

## **Neuropharmacology of Cannabinoids**

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anandamide, 2-arachidonoylglycerol, cannabinoids, CB<sub>1</sub> receptor, cognition, emotional behavior, endocannabinoid, fatty acid amide hydrolase, locomotor activity, monoacylglycerol lipase, nociception, reward processing

## Glossary

<b>Acoustic startle reflex (ASR)</b>	The ASR is a protective reflex that consists of a fast twitch of facial and body muscles, elicited by sudden and intense acoustic stimuli.
<b>Attentional set shift task (ASST)</b>	The ASST was established in rodents as an equivalent of the human WCST and involves a series of compound perceptual discriminations (e.g. odor and digging medium) that require subjects either to maintain attention and discriminate between two stimuli within one modality, or shift attention between two stimuli from two different modalities.
<b>Depolarisation-induced suppression of inhibition (DSI) or excitation (DSE)</b>	DSI and DSE are two related forms of short-term synaptic plasticity of GABAergic and glutamatergic transmission, respectively. They are induced by postsynaptic depolarisation and calcium-dependent synthesis of a retrograde acting endocannabinoids, which reversibly inhibit neurotransmitter release via CB <sub>1</sub> cannabinoid receptor mediated presynaptic mechanisms. DSI and DSE are thought to reflect two main mechanisms of endocannabinoid signaling.
<b>Elevated plus maze (EPM)</b>	The EPM is a classical paradigm for measuring anxiety-related behaviors in rodents. It consists of a plus-shaped, elevated apparatus with two opposed open, highly illuminated arms and two opposed closed arms. Exploration of the aversive open arms serves as an index for emotional reactivity.
<b>Inverse agonist</b>	An inverse agonist (e.g. the CB <sub>1</sub> receptor antagonist/inverse agonist Rimonabant) is an agent that binds to the same receptor binding-site as an agonist, but exerts the opposite pharmacological effects.
<b>Light/dark emergence test (EMT)</b>	Paradigm for measuring anxiety-related behaviors in rodents. The apparatus consists of a dark, enclosed and a highly illuminated open compartment. Exploration of the aversive open compartment serves as an index for emotional reactivity.
<b>Prepulse inhibition (PPI) of the ASR</b>	PPI is the natural reduction of the ASR if an acoustic, non-startling prestimulus is presented shortly (30 - 500 msec) before the startling stimulus. PPI is used as an operational measure for sensorimotor gating mechanisms.
<b>Progressive ratio (PR)</b>	PR tasks serve as a measure in rodents for the motivational value of a reinforcer. Testing usually occurs in a Skinner box where animals perform a specific operant action (e.g. lever pressing) in order to receive reinforcement. During testing, the operant requirements for reinforcement are steadily increased and animals are monitored for performance consistency.
<b>Tetrad of cannabinoid effects</b>	A series of physiological and behavioral tests used to measure the pharmacological effects of cannabinoids; including hypokinesia, hypothermia, catalepsy and antinociception.
<b>Wisconsin card sorting test (WCST)</b>	The WCST is a neuropsychological test that assesses the ability to display flexibility in the face of changing schedules of reinforcement. The participants respond to a series of stimuli where the rules about how to perform the task change from time to time.

## **Abstract**

The identification of  $\Delta^9$ -tetrahydrocannabinol as the major psychoactive constituent of *Cannabis sativa* was a milestone in cannabinoid research that led to the discovery of the cannabinoid receptors CB<sub>1</sub> and CB<sub>2</sub> and the endocannabinoid signaling system. This evolutionarily ancient and widely distributed modulatory system participates in a multitude of neurophysiological processes such as reward-related behaviors, pain perception, emotional homeostasis, memory storage or motor control. The development of potent synthetic cannabinoid receptor agonists as well as antagonists/inverse agonists have contributed to a better understanding of cannabinoid pharmacology and the neurobiological mechanisms involved in behavioral effects of cannabinoids. This chapter introduces the endocannabinoid system and its role as a mediator of mechanisms of synaptic plasticity in the nervous system. Cannabinoid behavioral neuropharmacology is then reviewed and discussed, highlighting the challenges associated with mechanistic interpretation of the effects of cannabinoids on behavior.

## 1. Cannabinoids and cannabinoid receptors

In 1964  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) was identified as the main psychoactive constituent of the drug cannabis, which enabled investigation of the behavioral actions of “cannabinoids” in animal models. For example, in dogs  $\Delta^9$ -THC causes static ataxia and in mice  $\Delta^9$ -THC causes hypokinesia, hypothermia, catalepsy and antinociception. This “tetrad” of effects of  $\Delta^9$ -THC on mice has been formalised as a behavioral assay for cannabinoid-type compounds. Accordingly, the effects of  $\Delta^9$ -THC in the tetrad assay are mimicked by a variety of synthetic  $\Delta^9$ -THC analogues (e.g. CP 55940, HU-210) and by other compounds with cannabinoid-type pharmacology (e.g. WIN 55,212-2). Furthermore, investigation of the structure-activity relationships of  $\Delta^9$ -THC and other cannabinoids in the tetrad assay revealed stereoselectivity indicative of a mechanism of action involving interaction with specific receptor binding sites – hence the concept of “cannabinoid receptors” emerged.

Definitive evidence for the existence of cannabinoid receptors in the brain was first obtained from membrane binding assays employing a radiolabelled cannabinoid - [ $^3$ H]CP 55940. Furthermore, the pharmacological properties of cannabinoid binding sites in brain membranes *in vitro* correlate with the structure-activity relationships of cannabinoids *in vivo*, indicating that the behavioral actions of cannabinoids are mediated by a distinct receptor. The molecular identity of this receptor was determined in 1990 with the cloning and sequencing of a G-protein coupled receptor, which when expressed in cells confers responsiveness to  $\Delta^9$ -THC and other cannabinoids. This brain cannabinoid receptor is now known as CB<sub>1</sub> to distinguish it from a structurally related cannabinoid receptor (CB<sub>2</sub>), which is predominantly associated with immune cells. Important evidence that CB<sub>1</sub> is largely responsible for mediating the behavioral effects of cannabinoids has come from the finding that the classic “tetrad” of cannabinoid actions observed in wild-type mice (see above) are not observed in mice where the CB<sub>1</sub> gene has been deleted (“CB<sub>1</sub>-knockout mice”).

Consistent with the diverse behavioral effects of cannabinoids in mice, the CB<sub>1</sub> receptor is both widely and abundantly expressed in the mammalian central nervous system (CNS). For example, high levels of CB<sub>1</sub> expression are particularly noteworthy in the dorsal striatum and in neurons that project from the striatum to the substantia nigra, which probably explains why cannabinoids affect locomotor activity in mice and other mammals. Likewise, CB<sub>1</sub> receptor expression in the dorsal horn of the spinal cord and in peripherally projecting neurons of the dorsal root ganglia has been linked with the anti-nociceptive actions of cannabinoids. Detailed analysis of CB<sub>1</sub> expression in the CNS using immunocytochemical techniques has revealed that CB<sub>1</sub> receptors are specifically targeted to the axons and axon terminals of neurons that express the CB<sub>1</sub> gene. This pattern of expression at a sub-cellular level is consistent with the inhibitory effects of cannabinoids on neurotransmitter release *in vitro*. Thus, cannabinoid activation of pre-synaptic CB<sub>1</sub> receptors causes G-protein mediated

inhibition of voltage-gated calcium channels, resulting in a transient reduction in neurotransmitter release. Longer-term inhibitory effects of cannabinoids on neurotransmitter release appear to be mediated via mechanisms resulting from CB<sub>1</sub>-mediated inhibition of cAMP-dependent protein kinase signaling.

Our now detailed understanding of the molecular and cellular mechanisms by which cannabinoids affect neural activity and behavior has provided an important basis for assessing the risks associated with recreational use of cannabis. It has also informed strategies to develop cannabinoid compounds with potential therapeutic properties; for example, use of CB<sub>1</sub> receptor agonists as analgesics or use of CB<sub>1</sub> receptor antagonists (e.g. SR141716A or “rimonabant”) as a treatment for obesity. However, equally importantly, research on cannabinoid action in the nervous system has revealed the existence of an endogenous cannabinoid signaling system with fundamental roles in mechanisms of synaptic plasticity. Thus, research on cannabinoid neuropharmacology now takes up a centre-stage position in 21<sup>st</sup> century neuroscience.

## **2. Endocannabinoids: discovery, biosynthesis and inactivation.**

The discovery of the G-protein coupled receptors CB<sub>1</sub> and CB<sub>2</sub> indicated that endogenous ligands for these receptors must exist and two derivatives of arachidonic acid were identified as candidate “endocannabinoids” in the 1990s – N-arachidonylethanolamide (“anandamide”, AEA) and 2-arachidonoylglycerol (2-AG). Both AEA and 2-AG are present in the CNS, but 2-AG is much more abundant than AEA. Furthermore, obtaining evidence that these molecules bind to and activate CB<sub>1</sub> receptors *in vivo* during normal brain function has been facilitated by molecular characterisation of enzymes that catalyse the synthesis or degradation of these molecules.

The mechanisms by which AEA is synthesized in the brain are currently not known, although several candidate pathways have been proposed. However, an enzyme that catalyses degradation of AEA has been identified – fatty acid amide hydrolase (FAAH). Importantly, the brain content of AEA in FAAH-knockout mice is 15 fold higher than in wild-type mice, providing compelling evidence that FAAH has a pivotal role in regulating AEA levels in the CNS. Furthermore, the enhanced basal level of AEA in the CNS of FAAH-knockout mice causes hypoalgesia, which is at least in part mediated by CB<sub>1</sub> receptors. The elevated levels of AEA in the CNS of FAAH-knockout mice does not appear to alter expression of CB<sub>1</sub> receptors, but interpreting the physiological significance of phenotypes observed in FAAH-knockout mice is nevertheless complicated. Therefore, use of FAAH inhibitors to transiently inhibit FAAH activity is an attractive complementary approach for analysis of the role FAAH in regulation of endocannabinoid signaling. A wide-range of compounds that inhibit FAAH have been developed but potential off-target effects of some of

these compounds (e.g. URB597) has complicated interpretation of their actions *in vivo*. However, some highly selective FAAH inhibitors have been developed (e.g. PF-3845), which has enabled investigation of the physiological and behavioral consequences of pharmacological inhibition of FAAH activity *in vivo*. Interestingly, administration of PF-3845 to mice does not mimic the tetrad of effects observed with  $\Delta^9$ -THC (see above) but it does cause elevation of AEA levels and CB<sub>1</sub>-mediated inhibition of neuropathic pain. Accordingly, FAAH inhibitors are considered to be potentially therapeutically useful because they may lack the psychoactive properties of  $\Delta^9$ -THC and other cannabinoids that bind directly to CB<sub>1</sub> receptors.

The endocannabinoid 2-AG is synthesized in the brain by the enzyme diacylglycerol lipase alpha (DAGL $\alpha$ ), which catalyses formation of 2-AG from arachidonic acid containing diacylglycerol. Evidence that DAGL $\alpha$  is the principal enzyme involved in biosynthesis of 2-AG in the brain has come from analysis of DAGL $\alpha$ -knockout mice, which have ~5-fold lower levels of 2-AG than wild-type mice. Furthermore, proof that 2-AG synthesized by DAGL $\alpha$  binds to CB<sub>1</sub> receptors *in vivo* has been provided by the finding that CB<sub>1</sub>-mediated mechanisms of synaptic plasticity in several regions of the brain (see below) are absent in DAGL $\alpha$ -knockout mice.

The principal enzyme in the brain responsible for inactivation of 2-AG is monoacylglycerol lipase (MAGL) and the key evidence for this has come from analysis of MAGL-knockout mice, which have ~10-fold higher CNS levels of 2-AG than wild-type mice. Furthermore, the elevation of 2-AG levels in the CNS of MAGL-knockout mice causes cross-tolerance to the antinociceptive and hypothermic effects CB<sub>1</sub> receptor agonists, providing further evidence that 2-AG synthesized *in vivo* binds to CB<sub>1</sub> receptors. Importantly, the effects of MAGL gene knockout are to a large extent phenocopied by administration of selective MAGL inhibitors such as JZL184, which causes an 8-10 fold elevation in brain 2-AG levels when administered to mice, without affecting brain AEA levels. JZL184 causes hypomobility, hypothermia and analgesia in mice, partially mimicking the tetrad effects of  $\Delta^9$ -THC, although the hypothermic and analgesic effects of JZL184 are lower in magnitude than for direct CB<sub>1</sub> agonists and JZL184 does not induce catalepsy. Thus, 2-AG appears to have a widespread role in the brain as an endogenous agonist for CB<sub>1</sub> receptors and accordingly the behavioral effects of  $\Delta^9$ -THC in mice could be considered equivalent, at least in part, to super-stimulation of endogenous 2-AG – CB<sub>1</sub> signaling.

### 3. Endocannabinoid signaling as a mechanism of synaptic plasticity

Characterisation of the molecular components that mediate and regulate endocannabinoid signaling in the CNS has not only enabled interpretation of the behavioral effects of cannabinoids, it has also provided the basis for discovery of mechanisms of synaptic plasticity at a cellular and sub-cellular level. Thus in 1998, based upon what was known at the time about the molecular neuroanatomy of the endocannabinoid (ECB) system, it was first proposed that endocannabinoids may mediate a particular form of synaptic plasticity in which endocannabinoids are synthesized post-synaptically but act on pre-synaptic CB<sub>1</sub> receptors to inhibit neurotransmitter release – i.e. retrograde synaptic signaling. In 2001/2002 an elegant series of experimental studies demonstrated that this hypothesis was indeed correct. Thus, transient depolarisation of principal neurons in several regions of the brain causes CB<sub>1</sub>-mediated inhibition of pre-synaptic release of the inhibitory neurotransmitter GABA (depolarisation-induced suppression of inhibition or DSI) and/or CB<sub>1</sub>-mediated inhibition of pre-synaptic release of the excitatory neurotransmitter glutamate (depolarisation-induced suppression of excitation or DSE). Furthermore, DSI and DSE are completely abolished in DAGL $\alpha$ -knockout mice, indicating that it is 2-AG that mediates these particular forms of synaptic plasticity. Consistent with the notion that 2-AG is synthesized post-synaptically but acts pre-synaptically, its biosynthetic enzyme DAGL $\alpha$  is concentrated post-synaptically in dendritic spines apposed to CB<sub>1</sub>-expressing axon terminals. Conversely, the degradative enzyme MAGL is localised pre-synaptically in axons and the duration of DSI and DSE in MAGL-knockout mice is prolonged when compared to wild-type mice, indicating that MAGL controls the time course of 2-AG/CB<sub>1</sub>-mediated retrograde synaptic signaling. Accordingly, the MAGL inhibitor JZL184 also prolongs the duration of DSI and DSE in wild-type mice. In contrast, FAAH inhibitors do not affect the duration of DSI and DSE, indicating that it is only 2-AG and not AEA that mediates these particular forms of endocannabinoid-mediated synaptic plasticity. Clearly, transient post-synaptic depolarisation of neurons that is induced experimentally using electrodes in DSI/DSE protocols may only partially recapitulate synaptic phenomena that occur physiologically. Nevertheless, it is thought that DSI and DSE are manifestations of Ca<sup>2+</sup> stimulation of basal DAGL $\alpha$ -dependent 2-AG synthesis, whilst basal DAGL $\alpha$ -dependent 2-AG synthesis is thought to be driven by metabotropic receptors that couple via G<sub>q/11</sub>-type proteins to stimulate phospholipaseC $\beta$ -mediated formation of DAG.

In addition to short-term mechanisms of synaptic plasticity such as DSI and DSE, there is evidence that endocannabinoid signaling also mediates long-term depression (LTD) of synaptic transmission. This was first observed in the striatum, where stimulation of cortical glutamatergic input causes activation of postsynaptic metabotropic glutamate receptors, leading to endocannabinoid/CB<sub>1</sub>-mediated long-term depression of transmission at excitatory cortico-striatal synapses. Endocannabinoid/CB<sub>1</sub>-mediated LTD has subsequently



been reported in other regions of the brain. Furthermore, there is evidence that again it is postsynaptic formation of 2-AG that mediates this particular form of long-term synaptic plasticity. However, the role of DAGL $\alpha$  as the source of 2-AG in endocannabinoid/CB $_1$ -mediated LTD has as yet, to the best of our knowledge, not been definitively proven using DAGL $\alpha$ -knockout mice and/or DAGL $\alpha$  inhibitors.

The physiological roles of AEA as an endogenous agonist for CB $_1$  receptors in the CNS are currently less well characterised when compared to 2-AG. This in part reflects incomplete knowledge of the mechanisms by which AEA is synthesized in the brain. However, we do have detailed information on anatomical distribution of the AEA-degrading enzyme FAAH. FAAH is widely expressed in the brain and is located in the somatodendritic compartment of principal neurons in many regions of the brain, including the olfactory bulb, neocortex, hippocampus, amygdala, thalamus and cerebellum. FAAH is also expressed in oligodendrocytes and ventricular ependymal cells, but it seems unlikely that expression of FAAH in these cell types directly impacts on mechanisms of synaptic plasticity. The functional significance of postsynaptic neuronal expression of FAAH in relation to retrograde synaptic signaling mediated by endocannabinoids is not known. One possibility is that FAAH-mediated regulation of postsynaptic AEA biosynthesis influences the temporal and spatial dynamics of retrograde endocannabinoid signaling, but arguing against such a role is the finding that FAAH inhibitors, unlike MAGL inhibitors, do not enhance the duration of endocannabinoid-mediated mechanisms of synaptic plasticity such as DSI and DSE. However, it has been found that overexpression of FAAH in cultured neurons shortens the duration of DSE. Furthermore, there is also evidence that AEA may mediate mechanisms of synaptic plasticity via CB $_1$ -independent molecular pathways. Thus, postsynaptic elevation of intracellular AEA levels is thought to cause LTD via a mechanism mediated by the cation channel TRPV1, which results in internalisation of post-synaptic AMPA-type glutamate receptors. Clearly, our understanding of the physiological roles of AEA in the brain is far from complete and further research is needed.

Having reviewed the molecular and cellular basis of cannabinoid action in the nervous system and the physiological mechanisms of endocannabinoid signaling, we have a basic framework for understanding the effects that cannabinoids have on whole-animal behavior. However, given the widespread distribution of CB $_1$  receptor expression in the CNS and the complex biochemistry of endogenous cannabinoid signaling, acquiring a mechanistic understanding of the behavioral actions of cannabinoids is challenging. Nevertheless, in the following section of this article we will review a variety of behavioral effects of cannabinoids that have been reported and discuss these with reference to the molecular and cellular level processes outlined above.

## **4. Behavioral effects of cannabinoids**

The neuropharmacological effects of cannabinoids are as diverse as the expression of cannabinoid receptors in the CNS. The regional distribution of CB<sub>1</sub> receptors throughout the mammalian CNS corresponds well with the behavioral effects of cannabinoids observed in animal experiments and in human cannabis users. The role of the CB<sub>2</sub> receptor in the brain has recently received increasing attention and its possible function in CNS processes is heavily debated. However, although most cannabinoid receptor agonists exhibit non-selective affinity for CB<sub>1</sub>/CB<sub>2</sub> receptors, the specific central pharmacological effects of CB<sub>2</sub> receptor agonists/antagonists are not well studied yet. We are therefore going to focus on the neuropharmacology of the CB<sub>1</sub> receptor for the present chapter. Various studies indicate that aside from dosage or route of administration, cannabinoid effects might vary greatly with the developmental stage and age (e.g. childhood, puberty). The following section will therefore exclusively review the most prominent neurobehavioral effects of cannabinoids in adult organisms.

### **4.1 Locomotor activity**

The initiation of locomotor activity depends upon processing of internal motivational and external sensoric stimuli and is mediated by interactions between limbic and motor systems. Cannabinoids profoundly affect locomotion, which is consistent with the abundant expression of the CB<sub>1</sub> receptor in neurons of the cerebellum and the basal ganglia. In particular, the basal ganglia represent an important structure for the regulation and initiation of motor activity, since they integrate cortical information into the coordination and organization of motor sequences and complex behaviors. Glutamate, GABA, and dopamine are among the most important neurotransmitters that participate in the control of basal ganglia function, and all three transmitter systems are modulated by cannabinoids. By regulating glutamatergic and GABAergic systems within the same neuronal network, cannabinoid receptors can modulate both inhibitory and excitatory neuronal transmission in the basal ganglia and may thus provide dual regulation of movement. CB<sub>1</sub> receptors are abundantly expressed on striatal GABAergic medium-spiny projection neurons but are also expressed on the terminals of glutamatergic cortical inputs to the striatum. In the cerebellar cortex, CB<sub>1</sub> receptors are abundantly expressed on glutamatergic and GABAergic inputs to Purkinje cells.

In humans, cannabis ingestion clearly affects motor performance, in particular balance and psychomotor control, and higher doses have been shown to induce hypokinesia, catalepsy, and ataxia. From experimental research in rodents (and dogs) it is known that synthetic and natural cannabinoid agonists (e.g.  $\Delta^9$ -THC, WIN 55,212-2, CP 55940) exert dose-dependent biphasic (or even triphasic) effects on locomotor activity in an

open field (see table 1). While very low doses appear to decrease activity in rodents, moderate to low doses have been found to stimulate activity and high doses induce catalepsy and inhibit locomotor activity. Additionally, administration of phytocannabinoid agonists (e.g.  $\Delta^9$ -THC) was found to induce circling behavior and hyperreflexia. These effects appear to be mediated directly by the CB<sub>1</sub> receptor since the CB<sub>1</sub> receptor antagonist/inverse agonist SR141716A counteracts most of the alterations in locomotor activity induced by application of CB<sub>1</sub> receptor agonists.

Conflicting results have also been reported for the pharmacological effects of CB<sub>1</sub> receptor antagonists (e.g. SR141716A, AM251) on locomotor activity. SR141716A was found not to affect activity levels on its own in rats and dogs, whereas one study in mice reported the induction of hyperactivity. Additionally, further studies in rats even demonstrated decreased activity in rats after application of high doses of AM251. These diverging behavioral effects might partially emerge from the inverse agonistic properties of these substances, which might induce similar biphasic dose-dependent effects as have been observed for CB<sub>1</sub> receptor agonists.

Aside from its effects on the activity level, SR141716A was also found to increase self-grooming behavior and scratching, and to reduce exploratory behavior. These findings raise an important issue for the behavioral testing of cannabinoid effects. Most studies investigating locomotor activity assess the performance of the animals in an open field. However, since cannabinoids are well known to modulate emotional behavior, the decrease in locomotion might not always be related to an inhibition of locomotor control, but might also vary with increased or decreased anxiety and the exploratory drive of the animals.

In line with the behavioral effects of synthetic and phytocannabinoid agonists, application of the endocannabinoid AEA was also found to induce biphasic effects on locomotor activity. Surprisingly, administration of pharmacological compounds that inhibit FAAH (e.g. URB597, PF-3845), and thereby increase the availability of AEA, do not affect locomotor behavior in rodents. In contrast, systemic injections of MAGL inhibitors (e.g. JZL184) or combined FAAH/MAGL inhibitors (e.g. JZL195) were found to attenuate locomotor activity and induce hyperreactivity, suggesting a main modulatory role for 2-AG in the regulation of locomotor behavior. Finally, catalepsy was only observed after combined pharmacological inhibition of FAAH and MAGL.

Taken together, a multitude of studies demonstrate that cannabinoids exert distinct modulatory effects on locomotor activity that vary with dosage and test conditions, but the detailed underlying mechanisms for cannabinoid-mediated effects on motor control remain yet to be identified.

## 4.2 Reward-related behavior

From an evolutionary perspective it is highly important to reinforce processes that are crucial for survival and reproduction (e.g. feeding and sexual behavior). Events, behavioral actions or objects that satisfy these basic needs are therefore generally considered as natural (non-drug) rewards. These processes are so elementary for survival of an individual that it is not surprising at all for a phylogenetically ancient signaling system, such as the ECB system, to be strongly involved in reward processing. Several brain structures, neurocircuits and related transmitter systems, known as the “reward system”, can be assigned to the main sub-components of reward: learning, hedonic/pleasurable experiences, and motivation. This brain reward system is not only crucial for the processing of natural rewards, but at the same time provides the basis for drug abuse and drug addiction. Along with the dopaminergic, the glutamatergic, and the endogenous opioid system, the ECB system has emerged recently as a key neurochemical mediator of reward processes. Although cannabinoids have been shown to affect and interact with all naturally rewarding processes (feeding, sexual behavior, social behavior, maternal behavior etc.) as well as with a variety of drugs of abuse (ethanol, nicotine, psychostimulants, opioids etc.), a complete description of all these pharmacological processes would be beyond the scope of the present chapter. We therefore focus here exemplarily on the modulatory role of cannabinoids on the rewarding effects palatable food.

CB<sub>1</sub> receptors are widely distributed throughout the brain reward circuits and exert an important modulatory influence on all other neurotransmitter systems involved in the mediation of reward-related behaviors. A close interaction between the ECB system and the glutamatergic system is well established in the brain reward system, since CB<sub>1</sub> receptors are densely located on glutamatergic synapses. Likewise, CB<sub>1</sub> and  $\mu$ -opioid receptors share a similar distribution throughout the reward circuits and a co-localization of both receptors has been shown for example in the nucleus accumbens and the dorsal striatum. Additionally, heterodimerization of CB<sub>1</sub> receptors with  $\mu$ -,  $\kappa$ -, and  $\delta$ -opioid receptors has been reported. Although the question as to whether CB<sub>1</sub> receptors are located directly on dopaminergic neurons is still up for debate, an indirect cannabinoid-mediated stimulation of dopaminergic signaling, mainly by disinhibition of GABAergic negative control over dopaminergic neurons in the ventral tegmental area (VTA) has been described in various studies. Furthermore, endocannabinoids are also necessary for the induction of several dopamine-dependent or independent long-term forms of synaptic plasticity in the VTA and in the terminal regions of dopaminergic neurons.

It has been well known for centuries that cannabinoids can induce euphoric and rewarding effects in humans and animals. One of the most prominent features of cannabis consumption is an initial period of euphoria and relaxation. These pleasurable subjective effects also contribute to its abuse. Aside from the euphoric effects, ingestion of cannabis

preparations is well known to induce a ravenous appetite, particularly for sweet and palatable food, termed “the munchies”. Many of these more anecdotal reports on the rewarding properties of cannabis and cannabinoids have been confirmed by recent scientific studies in humans and animals and therefore growing evidence indicates that the ECB system is a strong modulator of various aspects of reward processing. The following section will focus on pharmacological effects of cannabinoids on motivational, consummatory and hedonic aspects of reward-related behaviors for palatable food rewards (see table 2).

Stimulatory effects on (palatable) food ingestion have been described for different cannabinoid agonists and endocannabinoids in various studies. For example,  $\Delta^9$ -THC, AEA and 2-AG increase the preference for and intake of food or sucrose and the synthetic cannabinoid agonist CP 55940 increases the consumption of palatable solutions in rats. Furthermore, the motivation to actively respond for a palatable food reward, as measured by progressive-ratio (PR) schedules in a Skinner box, appears to be increased by administration of lower doses of cannabinoid agonists, but was also found to be decreased at higher doses. Additionally, cannabinoid effects on the hedonic value of food were addressed by taste reactivity studies which provide important information on the liking of palatable food rewards. Here it was shown that administration of AEA and  $\Delta^9$ -THC increases consumption and oral “liking” responses for palatable liquids.

CB<sub>1</sub> receptor antagonists, such as SR141716A or AM251, have been shown to inhibit palatable food intake. However, the precise mechanism through which CB<sub>1</sub> antagonism inhibits feeding has not been completely clarified thus far. Specifically, it is not known whether reduced feeding is induced by decreased appetite and attenuated hedonic value of food, or if side effects, such as motor slowing, incoordination, nausea, or substitute behaviors play a role. While SR141716A does not produce overt signs of sedation or motor slowing, other effects, such as induction of grooming, scratching, and head twitching, as well as reductions in spontaneous locomotion have been found at higher doses. However, an inhibition of CB<sub>1</sub> receptors has been found to decrease oral liking responses in taste reactivity studies.

The pharmacological effects of FAAH and MAGL inhibition on food reward are not well studied yet but simultaneous administration of the FAAH inhibitors URB597 or AM374 together with AEA seems to potentiate AEA effects.

### 4.3 Cognition

Cognition refers to all mental processes involved in processing and gaining of information, knowledge and comprehension. These processes include attention, thinking, remembering, problem solving, planning, behavioral flexibility and decision-making. Higher-order cognitive (or executive) functions are mediated mainly by fronto-striatal brain areas in both humans as well as rodents and high densities of CB<sub>1</sub> receptors have been described in frontal cortical and striatal regions. More specifically, CB<sub>1</sub> receptors have been identified on GABAergic, glutamatergic, noradrenergic as well as serotonergic neurons throughout frontal cortical regions and have been reported to enhance dopamine transmission in the medial prefrontal cortex and the nucleus accumbens in an indirect manner. In particular the ability of cannabinoids to modulate dopaminergic neurotransmission appears to be highly important, since dopamine signaling is crucially involved in executive functioning. A very high density of CB<sub>1</sub> receptors is also present in the hippocampus, where cannabinoids might exert their adverse effects on (spatial) memory performance. They are abundantly expressed on the terminals of hippocampal GABAergic basket cell interneurons in the CA1-CA4 field, as well as on GABAergic neurons in the dentate gyrus and to a lesser extent in glutamatergic hippocampal pyramidal cells and mossy cells.

It is well established that the ECB system plays a major role in cognitive processing. The following section will provide an overview of the pharmacological effects of cannabinoids on executive functions, including short-term mnemonic and attentional processing, as well as behavioral flexibility (see table 3).

Disruptions of attentional and mnemonic processing are the most consistent observations of cannabis intoxication in humans. These findings are consistent with a number of studies in laboratory rodents demonstrating that various cannabinoid agonists impair attention and memory functioning. Memory deficits have been reported after administration of phytocannabinoids (e.g.  $\Delta^9$ -THC), synthetic cannabinoids (e.g. WIN 55,212-2) and endocannabinoids for working memory tasks, spatial learning as well as recognition memory abilities. It appears that cannabinoids interfere mainly with memory acquisition, early consolidation and facilitate memory extinction, rather than affecting memory retrieval. This would be consistent with the observation that pharmacological inactivation of CB<sub>1</sub> receptors facilitates induction and maintenance of hippocampal long-term potentiation. With respect to the modulation of attention, the pharmacological effects of cannabinoids are not as conclusive as for memory processing, which might be related to the fact that most tasks applied are susceptible to disturbances in locomotor control. Dose-dependent inhibitory effects (or in some cases no effects) on attentional processing have been reported in different paradigms, such as prepulse inhibition (PPI) of the acoustic startle reflex (ASR) or reaction time tasks (RTT).

Behavioral flexibility, the ability to adapt to changing environments, is an important cognitive skill, and requires the capacity to adjust behavioral strategies and to suppress acquired response patterns. An important role of the ECB system in these processes has been suggested by various studies in humans and rodents. In humans, heavy cannabis use was shown to be associated with deficits in behavioral flexibility (or reversal learning) measured in a Wisconsin card sorting test (WCST). Likewise, administration of different cannabinoid agonists in laboratory rodents has also been found to impair cognitive flexibility in attentional set shifting tasks (ASST), developed as an equivalent to the human WCST, and in an olfactory go/no-go discrimination task and a cross maze paradigm.

CB<sub>1</sub> receptor antagonists have been found to enhance attentional processing, short-term memory functioning and behavioral flexibility, but also no effects on these cognitive processes were reported. In particular, memory acquisition appears to be improved by SR141716A or AM251, but controversial findings were reported on memory consolidation.

Surprisingly, FAAH inhibitors have been found to enhance learning in several procedures, although AEA inhibits memory functioning. The FAAH inhibitor OL-135 enhanced the acquisition rate in a water maze test (although this effect was not found in an earlier study). Additionally, administration of URB597 was shown to enhance the acquisition of passive-avoidance learning, without affecting consolidation or retrieval. Interestingly, the enhancing effects of FAAH inhibition on passive-avoidance learning could be blocked not only by SR141716A but also by an antagonist of the PPAR-receptor, suggesting that FAAH inhibition might enhance memory by increasing the levels of the endogenous PPAR- $\alpha$  ligands N-oleoylethanolamine (OEA) and palmitoylethanolamide (PEA). In contrast, memory-disrupting effects of URB597 have been reported in a delayed-non-match-to-sample task. The pharmacological effects of MAGL inhibitors or 2-AG on cognitive processing are not well studied yet and in particular the effects of both endocannabinoids on attentional processing and behavioral flexibility have to be further examined. Therefore, more studies are needed for a better understanding of the detailed role of AEA/2-AG and their degrading enzymes in cognitive processing.

#### 4.4 Emotional behavior

As described above, the main features of recreational cannabis use in humans are the euphoric and relaxing effects of the drug. However, aside from these pleasurable experiences, cannabis can also induce dysphoric reactions, including severe anxiety, panic and paranoia. Fear and anxiety are crucial and adaptive components of the overall stress response to threatening situations that might perturb homeostasis. Transient anxiety therefore elicits an appropriate response (e.g. escape or avoidance) and is of fundamental importance as a survival strategy for mammals. Anxious states are, thus, controlled by a highly complex system of both inhibitory and facilitatory mechanisms. Numerous interconnected limbic and cortical structures have been implicated in the modulation of anxious states that all express CB<sub>1</sub> receptors (e.g. frontal cortex, amygdala, thalamus, nucleus accumbens, hippocampus etc.).

In animal experiments the term 'emotionality' is classically used to conceptualize behavioral changes in an arousing context such as novel or anxiogenic environments or situations. Behavioral paradigms for emotional behavior in rodents therefore mainly assess innate (unconditioned) avoidance or conflict behaviors as well as conditioned aversion. The involvement of ECB signaling in the mediation of anxiety-related behaviors is very complex and only partially understood. Similar as in cannabis users, administration of cannabinoid agonists in rodents has been reported to induce anxiogenic as well as anxiolytic-like responses (see table 4). We are going to review pharmacological effects of cannabinoids on unconditioned anxiety-related behaviors in classical paradigms such as the elevated plus maze or the light/dark emergence test.

Synthetic and phytocannabinoid agonists have been reported in various studies to induce either anxiolytic- or anxiogenic-like reactions, depending upon dosage, test paradigm, the test context and conditions (e.g. light intensity; familiar vs, unfamiliar environment), species or genetic strain. Generally, low doses tend to reduce and high doses tend to increase, anxiety-like behaviors. However, in particular in mice, the genetic background of the animals seems to interfere with pharmacological effects on anxiety-related behaviors.

Similar conflicting effects have been reported for CB<sub>1</sub> receptor antagonists, which have been found to induce anxiogenic as well as anxiolytic behavioral responses. Thus, in paradigms based on innate fear reactions, cannabinoid pharmacology may either enhance or attenuate anxiety-like behavior.

Central administration of methanandamide (a metabolically stable analogue of AEA) directly into the PFC revealed anxiolytic-like responses in rats in the EPM test for low doses, whereas high doses induced anxiogenic effects. Central administration of AEA in the amygdala revealed no effects on emotional behavior. Inhibition of FAAH or MAGL



has been shown to reduce anxiety-like behaviors in different paradigms without affecting locomotor activity in rats and mice, indicating a possible (region-specific) anxiolytic-like role of endocannabinoids.

#### **4.5 Nociception**

Pain perception, an unpleasant sensory and/or emotional experience associated with actual or potential tissue damage, is an important component of the body's defense system that is essential for survival. Acute pain does not outlast the initiating painful stimulus (e.g. superficial wounds, chemicals or burns, ischemia and inflammation), whereas chronic pain outlasts the initiating (often unknown) stimulus. Cannabinoids have been used therapeutically for pain relief for many thousands of years, clearly indicating an involvement of the ECB system and cannabinoid pharmacology in nociception.

Endocannabinoids and CB<sub>1</sub> receptors are present in the major pain pathways and strongly modulate pain processing through central (both spinal and supraspinal) and peripheral mechanisms, most probably through a close interaction with the endogenous opioid system but also opioid-independent mechanisms. Although CB<sub>2</sub> receptors appear to be also important for pain processing, a description of these mechanisms would be beyond the scope of the present chapter, and we are therefore going to focus on CB<sub>1</sub> receptor mediated pharmacological effects of cannabinoids on nociception.

CB<sub>1</sub> receptor agonists exert antinociceptive and antihyperalgesic effects in various animal models of neuropathic and inflammatory pain, and are also effective against acute noxious stimuli (e.g. the tail-flick and hot-plate test). The antinociceptive effects produced by systemic administration of cannabinoids are attenuated following spinal transection, indicating an important role for supraspinal brain sites. In particular, the periaqueductal gray, the thalamus, the rostral ventromedial medulla and the amygdala appear to be important brain regions for cannabinoid analgesic action. Activation of these sites by endocannabinoids may, therefore, produce antinociception under physiological conditions.

The pharmacological effects of CB<sub>1</sub> receptor antagonists are quite controversial. Initial studies with CB<sub>1</sub> receptor antagonists, such as SR141716A, found no alterations on acute pain sensitivity (e.g. tail flick test or hot plate). In contrast, other studies reported hyperalgesic effects in the same test paradigms. In the formalin test of persistent pain, similar conflicting results have been described, as initial studies described a hyperalgesic activity for CB<sub>1</sub> receptor antagonism which could not be confirmed in further studies. Therefore, the nociceptive properties of CB<sub>1</sub> receptor antagonists/inverse agonists still need to be clarified, although the existing data indicate that differences in the level of

endogenous analgesic tone (dependent on stress effects, or other environmental factors of the pain models used) may contribute to the differences observed on pain processing.

Various studies indicate that administration of AEA or FAAH inhibitors (e.g. URB597, URB532, OMDM122) promotes analgesia, although the question of whether AEA exerts its antinociceptive effects via CB<sub>1</sub> receptor-dependent or independent mechanisms is not completely clarified. Inhibition of FAAH induces CB<sub>1</sub> receptor-dependent antinociceptive activity in several rodent pain models, including the formalin test, the carrageenan paw inflammation test, neuropathic pain and the hot-plate test. Similar anti-nociceptive effects have also been reported for MAGL inhibitors (e.g. OMDM169, URB602).

## **5. Conclusions**

The complexity of the neuropharmacological effects of cannabinoids, some of which were reviewed exemplarily in the present chapter, reflects the importance and extensive modulatory role of the CB<sub>1</sub> receptor and the ECB system in a multitude of CNS functions. However, this plethora of cannabinoid-mediated behavioral effects also emphasizes one of the major problems of neuropharmacological studies as well as limitations of the therapeutical use of cannabinoids, since it is not yet possible to clearly separate or independently investigate pharmacological effects of cannabinoids on specific behaviors. This is especially the case for pharmacological studies in rodents. Alterations in locomotor activity will always interfere with other behavioral tasks such as motivation, consumption, pain response, emotional reactions or cognitive behavior. Likewise, the emotional state of an animal might simply affect exploratory behavior and therefore reduce performance in motivational tasks or tests for cognitive function. Additionally, the modulatory influence of cannabinoids on food reward or pain processing might be problematic for the testing of cognitive behavioral tasks using either food or aversive events (e.g. foot shocks) as reinforcers. It is therefore absolutely crucial to be mindful of such possible confounding behavioral influences when performing neuropharmacological experiments with cannabinoids.

A further important issue in cannabinoid pharmacology are apparent conflicting findings (e.g. dose-dependent bi- or even triphasic effects, context-specificity), which have been best described for emotional behavior and locomotor control. A possible explanation for the complexity of cannabinoid effects might be provided by the 'on-demand' functions of the ECB system (depending on environmental stimuli and on the emotional state of an individual), and also by its fine-tuning of inhibitory and excitatory neuronal activity. Thus, the biphasic effects observed after CB<sub>1</sub> receptor-activation are not necessarily contradictory, since the ECB system functions as a neuromodulator of excitatory and inhibitory

neurotransmission by modulating the activity of both GABA- and glutamate-release, which represent the two major opposing systems that control many neurophysiological processes. It therefore appears that ECB signaling has an important role in maintaining homeostasis by dampening excessive neuronal responses induced by environmental challenges, and is therefore activated by relatively high levels of synaptic activity. As a result, cannabinoids selectively affect heterogeneous neurons that may have differential effects on the behavioral response. Interfering with such a complex regulatory process might therefore lead to complex and situation-dependent effects.

Taken together, more research on the complex pharmacology of cannabinoids is still needed to further clarify the detailed neurophysiological effects and most importantly to shed light on the underlying neurobiological mechanisms. Gaining greater knowledge of the functionality and neuropharmacology of this important neuromodulatory system is also mandatory in order to fully benefit from the valuable therapeutic potential of cannabinoids whilst minimizing adverse side effects.

### **Further reading**

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#### **Suggestings for cross references**

Philippe De Witte	<b>Behavioral biology, preclinical animal studies of addiction</b>	<b>37</b>	Animal models of drug addiction: Cannabinoids
Philippe De Witte	<b>Behavioral biology, preclinical animal studies of addiction</b>	<b>44</b>	Preclinical animal studies: Cannabinoid
Rainer Spanagel	<b>Neuropharmacology/Imaging/Genetics</b>	<b>278</b>	Neuroimaging in cannabis users