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INVITED REVIEW

Cannabis and the brain

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Summary

The active compound in herbal cannabis, Δ^9-tetrahydrocannabinol, exerts all of its known central effects through the CB_1 cannabinoid receptor. Research on cannabinoid mechanisms has been facilitated by the availability of selective antagonists acting at CB_1 receptors and the generation of CB_1 receptor knockout mice. Particularly important classes of neurons that express high levels of CB_1 receptors are GABAergic interneurons in hippocampus, amygdala and cerebral cortex, which also contain the neuropeptides cholecystokinin. Activation of CB_1 receptors leads to inhibition of the release of amino acid and monoamine neurotransmitters. The lipid derivatives anandamide and 2-arachidonylglycerol act as endogenous ligands for CB_1 receptors (endocannabinoids). They may act as retrograde synaptic mediators of the phenomena of depolarization-induced suppression of inhibition or excitation in hippocampus and cerebellum. Central effects of cannabinoids include disruption of psychomotor behaviour, short-term memory impairment, intoxication, stimulation of appetite, antinociceptive actions (particularly against pain of neuropathic origin) and anti-emetic effects. Although there are signs of mild cognitive impairment in chronic cannabis users there is little evidence that such impairments are irreversible, or that they are accompanied by drug-induced neuropathology. A proportion of regular users of cannabis develop tolerance and dependence on the drug. Some studies have linked chronic use of cannabis with an increased risk of psychiatric illness, but there is little evidence for any causal link. The potential medical applications of cannabis in the treatment of painful muscle spasms and other symptoms of multiple sclerosis are currently being tested in clinical trials. Medicines based on drugs that enhance the function of endocannabinoids may offer novel therapeutic approaches in the future.

Keywords: cannabinoid CB_1 receptor; Δ^9-tetrahydrocannabinol; rimonabant (SR141716A); anandamide; 2-arachidonylglycerol

Abbreviations: 2-AG = 2-arachidonylglycerol; DSI = depolarization-induced suppression of inhibition; FAAH = fatty acid amide hydrolase; Gi/o = G-proteins negatively linked to adenylate cyclase or to inositol phosphates; LTD = long-term depression; LTP = long-term potentiation; mGlu = metabotropic glutamate; NMDA = *N*-methyl-D-aspartate; THC = Δ^9-tetrahydrocannabinol

Introduction

A large literature exists on the effects of cannabis, with many of the earlier studies conducted in human subjects (Mendelson *et al*., 1976; Jones, 1978; Hollister, 1986). Unfortunately, much of this research would now be regarded as inadequately controlled and poorly designed. However, research on cannabis has been stimulated in recent years by the recognition that specific receptors exist in the brain that recognize cannabinoids, and by the discovery of a series of endogenous cannabinoids that act as ligands for these receptors. As was the case with opiate research in the 1970s, research on a psychoactive drug of plant origin has revealed a hitherto unknown physiological control mechanism. This review will focus mainly on the more recent literature in this field.

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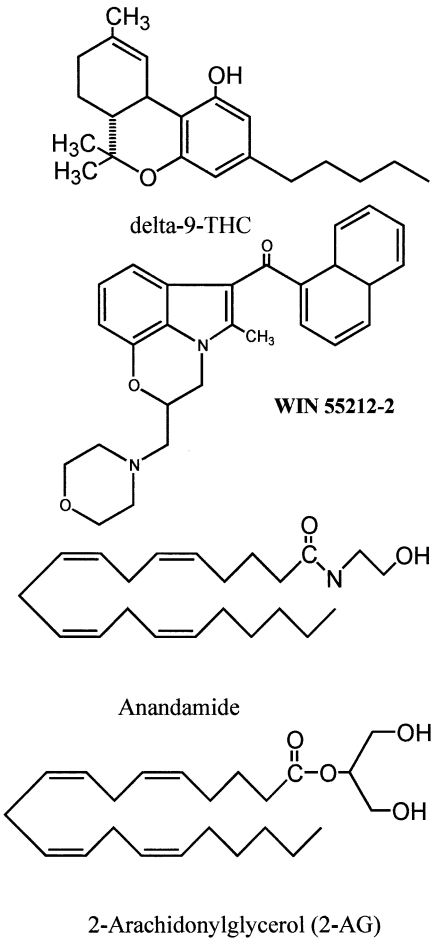

Fig. 1 Chemical structures of THC, the synthetic CB_1 receptor agonist WIN 55,2122 and the endocannabinoids.

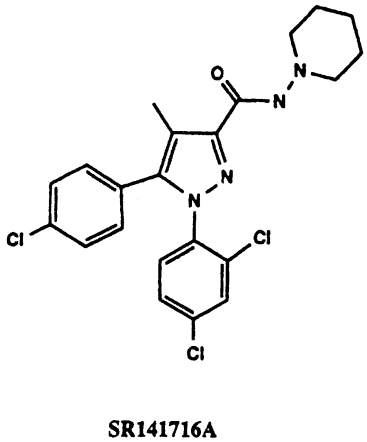

Fig. 2 Chemical structure of the CB_1 selective antagonist drug rimonabant (SR141716A).

The cannabinoid system in brain

Exogenous cannabinoids and their receptors

The principal active component in the complex mixture of cannabinoids present in extracts of the plant *Cannabis sativa* is Δ^9-tetrahydrocannabinol (THC) (Mechoulam, 1970) (Fig. 1). THC is a sticky resin that is not soluble in water. Smoking remains the most efficient means of delivering the drug and experienced users can titrate the dose by adjusting the frequency and depth of inhalation (Iversen, 2000). THC or cannabis extracts can also be taken orally in fat-containing foods or dissolved in a suitable pharmaceutical oil, but absorption is delayed and variable (Iversen, 2000). A series of man-made synthetic cannabinoids, some of which are more potent and more water soluble than THC, is also available (Pertwee, 1999) (Fig. 1). All of these compounds act as agonists at the CB_1 cannabinoid receptor (Matsuda *et al*., 1990), which is the only one known to be expressed in the brain. A second cannabinoid receptor, CB_2, is expressed only in peripheral tissues, principally in the immune system (Munro *et al*., 1993; Felder and Glass, 1998; Pertwee, 1999). THC and the synthetic cannabinoids also act to some extent as agonists at the CB_2 receptor. Both cannabinoid receptors are members of the G-protein coupled class, and their activation is linked to inhibition of adenylate cyclase activity (Howlett *et al*., 1988). A series of synthetic drugs is also now available that act as specific antagonists at CB_1 or CB_2 receptors (D'Souza and Kosten, 2001). One of these compounds, rimonabant(SR141716A), which acts selectively to block CB_1 receptors (Rinaldi-Carmona *et al*., 1994; Compton *et al*., 1996), has been widely used in studies of the actions of cannabinoids in the CNS (Fig. 2).

Endogenous cannabinoids

Following the discovery of specific cannabinoid receptors, a search was made for naturally occurring ligands of these receptors in mammalian tissues. This led to the discovery of a series of arachidonic acid derivatives with potent actions at cannabinoid receptors. These are: anandamide (*N*-arachidonyl-ethanolamine; Devane *et al*., 1992), 2-arachidonylglycerol (2-AG; Mechoulam *et al*., 1995; Sugiura *et al*., 1995; Stella *et al*., 1997) and 2-arachidonylglyceryl ether (Hanuš *et al*., 2001) (Fig. 1). Of these, anandamide is the ligand that has been most extensively studied so far. The endogenous cannabinoids known as 'endocannabinoids' are present only in small amounts in the brain or other tissues. Like other lipid mediators (e.g. prostaglandins) they appear to be synthesized and released locally on demand (see below). Anandamide and the other endogenous cannabinoids are rapidly inactivated by a combination of a transporter mechanism and by the enzyme fatty acid amide hydrolase (FAAH) (Di Marzo *et al*., 1994; Piomelli *et al*., 1998; Giuffrida *et al*., 2001). Genetically engineered mice lacking FAAH displayed elevated levels of anandamide in brain and were supersensitive to the biological actions of anandamide (Cravatt *et al*., 2001). The discovery of agents that could interfere with the inactivation of endogenous cannabinoids may provide a novel means of pharmaco-

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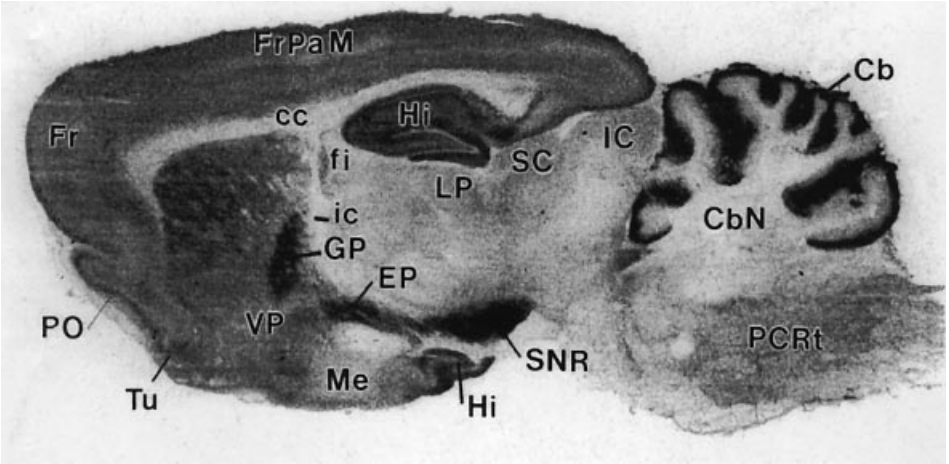

Fig. 3 Distribution of cannabinoid CB_1 receptors in rat brain revealed by an autoradiograph of the binding of radioactively labeled CP-55940 (a high affinity agonist ligand) to a sagittal brain section. The brain regions labelled are: Cb = cerebellum; CbN = deep cerebellar nucleus; cc = corpus callosum; 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 reticulate; Tu = olfactory tubercle; VP = ventroposterior thalamus. Photograph kindly supplied by Dr Miles Herkenham, National Institute of Mental Health, USA.

logically modifying cannabinoid function in the brain (Piomelli *et al*., 2000).

Neuroanatomical distribution of CB_1 receptors in brain

The distribution of cannabinoid receptors was first mapped in rat brain in autoradiographic studies, using the radioligand [H^3]CP-55,940, which binds with high affinity to CB_1 sites (Herkenham *et al*., 1991) (Fig. 3). The validity of using this radioligand was confirmed by autoradiographic studies in CB_1 receptor knockout mice, in which no detectable [H^3]CP-55,940 binding sites were observed (Zimmer *et al*., 1999). More recently, antibodies that target the C- or N-terminal regions of the CB_1 receptor protein have been used for immunohistochemical mapping studies (Egertová *et al*., 1998; Pettit *et al*., 1998; Egertová and Elphick, 2000). Immunohistochemistry provides a superior degree of spatial resolution to autoradiography, but the overall pattern of distribution of CB_1 receptors revealed by the two approaches is very similar (Elphick and Egertová, 2001).

The mapping studies in rat brain showed that CB_1 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).

In both animals and man the cerebral cortex, particularly frontal regions, contains high densities of CB_1 receptors. There are also very high densities in the basal ganglia and in the cerebellum (Fig. 3). In the limbic forebrain CB_1 receptors are found particularly in the hypothalamus and in the anterior cingulate cortex. The hippocampus also contains a high density of CB_1 receptors. The relative absence of the cannabinoid receptors from brainstem nuclei may account for the low toxicity of cannabinoids when given in overdose.

The regional distribution of the CB_1 receptor in brain correlates only poorly with the levels of anandamide and other endocannabinoids in different brain regions (Felder *et al*., 1996; Bisogno *et al*., 1999). However, measurements of endocannabinoids have yielded variable results, and a strict correlation would not be expected for ligands that are only produced on demand. There is a better correlation between the regional distribution of CB_1 receptors and the enzyme FAAH. FAAH is widely distributed in CNS and other tissues, suggesting that its role is not confined to inactivating endogenous cannabinoids. Nevertheless, particularly high levels of FAAH were found in brain regions that are enriched in CB_1 receptors, and immunohistochemical staining suggested a complementary relationship between FAAH and CB_1 receptors at the synaptic level (Egertová *et al*., 1998; Elphick and Egertová, 2001). In cerebellum, hippocampus and neocortex FAAH was expressed at high levels in the somato-dendritic regions of neurons that were postsynaptic to CB_1-positive axon terminals. The close and complementary relationship between CB_1 receptors and FAAH led to the hypothesis that FAAH may participate in the inactivation of endogenous cannabinoids released locally at synapses

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(Elphick and Egertová, 2001). These authors postulated a retrograde cannabinoid signalling mechanism, whereby endogenous cannabinoids are released in response to synaptic activation, feedback to presynaptic receptors on these axon terminals, and are subsequently inactivated by FAAH after their uptake into the postsynaptic compartment. This hypothesis has been supported independently by neurophysiological findings, as described below.

Effects of cannabinoids on synaptic function

Inhibition of neurotransmitter release

The presynaptic localization of CB_1 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, GABA, noradrenaline, dopamine, 5-HT and acetylcholine. The brain regions most often studied *in vitro*, usually in tissue slice preparations, have been cerebellum, hippocampus or neocortex. Neurotransmitter release has been studied directly in superfused preparations, and 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 CB_1 receptor agonists WIN552123, HU210 or CP55-2940 were 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 CB_1-selective antagonist rimonabant.

The cellular mechanisms involved in the inhibition of neurotransmitter release by cannabinoids remain unclear. Some have suggested that there is a direct inhibitory effect of CB_1 receptor activation on N-type Ca^{2+} currents (Caulfield and Brown, 1992; MacKie and Hill, 1992). However, the effect appears more likely to involve sites downstream of voltage-dependent Ca^{2+} channels, since a number of studies have shown that cannabinoids reduce the frequencies of miniature excitatory or inhibitory synaptic currents, which are Ca^{2+} independent, rather than altering their amplitude, which is Ca^{2+} sensitive (Schlicker and Kathmann, 2001). Deadwyler *et al*. (1995) suggested that the inhibitory effect of CB_1 receptor activation on adenylate cyclase activity causes a decreased phosphorylation of A-type K^+ channels by the cAMP-dependent enzyme protein kinase A. This, in turn, would activate the A-type K^+ channels and cause a shortening of the duration of presynaptic action potentials as they invade axon terminals.

Biosynthesis of endocannabinoids

Despite their similar chemical structures, the endocannabinoids are produced through distinct biochemical pathways. The formation of anandamide is thought to result from the hydrolysis of the precursor *N*-arachidonoyl phophatidylethanolamine, catalysed by the phosphodiesterase enzyme phospholipase D (Di Marzo *et al*., 1994; Cadas *et al*., 1997). 2-AG, on the other hand, is produced by cleavage of an inositol-1,2-diacylglycerol, catalysed by phospholipase C. Although both anandamide and 2-AG can activate CB_1 receptors, it is not clear whether both function as endocannabinoids, and whether their synthesis and release are independently controlled. The levels of 2-AG found in brain (2–10 nmol/g) are 50–1000 times higher than those of anandamide (10–50 pmol/g). There is some evidence for separate control of their biosynthesis. Stimulation of glutamate release from Schaffer collaterals in rat hippocampal slices increased levels of 2-AG, but not anandamide (Stella *et al*., 1997). On the other hand, another study using *in vivo* microdialysis probes showed that local administration of the dopamine D2 receptor agonist quinpirole caused an increased release of anandamide from rat striatum without affecting levels of 2-AG (Giuffrida *et al*., 1999). Indeed, despite the much higher tissue levels of 2-AG relative to anandamide and the availability of a very sensitive assay, no 2-AG could be detected at all in the striatal dialysate samples. In cultured rat cortical neurons activation of Ca^{2+} influx by stimulation of glutamate *N*-methyl-D-aspartate (NMDA) receptors caused an increase in 2-AG formation but not anandamide (Stella and Piomelli, 2001). However, if NMDA activation was combined with a cholinergic agonist (carbachol) the formation of both endocannabinoids was increased. In both cases Ca^{2+} influx was required for endocannabinoid synthesis. It is clear that much remains to be learned about the relative roles played by the different endocannabinoids. The biosynthesis of the most recently discovered third endocannabinoid, 2-arachidonylglyceryl ether, remains to be characterized.

Endogenous cannabinoids act as retrograde signal molecules at synapses

Important new insights into the physiological role of cannabinoids has emerged from neurophysiological studies published independently by three different research groups 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 signalling from postsynaptic neurons back to inhibitory cells that innervate them, and is particularly prominent in the hippocampus and cerebellum. Three prop-

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erties of DSI 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). Secondly, DSI is probably presynaptic, since 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 Gi-proteins negatively linked to adenylate cyclase or to inositol phosphates (Gi/o) protein to which the CB_1 receptor is coupled (Pitler and Alger, 1994). Wilson and Nicoll (2001) used slice preparations of rat hippocampus and induced DSI by brief depolarizing steps in the holding potential of voltage clamped CA1 pyramidal neurons. They found that DSI was completely blocked by the cannabinoid CB_1 receptor antagonists AM251 or rimonabant and could be mimicked by application of the CB_1 receptor agonist WIN55,2122, 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 sphere of influence some 40 μm in diameter.

Ohno-Shosaku *et al*. (2001) came to a similar conclusion using a different experimental paradigm. They recorded from pairs of cultured hippocampal neurons with inhibitory synaptic connections. They found that depolarization of the postsynaptic neurons lead 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 CB_1 receptor agonist WIN55,2122, which mimicked the inhibitory effect of DSI. Both DSI and the cannabinoid effect could be blocked by the CB_1 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 CB_1 receptor knockout mice (Ledent *et al*., 1999). Varma *et al*. (2001) also reported that agonists which stimulate metabotropic glutamate (mGlu) receptors enhanced DSI, whereas the broad-spectrum antagonist of mGlu receptors, LY341495, tended to reduce DSI, suggesting that glutamate may also be involved. Interestingly, Varma *et al*. (2001) found that mGlu agonists failed to have any effect on DSI in the CB_1 knockout animals, suggesting that glutamate acts to enhance the endocannabinoid signal.

Retrograde signalling by endocannabinoids is not restricted to the inhibitory inputs to postsynaptic neurons. Kreitzer and Regehr (2001*a*) showed that depolarization of rat cerebellar Purkinje cells leads to a transient inhibition of excitatory inputs from parallel fibre and climbing fibre inputs, a phenomenon described as depolarization-induced suppression of excitation (DSE). They found that DSE was triggered by Ca^{2+} influx into the Purkinje cells, and could be completely blocked by the CB_1 antagonist AM-251, and mimicked and occluded by the CB_1 receptor agonist WIN55,2122. Kreitzer and Regehr (2001*b*) 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 WIN55,2122. 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 CB_1 antagonists.

These findings suggest that endocannabinoids are involved in the rapid modulation of synaptic transmission in CNS by a retrograde signalling system that can influence synapses in a local region of some 40 μm diameter, causing inhibitory effects on both excitatory and inhibitory neurotransmitter release that persist for tens of seconds. This may play an important role in the control of neural circuits, particularly in cerebellum and hippocampus (see below). Exogenously administered THC or other cannabinoids cannot mimic the physiological effects of locally released endocannabinoids. Since they cause long-lasting activation of CB_1 receptors in all brain regions, their overall effect is to cause a persistent inhibition of neurotransmitter release from those nerve terminals that express CB_1 receptors, and as a consequence they temporarily occlude and prevent the phenomena of DSI and DSE.

Effects of cannabinoids on CNS function

Psychomotor control

CB_1 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 Rodríguez 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*., 2000). Similarly in mice, Adams and Martin (1996) described a 'popcorn effect' in animals treated with THC. Groups of mice are sedated by the drug, but will jump in response to auditory or tactile stimuli, as they fall into other animals these in turn jump, resembling corn popping in a popcorn machine. Interestingly, the CB_1 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 due, in part, to actions at cerebellar or striatal receptors. Patel and Hillard (2001) used tests of specific cerebellar functions to show that

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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 olgonucleotide directed to a sequence in the CB_1 receptor.

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 number of authors have attempted to combine what is known of the neuroanatomical distribution of the cannabinoid system and the results of behavioural and electrophysiological studies to speculate on the mechanisms underlying cannabinoid modulation of psychomotor function (Breivogel and Childers, 1998; Sañudo-Peña *et al*., 1999; Giuffrida *et al*., 2000; Elphick and Egertová, 2001). The CB_1 receptor is expressed particularly by striatal GABAergic medium-spiny projection neurons, and is abundant in regions contaning 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). CB_1 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. Sañudo-Peña *et al*. (1999) suggested that the primary role of the endocannabinoid system may be to inhibit tonic release of glutamate in the substantia nigra, regulating levels of basal motor activity. Exogenous cannabinoids also lead to decreased GABA release in substantia nigra, which could lead to a disinhibition of the inhibitory nigral input to the thalamocortical pathway, resulting in inhibition of movement. To what extent the effects of cannabinoids on motor function are due to actions in the cerebellum remains unclear, although as described above it is likely that effects on posture and balance are mediated in this brain region. As described previously, CB_1 receptors are known to occur abundantly on nearly all of the principal excitatory (glutamatergic) and inhibitory (GABAergic) inputs to cerebellar Purkinje cells.

The results of eliminating the expression of CB_1 receptors in knockout mice have yielded conflicting results. The knockout animals studied by Zimmer *et al*. (1999) displayed reduced levels of basal activity, in support of the hypothesis put forward by Sañudo-Peña *et al*. (1999), suggesting that tonic activation of CB_1 receptors promotes movement. However, the CB_1 knockout animals studied by Ledent *et al*. (1999) showed no change in spontaneous activity, and in some tests they exhibited increased motor activity. This is in line also with the observations of Compton *et al*. (1996) that the CB_1 antagonist SR141716 caused an increase in locomotor activity. The reasons for the discrepant findings in different strains of CB_1 knockout mice are unknown. Clearly, there is as yet only a poor understanding of the actions of cannabinoids in the basal ganglia and cerebellum. Interactions with other chemical signalling systems in the brain are likely to be important. Giuffrida *et al*. (1999) showed, for example, that dopamine D2 receptor agonists caused an increase in anandamide synthesis and release in striatum. Deadwyler *et al*. (1995) described the convergence of multiple presynaptic controls on the terminals of granule cells in cerebellum. In addition to the CB_1 receptor, these terminals also express high densities of kappa opioid, adenosine A_1 and GABA-B receptors, all of which are coupled through a similar Gi/o type G-protein to inhibit adenylate cyclase and are capable of inhibiting glutamate release. Such complexities are likely to prove the norm.

There is anecdotal evidence that cannabis can relieve muscle pain and spasticity in patients suffering from multiple sclerosis (Consroe *et al*., 1996). Experimental data obtained by Baker *et al*. (2000) in an animal model of multiple sclerosis appears to support such claims. Mice immunized with myelin antigens develop spasticity and tremor. Both symptoms were ameliorated by administration of cannabinoids, and the symptoms were exacerbated by rimonabant, suggesting the involvement of CB_1 receptors and tonic activity in the endocannabinoid system. Controlled clinical trials of cannabis-based medicines for the treatment of multiple sclerosis are currently under way.

Cannabinoid mechanisms in the hippocampus and effects on memory

One of the well established effects of acute intoxication with cannabis in man is an impairment of short-term memory (the extensive literature on human studies is reviewed by Jones, 1978; Miller and Branconnier, 1983; Solowij, 1998; Earleywine, 2002). 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 a review see Hampson and Deadwyler, 1999). These include delayed matching or non-matching tests in rodents (Mallet and Beninger, 1998; Hampson and Deadwyler, 1999), 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 CB_1 receptor.

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A probable site for these effects is the hippocampus. Hampson and Deadwyler (1999) claimed that the effects of the 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. CB_1 receptors are expressed at high densities in the hippocampus. They are particularly abundant on the terminals of a sub-set of GABAergic basket cell interneurons, which also contain the neuropeptide cholecystokinin (Katona *et al*., 1999), and this is also the case in 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. GABAergic neurons in the dentate gyrus also express CB_1 receptors, with terminals concentrated at the boundary of the molecular and granule cell layers (Egertová and Elphick, 2000). In addition CB_1 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.

The mechanisms underlying synaptic plasticity have been studied more intensely in the hippocampus than in any other brain region. 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 clearly that cannabinoids inhibit the induction of both LTP and LTD (for review see Elphick and Egertová, 2001). Cannabinoids appear to work by reducing glutamate release below the level needed to activate 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 might have been expected to increase the level of excitability of hippocampal pyramidal cells, it seems that the cannabinoid-induced reduction in glutamate release predominates. 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. CB_1 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 CB_1-containing GABergic 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). CB_1 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).

One approach to answering the question of what role the tonic release of endocannabinoids may play in hippocampal function has been to examine the effects of CB_1 receptor knockout or of selective CB_1 receptor antagonists. Unfortunately, these studies have so far yielded conflicting results. Bohme *et al*. (2000) reported a significant enhancement of LTP in CB_1 knockout mice, and Reibaud *et al*. (1999) found a significant enhancement of memory in such animals. However, tests with the CB_1 antagonist rimonabant showed no effects on LTP (Terranova *et al*., 1995) or on learning and memory in a spatial learning task (Mallet and Beninger, 1998), although Terranova *et al*. (1996) reported that rimonabant enhanced memory in a short-term olfactory memory test in rats (social recognition test).

Cannabinoids and the neocortex

Like other intoxicant drugs cannabis causes profound changes in a variety of higher brain functions. The literature on the acute effects of the drug in human subjects is large, and can only be summarized here (for reviews see Jones, 1978; Solowij, 1998; Iversen, 2000; Earleywine, 2002). The distribution of CB_1 receptors in the neocortex has been described in detail (Herkenham *et al*., 1991; Egertová and Elphick, 2000). As in the hippocampus, the majority of cortical interneurons expressing high levels of CB_1 receptor are GABAergic cells, which also express cholecystokinin (Marsicano and Lutz, 1999). CB_1-positive terminals are concentrated in layers II–III and layers V–VI, with few in layers I or IV. Despite the obvious importance of the abundant CB_1 receptors in the neocortex there have so far been few electrophysiological studies of their effects on neural activity.

The earlier literature, however, contains several reports of the effects of acute and chronic cannabis use on EEG activity, both in man and animals (reviewed by Adams and Martin, 1996; Solowij, 1998). Most studies in man have observed changes consistent with a state of drowsiness, with increases in relative and absolute α power particularly in frontal regions of cortex. In contrast, the CB_1 antagonist rimonabant was shown to induce EEG changes characteristic of arousal in rats, and increased the time spent in wakefulness as opposed to sleep (Santucci *et al*., 1996). Mechoulam *et al*. (1997) have suggested that anandamide may play a role in the control of the sleep–waking cycle.

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

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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; Mathew *et al*., 1998). This curious effect can also be seen in rats trained to respond for food reward using a fixed interval schedule. When treated with THC or WIN55,2122 the animals shortened their response interval, whereas the antagonist rimonabant lengthened this interval (Han and Robinson, 2001).

There have been many studies of the acute and chronic effects of cannabis on human cognitive function (Jones, 1978; Solowij, 1998; Earleywine, 2002). Performance on a variety of tests of cognitive function is impaired by the drug, but by comparison with alcohol the effects of cannabis are subtle. Whereas even moderate doses of alcohol, for example, impair reaction time, most studies with cannabis have failed to show consistent effects on measures of simple reaction time. Thus the drug's ability to disrupt cognitive function cannot be due to an inability to respond promptly. Among the impairments of cognitive function that have been observed in many, but not all, human studies are: decreased ability to inhibit responses, decreased vigilance, especially for long and boring tasks, decreased ability to perform complex mental arithmetic and impairments in tests of complex reaction times. On the other hand, intoxicated subjects can perform simple arithmetic, learn simple lists of words and recall memories laid down earlier.

Other studies have addressed the question of whether more severe deficits in cognitive function might develop in chronic heavy users of cannabis, or in animals treated for prolonged periods with the drug. The human studies are fraught with difficulties, as described in detail by Earleywine (2002). Among the confounding factors in human studies are that comparisons have to be made between groups of drug users versus non-users, but it is usually impossible to compare the baseline performance of these groups prior to cannabis use to see if they are properly matched. Statistical analysis of such data has often been poor, common errors being the use of so many different tests that the likelihood of finding some significant differences is increased, or the use of inadequate sample sizes. Other drug use can also confound the data. Results have been very variable. Some studies in long-term very heavy users of cannabis (10–20 joints per day for more than 10 years) in Jamaica (Bowman and Pihl, 1973) and Costa Rica (Satz *et al*., 1976) failed to show any significant difference between users versus non-users using a battery of test assessments of cognitive function, and similar negative results were reported in some studies of US college students (Earleywine, 2002). However, most reports have shown that there are deficits in the performance of complex cognitive tasks in long-term cannabis users, although there is little evidence that these are qualitatively or quantitatively more severe than those seen after acute use of the drug (Earleywine, 2002).

Even more controversial is the question of whether long-term cannabis use can cause irreversible deficits in higher brain function that persists after drug use stops. Many studies have suffered from poor design. It is not sufficient to identify a group of cannabis users and simply to test them after stopping cannabis use. Pope *et al*. (2001), for example, recruited 63 current heavy users, who had smoked cannabis at least 5000 times in their lives, and 72 control subjects. Subjects underwent a 28-day washout from cannabis use, monitored by urine assays. At days 0, 1 and 7 the heavy users scored significantly below control subjects on a battery of neuropsychological tests, particularly in recall of word lists. However, by day 28 there were virtually no differences between the groups on any of the test results, and no significant association between cumulative lifetime cannabis use and test scores. The fact that drug-induced effects on cognitive performance can persist for up to a week after stopping the drug (perhaps because of the persistence of THC in the body, or because of a subtle withdrawal syndrome) means that many earlier studies that did not allow a sufficiently long washout period may be invalid. On the other hand, some well designed studies have shown subtle persistent cognitive deficits in ex-cannabis users. Solowij (1998) recruited a group of people who had used cannabis regularly for at least 5 years but who had stopped on average 2 years before the experiment. The subjects were given a very difficult task. They had to listen to a series of tones, some in the right ear some in the left; the tones were long or short (but differing by only 51 ms) and high or low pitch (but differing very little). Participants had to press a button as fast as possible in response to longer tones of a specified pitch in the correct ear. Previous research using this paradigm showed that current regular cannabis users had difficulty in discriminating between the tones. Measurements of event-related potentials also revealed small but significant abnormalities in the P300 wave (Solowij, 1998). The ex-users continued to make significant errors in the discrimination task, but they showed normal P300 waves. The conclusion of these and many other studies in ex-users seems to be that regular cannabis use can cause small but significant impairments in cognitive function that may persist after drug use stops. Such impairments appear to be associated with long-term heavy use of the drug and are unlikely to affect most recreational users.

Effects of cannabinoids on hypothalamic control of appetite

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. 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 the 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.

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THC also stimulates food intake in experimental animals, and 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). Conversely the CB_1 antagonist rimonabant given on its own suppressed food intake and led to reduced body weight in adult non-obese rats (Colombo *et al*., 1998). These results suggest that cannabinoids may play a role in the regulation of food intake and body weight (Mechoulam and Fride, 2001). A possible reciprocal link between endocannabinoid mechanisms and the appetite-suppressing hormone leptin was suggested by Di Marzo *et al*. (2001*a*). They found that food-deprived CB_1 receptor knockout mice eat less than their wild-type litter mates, and the CB_1 antagonist rimonabant reduced food intake in the wild-type animals but not in the knockouts. Animals with defective leptin signalling (obese db/db or ob/ob mice and Zucker rats) exhibited elevated hypothalamic levels of anandamide and 2-AG. On the other hand, treatment of normal rats or ob/ob (leptin deficient) mice with leptin caused decreases in hypothalamic levels of the endocannabinoids. These findings suggest that hypothalamic endocannabinoids may play an important role in mediating the appetite-suppressant effects of leptin. At some stages during development these effects of endocannabinoids may be of critical importance. Fride *et al*. (2001) found that administration of the CB_1 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 within 4–8 days. The effect of rimonabant could be almost fully reversed by co-administering THC.

Cannabinoids as anti-emetic agents

The ability of THC and the synthetic cannabinoid nabilone to control the nausea and vomiting associated with cancer chemotherapy is one of the few well documented medical applications for these drugs (for reviews of the controlled clinical trials see Vincent *et al*., 1983; British Medical Association, 1997; Joy *et al*., 1999; and the meta-analysis reported by Tramèr *et al*., 2001). THC (dronabinol) and nabilone were approved for medical use in the USA, although neither drug has found much utility. The narrow window between the anti-emetic dose and that causing unwanted psychic effects made these drugs difficult to use. The advent of serotonin $5\text{-}HT_3$ receptor antagonists as new and more powerful anti-emetic drugs that were free of unwanted psychic effects during the 1980s also made the cannabinoids less attractive.

Studies in experimental animals have confirmed that the anti-emetic effects of cannabinoids are mediated through CB_1 receptors (Darmani, 2002), and in some susceptible species (e.g. the least shrew) the CB_1 antagonist rimonabant is emetic, an effect that can be blocked by THC or WIN55,2122 (Darmani, 2001).

Cannabinoids and pain

Cannabis was widely used in 19th century medicine for pain relief and there is renewed interest in cannabis-based medicines, with pain as one of the key therapeutic targets (British Medical Association, 1997; Joy *et al*., 1999). Endogenous cannabinoids and cannabinoid receptors exist at various levels in the pain pathways, from peripheral sensory nerve endings to spinal cord and supraspinal centres, in a system that is parallel to but distinct from that involving endorphins and opiate receptors.

Systemically administered THC and synthetic cannabinoids have anti-nociceptive and anti-hyperalgesic effects in a variety of animal models of acute and inflammatory pain (for reviews see Pertwee, 2001; Iversen and Chapman, 2002). Since cannabinoids inhibit motor activity this could prevent animals from exhibiting the normal behavioural reactions in analgesic tests; however, a number of studies have also shown that cannabinoids suppress electrophysiological responses of spinal cord neurons to noxious stimulation, and block spinal c-fos expression in response to such stimulation (Walker *et al*., 1999; Pertwee, 2001; Iversen and Chapman, 2002). Cannabinoids and anandamide also exert anti-nociceptive effects in animal models of inflammatory pain when injected directly into spinal cord, brain stem or thalamus (Pertwee, 2001). Behavioural studies have shown that cannabinoids reduce thermal and mechanical allodynia in rat models of neuropathic pain (Herzberg *et al*., 1997; Fox *et al*., 2001; Iversen and Chapman, 2002). Furthermore, noxious stimulation evoked an increased release of anandamide in the periaqueductal grey region of brainstem, a key site for modulating nociceptive information (Walker *et al*., 1999). The anti-nociceptive effects of cannabinoids are blocked by the CB_1 antagonist rimonabant, but the antagonist itself does not alter basal pain thresholds, suggesting that these are not controlled by tonic activity in the endocannabinoid system (Compton *et al*., 1996).

Results obtained with CB_1 receptor knockout mice, however, suggest that not all of the anti-nociceptive effects of THC or anandamide are mediated via CB_1 receptors. Thus, although Di Marzo *et al*. (2000) found that the anti-nociceptive effects of THC were virtually absent in the knockout animals, anandamide continued to show analgesic activity in the hot-plate test. It is possible that the analgesic effects of anandamide are mediated in part through an action at other as yet ill-defined cannabinoid receptors (Breivogel *et al*., 2001; Hájos *et al*., 2001). Alternatively, it has been proposed that the effects of anandamide might be mediated through its ability to bind to the vanilloid VR1 receptor, which is present in primary afferent neurons and known to play an important role in nociceptive responses (Di Marzo *et al*., 2001*b*). To complicate matters further, Zimmer *et al*. (1999), in a different strain of CB_1 receptor knockout mice, found that THC continued to exert some anti-nociceptive actions in hot-plate and formalin tests in the knockout animals. The reasons for the discrepant results obtained

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with different strains of CB_1 receptor knockout mice are unknown.

There is 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 synergically—one potentiated the anti-nociceptive actions of the other. This potentiation could be blocked by either rimonabant or by naloxone, indicating that both CB_1 and opiate receptors were involved (Fuentes *et al*., 1999). Meng *et al*. (1998) showed that temporary inactivation of neural activity in the rostral ventromedial medulla (RVM) in rat brainstem prevented the analgesic effects of systemically administered cannabinoids, while leaving their effects on motor activity unaffected. An electrophysiological analysis of the effects of cannabinoids on single cell firing patterns in RVM 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.

Basic research into the role of cannabinoids and endocannabinoids in pain mechanisms is progressing rapidly. Clinical progress, however, has been slow. A meta-analysis of clinical trials of cannabinoids as analgesics concluded that there was not enough evidence to justify their use in this indication (Campbell *et al*., 2001). However, this may merely reflect the paucity of data from adequately sized controlled clinical trials, and cannabis-based medicines may yet find genuine medical applications in this field.

Cannabis as an intoxicant and drug of dependence

Cannabis intoxication

Despite being illegal, cannabis is one of the most widely used intoxicants; almost half of all 18 year olds in the USA and in most European countries admit to having tried it at least once, and ~10% of that age group are regular users (Iversen, 2000). There have been many subjective accounts of the cannabis 'high' (see Iversen, 2000; Earleywine, 2002). 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 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. The feelings of heightened perception, increased appetite and distortion of the sense of time have already been referred to. A survey of 1333 young British cannabis users (Atha and Blanchard, 1997) reported that the most common positive benefits reported were relaxation and relief from stress (25.6%), insight/personal development (8.7%) and euphoria (4.9%); more than half reported some positive benefits. But 21% of the users also attributed some adverse effects to cannabis use, including impaired memory (6.1%), paranoia (5.6%) and amotivation/laziness (4.8%).

As with other intoxicant drugs, little is known about the brain mechanisms that underlie the cannabis 'high'. The intoxicant effects are clearly mediated via CB_1 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 CB_1 antagonist blocked the acute psychological effects of the active cigarettes. Interestingly rimonabant itself when given alone (with placebo cigarette) produced no significant psychological effects. Mathew *et al*. (1997) used $H_2{}^{15}O$ and PET to measure changes in regional cerebral blood flow in a double blinded study in 32 volunteers comparing THC with placebo. Self ratings of cannabis intoxication correlated most markedly with increased blood flow in the right frontal region.

Endocannabinoids and CB_1 receptors are present in many regions of the limbic forebrain. For example, Katona *et al*. (2001) reported that CB_1 receptors were expressed in high densities in lateral and basal nuclei in the rat amygdala. As in hippocampus, the CB_1 receptors in these regions were located presynaptically on the terminals of cholecystokinin-containing GABAergic interneurons. Electrophysiological experiments showed that cannabinoids modulated GABAergic synaptic transmission. The authors suggested that such effects might underlie some of the actions of cannabinoids on emotional behaviour. Other experiments have revealed that, in common with other euphoriant drugs, THC selectively activates dopaminergic neurons in the ventral tegmental area. 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 intravenously) caused an increased 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. Tanda *et al*. (1997) found that the increased release of dopamine provoked by THC could be blocked by administration of the μ-opiate receptor antagonist naloxonazine, suggesting the involvement of an opioid mechanism.

Tolerance and dependence

Many animal studies have shown that tolerance develops to most of the behavioural and physiological effects of THC (for review see Pertwee, 1991). The earlier clinical literature suggested that tolerance also occurs after repeated administration of THC in man, although many of these studies were poorly controlled (for reviews see Jones, 1978, 1987;

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Hollister, 1986). 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 behaviour 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 to be positive by these definitions. Swift *et al*. (2001) undertook a survey of 10 641 Australians aged 18 years and older. They reported that almost one-third of regular cannabis users fell within the definitions of 'substance abuse' (10.7%) or 'substance dependence' (21%). In the USA, Anthony *et al*. (1994) reported the results obtained from a large scale survey which indicated that some 46% of those interviewed had ever used cannabis and 9% of users became dependent. 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 (Budney *et al*., 2001).

The existence of dependence on cannabinoids in animals is also much more clearly observable because of the availability of CB_1 receptor antagonist drugs that can be used to precipitate withdrawal. Thus, Aceto *et al*. (1996) described a behavioural 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 CP-55,940 (Rubino *et al*., 1998) or WIN55,2122 (Aceto *et al*., 2001). Rimonabant-induced withdrawal after 2 weeks of treatment of rats with the cannabinoid HU-120 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 (Rodríguez 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 indicate clearly 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 behaviour with THC were unsuccessful (Pertwee, 1991), some success has been achieved recently. Squirrel monkeys were trained to self-administer low doses of THC (2 μg/kg per injection), but only after the animals had first been trained to self-administer cocaine (Tanda *et al*., 2000). THC is difficult to administer intravenously and these authors succeeded perhaps in part because they succeeded in delivering the drug intravenously in doses comparable to those to which human cannabis users are exposed. The potent synthetic cannabinoids are far more water soluble than THC, which makes intravenous administration easier. Mice could be trained to self-administer intravenous WIN55,2122, but CB_1 receptor knockout animals failed to exhibit this behaviour (Ledent *et al*., 1999). Another 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).

A number of studies have suggested that there may be links between the development of dependence to cannabinoids and to opiates (Manzanares *et al*., 1999). Some of the behavioural 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 WIN55,2122 became sensitized to the behavioural 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. THC also facilitated the anti-nociceptive effects of RB 101, 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. CB_1 receptor knockout mice exhibited greatly reduced morphine self-administration behaviour 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*., 2000). Knockout of the μ-opioid receptor also reduced rimonabant-induced withdrawal signs in THC-treated mice, and there was an attenuated naloxone withdrawal syndrome in morphine dependent CB_1 knockout mice (Lichtman *et al*., 2001*a*, b).

These findings point clearly to interactions between the endogenous cannabinoid and opioid systems in CNS, although the neural circuitry involved remains unknown. Whether this relationship is relevant to the so-called 'gateway' theory is unclear. The US National Household survey of Drug Abuse (US Department of Health and Human Services,

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1999) indicated that respondents aged 22 years or older who had started cannabis use before the age of 21 years were 24 times more likely than non-cannabis users to initiate use of hard drugs. But the proportion of cannabis users who progress in this way remains very small (~1% or less), and mathematical modelling using the Monte Carlo method suggested that the association between cannabis use and hard drug use need not be causal but could relate to some common predisposing factor, e.g. 'drug-use propensity' (Morral *et al*., 2002).

Adverse effects of cannabis on the CNS

Is cannabis neurotoxic?

Although there have been claims that chronic cannabis use may permanently damage the brain, there is little scientific evidence to support these claims (for reviews see Dornbush *et al*., 1976; Hollister, 1986, 1998; Zimmer and Morgan, 1997). As described above, some studies have revealed a modestly impaired ability to focus attention and filter out irrelevant information in ex-cannabis users (Solowij, 1998), but other studies failed to find any impairments in cognitive function (Pope *et al*., 2001). There is little evidence that cannabis use impairs work performance or leads to an 'amotivational syndrome' (Dornbush *et al*., 1976; Hollister, 1986; Abood and Martin, 1992), nor is there any convincing evidence for neuropathological changes in the brains of cannabis users (Hollister, 1986). The earlier studies have been complemented by the application of powerful modern neuroimaging methods. For example, an MRI study compared 18 current, frequent, young adult cannabis users with 13 comparable non-users and found no evidence of cerebral atrophy or regional changes in tissue volumes (Block *et al*., 2000).

Animal studies have yielded conflicting results. Treatment of rats with high doses of THC given orally for 3 months (Scallet *et al*., 1987) or subcutaneously for 8 months (Landfield *et al*., 1988) was reported to lead to neural damage in the hippocampal CA3 zone, with shrunken neurons, reduced synaptic density and loss of cells. However, in another study the potent synthetic cannabinoid WIN55,2122 was administered twice daily (2 mg/kg) to rats and led to an apparent increase in hippocampal granule cell density, and increased dendritic length in the CA3 zone. In perhaps the most severe test of all, rats and mice were treated with THC 5 days each week for 2 years and no histopathological changes were observed in brain, even after 50 mg/kg/day (rats) or 250 mg/kg/day (mice) (Chan *et al*., 1996). Although claims were made that exposure of a small number of rhesus monkeys to cannabis smoke led to ultrastructural changes in septum and hippocampus (Harper *et al*., 1977; Heath *et al*., 1980), subsequent larger scale studies failed to show any cannabis-induced histopathology in monkey brain (Scallet, 1991).

Studies of the effects of cannabinoids on neurons *in vitro* have also yielded inconsistent results. Exposure of rat cortical neurons to THC was reported to decrease their survival, with twice as many cells dead after 2 h exposure to 5 μM THC than in control cultures (Downer *et al*., 2001). Concentrations of THC as low as 0.1 μM had a significant effect. The effects of THC were accompanied by release of cytochrome *c*, activation of caspase-3 and DNA fragmentation, suggesting an apoptotic mechanism. All of the effects of THC could be blocked by the antagonist AM-251 or by pertussis toxin, suggesting that they were mediated through CB_1 receptors. Toxic effects of THC have also been reported on hippocampal neurons in culture, with 50% cell death after 2 h exposure to 10 μM THC or after 5 days exposure to 1 μM drug (Chan *et al*., 1998). The antagonist rimonabant blocked these effects, but not pertussis toxin. The authors proposed a toxic mechanism involving arachidonic acid release and formation of free radicals. However, other authors failed to observe any damage in rat cortical neurons exposed for up to 15 days to 1 μM THC, although they found that this concentration of THC killed rat C6 glioma cells, or human astrocytoma U373MG and mouse neuroblastoma N18TG12 cells (Sánchez *et al*., 1998). In a remarkable study injections of THC into solid tumours of C6 glioma in rodent brain led to increased survival times, and a complete eradication of the tumours was evident in 20–35% of the treated animals (Galve-Roperh *et al*., 2000). The anti-proliferative effects of cannabinoids has suggested a potential utility for such drugs in cancer treatment (Guzmán *et al*., 2001).

Some studies have reported neuroprotective actions of cannabinoids. Administration of WIN55,2122 was found to reduce cerebral damage in rat hippocampus or cerebral cortex after global ischaemia or focal ischaemia models *in vivo* (Nagayama *et al*., 1999). The endocannabinoid 2-AG protected against damage elicited by closed head injury in mouse brain, and the protective effects were blocked by rimonabant (Panikashvili *et al*., 2001). THC had a similar effect *in vivo* in protecting against damage elicited by ouabain (Van der Stelt *et al*., 2001). Rat hippocampal neurons in tissue culture were protected against glutamate-mediated damage by low concentrations of WIN55,2122 or CP-55,940 and these effects were mediated through CB_1 receptors (Shen and Thayer, 1998). But not all of these effects seem to require mediation via cannabinoid receptors. Nagayama *et al*. (1999) reported protective effects of WIN55,2122 that did not require either cannabinoid receptor in cortical neurons exposed to hypoxia, and similar findings were reported for the protective actions of anandamide and 2-AG in cortical neuron cultures (Sinor *et al*., 2000). Both THC and cannabidiol, which is not active on cannabinoid receptors, protected rat cortical neurons against glutamate toxicity (Hampson *et al*., 1998) and these effects, were also independent of CB_1 receptors. The authors suggested that the protective effects of THC in their studies might be due to the antioxidant properties of these polyphenolic molecules, which have redox potentials higher than those of known antioxidants (e.g. ascorbic acid).

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The mixed reports of neurotoxic and neuroprotective effects of cannabinoids are confusing. While it may be possible to demonstrate neurotoxic actions after exposure of neurons to high concentrations of cannabinoids *in vitro*, there is little evidence for any significant neural damage *in vivo* after the administration of pharmacologically relevant doses of these drugs.

Cannabis and psychiatric illness

A temporary form of drug-induced psychosis can occur in some cannabis users. In some of the psychiatric literature this is referred to as 'cannabis psychosis' (or 'marijuana psychosis'). Research psychiatrists, particularly in Britain (Thomas, 1993; Hall and Degenhardt, 2000; Johns, 2001), have studied this condition carefully. It nearly always results from taking large doses of the drug, often in food or drink, and the condition may persist for some time, perhaps as the accumulated body load of THC is washed out. The acute toxic psychosis that is sometimes caused by cannabis can be sufficiently serious to lead to the subject being admitted to hospital, and the initial diagnosis can be confused with schizophrenia, since the patients may display some of the characteristic symptoms of schizophrenic illness. These include delusions of control (being under the control of some outside being or force), grandiose identity, persecution, thought insertion, auditory hallucinations (hearing sounds, usually non-verbal in nature), changed perception and blunting of the emotions. Not all symptoms will be seen in every patient, but there is a considerable similarity to paranoid schizophrenia. This has led some to propose a 'cannabinoid hypothesis of schizophrenia', suggesting that the symptoms of schizophrenic illness might be caused by an abnormal over-activity of endogenous cannabinoid mechanisms in the brain (Emrich *et al*., 1997).

A number of studies have addressed the more contentious question of whether cannabis use can precipitate long-term psychiatric illness. The strongest evidence seemed to come from a study in Sweden that involved taking detailed medical records and information about the social background and drug-taking habits of 45 570 conscripts on entry to the Swedish army at age 18 years and following up of their subsequent medical history over a 15-year period (Andreasson *et al*., 1987). A total of 4293 of the conscripts admitted having taken cannabis at least once, but the cannabis users accounted for a disproportionate number of the 246 cases of schizophrenic illness diagnosed in the overall group on follow-up. The relative risk of schizophrenia in those who had used cannabis was 2.4 times greater than in the non-users. In the small number of heavy users (who had taken the drug on more than 50 occasions) the relative risk of schizophrenia increased to 6.0. The authors concluded that cannabis was an independent risk factor for schizophrenia. There have been other similar reports (Mathers and Godse, 1992; Hall and Degenhardt, 2000; Johns, 2001). Hambrecht and Hafner (2000), for example, studied 232 patients in Germany with first-episode schizophrenia. They found that 13% of these had a history of cannabis use, a rate twice that of matched normal controls. At first viewing these findings seem convincing, but they do not prove any cause-and-effect relationship with cannabis. It may simply be that both cannabis use and schizophrenia are related to some common predisposing factor, such as personality. Indeed some psychologists and psychiatrists believe that they can identify psychological traits that are described as 'schizotypy' and which may predict an increased risk of developing clinical psychosis. Some studies in healthy adults have reported that those subjects who used cannabis scored higher on schizotypy scales than non-users (Williams *et al*., 1996; Skosnik and Spatz, 2001). Half of the cannabis-using subjects in the original Swedish study had used cannabis more than 10 times and subsequently developed schizophrenia had also taken amphetamine, a drug known to be capable of inducing a schizophrenia-like psychosis. The cannabis users also came from deprived social backgrounds, another known risk factor of schizophrenia. More detailed follow-ups of some of the original Swedish cohort, however, claimed to have answered some of these criticisms (Andreasson *et al*., 1989; Zammit *et al*., 2002). In addition, further reports from New Zealand (Arseneault *et al*., 2002; Fergusson *et al*., 2003), Australia (Patton *et al*., 2002) and France (Verdoux *et al*., 2003) add weight to the hypothesis that the development of cannabis dependence in young people is associated with increased rates of psychiatric symptoms, both of psychosis and depression and anxiety (Patton *et al*., 2002).

Nevertheless, the existence of a causative relationship between cannabis use and long-term psychotic illness remains unproven. If cannabis use did precipitate schizophrenia one might expect to have seen a large increase in the numbers of sufferers from this illness as cannabis use became more common in the West during the past 30 years. However, a detailed review of the epidemiological evidence up to 1990 appeared to show that this has not been the case (Thornicroft, 1990).

On the other hand, it is clear that cannabis can exacerbate the symptoms of existing psychotic illness. While schizophrenic patients seem to use cannabis and other psychoactive drugs as a form of 'self-medication', cannabis can make the key symptoms of delusions and hallucinations worse and it tends to counteract the anti-psychotic effects of the drugs used to treat the illness (Negrete *et al*., 1986; Linzen *et al*., 1994). On the other hand, one Swedish study reported that cannabis use made schizophrenic patients less withdrawn and more likely to speak (Peralta and Cuesta, 1992). It would seem prudent, nevertheless, to discourage the use of cannabis in patients with existing psychotic illness.

Conclusion

The discovery of the endocannabinoids and the availability of new pharmacological tools, together with the development of strains of genetically engineered knockout mice that lack

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functional cannabinoid receptors, has revitalized the field of cannabis research in the past few years. The effects of administering THC or other cannabinoids can never simulate the highly localized function of the endocannabinoids, which appear to act as a fine control system to regulate neurotransmitter release at the synaptic level. There has been renewed interest in the potential therapeutic applications of cannabis-based medicines (British Medical Association, 1997; Joy *et al*., 1999; Robson, 2001). THC or other cannabinoid agonists all suffer from the problem of a narrow therapeutic window between the desired clinical benefits and the unwanted psychic side-effects. It is possible that the pharmacological manipulation of the endocannabinoid system, boosting function, for example by drugs that inhibited the inactivation of the endocannabinoids, may offer a safer and more subtle approach to cannabis-based medicines in the future (Piomelli *et al*., 2000).

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