoyak, S.R., Verrico, C.D. and Lewis, D.A. (2010) Cannabinoid CB1 Receptor Immunoreactivity in the Prefrontal Cortex: Comparison of Schizophrenia and Major Depressive Disorder. *Neuropsychopharmacology*, **35**:2060–71.

Elphick, M.R. and Egertova, M. (2001) The neurobiology and evolution of cannabinoid signalling. *Phil Trans R Soc Lond B*, **356**:381–408.

Glass, M., Dragunow, M. and Faull, R.L. (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the foetal, neonatal and adult human brain. *Neuroscience*, **77**:299–318.

Glass, M., Dragunow, M. and Faull, R.L. (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience*, **97**:505–19.

Glass, M., Faull, R.L. and Dragunow, M. (1993) Loss of cannabinoid receptors in the substantia nigra in Huntington's disease. *Neuroscience*, **56**:523–7.

Harrison, P.J. and Weinberger, D.R. (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol Psychiatry*, **10**:40–68.

Hashimoto, T., Arion, D., Unger, T., *et al.* (2008) Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Mol Psychiatry*, **13**:147–61.

Herkenham, M., Lynn, A.B., Little, M.D., *et al.* (1990) Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA*, **87**:1932–6.

Hoehe, M.R., Caenazzo, L., Martinez, M.M., *et al.* (1991) Genetic and physical mapping of the human cannabinoid receptor gene to chromosome 6q14-q15. *New Biol*, **3**:880–5.

Hoffman, A.F. and Lupica, C.R. (2000) Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *J Neurosci*, **20**:2470–9.

Kim, J. and Alger, B.E. (2004) Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. *Nat Neurosci*, **7**:697–8.

Koethe, D., Llenos, I.C., Dulay, J.R., *et al.* (2007) Expression of CB1 cannabinoid receptor in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression. *J Neural Transm*, **114**:1055–63.

Mailleux, P., Parmentier, M. and Vanderhaeghen, J.J. (1992) Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides. *Neurosci Lett*, **143**:200–4.

Makara, J.K., Mor, M., Fegley, D., *et al.* (2005) Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. *Nat Neurosci*, **8**:1139–41.

Marco, E.M., Granstrem, O., Moreno, E., *et al.* (2007) Subchronic nicotine exposure in adolescence induces long-term effects on hippocampal and striatal cannabinoid-CB1 and mu-opioid receptors in rats. *Eur J Pharmacol*, **557**:37–43.

Matsuda, L.A. (1997) Molecular aspects of cannabinoid receptors. *Crit RevNeurobiol*, **11**:143–66.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C. and Bonner, T.I. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, **346**:561–4.

Mechoulam, R. and Hanus, L. (2000) A historical overview of chemical research on cannabinoids. *Chem Phys Lipids*, **108**:1–13.

Newell, K.A., Deng, C. and Huang, X.F. (2006) Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. *Exp Brain Res*, **172**:556–60.

Newell, K.A., Zavitsanou, K., Jew, S.K. and Huang, X.F. (2007) Alterations of muscarinic and GABA receptor binding in the posterior cingulate cortex in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*, **31**:225–33.

Nunez, E., Benito, C., Pazos, M.R., *et al.* (2004) Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. *Synapse*, **53**:208–13.

Ong, W.Y. and Mackie, K. (1999) A light and electron microscopic study of the CB1 cannabinoid receptor in primate brain. *Neuroscience*, **92**:1177–91.

Pertwee, R.G. (2010) Receptors and channels targeted by synthetic cannabinoid receptor agonists and antagonists. *Curr Med Chem*, **17**:1360–81.

Piomelli, D. (2003) The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci*, **4**:873–84.

Schlicker, E. and Kathmann, M. (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci*, **22**:565–72.

Shire, D., Carillon, C., Kaghad, M., *et al.* (1995) An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. *J Biol Chem*, **270**:3726–31.

Sundram, S., Copolov, D. and Dean, B. (2005) Clozapine decreases [3H] CP 55940 binding to the cannabinoid 1 receptor in the rat nucleus accumbens. *Naunyn Schmiedebergs Arch Pharmacol*, **371**:428–33.

Tsou, K., Brown, S., Sanudo-Pena, M.C., Mackie, K. and Walker, J.M. (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience*, **83**:393–411.

Tsou, K., Mackie, K., Sanudo-Pena, M.C. and Walker, J.M. (1999) Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience*, **93**:969–75.

Uriguen, L., Garcia-Fuster, M.J., Callado, L.F., *et al.* (2009) Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: effect of antipsychotic treatment. *Psychopharmacology (Berl)*, **206**:313–24.

Van Sickle, M.D., Duncan, M., Kingsley, P.J., *et al.* (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*, **310**:329–32.

Vogt, B.A., Pandya, D.N. and Rosene, D.L. (1987) Cingulate cortex of the rhesus monkey: I. Cytoarchitecture and thalamic afferents. *J Comp Neurol*, **262**:256–70.

Westlake, T.M., Howlett, A.C., Bonner, T.I., Matsuda, L.A. and Herkenham, M. (1994) Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience*, **63**:637–52.

Wiley, J.L., Kendler, S.H., Burston, J.J., Howard, D.R., Selley, D.E. and Sim-Selley, L.J. (2008) Antipsychotic-induced alterations in CB1 receptor-mediated G-protein signaling and in vivo pharmacology in rats. *Neuropharmacology*, **55**:1183–90.

Wilson, R.I. and Nicoll, R.A. (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature*, **410**:588–92.

Zavitsanou, K., Garrick, T. and Huang, X.F. (2004) Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*, **28**:355–60.

Zavitsanou, K. and Huang, X.F. (2002) Decreased [(3)H] spiperone binding in the anterior cingulate cortex of schizophrenia patients: an autoradiographic study. *Neuroscience*, **109**:709–16.

Zavitsanou, K., Ward, P.B. and Huang, X.F. (2002) Selective alterations in ionotropic glutamate receptors in the anterior cingulate cortex in schizophrenia. *Neuropsychopharmacology*, **27**:826–33.

Chapter

17

The endocannabinoid system in schizophrenia

Paul Morrison

It must be recognized that the brain is not a chemical factory…

In order to bring all the forthcoming biochemical observations into a meaningful framework it will prove necessary to emphasize more strongly aspects of neurocircuits and connectivity and to do so both at the microscopic and macroscopic level.

(Carlsson, 2001), Nobel Lecture

There has been talk of an endocannabinoid (eCB) hypothesis of psychosis/schizophrenia, mirroring the dopamine (DA), glutamate and gamma-aminobutyric acid (GABA) hypotheses. All these neurochemical hypotheses in fact share the same basic structure, wherein an excess/deficit of a particular neurotransmitter or receptor substance is postulated to be a central event in the emergence of psychopathology. Studies examining the eCB system (ECS) in schizophrenia have focused on two areas, eCB levels and CB1 receptor availability. In both cases, deviations from normality have been observed. Specifically, there have been consistent reports of increased anandamide (AEA) concentrations in schizophrenia. Rather than being pathological, it has been postulated that raised AEA serves to limit psychosis and provide protection. There is some evidence to support a positive effect of excess AEA on general well-being, but the data at present do not indicate that it limits positive psychotic symptoms. Indeed, any account is made more complex because AEA is an agonist, not just at cannabinoid receptors but also at TRPV1 (transient receptor potential, vanilloid) receptors.

For CB1 receptor density, findings have been inconsistent (see Chapter 16 for a detailed review of these studies). Increases, decreases, no change and effects due to medication have been reported in schizophrenic brains at post-mortem. Some have proposed that CB1 receptor down-regulation is a compensatory adaption in schizophrenia or is secondary to antipsychotic treatment. Data from in-vivo imaging studies involving drug naïve, first-episode patients and newly developed CB1 ligands may resolve this issue. Overall, there is increasing evidence that, in biochemical terms, the ECS is altered in schizophrenia. Whether such changes are part of the pathology in schizophrenia or a compensatory response aimed at restoring health is unclear. The challenge remains as to how biochemical changes in the ECS impact upon neurocircuits and information processing within the central nervous system.

Endocannabinoid concentrations in schizophrenia

Studies to date have focussed on AEA. It remains unknown whether there are changes in the other prominent eCBs, 2-arachidonoyl glycerol (2-AG). This gap in our knowledge is potentially problematic because, whereas AEA is a dual-purpose signal with endovanilloid and endocannibinoid properties, 2-AG and Δ^9-tetrahydrocannabinol (THC), have no activity at TRPV1 receptors (Pertwee, 2006).

Earlier studies reported increased AEA concentrations in blood samples from schizophrenia patients (De Marchi *et al*., 2003). These findings carried the drawback that AEA is synthesized in peripheral tissues, as well as centrally, making identification of the source of excess AEA impossible. To circumvent this problem, some studies have used cerebrospinal fluid (CSF) sampling. Similarly, elevated AEA concentrations in schizophrenia have been observed (Leweke *et al*., 1999; Giuffrida *et al*., 2004).

Giuffrida and colleagues measured CSF anandamide levels in samples of: first-episode, antipsychotic-naive schizophrenia patients (n = 47); medicated schizophrenic patients (typicals n = 36, atypicals

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n = 31); patients suffering from an affective disorder (*n* = 22); dementia (*n* = 13); and healthy controls (*n* = 81) (Giuffrida *et al.*, 2004). The major finding was that AEA levels were approximately eight times higher in the CSF of schizophrenia patients compared with healthy controls. In contrast, AEA levels were not elevated in affective disorders or in dementia. Anandamide levels appeared to "normalize" in the group of schizophrenia patients who were prescribed typical D2 blocking drugs (haloperidol, phenothiazenes). In contrast, AEA remained elevated in those schizophrenia patients treated with atypical antipsychotics (risperidone, olanzapine, quetiapine, clozapine).

Based on previous animal work suggesting that AEA serves as a negative-feedback signal on dopaminergic drive, the authors hypothesized that, in schizophrenia patients, AEA levels would show an inverse relationship with psychosis as rated by the Positive and Negative Syndrome Scale (PANSS) scale (Giuffrida *et al.*, 2004). Overall, following the removal of three outliers, AEA levels were inversely related to cumulative PANSS scores (r = –0.4), negative symptom scores (r = –0.3) and general symptom scores (r = –0.4), but showed *no* relationship with ratings of positive psychotic symptoms. So, although elevations in CSF anandamide appeared to be beneficial for patients most significantly on the general PANSS scale, the findings did not support negative-feedback inhibition of dopamine-driven positive psychotic symptoms.

In a subsequent study, CSF anandamide levels were compared between prodromal patients (*n* = 27) and healthy controls (*n* = 81) (Koethe *et al.*, 2009). Anandamide concentrations were found to be approximately six times greater in the prodromal group. In a subsequent step, the prodromal group were split at the median into high versus low AEA groups. The relative risk (RR) of transition into frank psychosis was lower in the high AEA group (RR 0.33, 95% confidence intervals [CI]: 0.09–1.29), although only at a trend level (*p* = 0.09). The authors suggested that AEA mobilization may play a protective role in at least a sub-group of patients with early stage schizophrenia. Further work in a larger sample of prodromal patients will be necessary to confirm or refute this. If true, this offers the possibility that pharmacological manipulations aimed at increasing AEA concentrations may be beneficial in schizophrenia.

Endocannabinoids and dopamine in animal studies.

Neurochemical assays

There have been several animal studies in which forebrain AEA levels were measured following treatment with psychostimulants or direct dopamine receptor agonists. Using microdialysis, Giuffrida and colleagues reported increases in striatal AEA levels following treatment with the D2 agonist quinpirole (Giuffrida *et al.*, 1999). Patel *et al.* found that treatment with low-dose quinpirole increased AEA levels in homogenized forebrain (cortex and striatum) tissue, an effect lost at higher doses of quinpirole (Patel *et al.*, 2003).

Tzavara and colleagues measured AEA levels in the brains of mice made deficient for the dopamine transporter (DAT) (Tzavara *et al.*, 2006). Such DAT knockout mice are constitutively hyperdopaminergic. They are hyperactive and display abnormalities in sensorimotor gating and cognition (Gainetdinov *et al.*, 2002). As such they are believed to resemble major neuropsychiatric conditions such as ADHD and schizophrenia. In DAT knockout mice marked reductions of AEA were observed in the striatum, but not in the cortex, cerebellum or hippocampus (Tzavara *et al.*, 2006). Hyperactive behaviors showed improvement following treatment with inhibitors of AEA uptake or enzymatic degradation. Interestingly, the beneficial effect of such treatments in DAT knockout mice was inhibited, not by antagonists at the CB1 receptor, but by blockers of TRPV1 receptors. In agreement, TRPV1, but not CB1, receptor binding was shown to be increased in the striatum of DAT knockout mice (Tzavara *et al.*, 2006).

Electrophysiological assays

There has been a surge of knowledge into the roles of the ECS and dopamine in regulating neurotransmission within the striatum (Calabresi *et al.*, 2007; Wickens, 2009). This has come from electrophysiological studies, incorporating methods for the unambiguous identification of specific cell types (Shen *et al.*, 2008). Dopamine and the eCBs regulate the strength of individual synapses within the striatum. Such processes are now known to be fundamental for new learning (Yin *et al.*, 2009). Numerous studies have shown that long-term depression (LTD) of corticostriatal fibers is mediated by retrograde eCB signaling (Ronesi *et al.*, 2004; Gerdeman

et al., 2002; Robbe *et al*., 2002; Kreitzer and Malenka, 2007). At synapses belonging to the indirect pathway, D2 receptor stimulation is one of three co-incident signals required for the mobilization of eCBs, the other two being activation of post-synaptic mGLU receptors and calcium channels (Shen *et al*., 2008). For synapses belonging to the direct pathway, eCB-dependent LTD has no requirement for dopamine. Indeed, at these synapses stimulation of D1 receptor stimulation occludes eCB-dependent LTD, and is instead a requirement for long-term potentiation (Shen *et al*., 2008).

There is strong evidence that the eCB responsible for retrograde signaling within the striatum is 2-AG (Jung *et al*., 2005; Uchigashima *et al*., 2007). Previously it was believed that AEA and 2-AG were interchangeable as retrograde signals. However, the picture is more complex. Anandamide, as well as having additional effects at TRPV1 receptors, can also oppose the mobilization of 2-AG. Using striatal tissue, Maccarrone and colleagues demonstrated that elevation of AEA concentrations (by genetic or pharmacological manipulations) resulted in concomitant reductions in the levels and physiological effects of 2-AG (Maccarrone *et al*., 2008). Thus AEA appeared to down-regulate the other eCB. Furthermore, the effect of AEA in this paradigm was mediated via TRPV1 (rather than CB1) receptors. The same group has now shown that TRPV1 agonists increase glutamate release within the striatum by stimulating pre-synaptic terminals (Musella *et al*., 2009). Thus, at cortico-striatal synapses, TRPV1 and CB1 receptors modulate glutamate release, but in opposite directions.

CB1 receptors in schizophrenia: post-mortem findings

As discussed in Chapter 16, three post-mortem studies have reported an increase in central CB1 receptor density in schizophrenia (Dean *et al*., 2001; Zavitsanou *et al*., 2004; Newell *et al*., 2006), whereas one study reported no change (Koethe *et al*., 2007), and one found a decrease (Eggan *et al*., 2008). In the most recently published study there was a decrease in CB1 density in anti-psychotic-treated schizophrenia patients, whereas untreated patients showed no differences from controls (Uriguen *et al*., 2009). A brief synopsis of these studies is provided here.

Using quantitative autoradiography, Dean and colleagues reported an approximate 20% *increased* binding of the CB1 agonist [^{3}H]CP55 940 in the dorsolateral prefrontal cortex (DLPFC) from schizophrenic patients compared with controls (n = 14 in each group) (Dean *et al*., 2001). Similarly, Zavitsanou *et al.* found a 64% *increased* binding of the CB1 antagonist [^{3}H]SR141716A in the left anterior cingulate cortex (ACC) from patients with schizophrenia (n = 10) compared with controls (n = 9) (Zavitsanou *et al*., 2004). Finally, Newell and colleagues reported a 25% *increased* binding of [^{3}H]CP55 940 in layers I and II of the posterior cingulate cortex (PCC) from schizophrenia patients (n = 8) versus controls (n = 8) (Newell *et al*., 2006).

Koethe and colleagues quantified CB1 receptors in the ACC using immunochemistry. *No difference* in CB1 density was found in tissue from schizophrenia patients, bipolar patients and controls (n = 15 in all groups) (Koethe *et al*., 2007).

Using in-situ hybridization and immunochemistry, Eggan and colleagues measured CB1 mRNA and protein in the DLPFC from schizophrenia subjects versus controls (n = 23 in each group). In contrast to the findings above, CB1 mRNA and protein levels were *decreased* in tissue from schizophrenia patients. The authors proposed that CB1 down-regulation constitutes a compensatory mechanism in schizophrenia aimed at restoring "normal" network dynamics (Eggan *et al*., 2008).

In the most recently published study, Uriguen and colleagues measured CB1 (as well as dopamine D2 and adenosine A_{2A}) mRNA and protein in the DLPFC from schizophrenia patients (n = 31) and controls (n = 46) (Uriguen *et al*., 2009). Anti-psychotic status was established by toxicological screening at post-mortem. The detected anti-psychotics were quetiapine (n = 4), olanzapine (n = 3), clozapine (n = 3) risperidone (n = 1) and typical antipsychotics (n = 2). They reported that, in schizophrenia, the density of D2 or A_{2A} receptors in the DLPFC *did not differ* from controls and nor was D2 or A_{2A} density affected by anti-psychotic treatment. The major finding was that CB1 density was significantly *decreased* in anti-psychotic treated schizophrenia patients, but not in drug-free subjects. No differences in mRNA amounts encoding for A_{2A}, D2 or CB1 receptors were found. The authors suggested that anti-psychotics induce down-regulation of CB1 receptors in the brain (Uriguen *et al*., 2009).

Overall, it is unclear whether CB1 receptor density is altered in schizophrenia: inconsistency dominates. Future studies will take advantage of newly developed radioligands to assay CB1 receptor availability in vivo. Such methods make it feasible to measure CB1

in first-episode patients before anti-psychotic treatment. A word of caution is required however. A similar endeavor to assay D2 in schizophrenia occurred over more than 8 years, findings were mixed and controversy ensued. Ultimately meta-analytic studies were necessary; and concluded that although D2 elevations were statistically significant, the effect size was so small as to be clinically insignificant (McKenna, 2007).

Conclusions

There are parallels between an eCB and DA hypothesis of psychosis/schizophrenia. Both hypotheses rest on the pro-psychotic properties of stimulant drugs and THC, while the status of the DA hypothesis is further cemented by the anti-psychotic properties of D2 antagonists. Whether eCB manipulation will also display potent anti-psychotic properties is less certain, but if true will undoubtedly strengthen any eCB claims.

For both systems, measurement of receptor proteins and endogenous transmitter levels (either directly, or as indexed by a metabolite or uptake of a pre-cursor), has been undertaken repeatedly. As the archetypal neurochemical hypothesis, this is particularly true of DA. What has emerged is that whether receptors or transmitters are measured, there is a considerable overlap between schizophrenia patients and healthy controls. Any observed differences appear to be slight and their clinical relevance is not entirely clear (McKenna, 2007).

Measurement of CB1 receptor numbers in schizophrenia post-dates similar work in D2 by over 20 years. At present the picture is confused. It remains to be seen whether in-vivo work bears fruit or follows a similar path to that of D2.

Measurement of eCB levels has yielded more consistent data. Raised AEA in schizophrenia has been a replicable observation. So far, it has been claimed that elevated AEA constitutes a protective response. Weak effects on general symptom scores have been reported, but any inhibitory effect on positive psychotic symptoms is less convincing. Further work in larger samples is necessary to ascertain whether AEA has any effect on transition from an at-risk mental state to frank illness. Animal studies have shown that many of the effects of AEA are mediated via TRPV1 as opposed to CB1 receptors. Thus it is unclear how THC-associated psychoses (mediated via CB1) and changes in AEA levels fit together.

In the quote that began this chapter, Carlsson called for more attention into micro and macro-circuit organization, rather than purely biochemical observation. In keeping with this proposal, the most notable advances in understanding the neurochemistry of eCBs have come, not from assays of eCB release, but from electropharmacological studies. The role of the ECS in, for example, retrograde signaling, LTD and spike-timing dependent plasticity could not have been uncovered by purely biochemical assays. If eCBs are involved in schizophrenia, it is safe to say that such processes will be affected and would manifest as abnormalities in information-processing.

References

Calabresi, P., Picconi, B., Tozzi, A. and Di Filippo, M. (2007) Dopamine-mediated regulation of corticostriatal synaptic plasticity. *Trends Neurosci*, **30**:211–19.

Carlsson, A. (2001) A half-century of neurotransmitter research: impact on neurology and psychiatry. Nobel lecture. *Biosci Rep*, **21**:691–710.

De Marchi, N., De Petrocellis, L., Orlando, P., Daniele, F., Fezza, F. and Di Marzo, V. (2003) Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids Health Dis*, **2**:5.

Dean, B., Sundram, S., Bradbury, R., Scarr, E. and Copolov, D. (2001) Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience*, **103**:9–15.

Eggan, S.M., Hashimoto, T. and Lewis, D.A. (2008) Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. *Arch Gen Psychiatry*, **65** : 772–84 .

Gainetdinov, R.R., Sotnikova, T.D. and Caron, M.G. (2002) Monoamine transporter pharmacology and mutant mice. *Trends Pharmacol Sci*, **23**:367–73.

Gerdeman, G.L., Ronesi, J. and Lovinger, D.M. (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nature Neuroscience*, **5**:446–51.

Giuffrida, A., Leweke, F.M., Gerth, C. W., et al. (2004) Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology*, **29**:2108–14.

Giuffrida, A., Parsons, L.H., Kerr, T.M., Rodriguez de Fonseca, F., Navarro, M. and Piomelli, D. (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nature Neuroscience*, **2**:358–63.

Jung, K.M., Mangieri, R., Stapleton, C., et al. (2005) Stimulation of endocannabinoid formation in brain slice cultures through activation of group I metabotropic glutamate receptors. *Mol Pharmacol*, **68**:1196–202.

Koethe, D., Giuffrida, A., Schreiber, D., et al. (2009) Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. *Br J Psychiatry*, **194**:371–2.

Koethe, D., Llenos, I. C., Dulay, J. R., et al. (2007) Expression of CB1 cannabinoid receptor in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression. *J Neural Transm*, **114**:1055–63.

Kreitzer, A.C. and Malenka, R.C. (2007) Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature*, **445**:643–7.

Leweke, F.M., Giuffrida, A., Wurster, U., Emrich, H.M. and Piomelli, D. (1999) Elevated endogenous cannabinoids in schizophrenia. *Neuroreport*, **10**:1665–9.

Maccarrone, M., Rossi, S., Bari, M. et al. (2008) Anandamide inhibits metabolism and physiological actions of 2-arachidonoyl glycerol in the striatum. *Nature Neuroscience*, **11**:152–9.

McKenna, P. (2007) *Schizophrenia and Related Syndromes*. Routledge, London and New York.

Musella, A., De Chiara, V., Rossi, S., et al. (2009) TRPV1 channels facilitate glutamate transmission in the striatum. *Mol Cell Neurosci*, **40**:89–97.

Newell, K.A., Deng, C. and Huang, X.F. (2006) Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. *Exp Brain Res*, **172**:556–60.

Patel, S., Rademacher, D.J. and Hillard, C. J. (2003) Differential regulation of the endocannabinoids anandamide and 2-arachidonoylglycerol within the limbic forebrain by dopamine receptor activity. *J Pharmacol Exp Ther*, **306**:880–8.

Pertwee, R. G. (2006) Cannabinoid pharmacology: the first 66 years. *Br J Pharmacol*, **147** Suppl 1:S163–71.

Robbe, D., Kopf, M., Remaury, A., Bockaert, J. and Manzoni, O.J. (2002) Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc Natl Acad Sci USA*, **99**:8384–8.

Ronesi, J., Gerdeman, G.L. and Lovinger, D.M. (2004) Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. *J Neurosci*, **24**:1673–9.

Shen, W., Flajolet, M., Greengard, P. and Surmeier, D.J. (2008) Dichotomous dopaminergic control of striatal synaptic plasticity. *Science*, **321**:848–51.

Tzavara, E.T., Li, D.L., Moutsimilli, L., et al. (2006) Endocannabinoids activate transient receptor potential vanilloid 1 receptors to reduce hyperdopaminergia-related hyperactivity: therapeutic implications. *Biol Psychiatry*, **59**:508–15.

Uchigashima, M., Narushima, M., Fukaya, M., Katona, I., Kano, M. and Watanabe, M. (2007) Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J Neurosci*, **27**:3663–76.

Uriguen, L., Garcia-Fuster, M.J., Callado, L.F. et al. (2009) Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: effect of antipsychotic treatment. *Psychopharmacology (Berl)*, **206**:313–24.

Wickens, J.R. (2009) Synaptic plasticity in the basal ganglia. *Behav Brain Res*, **199**:119–28.

Yin, H.H., Mulcare, S.P., Hilario, M.R., et al. (2009) Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nature Neuroscience*, **12**:333–41.

Zavitsanou, K., Garrick, T. and Huang, X.F. (2004) Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*, **28**:355–60.

Chapter

18

The acute effects of cannabinoids in patients with psychotic illness

Cécile Henquet, Andrew Sewell, Rebecca Kuepper, Mohini Ranganathan and Deepak Cyril D'Souza

As discussed in other chapters in this book, the acute effects of cannabis and cannabinoids in healthy humans have been well characterized. This chapter will review the acute effects of cannabis and cannabinoids in people with psychotic illness.

Epidemiology

Schizophrenia patients have a higher lifetime risk of having substance misuse (abuse or dependence) than the general population (Regier *et al*., 1990; Cantor-Graae *et al*., 2001; McCreadie, 2002; Kessler *et al*., 2005; Swartz *et al*., 2006; Ringen *et al*., 2008). The three substances most commonly misused by schizophrenia patients are tobacco, alcohol, and cannabis (McCreadie, 2002; Margolese *et al*., 2004); of illicit drugs, cannabis is the most frequently used (Mueser *et al*., 1992; Cuffel *et al*., 1993; Linszen *et al*., 1994; Kessler *et al*., 1995; Hambrecht and Hafner, 1996; Farrell *et al*., 1998; Fowler *et al*., 1998; Jablensky *et al*., 2000; Bersani *et al*., 2002; Buhler *et al*., 2002; McCreadie, 2002; Green *et al*., 2005). Data from 53 treatment studies of schizophrenia patients revealed that 12-month prevalence estimates of use and misuse of cannabis were 29% and 19%, respectively, and lifetime use and misuse estimates were 42% and 23%, respectively (Green *et al*., 2005). In a recent meta-analysis (see Figure 18.1), median prevalence rate of current cannabis abuse in schizophrenia patients was 20% and that of cannabis dependence 31%, while the lifetime rates were 12% for abuse and 26% for dependence (Koskinen *et al*., 2009). The median rate of cannabis misuse was higher in first-episode (current 28.6%, lifetime 44.4%) than chronic patients (current 22.0%, lifetime 12.2%). The authors concluded that about 25% of schizophrenia patients in clinical samples carry a diagnosis of cannabis misuse. In contrast, the rate of cannabis abuse and dependence in the general population has been estimated to be 1.13% and 0.32%, respectively (Compton *et al*., 2004).

Information about the acute effects of cannabis and cannabinoids on individuals with, without or at risk for schizophrenia comes from retrospective self-reports, real-time self-reports and experimental studies.

Effects of cannabis in high-risk groups and recently diagnosed patients

Retrospective self reports

High levels of schizotypy overlap with less severe states of psychotic illness. Barkus *et al*. investigated cannabis effects in individuals with high versus average "psychosis-proneness" (Barkus and Lewis, 2008). Psychosis proneness was determined psychometrically using the Schizotypal Personality Questionnaire (SPQ) (Raine, 1991), and the authors developed a self-report questionnaire, the Cannabis Experiences Questionnaire (CEQ), to investigate acute "pleasurable experiences" and "psychosis-like experiences" as well as "after-effects" following cannabis use (Barkus *et al*., 2006). They showed that individuals with high psychosis-proneness reported higher levels of both pleasurable experiences and psychosis-like experiences when smoking cannabis than did controls. Stirling *et al*. further investigated the effects of high schizotypy on Δ^9-tetrahydrocannabinol (THC) sensitivity and found that high-scoring schizotypes were more likely to report psychosis-like experiences and unpleasant after-effects following cannabis exposure (Stirling *et al*., 2008).

Another study examined the effects of cannabis use in patients recently diagnosed or at high risk for schizophrenia (Peters *et al*., 2009). The authors developed an in-house "yes-no" questionnaire of acute effects of cannabis compiled from previous studies on subjective

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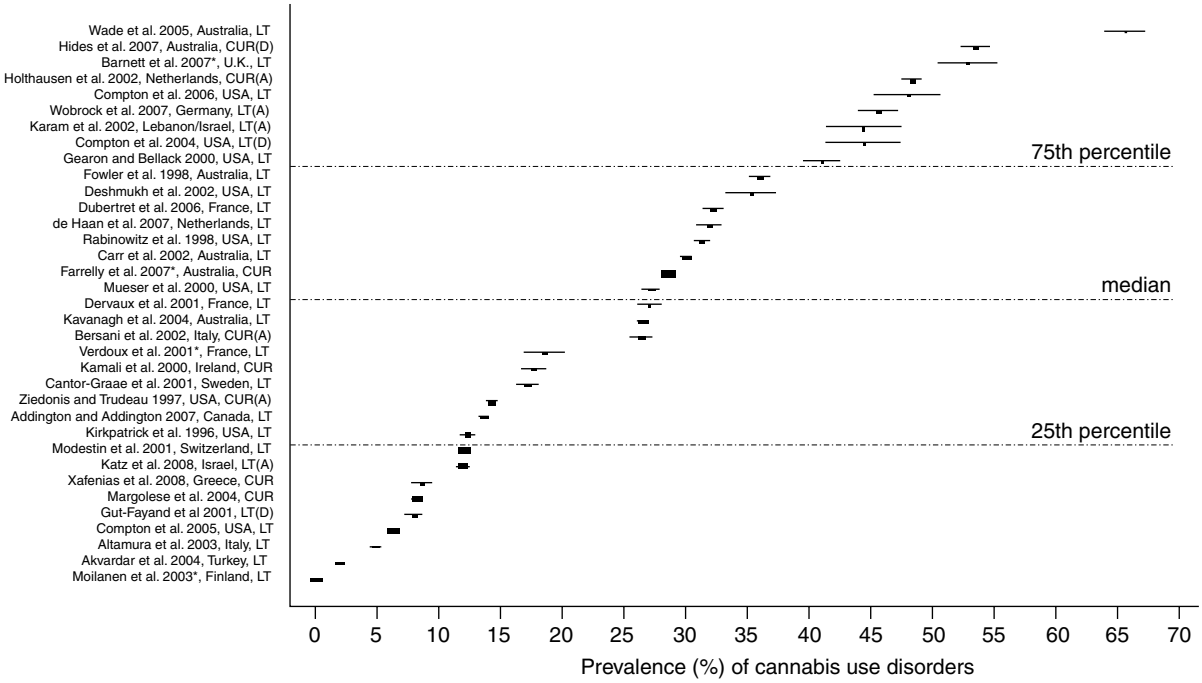


Figure 18.1. Meta-analysis of prevalence of cannabis use disorders in schizophrenia.

effects of cannabis or drugs in general in schizophrenia patients, and a list of chronic effects of cannabis relating to prodromal signs of schizophrenia from the literature on long-term effects of frequent cannabis use in subjects without major psychiatric illness. Recent-onset subjects (diagnosed using the Inventory for the Retrospective Assessment of the Onset of Schizophrenia) were asked to rate only those effects that they had experienced before the first onset of their psychosis. High-risk patients reported feeling more anxious, depressed and suspicious soon after cannabis use, but some also felt less depressed. Recently diagnosed schizophrenia patients also reported increased visual and auditory hallucinations and confusion after cannabis use. Both patient groups reported the long-term effects of cannabis use to be depression, less control over thoughts and social problems. Finally, a large proportion (37%) of recent-onset schizophrenia patients reported that their very first psychotic symptoms occurred during cannabis intoxication.

One recent report described repeated paranoid psychosis precipitated by smoking "Spice," a recently available blend of the synthetic cannabinoids CP47 497 and JWH-018. A 25-year-old man with a strong family history of schizophrenia and a psychotic break at age 18 years precipitated by smoking cannabis, with several further psychotic episodes thereafter, all triggered by smoking cannabis, smoked spice on three separate occasions and developed psychosis on each occasion, marked by auditory command hallucinations and paranoid delusions, which were new symptoms for him (Muller *et al.*, 2010). Other reports have noted psychotic episodes induced by these synthetic cannabinoids in healthy people (Missouri Department of Health and Senior Services, 2010; Vearrier and Osterhoudt, 2010).

Retrospective clinical data

Self-reported cannabis use is high among patients who describe positive effects from cannabis use, such as improved mood and sleep, and lessened social anxiety, even though cannabis use seems to worsen psychotic symptoms during the course of illness and negatively impacts the disease course (Grech *et al.*, 2005; Linszen and van Amelsvoort, 2007 and see Chapter 9). Most studies of first-episode patients demonstrate that substance misuse typically precedes psychosis onset, often by several years (Silver and Abboud, 1994; Rabinowitz *et al.*, 1998; Buhler *et al.*, 2002; Mauri *et al.*, 2006), and this is particularly true of cannabis misuse (Allebeck *et al.*, 1993;

Linszen *et al.*, 1994). It is less clear the extent to which substance misuse precedes prodromal symptoms, but two studies found that pre-prodromal substance misuse occurred in 28% to 34% of schizophrenia patients (Hambrecht and Hafner, 1996; Veen *et al.*, 2004). Several studies of first-episode psychosis have found an earlier age at onset for individuals with a history of comorbid substance use (Rabinowitz *et al.*, 1998; Van Mastrigt *et al.*, 2004; Veen *et al.*, 2004) although others have not (Cantor-Graae *et al.*, 2001; Sevy *et al.*, 2001). In a study of first-episode psychosis, Compton *et al.* showed that *progression* to daily cannabis use increases the risk for prodromal symptoms and psychotic illness, although daily cannabis use in itself does not (Compton *et al.*, 2009). The authors concluded that an increase in cannabis use may hasten the onset of prodromal as well as psychotic symptoms.

Acute effects of cannabis in established schizophrenia patients

Retrospective self reports

Few studies have investigated patients' subjective experiences following cannabis use. The acute effects of cannabis in schizophrenia patients are reported to include no effects (Peralta and Cuesta, 1992), reductions in anxiety, depression and negative symptoms, increased suspiciousness and variable effects on hallucinations (Dixon *et al.*, 1990; Arndt *et al.*, 1992; Peralta and Cuesta, 1992). Dixon *et al.* assessed the self-reported acute effects of alcohol, cannabis and cocaine in 40 patients with schizophrenia or schizophreniform psychosis. The majority reported decreased dysphoria from all three drugs, decreased anxiety from cannabis and alcohol, increased anxiety from cocaine, and increased paranoia and hallucinations from cannabis and cocaine but not from alcohol. Weil found that many patients reported derealization from cannabis (Weil, 1970). In contrast, Hekimian and Gershon described favorable subjective responses in cannabis-using patients with schizophrenia (Hekimian and Gershon, 1968). Knudsen and Vilmar found that patients reported feeling "inspired, relaxed, energized or active" following cannabis use, and also used cannabis to reverse the side effects of antipsychotic medication (Knudsen and Vilmar, 1984). After these initial positive effects, however, patients described an exacerbation of positive psychotic symptoms and increases in dysphoria and aggression.

Reports of the subjective effect of cannabis use on psychotic symptoms have been contradictory. Cannabis was reported by some patients to be unpleasant and to cause adverse psychic effects (Negrete *et al.*, 1986). Treffert followed four schizophrenia patients longitudinally and found severe exacerbations of psychosis and functional deterioration after periods of moderate cannabis use (Treffert, 1978), and more than 50% of subjects in a later study found that cannabis increased their positive symptoms (Addington and Duchak, 1997). By others, cannabis was reported to reverse neuroleptic effects and inspire, energize or relax (Knudsen and Vilmar, 1984); in another study, one half of 45 participants (50.9%) reported that they were using drugs (including cannabis) or alcohol to cope with or reduce auditory hallucinations (Gregg *et al.*, 2009). Slightly more (57.4%) were using drugs to abate feelings of suspiciousness or paranoia, and two out of five (38.7%) were using drugs when they were experiencing medication side effects. In another study, chronically treated psychotic patients were asked a series of questions on how their antipsychotic medication affected their psychosis (Kapur *et al.*, 2005). Among the most common reported effects was that the medication "helps me stop thinking" so that "the symptoms do not bother me so much." It is possible that from the patients' viewpoint cannabis use is beneficial because it decreases their preoccupation with psychotic symptoms, even as it increases symptoms as measured objectively. Alternatively, while cannabis may increase psychotic symptoms, cannabis may also reduce symptom-related distress such that schizophrenia patients may experience the overall effects of cannabis as "beneficial."

One area that remains largely unexplored is drug users' *stated reasons for drug use* – what they *believe* leads them to use. These perceptions, however inaccurate, may themselves drive drug-taking behavior and thus merit further investigation (Dixon *et al.*, 1991). Behavior is often based on attitudes that are shaped by beliefs (Fishbein, 1980). Beliefs that are based on personal experience have a stronger influence in the formation of attitudes than information gained in other ways, and better predict later behavior (Fazio and Zanna, 1981). Thus, what drugs users believe are their reasons for using drugs may be a crucial determinant of their drug-use behavior, including whether they continue to use or relapse.

Numerous qualitative studies have been conducted in North America, Australia and the United Kingdom

investigating self-reported reasons for drug use in patients with psychotic disorders. Some protocols involve patients selecting reasons for use from predetermined lists (Test *et al*., 1989; Dixon *et al*., 1991; Warner *et al*., 1994; Addington and Duchak, 1997); whereas others ask open-ended questions (Baigent *et al*., 1995; Fowler *et al*., 1998). Despite differences in methodology, results are similar. There are three main motives for drug use, regardless of the drug type:

1. to enhance positive mood or achieve intoxication: "get high" (Dixon *et al*., 1991) or "feel good" (Fowler *et al*., 1998);
2. to cope with negative emotions: "decrease depression" or "relax" (Dixon *et al*., 1991; Addington and Duchak, 1997; Fowler *et al*., 1998; Gearon *et al*., 2001; Baker *et al*., 2002; Spencer *et al*., 2002; Goswami *et al*., 2004; Green *et al*., 2004; Schofield *et al*., 2006);
3. for social reasons: "something to do with friends" (Test *et al*., 1989), and "to face people better" (Fowler *et al*., 1998).

In a review of 14 studies specifically addressing self-reported reasons for cannabis use in patients with psychotic disorders, Dekker concluded that these three reasons were the most cited, with only a minority (12.9%) reporting cannabis use as a means to relieve medication side effects or symptoms of psychosis such as hallucinations and suspiciousness (Dekker *et al*., 2009). People with psychosis appear more likely to use cannabis for mood elevation than for relaxation, less likely to use out of habit or for social reasons and more likely to use in order to cope with boredom or other negative affective states (Green *et al*., 2004).

However, retrospective self-report data are subject to distortion. Individuals who misuse substances typically use denial and rationalization to justify their use. In addition, cannabis alters perception and has amnestic effects that may influence the interpretation of events and therefore interfere with the accurate recall of cannabis effects. Cannabis is often used in combination with nicotine, alcohol and other illicit drugs, so it is difficult to attribute consequences solely to cannabis in naturalistic studies. Finally, it is possible that the positive and negative effects of cannabis may be dose-related, and dose–response relationships are almost impossible to assess in naturalistic studies because cannabis dose is seldom measured, and its principal psychoactive ingredient (THC) is not assayed. Some limitations of self report can be addressed through experimental studies as well as experience sampling (see Table 18.1).

Experience sampling

Although some studies have consistently shown that patients are more sensitive to the acute negative effects of cannabis, the beneficial effects of cannabis use that patients themselves report, often lead them to continue to use cannabis despite long-term negative consequences. Evidence that patients may use cannabis to "self-medicate" distress associated with their illness comes from a population-based study that linked vulnerability for psychosis in cannabis-naïve individuals with future cannabis use (Ferdinand *et al*., 2005). However, other population-based studies found no such evidence for self-medication effects (Stefanis *et al*., 2004; Henquet *et al*., 2005). Recently, Henquet *et al*. conducted a *momentary assessment study* to investigate the complicated dynamics of cannabis use and its varied effects in psychotic patients in the context of daily life by using the Experience Sampling Method (ESM) (Myin-Germeys *et al*., 2001, 2002). The ESM is a pseudo-random time-sampling self-assessment technique. Subjects receive a digital wristwatch, and a paper-and-pen ESM booklet. Twelve times a day on six consecutive days, the watch beeps randomly once in each 90-minute time block between 7:30 a.m. and 12:30 p.m. After each beep, subjects complete 7-point Likert scales on affect, thoughts, symptom severity, and activity at the moment of the beep. This permits a systematic observation of recreational cannabis use in daily life. Previous studies using ESM have demonstrated its feasibility, validity, and reliability in schizophrenia patients. The study investigated the acute effects of cannabis on mood and psychotic symptoms in the daily life of 42 patients with a psychotic disorder and 38 healthy controls who were all regular cannabis users. Not only was the frequency of cannabis use significantly higher in patients than in controls, but there was no evidence for self-medication, as neither positive nor negative affect predicted cannabis use at the next sampling point. Similarly, no associations were found between delusions or hallucinations and subsequent cannabis use. Cannabis acutely induced hallucinatory experiences in patients but not healthy controls, and decreases in negative affect were observed after cannabis use in patients but not in controls, indicating that patients were also much more sensitive to the mood-enhancing effects of cannabis. In addition, patients were more sensitive to the increased sociability seen

Table 18.1. Effects of cannabis use on subsequent symptom levels, patients versus controls.

		Cannabis – mean (SD)	Cannabis + mean (SD)	Cannabis effect size [1]	Group X cannabis [2]
Positive affect	controls	4.99 (1.14)	5.12 (1.17)	β = 0.18, 95% CI: 0.07, 0.28; $p = 0.01$	$\chi^2 = 0.98°$; $p = 0.32$
	patients	4.30 (1.26)	4.46 (1.32)	β = 0.24, 95% CI: 0.15, 0.35; $p < 0.001$	
Negative affect	controls	1.35 (0.65)	1.29 (0.70)	β = 0.03, 95% CI: –0.05, 0.10; $p = 0.47$	$\chi^2 = 6.43°$; $p = 0.011$
	patients	1.96 (1.16)	1.78 (0.96)	β = –0.10, 95% CI: –0.17, –0.03; $p = 0.0043$	
Delusions	controls	1.87 (0.82)	1.87 (0.89)	β = 0.02, 95% CI: –0.06, 0.10; $p = 0.70$	$\chi^2 = 1.11°$; $p = 0.28$
	patients	2.47 (1.25)	2.45 (1.26)	β = –0.05, 95% CI: –0.12, 0.03; $p = 0.25$	
Hallucinations	controls	1.00 (0.07)	1.00 (0.13)	β = 0.01, 95% CI: –0.04, 0.06; $p = 0.75$	$\chi^2 = 3.66°$; $p = 0.056$
	patients	1.38 (0.88)	1.40 (0.95)	β = 0.08, 95% CI: 0.03, 0.13; $p = 0.002$	
Auditory hallucinations	controls	1.00 (0.08)	1.01 (0.25)	β = 0.01, 95% CI: –0.06, 0.08; $p = 0.72$	$\chi^2 = 3.36°$; $p = 0.067$
	patients	1.40 (1.03)	1.50 (1.21)	β = 0.11, 95% CI: 0.04, 0.17; $p = 0.003$	

[1] Regression coefficient indicates change in symptom score associated with no use (cannabis –) versus use (cannabis +), analyses adjusted for age, gender, alcohol use, overall level of cannabis use during the experience sampling week and symptom level at the previous beep;
[2] Chi-squared (df = 1) test for the interaction term, adjusted for age, gender, alcohol use, overall level of cannabis use during the experience sampling week and symptom level at the previous beep.

with cannabis – preference for being alone decreased under the influence of cannabis, while no such effect was observed in the control group.

Post-hoc analysis assessing the duration of cannabis' effects on mood and hallucinations in the patient group suggested that cannabis may have biphasic effects on mood and psychotic symptoms, with increases in positive affect observed in the short-term (immediately after cannabis use), but not in the long term (several hours later). The fact that pro-hallucinatory effects of cannabis did not occur immediately shows that cannabis may have immediate positive effects on mood followed by later negative effects on psychotic symptoms. The authors concluded that this delay between immediate reward and negative consequences might explain why schizophrenia patients continue to use cannabis.

The experience-sampling approach addresses some of the limitations of retrospective self-report. Experimental approaches address some of the limitations of the experience-sampling approach by using standardized doses, standardized delivery, objective assessments of symptoms and performance-based measures of memory, attention and executive function.

Experimental exposure to THC

Only two published studies have administered THC to schizophrenia patients directly in order to study acute effects on psychosis outcomes, cognition and side effects. D'Souza *et al.* conducted a randomized, double-blind, placebo-controlled study in order to investigate whether schizophrenia patients were more vulnerable than healthy controls to the effects of THC on cognition and psychotic symptoms (D'Souza *et al.*, 2005). The study included subjects with past cannabis experience but without lifetime cannabis misuse or lifetime misuse of drugs other than nicotine. Controls were healthy subjects; neither they nor their immediate family had any history of DSM-IV axis I disorders. Thirteen stable antipsychotic-medicated schizophrenia patients and 22 healthy controls were given 0 mg,

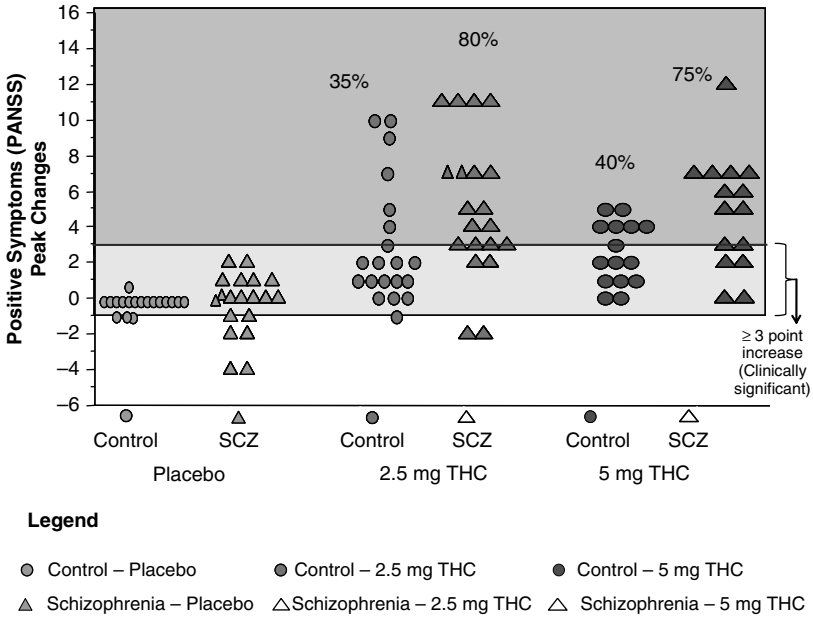


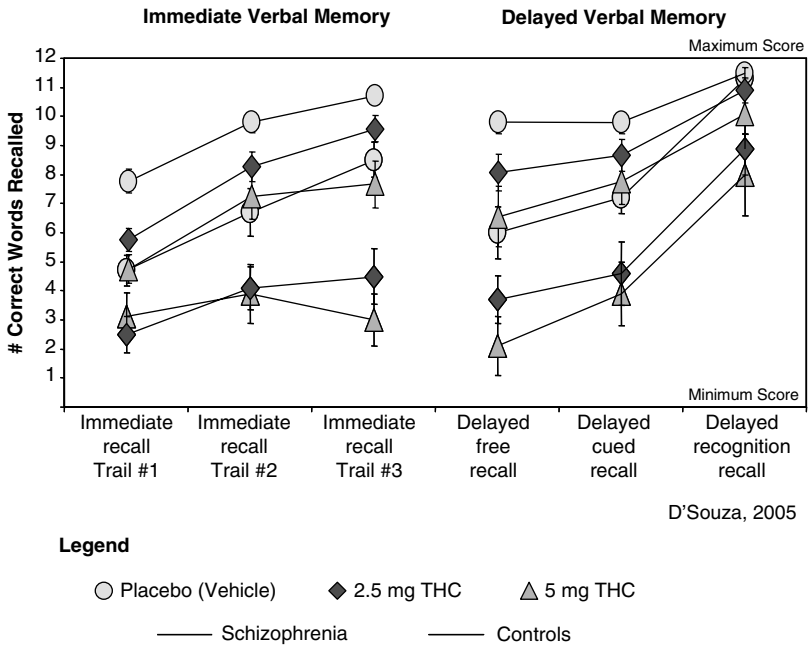
Figure 18.2. Enhanced sensitivity to the amnestic effects of Δ^9-tetrahydrocannabinol (THC) in schizophrenia. See also color plate section.

Peak increase in positive symptoms measured by the positive symptoms subscale of the Positive and Negative Symptoms Scale (PANSS) (group means ± 1 SD).

Clinically significant increase = 3 point or greater increase in PANSS positive symptom subscale score.

2.5 mg and 5 mg THC intravenously in a three-day, double-blind, randomized, counterbalanced study. Test days were separated by at least a week (more than three times the elimination half-life of THC) in order to minimize carryover effects. Subjects refrained from consuming caffeine, alcohol and illicit drugs for two weeks before testing until study completion and urinary testing confirmed self-reported abstinence. Symptoms of schizophrenia were assessed at several time points following THC or placebo administration by means of the Positive and Negative Syndrome Scale (PANSS) (Kay *et al*., 1986). A three-point or greater increase on the PANSS positive symptom subscale was considered a clinically significant response. Acute THC effects on neuropsychological functioning were tested using a verbal fluency test (Corkin *et al*., 1964), the Hopkins Verbal Learning Test (Brandt, 1991) for learning and immediate and delayed recall, and a continuous performance test (Gordon, 1986) to measure attention. Motor side effects were measured using the Abnormal Involuntary Movement Scale (AIMS) for dyskinesias, the Barnes Akathisia Scale for akathisisa and the Simpson Angus Scale (SAS) for parkinsonism.

There were similarities between the two groups (see Figure 18.2), as well as differences. Δ^9-tetrahydrocannibinol acutely impaired immediate recall, delayed-free recall and delayed-cued recall in a dose-dependent fashion, as well as increasing omission errors in the attention task. Schizophrenia patients were more sensitive to the cognitive effects of cannabis, particularly impairment of memory and attention, although there were no significant group-by-dose interactive effects. Δ^9-tetrahydrocannibinol had no effects on verbal fluency, nor did it make subjects more calm and relaxed. Δ^9-tetrahydrocannibinol transiently increased positive symptoms in both schizophrenia patients and matched healthy controls. These effects were dose-related, occurred 10 to 20 minutes after THC administration, and resolved within four hours. Eighty percent of the schizophrenia patients but only 35% of control subjects had a clinically significant increases in psychosis in response to 2.5 mg THC and 75% of schizophrenia patients but only 50% of control subjects had a suprathreshold response to 5 mg (see Figure 18.3). There was no interaction between group, dose and time, nor was there a difference between



Effects of THC on the learning, immediate free recall, delayed free recall, delayed cued and recognition recall measured by a 12-word learning task (Hopkins Verbal Learning Test)

Figure 18.3. Enhanced sensitivity to the psychotomimetic effects of Δ^9-tetrahydrocannabinol (THC) in schizophrenia. See also color plate section.

groups in the effect of THC on other measures, such as feeling high (VAS) or perceptual alterations (on the Clinician-Administered Dissociative States Scale). Δ^9-tetrahydrocannabinol increased total scores on the AIMS (dyskinesia) as well as on the SAS (rigidity). Plasma THC and 11-nor-Δ^9-carboxy-THC levels were the same in both patient and control groups.

To summarize, THC transiently exacerbated a range of positive and negative psychotic symptoms, perceptual alternations, cognitive deficits and medication side effects associated with schizophrenia without producing clear beneficial effects. More schizophrenia patients than controls had clinically significant psychotic exacerbation in response to THC administration, and schizophrenia patients were more vulnerable to the negative effects of THC on learning and memory, despite maintenance on stable therapeutic doses of antipsychotic medications. This study indicated that individuals with an established psychotic disorder show abnormal sensitivity to the cognitive and the psychotogenic effects of THC, a finding that has been described in epidemiological studies as well (van Os *et al*., 2002; Henquet *et al*., 2005).

A second study also investigated differential sensitivity to the acute effects of THC in psychotic patients (Henquet *et al*., 2006). In a double-blind, placebo-controlled cross-over study, subjects smoked a tobacco cigarette containing either 300 μg/kg THC or 0 μg THC during two test sessions separated by one week. Thirty patients with a DSM-IV diagnosis of a psychotic disorder, 12 relatives of patients with a psychotic disorder and 32 healthy controls were enrolled; excluded were cannabis-naïve subjects, subjects with weekly use of drugs other than cannabis and subjects drinking more than five units/day of alcohol. Fifteen minutes after cigarette inhalation, subjects took a neuropsychological test battery that included a visual verbal learning test and the Abstract Visual Patterns Learning test (which measures memory storage and retrieval of verbal information), a continuous performance test (CPT), the Stroop Color-Word test (measuring attention) and the Digit Symbol Substitution Test (measuring speed). Transient psychotic experiences were assessed using the 40-item Community Assessment of Psychic Experiences (CAPE), a self-report instrument that was developed to capture variation in the positive and negative dimensions of psychotic experiences as well as variation in depression in the general population (Konings and Maharajh, 2006) (http://cape42.homestead.com). As subclinical psychotic experiences

measured with the CAPE show continuity with less severe states of psychosis seen in psychotic illness and are transmitted in families, CAPE scores are considered a proxy for underlying psychosis liability. In this experimental study, the items of the CAPE were modified to measure momentary psychotic experiences during THC intoxication (CAPE-state). Catechol-O-methyltransferase (COMT) *Val*^{158}*Met* genotypes were also measured.

In both patients and controls, THC acutely impaired memory and attention. Interestingly, further comparison between patients and controls (unpublished data) showed only minor differences between groups in sensitivity to the cognitive effects of cannabis. In fact, patients seemed to be less sensitive to the verbal memory effects of THC than healthy controls, and there were no significant between-group differences in delayed free recall, delayed recognition or visual memory. Only the acute effects of THC on attention were more pronounced in patients than in controls. Reaction time on a CPT increased (i.e. attention deteriorated) in patients ($\beta = 1.25$, 95% CI: 0.002, 0.16, $p = 0.04$), but not in controls ($\beta = 0.18$, 95% CI: 0.35, 2.15, $p = 0.007$). Patients also had a significantly larger increase in Stroop interference score (the time needed to complete Card III relative to Cards I and II) after THC exposure than did controls ($\beta = 0.16$, 95% CI: 0.05, 0.27, $p = 0.004$ for patients and $\beta = 0.01$, 95% CI: –0.08, 0.11, $p = 0.8$ for controls). In addition, the *Val*^{158}*Val* genotype predicted increased sensitivity to the acute effects of THC on cognition. The *Val*^{158}*Met* genotype moderated the acute effects of THC on psychotic symptoms, but only in individuals with pre-existing elevated scores on the CAPE-trait questionnaire.

These seemingly contradictory findings of the effects of THC on cognition may explain why only a minority of the individuals exposed to THC develop psychotic symptoms. In addition, available data seem to suggest that higher-order interactions are necessary to explain individual differences in sensitivity to the acute effects of THC on cognition and psychosis. Δ^9-tetrahydrocannabinol sensitivity in patients and controls alike may be restricted to individuals with the *Val*^{158}*Val* genotype or specific variations in other genes.

D'Souza *et al.* reported that chronic cannabis smoking affects acute responses to THC in healthy subjects (D'Souza *et al.*, 2008). In a three-day, double-blind, placebo-controlled study, the dose-related effects of 0 mg, 2.5 mg and 5 mg intravenous THC were studied in 30 frequent users of cannabis and 22 healthy controls. Frequent users showed the same euphoric effects as controls, but fewer psychotomimetic, perceptual altering, cognitive impairing and anxiogenic effects. Their cortisol levels increased less and their prolactin levels were lower. These data suggest that frequent cannabis users either are: inherently tolerant to the psychotomimetic, perceptual altering, amnestic, endocrine and other effects of cannabinoids; develop tolerance; or both.

Similarly, Ramaekers *et al.* investigated the effects of high doses of THC (500 μg/kg) on neuropsychological performance in 12 heavy (more than four days a week) and 12 occasional (weekly use or less) users and found THC-induced performance decrements only in the occasional users (Ramaekers *et al.*, 2009). Di Forti *et al.* recently showed that patients are more likely to use cannabis for longer periods and with greater frequency than healthy controls; this increased use might actually mitigate the negative effects of cannabis smoking in some patients (Di Forti *et al.*, 2009). The authors investigated patterns of cannabis use in 280 patients with first-episode psychosis and 174 healthy controls. There were no between-group differences in age of first use, but patients were more likely to be current daily users and to have smoked cannabis for more than five years. In addition, the patient group used more "skunk" (high-potency cannabis) than controls. Mason also investigated the moderating effects of prior exposure to cannabis on psychosis outcome (PSI scores) and found, in line with D'Souza's work, that lower-frequency cannabis users showed greater acute psychotomimetic responses to cannabis (Mason *et al.*, 2008).

In a recent case series, Schwarcz *et al.* administered dronabinol to six treatment-resistant schizophrenia patients who reported improvement in symptoms with past use of cannabis (Schwarcz *et al.*, 2009). Using a fixed schedule (5 mg dronabinol daily for the first week, 10 mg daily during the second week, and 20 mg daily during the third week) they documented clinical improvement on the Brief Psychiatric Rating Scale items of *conceptual disorganization*, *hallucinatory behavior*, *suspiciousness* and *unusual thought content*, as well as improvement in overall functioning measured with the Clinical Global Impression scale. In eight more chronic treatment-refractory schizophrenia patients treated with dronabinol, four showed no change, but four showed remarkable improvement in paranoia and agitation (Schwarcz, personal communication, 2010). Although the study lacked a control

group, this suggests that there is a subset of schizophrenia patients for whom THC does not worsen symptomatology and that patients differ in their sensitivity to the acute effects of THC.

The psychoactive effects of cannabis increased with THC content. It is important to note, however, that THC is not equivalent to cannabis, which contains nearly 70 other cannabinoids (ElSohly and Slade, 2005), of which THC is the most active. All the experimental studies administered THC, albeit using differing routes of administration: intravenous (D'Souza *et al*., 2005), smoked (Henquet *et al*., 2006) and oral (Schwarcz *et al*., 2009). However, more recently, some of the other constituents of cannabis are receiving greater attention. One such compound is cannabidiol.

Effects of cannabidiol

Cannabidiol is not pro-psychotic and appears to ameliorate the psychotomimetic effects of herbal cannabis (Rottanburg *et al*., 1982; Solomons *et al*., 1990; Morgan and Curran, 2008). Cannabidiol has been shown to have anxiolytic and antipsychotic effects (Leweke *et al*., 2005; Zuardi *et al*., 2006), leading to the suggestion that cannabidiol may offset some of the adverse effects of THC. In patients with psychotic illness, cannabidiol appears to act like an antipsychotic. Zuardi *et al*. reported that open-label cannabidiol treatment of four schizophrenia patients for four weeks resulted in a 50% response rate (Zuardi *et al*., 1995; Zuardi *et al*., 2006) and the two patients who did not respond to cannabidiol were also refractory to clozapine. A double-blind, active-controlled, clinical trial comparing cannabidiol with the prototypical antipsychotic amisulpiride in the treatment of forty schizophrenia patients suggested that cannabidiol was well tolerated, with antipsychotic efficacy equal to amisulpiride (Leweke *et al*., 2005). Finally, a recent open-label, four-week study in six outpatients with Parkinsons Disease and psychosis showed that cannabidiol was well tolerated and markedly reduced the psychotic symptoms (Zuardi *et al*., 2009). Thus, preclinical data and a small body of clinical data suggest that cannabidiol may be a well-tolerated and efficacious antipsychotic medication (see also Chapter 2).

Conclusions

Cannabis use and misuse is common in people with schizophrenia. Individuals with a history of comorbid cannabis use may have an earlier age at onset of psychosis. Furthermore, an increase in cannabis use may hasten the onset of prodromal as well as psychotic symptoms.

Collectively, momentary assessment data, epidemiological findings and laboratory experiments suggest that patients with a psychotic disorder show increased sensitivity to the pro-psychotic effects of cannabis and THC. Self-report data indicates that patients experience rewarding effects of cannabis immediately and negative effects on psychotic symptoms only later. The combination of delayed negative effects and increased sensitivity to immediate rewarding effects may explain psychotic patients' persistent cannabis use. According to this model, proposed by Spencer *et al*., use of cannabis is driven by expectations about the acute effects of cannabis (Spencer *et al*., 2002). Subacute negative psychotic effects are then experienced as evidence that more use is necessary in order to bring about the anticipated rewarding effects. This motivation to improve mood reinforces use and promotes cannabis dependence, despite the long-term negative impact cannabis may have on functional outcome. There is little evidence to support the "self-medication" hypothesis, at least in its original form.

Finally, tantalizing evidence suggests that the beneficial effects of cannabis that patients report, may in part be due to a heretofore unrecognized and little-studied component of cannabis: cannabidiol. Ongoing clinical trials are likely to provide more definitive evidence regarding the effects of cannabidiol in schizophrenia.

References

Addington, J. and Duchak, V. (1997) Reasons for substance use in schizophrenia. *Acta Psychiatr Scand,* **96**: 329–33.

Allebeck, P., Adamsson, C., Engström, A. and Rydberg, U. (1993) Cannabis and schizophrenia: a longitudinal study of cases treated in Stockholm County. *Acta Psychiatr Scand*, **88**:21–4.

Arndt, S., Tyrrell, G., Flaum, M. and Andreasen, N.C. (1992) Comorbidity of substance abuse and schizophrenia: the role of pre-morbid adjustment. *Psychol Med,* **22**:379–88.

Baigent, M., Holme, G. and Hafner, R.J. (1995) Self reports of the interaction between substance abuse and schizophrenia. *Aust N Z J Psychiatry,* **29**:69–74.

Baker, A., Lewin, T., Reichler, H., *et al*. (2002). Motivational interviewing among psychiatric in-patients with substance use disorders. *Acta Psychiatr Scand,* **106**:233–40.

Barkus, E. and Lewis, S. (2008) Schizotypy and psychosis-like experiences from recreational cannabis in a non-clinical sample. *Psychol Med,* **38**:1267–76.

Barkus, E., Stirling, J., Hopkins, R.S. and Lewis, S. (2006) Cannabis-induced psychosis-like experiences are associated with high schizotypy. *Psychopathology,* **39**:175–8.

Bersani, G., Orlandi, V., Kotzalidis, G.D. and Pancheri, P. (2002) Cannabis and schizophrenia: impact on onset, course, psychopathology and outcomes. *Eur Arch Psychiatry Clin Neurosci,* **252**:86–92.

Brandt, J. (1991) The hopkins verbal learning test: Development of a new memory test with six equivalent forms. *The Clinical Neuropsychologist,* **5**:125–42.

Buhler, B., Hambrecht, M., Löffler, W., an der Heiden, W. and Häfner, H. (2002) Precipitation and determination of the onset and course of schizophrenia by substance abuse – a retrospective and prospective study of 232 population-based first illness episodes. *Schizophr Res*, **54**:243–51.

Cantor-Graae, E., Nordstrom, L. and McNeil, T.F. (2001). Substance abuse in schizophrenia: a review of the literature and a study of correlates in Sweden. *Schizophr Res,* **48**:69–82.

Compton, M.T., Kelley, M.E., Ramsay, C.E., *et al*. (2009) Association of pre-onset cannabis, alcohol, and tobacco use with age at onset of prodrome and age at onset of psychosis in first-episode patients. *Am J Psychiatry,* **166**:1251–7.

Compton, W.M., Grant, B.F., *et al*. (2004) Prevalence of marijuana use disorders in the United States: 1991–1992 and 2001–2002. *JAMA,* **291**:2114–21.

Corkin, S., Milner, B., Colliver, J.B, Glantz, M.D. and Stinson, F.S. (1964) Effects of different cortical excisions on sensory thresholds in man. *Trans Am Neurol Assoc,* **89**:112–16.

Cuffel, B., Heithoff, K. and Lawson, W. (1993) Correlates of patterns of substance abuse among patients with schizophrenia. *Hosp Community Psychiatry,* **44**:247–51.

D'Souza, D.C., Abi-Saab, W.M., Madonick, S., *et al*. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biol Psychiatry*, **57**:594–608.

D'Souza, D.C., Ranganathan, M., Braley, G., *et al*. (2008) Blunted psychotomimetic and amnestic effects of delta-9-tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology*, **33**:2505–16.

Dekker, N., Linszen, D. and De Haan, L. (2009) Reasons for Cannabis Use and Effects of Cannabis Use as Reported by Patients with Psychotic Disorders. *Psychopathology,* **42**:350–60.

Di Forti, M., Morgan, C., Dazzan, P., *et al*. (2009) High-potency cannabis and the risk of psychosis. *Brit J Psychiatry,* **195**: 88–491.

Dixon, L., Haas, G., Weiden, P., Sweeney, J. and Francis, A. (1990) Acute effects of drug abuse in schizophrenic patients: clinical observations and patients' self-reports. *Schizophr Bull,* **16**:69–79.

Dixon, L., Haas, G., Weiden, P., Sweeney, J. and Francis, A. (1991) Drug abuse in schizophrenic patients: clinical correlates and reasons for use. *Am J Psychiatry,* **148**:224–30.

Elsohly, M.A. and Slade, D. (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sci,* **78**:539–48.

Farrell, M., Howes, S., Taylor, C., *et al*. (1998). Substance misuse and psychiatric comorbidity: an overview of the OPCS National Psychiatric Morbidity Survey. *Addict Behav,* **23**:909–18.

Fazio, R. and Zanna, M. (1981) Direct Experience and Attitude-Behavior Consistency. *Advances in Experimental Social Psychology*. L. Berkowitz. Chicago, University of Chicago Press: 161–186.

Ferdinand, R.F., Sondeijker, F., van der Ende, J., Selten, J.P., Huizink, A. and Verhulst, F.C. (2005) Cannabis use predicts future psychotic symptoms, and vice versa. *Addiction,* **100**:612–8.

Fishbein, M. (1980). A theory of reasoned action: some applications and implications. *Nebr Symp Motiv,* **27**:65–116.

Fowler, I., Carr, V., Carter, N.T. and Lewin, T.J. (1998) Patterns of current and lifetime substance use in schizophrenia. *Schizophr Bull,* **24**:443–55.

Gearon, J. S., Bellack, A.S., Rachbeisel, J. and Dixon, L. (2001) Drug-use behavior and correlates in people with schizophrenia. *Addict Behav,* **26**:51–61.

Gordon, M. (1986) Microprocessor-based assessment of attention deficit disorders (ADD). *Psychopharmacol Bull,* **22**:288–90.

Goswami, S., Mattoo, S.K., Basu, D. and Singh, G. (2004) Substance-abusing schizophrenics: do they self-medicate? *Am J Addict,* **13**:139–50.

Grech, A., Van Os, J., Jone, P.B, Lewis, S.W. and Murray, R.M. (2005) Cannabis use and outcome of recent onset psychosis. *Eur Psychiatry,* **20**:349–53.

Green, A.I., Tohen, M.F., Hamer, R.M., *et al*. (2004) First episode schizophrenia-related psychosis and substance use disorders: acute response to olanzapine and haloperidol. *Schizophr Res,* **66**:125–35.

Green, B., Kavanagh, D.J., and Young, R.M. (2004) Reasons for cannabis use in men with and without psychosis. *Drug Alcohol Rev,* **23**:445–53.

Green, B., Young, R., and Kavanagh, D. (2005) Cannabis use and misuse prevalence among people with psychosis. *Br J Psychiatry,* **187**:306–13.

Gregg, L., Barrowclough, C., and Haddock, G. (2009) Development and validation of a scale for assessing reasons for substance use in schizophrenia: the ReSUS scale. *Addict Behav,* **34**:830–7.

Hambrecht, M. and Hafner, H. (1996) Substance abuse and the onset of schizophrenia. *Biol Psychiatry,* **40**:1155–63.

Hekimian, L.J. and Gershon, S. (1968) Characteristics of drug abusers admitted to a psychiatric hospital. *JAMA,* **205**:125–30.

Henquet, C., Krabbendam, L., Spauwen, J., *et al.* (2005) Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *BMJ,* **330**:11.

Henquet, C., Rosa, A., Krabbendam, L., *et al.* (2006) An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology,* **31**:2748–57.

Jablensky, A., McGrath, J., Herrman, H., *et al.* (2000) Psychotic disorders in urban areas: an overview of the Study on Low Prevalence Disorders. *Austr NZ J Psychiatry,* **34**:221–36.

Kapur, S., Mizrahi, R., and Li, M. (2005) From dopamine to salience to psychosis – linking biology, pharmacology and phenomenology of psychosis. *Schizophr Res,* **79**:59–68.

Kay, S.R., Opler, L.A., and Fizbein, A. (1986) Significance of positive and negative syndromes in chronic schizophrenia. *Br J Psychiatry,* **149**:439–48.

Kessler, R., Foster, C., Saunders, W.B. and Stang, P.E. (1995) Social consequences of psychiatric disorders, I: Educational attainment. *Am J Psychiatry,* **152**:1026–32.

Kessler, R.C., Chiu, W.T., Demler, O., Merikangas, K.R and Walters, E.E. (2005) Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry,* **62**:617–27.

Knudsen, P. and Vilmar, T. (1984) Cannabis and neuroleptic agents in schizophrenia. *Acta Psychiatr Scand,* **69**:162–74.

Konings, M. and Maharajh, H.D. (2006) Cannabis use and mood disorders: patterns of clinical presentations among adolescents in a developing country. *Int J Adolesc Med Health,* **18**:221–33.

Koskinen, J., Lohonen, J., Koponen, H., Isohanni, M and Mietunnen, J. (2009) Rate of Cannabis Use Disorders in Clinical Samples of Patients With Schizophrenia: A Meta-analysis. *Schizophr Bull*, **36**:1115–30.

Leweke, F., Koethe, D., *et al.* (2005) Cannabidiol as an Antipsychotic: A Double-Blind, Controlled Clinical Trial of Cannabidiol versus Amisulpiride in Acute Schizophrenia. *15th Annual Symposium on Cannabinoids*. Clearwater Beach, FL, Cannabinoid Research Society.

Linszen, D. and van Amelsvoort, T. (2007) Cannabis and psychosis: an update on course and biological plausible mechanisms. *Curr Opin Psychiatry,* **20**:116–20.

Linszen, D.H., Dingemans, P.M. and Lenior, M.E. (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. *Arch Gen Psychiatry,* **51**:273–9.

Margolese, H., Malchy, L., Negrete, J.C., Tempier, R. and Gill, K. (2004) Drug and alcohol use among patients with schizophrenia and related psychoses: levels and consequences. *Schizophr Res,* **67**:157–66.

Mason, O., Morgan, C.J., Dhiman, S.K., *et al.* (2008) Acute cannabis use causes increased psychotomimetic experiences in individuals prone to psychosis. *Psychol Med,* **39**:951–6.

Mauri, M., Volonteri, L., De Gaspari, I.F., Colasanti, A., Brambilla, M.A. and Cerruti, L. (2006) Substance abuse in first-episode schizophrenic patients: a retrospective study. *Clin Pract Epidemiol Ment Health,* **2**:4.

McCreadie, R.G. (2002) Use of drugs, alcohol and tobacco by people with schizophrenia: case-control study. *Br J Psychiatry,* **181**:321–5.

Missouri Department of Health and Senior Services (2010) Health Advisory: K2 Synthetic Marijuana Use Among Teenagers and Young Adults in Missouri. Jefferson City, MO, Missouri Department of Health and Senior Services.

Morgan, C.J. and Curran, H.V. (2008) Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *Br J Psychiatry,* **192**:306–7.

Mueser, K., Bellack, A. and Blanchard, J.J. (1992) Comorbidity of schizophrenia and substance abuse: implications for treatment. *J Consult Clin Psychol,* **60**:845–56.

Muller, H., Sperling, W., Köhrmann, M., Huttner, H.B., Kornhuber, J. and Maler, J.M. (2010) The synthetic cannabinoid Spice as a trigger for an acute exacerbation of cannabis induced recurrent psychotic episodes. *Schizophr Res,* **118**:309–10.

Myin-Germeys, I., Krabbendam, L., Jolles, J., Delespaul, P.A and van Os, J. (2002) Are cognitive impairments associated with sensitivity to stress in schizophrenia? An experience sampling study. *Am J Psychiatry,* **159**:443–9.

Myin-Germeys, I., van Os, J., Schwartz, J.E., Stone, A.A. and Delespaul, P.A. (2001) Emotional reactivity to daily life stress in psychosis. *Arch Gen Psychiatry,* **58**:1137–44.

Negrete, J.C., Knapp, W.P., Douglas, D.E. and Smith, W.B. (1986) Cannabis affects the severity of schizophrenic symptoms: results of a clinical survey. *Psychol Med,* **16**:515–20.

Peralta, V. and Cuesta, M.J. (1992) Influence of cannabis abuse on schizophrenic psychopathology. *Acta Psychiatr Scand,* **85**:127–30.

Peters, B., de Koning, P., Dingemans, P., Becker, H., Linszen, D.H. and de Haan, L. (2009) Subjective effects of

cannabis before the first psychotic episode. *Aust N Z J Psychiatry,* **43**:1155–62.

Rabinowitz, J., Bromet, E.J., Lavelle, J., Carlson, G., Kovasznay, B. and Schwartz, J.E. (1998) Prevalence and severity of substance use disorders and onset of psychosis in first-admission psychotic patients. *Psychol Med,* **28**:1411–9.

Raine, A. (1991) The SPQ: a scale for the assessment of schizotypal personality based on DSM-III-R criteria. *Schizophr Bull,* **17**:555–64.

Ramaekers, J.G., Kauert, G., Theunissen, E.L., Toennes, S.W. and Moeller, M.R. (2009) Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *J Psychopharmacol,* **23**:266–77.

Regier, D., Farmer, M., Rae, D.S., *et al.* (1990) Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) study.[comment]. *JAMA,* **264**:2511–8.

Ringen, P., Lagerberg, T., Birkenaes, A.B., *et al.* (2008) Differences in prevalence and patterns of substance use in schizophrenia and bipolar disorder. *Psychol Med,* **38**:1241–9.

Rottanburg, D., Robins, A.H., Ben-Arie, O., Teggin, A. and Elk, R. (1982) Cannabis-associated psychosis with hypomanic features. *Lancet,* **2**:1364–6.

Schofield, D., Tennant, C., Nash, L., *et al.* (2006) Reasons for cannabis use in psychosis. *Aust N Z J Psychiatry,* **40**:570–4.

Schwarcz, G., Karajgi, B. and Macarthy, R. (2009) Synthetic delta-9-tetrahydrocannabinol (dronabinol) can improve the symptoms of schizophrenia. *J Clin Psychopharmacol,* **29**:255–8.

Sevy, S., Robinson, D.G., Holloway, S., *et al.* (2001) Correlates of substance misuse in patients with first-episode schizophrenia and schizoaffective disorder. *Acta Psychiatr Scand,* **104**:367–74.

Silver, H. and Abboud, E. (1994) Drug abuse in schizophrenia: comparison of patients who began drug abuse before their first admission with those who began abusing drugs after their first admission. *Schizophr Res,* **13**:57–63.

Solomons, K., Neppe, V.M. and Kuyl, J.M. (1990) Toxic cannabis psychosis is a valid entity. *S Afr Med J,* **78**:476–81.

Spencer, C., Castle, D. and Michie, P.T. (2002) Motivations that maintain substance use among individuals with psychotic disorders. *Schizophr Bull,* **28**:233–47.

Stefanis, N.C., Delespaul, P., Henquet, C., Bakoula, C., Stefanis, C.N. and van Os, J. (2004) Early adolescent cannabis exposure and positive and negative dimensions of psychosis. *Addiction,* **99**:1333–41.

Stirling, J., Barkus, E.J., Nasobi, L., *et al.* (2008) Cannabis-induced psychotic-like experiences are predicted by high schizotypy. Confirmation of preliminary results in a large cohort. *Psychopathology,* **41**:371–8.

Swartz, M., Wagner, H., Swanson, J.W., *et al.* (2006) Substance use in persons with schizophrenia: baseline prevalence and correlates from the NIMH CATIE study. *J Nerv Ment Dis,* **194**:164–72.

Test, M.A., Wallisch, L.S., Allness, D.J. and Ripp, K. (1989) Substance use in young adults with schizophrenic disorders. *Schizophr Bull,* **15**:465–76.

Treffert, D.A. (1978) Marijuana use in schizophrenia: a clear hazard. *Am J Psychiatry,* **135**:1213–5.

Van Mastrigt, S., Addington, J. and Addington, D. (2004) Substance misuse at presentation to an early psychosis program. *Soc Psychiatry Psychiatr Epidemiol,* **39**:69–72.

van Os, J., Bak, M., Hannsen, M., Bijl, R.V., de Graaf, R. and Verdoux, H. (2002) Cannabis use and psychosis: a longitudinal population-based study. *Am J Epidemiol,* **156**:319–27.

Vearrier, D. and Osterhoudt, K.C. (2010) A teenager with agitation: higher than she should have climbed. *Pediatr Emerg Care,* **26**:462–5.

Veen, N.D., Selten, J.P., van der Tweel, I., Feller, W.G., Hoek, H.W. and Kahn, R.S. (2004) Cannabis use and age at onset of schizophrenia. *Am J Psychiatry,* **161**:501–6.

Warner, R., Taylor, D., Wright, J., *et al.* (1994) Substance use among the mentally ill: prevalence, reasons for use, and effects on illness. *Am J Orthopsychiatry,* **64**:30–9.

Weil, A.T. (1970) Adverse reactions to marihuana. Classification and suggested treatment. *N Engl J Med,* **282**:997–1000.

Zuardi, A., Crippa, J.A., Hallak, J.E., *et al.* (2009) Cannabidiol for the treatment of psychosis in Parkinson's disease. *J Psychopharmacol,* **23**:979–83.

Zuardi, A.W., Hallak, J.E., Dursun, S.M., *et al.* (2006) Cannabidiol monotherapy for treatment-resistant schizophrenia. *J Psychopharmacol,* **20**:683–6.

Zuardi, A.W., Morais, S.L., Guimarães, F.S. and Mechoulam, R. (1995) Antipsychotic effect of cannabidiol. *J Clin Psychiatry,* **56**:485–6.

Chapter

19

Cannabis abuse and the course of schizophrenia

Don Linszen and Thérèse van Amelsvoort

As detailed elsewhere in this book, cannabis has been used for centuries to induce euphoria and relaxation as desired mental effects. However, adverse effects of intoxication with cannabis include anxiety and panic (Thomas, 1996; Reilly *et al*., 1998), depression (Bovasso, 2001; Patton *et al*., 2002; see Chapter 10) and impairment in certain domains of cognitive function (see Chapters 8 and 9). Psychosis, including paranoid delusions and hallucinations, has been found to be an effect of cannabis use in cohort studies from Vietnam (Talbott and Teague, 1969), India (Chopra and Smith, 1974), Pakistan (Chaudry *et al*., 1991) and New Zealand (Thomas, 1996) (Chapter 18). This work suggests that cannabis, especially in high doses, can produce a "toxic" psychosis in people without mental disorders. Evidence for cannabis (and especially heavy abuse) as a causal risk factor for psychotic disorders comes from epidemiological studies as reviewed in Chapter 15 (and see also Miller *et al*., 2009), including the seminal study of Swedish conscripts (Andreasson *et al*., 1987), the study from Zammit *et al*. (2002), the Dunedin study from New Zealand (Arsenault *et al*., 2000), the Dutch NEMESIS sample (Van Os *et al*., 2002) and a study of Israeli conscripts (Weiser *et al*., 2003).

Schizophrenia and related psychotic disorders are clinical syndromes with a wide variation in symptoms between individuals (Thaker and Carpenter, 2001). Factor-analytic studies of schizophrenia have revealed that the disease is best described by three dimensions or syndromes, namely reality distortion (positive symptoms), psychomotor poverty (negative symptoms) and disorganization. Central features of the reality distortion dimension are hallucinations and delusions; psychomotor poverty symptoms include loss of motivation and restricted emotional experience; and disorganization symptoms, which encompass disorganized thought, incongruity of affect and bizarre behavior (Liddle *et al*., 1987).

Our group found a fourth dimension with depression-related symptoms (van der Does *et al*., 1995). Cognitive impairments have been established as central features of schizophrenia as well; deficits have been established in attention, short-term memory, verbal memory, concentration and planning, and problem-solving tasks (Bilder *et al*., 2000).

Given that cannabis can cause a wide variety of effects that resemble the extensive and varied symptomatology of schizophrenia itself, questions rise such as: What is the impact of cannabis use on psychotic relapse and the symptomatic course of schizophrenia? Is there evidence for aggravation of the course of all symptom dimensions? Do positive consequences such as relief of negative symptoms occur with cannabis use?

These questions have become more urgent since: (1) cannabis is the most used drug in the general population and cannabis use has increased, especially among young people (Miller and Plant, 2002); (2) cannabis use and schizophrenia both have their onset in adolescence and young adulthood; and (3) cannabis is clearly the most used illicit drug among individuals with schizophrenia (Bersani *et al*., 2002) and is more often used by patients with schizophrenia and other psychotic disorders compared with people without psychoses (Mueser *et al*., 1990; Dixon *et al*., 1991; Hall and Degenhardt, 2000). Clarification of the relationship between schizophrenia and cannabis abuse would have the theoretical benefits of elucidating the mechanisms of psychotic relapse and the symptomatic course, as well as having practical impact for the treatment of substance abusers with schizophrenia or related psychotic disorders (see also Chapters 20, 21).

This chapter reviews studies that have examined the effects of cannabis use on the course of schizophrenia. Studies that examined the relation of poly-drug abuse and schizophrenia were excluded when they did not examine the independent effects of cannabis (Zisook

Marijuana and Madness, Second Edition, ed. David Castle, Robin M. Murray and Deepak Cyril D'Souza. Published by Cambridge University Press.

et al., 1992; Gupta *et al.*, 1996; Bersani *et al.*, 2002; Hunt *et al.*, 2002).

Cannabis abuse and the course of schizophrenia

Until the 1990s, studies examining the relationship between cannabis use and schizophrenia consisted of case series, in which possible relationships between cannabis abuse and psychotic symptoms were difficult to test. In a few case–control studies, psychotic symptoms were evaluated retrospectively, using hospital files (Negrete *et al.*, 1986). Also, the observation period was typically only 1 week and schizophrenic symptoms were evaluated once, on a cross-sectional basis (Peralta and Cuesta, 1992).

An increase of psychotic symptoms in cannabis-abusing schizophrenia patients has been found repeatedly (Weil, 1970; Chopra and Smith, 1974; Treffert, 1978; Knudsen and Vilmar, 1984; Cleghorn *et al.*, 1991). Cleghorn *et al.* (1991), in a controlled study, reported that patients with schizophrenia and prominent cannabis abuse had significantly more hallucinations, delusions and thought disorder than controls. In terms of negative symptoms, Knudsen and Vilmar (1984) found negative symptoms overall, and affective flattening in particular, to be less pronounced in cannabis-abusing schizophrenia patients compared with those not using cannabis. Peralta and Cuesta (1992) found no aggravation of positive psychotic symptoms in patients with schizophrenia when exposed to cannabis, but an exacerbation of alogia as a negative symptom was established. In another case–control study (Dixon *et al.*, 1991), fewer positive and negative symptoms were found in a sample of drug-abusing patients with schizophrenia (cannabis being the drug of choice) compared with non-users.

These cross-sectional and retrospective studies thus give somewhat conflicting results, perhaps reflecting the limitations of the methodology. Much more robust are prospective studies that allow the tracking of the effects of cannabis on psychotic symptoms over time.

Short-term prospective studies

The first large prospective cohort study that examined the relationship between cannabis abuse and the symptomatic course of recent-onset schizophrenia and related disorders (Linszen *et al.*, 1994) was conducted over the course of a year using monthly Brief Psychiatric Rating Scale (BPRS) assessments. Psychopathological symptoms were assessed by an independent evaluator who was blind to cannabis abuse. Twenty-four young cannabis-abusing patients were compared with 69 non-abusers. The mean age when they started cannabis abuse was 16 years, and the mean duration of abuse before admission was 3.9 years.

All but one of the cannabis-abusing patients started their habit at least one year before their first psychotic symptoms (mean = 3 years, range = 0–7 years). Within the group of 24 cannabis abusers, 13 heavy users (54%) could be identified, this group being defined as using more than one cigarette a day. The mild abusing group (n = 11) consumed between one cigarette a week and one a day. Hard drug abuse was rare (two patients used cocaine and ecstacy; one of these patients used hard drugs sporadically in combination with heavy cannabis abuse).

Perhaps the most striking finding of this prospective study was the occurrence of significantly more, and earlier, psychotic relapses or exacerbations in the total group of cannabis-abusing patients over a 12 month period. When a distinction was made with respect to the intensity of abuse, the association became even stronger: it appeared that particularly heavy cannabis-abusing patients relapsed more frequently and earlier. This finding was confounded neither by exposure to alcohol and/or any other (psychoactive) drugs, nor by differences in anti-psychotic medication adherence and dosage. Baseline symptom status, prognostic scale score and history of prior relapse rates were similar for cannabis-abusers and non-abusers. No other demographic or clinical factors could be identified that affected this relationship.

Two additional findings indicated a possible causal relation between cannabis and psychotic relapse. First, 14 out of 24 cannabis-abusing patients reported an immediate increase of psychotic symptoms, after resuming cannabis abuse. Thirteen of these 14 patients were clinically in remission when they reported the increase of psychotic symptoms. Six patients noted no such exacerbation of symptoms, whereas one further patient reported a decrease in psychotic symptoms when using cannabis. Second, in all but one patient cannabis abuse preceded the initial onset of psychotic episodes by at least a year.

In addition to psychotic relapse, we also examined the relationship between cannabis abuse and symptom dimensions of recent-onset schizophrenic disorders over a 12-month period. Positive, negative, disorganization and depressive symptom dimensions were

compared between the cannabis-abusing patients and non-abusers. No effect was found for the positive syndrome ($p = 0.43$), the negative syndrome ($p = 0.23$) or the depression syndrome ($p = 0.27$). In the mild-abusing group, symptoms of anxiety and depression tended to be less prevalent than in the non- and heavy-abusing group, suggesting that those with mild cannabis abuse were using cannabis to "self-medicate." We could not confirm the existence of an amotivational syndrome, and there was no apparent exacerbation of negative symptoms in the cannabis-abusing group. However, in a re-analysis of the data a main effect of cannabis abuse was found for the course of the symptoms of the disorganization dimension ($p = 0.01$), with the scores tending to increase over the 12-month period ($p < 0.01$) (Linszen *et al*., 1995).

In a one-year follow-up study from Spain (Martinez-Arevalo *et al*., 1994) data were analyzed from 62 young adults with schizophrenia who had suffered from at least one relapse. No definition of relapse was provided. Cannabis consumption was found to be the best predictor of relapse over the follow-up period, with evidence strongest for continued use during follow-up (64% relapse in individuals using cannabis at baseline and follow-up, compared with 17% in non-users). In that study, hospitalization rates at baseline were similar in individuals who used cannabis regularly compared with controls (13% and 17% respectively); at follow-up there was evidence for increased hospitalization in those using cannabis regularly (43% versus 17%, $p = 0.08$). Patients had a history of psychoactive substance abuse before the study and misused alcohol during the follow-up period. These data could have confounded the results, since the outcome of the study was adjusted for measures of illness severity at baseline, but not for alcohol or substance use.

A US study by Kovasznay *et al*. (1997) examined the relationship between substance use and psychotic disorders, and found that patients with schizophrenia reported significantly more cannabis use than patients with an affective psychotic disorder over a six-month period. Enduring cannabis abuse was associated with exacerbation of overall symptoms scored on the BPRS.

Hides *et al*. (2006) in Brisbane, Australia, sought to examine the influence of cannabis use on psychotic symptom relapse and the influence of psychotic symptom severity on relapse in cannabis use in the six months following hospital admission. At baseline, 84 participants with recent-onset psychosis were assessed and 81 were followed up weekly for six months, using telephone and face-to-face interviews. A higher frequency of cannabis use (days per week) was associated with an increase in psychotic relapses based on the BPRS. Analyses controlled for medication adherence, alcohol and other substance use at baseline and duration of untreated psychosis. The authors concluded that the relationship between cannabis use and psychosis could be bidirectional, highlighting the need for early intervention programs to target both cannabis use and psychotic symptoms.

A strong association between cannabis abuse and relapse (BPRS-rated) was reported in a study from Melbourne, Australia. In this 15-month prospective follow-up study of first-episode psychosis, cannabis use was adjusted for measures of illness severity at baseline and for both alcohol and other drug use. The adjustment reduced the association by an estimated 15%.

A study in Madrid, Spain (Arias *et al*., 2002) reported weak evidence for an association between cannabis dependence and increased relapse after adjusting for alcohol and other drug use. No definition of relapse was provided. Cannabis was not associated with the positive symptom severity according to the Positive and Negative Symptoms Scale (PANSS), but at follow-up an association was observed between cannabis dependence and a reduced score on the PANSS negative symptom sub-scale. Cannabis abuse was also associated with a greater number of admissions.

Pencer and co-workers (2005) examined symptomatic and functional outcome in adolescents experiencing their first episode of psychosis in Calgary, Canada. The adolescents ($n = 69$) were compared with adults ($n = 69$), all presenting for treatment for the first time to a specialized early psychosis program. Assessments were conducted at the initial presentation, and at 1- and 2-year follow-up. Assessments included positive and negative symptoms, depression, number of relapses, substance use, cognitive functioning, age-appropriate productivity (employment or being in school) and quality of life. Compared with adults, the adolescents had similar clinical and functional outcomes but used more cannabis and had an increased number of relapses. Cannabis use at baseline was associated with decreased levels of productivity or employment, as well as reduced quality-of-life measures at follow-up.

In a study of recent onset psychosis patients in South London, England, Grech *et al*. (2005) found that regular cannabis use at baseline was associated with increased level of positive symptoms at follow-up. Cannabis use was not associated with negative symptoms scores;

there was some evidence that individuals who had used cannabis frequently at baseline had a more continuous course of illness than people who had not used cannabis regularly (odds ratio [OR] = 2.4, 95% CI: 0.9, 6.9).

In a treatment trial, Green *et al*. (2004) examined the effects of olanzapine versus haloperidol in a cohort with patients with first-episode schizophrenia-related psychosis and substance-use disorders. This study measured change in symptom scores from baseline to follow-up, and adjusted for baseline scores. Cannabis misuse was found not to be significantly associated with a change in PANSS total score. However, the change score was less in both arms for the cannabis abuse group compared with non-users. No combined analysis of the two trial groups was presented, because of low statistical power.

Degenhardt *et al*. (2007) sought to examine the temporal dynamics of relationships between cannabis, psychosis and depression among young adults with psychotic disorders in a 10-month prospective study in Sydney, Australia. The study measured change in symptom scores from baseline to follow-up and made adjustments for illness severity at baseline. Cannabis use was associated with an increase in BPRS score that persisted after adjusting for the prior BPRS scores, but not with depression scores.

The study of Miller *et al*. (2009) was designed to follow patients' use of cannabis and treatment adherence in a naturalistic setting over the first 12 months of service engagement. It examined whether cannabis use is a risk factor for two distinct types of non-adherence: medication and treatment dropout. Participants were 112 first-episode schizophrenia patients, enroled in a study of differential effectiveness of two second-generation antipsychotic medications. Multiple indicators were used to assess cannabis use and adherence to medication. Patients were encouraged to continue in the study even after periods of treatment refusal, or to change from study to standardized medication. After 12 months, 23 had dropped out and 37 had at some point been non-adherent to medication. Of 34 participants who used cannabis during treatment, 32 had a prior diagnosis of cannabis abuse/dependence and 30 were male. Independent of age, race, socioeconomic status, gender, site and medication assignment, cannabis use significantly increased the hazard of non-adherence by a factor of 2.4 ($p < 0.001$) and the hazard of dropout by a factor of 6.4 ($p = 0.034$). The authors concluded that treatment for first-episode schizophrenia may be more effective if providers address the issue of cannabis use with patients throughout the early years of treatment, especially for those with existing cannabis abuse/dependence.

A recent study by Henquet and colleagues (2010) examined the effects of cannabis on psychotic symptoms and mood in patients with psychosis ($n = 42$) and healthy controls ($n = 38$). Participants were followed in their daily lives using a structured time-sampling technique. Daily life cannabis use predicted subsequent increases in positive symptoms and increased levels of hallucinatory experiences affect and decreases of negative affect. Mood-enhancing properties of cannabis were acute, whereas psychosis-inducing effects were sub-acute. There was no direct evidence for self-medication effects in daily life. Patients with psychosis were found to be more sensitive to both the psychosis-inducing and mood-enhancing effects of cannabis. The authors concluded that the temporal dissociation between acute rewarding effects and sub-acute toxic influences might be instrumental in explaining the vicious circle of deleterious use in these patients. The reader is referred to Chapter 18 for a more detailed account hereof.

Previous studies have been contradictory regarding the effects of regular cannabis use on mood. Denson and Earleywine (2006) found that regular users reported less depressed mood and more positive affect than non-users, whereas Degenhardt and colleagues (2003) reported an association between heavy cannabis use and depression (see Chapter 10). The reason for these differences is not clear, but may be due to differences in cannabis composition, as pure Δ^9-tetrahydrocannabinol is anxiogenic when given acutely, whereas cannabidiol appears to ameliorate these effects (Bhattacharyya *et al*., 2010).

Longer-term prospective studies

A shortcoming of the aforementioned studies is the relative short follow-up period, given the long-term course of schizophrenia. Longer-term studies of the impact of cannabis on the course of schizophrenia are now emerging and are reviewed here.

A prospective case–control study from Germany (Caspari, 1999) followed a representative sample of 39 schizophrenia patients with cannabis use for 68 months after their first hospital admission. Patients with cannabis abuse showed a significantly higher index of re-hospitalization in the follow-up period (0.98 re-admissions per year compared with 0.35 for

the non-abuse group) and tended to have poorer psychosocial functioning than the non-abusing controls. They also scored higher on the "thought disturbance" and "hostility" subscales of the BPRS. Regular cannabis use was associated neither with negative symptoms scores, nor with anxiety or depression sub-scale scores. Shortcomings of this study included a lack of repeated measurements of the symptomatic course during the follow-up period (the BPRS was only assessed at the end of the study); thus it remains uncertain whether aggravation of symptoms and re-hospitalization were related to cannabis abuse.

In a further German case–control study of the effects of substance abuse (Bühler *et al*., 2002), 115 patients with first episodes of schizophrenia were studied over a 5-year period with six assessments. The number of cannabis using patients was small ($n = 4$) and had to be combined with patients with alcohol use ($n = 12$) and alcohol alone ($n = 12$) for analysis. The comorbid patients were compared with 29 non-comorbid patients, matched for age and sex. At each assessment the substance-abusing group showed a higher positive symptom score than the non-abusers; there was a trend toward lower negative symptom scores (notably affective flattening) in the substance users. Subjects with substance abuse also exhibited poorer treatment adherence, lower utilization of rehabilitation and a higher rate of unemployment than non-users after 5 years.

In contrast with the two aforementioned studies, a prospective 10-year follow-up study from Manchester, UK (Stirling *et al*., 2005) found no association between regular cannabis use and psychotic symptoms scores on the Scale for the Assessment of Positive Symptoms (SAPS). Cannabis use was not associated with negative symptoms on the SAPS either. Neurocognitive ability at follow-up was found to be greater on five out of nine sub-scales in people who had used cannabis at baseline.

Addington and Addington (2007) examined 203 subjects that were consecutively admitted to an early psychosis program to determine the prevalence of substance use and its impact on 3-year outcomes. Assessments were performed at baseline and 1-, 2- and 3-year follow-ups. The prevalence of substance misuse was high, with 51% having a substance-use disorder (SUD) (33% with cannabis and 35% alcohol). Numbers with an alcohol SUD declined considerably after 1 year and for cannabis SUD after 2 years. Substance misuse was significantly associated with male gender, young age and early age of onset and cannabis misuse was associated with higher positive symptom scores.

In Madrid, Spain, Gonzalez-Pinto *et al*. (2009) examined the influence of cannabis use in an 8-year follow-up after a first psychotic episode. Patients who had never used cannabis ($n = 40$) were compared with: (1) those who used cannabis before the first episode but discontinued their use during follow-up ($n = 27$); and (2) those who used cannabis both before the first episode and during follow-up ($n = 25$). The three groups did not differ significantly in terms of symptoms or functioning, or on functional outcomes at baseline or during short-term follow-up. However, the group who had used but stopped showed better long-term functional outcome compared with the other two groups (effect size 1.26, 95% confidence intervals [CI]: 0.65, 1.86) and had fewer negative symptoms than the continuous cannabis-using group, even after adjusting for potential confounders (effect size 0.72, 95% CI: −1.27, −0.14). All patients experienced improvements in positive symptoms during long-term follow-up. The authors concluded that cannabis had a deleterious effect, but stopping the habit after the first psychotic episode contributed to an improvement in outcome that became more obvious as time progressed.

A methodological flaw in studies examining the influence of cannabis use in clinical samples is selection bias, for example from hospital-based recruitment. To avoid such bias, van Os *et al*. (2002) used a population-based sample of individuals with a vulnerability to psychosis, to establish whether alcohol and cannabis use influenced outcome. Of 59 subjects with Diagnostic and Statistical Manual of Mental Disorders *(DSM*-III-R) (American Psychiatric Association, 1987), diagnosis of any psychosis at baseline for whom follow-up data were available, nine reported cannabis use. A strong interaction was found between cannabis use and established psychotic symptoms, such that the elevated risk of psychosis associated with cannabis use was much stronger for those with an established vulnerability at baseline.

Pooled analysis

In a recent systematic review of studies published on this topic till the end of 2006 Zammit *et al*. (2008) concluded that the lack of uniformity in outcome and exposure measurements, as well as the content of statistical results presented, precluded meta-analysis. Overall, of the 52 outcomes reported from 13 included

studies, cannabis was associated with statistical evidence of a worse outcome in 14, and a better outcome in 7. There was no evidence of association in either direction for the other 31 outcomes. Seven studies looked at people with schizophrenia (or related spectrum disorders) only, while the other six included people with other psychoses as well. All of the seven associations with better outcomes in cannabis users were in studies that included individuals with any psychosis rather than specifically schizophrenia or related spectrum disorders. The authors concluded that cannabis use was consistently associated with increased relapse and non-adherence to treatment in psychotic patients, but that associations with elevated psychotic symptom scores were too inconsistent to conclude definitively that cannabis use has a detrimental effect on outcomes in people with psychotic illnesses.

Conclusions

The most relevant finding of this review is that cannabis use is an independent risk factor for increased relapse and decreased treatment adherence in schizophrenia. However, in prospective studies of patients with schizophrenia and related disorders, links with increases in psychotic and disorganization symptoms are more varied, making it difficult to conclude definitively that cannabis use leads unambiguously to poor outcomes in people with a psychotic illness. When a distinction with respect to the intensity of abuse is made, however, it appears that particularly heavy cannabis-abusing patients suffered more from relapses and experience more florid psychotic and disorganization symptoms. These findings could have been more convincing had all the studies included systematic laboratory confirmation of cannabinoid derivatives in urine. Having said this, Martin *et al.* (1988) found that the information on use of soft drugs given by patients is generally reliable. Moreover, evaluation in most studies included self-reports of patients and by experienced clinicians; also, use of cannabis is not illegal in one country, i.e. the Netherlands.

Two additional findings indicate a possible causal relation between cannabis exposure and psychotic relapse. First, most of the cannabis-abusing patients reported an immediate increase of psychotic symptoms after resuming cannabis abuse. Second, in most studies cannabis abuse preceded the onset of the first psychotic episode by at least 1 year. This finding is congruent with the observations of epidemiological studies that consistently reveal cannabis abuse before illness onset to be an independent risk factor for schizophrenia (see Chapter 15).

Some support exists for the self-medication hypothesis of schizophrenia and cannabis (see Chapter 20), as schizophrenia patients successfully reduced their negative symptoms (Peralta and Cuesta, 1992), affective symptoms (Dixon *et al.*, 1991), anxiety and depression with mild abuse (Linszen *et al.*, 1994). Some patients reportedly use cannabis to decrease side effects of antipsychotic medications (Knudsen and Vilmar, 1984) although pathways are not clear.

A biological explanation of the demonstrated relation between psychotic symptoms of schizophrenia patients and cannabis abuse may be found in recent pharmacological studies. Δ^9-Tetrahydrocannabinol (THC), the principal psychoactive constituent of cannabis, acts as a dopamine agonist in dopaminergic projections of the medial forebrain bundles (see Chapter 1). Dopaminergic hyperactivity is generally thought to relate to the presence of psychotic symptoms of schizophrenia, although other neurotransmitters may also be involved. An increase of dopamine could undo the dopamine receptor blockade of antipsychotic medication. This review suggests that intensity of abuse is correlated with an increase of psychotic relapses, suggesting THC interferes with dopaminergic neurotransmission in the medial forebrain of patients. Future studies with brain imaging techniques applied to heavy and non-abusing schizophrenic patients with standard antipsychotic medication may reveal these differences in dopamine receptor blockade or in other neurotransmission systems (e.g. glutamate). Cannabis abuse may also influence antipsychotic drug metabolism, lowering plasma levels of active metabolites. Thus, theoretically cannabis abusers with schizophrenia could be relatively undertreated.

It is also possible that young persons who use cannabis regularly are more vulnerable to, or have less effective coping mechanisms for dealing with, stressful life events. This same vulnerability to stress may produce a lower threshold for recurrence of psychotic symptoms, even if they discontinued cannabis use. A further interesting possibility is that there may be some common genetic basis for cannabis abuse, schizophrenia and underlying neuropsychological and neurobiological vulnerabilities of both disorders.

Further studies are needed to elucidate the relationship between cannabis abuse and psychotic symptoms in schizophrenia. These studies should include quantitative estimations of cannabis abuse repeated over time;

laboratory confirmation of single, or poly-cannabis abuse; repeated assessments of dose–response effects; and repeated assessments of potential confounding variables, notably adherence with antipsychotic medication.

References

Addington, J. and Addington, D. (2007) Patterns, predictors Effects of cannabis use on outcomes of psychotic disorders: systematic review. *Br J Psychiatry*, **193**:357–63.

American Psychiatric Association (1987) Diagnostic and Statistical Manual of Mental Disorders (*DSM*-III-R), 3rd edn. Washington, DC: American Psychiatric Association.

Andreasson, S., Allebeck, P., Engström, A., and Rydberg, U. (1987) Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet*, **2**:1483–6.

Arias Horcajadas, F., Romero, S.S. and Calo, J.J.P. (2002) Relevance of drug use in clinical manifestations of schizophrenia [Spanish]. *Actas Esp Psiquiatr,* **30**:65–73.

Arseneault, L., Moffitt, T.E., Caspi, A., Taylor, P.J. and Silva P.A. (2000) Mental disorders and violence in a total birth cohort: results from the Dunedin Study. *Arch Gen Psychiatry,* **57**:979–86.

Bersani, G., Orlandi, V., Kotzalidis, G.D. and Pancheri, P. (2002) Cannabis and schizophrenia: impact on onset, course, psychopathology and outcomes. *Eur Arch Psychiatry Clin Neurosci*, **252**:86–92.

Bhattacharyya, S., Morrison, P.D., Fusar-Poli, P., *et al.* (2010) Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology,* **35**:764–74.

Bilder, R.M., Goldman, R.S., Robinson, D., *et al.* (2000) Neuropsychology of first-episode schizophrenia: initial characterization and clinical correlates. *Am J Psychiatry*, **157**:549–59.

Bovasso, G.B. (2001) Cannabis abuse as a risk factor for depressive symptoms. *Am J Psychiatry*, **158**:2033–7.

Bühler, B., Hambrecht, M., Löffler, W., Heiden an der, W. and Häfner, H. (2002) Precipitation and determination of the onset and course of schizophrenia by substance abuse – a retrospective and prospective study of 232 population-based first illness episodes. *Schizophr Res*, **54**:243–51.

Caspari, D. (1999) Cannabis and schizophrenia: results of a follow-up study. *Eur Arch Psychiatry Clin Neurosci*, **249**:45–9.

Chaudry, H.R., Moss, H.B., Bashir, A., *et al.* (1991) Cannabis psychosis following bhang ingestion. *Brit J Addiction*, **86**:1075–81.

Chopra, G.S., and Smith, J.W. (1974) Psychotic reactions following cannabis use in East Indians. *Arch Gen Psychiatry,* **30**:24–7.

Cleghorn, J.M., Kaplan, R.D., Szechtman, B., Szechtman, H., Brown, G.M. and Franco, S. (1991) Cannabis abuse and schizophrenia: Effect on symptoms but not on neurocognitive function. *J Clin Psychiatry*, **52**:26–30.

Degenhardt, L., Hall, W. and Lynskey, M. (2003) Exploring the association between cannabis use and depression. *Addiction*, **98**:1493–504.

Degenhardt, L., Tennant, C., Gilmour, S., *et al.* (2007) The temporal dynamics of relationships between cannabis, psychosis and depression among young adults with psychotic disorders: findings from a 10-month prospective study. *Psychol Med*, **37**:1–8.

Denson, T.F. and Earleywine, M. (2006) Decreased depression in marijuana users. *Addict Behav*, **31**:738–42.

Dixon, L., Haas, G., Weiden, P.J., Sweeney, J., and Frances A.J. (1991) Drug abuse in schizophrenic patients: Clinical correlates and reasons for use. *Am J Psychiatry* **148**:224–30.

González-Pinto, A., Alberich, S., Barbeito, S., *et al.* (2009) Cannabis and first-episode psychosis: different long-term outcomes depending on continued or discontinued use. *Schizophr Bull*, **37**:631–9.

Grech, A., van Os, J., Jones, P.B., Lewis, S.W. and Murray, R.M. (2005) Cannabis use and outcome of recent onset psychosis. *Eur Psychiatry*; **20**:349–53.

Green, A.I., Tohen, M.F., Hamer, R.M., *et al.*; HGDH Research Group (2004) First episode schizophrenia-related psychosis and substance use disorders: acute response to olanzapine and haloperidol. *Schizophr Res,* **66**:125–35.

Gupta, S., Hendricks, S., Kenkel, A.M., Bhatia, S.C. and Haffke, E.A. (1996) Relapse in schizophrenia: is there a relationship to substance abuse? *Schiz Res*, **20**:153–6.

Hall, W. and Degenhardt, L. (2000) Cannabis use and psychosis: a review of clinical and epidemiological evidence. *Aust NZ J Psychiatry*, **34**:26–34.

Henquet, C., van Os, J., Kuepper, R., *et al.* (2010) Psychosis reactivity to cannabis use in daily life: an experience sampling study. *Br J Psychiatry,* **96**:447–53.

Hides, L., Dawe, S. and Kavanagh, D.J. (2006) A prospective study of psychotic symptom and cannabis relapse in recent onset psychosis. *Br J Psychiatry*, **189**:137–43.

Hunt, G.E., Bergen, J. and Bashir, M. (2002) Medication compliance and comorbid substance abuse in schizophrenia: impact on community survival 4 years after a relapse. *Schiz Research*, **54**:253–64.

Knudsen, P. and Vilmar, T. (1984) Cannabis and neuroleptic agents in schizophrenia. *Acta Psychiatrica Scandinavica*, **69**:162–74.

Kovasznay, B., Fleischer, J., Tanenberg-Karant, M., Jandorf, L., Miller, A.D. and Bromet, E. (1997) Substance use disorder and the early course of illness in

schizophrenia and affective psychosis. *Schizophr Bull*, **23**:195–201.

Liddle, P. (1987) The symptoms of chronic schizophrenia. A re-examination of the positive-negative dichotomy. *Br J Psychiatry*, **151**:145–51.

Linszen, D.H., Dingemans, P.M. and Lenior, M.E. (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. *Arch Gen Psychiatry*, **51**:273–9.

Linszen, D.H., Dingemans, P.M. and Lenior M.E. (1995) Symptom dimensions of schizophrenia disorders and cannabis abuse: a longitudinal study. *Schizophr Res,* **15**:16.

Martin, G.W., Wilkinson, A. and Kapur, B.M. (1988) Validation of self-reported cannabis use by urine analysis. *Addictive Behavior,* **13**:147–50.

Martinez-Arevalo, M.J., Calcedo-Ordoñez, A. and Varo-Prieto, J.R. (1994) Cannabis consumption as prognostic factor in schizophrenia. *Br J Psychiatry*, **164**:679–81.

Miller, P. and Plant, M. (2002) Heavy cannabis use among UK teenagers: an exploration. *Drug Alcohol Depend*, **65**:235–42.

Miller, R., Ream G., McCormack, J., Gunduz-Bruce, H., Sevy, S. and Robinson, D. (2009) A prospective study of cannabis use as a risk factor for non-adherence and treatment dropout in first-episode schizophrenia. *Schiz Research*, **113**:138–44.

Mueser, K.T., Yarnold, P.R., Levinson, D.F., *et al.* (1990). Prevalence of cannabis abuse in schizophrenia: demographic and clinical correlates. *Schizophr Bull,* **16**:31–56.

Negrete, J.C., Knapp, W.P., Douglas, D.E. and Smith, B. (1986) Cannabis affects the severity of schizophrenic symptoms: Results of a clinical survey. *Psychol Med*, **16**:515–20.

Patton, G.C., Coffey, C., Carlin, J.B., Degenhardt, L., Lynskey, M. and Hall, W. (2002) Cannabis use and mental health in young people: cohort study. *BMJ*, **325**:1195–8.

Pencer, A., Addington, J. and Addington, D. (2005) Outcome of a first episode of psychosis in adolescence: a 2-year follow-up. *Psychiatry Res* **133**:35–43.

Peralta, V. and Cuesta, M.J. (1992) Influence of cannabis abuse on schizophrenic psychopathology. *Acta Psych Scand*, **85**:127–30.

Reilly, D., Didcott, R., Swift, W., *et al.* (1998) Longterm cannabis use: characteristics of users in Australian rural areas. *Addiction*, **93**:837–46.

Stirling, J., Lewis, S., Hopkins, R. and White, C. (2005) Cannabis use prior to first onset psychosis predicts spared neurocognition at 10-year follow-up. *Schizophr Res,* **75**:135–7.

Talbott, J.A. and Teague, J.W. (1969) Marijuana psychosis. *JAMA*, **210**:299–302.

Thaker, G.K. and Carpenter, W.T. (2001) Advances in schizophrenia. *Nature Medicine,* **7**:667–71.

Thomas, H. (1996) A community survey of adverse effects of cannabis use. *Drug and Alcohol Dependence*, **42**:201–7.

Treffert, D.A. (1978) Marijuana use in schizophrenia: A clear hazard. *Am J Psychiatry*, **135**:1213–5.

van der Does, A.J.W, Dingemans, P.M.A.J., Linszen, D.H., Nugter, M.A. and Scholte, W.F. (1995) Dimensions and subtypes of recent-onset schizophrenia: a longitudinal analysis. *J Nerv Ment D is*, **183**:681–7.

van Os, J., Bak, M., Hanssen, M., Bijl, R.V., Graaf de, R. and Verdoux, H. (2002) Cannabis use and psychosis: a longitudinal population-based study. *Am J Epidemiology*, **156**:319–27.

Weil, A.T. (1970) Adverse reactions to marijuana, classification and suggested treatment. *New Eng J Med,* **282**:997–1000.

Weiser, M., Reichenberg, A., Rabinowitz, J., *et al.* (2003) Self-reported drug abuse in male adolescents with behavioral disturbances, and follow-up for future schizophrenia. *Biol Psychiatry*, **54**:655–60.

Zammit, S., Allebeck, P., Andreasson, S., Lundberg, I. and Lewis, G. (2002) Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. *British Medical Journal*, **325**:1199–201.

Zammit, S., Moore, T.H., Lingford-Hughes, A., *et al.* (2008) Effects of cannabis use on outcomes of psychotic disorders: systematic review. *Br J Psychiatry*, **193**:357–63.

Zisook, S., Heaton, R., Moranville, J., Kuck, J., Jernigan, T. and Braff, D. (1992) Past substance abuse and clincal course of schizophrenia. *Am J Psychiatry*, **149**:552–3.

Chapter

20

Understanding cannabis use in schizophrenia

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Large-scale international surveys have found consistently high rates of cannabis use in schizophrenia and other psychotic populations (Merikangas *et al*., 1998; Degenhardt *et al*., 2001; Farrell *et al*., 2001). Despite this we still have little understanding of the aetiology of the increased rates of cannabis use in psychosis. Three models for understanding the relationship between cannabis and psychosis have been proposed (Thornicroft, 1990; Khantzian, 1997; Mueser *et al*., 1998). The *vulnerability* model posits that cannabis use contributes to the onset, symptom severity and relapse of psychosis. The *symptom alleviation* model proposes that individuals with psychosis use cannabis in an attempt to alleviate positive and negative symptoms, as well as depression and anxiety. The *common factor* model proposes that some of an individual's vulnerability to cannabis use and psychosis is caused by shared underlying factors, such as genetic vulnerability or conduct disorder/antisocial personality disorder.

Consistent with the vulnerability model, cannabis use has been strongly associated with the onset of psychotic symptoms and disorders in a growing number of large-scale longitudinal studies (Moore *et al*., 2007 and see Chapter 15). Little evidence for the symptom alleviation model has emerged from these studies (Arseneault *et al*., 2002; Fergusson *et al*., 2003), although individuals do report improvements in positive affect (Henquet *et al*., 2010). Despite self-reports of improved affect, a growing number of prospective studies have found cannabis use is predictive of a more severe psychotic symptom course and increased risk of psychotic relapse (Verdoux *et al*., 2003; Ferdinand *et al*., 2005; Hides *et al*., 2006; Degenhardt, 2007; Zammit *et al*., 2008; and see Chapter 19). However, there is also evidence of increased cannabis use during periods when psychotic symptoms are worse, suggesting that relationships between cannabis and psychosis may be bidirectional (Ferdinand *et al*., 2005; Hides *et al*., 2006; Degenhardt, 2007). Conduct disorder and antisocial personality disorder are related to an increased risk of schizophrenia (Hodgins *et al*., 1996; Robins, 1966) and drug use disorders, in both the general population (Anthony and Helzer, 1991) and in people with psychotic disorders (Mueser *et al*., 2000), suggesting this may account for some of the increased comorbidity between cannabis-use disorder and psychosis.

While tests of the vulnerability and symptom alleviation models have provided increasing evidence for the relationship between cannabis use and psychosis, these models do not elucidate the mechanisms underlying their associations. For example, influences of symptoms on cannabis use do not necessarily imply that cannabis is effective in addressing psychotic symptoms, or that those symptoms were the target; users often report smoking cannabis to alleviate distress (Henquet *et al*., 2010). Furthermore, explanations other than symptom alleviation are possible in some cases (e.g. loss of behavioral control). This chapter briefly reviews potential biological, personality and cognitive mechanisms underpinning the relationship between cannabis use and psychosis.

Personality models of cannabis and psychosis

A number of personality traits have been identified as potential moderators of the relationship between cannabis use and psychosis (Blanchard *et al*., 2000b). Several studies have found cannabis users with psychosis proneness or schizotypy are more likely to report psychotic-like experiences (PLEs) when they had smoked cannabis (Verdoux *et al*., 2003; Barkus *et al*., 2006; Barkus and Lewis, 2008; Stirling *et al*., 2008). Cannabis users with high psychosis proneness have also been found to report more PLEs during acute cannabis intoxication (Mason *et al*., 2009). These findings have

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led to suggestions that schizotypy may moderate the effects of cannabis use on PLEs in non-clinical populations, which are consistent with a vulnerability model of cannabis use and psychosis. However, this research is based on non-clinical samples and researchers have yet to determine whether schizotypy may be a mechanism underlying the relationship between cannabis use and psychosis among individuals with an established psychotic disorder. Findings that schizotypy was not associated with cannabis use per se in these studies, suggest that schizotypy is not a common factor underlying both psychosis and cannabis use.

The personality traits of neuroticism (N; a general disposition to experience negative mood states, decreased stress tolerance and difficulty in controlling one's mood) and impulsivity (IMP; behavioral disinhibition, sensation-seeking, risk-taking and a lack of constraint) are also potential moderators of the relationship between cannabis use and psychosis (Blanchard *et al*., 2000b). Cross-sectional studies have found impulsivity, sensation seeking and novelty seeking to be associated with a lifetime history of cannabis use among patients with psychotic disorders (Kwapil, 1996). Neuroticism has also been associated with greater substance use problems and severity in comorbid psychotic populations (Blanchard *et al*., 1998) and to predict psychotic-symptom relapse over a 12-month follow-up (Gleeson, 2001). However, the cross-sectional nature of these data limits the conclusions that can be made and prospective studies are required to determine the influence of N, IMP and schizotypy on cannabis use, psychotic symptoms and relapse in psychotic populations.

The mechanisms by which personality traits such as N, IMP or schizotypy exert their influence on cannabis use and psychosis also remain unclear. Using a stress-vulnerability-coping model of psychosis, Blanchard *et al.* (2000b) proposed that personality variables may influence affective outcomes and increase risk for substance use via their influence on stress and coping. Neuroticism has been found to exert an influence on both exposure to stressful life events and reactivity to them via the choice and effectiveness of coping strategies (Blanchard *et al*., 2000a). In support of this model, elevated stress levels and life events have been found to be associated with substance-use severity and the use of maladaptive-coping strategies in psychotic populations (Blanchard *et al*., 2000a).

Personality traits have also been found to have a differential effect on the selection of coping strategies in a comorbid group, in which extraversion was associated with more adaptive coping strategies whereas N and IMP were related to the selection of maladaptive-coping skills (Ventura *et al*., 1992; Pallanti *et al*., 1997, Sinha, 2001). However, research has yet to consider the influence of cognitive variables including motives (reasons for cannabis use) and expectancies (beliefs about the outcomes or consequences of cannabis use) for cannabis use on the relationship between personality variables, affect, stress and coping among individuals with cannabis use and psychosis. Furthermore, identifying a personality trait as a moderator of the relationship between cannabis use and psychotic symptoms does not exclude genetic or other biological mechanisms that may underlie the personality trait or convey its effects. Both schizotypy and N appear to have strong genetic components (Jang *et al*., 2001; Hettema *et al*., 2004; Lenzenweger, 2010), although the precise genetic loci remain to be definitively identified.

Motives and expectancies for cannabis use in psychosis

A growing number of studies have examined motives and expectancies for cannabis use among individuals with psychosis (see Green *et al*., 2003; Gregg *et al*., 2007 for reviews). Qualitative studies using checklists/questionnaires have found that people with psychosis primarily report using cannabis to relieve anxiety and dysphoria (Test *et al*., 1989; Dixon *et al*., 1991; Warner *et al*., 1994; Baigent *et al*., 1995; Addington and Duchak, 1997; Fowler *et al*., 1998; Schofield *et al*., 2006). However, several studies also found cannabis use to be associated with increased psychotic symptoms, leading several authors to suggest psychotic individuals may use cannabis to reduce negative affect at the expense of an increase in psychotic symptoms (Dixon *et al*., 1989; Warner *et al*., 1994; Baigent *et al*., 1995). This conclusion is supported by a recent experience sampling study, in which individuals with psychosis reported that cannabis use was associated with subsequent increases in positive mood, but also an increased levels of psychotic symptoms (Henquet *et al*., 2010).

Quantitative studies using valid and reliable measures of motives and expectancies for substance use in psychosis report similar findings. Two studies found psychotic patients with a substance-use disorder (SUD; excluding alcohol) were significantly more likely to report using substances to reduce or regulate negative emotions (coping motives) and to enhance

positive mood or well-being (enhancement motives) than those without a SUD (Mueser *et al*., 1995; Spencer *et al*., 2002). Cannabis using patients were also more likely to endorse expectancies that cannabis use would improve their social and sexual functioning and their ability to reason and think clearly (perceptual and cognitive enhancement), but no differences were found on cannabis-use expectancies concerning relaxation and tension reduction (Mueser *et al*., 1995).

Together these studies indicate that people with psychosis report using cannabis primarily to reduce negative affect or increase positive affect. While these findings are consistent with a variant of the symptom alleviation model, research indicating that this reduction in negative affect may come at the expense of increased positive psychotic symptoms is more consistent with vulnerability models of cannabis use in psychosis. Nonetheless, motives and expectancies for cannabis use have been identified as important predictors of the frequency of cannabis use in psychotic populations in cross-sectional research and in one prospective study over four weeks, indicating they are important treatment targets in this comorbid population (Spencer *et al*., 2002; Green *et al*., 2007; Hides *et al*., 2009).

Cannabis use expectancies and motives may be important mediators of the influence of affective symptoms on cannabis use. For example, Spencer *et al*. (2002) found substance-use motives mediated the influence of global symptom severity and negative psychotic symptoms on the severity of substance dependence among 69 psychotic patients. However, a second study of 101 inpatients with psychosis and cannabis use found no association between cannabis-use expectancies and positive, negative or general psychopathology symptoms (Hides *et al*., 2008). More prospective research is required to increase understanding of the relationships between cannabis use, symptom variables and motives and expectancies for cannabis use. Research exploring how these cognitive variables interact with personality, stress and coping variables is also warranted, due to increasing evidence of possible mediational relationships between these variables influencing substance use in normal populations (Wills *et al*., 1999; Kuntsche *et al*., 2008; Mezquita *et al*., 2010)

A number of other cognitive factors have also been identified as potentially important variables in the relationship between cannabis use and psychosis. For example, a recent qualitative study identified four key themes that motivated and maintained substance use in 19 current substance users with psychosis (Lobbana *et al*., 2010). The first theme focused on the perception of drug use as "normal" in the individual's environment, while the second focused on whether the individual had internal or external attributions for their drug use. Those with internal attributions tended to report using drugs to have fun, reduce social anxiety and negative affect and improve interpersonal relationships, while those who made external attributions tended to focus on the influence of others or situational variables on their drug use. A third theme, changes in life goals affecting drug use, showed that changes in drug use could be motivated either by key life events or changes in the values of the individual's peer group toward pro-social goals. The final theme focused on beliefs about the relationship between drugs and mental health and focused on drugs being associated with the onset of psychosis, using drugs to cope with mental health symptoms (primarily depression and anxiety) and there being no relationship between drugs and psychosis.

How explicit motives or expectancies for cannabis use interact with implicit cognitions has also yet to be determined. Such implicit automatic processes are thought to underlie why people with psychosis continue to use cannabis, despite knowledge of its harms (Wiers *et al*., 2002). Implicit cognitions exert their influence outside of conscious control through two dual systems: an impulsive system in which stimuli are evaluated automatically according to their motivational and emotional significance; and a slower reflective system involving controlled processes related to conscious deliberations and emotion regulation (Stacy and Wiers, 2010). Studies have shown both implicit and explicit cognitions to be associated with alcohol use in adolescents, undergraduates and adults, but implicit associations predict more of the variance in alcohol use (Rooke *et al*., 2008; Stacy and Wiers, 2010). Implicit association tasks among high-risk adolescents have also been found to predict unique variance in cannabis use (Ames *et al*., 2007) and prospectively to predict alcohol use in college students over 6 months (Kelly *et al*., 2005). Implicit alcohol-related cognitions have been more strongly associated with the alleviation of negative affect than the enhancement of positive affect in a number of studies (Stacy and Wiers, 2010), and a recent study found explicit expectancies of negative affect moderated the association between depression and implicit alcohol-related memory among university students

(Kelly *et al.*, 2011). Together, this research suggests that automatic associations may play an important role in substance-use behavior as a result of complex interaction between cognitive, behavioral and affective processes, but the influence of these variables in psychotic populations is yet to be examined.

Biological models of cannabis use in psychosis

Finally, there has been increasing interest in biological models of the relationship between cannabis use and psychosis. For example, as discussed in Chapters 12 and 15, functional polymorphisms of the catechol-O-methyltransferase (*COMT Val^{158}Met*) gene, which reduces the capacity to metabolize dopamine among cannabis users, has been identified as a potential moderator of the onset of psychosis among cannabis users under the age of 18 years, providing evidence for a vulnerability model of cannabis use in psychosis (Caspi *et al.*, 2005). However, no association between the *COMT Val^{158}Met* and the age of onset of cannabis use or psychosis was found in a retrospective study of 493 patients with an established diagnosis of schizophrenia (Zammit *et al.*, 2007). These findings suggest that once a person has an established disorder, other gene-environment interactions or mechanisms may come into play (e.g. increased sensitivity to acute dopamine release after cannabis use). For example, van Winkel *et al.* (2008) found that *COMT Val^{158}Met* moderated the effect of stress on psychotic symptoms and negative affect in cannabis users with a psychotic disorder compared with cannabis-using controls, suggesting there may be complex interactions between this genetic variable and a range of environmental factors among cannabis users with psychosis. The reader is referred to Chapter 12 for a further discussion of these issues.

A number of biological models have focused on the role of the endogenous cannabinoid system on neurodevelopment and brain functioning via activation of the dopamine and glutaminergic systems and suppression of gamma-aminobutyric acid (GABA) functions (Cohen *et al.*, 2008 and see Chapter 6). Evidence suggests that acute cannabis use increases dopamine initially and improves cognitive functioning, while repeated cannabis exposure suppresses dopamine release in the prefrontal cortex (PFC) resulting in cognitive deficits and negative symptoms (Cohen *et al.*, 2008). Such changes in the neurocognitive effects of cannabis use over time may account for the inconsistent results of neuropsychological studies. For example, a recent review identified three studies reporting that cannabis use was associated with deficits in executive functioning, memory and attention, and four studies that found cannabis was associated with improvements in both those variables, and psychomotor speed and visual spatial construction (Coulston *et al.*, 2007). The impact of cannabis-mediated increases in mesolimbic dopaminergic activity in the ventral tegmental area and PFC that regulate motivation, salience attribution and reward-related behaviors also requires further investigation due to the implications of these neurocognitive changes on an individual's motivation and ability to change their cannabis use (Cohen *et al.*, 2008). Clearly, more work on how these neurocognitive changes impact on the cognitive, affective, behavioral and neuropsychological functioning of individuals with cannabis use and psychosis is required.

Conclusions

The relationship between cannabis and psychosis is likely to involve a complex interaction between biological, psychological and environmental variables. Dual process theories of addiction and other biologically based personality theories (e.g. the work of Eysenck, Cloninger and Gray), suggest that approach and avoidance personality traits may exert a strong influence on cannabis use in psychosis. Research has also highlighted the potential importance of genetic and biological factors, stress, coping style, negative affect as well as cannabis-use motives and expectancies. The role of a number of other cognitive (e.g. attributional style), affect (e.g. negative versus positive temperament) and behavioral (e.g. behavioral self-control) factors in the relationship between cannabis use and psychosis is yet to be determined. This includes the role of implicit cognitions for cannabis use in psychosis, as they have been associated with both substance-use behavior and the reduction of negative affect among normal populations. However, stress-vulnerability-coping models incorporating how these psychological variables interact (i.e. as moderators or mediators) with biological and environmental variables to influence cannabis use among individuals with psychosis are yet to be applied. Such studies could provide valuable insights into the aetiology of cannabis use in psychosis and assist with the development of innovative treatment interventions.

References

Addington, J. and Duchak, V. (1997) Reasons for substance use in schizophrenia. *Acta Psychiatr Scand,* **96**:329–33.

Ames, S.L., Grenard, J.L., Thush, C., Sussman, S., Wiers, R.W. and Stacey, A.W. (2007) Comparison of indirect assessments of association as predictors of marijuana use among at-risk adolescents. *Exp Clin Psychopharmacol,* **15**:204–18.

Anthony, J.C. and Helzer, J.E. (1991) Syndromes of drug abuse and dependence. *In*: Robins, L. N. and Regir, D. A. (eds.) *Psychiatric Disorders in America: The Epidemiologic Catchment Area Study* New York: Free Press.

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Avshalam, C. and Moffitt, T.E. (2002) Cannabis use in adolescence and risk for adult psychosis: Longitudinal prospective study. *BMJ,* **325**:1195–9.

Baigent, M., Holme, G. and Hafner, R.J. (1995) Self reports of the interaction between substance abuse and schizophrenia. *Aust NZ J Psychiatry,* **29**:69–74.

Barkus, E. and Lewis, S. (2008) Schizotypy and psychosis-like experiences from recreational cannabis in a non-clinical sample. *Psychological Medicine,* **38**:1267–76.

Barkus, E., Stirling, J., Hopkins, R. and Lewis, S. (2006) Cannabis-induced psychosis-like experiences are associated with high schizotypy. *Psychopathology,* **39**:175–8.

Blanchard, J.J., Brown, S.A., Horan, W.P. and Sherwood, A.R. (2000a) Substance use disorders in schizophrenia: Review, integration and a proposed model. *Clin Psychol Rev,* **20**:207–34.

Blanchard, J.J., Brown, S.A., Horan, W.P. and Sherwood, A.R. (2000b) Substance use disorders in schizophrenia: Review, integration, and a proposed model. *Clin Psychol Rev,* **20**:207–34.

Blanchard, J.J., Mueser, K.T. and Bellack, A.S. (1998) Anhedonia, positive and negative affect, and social functioning in schizophrenia. *Schizophr Bull,* **24**:413–24.

Caspi, A., Moffitt, T.E., Cannon, M., *et al.* (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene x environment interaction. *Biol Psychiatry,* **57**:1117–27.

Cohen, M., Solowij, N. and Carr, V. (2008) Cannabis, cannabinoids and schizophrenia: integration of the evidence. *Aust NZ J Psychiatry,* **42**:357–68.

Coulston, C.M., Perdices, M. and Tennant, C.C. (2007) The neuropsychology of cannabis and other substance use in schizophrenia: Review of the literature and critical evaluation of methodological issues. *Aust NZ J Psychiatry,* **41**:869–84.

Degenhardt, L., Hall, W. and Lynskey, M. (2001) Alcohol, cannabis and tobacco use among Australians: A comparison of their associations with other drug use and use disorders, affective and anxiety disorders, and psychosis. *Addiction,* **96**:1603–14.

Degenhardt, L., Tennant, C., Gilmour, S., *et al.* (2007) The temporal dynamics of relationships between cannabis, psychosis and depression among young adults with psychotic disorders: findings from a 10-month prospective study. *Psychol Med,* **37**:927–34.

Dixon, L., Haas, G., Weiden, P.J., Sweeney, J. and Frances, A.J. (1991) Drug abuse in schizophrenic patients: Clinical correlates and reasons for use. *Am J Psychiatry,* **148**:224–30.

Dixon, L., Haas, G.H., Dulit, R.A., Weiden, P.J., Sweeney, J. and Hien, D. (1989) Substance use in schizophrenia: Preferences, predictors and psychopathology. *Schizophr Res,* **2**:6.

Farrell, M., Howes, S., Bebbington, P., *et al.* (2001) Nicotine, alcohol and drug dependence and psychiatric comorbidity: Results of a national household survey. *Brit J Psychiatry,* **179**:432–7.

Ferdinand, R.F., Sondeijker, F., Van Der Ende, J., Selten, J.P., Huizink, A. and Verhulst, F.C. (2005) Cannabis use predicts future psychotic symptoms, and vice versa. *Addiction,* **100**:612–17.

Fergusson, D.M., Horwood, L.J. and Swain-Campbell, N.R. (2003) Cannabis dependence and psychotic symptoms in young people. *Psychol Med,* **33**:15–21.

Fowler, I.L., Carr, V.J., Carter, N.T. and Lewin, T.J. (1998) Patterns of current and lifetime substance use in schizophrenia. *Schizophr Bull,* **24**:443–55.

Gleeson, J.F., Rawlings, D., Jackson, H.J., *et al.* (2005) Agreeableness and neuroticism as predictors of relapse after first-episode psychosis: A prospective follow-up study. *Journal of Nervous and Mental Disease,* **193**:160–169.

Green, B., Kavanagh, D. and Young, R. (2003) Being stoned: A review of self-reported cannabis effects. *Drug Alcohol Rev,* **22**:453–60.

Green, B., Kavanagh, D.J. and Young, R.M. (2007) Predictors of cannabis use in men with and without psychosis. *Addict Behav,* **32**:2879–87.

Gregg, L., Barrowclough, C. and Haddock, G. (2007) Reasons for increased substance use in psychosis. *Clin Psychol Rev,* **27**:494–510.

Henquet, C., Van Os, J., Kuepper, R., *et al.* (2010) Psychosis reactivity to cannabis use in daily life: An experience sampling study. *Brit J Psychiatry,* **196**:447–53.

Hettema, J.M., Prescott, C.A. and Kendler, K.S. (2004) Genetic and environmental sources of covariation between generalized anxiety disorder and neuroticism. *Am J Psychiatry,* **161**:1581–7.

Hides, L., Dawe, S., Kavanagh, D.J. and Young, R.M. (2006) Psychotic symptom and cannabis relapse in recent onset psychosis: Prospective study. *Brit J Psychiatry,* **189**:137–43.

Hides, L., Kavanagh, D.J., Dawe, S. and Young, R.M. (2009) The influence of cannabis use expectancies on cannabis use and psychotic symptoms in psychosis. *Drug Alcohol Rev,* **28**:250–6.

Hodgins, S., Toupin, J. and Côté, G. (1996) Schizophrenia and antisocial personality disorder: A criminal combination. *In*: Schlesinger, L. B. (ed.) *Explorations in Criminal Psychopathology: Clinical Syndromes with Forensic Implications*. Springfield, IL: Charles C. Thomas.

Jang, K.L., Hu, S., Livesley, W.J., *et al.* (2001) Covariance structure of neuroticism and agreeableness: A twin and molecular genetic analysis of the role of the serotonin transporter gene. *J Pers Soc Psychol,* **81**:295–304.

Kelly, A.B., Masterman, P.W. and Marlatt, G.A. (2005) Alcohol related associative strength and drinking behaviors: concurrent and prospective relationships. *Drug Alcohol Rev,* **24**:489–98.

Kelly, A.B., Masterman, P.W. and Young, R.M. (2011) Negative mood, implicit alcohol-related memory, and alcohol use in young adults: The moderating effect of alcohol expectancy. *Addictive Behaviors*, **36**:148–157.

Khantzian, E.J. (1997) The self-medication hypothesis of substance use disorders: A reconsideration and recent applications. *Harv Rev Psychiatry,* **4**:231–44.

Kuntsche, E., Von Fischer, M. and Gerhard, G. (2008) Personality factors and alcohol use: A mediator analysis of drinking motives. *Pers Individ Dif,* **45**:796–800.

Kwapil, T.R. (1996) A longitudinal study of drug and alcohol use by psychosis-prone and impulsive-nonconforming individuals. *J Abnorm Psychol,* **105**:114–23.

Lenzenweger, M.F. (2010) *Schizotypy and Schizophrenia: The View from Experimental Psychopathology,* New York, Guilford Press.

Lobbana, F., Barrowclough, C., Jeffery, S., *et al.* (2010) Understanding factors influencing substance use in people with recent onset psychosis: A qualitative study. *Soc Sci Med,* **70**:1141–7.

Mason, O., Morgan, C., Dhiman, S., *et al.* (2009) Acute cannabis use causes increased psychotomimetic experiences in individuals prone to psychosis. *Psychol Med,* **39**:951–6.

Merikangas, K.R., Mehta, R.L., Molnar, B.E., *et al.* (1998) Comorbidity of substance use disorders with mood and anxiety disorders: Results of the international consortium in psychiatric epidemiology. *Addict Behav,* **23**:893–908.

Mezquita, L., Stewart, S.H. and Ruipérez, M. (2010) Big-five personality domains predict internal drinking motives in young adults. *Personality and Individual Differences,* **49**:240–5.

Moore, T. H., Zammit, S., Lingford-Hughes, A., *et al.* (2007) Cannabis use and risk of psychotic or affective mental health outcomes: A systematic review. *Lancet,* **370**:319–28.

Mueser, K.T., Drake, R.E. and Wallach, M.A. (1998) Dual diagnosis: A review of etiological theories. *Addict Behav,* **23**:717–34.

Mueser, K.T., Nishith, P., Tracy, J.I., Degirolamo, J. and Molinaro, M. (1995) Expectations and motives for substance use in schizophrenia. *Schizophr Bull,* **21**:367–78.

Mueser, K.T., Yarnold, P.R., Rosenberg, S.D., Swett, C., Miles, K.M. and Hill, D. (2000) Substance use disorder in hospitalized severely mentally ill psychiatric patients: prevalence, correlates, and subgroups. *Schizophr Bull,* **26**:179–92.

Pallanti, S., Quercioli, L. and Pazzagli, A. (1997) Relapse in young paranoid schizophrenic patients: A prospective study of stressful life events, P300 measures, and coping. *Am J Psychiatry,* **154**:792–8.

Robins, L.N. (1966) *Deviant Children Grown Up*, Huntington, NY, Robert E. Krieger Publishing Company.

Rooke, S.E., Hine, D.W. and Thorsteinsson, E.B. (2008) Implicit cognition and substance use: A meta-analysis. *Addict Behav,* **33**:1314–28.

Schofield, D., Tennant, C.C., Nash, L., *et al.* (2006) Reasons for cannabis use in psychosis. *Aust NZ J Psychiatry,* **40**:570–4.

Sinha, R. (2001) How does stress increase risk of drug abuse and relapse? *Psychopharmacology,* **158**:343–59.

Spencer, C., Castle, D. and Michie, P.T. (2002) Motivations that maintain substance use among individuals with psychotic disorders. *Schizophr Bull,* **28**:233–47.

Stacy, A.W. and Wiers, R.W. (2010) Implicit cognition and addiction: A tool for explaining paradoxical behavior. *Ann Rev Clin Psychol,* **6**:551–75.

Stirling, J., Barkus, E., Nabosi, L., Irshad, S., Roemer, G. and Schreudergoidheijt, B. (2008) Cannabis-induced psychotic-like experiences are predicted by high schizotypy: Confirmation of preliminary results in a large cohort. *Psychopathology,* **41**:371–8.

Test, M.A., Wallisch, L.S., Allness, D.J. and Ripp, K. (1989) Substance use in young adults with schizophrenic disorders. *Schizophr Bull,* **15**:465–76.

Thornicroft, G. (1990) Cannabis and psychosis: Is there epidemiological evidence for an association? *Brit J Psychiatry*, **157**:25–33.

van Winkel, R., Henquet, C., Rosa, A., *et al*. (2008) Evidence that the COMTVal158Met polymorphism moderates sensitivity to stress in psychosis: an experience-sampling study. *Am J Med Genet B (Neuropsychiatr Gen),* **147B**:10–17.

Ventura, J., Nuechterlein, K.H., Hardesty, J.P. and Gitlin, M. (1992) Life events and schizophrenic relapse after withdrawal from medication. *Brit J Psychiatry,* **161**:615–20.

Verdoux, H., Gindre, C., Sorbora, F., Tournier, M. and Swendsen, J.D. (2003) Effects of cannabis and psychosis vulnerability in daily life: An experience sampling test study. *Psychol Med,* **33**:23–32.

Warner, R., Taylor, D., Wright, J., *et al*. (1994) Substance use among the mentally ill: Prevalence, reasons for use, and effects on illness. *Am J Orthopsychiatry,* **64**:30–9.

Wiers, R.W., Stacy, A.W., Ames, S.L., *et al.* (2002) Implicit and explicit alcohol-related cognitions. *Alcoholism Clin Exp Res,* **26**:129–37.

Wills, T.A., Sandy, J.M., Shinar, O. and Yaeger, A. (1999) Contributions of positive and negative affect to adolescent substance use: Test of a bidimensional model in a longitudinal study. *Psychol Addict Behav,* **13**:327–38.

Zammit, S., Moore, T.H., Lingford-Hughes, A., *et al.* (2008) Effects of cannabis use on outcomes of psychotic disorders: systematic review. *Brit J Psychiatry,* **193**:357–63.

Zammit, S., Spurlock, G., Williams, H., *et al*. (2007) Genotype effects of CHRNA7, CNRI and COMT in schizophrenia: interactions with tobacco and cannabis use. *Brit J Psychiatry*, **191**:402–7.

Chapter

21

Addressing cannabis use in people with psychosis

Wynne James and David Castle

As detailed elsewhere in this book, cannabis use is common among people with schizophrenia, and regular use – even at relatively low levels – can have a serious impact on illness course (Grech *et al*., 2005; Degenhardt *et al*., 2007). Understanding which interventions can reduce the impact of cannabis and other drug use and support recovery from psychotic illness is increasingly the focus of psychiatric practice and research.

This chapter reviews the current evidence for addressing cannabis use among people with a psychotic illness, focusing specifically on screening, assessment (including evaluating the nature and extent of use, reasons for use, and readiness to change), models of service delivery and treatment interventions. It must be acknowledged that there remains a limited body of knowledge from which to draw recommendations about specific treatment interventions for this group of patients. These limitations aside, consolidation and synthesis of what is currently known is important to advance practice, stimulate further debate and provide direction for future research.

Screening

An awareness of past or present substance use is essential when determining a patient's diagnosis; deciding on the most appropriate treatment path; and planning their future care (Zeidonis and Fisher, 1994). Undetected substance use can confuse the interpretation of important signs and symptoms of psychosis, possibly leading to over-management with medication, as well as rendering other psychosocial interventions less effective (Drake *et al*., 1993). Conversely, the early detection of substance use can lead to better treatment outcomes for patients and contribute to an overall reduction in healthcare costs by facilitating timely and appropriate treatment interventions (Tiet *et al*., 2008).

Despite very clear evidence that supports the routine use of screening measures within mental health services, it remains disappointing that substance use all too often goes unconsidered, undetected and therefore untreated (Graham, 2004; Green *et al*., 2007). This situation stems from a number of complex factors that include: an under-appreciation among some clinicians of the prevalence and potential implications of cannabis and other substance use in patients with psychosis (Drake *et al*., 1993b); a lack of awareness regarding different approaches to screening and detection of substance use (Seigfried, 1998); and an absence of systematic screening processes within mental health services to facilitate detection (Ananth *et al*., 1989; Drake and Wallach, 1989).

Improved rates of substance-use detection within mental health services has been found through the introduction of formal screening programs that are integrated into everyday clinical practice (Appleby *et al*., 1997). Although there is only limited evidence that identifies which are the essential elements of successful screening programs, the following have been highlighted: staff education on contemporary evidence-based approaches to screening and treatment (Baker *et al*., 2002; Schulte *et al*., 2008); the routine inclusion of questions about past or current substance use within relevant assessment documentation in psychiatric settings (Ley *et al*., 2002); and the use of self-report screening instruments where substance use is suspected and more targeted investigation is required (Crome *et al*., 2006).

Ideally, self-report instruments for this cohort should be brief and screen for multiple substances, as poly-drug use is common (Griffin *et al*., 2009). As many self-report measures were designed for application within the general population, they must also have demonstrated reliability and validity within the population of people with psychotic disorders, as well as for younger and older people, and both men and women (Bennett *et al*., 2006). Tiet *et al*. (2008) provide a comprehensive

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review identifying the strengths and weaknesses of many common screening tools for substance-use disorders among people with mental illness, including: the Dartford Assessment of Life Instrument (Rosenberg *et al*., 1998); the Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST; WHO, 2002); and the Drug Abuse Screening Test (DAST; Skinner, 1982). Inherent shortcomings identified within each instrument prompted the reviewers to conclude that the search for a gold standard screening tool continues.

Concordance between self-report and objective clinical findings can prove suspect when patients deny or under-report their use of illicit substances (Weiss *et al*., 1998; de Beaurepaire *et al*., 2007). Therefore, to produce the highest rate of detection, routine screening instruments and self-report measures should ideally be used in conjunction with biochemical assays such as urinalysis and collateral information from family members, significant others and clinicians (Essock *et al*., 2006).

While screening instruments should be routinely used for all patients, there are a number of clinical correlates of cannabis use among the psychiatric population that can alert staff to which patients should be treated with a higher degree of suspicion. As with the general population, young single males who smoke, consume higher levels of alcohol and attain poorer educational achievement are over-represented among cannabis users and should be assessed particularly carefully (Dixon *et al*., 1991; Hall and Degenhardt, 2000). Other factors such as homelessness, legal and financial problems, violence and non-compliance with treatment may also be predictors of ongoing substance use (Zeidonis and Fisher, 1994). Finally, attention should be paid to any patients who remain unresponsive to conventional treatments or who suffer illness relapses frequently owing to unexplained circumstances (Linszen *et al*., 1994).

Improving rates of detection:

- include questions addressing substance use within admission and review documentation;
- routine use of appropriate screening tools;
- laboratory findings, including urinalysis;
- collateral information from relatives and significant others;
- an awareness of signs and symptoms of regular use, intoxication and withdrawal;
- clinical correlates.

Assessment

Whereas screening is concerned with case-finding and triage, assessment refers to the structured collection of relevant information essential for determining diagnosis and treatment, ascertaining current need and monitoring change. Understanding the interplay between cannabis use and mental illness, and then deciding on which interventions to employ, requires careful analysis of a number of complex factors that include current mental state, the nature and extent of cannabis use, reasons for use and readiness to change. Other factors such as housing, employment and relationships can also have a significant bearing on the course of recovery and require due consideration (Carey and Correira, 1998).

The components of assessment vary widely from service to service and setting to setting, depending on the information sought by the clinician and/or researcher. What follows are some examples of structured assessment tools useful for understanding the nature and extent of cannabis use and for evaluating motivational factors such as reasons for cannabis use and readiness to change. These are all insights deemed essential for the development of individualized care plans and for informing any subsequent treatment interventions (Drake and Meuser, 2000).

Assessing the nature and extent of cannabis use

A number of diagnostic interview schedules exist to assess the presence of both cannabis-use disorders and mental disorders in a single assessment. These include: the Composite International Diagnostic Interview (CIDI, Semler *et al*., 1987); the Structured Clinical Interview for DSM–IV (SCID, First *et al*., 1992); the Psychiatric Research Interview for Substance and Mental Disorders (PRISM: Hasin *et al*., 1996); and the Schedule for Clinical Assessment in Neuropsychiatry (SCAN, Wing *et al*., 1990). However, although these instruments are routinely used to add rigor and provide consistency to research enquiry, they are often deemed unsuitable for day-to-day clinical practice, as they take considerable time to complete and require staff to have undertaken specific training in their use (Crome *et al*., 2006).

The Addiction Severity Index (ASI) (McLellan *et al*., 1992) is probably the most commonly used brief semi-structured instrument that assesses the nature

and severity of alcohol and substance use among drug-using patients within a clinical setting (Nidecker *et al.*, 2008). The ASI evaluates information concerning history, frequency and consequences of alcohol and substance use, as well as medical, legal, employment, social/family and psychological functioning. Although a number of studies have raised concerns about the overall performance of the ASI when used in isolation with patients with severe mental illness (Lehman *et al.*, 1996; RachBeisal *et al.*, 1999), the drug and alcohol severity and family/social domains of the instrument demonstrate good psychometric properties when administered with such patients (Gearon *et al.*, 2001; Meuser *et al.*, 2009).

The Severity of Dependence Scale (SDS) (Gossop *et al.*, 1995) is another brief self-report measure suitable to determine the degree of a patient's principle substance of use. The SDS is brief (five items), requires minimal training and has sound psychometric properties when used to identify cannabis dependence among people with psychotic illness (Hides *et al.*, 2009).

Finally, the Cannabis and Substance Use Assessment Schedule (CASUAS) has been used in a number of studies to measure cannabis use and its impact on patients (Edwards *et al.*, 2006; Hinton *et al.*, 2007). The tool, derived from the SCAN and the ASI, quantifies frequency of cannabis use and provides a measure of severity based on a detailed assessment of amount used, withdrawal/dependence, cannabis related problems, risk-taking, interference with functioning, craving and confidence to quit.

Reasons for use

As detailed in Chapter 20, cannabis use within the psychiatric population is reported to be motivated by many of the same reasons as those found within the general population – to relieve depression, reduce anxiety or boredom, and to help users relax and socialize (Schofield *et al.*, 2006; Schaub *et al.*, 2008; Horsfall *et al.*, 2009). Motives related to self-medication – that is, to relieve or cope with hallucinations or reduce paranoia and suspicion – have also been reported (Khantzian, 1997). Gaining a better understanding of a patient's underlying motives for use is necessary to inform treatment planning, as the reasons for use may predict patterns of use and also mediate the relationship between symptoms and substance dependence.

The Substance Use Scale for Psychosis (SUSP, Spencer *et al.*, 2002) is a 26-item self-report instrument that contains five subscales: social use, enhancement, coping with unpleasant affect, conformity/acceptance and coping with positive symptoms or side effects from medication. The scale includes a number of items from the Drinking Motives Questionnaire (Cooper *et al.*, 1995), as well as additional motives specific to symptoms of severe mental illness. Its reliability and validity have been demonstrated in individuals with psychotic disorders (Spencer *et al.*, 2002).

More recently, Gregg *et al.* (2009) developed and tested the Reasons for Substance Use in Schizophrenia scale (ReSUS) to further explore reasons for use against demographic variables, symptomatology, patterns of use and motivation to change. The ReSUS contains 40 items describing situations in which people drink or use drugs. Respondents are asked to indicate for each situation whether they used their most problematic substance as "never," "sometimes," "often" or "always." Gregg *et al.* (2009) found the ReSUS to be a valid and reliable measure for assessing reasons for substance use in people with schizophrenia.

Findings from measures such as these can then be used to select the most appropriate management strategies and tailor treatment interventions for individual patients. For instance, individuals who use cannabis to cope with negative affect may benefit from interventions designed to reduce or manage stress more effectively. For those who use cannabis to enhance emotional experiences, other sources of pleasure can be explored and developed.

Readiness to change

Assessment of readiness to change is another crucial consideration when treating substance use among people with psychotic illness (Pantalon and Swanson, 2003). The Transtheoretical Model (TTM) is a commonly used, conceptual framework for understanding a patient's readiness to change their drug-using behavior (Prochaska and Velicer, 1997). The TTM identifies five different stages of change: (1) precontemplation – where a patient expresses no intention to change within the next 6 months; (2) contemplation – where change is intended sometime in the future (usually defined as between 1 and 6 months); (3) preparation – where change is intended in the immediate future (1 month) and steps are taken to help prepare for change; (4) action – where the target behavior has been modified for less than 6 months; and finally (5) maintenance – where the change in target behavior has extended beyond 6 months. The

first three stages are viewed as motivational, while the latter two stages are viewed as actional. Progression through the stages is seen as sequential and dependent upon the individual successfully tackling a range of different barriers, while acknowledging that relapse to an earlier stage can, and often does, occur.

Nidecker *et al.* (2008) recently evaluated five leading assessment measures that utilize the TTM conceptual framework in patients with co-occurring substance abuse and severe mental illness: the University of Rhode Island Change Assessment, Maryland; the Decision Balance Scale; the Process of Change Scale; the Temptation to Use Scale; and the Abstinence Self-Efficacy Scale. Results revealed good reliability and validity across the five measures and supported their use in people with co-occurring severe mental illness and substance abuse. Importantly, they concluded that people with severe mental illness use a similar process of change as those without severe mental illness, thus lending further support to motivational interventions for this group tailored to an individual's current stage of change (DiClemente *et al.*, 2008).

As with screening instruments, the reliability of findings from any assessment tool is reliant on each patient's willingness to acknowledge and talk about their substance use. As such, assessors need to do as much as they can to engage patients, build rapport and develop therapeutic relationships so that patients feel that disclosure about their use may result in positive change, rather than punitive action. It is also important to acknowledge that none of the areas identified above are static: the nature and extent of use, reasons for use and readiness to change may require repeated assessment over time, especially when reviewing outcomes following the implementation of specific treatment interventions (see below).

Assessment:

- address current circumstances and extent of use;
- history of use;
- past treatment;
- impact current use has on illness;
- motives for ongoing use;
- readiness to change;
- support.

Models of service delivery

The literature identifies three broad service delivery approaches for the treatment of people with co-occurring severe mental illness and substance abuse: sequential, parallel and integrated (see below). Within the *sequential* model, either the psychiatric illness or the substance misuse is treated before the other. An example would be addressing alcohol dependency before offering treatment for depression. This approach has been criticized for being fragmented and for placing the burden of integration on the patient (Drake and Meuser, 2000). Sequential approaches are often the result of psychiatric and drug services being organizationally separated, with each holding inflexible admission criteria that prevent entry by patients with dual problems.

Models of service delivery:

- serial: the treatment of one condition followed by treatment of the other;
- parallel: the concurrent treatment of both conditions by different services;
- integrated: the treatment of both conditions at the same time within one setting.

The *parallel* model refers to the concurrent but separate treatment of both disorders by different specialist teams. For example, having a patient's psychosis managed by psychiatric services, while at the same time their cannabis use is being addressed by drug services. The benefit of the parallel model is that both disorders are treated simultaneously by experts in their field (Kavanagh and Connolly, 2009). However, there are also a number of disadvantages to this model, including the expectation that the patient will attend two different services and engage in two often very different treatment paradigms. Treatment drop-out rates are often high with this sort of approach, and any positive outcomes rely heavily on pre-agreed collaboration and communication between different services.

Finally, the *integrated* model emphasizes the importance of treating both substance abuse and mental illness at the same time by the same service (preferably by the same clinician or team of clinicians) (Meuser *et al.*, 2003). This approach recognizes that the responsibility for integrating services lies with the service provider and not with the patient (Drake *et al.*, 2004). Integrated services have a number of common elements that include case management, an assertive style of engagement, comprehensive services (including inpatient, community, day hospital and outpatient care) and a long-term optimistic perspective of recovery (Green *et al.*, 2007).

The integrated treatment model is widely accepted as the preferred model of care for people with co-occurring problems (Drake *et al*., 2004b; Essock *et al*., 2006; Green, 2007). Implementing integrated models of care requires strong and clear leadership at both clinical and organizational levels, staff training in the skills necessary to manage dual problems, clinical supervision that ensures fidelity to the principals of integrated service delivery and secure funding that recognizes the increased costs associated with providing intensive longer-term integrated community care (Brunette *et al*., 2008). However, while integrated models of care are currently favored over sequential or parallel approaches in the limited literature available, support for integration is not without criticism. Integration has significant leadership, resource, training and treatment delivery implications, which are likely contributing factors to the current paucity of integrated services available within public mental health settings (Ducharme *et al*., 2006; Schulte *et al*., 2008).

Arguably, the lack of conclusive evidence for the effectiveness of integration must raise the question of whether such effort and investment are fully justified (Ley *et al*., 2002). Mental health services working toward integration should move cautiously, evaluating the impact these changes have on patient outcomes and on the confidence and capacity of staff to manage both disorders simultaneously. Few mental health staff are trained to competently manage both disorders simultaneously. Therefore to facilitate integration and to up-skill staff, mental health services should consider utilising an interim parallel model, where closer links between drug and alcohol services are developed and where memoranda of understanding and pathways of care are agreed upon. Training and supervision should be offered between services to increase the capacity of both sectors to respond effectively. Finally, mental health teams should be strengthened with specialist dual-diagnosis workers who work alongside staff in helping manage patients with dual problems.

Psychosocial treatment interventions

Early treatment interventions for people with a dual diagnosis frequently utilized a stage-wise approach that relied heavily on the traditional 12-step model of drug treatment as used by Alcoholics Anonymous (Drake *et al*., 2004b; Osher and Kofoed, 1989). The 12-step philosophy advocates total abstinence and uses confrontation as a technique to break through denial. However, this approach may have proven too stressful for many patients as drop-out rates for people with dual diagnosis are high and other related outcomes were poor (Drake *et al*., 2001).

More recently a wider range of psychosocial interventions have been trialed. Many of these interventions are based on the premise that what benefits substance users more generally may, with some enhancements, also benefit substance users with severe mental health issues (Barrowclough, 2006). Significant differences exist across these interventions in terms of intensity, duration, theoretical model used, mode and location of administration, the target group to which they have been applied and the way that they have been evaluated (Brunette and Meuser, 2006). These differences make comparison between studies difficult and also limit the generalizability of findings across groups and settings (Drake *et al*., 2008).

These issues aside, findings from studies thus far provide valuable insights into what does and what does not work, as well as providing directions for future research. While conclusive randomized controlled-trial evidence that supports one intervention over another is not yet available (Cleary *et al*., 2009), recent comprehensive reviews of the emerging evidence do provide support for some psychosocial interventions over others (Drake *et al*., 2008; Tiet and Mausbach, 2007; Hjorthøj *et al*., 2009; Rathbone *et al*., 2009).

Barrowclough *et al*. (2001) demonstrated positive results in a randomized controlled trial comparing routine psychiatric care with a program of routine psychiatric care augmented with a comprehensive package of motivational interviewing, cognitive-behavioral therapy and family/care-giver psychoeducation interventions. This intervention was delivered within participants' homes over a 9-month period and required the involvement of family or care-givers to ensure consistency of intervention. Findings suggested that integrated comprehensive care can generate significant improvements in general functioning, reduce positive symptoms and lead to an increase in the number of days abstinent from drugs and alcohol. Cannabis was the illicit substance most commonly used by participants in this study. Importantly, significant improvements were maintained in the treatment group at 18-month follow-up (Haddock, 2003).

James *et al*. (2004) also observed favorable results across several domains in a group-based intervention aimed at reducing substance use and improving mental

health among cannabis users with psychosis. The intervention was tailored to each participant's motivates for drug use and readiness to change, and encompassed aspects of psychoeducation, MI, relapse prevention and harm reduction. The intervention was guided by a comprehensive treatment manual that outlined each of the weekly sessions and covered all other aspects of the 6-week programme (James *et al.*, 2002). Sixty-eight participants were enrolled and randomly allocated to routine psychiatric care or routine care plus the group intervention. Significant improvements were observed within the intervention group regarding psychopathology, chlorpromazine-equivalent doses of medication and reductions in cannabis use and polydrug use (James *et al.*, 2004).

Kavanagh *et al.* (2004) evaluated a motivational enhancement approach for addressing substance use among people with recent onset psychosis and also showed positive results. Twenty-five inpatients aged 18–35 years with early psychosis and current misuse of non-opioid drugs, were allocated randomly to Standard Care (SC) or SC plus Start Over and Survive (SOS). Start Over and Survive is an intervention based on the principles of MI and relapse prevention, with aspects of psycho-education and harm reduction included. The intervention was manualized and comprised three hours of individual treatment over six to nine sessions, that was usually completed within 7–10 days. Participant goals for change were selected in consultation with therapists and included both reduced use and abstinence from substance use. Substance use and related problems were assessed at baseline, 6 weeks and at 3, 6 and 12 months. Final assessments were undertaken blind to the condition the participant was allocated. Participants who received the treatment intervention reported less substance use than controls. Effects were well maintained at 12 months post-intervention.

A recent Cochrane review (Rathbone *et al.*, 2009) identified just one randomized controlled trial that evaluated treatment interventions for cannabis use specifically among people with psychotic illness. Edwards *et al.* (2006) developed a cannabis-focused intervention for young people with first-episode psychosis. The intervention commenced with a comprehensive assessment followed by MI, goal setting, relapse prevention and education about the possible effects of cannabis in relation to psychosis. The intervention was delivered to 23 participants in weekly sessions by trained clinicians over a 3-month period, each session lasting between 20 and 60 minutes. The control group of 24 was provided with standard care plus psycho-education only. No significant differences were found between the treatment and control group at the end of treatment and at 6-month follow-up in terms of drug use, psychopathology and functioning. However, both the treatment and the control group had significantly reduced their cannabis use over this time. The authors concluded that as neither intervention was found to be superior, relatively simple, general interventions should be considered in the first instance to reduce cannabis use among this population.

Conclusions

As our understanding improves about the negative impact cannabis use can have on severe mental illness, the issue of how best to respond to this problem becomes an ever more pertinent and important question. Deficiencies regarding the way services identify and detect cannabis use among psychiatric populations can be significantly improved through the introduction of routine screening and assessment procedures. Such changes can improve the rate of detection; ensure that those who are using are identified and informed about the possible consequences to their health; and ensure the most appropriate treatment intervention is tailored to each patient.

In terms of models of care and therapeutic interventions, we are increasingly able to define the most optimal shape and content of specific treatment programmes. While much more work still needs to be done in this area, an encouraging start has been made.

References

Ananth, J., Vanderwater, S., Kamal, M. *et al.* (1989) Missed disgnosis of substance abuse in psychiatric patients. *Hosp Commun Psychiatry*, **40**:297–9.

Appleby, L., Dyson, V., Luchins, D.J., and Cohen, L.S. (1997) The impact of substance use screening on a public psychiatric inpatient population. *Psychiatric Serv,* **48**:1311–6.

Baker, A., Lewin, T., Reichler, H., Clancey, R., *et al.* (2002) Motivational interviewing among psychiatric in-patients with substance use disorders. *Acta Psychiatr Scand,* **106**:233–40.

Barrowclough, C., Haddock, G., and Tarrier, N. (2001) Randomized controlled trial of motivational interviewing, cognitive behavior therapy, and family intervention for patients with co-morbid

schizophrenia and substance use disorders. *Am J Psychiatry*, **158**:1706–13.

Barrowclough, C., Haddock, G., Lobban, F., *et al.* (2006) Group cognitive therapy for schizophrenia. *Brit J Psychiatry,* **189**:527–32.

Bennett, M.E., Bellack, A.S. and Gearon, J.S. (2006). Development of a comprehensive measure to assess clinical issues in dual diagnosis patients: The Substance Use Event Survey for Mental Illness. *Addict Behav,* **31**:2249–67.

Brunette, M.F., and Mueser, K.T. (2006) Psychosocial interventions for the long-term management of patients with severe mental illness and co-occurring substance use disorder. *J Clin Psychol,* **67**:10–17.

Brunette, M.F., Asher, D., Whitley, R., *et al.* (2008) Implementation of integrated dual disorder treatment: A qualitative analysis of facilitators and barriers. *Psychiatric Serv,* **59**:989–95.

Carey, K.B. and Correia, C.J. (1998) Severe mental illness and addiction: assessment considerations. *Addict Behav,* **23**:735–48.

Cleary, M., Hunt, G.E., Matheson, S.L., *et al.* (2009) *Psychosocial interventions for people with both severe mental illness and substance misuse (Cochrane Review*). Cochrane Database of Systematic Reviews. Issue 4.

Cooper, M.L., Frone, M.R., Russell, M., *et al.* (1995) Drinking to regulate positive and negative emotions: a motivational model of alcohol use. *J Personal Soc Psych,* **69**:990–1005.

Crome, I.B., Bloor, R., and Thorn, B. (2006) Screening for illicit drug use in psychiatric hospitals: whose job is it? *Advances in Psychiatric Treatment,* **12**:375–83.

de Beaurepaire, R., Lukasiewicz, M., Beauverie, P., *et al.* (2007) Comparison of self-reports and biological measures for alcohol, tobacco and illicit drugs consumption in psychiatric inpatients. *Eur Psychiatry,* **22**:540–8.

Degenhardt, L., Tennant, C., Glimour, S., *et al.* (2007) The temporal dynamics of relationships between cannabis, psychosis and depression among young adults with psychotic disorders: findings from a 10-month prospective study. *Psychol Med,* **37**:927–34.

DiClemente, C.C., Nidecker, M., and Bellack, A.S. (2008) Motivation and the stages of change among individuals with severe mental illness and substance use disorders. *J Subst Abuse Treat*, **34**:25–35.

Dixon, L., Haas, G., Weldon, P.J., Sweeney, J., and Frances, A.J. (1991). Drug abuse in schizophrenic patients: clinical correlates and reasons for use. *Am J Psychiatry,* **148**:224.

Drake, R.E., Altereman, A.L., and Rosenberg, S.R. (1993) Detection of substance use disorders in severely mentally ill patients. *J Nerv Ment Dis,* **24**:589–608.

Drake, R.E., Altereman, A.L., and Rosenberg, S.R. (1993b) Detection of substance use disorders in severely mentally ill patients. *Community Ment Health J,* **29**:175–92.

Drake, R.E. and Wallach, M.A. (1989). Substance use among the chronically mentally ill. *Commun Psychiatry* **40**:1041–16.

Drake, R.E. and Mueser, K.T. (2000) Psychosocial approaches to dual diagnosis. *Schizophr Bull*, **26**:105–18.

Drake, R.E., Essock, S.M., Shaner, A. *et al* (2001) Implementing dual diagnosis services for clients with severe mental illness. *Psychiatric Serv,* **52**:469–76.

Drake, R.E., Morse, G., Brunette, M.F., Torrey, W.C., *et al.* (2004a) Evolving US service model for patients with severe mental illness and co-occurring substance use disorder. *Acta Neuropsychiatrica,* **16**:36–40.

Drake, R.E., Mueser, K.T., Brunette, M.F., and McHugo, G.J. (2004b) A review of treatments for people with severe mental illness and co-occurring substance abuse. *Psychiatr Rehabil J,* **27**:360–74.

Drake, R.E., O'Neal, E.L. and Wallach, M.A. (2008) A systematic review of psychosocial interventions for people with co-occurring severe mental and substance use disorders. *J Subst Abuse Treat,* **34**:123–38.

Ducharme, L.J., Knudsen, H.K., and Roman, P.M. (2006). Availability of Integrated care for co-occurring substance abuse and psychiatric conditions. *Community Ment Health J,* **42**:363–75.

Edwards, J., Elkins, K., Hinton, M. *et al.* (2006) Randomized controlled trial of a cannabis-focused intervention for young people with first-episode psychosis. *Acta Psychiatr Scand,* **114**:109–17.

Essock, S.M., Mueser, K.T., Drake, R.E., *et al.* (2006) Comparison of ACT and Standard Case Management for Delivering Integrated Treatment for Co-occurring Disorders. *Psychiatric Serv,* **57**:185–96.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B.W., and Benjamin, I. (1994) Structured clinical interview for DSM IV Axis II Personality Disorders (SCID – II. Version 2.0. New York: Biometric Research Department, New York Psychiatric Hospital.

Flynn, P.M. and Brown, B.S. (2008) Co-occurring disorders in substance abuse treatment: Issues and prospects. *J Subst Abuse Treat*, **34**:36–47.

Gearon, J.S., Bellack, A.S., RachBeisel, J., and Dixon, L. (2001) Drug-use behavior and correlates in people with schizophrenia. *Addict Behav,* **26**:51–61.

Graham, H.L. (2004) Implementing integrated treatment for coexisting substance use and severe mental health problems in assertive outreach teams: training issues. *Drug Alcohol Rev,* **23**:463–70.

Grech, A., van Os, J., Jones, P.B., Lewis, S.W. *et al.* (2005) Cannabis use and outcome of recent onset psychosis. *Eur Psychiatry,* **20**:349–53.

Green, A.L., Drake, R.E., Brunette, M.F. and Noordsy, D.L. (2007) Schizophrenia and Co-occurring Substance use disorder. *Am J Psychiatry,* **164**:402–8.

Gregg, L., Barroclough, C. and Haddock, G. (2009) Development and validation of a scale for assessing reasons for substance use in schizophrenia: The ReSUS scale. *Addict Behav,* **34**:830–7.

Griffin, M.L., Kolodziej, M.E. and Weiss, R.D. (2009) Measuring principle substance of abuse in comorbid patients for clinical research. *Addict Behav,* **34**:826–9.

Gossop M., Darke, S., Griffiths, P., *et al.* (1995) The Severity of Dependence Scale (SDS): psychometric properties of the SDS in English and Australian samples of heroin, cocaine and amphetamine users. *Addiction,* **90**:607–14.

Haddock, G., Barrowclough, C., Tarrier, N., *et al.* (2003) Randomized controlled trial of cognitive behavior therapy and motivational interviewing for schizophrenia and substance use: 18-month, carer and economic outcomes. *Brit J Psychiatry*, **183**:418–24.

Hall, W. and Dagenhardt, L. (2000) Cannabis use and psychosis: a review of clinical and epidemiological evidence. *Aust NZ J Psychiatry,* **34**:26–34.

Hasin, D.S., Trautmen, K.D., Miele, G.M. *et al.* (1996) Psychiatric Research Interview for Substance and Mental Disorder (PRISM). Reliability for substance abusers. *Am J Psychiatry*, **153**:1195–201.

Hides, L., Cotton, S.M., Berger, G., Gleeson, J., *et al.* (2009) The reliability and validity of the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) in first-episode psychosis. *Addict Behav,* **34**:821–5.

Hinton, M., Edwards, J., Elkins, K., *et al.* (2007) Reductions in cannabis and other illicit substance use between treatment entry and early recovery with first-episode psychosis. *Early intervention in Psychiatry*, **1**:259–66.

Hjorthoj, C., Fohlmann, A. and Nordentoft, M. (2009) Treatment of cannabis use disorders in people with schizophrenia spectrum disorders – A systematic review. *Addict Behav,* **34**:846–51.

Horsfall, J., Cleary, M., Hunt, G.E., and Walter, G. (2009) Psychosocial treatments for people with co-occurring severe mental illness and substance use disorders (dual diagnosis): a review of empirical evidence. *Harv Rev Psychiatry*, **17**:24–34.

James, W., Koh, G., Spencer, C., *et al.* (2002) *Managing Mental Health and Drug Use*. Perth, Western Australia: Uniprint.

James, W., Preston, N., Koh, G., *et al.* (2004) A group intervention which assists patients with dual diagnosis reduce their drug use: A randomized controlled trial. *Psychol Med*, **34**:983–90.

Kavanagh, D.J., Young, R., White, A., *et al.* (2004) A brief motivational intervention for substance misuse in recent-onset psychosis. *Drug Alcohol Rev,* **23**:151–5.

Kavanagh, D.J. and Connolly, M.C. (2009) Interventions for co-occurring addictive and other mental disorders. *Addict Behav,* **24**:838–45.

Khantzian, E.J. (1997) The self-medication hypothesis of substance use disorders: a reconsideration and recent applications. *Harv Rev Psychiatry*, **4**:231–44.

Lehman, A.F., Myers, C.P., Dixon, L.B., *et al.* (1996) Detection of substance use disorders among psychiatric inpatients. *Journal of Nervous and Mental Disease,* **184**:228–33.

Ley, A., McLaren, S., and Siegfried, N., (2000) *Treatment programmes for people with both severe mental illness and substance misuse (Cochrane Review)*. Cochrane Library, Issue 1.

Ley, A., Jeffrey, D., Ruiz, J., McLaren, S., and Gillespie, C. (2002) Under detection of co-morbid drug use at acute psychiatric admission. *The Psychiatrist,* **26**:248–51.

Linszen, D.H., Dingemans, P.M. and Lenior, M.E. (1994) Cannabis abuse and the course of recent-onset schizophrenic disorders. *Arch Gen Psychiatry*, **51**:273–9.

McLellan, A.T., Kushner, H., Metzger, D., *et al.* (1992) The fifth edition of the Addiction Severity Index. *J Subst Abuse Treat,* **9**:199–213.

Meuser, K.T., Noordsy, D.L., Drake, R.E., *et al.* (2003) *Integrated Treatment for Dual Diagnosis: A Guide to Effective Treatment*. New York, Guildford.

Meuser, K.T., Glynn, S.M., Cather, C., Zarate, R., *et al.* (2009) Family intervention for co-occuring substance use and severe psychiatric disorders: participant characteristics and correlates of initial engagement and more extended exposure in a randomized controlled trial. *Addict Behav*, **34**:867–77.

Nidecker, M., DiClemente, C.S., Bennett, M.E. and Bellack, A.S. (2008) Application of the transtheoretical Model of Change: Psychometric properties of leading measures in patients with co-occurring drug abuse and severe mental illness. *Addict Behav,* **33**:1021–30.

Osher, F.C. and Kofoed, L.L. (1989) Treatment of patients with psychiatric and psychoactive substance abuse disorders. *Hosp Commun Psychiatry*, **40**:1025–30.

Pantalon, M.V. and Swanson, A.J. (2003) Use of the University of Rhode Island Change Assessment to measure motivational readiness to change in psychiatric and dually diagnosed individuals. *Psychol Addict Behav*, **17**:91–7.

Prochaska, J.O. and Velicer, W.F. (1997). The transtheoretical model of health behavior change. *Am J Health Promot*, **12**:38–48.

RachBeisel, J., Scott, J., and Dixon, L. (1999) Co-occurring severe mental illness and substance use disorders: a review of recent research. *Psychiatr Serv*, **50**:1427–34.

Rathbone, J., Variend, H., and Mehta, H. (2009) Cannabis and schizophrenia. Cochrane Database of Systematic Reviews, Issue 4.

Rosenberg, S.D., Drake, R.E. and Wolford, G.I. (1998) Dartmouth Assessment of Lifestyle Inventory (DALI): a substance use disorder screen for people with severe mental illness. *Am J Psychiatry*, **155**:232–8.

Schaub, M., Franghaenal, K. and Stohler, R. (2008) Reasons for cannabis use: patients with schizophrenia verses matched healthy controls. *Aust NZ J Psychiatry,* **42**:1060–5.

Schofield, D., Tennant, C., Nash, L., Dagenhardt, L., *et al.* (2006) Reasons for cannabis use in psychosis. *Aust NZ J Psychiatry*, **40**:570–4.

Schulte, S.J., Meier, P.S., Stirling, J. and Berry, M. (2008) Treatment approaches for dual diagnosis clients in England. *Drug Alcohol Rev,* **27**:650–8.

Semler, G., Wittchen, H.U., Loschke, K., *et al.* (1987) Test-retest reliability of a standardized psychiatric interview(DIS/CIDI). *Eur Arch Psychiatry Neurol Sci*, **236**:214–22.

Siegfried, N. (1998) A review of co morbidity; major mental illness and problematic substance use. *Aust NZ J Psychiatry,* **32**:707–17.

Skinner, H.A. (1982) The Drug Abuse Screening Test. *Addict Behav,* **7**:263–371.

Spencer, C., Castle, D.J. and Mitchie, P. (2002) An examination of the validity of a motivational model for understanding substance use among individuals with psychotic disorders. *Schizophr Bull*, **28**:233–47.

Tiet, Q.Q. and Mausbach, B. (2007) Treatments for patients with dual diagnosis: A review. *Alcoholism: Clin Exp Res,* **31**:513–36.

Tiet, Q.Q., Finney, J.W. and Moos, R.H. (2008) Screening psychiatric patients for illicit drug use disorders and problems. *Clin Psychol Rev,* **28**:578–91.

Wing, J.K., Babor, T., Brugha, T. *et al.* (1990). SCAN: Schedules for Clinical Assessment in Neuropsychiatry. *Arch Gen Psychiatry,* **47**:589–93.

Weiss, R.D., Najavitis, I.M., Greenfield, S.M., *et al.* (1998) Validity of substance use self reports in dually diagnosed outpatients. *Am. J. Psychiatry,* **155**:127–8.

WHO ASSIST Working Group (2002) The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): development, reliability and feasibility. *Addiction,* **97**:1183–94.

Zeidonis, D. and Fisher, W. (1994) Assessment and treatment of co morbid substance abuse in individuals with schizophrenia. *Psychiatric Ann*, **24**:477–83.

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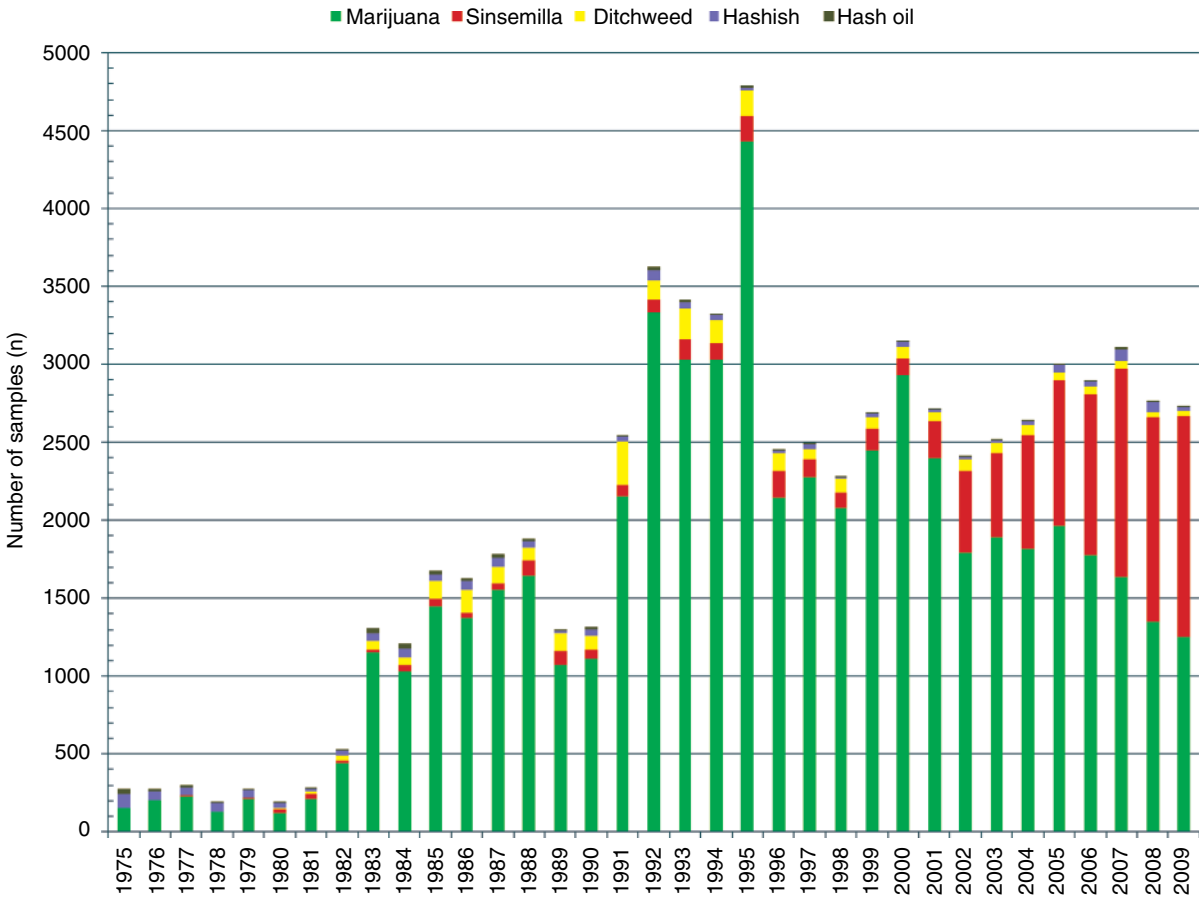


Figure 4.1. Number of cannabis seizures analyzed by type and year in the United States (1975–2009).

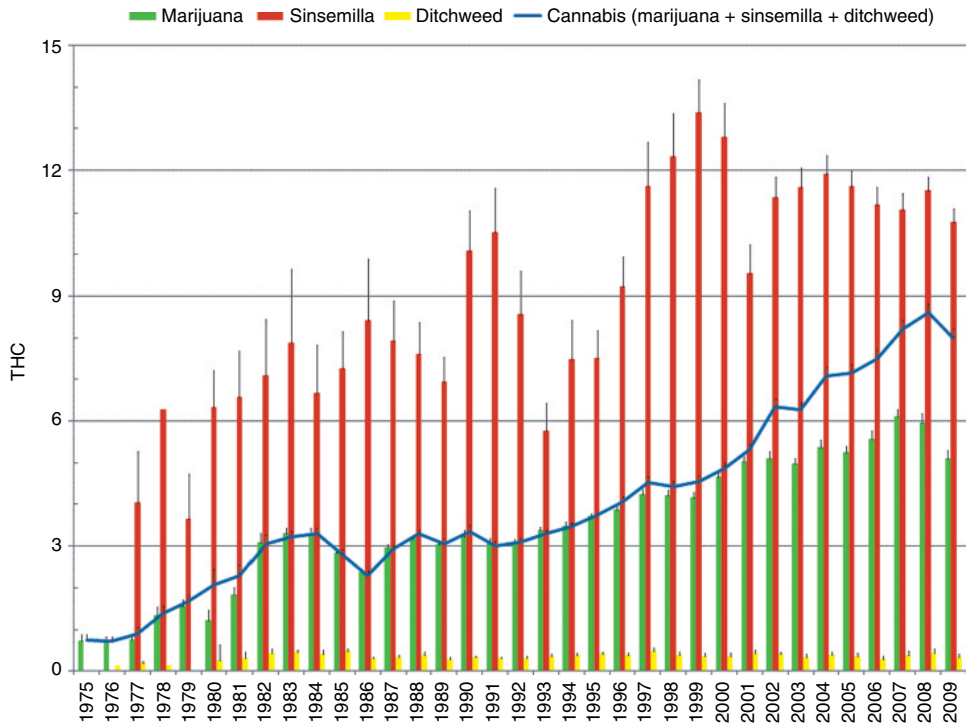


Figure 4.2. Mean THC content (%) with 95% confidence intervals for marijuana, sinsemilla and ditchweed seizures in the United States (1975–2009). THC, Δ^9-tetrahydrocannabinol.

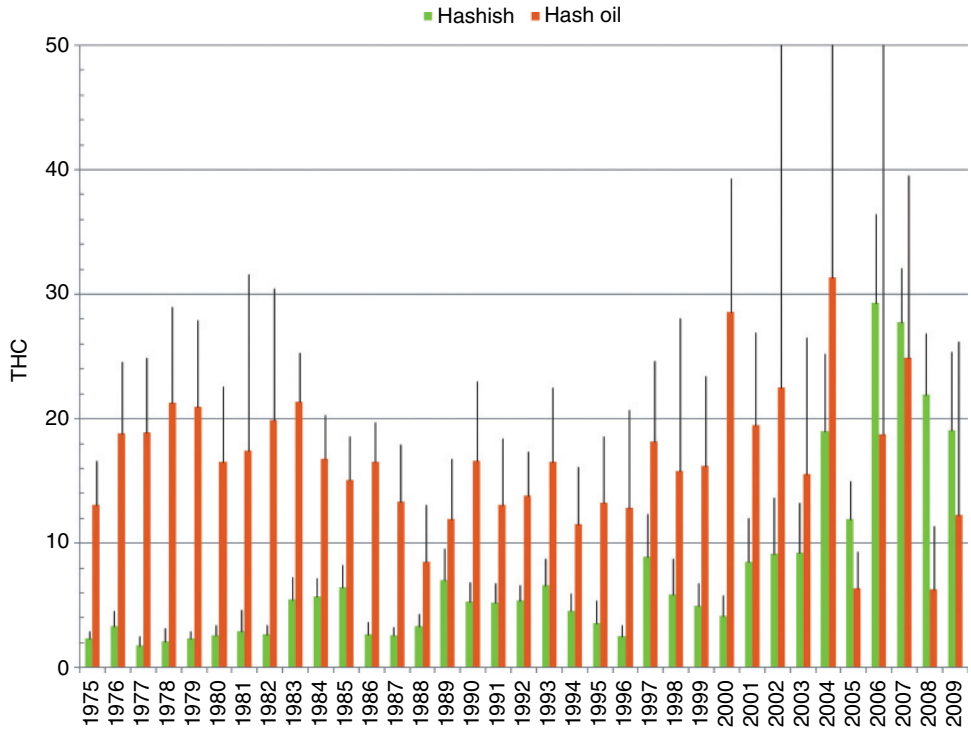


Figure 4.3. Mean THC content (%) with 95% confidence intervals for hashish and hash oil seizures in the United States (1975–2009). THC, Δ^9-tetrahydrocannabinol.

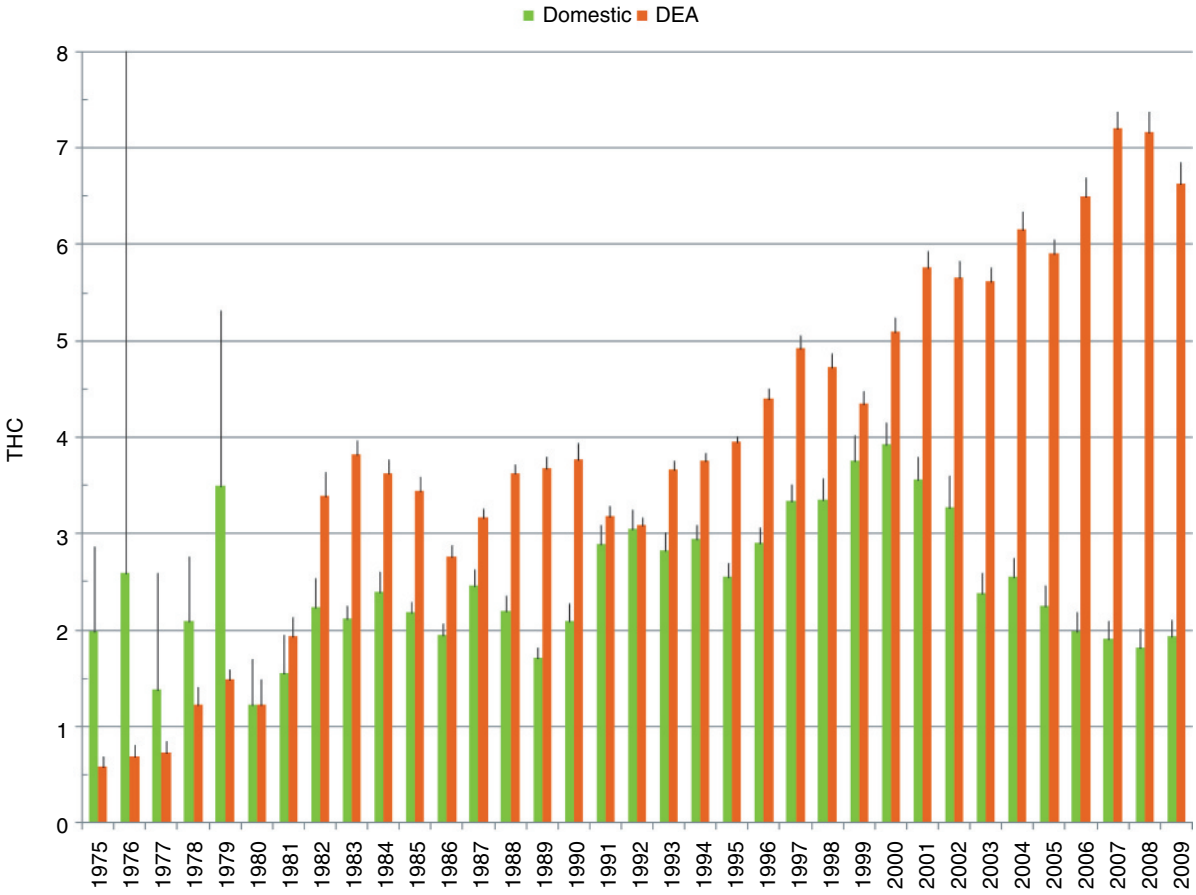


Figure 4.4. Mean THC content (%) with 95% confidence intervals for domestic and DEA marijuana seizures in the United States (1975–2009). DEA, Drug Enforcement Administration; THC, Δ^9-tetrahydrocannabinol.

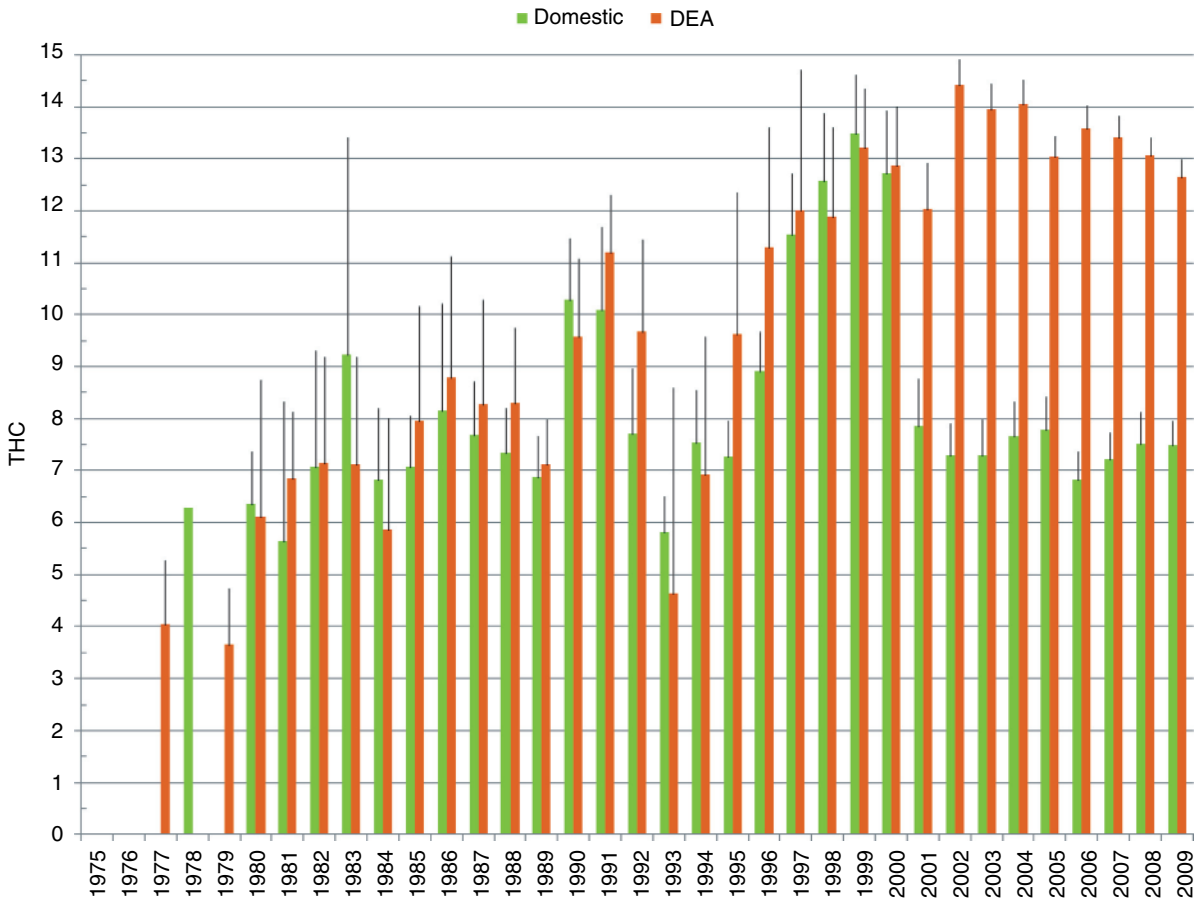


Figure 4.5. Mean THC content (%) with 95% confidence intervals for domestic and DEA sinsemilla seizures in the United States (1975–2009). DEA, Drug Enforcement Administration; THC, Δ^9-tetrahydrocannabinol.

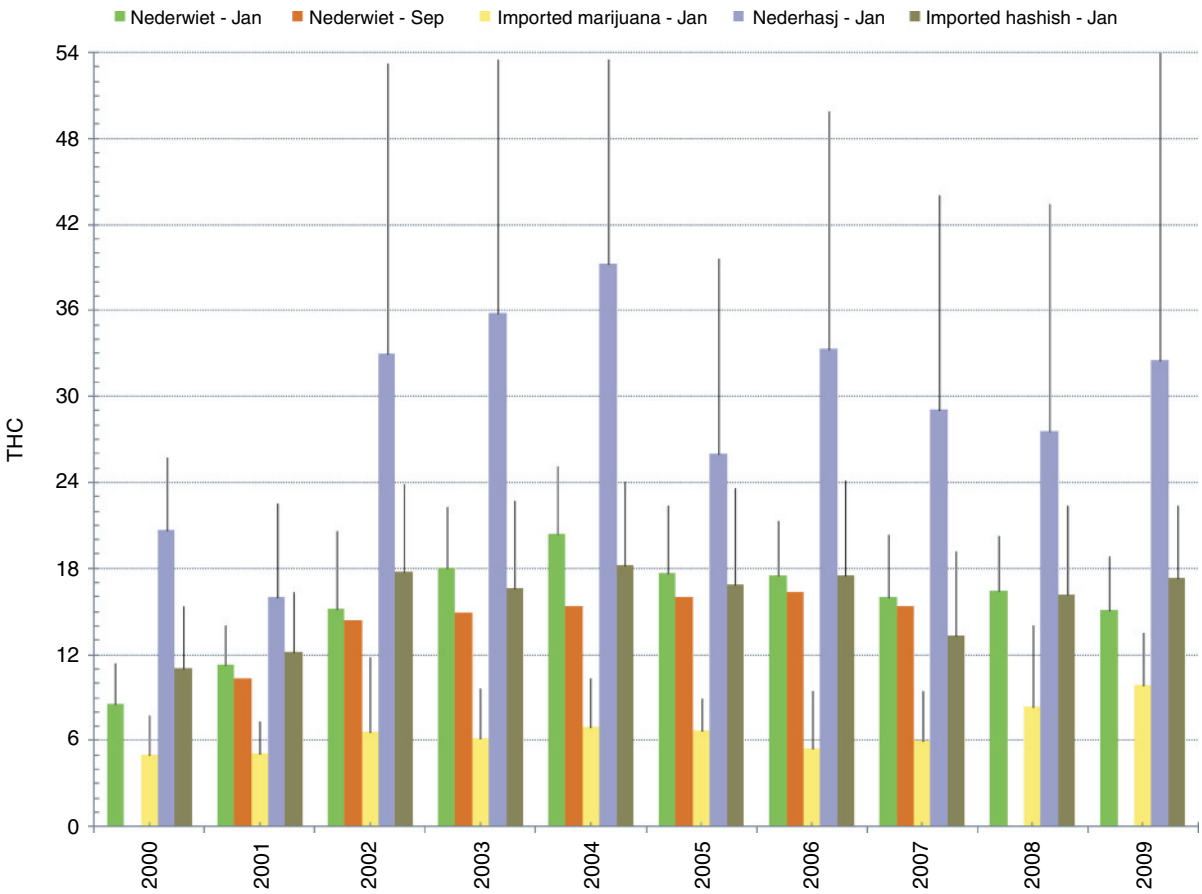


Figure 4.6. THC content (mean ± SD) (%) of cannabis products purchased in coffee shops in The Netherlands (2000–2009). THC, Δ^9-tetrahydrocannabinol.

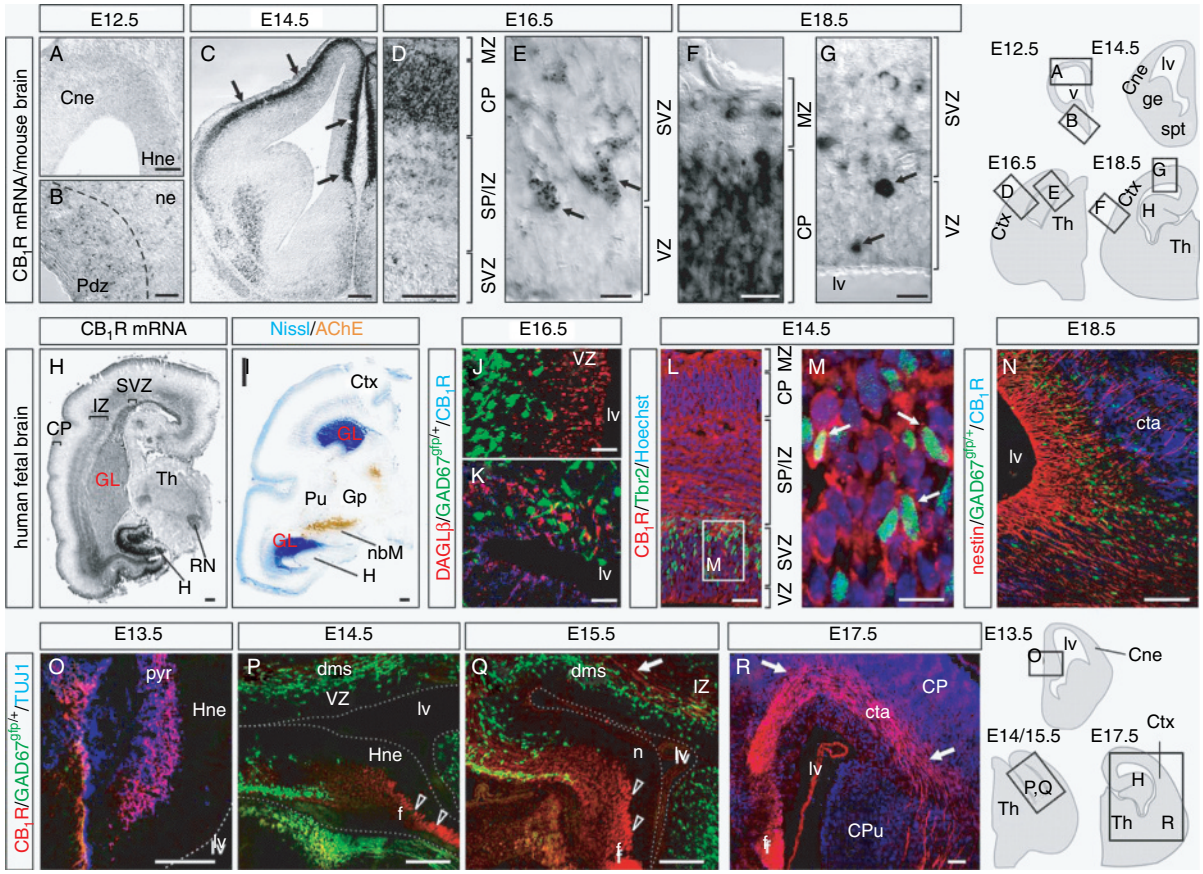


Figure 6.2. CB1 receptor expression in the developing neocortex. A–G show in-situ hybridization of CB1 mRNA at the indicated developmental stages. C, D and F show the presence of CB1 transcripts in pyramidal neurons, and E and G in VZ/SVZ neural progenitors. L–N reveal CB1 receptor expression in Tbr-2-positive intermediate progenitors. O–R, CB1 receptors are enriched in projecting axons of cortical neurons. Arrows indicate corticothalamic axons and arrowheads indicate axons commited to the fimbria. See original article for further details. Reproduced with permission from Mulder *et al.*, 2008. Copyright 2008 National Academy of Sciences, USA.

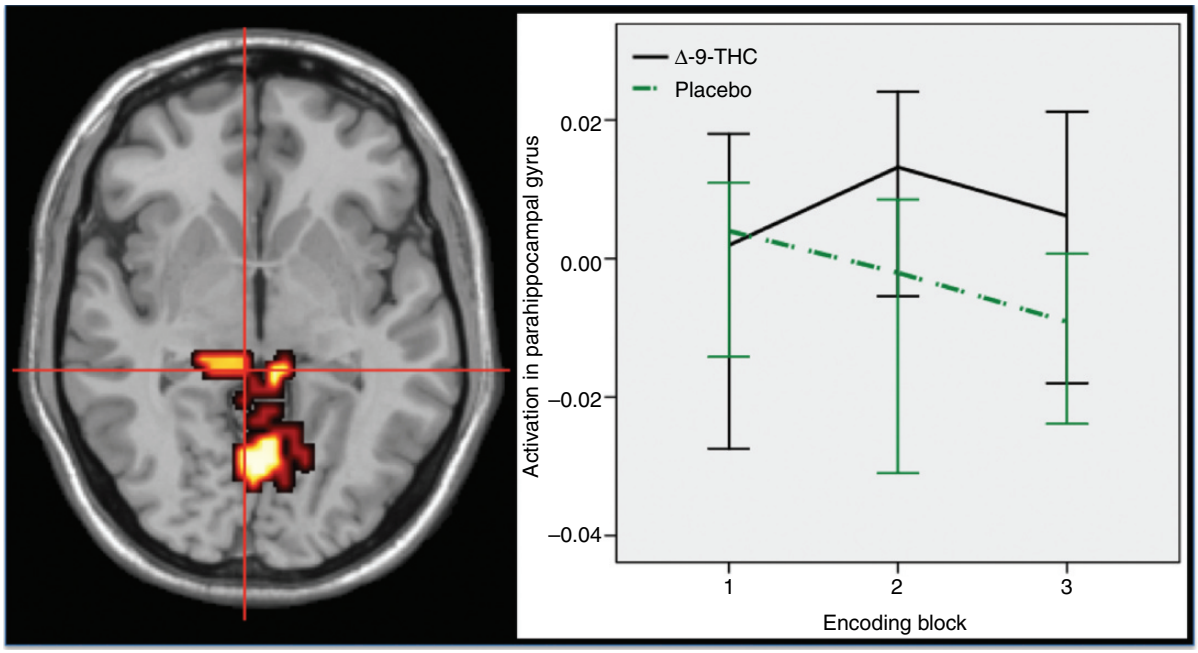


Figure 14.1. Effect of Δ^9-tetrahydrocannabinol (THC) on activation in the parahippocampal gyri (cross-hair in the transverse section of brain on the left of the panel) bilaterally extending to the midbrain and cerebellum during verbal learning. The left side of the brain is shown on the left side of the image. The plot on the right side of the panel shows the mean magnitude of activation (in aribitrary units; error bars show standard error of mean) in the parahippocampal gyral cluster on the left during each encoding block (x-axis) following administration of THC (solid line) and placebo (dashed line).

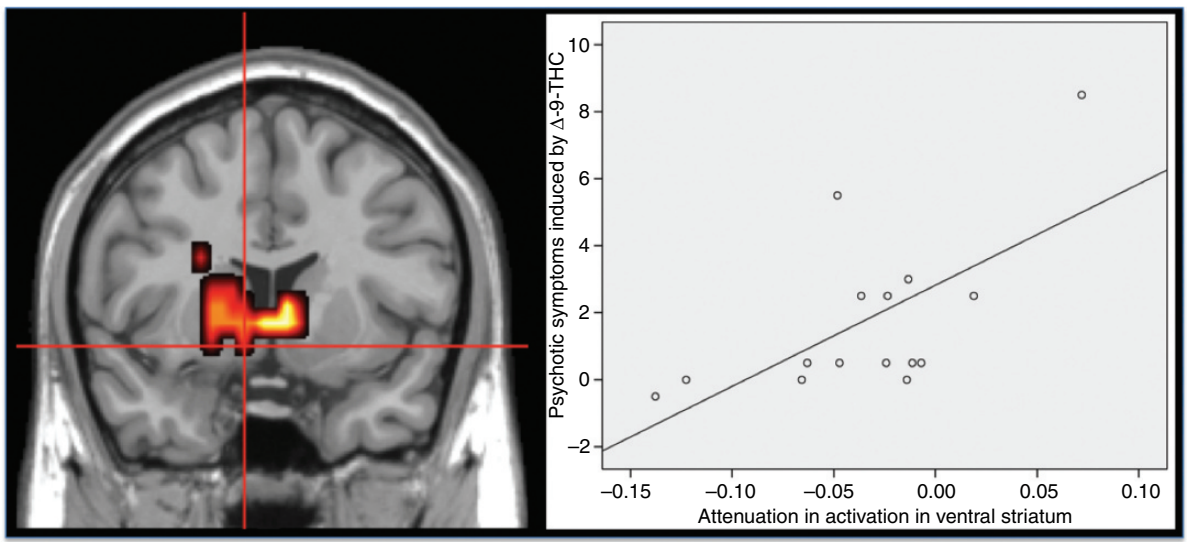


Figure 14.2. Effect of Δ^9-tetrahydrocannabinol (THC) on activation in the ventral striatum (cross-hair in the coronal section of brain on the left of the panel) during repeated recall trials. The plot on the right of the panel shows the correlation between attenuation of activation in the ventral striatum (in arbitrary units) caused by THC across repeated recall blocks and psychotic symptoms (y-axis) induced by it.

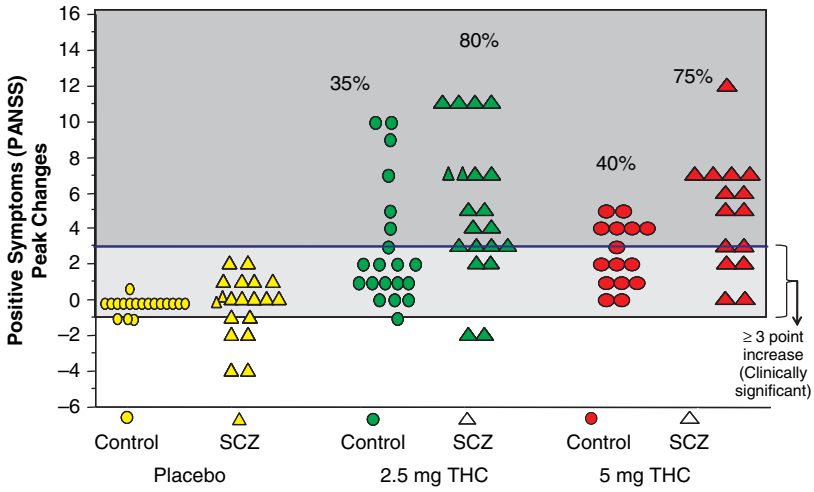


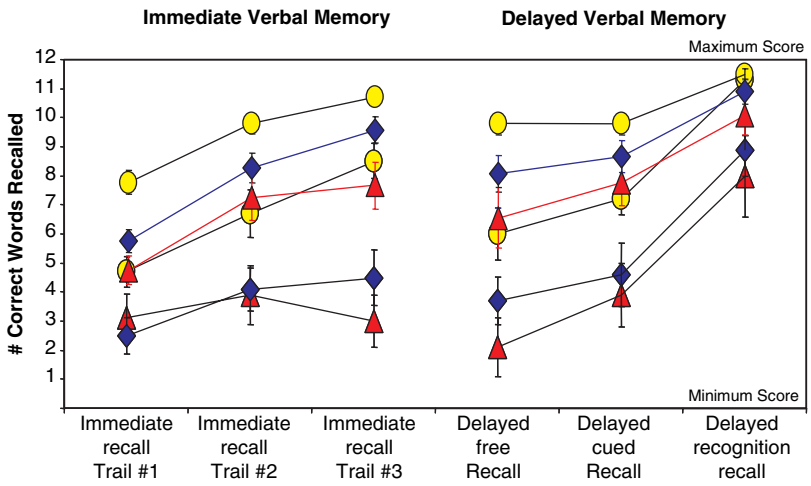
Figure 18.2. Enhanced sensitivity to the amnestic effects of Δ^9-tetrahydrocannabinol (THC) in schizophrenia.

Legend

Control – Placebo
Control – 2.5 mg THC
Control – 5 mg THC
Schizophrenia – Placebo
Schizophrenia – 2.5 mg THC
Schizophrenia – 5 mg THC

Peak increase in positive symptoms measured by the positive symptoms subscale of the Positive and Negative Symptoms Scale (PANSS) (group means ± 1 SD).

Clinically significant increase = 3 point or greater increase in PANSS positive symptom subscale score.



D'Souza, 2005

Figure 18.3. Enhanced sensitivity to the psychotomimetic effects of Δ^9-tetrahydrocannabinol (THC) in schizophrenia.

Legend

Placebo (Vehicle)
2.5 mg THC
5 mg THC
Schizophrenia
Controls

Effects of THC on the learning, immediate free recall, delayed free recall, delayed cued and recognition recall measured by a 12-word learning task (Hopkins Verbal Learning Test)