

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 cannabinoids 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

Chapter 1: How cannabis works in the brain

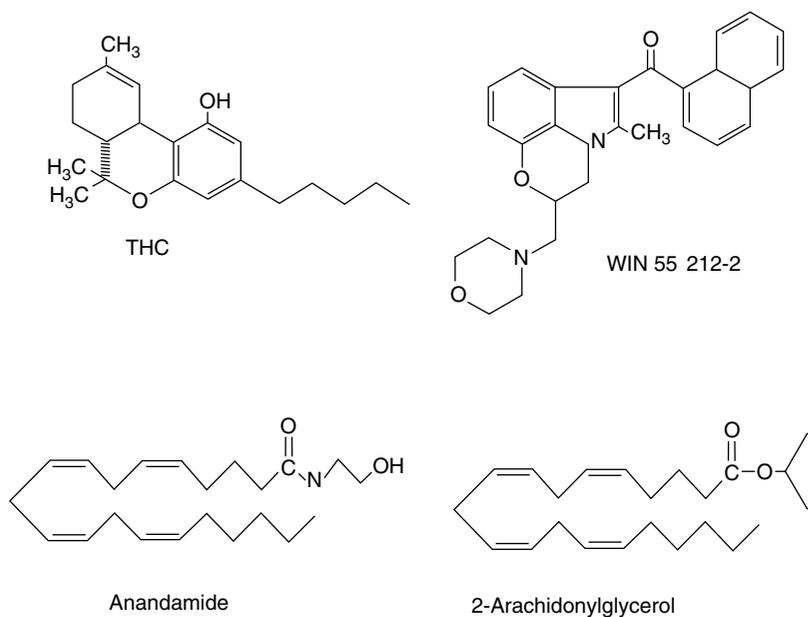


Figure 1.1. Chemical structures of THC (Δ^9 -tetrahydrocannabinol), the synthetic CB1 receptor agonist WIN55 212-2 and the endocannabinoids. Reproduced with permission from Murray *et al.*, 2007.

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 D₂ receptors in rat nucleus accumbens and found many examples of overlapping distributions, with CB1-positive terminals contacting D₂-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

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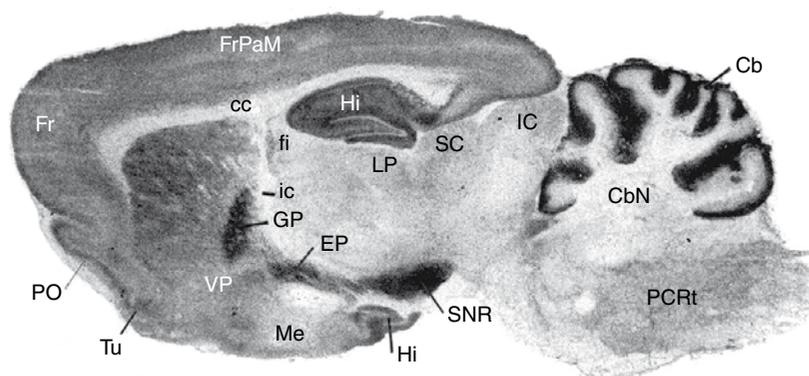
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Figure 1.2. Distribution of cannabinoid CB1 receptors in rat brain revealed by an autoradiograph of the binding of radioactively labeled CP55 940 (a high-affinity agonist ligand) to a sagittal brain section. The brain regions labeled are: Cb, cerebellum; 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 thalamus; Me, medial amygdaloid nucleus; PO, primary olfactory cortex; PCrt, parvocellular reticular nucleus; SNR, substantia nigra reticulata; Tu, olfactory tubercle; VP, ventroposterior thalamus. 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

Chapter 1: How cannabis works in the brain

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 pertussis 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

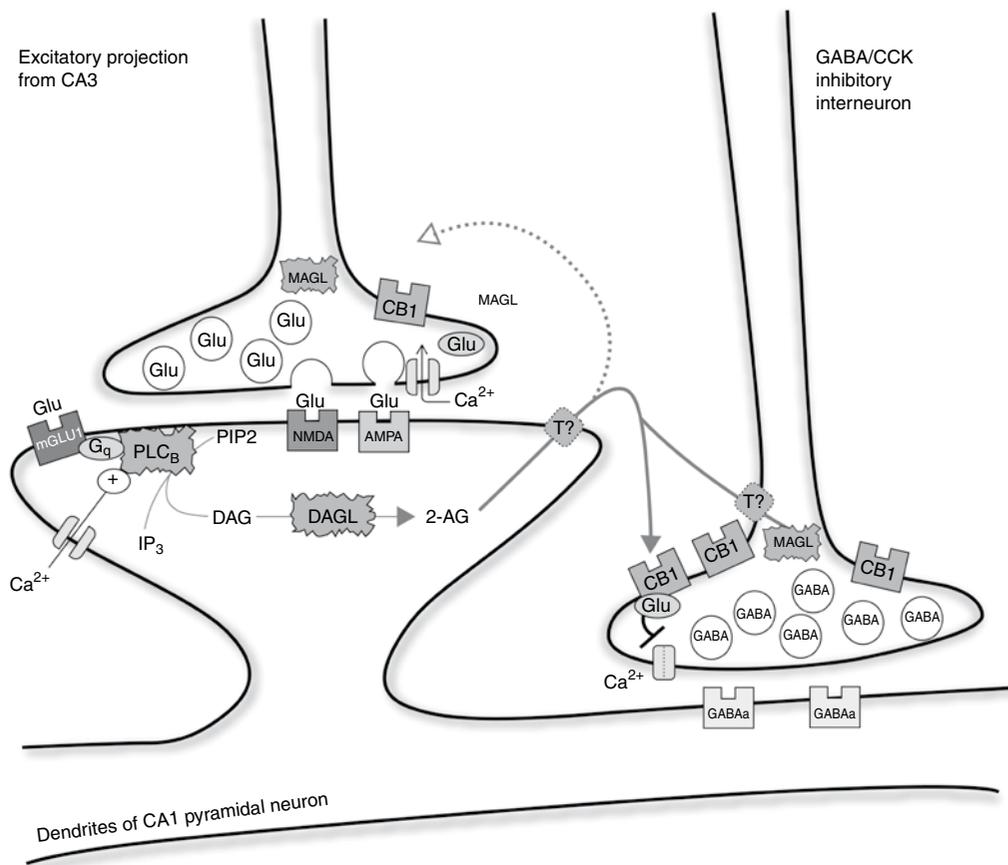


Figure 1.3. (A) Cannabinoids “fine tune” neurotransmission in the hippocampus. In area CA1 of the hippocampus, activation of pyramidal neurons stimulates the synthesis and release of the endocannabinoid 2-AG, which acts at CB1 receptors on adjacent GABAergic nerve terminals to suppress GABA release. 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 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 G $_q$, G-proteins; IP $_3$, inositol triphosphate; LTP, long-term potentiation; MAGL, monoacylglycerol lipase; mGlu1, metabotropic glutamate receptors; NMDA, N-methyl-D-aspartate receptor; PIP $_2$, phosphatidylinositol; 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 endocannabinoids 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).

Chapter 1: How cannabis works in the brain

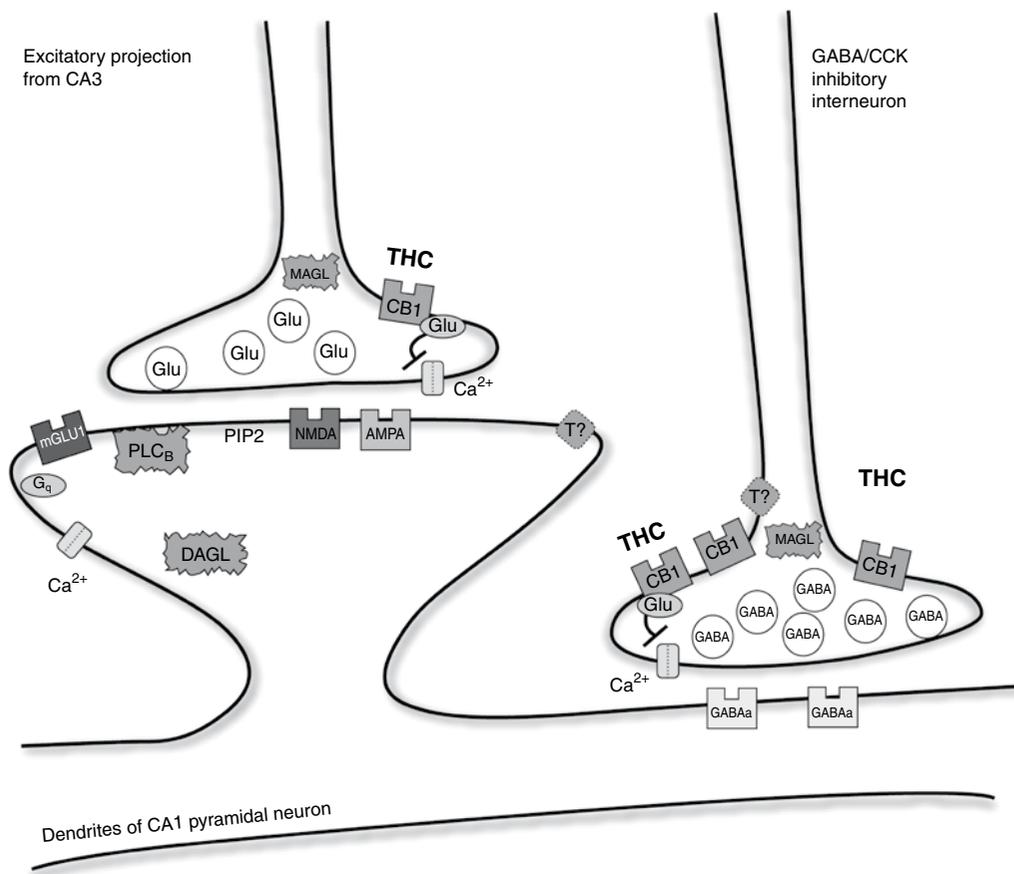


Figure 1.3. (cont.)

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 (Sañudo-Peña *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 anti-sense 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 (Castañé *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

Chapter 1: How cannabis works in the brain

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 4–8 days. 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). CB1 receptors 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 1.5–1.8 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

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Chapter 1: How cannabis works in the brain

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 WIN55212-2 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

Chapter 1: How cannabis works in the brain

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