Cannabinoid Function in Learning, Memory and Plasticity

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Abstract Marijuana and its psychoactive constituents induce a multitude of effects on brain function. These include deficits in memory formation, but care needs to be exercised since many human studies are flawed by multiple drug abuse, small sample sizes, sample selection and sensitivity of psychological tests for subtle differences. The most robust finding with respect to memory is a deficit in working and short-term memory. This requires intact hippocampus and prefrontal cortex, two brain regions richly expressing CB_1 receptors. Animal studies, which enable a more controlled drug regime and more constant behavioural testing, have confirmed human results and suggest, with respect to hippocampus, that exogenous cannabinoid treatment selectively affects encoding processes. This may be different in other brain areas, for instance the amygdala, where a predominant involvement in memory consolidation and forgetting has been firmly established. While cannabinoid receptor agonists impair memory formation, antagonists reverse these deficits or act as memory enhancers. These results are in good agreement with data obtained from electrophysiological recordings, which reveal reduction in neural plasticity following cannabinoid treatment, and increased plasticity following antagonist exposure. The mixed receptor properties of the pharmacological tool, however, make it difficult to define the exact role of any CB_1 receptor population in memory processes with any certainty. This makes it all the more important that behavioural studies use selective administration of drugs to specific brain areas, rather than global administration to whole animals. The emerging role of the endogenous cannabinoid system in the hippocampus may be to facilitate the induction of long-term potentiation/the encoding of information. Administration of exogenous selective CB_1 agonists may therefore disrupt hippocampus-dependent learning and memory by 'increasing the noise', rather than 'decreasing the signal' at potentiated inputs.

Keywords Endocannabinoids · CB_1 receptors · Perception · Cognition · Memory formation · Hippocampus · In vitro slice · Synaptic plasticity · LTP · LTD · DSI

1 Introduction

The resin made from flowers and leaves of the hemp plant *Cannabis sativa,* commonly known as cannabis or marijuana, comprises approximately 60 terpenophenolic compounds, which are referred to as plant cannabinoids. The primary psychoactive constituent is Δ^9-tetrahydrocannabinol (Δ^9THC) (Gaoni and Mechoulam 1964). Marijuana has been used for hundreds of years all over the world for both recreational and medicinal purposes, but has always been known for both positive effects, including relaxation, calming and stress relief, and negative effects, such as nausea, sickness, vomiting, dizziness and headaches. Like most drugs of abuse, cannabis is known for its ability to induce euphoria, lethargy, confusion, depersonalisation, altered time sense, impaired motor performance, memory defects, paranoia, depression, fear, anxiety and hallucinations. Given that most of these effects are mediated through specific receptors, it makes cannabinoids and their

receptors an interesting target for the development of treatment strategies in the clinical setting.

In this chapter we will focus on the cognitive effects that have been described after cannabinoid use in humans and more recently in animals. We rely on many other chapters of this handbook in which details about the pharmacology and physiology, molecular and cell biology, and medicinal chemistry of cannabinoids are reviewed in detail (see chapters by Pertwee, Howlett, Abood, Reggio, Mackie, and Szabo and Schlicker). In the first part we will summarise effects of marijuana smoking on cognition in humans, and this will be followed by a brief introduction to the physiology of these effects. This will mainly concentrate on functional imaging data and electroencephalographic (EEG) recordings in humans. The main focus, however, will review work on animals and cognition (learning and memory) as this provides, to date, the best and most detailed data of the basic behavioural pharmacology of cannabinoids and cannabinoid receptors. Finally, we will try to explain the behavioural results in terms of ex vivo and in vitro physiology and synaptic plasticity.

2
Marijuana and Cognition in Man

Cannabis use alters both motor and cognition-based behaviour in man. Collectively, data strongly indicate acute intoxication to be more effective in disrupting memory than chronic use, probably due to long-term habituation and related changes in brain function. While simple cognitive tasks can be performed normally, the severity of cognitive impairment correlates with task difficulty, and this may be the direct consequence of deficits in attention and goal-directed learning. Importantly, there are few if any gross motor impairments, even after chronic cannabis smoking over many years.

Acute cannabis intoxication leads to multiple effects, including changes in reaction time and perception. Simple reaction times are recorded such that test subjects have to press a button in response to a tone or light. This merely requires motor execution; such tasks are devoid of complex cognitive processing. Several studies have reported that reaction times increase after marijuana use (Borg et al. 1975; Dornbush et al. 1971), but this has not been confirmed by others (Braden et al. 1974; Evans et al. 1976) despite comparable sample sizes and drug doses. Increasing the complexity of the task (pressing different buttons in response to different stimuli) consistently leads to up to 50% longer reaction times in users relative to controls, and there seems to be a strong correlation between task complexity and cannabis-induced impairment (Clark and Nakashima 1968; Chait and Pierri 1992).

Stronger evidence supports the notion that cannabis use alters perception, such as taste, smell, hearing and vision. In users there are clear problems of colour discrimination (Adams et al. 1976) and identification of figures hidden in pictures (Pearl et al. 1973). Perceptual changes also pertain to time sense, which is generally altered in cannabis users. As they estimate time to pass more slowly than control subjects (Tart 1971; Chait and Pierri 1992), this could explain

why they are prepared to take greater risks, for example in driving faster and more dangerously (Bech et al. 1973). Such drug effects are of importance when investigating complex behaviours. Hasty reactions and perceptual deficits could easily explain impairments in memory tasks and need to be excluded.

Despite perceptual effects, it is still possible to identify cannabis-induced memory problems. A type of memory highly sensitive to marijuana intoxication is recognition memory. Typically, test subjects are presented with a series of words. After a delay period, a second series is presented containing some words from the original series, but also some new ones. Cannabis users have no problem identifying the words from the original list, but they often recognise some words that are actually new (Dornbush 1974). Such memory intrusions may reflect problems in distinguishing between relevant and irrelevant words, a hypothesis that is supported by observations on free recall. Here, participants write down as many words as they remember from the original list without being primed. This is a more complex paradigm, and users not only remember fewer words than controls (Dornbush et al. 1971), they also have memory intrusions, inserting words that were not presented (Miller and Cornett 1978).

Determinations of cognitive alterations in *chronic* marijuana users are more difficult. Classical studies of Jamaican (Bowman and Pihl 1973) and Costa Rican (Satz et al. 1976) subjects did not reveal any cognitive impairment, despite a battery of psychological tests and the fact that chronic users had been smoking more than nine joints per day for more that 10 years. These results were confirmed in a recent report on 1,300 residents in Baltimore that had been followed in a longitudinal study over 11 years. Mini-Mental State Examination was applied to investigate any changes in mental functioning, yet no significant difference was observed between chronic marijuana consumption and controls (Lyketsos et al. 1999).

Cognitive differences were revealed in a study on 1,600 Egyptian prisoners (Soueif 1976). In this study, 16 different measures were recorded, of which 10 revealed impairment in the user group, while 2 showed better performance. However, the selected groups were not well controlled and many of the critiques listed below apply to this investigation. Similarly, deficits in IQ, memory, time estimation and reaction times were reported in several studies performed in India (Wig and Varma 1977; Menhiratta et al. 1978). Finally, investigations on college students with at least twice weekly marijuana consumption revealed deficits in memory formation, specifically deficits in information transfer into long-term memory (Gianutsos and Litwack 1976; Entin and Glodzung 1973). However, a later study did not confirm these memory impairments (Rochford et al. 1977). More recent studies on cognitive deficits in marijuana users collectively suggest that impairments are (1) predominant for the attentional/executive system related to prefrontal cortex, and (2) increase with the length of cannabis use (Pope and Yurgelun-Todd 1996; Fletcher et al. 1996; Elwan et al. 1997). Such deficits can readily explain impairments in short-term memory, which are frequently reported for cannabis users (Schwartz et al. 1989).

Many of these studies, however, are flawed and do not reveal the true extent to which long-term cannabis use affects human cognition. Especially, early studies from the 1970s and 1980s were conducted on small sample sizes, and it has been

calculated that an $n = 25$ per group is necessary to attain reliable results (Cohen 1990). Moreover, subjects that feel less affected by drug use are more likely to sign up for trials, while those experiencing severe problems may feel less eager to participate, even for pay (Strohmetz et al. 1990). As a consequence, the finding of subtle differences may not be a true reflection of the effects of chronic cannabis use. Psychological testing has seen considerable refinement, and the emergence of novel, increasingly sensitive tasks has helped to reveal differences between long-term marijuana smokers and controls. This suggests that tests used in the original studies, which have not found differences between test and control groups, were insensitive and might have been too simple.

Another critique frequently raised with respect to chronic use is the idea that users may have already been different from non-users prior to ever smoking marijuana. This is a valid point, as one might argue that (1) people of lower IQ may be more prone to drug use and (2) any intellectual difference may have preceded any cannabis smoking habits. Randomised control studies in which non-users are signed up for chronic smoking, however, are ethically difficult to justify. Another potential confounder is the use of multiple drugs. Many marijuana smokers are likely to use other and more drugs than controls (Earleywine and Newcomb 1997). Multi-drug effects can only be assessed in the context of each drug alone. Subjects who meet this criterion do not normally form part of studies. Consequently, multi-drug use will make the sample group heterogeneous so that results may not reflect the typical cannabis user.

In contrast, animal research is devoid of many of the above critiques and results are thus not confounded by, for example, polydrug use, low sample sizes, pre-treatment differences, etc. Consequently, the main focus of this chapter rests on such animal models and the effects of acute and chronic cannabis administration on learning, memory, and related brain physiology.

3 Effects of Cannabinoids on the Brain

To date, there is no evidence for gross morphological and structural changes in brain following short-term or long-term marijuana smoking. Although this has been investigated over many years, of particular interest here are studies that have utilised modern imaging techniques such as magnetic resonance imaging (MRI). There were no regional or global changes in brain tissue volume or composition in cannabis users (Block et al. 2000). More subtle changes can be determined through post-mortem analysis using radiolabelled compounds, or measurement of endocannabinoid levels. Such work showed reduced cannabinoid binding in caudate and hippocampus of Alzheimer's brains (Westlake et al. 1994), and in normal ageing (Biegon and Kerman 1995). No such studies have been reported on chronic marijuana smokers yet.

Alterations in brain function following acute and chronic use of cannabis is nevertheless detectable using cerebral blood flow (CBF) measurements such as positron emission tomography (PET) and multi-site EEG. Although very impor-

tant for the understanding of brain regions associated with behavioural changes, CBF measurements are not ideal for determination of marijuana-induced changes in brain function (Mathew and Wilson 1991; also see chapter by Lindsey et al. in this handbook). This is mainly due to contaminating effects of cannabis on vascular smooth muscles and altered vasomotor tone, but also due to alterations in respiration and general circulation (for details see chapter by Pacher et al. in this volume). Since such circulation-related effects cannot be controlled for properly, they may lead to increased variability and make interpretations of CBF studies in marijuana users difficult. Fortunately, there is no conclusive evidence to suggest these peripheral effects impact significantly on blood circulation in brain. PET, for instance, makes use of a radiotracer (^{11}C, ^{13}N or ^{15}O) followed by reconstruction of tomographic slices depicting isotope concentrations in different brain regions. A shortcoming of PET is, however, its low resolution. Areas of less than 2 mm cannot be resolved properly. As with psychological testing, results of PET tests have been ambiguous; some reported decreased CBF, others increased CBF; some found no difference (see Wilson and Mathew, 2002 for review). Yet, it remains elusive as to why this variability is observed.

Collectively, data from CBF studies confirm the contention that alterations in brain function predominate in areas with high levels of cannabinoid receptor sites (Pertwee 1997). However, global marijuana intoxication will induce multiple effects at the same time, making it difficult to correlate any particular effect and CBF change. Overall, it has been found that chronic cannabis users have a lower resting level of brain blood flow than controls and that marijuana smoking or intravenous administration increases CBF in most cortical areas in a dose- and time-dependent manner (Wilson and Mathew 2002). Increases in CBF peaked at 30 min and returned to near-baseline levels 2 h after smoking. Subcortical areas including basal ganglia, thalamus, hippocampus and amygdala showed reduced CBF relative to placebo and both hemispheres were affected to the same extent. In addition, cerebellar blood flow increased by at least 1 standard error of the mean in about 60% of subjects. It should be obvious from these results that systemic administration of marijuana may not help to resolve the question as to what the function of individual subpopulations of receptors located in specific brain areas might be. CBF will provide important information as to global changes related to drug treatment.

An interesting approach in utilising PET is its combination with cognitive tasks. While subjects perform verbal memory recall tasks, they are monitored in the scanner. Relative to controls, frequent marijuana users presented with reduced memory-related blood flow in prefrontal cortex, but increased CBF in hippocampus and cerebellum (Block et al. 2002). These alterations were paralleled by an increased recency effect, suggesting that users rely on short-term memory and thus fail in multiple trial learning tasks, while control subjects encode and retrieve episodic memory. Consequently, it may be argued that chronic marijuana use leads to a reconfiguration of memory processing. Reductions in prefrontal CBF are consistent with deficits in working memory.

Another functional approach is the use of multiple recording sites on the skull to detect global changes in cortical activity through EEG. Event-related potentials (ERPs) derived from EEGs recorded during complex cognitive tasks have been

recorded in a number of studies by Solowij and colleagues (see Solowij 1998 for review). Collectively, data in this area can be summarised as follows. Independent of frequency of marijuana smoking, ERPs in frontal regions progressively decline with the number of years of use. This suggests a physiological mechanism for the reduced ability to focus attention and filter out irrelevant information. Interestingly, the deficit was maintained even after several months of abstinence. The speed of information processing can be measured as positive wave at 300 ms (P300) of the ERP. Similar to reaction times, P300 was impaired with increasing frequency and length of marijuana use. Long-term marijuana use manifests in elevated absolute power and interhemispheric coherence of alpha and theta rhythm of the EEG (Struve et al.1994) and reduces the P50 auditory sensory gating response (Patrick et al. 1999).

4
Cannabinoids Modulate Cognition in Animal Models

Guided by the older work from humans, research into the behavioural effects of cannabinoids concentrated on the disruption of working and short-term memory formation. This is in agreement with data suggesting marijuana-induced increases in CBF in paralimbic regions of the frontal lobes and the cerebellum, but reduced blood flow in the temporal lobe (O'Leary et al. 2002). Hypoactivity in the temporal lobe may constitute the neural basis of cognitive alterations seen in cannabis users and has prompted the search for the underlying mechanisms using behavioural paradigms that specifically activate the medial temporal lobe, or using electrophysiological recording protocols in medial temporal lobe structures. It is also in line with reductions of the cortical P300 amplitude in marijuana addicts. The P300 is an ERP reflecting attentional resource allocation and active working memory (Johnson et al. 1997). Similarly, monkeys treated with Δ^9THC chronically have predominantly slow-wave EEGs (1–2 Hz) in hippocampus, amygdala and septum (Stadnicki et al. 1974) and present with similar deficits as human subjects (Aiger 1988; Branch et al. 1980; Evans and Wenger 1992; Gluck et al. 1973; Nakamura-Palacios et al. 2000; Schulze et al. 1988; Winsauer et al. 1999). Increased sophistication in pharmacological and physiological techniques applicable to rodents has now considerably increased our understanding of cannabinoid mechanisms in different types of memory, suggesting a modulatory role of cannabinoids and cannabinoid receptors in encoding, memory consolidation and even forgetting.

4.1
Spatial Learning

4.1.1
Water Maze

With respect to rodents such as rats and mice, training in the open-field water maze, in which animals search for a submerged and non-visible platform, is probably the most popular learning paradigm tackling spatial and thus hippocampus-

dependent memory (Morris 1984). Animals learn to find the submerged platform in opaque water in relation to distal cues; a progressive reduction of the latency to swim to and climb onto the platform is an index for learning. If the platform is kept in the same place, this is a reference memory task while changing the platform location to a new position every day reflects a working memory paradigm.

Despite the paradigm's popularity as a spatial learning task, reports on the effects of cannabinoids are relatively recent. The initial report by Ferrari and colleagues (1999) revealed that HU210 induced a learning deficit in rats trained in a reference memory task. Animals treated with doses of up to 100 mg/kg i.p. were unable to acquire the spatial location of the submerged platform on four consecutive days. By contrast, learning to swim to a visible platform was not different between drug groups and controls, thus excluding sensory perception as a factor to explain the deficit. This has recently been confirmed with Δ^9THC and Δ^8THC in rats and mice (Da Silva and Takahashi 2002; Mishima et al. 2001; Varvel et al. 2001; Diana et al. 2003), but not with nabilone (Diana et al. 2003). Once spatial memory is acquired, consolidation and recall is no longer sensitive to cannabinoid treatment (unless drug doses are extremely high and cause considerable motor side-effects). However, the learning deficit in the water maze may not be due to memory problems. Robinson and co-workers (2003) revealed that Δ^9THC induced place aversion in a novel place preference/aversion task conducted in the water maze. This aversion would in itself account for the observed spatial learning deficits in the drug groups.

When exposed to a working memory paradigm, in which the location of the platform was changed on a daily basis, Δ^9THC-treated mice were impaired in finding the platform despite extensive pre-training over weeks. Consequently, Varvel and co-workers (2001; Lichtman et al. 2002) claimed that spatial working memory in mice is more sensitive to cannabinoid treatment. Despite extensive pre-training of the mice to the working memory task, animals were unable to remember the new platform location when under Δ^9THC. However, mice in the reference memory paradigm were also extensively pre-trained, and a lack of deficit with low doses of Δ^9THC may simply be due to the fact that cannabinoid receptor-dependent mechanisms are not active during memory recall. If animals are naïve as to the exact platform location, acquisition learning is still impaired with Δ^9THC (Da Silva and Takahashi 2002).

Interestingly, Varvel et al. (2001) used a working memory paradigm, in which animals were released from the same location on each day; but this release site was altered between days. This protocol, which was also used more recently for the testing of CB_1-null mutants (Varvel and Lichtman 2002), therefore has a strong egocentric, and thus hippocampus-independent (Jarrard 1993), component; animals may have acquired the task without the use of distal cues and allocentric strategies. It remains uncertain whether cannabinoids selectively interfere with egocentric spatial tasks or whether the deficit is ubiquitous for all forms of spatial acquisition.

Unexpected was the finding that CB_1 knockout mice acquired a spatial reference memory task in the water maze normally. This was unexpected since pharmacological studies had predicted that CB_1 knockout would facilitate learning and memory

formation (for radial maze, see Lichtman 2000). Mice were, however, impaired in reversal learning, suggesting a deficit in task flexibility (Varvel and Lichtman 2002). $CB_1^{-/-}$ mice were not different from wild-type littermates in a working memory version of the task, and were also insensitive to Δ^9THC, WIN55,212-2 or methanandamide treatment. By contrast, all these cannabinoids disrupted working memory in wild-type littermates in a manner that was sensitive to rimonabant (SR141716A).

4.1.2 Radial Arm Maze

Radial arm mazes come in different shapes and forms. The most common one consists of eight arms radiating from an octagonal central platform. Animals kept on 80%–85% of their free-feeding body weights learn to retrieve a food reward from the distal end of each arm or, in some cases, a predetermined selection of arms. Use of the eight-arm radial maze has significantly contributed to our understanding of cannabis effects on cognition in rodents, but has some complications. For example, cannabis, Δ^9THC or synthetic analogues can depress locomotor activity (see DeSanty and Dar 2001a,b; Rodriguez de Fonseca et al. 1998; chapter by Fernández-Ruiz and González in this volume for review). This may impact on task performance such that longer latencies to reach and consume the reward may decrease attention processes due to longer test sessions. Such an effect is in line with cannabis-induced alterations in human cognition and thus may not be ideally suited to measure learning/memory. Furthermore, cannabinoids are widely known for their stimulatory effects on appetite (for review, see chapter by Maccarrone and Wenger, this volume). This may lead to a difference in motivation of already hungry animals to perform in this food-rewarded task (Di Marzo et al. 2001; Mechoulam and Friede 2001).

Despite such limitations, numerous reports suggest that cannabinoids impair performance in the eight-arm radial maze, especially when all arms are baited and revisits to the same arms are recorded as working memory errors. Animals were trained to criterion performance with all eight arms baited. Systemic administration of cannabinoids increased the number of working memory errors with low doses not affecting the amount of time required to complete the visits. This was originally reported in chronic experiments with Δ^9THC administered for 3 or 6 months (Stiglick and Kalant 1982), and has been confirmed more recently for acute infusions of Δ^9THC (Hernandez-Tristan et al. 2000; Lichtman and Martin 1996; Lichtman et al. 1995; Mishima et al. 2001; Molina-Holgado et al. 1995; Nakamura et al.1991), synthetic CB_1 receptor agonists WIN55,212-2 and CP55,940, or local infusion of CP55,940 directly into the hippocampus (Lichtman et al. 1995). To increase task difficulty, some researchers have introduced a short delay between visits to arms 1–4 and 5–8 in these experiments. Cannabinoids also disrupt performance in this short-term memory task when delays were 5 s (Molina-Holgado et al. 1995), 30 s (Hernandez-Tristan et al. 2000), or 1 h long (Nakamura et al. 1991). It is, however, unclear whether animals prefer to revisit arms 1–4 or 5–8. In accordance with drug effects on locomotor activity, post-delay performance was prolonged in

the Δ^9THC group (Hernandez-Tristan et al. 2000; Nakamura et al. 1991). Mishima and co-workers (2001) employed a mixed reference and working memory protocol. Four predetermined arms were rewarded while four others were not. Entry into an arm that was never baited was counted as a reference memory error, re-entry into a previously baited arm as a working memory error. Δ^9THC (6 mg/kg) significantly impaired working memory but not reference memory. However, inspection of their data suggests that reference memory may have also been affected by drug treatment.

Confirmation that drug effects were mediated via CB_1 receptor activation was obtained through co-administration of the selective receptor antagonist rimonabant, which reversed deficits induced by Δ^9THC (Lichtman and Martin 1997; Mishima et al. 2001). Rimonabant alone had no effect when delays between entries 1–4 and 5–8 were short (Lichtman 2000; Lichtman and Martin 1997; Mishima et al. 2001), but there was a memory enhancement for delays of several hours (Lichtman 2000). This result suggests that blockade of CB_1 receptors may aid the development of short-term memory, and receptor antagonists may become important as cognitive enhancers not only for future animal research but also with respect to treatment of cognitive impairment in humans.

4.1.3 T- and Y-Maze Procedures

Rodents show spontaneous alternation behaviour when tested in simple T- or Y-shaped mazes. They can also be forced to alternate, i.e. when food reward is placed at the end of the goal arm of the T/Y. As pointed out for the eight-arm radial maze, this paradigm uses food or drinking of juice as reward and is contraindicated when using cannabinoids. Systemic administration of Δ^9THC prior to daily testing decreased the alternation score (Nava et al. 2000). In control animals, in vivo brain dialysis confirmed an alternation-induced release of acetylcholine in hippocampus, which was smaller in the Δ^9THC group. In addition, the alternation impairment and the acetylcholine release depression persisted in animals treated with Δ^9THC twice daily with 5 mg/kg Δ^9THC i.p. for up to 1 week (Nava et al. 2001). Both effects were fully reversed by rimonabant, suggesting that no tolerance developed after chronic 5-day Δ^9THC exposure.

Delayed alternation is another possible training protocol for the T/Y-maze, in which animals are rewarded for choosing any goal box in trial one. They are then returned to the start box and released after an inter-trial interval. In trial two, they have to enter the arm not visited in trial one (non-match) and are rewarded. Typically, animals acquire a criterion of 80% correct responses after a short training period. When tested in the presence of Δ^9THC, there was a significant drop in performance coupled with a reduction in monoamine turnover in their prefrontal cortex (Jentsch et al. 1997). Animals treated with a similar dose (5 mg/kg) Δ^9THC, however, were not impaired in brightness discrimination (Jentsch et al. 1996) or visual discrimination of forms procedures (Mishima et al. 2001) administered in the same apparatus.

4.1.4 Delayed Match-to-Position Tasks

Different from the short-term memory tested in the radial arm maze are the delayed match-to-position (DMTP) or delayed match-to-sample (DMTS) tasks that employ standard conditioning chambers. In the most frequently used version, rats learn to press a lever during the sample phase and press the same (match) or opposite (non-match) lever during the choice phase. Task difficulty is modulated by the introduction of a delay between sample and choice phase (0–45 s); performance falls to chance at delays exceeding 45 s (Deadwyler et al. 1996). The laboratories of Hampson and Deadwyler have extensively studied hippocampal involvement in this task (Hampson et al. 1999a) and determined learning-related single unit activity of ensembles of CA3 and CA1 neurones during the different phases of the task (Deadwyler and Hampson 1999; Deadwyler et al. 1996; Hampson and Deadwyler 1996; Hampson et al. 1993, 1999b). In summary, distinct hippocampal pyramidal cells fire during the sample, delay and match phases, respectively. In a series of elegant studies, Hampson, Deadwyler and their colleagues have provided compelling evidence for a modulatory role of cannabinoids in delayed match-to-sample performance. Acutely injected Δ^9THC (0.75–2 mg/kg i.p.) prior to testing resulted in dose- and delay-dependent performance deficits. Animals were able to perform the task at 0–5 s delays, but the severity of impairment increased with the inter-trial interval, suggesting that short-term memory had been compromised (Heyser et al. 1993). At the same time, hippocampal firing during the sample phase was greatly diminished, leading to ensemble miscodes that increase the probability for the occurrence of errors especially at long, but not very short delays. Behavioural tolerance to Δ^9THC developed after 35 days of daily Δ^9THC exposure and was followed by a short withdrawal period of 2 days (Deadwyler et al. 1995). Behavioural sensitisation, however, developed within 4 days of repeated treatment (Miyamoto et al. 1995). These initial results both in terms of behavioural performance and physiological responses have been confirmed for delayed nonmatch-to-position protocols (Hampson and Deadwyler 1998; Mallet and Beninger 1996) and extended to other cannabinoids such as WIN55,212-2 (Hampson and Deadwyler 1999, 2000; Han et al. 2000). A similar performance deficit was reported for exogenous administration of the endocannabinoid anandamide (Mallet and Beninger 1996), and deficits were reversed by the CB_1 receptor antagonist rimonabant (Hampson and Deadwyler 1999, 2000; Mallet and Beninger 1998). Rimonabant alone had no effect (Hampson and Deadwyler 2000; Mallet and Beninger 1998).

In their studies, Hampson and Deadwyler have made a strong case for a role of CB_1 receptors in encoding, since pyramidal cell firing was diminished during encoding in animals exposed to cannabinoids. Firing during delay and matching phases remained unchanged, suggesting that errors occur only when there is an encoding deficit. A non-memory-related explanation for this behavioural deficit could be derived from results obtained by Han and Robinson (2001), who showed that cannabinoids such as WIN55,212-2 or Δ^9THC can shorten time estimation

in the rat. This, however, would suggest an increase in response rate during the delay, which is difficult to observe given that the levers are not present during the delay. Also, Hampson and Deadwyler have not reported alterations in the amount or length of delay-related firing in cannabis-treated rats.

4.2 Conditioning of Fear

Auditory fear conditioning is a standard procedure used in animal research (for review, see Crawley 2000). In a typical experiment, the animal is placed in a small chamber and a tone is presented after a short habituation period. The tone co-terminates with a mild footshock delivered through the grid floor, which the animals cannot escape. Consequently, this procedure is also called 'learned helplessness'. It results in a typical freezing reaction consisting of a crouching posture and immobility. One trial often is sufficient for induction of lasting memory, which can be tested hours or days later by measuring the freezing response upon re-exposure to the chamber (contextual fear conditioning) and presentation of the tone (cued fear conditioning). Mice treated with the CB_1 receptor antagonist rimonabant or CB_1 knockout mice readily acquire this fear-conditioning paradigm (Marsicano et al. 2002). While 6 days of extinction training, in which no shock is delivered, reduced the amount of freezing in wild-type littermates, knockout mice or wild-types treated with rimonabant throughout extinction maintain their freezing levels. These data suggest that the endocannabinoid system is highly active during forgetting and the extinction of aversive memories (Marsicano et al. 2002). Re-exposure to the tone during extinction also induced release of the endocannabinoids anandamide and 2-arachidonoyl glycerol in the basolateral amygdala, and this not only confirms the importance of the amygdala in fear conditioning, but also that on-demand release of endocannabinoids controls extinction of the fear response (Marsicano et al. 2002).

The acoustic startle response is based on a naturally occurring startle reaction to loud noise. If this loud tone is presented 30–500 ms after a 20-ms pre-pulse (pure tone), the startle reaction is considerably reduced. This is termed pre-pulse inhibition, reflects a measure of sensory-motor gating and involves a multitude of brain stem areas and transmitter systems (Koch 1999). Rats injected with the synthetic cannabinoids WIN55,212-2 (1.2 mg/kg) or CP55,940 (0.1 mg/kg) show little if any pre-pulse inhibition relative to controls (Mansback et al. 1996; Schneider and Koch 2002). The CP55,940-induced deficit in sensory-motor gating was fully reversed by 10 mg/kg rimonabant, but the antagonist had no effect when given alone (Mansbach et al. 1996). Although these data strongly suggest that cannabinoids can modulate sensory-motor gating, they have not resolved the issue of whether acquisition and execution of a normal startle response, either to a single loud noise or in a pre-pulse paradigm, is under the control of the endocannabinoid system.

4.3 Avoidance Tasks

Similar to the fear conditioning paradigms described above, shock is used as a reinforcer when animals are trained to avoid certain compartments. Two different training procedures are widely used. Passive or inhibitory avoidance refers to the active inhibition of a response in order to avoid a footshock; by contrast, active avoidance refers to behavioural escape from a dangerous area in order to avoid punishment.

Passive or inhibitory avoidance can be performed in two protocols. In step-through passive avoidance tasks, animals are released into a brightly lit chamber and a door is opened to allow entry into a dark compartment. Once entering the dark compartment, the door is closed and a foot shock is delivered. Upon re-exposure, animals will prefer to stay in the bright chamber. Step-down inhibitory avoidance, on the other hand, uses an elevated platform, from which animals will step down onto a grid floor. This triggers shock delivery and escape back onto the platform. Memory is assessed in a test session as an increase in step-down latency relative to the latency observed during acquisition training. As with most shock-motivated tasks, only a few trials are given to induce long-lasting memory traces.

Mice and rats treated with the endocannabinoid anandamide (1.5–6 mg/kg) immediately post-training presented with a significant memory impairment (Castellano et al. 1997, 1999) when tested in a step-through variant. The block of memory formation is due to an effect of anandamide on memory consolidation, since injections 2 h post-training had no effect. Interestingly, this effect was specific to the CD1 and DBA strains, but memory facilitation was observed in C57Bl/6 mice (Castellano et al. 1999). It is likely that modulation of memory strength with cannabinoids affected the monoaminergic transmitter system, since both D_1 and D_2 receptor antagonists reversed deficits induced by anandamide. The memory deficit was also reversed by naltrexone, an opioid receptor antagonist, suggesting cross-talk between cannabinoid and opioid system.

Memory for the shock tested 15 min or 24 h later was also reduced in rats injected intracerebroventricularly with anandamide or arachidonic acid (3.6 nmol in 5 µl) immediately post-training (Rodriguez de Fonseca et al. 1998). While anandamide administration enhanced the amount of slow-wave and rapid eye movement sleep in the period between training and retention testing, arachidonic acid led to a reduction in slow wave sleep. It therefore remains uncertain whether drug-induced changes in sleep pattern have any bearing on the consolidation and expression of an inhibitory avoidance response. A more detailed time course with systemic pre-training, post-training and pre-test injection of Δ^9THC in rats revealed memory deficits independent of injection time (Mishima et al. 2001). Since no deficit in acquisition learning was reported, it is safe to assume that cannabinoids have modulated consolidation and retention processes of emotional memories. Any functional role for the endocannabinoid system, however, still remains elusive. Site-direct infusion of anandamide (100 µmol/0.5 µl) into hippocampal CA1 also induced anterograde amnesia in step-down inhibitory avoidance learning (Barros et al. 2004).

Similarly, an acquisition impairment was reported for rats tested in an active avoidance paradigm (Izquierdo and Nasello 1973). The weak CB_1 receptor ligand cannabidiol (3.5 mg/kg) reduced conditioned responding, but was not effective when administered immediately post-training. This result is in agreement with more recent active avoidance training in CB_1-null mutant mice, which showed increased conditioned responding consistent with memory enhancement (Martin et al. 2002). At odds with these results is the finding that rats chronically treated with Δ^9THC (20 mg/kg) for 3 months and subsequently left untreated for 30 or 118 days before training in a shuttle box outperformed controls (Stiglick et al. 1984). Animals that had been exposed to Δ^9THC attained asymptotic performance levels faster than controls. It remains to be shown whether this effect is mediated by the cannabinoid system. An interesting comparison can be made with hippocampally lesioned animals. Such rats also show enhanced active avoidance and outperform controls (Isaacson et al. 1961), suggesting that systemically administered Δ^9THC may induce a functional lesion of the hippocampus (Hampson and Deadwyler 1998).

4.4 Other Memory Paradigms

Olfactory memory traces can be assessed in a social recognition task in which an adult animal is brought together with a juvenile conspecific. Exploration of the juvenile's anus can be monitored as a dependent variable for periods of up to 5 min. Memory is assessed through re-exposure and a reduction in anogenital sniffing is taken as an index of recognition memory. Results obtained with cannabinoid agonists and antagonists are straightforward.

In the first investigation into the effects of rimonabant, Terranova and coworkers (1996) reported enhancement of social recognition memory at doses of 0.1–3 mg/kg administered subcutaneously within 5 min post-training. Memory was assessed 2 h post-presentation of the juvenile, and the enhancement was present in both aged rats and mice. Reversal of this enhancement was attained by simultaneous administration of scopolamine, suggesting a tight interaction between cholinergic and cannabinoid system. Terranova and colleagues' finding was the first to suggest a memory-related function of the endocannabinoid system, which is particularly important during consolidation and forgetting (Marsicano et al. 2002). In contrast to rimonabant, administration of the CB_1 receptor agonist WIN55,212-2 (0.6–1.2 mg/kg) impaired short-term (30 min) social recognition memory in a dose-dependent manner without affecting anogenital exploration per se (Schneider and Koch 2002).

Another test for short-term memory, but with a different psychological quality, is object recognition (Ennaceur and Delacour 1988). During acquisition, animals are exposed to a novel environment containing object A, and are tested during re-exposure to object A plus first-time exposure to novel object B, after minutes or hours. The time spent exploring each object in the test session is an index of short-term memory for A. Good memory is characterised by preferential exploration of B, bad memory by exploration of A. Brain structures involved in

object recognition include entorhinal and perirhinal cortex. Intraperitoneal injection of Δ^9THC (10 mg/kg: Ciccocioppo et al. 2002), WIN55,212-2 (0.6–1.2 mg/kg: Schneider and Koch 2002), CP55,940 (0.025–2.5 mg/kg: Kosiorek et al. 2003) or *R*-(+)-methanandamide (0.25–2.5 mg/kg: Kosiorek et al. 2003) impaired object recognition memory, and this deficit was reversed by 1 mg/kg rimonabant (Ciccocioppo et al. 2002). While this was interpreted as a CB_1 receptor-mediated action, caution should be exercised, since rimonabant alone was not tested, and this allows for the alternative interpretation that the endocannabinoid system might limit the length of the recognition memory. In agreement with this notion, CB_1-null mutant mice show normal exploration during acquisition, but enhanced memory when tested against object B (Maccarone et al. 2002; Reibaud et al. 1999).

A different, more complex learning protocol was used by Brodkin and Moerschbacher (1997). In a specifically modified conditioning box, rats were trained for 14 weeks to respond to a sequence of lights by pressing appropriate keys. Once asymptotic performance criteria were met, drugs like cannabidiol (100 mg/kg) and anandamide (18 mg/kg) were injected, but had no effect on performance. By contrast, Δ^9THC (3.2–18 mg/kg) and *R*-(+)-methanandamide (1–18 mg/kg) impaired performance in a dose-related manner. This impairment was reversed by rimonabant (1 mg/kg), but the antagonist had no effect on its own.

4.5 Summary

Collectively, the behavioural data suggest a modulatory role of cannabinoids in learning and formation of different forms of memory. In view of the numerous side-effects of both natural and synthetic cannabinoids, however, hard proof for this notion is difficult to obtain. Reinforcer modulation may include cannabinoid-induced increases in food consumption (see chapter by Maccarrone and Wenger, this volume); activity-related changes of Δ^9THC or anandamide include the reduction of ambulations in the open field (Järbe and Hiltunen 1987; Järbe et al. 2002; Navarro et al. 1993; see Fernández-Ruiz and González, this volume), induction of catalepsy (Teng and Craft 2004 for a recent example), and suppression of conditioned responding in a lever-press task (Arizzi et al. 2004); anxiogenic properties of cannabinoids would lead to higher levels of emotionality (Onaivi et al. 1990). Consequently, the observed deficit may not be a result of an impairment in acquisition or consolidation, but may be due to unrelated side-effects of drugs, such as reductions in reaction time or even signal detection (Presburger and Robinson 1999).

Despite all these problems, there is now strong evidence for a role of CB_1 receptors in memory formation. For delay-dependent short-term memory tasks, CB_1 receptors may be able to modulate the encoding processes. By contrast, CB_1 receptors may play a role in consolidation and even recall in memory formation of avoidance tasks. These effects are likely to be mediated by different CB_1 receptor populations located in different brain regions, and a better understanding of their function requires more localised administration of selective CB_1 agonists and antagonists.

5
Synaptic Plasticity

The anecdotal reports of effects of smoking cannabis on cognitive processes has naturally prompted investigation into the effects of cannabinoids on synaptic plasticity, and in particular long-term potentiation (LTP). To summarise 17 years of effort, it has been easy to show that cannabinoids have effects on LTP, and most commonly suppress it, but far more difficult to define the mechanisms by which this occurs. Emerging themes are that the commonly reported inhibition of LTP by synthetic cannabinoids may be mediated by non-CB_1 receptors, and that the function of the endogenous cannabinoid system may be to facilitate induction of LTP. The vast majority of studies have focussed on synaptic plasticity in the CA1 region of the rat hippocampal slice and we will start our review there.

5.1
LTP in the Hippocampus

The first reported investigation was that of Nowicky et al. (1987) who showed that pre-incubation of adult rat hippocampal slices with Δ^9THC could either inhibit or potentiate high-frequency stimulation-induced LTP, depending on the concentration used. High-frequency stimulation (200 Hz for 500 ms) induced a potentiation of the CA1 population spike amplitude that had a decay half-time of 280 min. Pre-incubation with 10 pM Δ^9THC increased this to 350 min, whereas pre-incubation with 100 or 1,000 pM Δ^9THC reduced it to 91 and 31 min, respectively. These changes in synaptic plasticity were associated with corresponding changes in the baseline population spikes, so that 10 pM Δ^9THC increased the population spike amplitude, and 100 or 1,000 pM Δ^9THC decreased it. Before delivering the high-frequency train though, stimulus strength was adjusted to counter any effect on baseline transmission. These experiments were performed before the dawn of the age of the cannabinoid receptor and few pharmacological tools were available. It is therefore possible that the effects could be a result of some non-specific mechanism of membrane perturbation.

5.1.1
Effects of Synthetic Cannabinoids on LTP Are Stereoselective

One of the cardinal features of a receptor-mediated effect is stereoselectivity. It was therefore significant that cannabinoids were shown to be stereoselective as inhibitors of LTP. HU-210 and HU-211 were the first to be tested, since their stereochemical purity is particularly high, and only HU-210 is psychoactive. The experiments by Collins et al. (1994) showed that pre-incubation of adult rat hippocampal slices with 100 nM HU-210, but not HU-211, blocked LTP of the CA1 field excitatory postsynaptic potential (fEPSP) induced by high-frequency stimulation (100 Hz for 500 ms). Slices were pre-incubated with the drugs so there was

no internal control to determine if HU-210 depressed the baseline fEPSP. Stereoselectivity of the effect has subsequently been confirmed (Paton et al. 1998) for another cannabinoid, where perfusion of 5 μM WIN55,212-2 but not WIN55,212-3 blocked LTP of the CA1 population spike induced by high-frequency stimulation (100 Hz for 500 ms). This inhibition occurred in the absence of any effect on the amplitude of the baseline population spike, but was associated with a decrease in paired pulse depression.

5.1.2 Effects of Cannabinoids Are Blocked by CB_1 Receptor Antagonism

A second cardinal feature of receptor-mediated actions is that they should be blocked by a suitable antagonist. The tools that have been used for the investigation of LTP have been rimonabant, AM251 and AM281. The properties of these, and specifically whether they are antagonists or inverse agonists at CB_1 receptors are discussed elsewhere (see the chapter by Pertwee, this volume). Rimonabant was the first to become available and has been shown to prevent the blockade of LTP in the CA1 region by HU-210 (Collins et al. 1995), and WIN55212-2 (Terranova et al. 1995; Paton et al. 1998; Misner and Sullivan 1999).

An alternative approach to the pharmacological manipulation of receptors is to use knockout animals. CB_1 knockout mice have been produced, and high-frequency stimulation (100 Hz for 250 ms) induced significantly larger LTP of the CA1 field EPSP in hippocampal slices prepared from $CB_1^{-/-}$ mice compared to wild-type controls (Bohme et al. 2000). This was not associated with any detectable change in the amplitude of the half-maximal field EPSP evoked in the two groups.

5.1.3 Prenatal Exposure to Cannabinoids Inhibits LTP

Chronic effects of administration of cannabinoids have been studied in slices prepared from 40-day-old rats born to mothers who received daily subcutaneous injections of WIN55,212-2 throughout gestation (Mereu et al. 2003). LTP in these slices was reduced as compared to control slices prepared from rats born to untreated mothers. The slices also showed impaired basal and K^+-stimulated glutamate release.

5.1.4 Putative Endogenous Cannabinoids Inhibit LTP

The range of putative endogenous cannabinoids have been outlined previously (see Di Marzo et al., this volume), and several studies have investigated the effects of application of these on LTP. In rat hippocampal slices, perfusion of 20 μM sn-2 arachidonylglycerol (2-AG) blocked LTP of CA1 field EPSPs induced by high-frequency stimulation (100 Hz for 1 s), with little or no effect on the baseline field

EPSP (Stella et al. 1997; Schweitzer et al. 1999), and the effect of 2-AG was blocked by 2 μM rimonabant (Stella et al. 1997). Similarly, perfusion of anandamide (3–10 μM) blocked LTP of CA1 population spikes, and this was prevented by rimonabant (10 μM) (Terranova et al. 1995).

5.1.5 Do Cannabinoids Suppress Baseline Excitatory Transmission in the CA1 Region?

Any drug that reduces excitatory drive would be expected to impair high-frequency stimulus-induced LTP by limiting the level of postsynaptic depolarisation achieved during the induction train. This would in turn reduce the relief of the voltage-dependent block of *N*-methyl-D-aspartate (NMDA) receptor-gated channels by Mg^{2+} and hence reduce the postsynaptic Ca^{2+} entry that is required to trigger the processes that lead to synaptic potentiation. The question of whether cannabinoids inhibit baseline excitatory transmission is therefore an important one. The suppression of inhibitory transmission by cannabinoids in the hippocampus has been well documented, but whether cannabinoids also suppress excitatory glutamatergic transmission (as they do in the cerebellum, see the chapter by Szabo and Schlicker, this volume) is less clear cut. Thus, there are reports stating explicitly that WIN55,212-2 either inhibits (Misner and Sullivan 1999; Al-Hayani and Davies 2000; Ameri and Simmet 2000; Hajos et al. 2001), or does not inhibit (Terranova et al. 1995; Paton et al. 1998; Al-Hayani and Davies 2000), excitatory synaptic transmission in the CA1 region. This apparent discrepancy has now been resolved by the demonstration that the most commonly used CB_1 receptor agonist, WIN55,212-2, at tenfold higher concentrations, also activates a TRPV1-like receptor, which is also sensitive to rimonabant (Hajos et al. 2001; Hajos and Freund 2002). Thus, in slices prepared from $CB_1^{+/+}$ mice, perfusion of WIN55,212-2 inhibited pharmacologically isolated excitatory postsynaptic currents (EPSCs) with an EC_{50} of 2.01 μM, and pharmacologically isolated inhibitory postsynaptic currents (IPSCs) with an EC_{50} of 0.24 μM (Hajos et al. 2001). In slices prepared from $CB_1^{-/-}$ mice, WIN55,212-2 no longer inhibited evoked IPSCs, but still inhibited evoked EPSCs. This inhibition of excitatory transmission was mimicked by the TRPV1 agonist capsaicin (10 μM), and was blocked by the TRPV1 antagonist, capsazepine (10 μM). The fact that the suppression of EPSCs (as well as IPSCs) by WIN55,212-2 is blocked by rimonabant is significant, since this is the criterion by which an effect would previously have been judged to be CB_1 receptor mediated. The tenfold concentration difference in the EC_{50} of WIN55,212-2 in blocking inhibitory, as opposed to excitatory, transmission also explains why the drug has been reported to selectively block paired-pulse depression of population spikes (an effect dependent on feedback inhibitory transmission) but not baseline synaptic transmission (Paton et al. 1998).

Whether this central TRPV1-like receptor has the same properties as the better characterised peripheral TRPV1 receptors (Szallasi and Di Marzo 2001), and whether it represents the same non-CB_1 non-CB_2 receptor characterised by Breivo-

gel et al. (2001; see chapter by Pertwee, this volume) remains to be determined. Equally, it is hard to resolve whether the effects of other cannabinoids are likely to be mediated by the TRPV1-like receptor. Available evidence suggests that the receptor is also activated by CP55,940, but that it is not blocked by AM251 (Hajos and Freund 2002). The answer is therefore that some cannabinoids do indeed inhibit excitatory transmission in the hippocampus, but via a TRPV1-like receptor. This in turn raises the question: Is inhibition of the induction of LTP by cannabinoids secondary to suppression of baseline excitatory transmission?

Several of the studies on LTP reported above have commented in passing on any associated effects of cannabinoids on baseline field EPSP or population spike responses, but answers have been contradictory. Thus, some have found no change (Terranova et al. 1995; Stella et al. 1997; Paton et al. 1988; Schweitzer et al. 1999), whereas others have found an inhibition of baseline excitatory responses (Nowicky et al. 1987; Misner and Sullivan 1999). The question was addressed directly by Misner and Sullivan (1999) who found that the EPSC was reduced by about 50% in slices prepared from neonatal rats. Perfusion of 5 μM WIN55,212-2 blocked the induction of LTP by high-frequency stimulation (100 Hz for 200 ms), but this could be overcome by manipulations designed to overcome the Mg^{2+}-block of NMDA receptor-gated channels, i.e. reducing the concentration of Mg^{2+} in the perfusion medium, or by slightly depolarising the recorded cell during the high-frequency train. They therefore concluded that the effect of WIN55,212-2 was to reduce the excitatory drive and therefore the extent of activation of NMDA receptors.

Note that lack of an effect of cannabinoids on the low-frequency-evoked synaptic responses in some of the experiments described above does not exclude the possibility that the drugs may have an effect on the high-frequency response required to induce LTP. It would therefore be important to establish the effects of cannabinoid receptor ligands on the response to repetitive high-frequency stimulation, as well as on the response to low-frequency stimulation. Though some of the experiments described above suggest that cannabinoids might inhibit induction of LTP by suppressing excitatory drive, none of them distinguishes the possibility that cannabinoids block LTP via an action on the TRPV1-like receptor rather than the CB_1 receptor. WIN55,212-2 is an agonist at both, and rimonabant inhibits both. Resolution of this question must await the development of ligands that will reliably differentiate the two receptors, and/or investigation of the effects of cannabinoids on the induction of LTP in $CB_1^{-/-}$ animals.

5.1.6
Long-Lasting Effects of Perfusion of Cannabinoid Receptor Ligands

Diana et al. (2002) argue that the apparent blockade of LTP by perfusion of WIN55,212-2 is in fact due to the very slow and gradual inhibition of the baseline synaptic response. Thus, when the potentiation of a high-frequency-stimulated pathway is compared to the gradual decay of a control un-tetanised pathway, it appears that administration of WIN55,212-2 does not inhibit LTP. From inspection of

individual figures it is hard to see that this could explain the results in all other reports; however, the interpretation of this experiment illustrates an important point. Cannabinoid ligands are very lipophilic, which makes them notoriously difficult to work with. They require use of some vehicle (e.g. DMSO, TWEEN 80, ethanol) to disperse them, they stick to glassware and tubing, they produce relatively slow effects, and they are very difficult to wash off. The last of these points makes it very difficult to distinguish between long-term effects [e.g. LTP, long-term depression (LTD)] caused by transient application of the drug, and a short-term effect that persists because the drug is still present in the tissue. Furthermore, the free concentration of drug available to the receptors is liable to vary widely between different preparations (e.g. cultured neurones vs slices) and different recording conditions. For instance, the observation that WIN55,212-2 inhibits excitatory transmission in slices prepared from neonatal, but not adult, rats (Al-Hayani and Davies 2000), may be due to improved drug access in the neonatal tissue. These considerations make any meaningful comparison of effective concentrations between reports very difficult.

5.1.7 Cannabinoid Involvement in Depolarisation-Induced Suppression of Inhibition

Depolarisation-induced suppression of inhibition (DSI) is a form of short-term plasticity which merits mention here. In the hippocampus, depolarisation of pyramidal neurones induces a short-term suppression of γ-aminobutyric acid (GABA)ergic IPSCs (Pitler and Alger 1992). This DSI is blocked by perfusion of CB_1 receptor antagonists, and it is absent in $CB_1^{-/-}$ mice (Ohno-Shosaku et al. 2001; Wilson and Nicoll 2001). These results cemented the role of cannabinoids as retrograde messengers in the nervous system (see chapter by Vaughan and Christie, this volume). Specifically, they are consistent with the notion that increased Ca^{2+} entry during the depolarising pulse triggers synthesis and release of an endocannabinoid from the pyramidal cell, which then activates CB_1 receptors on local inhibitory terminals and suppresses GABAergic inhibition of that cell. An additional trigger to the synthesis and release of endocannabinoids may be activation of group I metabotropic glutamate (mGlu) receptors (Varma et al. 2001; Ohno-Shosaku et al. 2002) and muscarinic acetylcholine receptors (Kim et al. 2002). Note that depolarisation-induced suppression of excitatory synaptic transmission (DSE) has been reliably demonstrated in cerebellar tissue (Kreitzer and Regehr 2001: Maejima et al. 2001), but not in the hippocampus (but see Ohno-Shosaku et al. 2002). This correlates with immunocytochemical studies that reveal CB_1 receptors are located on excitatory glutamate-containing terminals in the cerebellum, but not in the hippocampus.

DSI expressed in the hippocampus is transient, and suppression of the resulting inhibition could not account for long-term plasticity of synapses. However, it may be important in facilitating depolarisation, and therefore induction of LTP, during a high-frequency stimulus.

5.1.8 Release of Endogenous Cannabinoids During DSI Facilitates the Induction of LTP

Carlson et al. (2002) demonstrated that delivery of a subthreshold high-frequency train (i.e. one that would not normally induce LTP) during the phase of DSI could induce LTP. The subthreshold train (50 Hz for 400 ms) usually failed to induce LTP of the fEPSP recorded from a population of cells in the CA1 region, or of the EPSC recorded from a single CA1 pyramidal cell. However, a depolarising pulse (to 0 mV for 1 s) delivered to the single cell 3 or 13 s before the subthreshold train, resulted in LTP of the EPSC, but not of the fEPSP. Furthermore, perfusion of AM251 (2 μM) prevented this facilitation. It is therefore apparent that the release of endocannabinoids (in this instance by the depolarising pulse) causes a local facilitation of the induction of LTP.

It is intriguing to speculate on the consequences of extending this paradigm. High-frequency stimulation (100 Hz for 1 s) also induces release of endocannabinoids, specifically 2-AG (Stella et al. 1997). If synthesis and release of cannabinoids is sufficiently rapid, then the local release of endocannabinoids caused by the inducing train would tend to facilitate the induction of LTP at that particular site and therefore increase the signal-to-noise ratio of any potentiating synapses in that area. The role of endogenous cannabinoids in the CA1 region of the hippocampus may therefore be to cause a local facilitation of LTP. Assuming that LTP is an important neural basis of at least some forms of learning and memory, the physiological role of the endocannabinoid system would be to enhance hippocampal-dependent learning and memory. In contrast, smoking cannabis may impair learning and memory due to the inappropriate global facilitation of LTP at synapses throughout the brain, rather than at discrete local sites, leading to an elevation of the background noise and a reduction in the signal-to-noise ratio of potentiated synapses.

The findings of Carlson et al. (2002) also prompt the question of why administration of CB_1 receptor agonists is almost universally observed to cause suppression, rather than facilitation of high-frequency stimulus induced LTP. This may relate first to the experimental conditions, which have generally used supra-threshold induction protocols, which are liable to saturate LTP and would not allow any potentiation to be observed. Second, the synthetic agonist most commonly used (i.e. WIN55,212-2) may suppress excitatory transmission, and therefore LTP, via its action on the TRPV1-like rather than the CB_1 receptor. While this could also explain why anandamide (another agonist at the TRPV1-like receptor) blocks LTP (Terranova et al. 1995), note that it cannot explain why 2-AG (which is not an antagonist at the TRPV1-like receptor) also does (Stella et al. 1997).

5.2 LTD in the Hippocampus

Misner and Sullivan (1999) described that 5 μM WIN55,212-2 not only blocked the induction of LTP of CA1 field EPSPs (and EPSCs) by high-frequency stimulation

(100 Hz for 200 ms), but also that it blocked LTD induced by lower frequency stimulation (1 Hz for 15 min). This block of LTD was again relieved by reducing the concentration of Mg^{2+} in the perfusion medium, or by slightly depolarising the recorded cell during the low-frequency train. It was therefore interpreted as being secondary to a reduced excitatory drive resulting in any postsynaptic Ca^{2+} changes being insufficient to reach threshold to induce LTD.

There is also intriguing evidence that release of endogenous cannabinoids may contribute to the maintenance of LTD of IPSCs, and hence contribute to the E–S coupling that is observed after the induction of LTP. High-frequency stimulation (100 Hz for 1 s) or theta burst stimulation (10x100 Hz for 50 ms) caused LTD of pharmacologically isolated IPSCs recorded from the CA1 region of rat hippocampal slices (Chevaleyre and Castillo 2003). AM251 blocked the induction of this LTD, but had no effect if it was applied 10 min after the high-frequency train. LTD was blocked by group I mGlu receptor antagonists, and mimicked by group I mGlu receptor agonists. This suggests that activation of group I mGlu receptors during the high- frequency train stimulates transient release of an endocannabinoid that causes a persistent reduction of GABA release. In this scenario, endocannabinoids have a role, not only in regulating the induction of synaptic plasticity, but also in the expression of synaptic plasticity, during the maintenance phase. Specifically, they may contribute to the increased E–S potentiation, i.e. the ability of a given-sized EPSP to evoke an action potential. The mechanism by which transient release of an endocannabinoid can evoke long-lasting depression of the IPSC has not been determined.

5.3 LTP and LTD in Other Brain Regions

The differences in expression of CB_1 receptors in various brain regions suggest that cannabinoid receptor ligands may have different effects on synaptic plasticity in different synaptic pathways.

5.3.1 Cortex

Results from prefrontal cortex are generally consistent with those from the hippocampus, but carry the same caveat that they may reflect activity of cannabinoids at TRPV1-like receptors, rather than CB_1 receptors. High-frequency stimulation (100 Hz for 1 s) induced LTD, LTP, or no change in pharmacologically isolated EPSCs in approximately equal proportions of cells recorded. WIN55,212-2 (1 μM) increased the proportion of cells exhibiting LTD and decreased the proportion exhibiting LTP, whereas rimonabant increased the proportion of cells exhibiting LTP and decreased the proportion exhibiting LTD (Auclair et al. 2000; Barbara et al. 2003). These effects on synaptic plasticity could be explained as a secondary consequence of changes in the baseline EPSC, since WIN55,212-2 decreased, and

rimonabant increased, the baseline response. In this region, the effects of cannabinoids on pairing-induced LTP have also been investigated. LTP was induced between pairs of whole-cell recorded layer 5 cells by coincident pairing of strong pre- and postsynaptic depolarisation, and in the presence of AM251 this potentiation was significantly increased (213% vs 162% of control) (Sjöström et al. 2003).

5.3.2 Amygdala

In the amygdala, immunocytochemical labelling shows that CB_1 receptors are, just as in the hippocampus, selectively localised to the terminals of a subset of inhibitory neurones (Katona et al. 2001). In slices of mouse basolateral amygdala, LTP of the population spike induced by high-frequency trains (100 Hz for 1 s) was significantly greater in slices prepared from $CB_1^{-/-}$ mice than that of wild-type controls (147% vs 117% of control population spike amplitude, respectively). Rimonabant did not affect the baseline synaptic response in slices prepared from wild-type mice, suggesting that tonic activity of the endocannabinoid system normally inhibits high-frequency stimulus induction of LTP, but not via an inhibition of the baseline response (Marsciano et al. 2002). Low-frequency stimulation (1 Hz for 900 s) induced LTD of the population spike, but this was comparable in slices prepared from $CB_1^{-/-}$ and wild-type controls (depressed to 75% vs 80% of control population spike amplitude, respectively). The same study also investigated the effect of low-frequency stimulation on pharmacologically isolated IPSCs. Low-frequency stimulation (1 Hz for 100 s) induced LTD of the IPSC, which was blocked in wild-type mice by rimonabant (5 μM), and was absent in $CB_1^{-/-}$ mice. This study therefore indicates that CB_1 receptors are involved in synaptic plasticity in the amygdala, but does not determine whether the effects of synthetic cannabinoids on synaptic plasticity are mediated by CB_1 receptors.

5.3.3 Nucleus Accumbens

In the nucleus accumbens immunocytochemical labelling shows that, in contrast to the hippocampus, CB_1 receptors are located on glutamatergic nerve terminals (Robbe et al. 2001). In a mouse brain slice preparation, low-frequency stimulation (13 Hz for 10 min) evoked LTD of field EPSPs or EPSCs recorded in the nucleus accumbens (Robbe et al. 2002, 2003). This LTD apparently depended on the activity of endocannabinoids, since it was occluded by 0.3 μM WIN55,212-2, blocked by 0.1–1 μM rimonabant or 2 μM AM251, and critically, it was absent in $CB_1^{-/-}$ mice. While the use of $CB_1^{-/-}$ mice shows that CB_1 receptors are essential for this form of synaptic plasticity in the nucleus accumbens, the cannabinoid ligands were not tested in $CB_1^{-/-}$ animals, and so although it is likely, it is not certain that they produced their effects via the CB_1 receptor. In a similar study, low-frequency stimulation (10 Hz for 5 min) induced LTD of the population spike recorded in

slices of rat nucleus accumbens to about 70% of control. This was blocked by perfusion of rimonabant (1 μM), and by desensitisation of CB_1 receptors induced by chronic pre-treatment of the rats with WIN55,212-2 (Hoffman et al. 2003).

5.3.4 Striatum

In the striatum, CB_1 receptors are located on excitatory terminals. High-frequency stimulation (100 Hz for 1 s) induced LTD of EPSCs (to 60% of control) in striatal slices prepared from wild-type mice, but not in slices prepared from $CB_1^{-/-}$ mice (Gerdeman et al. 2002). HU-210 (1 μM) inhibited evoked EPSCs in slices prepared from wild-type mice, but had no effect in slices prepared from $CB_1^{-/-}$ mice. Preincubation of slices prepared from wild-type animals in rimonabant (3 μM) for 90 min blocked the induction of LTD by high-frequency stimulation. In the striatum, therefore, there is compelling evidence that HU-210 blocks LTP via an action on CB_1 receptors.

5.3.5 Cerebellum

In slices of rat cerebellum, LTD of parallel fibre inputs to Purkinje cells can be induced by pairing low-frequency stimulation (1 Hz for 5 min) with post-synaptic depolarisation. Both WIN55, 212-2 (1 μM) and CP55,940 (400 nM) reduced the EPSC by about 50%, and impaired the induction of LTD, an effect which was blocked by rimonabant (1 μM) (Levenes et al. 1998). In this pathway, therefore, it appears that cannabinoid effects on synaptic plasticity may be secondary to changes in baseline responses.

5.4 Future Questions

The finding that the most common pharmacological tools used to probe CB_1 receptors, WIN55,212-2 and rimonabant, also have actions on a TRPV1-like receptor requires that much of the existing literature be re-evaluated. It is often not clear whether effects are mediated by CB_1 receptors or the TRPV1-like receptor. The dearth of information on the pharmacological properties of the TRPV1-like receptor means that even results obtained using related drugs, e.g. CP55,940, AM251 and AM281, must also be treated with caution. Notwithstanding this, the current information suggests that the suppression of high-frequency stimulation-induced LTP in the CA1 hippocampal region by WIN55,212-2 is likely to be secondary to suppression of excitatory drive and is mediated by the TRPV1-like receptor. A big step towards resolving the receptors involved would be to establish whether WIN55,212-2 can inhibit high-frequency stimulation-induced LTP in the CA1 region of hippocampal slices prepared from $CB_1^{-/-}$ mice. Note that the situation

may be very different in other brain areas, such as nucleus accumbens where CB_1 receptors are also present on excitatory terminals, and so modulation of synaptic plasticity in these regions is more likely to be CB_1 receptor mediated. This makes it all the more important that behavioural studies use selective administration of drugs to specific brain areas, rather than global administration to whole animals.

In contrast to our original preconceptions, it is emerging that the role of the endogenous cannabinoid system in the hippocampus may be to facilitate the induction of LTP. Administration of exogenous-selective CB_1 agonists may therefore disrupt hippocampus-dependent learning and memory by 'increasing the noise', rather than 'decreasing the signal' at potentiated inputs.

An understanding of the role of the endogenous cannabinoid system in the regulation of synaptic plasticity will depend on identification of the physiological stimuli that trigger the synthesis and release of the endocannabinoid(s). There may be a background level of endocannabinoid activity (controlled by, for example, metabotropic, cholinergic and glutamatergic inputs) that sets the threshold for induction of LTP throughout the hippocampus. Alternatively, the very firing patterns that induce LTP may trigger synthesis and release of endocannabinoids and the time scale over which this occurs will determine any impact on the induction of LTP.

The situation is made more complicated by the diverse pharmacology of some of the putative endocannabinoids. 2-AG appears to exert its effects via the CB_1 receptor, but anandamide may activate both CB_1 and TRPV1-like receptors. The question of which endocannabinoid is released is therefore functionally important.

6 Where to Go from Here?

An obvious feature of this chapter is that, despite the increasing knowledge of the physiology and pharmacology of cannabinoid function in the brain, little transfer as to behavioural consequences has been achieved. While it is clear from the in vitro approach that there are numerous stimulation patterns able to trigger release of endocannabinoids, such conditions have yet to be identified in the behavioural setting. This must involve not only the definition of the psychological circumstances that lead to endogenous cannabinoid release, but also the characterisation of the cellular mechanisms that aid this release. It has also become clear from the in vitro approach that cannabinoid receptors are present only in particular neuronal cell types and that the types of neurone that express these receptors vary between brain areas in a manner that is expected to affect the outcome of cannabinoid receptor activation in vivo. In order to understand this variation in terms of behavioural outcome, it will be essential to apply cannabinoid agonists and antagonists in behaving animals in a more restricted and site-directed manner. In addition, local administration and behavioural testing should be combined with physiological assessment of neural changes induced in response to drug-treatment. This is achievable either by using ex vivo slice preparations of the area of interest (for example cerebellum, hippocampus, visual cortex, etc.) or by chronic implan-

tation of electrodes into the area of interest and in vivo recording of either EEG or single units during behavioural testing. Although this latter approach requires a high level of technical sophistication, especially when conducted using mice, it may yet lead to an unprecedented gain of information concerning the function of the endocannabinoid system in the brain.

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