Addiction Biology

REVIEW FOR SPECIAL ISSUE ON CANNABINOIDS doi:10.1111/j.1369-1600.2008.00104.x

The endocannabinoid system: emotion, learning and addiction

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ABSTRACT

The identification of the cannabinoid receptor type 1 (CB1 receptor) was the milestone discovery in the elucidation of the behavioural and emotional responses induced by the *Cannabis sativa* constituent Δ^9-tetrahydrocannabinol. The subsequent years have established the existence of the endocannabinoid system. The early view relating this system to emotional responses is reflected by the fact that *N*-arachidonoyl ethanolamine, the pioneer endocannabinoid, was named anandamide after the Sanskrit word '*ananda*', meaning 'bliss'. However, the emotional responses to cannabinoids are not always pleasant and delightful. Rather, anxiety and panic may also occur after activation of CB1 receptors. The present review discusses three properties of the endocannabinoid system as an attempt to understand these diverse effects. First, this system typically functions 'on-demand', depending on environmental stimuli and on the emotional state of the organism. Second, it has a wide neuro-anatomical distribution, modulating brain regions with different functions in responses to aversive stimuli. Third, endocannabinoids regulate the release of other neurotransmitters that may have even opposing functions, such as GABA and glutamate. Further understanding of the temporal, spatial and functional characteristics of this system is necessary to clarify its role in emotional responses and will promote advances in its therapeutic exploitation.

Keywords Anxiety, cannabinoids, depression, drug abuse, fear, stress.

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THE ENDOCANNABINOID SYSTEM AND EMOTIONS: IS IT ONLY 'BLISS'?

The proposal of a specific site of action for the *Cannabis sativa* constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) 20 years ago (Devane *et al.* 1988) provided the first step towards a molecular understanding of the emotional responses induced by this natural cannabinoid and its synthetic derivatives. A G protein-coupled receptor was subsequently cloned (Matsuda *et al.* 1990) and named cannabinoid type 1 (CB1) receptor. Next, two endogenous agonists (endocannabinoids), *N*-arachidonoyl ethanolamine (anandamide) and 2-arachidonoyl glycerol (2-AG), were identified. In addition, a second G protein-coupled cannabinoid receptor was cloned, the type 2 (CB2) receptor. The major components that terminate the actions of endocannabinoids were characterized, namely fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (reviewed in Howlett *et al.* 2002; Marsicano & Lutz 2006; Pacher, Batkai & Kunos 2006; Piomelli 2003). However, the endocannabinoid synthesizing machinery and the putative re-uptake mechanism by a membrane transporter need further clarifications.

For centuries, *Cannabis* preparations have been used for recreational purposes because of their diverse psychotropic effects, characterized by a state of euphoria, relaxation, happiness, laughter and 'high' (Hall & Solowij 1998; Huestis *et al.* 2001; Johns 2001). It is, therefore, not surprising that the first endocannabinoid identified (*N*-arachidonoyl ethanolamine) was nicknamed anandamide, after the Sanskrit word '*ananda*', for 'bliss' (Devane *et al.* 1992). Despite the early view on this endocannabinoid as a substance implying 'supreme happiness', activation of CB1 receptors does not always result in feelings of pleasure and delight. Rather, anxiety, panic and psychotic reactions may also occur after *Cannabis* smoking or Δ^9-THC administration to humans (Zuardi *et al.* 1982; Thomas 1996; Hall & Solowij 1998; Johns 2001; D'Souza *et al.* 2004). The same complex picture applies to studies with CB1 receptor agonists or antagonists in

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animal models of psychiatric disorders (reviewed in Millan 2003; Hill & Gorzalka 2005a; Viveros, Marco & File 2005). Thus, the endocannabinoid system is very likely to be relevant for emotional states related to anxiety, stress, depression and addiction. The question has remained whether its function is to aggravate or to alleviate them.

This is a major issue if one envisages the clinical use of cannabinoids as well as the exploitation of this system as a therapeutic target. Regarding psychiatric research, several studies have focused on a range of disorders, such as generalized anxiety, post-traumatic stress disorder, depression and drug abuse (reviewed in Hill & Gorzalka 2005a; Maldonado, Valverde & Berrendero 2006; Viveros *et al.* 2005; Wotjak 2005). Therefore, the aim of this review is to discuss the role of the endocannabinoid system in behavioural responses and to attempt to reconcile the intricate data on this subject. First, we will comment on the biphasic and often seemingly illogic effects of cannabinoids in relation to aversive responses and addiction. Next, three particular characteristics of the endocannabinoid system will be discussed as we will try to understand conflicting findings: Its 'on-demand' functioning (*when* the system works), its neuro-anatomical distribution (*where* it works) and its neuromodulatory activity (*what* it does). Further understanding of these properties may yield new interventions that could be of therapeutic interest. A thorough and comprehensive review on the neurobiology of emotions and psychiatric disorders is beyond the scope of this review. They were addressed elsewhere (Millan 2003; 2006; Charney 2004; Castren 2005; Everitt & Robbins 2005; Berton & Nestler 2006).

THE ENDOCANNABINOID SYSTEM AND BIDIRECTIONAL RESPONSES

The literature on *Cannabis* and emotion has abundantly reported 'contradictory' results. One reason for this could be the large number of substances present in the plant. Although Δ^9-THC is the main active constituent, other cannabinoids may alter its pharmacokinetics and pharmacodynamics, not to mention their own complex pharmacological profiles (Nadulski *et al.* 2005; Moreira, Aguiar & Guimaraes 2006; Zuardi *et al.* 2006; Pertwee *et al.* 2007). The administration of pure Δ^9-THC or synthetic cannabinoids is aimed at circumventing this complexity [for further details on the molecular pharmacology of natural and synthetic cannabinoids mentioned in this review, see Howlett *et al.* (2002); Pacher *et al.* 2006)]. However, Δ^9-THC administration induces the same diversity of results as *Cannabis*. Among them are the feelings of 'high', euphoria, and schizophrenia-like positive and negative symptoms in humans (Green, Kavanagh & Young 2003; D'Souza *et al.* 2004). These complex actions are not different when it comes to the study of aversive or rewarding responses in laboratory animals, as discussed below. The following paragraphs will summarize the data on these biphasic effects of cannabinoids, paving the way to a discussion attempting at proposing possible mechanisms underlying the diverse and even 'contradictory' findings. For this discussion, the understanding of how the endocannabinoid system functions is a prerequisite.

Responses to aversive stimuli

Several studies have evaluated the effects of cannabinoids in rodents exposed to models of aversive responses. They are grouped here in three distinct categories. First, models based on innate (unconditioned) aversion, predictive of anxiolytic-like activity. Second, models based on conditioned fear. Third, models based on inescapable or chronic stress, which may be predictive for antidepressant-like activity.

Numerous studies have addressed how cannabinoids influence anxiety-related behaviours in response to unconditioned stimuli. One of the most widely used assay is the elevated-plus maze. In this apparatus, Δ^9-THC increased anxiety-like behaviours in rats and mice in doses ranging from 1 to 10 mg/kg body weight (Onaivi, Green & Martin 1990; Patel & Hillard 2006), while anxiolytic-like effects were reported with doses of 0.5 or 0.75 mg/kg (Braida *et al.* 2007; Rubino *et al.* 2007b). Anxiolytic-like effects induced by low dose (0.3 mg/kg) were also described in mice exposed to another model, the light-dark box (Berrendero & Maldonado 2002; Valjent *et al.* 2002). In addition to Δ^9-THC, some studies have also addressed the activity of synthetic cannabinoids in these models. Adding intricacy to the picture, their effects did not always coincide with those induced by Δ^9-THC, even under the same experimental parameters (Patel & Hillard 2006). Thus, cannabinoids may induce either anxiolytic- or anxiogenic-like effects in particular models, depending on the dose administered (reviewed in Viveros *et al.* 2005). Generally, low doses tend to reduce and high doses tend to increase, anxiety-like behaviours.

An alternative approach to study the role of endocannabinoid system is the inhibition of either re-uptake (membrane transport) or intracellular hydrolysis. Importantly, these drugs do not necessarily share the properties of CB1 receptor agonists. While direct CB1 activation (agonists in high doses) induces hypolocomotion, catalepsy, hypothermia and analgesia (called the 'tetrad') in rodents (Compton *et al.* 1992; Martin *et al.* 1991), compounds that enhance the endogenous levels of endocannabinoids do not tend to modify locomotion (Kathuria *et al.* 2003). In this respect, inhibitors of either

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anandamide transport (e.g. AM404) or hydrolysis (e.g. URB597) reduce anxiety-like behaviours without motor impairment in rats and mice. This was observed in the elevated-plus and the elevated-zero maze, as well as in the light-dark box test (Kathuria *et al.* 2003; Bortolato *et al.* 2006; Patel & Hillard 2006; Rutkowska, Jamontt & Gliniak 2006; Naidu *et al.* 2007; Moreira *et al.* 2008). This feature points to an anxiolytic-like role of endocannabinoids. In line with this notion, mice lacking CB1 receptor (CB1 knock-out mice) or mice treated with the CB1 receptor antagonists rimonabant or AM251, exhibit increased anxiety-like behaviours, unravelling a tonic modulation by the endocannabinoid system (Navarro *et al.* 1997; Martin *et al.* 2001; Haller *et al.* 2004; Patel & Hillard 2006). Interestingly, the effects of CB1 receptor antagonism in humans seem to support this possibility, since clinical trials with rimonabant for the treatment of obesity and associated metabolic dysregulations revealed that this drug may induce anxiety- and depression-like symptoms (Van Gaal *et al.* 2005; Scheen *et al.* 2006). However, anxiolytic-like effects were also reported after the injection of CB1 receptor antagonists in rodents (Rodgers *et al.* 2003; Griebel, Stemmelin & Scatton 2005). Thus, in models based on innate fear reactions, there are evidences that the endocannabinoid system may either enhance or attenuate anxiety-like behaviour.

This system may also interfere with learned aversive behaviours, such as the conditioned fear response, a potential model for post-traumatic stress disorder. In this paradigm, endocannabinoids seem to be of major importance for the extinction of fear memories (Marsicano *et al.* 2002; Kamprath *et al.* 2006). Marsicano *et al.* (2002) showed that either genetic or acute pharmacological blockade of CB1 receptors in mice impaired the extinction of conditioned fear. Mice were exposed to a tone that was previously paired with a foot-shock and, as expected, exhibited fear-like reaction (i.e. freezing). Subsequent exposures to the tone extinguished freezing in wild-type animals, but this was strongly impaired in CB1 receptor-deficient mice. In addition, exposure to the conditioned stimulus (i.e. tone) increased endocannabinoid levels in the amygdala, suggesting that endocannabinoids initiate signalling processes favouring extinction of fear memories (Marsicano *et al.* 2002). Subsequent confirmatory investigations further strengthened the role of the endocannabinoid system in fear extinction. First, impaired extinction to contextual fear memory was observed after pharmacological blockade of the CB1 receptor in mice (Suzuki *et al.* 2004). Then, it was reported that inhibition of endocannabinoid re-uptake by AM404 was able to enhance extinction of conditioned fear potentiated startle in rats (Chhatwal *et al.* 2005). Enhanced extinction was also observed after direct activation of CB1 receptor with the potent agonist WIN-55,212-2 in rats exposed to fear conditioned to a context (Pamplona *et al.* 2006). Thus, experiments with either blockade or enhancement of the endocannabinoid system have supported a role for this system in fear extinction (reviewed also by Lafenêtre, Chaouloff & Marsicano 2007; Lutz 2007).

Other lines of evidence also point to diverse effects of cannabinoids and CB1 receptor antagonists in reactions to acute or chronic inescapable stressful events. These models are of relevance for studying the neurobiology of mood disorders and screening antidepressant drugs (Millan 2006). In rats exposed to the forced swim test, the CB1 receptor agonists HU210 and WIN-55,212-2 induced an antidepressant-like effect (Hill & Gorzalka 2005b; Bambico *et al.* 2007). The same was found with the endocannabinoid re-uptake inhibitor AM404 (Hill & Gorzalka 2005b). FAAH inhibition by URB597 also enhanced stress-coping behaviour both in this model and in the tail-suspension test in mice (Gobbi *et al.* 2005; Naidu *et al.* 2007). In line with these data, an increase in endocannabinoid levels is also efficient in alleviating later consequences of stressful events, such as the reduction in response to appetitive stimuli. This resembles anhedonia and reduced motivation, frequently present in human depression. Both URB597 and the CB1 agonist CP-55,940 selectively prevented attenuation in sucrose preference induced by restraint stress in mice (Rademacher & Hillard 2007). In addition, similar to antidepressant drugs, inhibition of anandamide hydrolysis prevented the anhedonia-like behaviour in rats exposed to chronic mild stressors (Bortolato *et al.* 2007). In this model, CB1 knock-out mice have an increased anhedonia-like behaviour as compared with their wild-type littermates (Martin *et al.* 2001). CB1 receptor knock-out mice also showed an impaired stress-coping behaviour and an increased activity in the hypothalamic-pituitary-adrenal axis, a key regulatory mechanism in response to stressful stimuli (Cota *et al.* 2007; Steiner *et al.* in press; 2008). These data raise the interesting point that the endocannabinoid system may have a tonic modulatory role on mood states, as it may have in anxiety. Finally, it is worth noting that a depressed mood was also among the side effects observed in the clinical studies with rimonabant (Van Gaal *et al.* 2005; Scheen *et al.* 2006).

However, high doses of cannabinoids may aggravate, rather than prevent, the consequences of stressful events (Patel, Cravat & Hillard 2005). In further complicating the picture, CB1 receptor antagonists may also exhibit antidepressant-like activity (Shearman *et al.* 2003; Griebel *et al.* 2005; Steiner *et al.* 2008). Therefore, as discussed for anxiety, data obtained from models of depression-related behaviours are still difficult to understand, and share similarities to the observation that the endocannabinoid system may modulate stress responses in opposite directions.

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Responses related to addiction

Paradigms relevant to the study of drug addiction may also offer insights as to whether cannabinoids are 'pleasant' or aversive. The fact that *Cannabis* is self-administered points obviously to a link between the endocannabinoid system and emotional responses related to addiction. Substances that have abuse-risk may induce hedonic states during the initial experiences, although as addiction develops, subsequent administration may represent a relief from aversion (withdraw syndrome), rather than 'pleasure'. This might be because of a modification in the 'hedonic set point', secondary to the chronic effects of these drugs (LeMoal & Koob 2007). Although the animal models currently used are unlikely to reflect these complex features of addictive behaviours, they may mimic specific aspects, such as the reinforcing properties of a substance, the memories related to the context of drug intake and the consequences of chronic administration and withdrawal (Sanchis-Segura & Spanagel 2006). Thus, using these models, it is possible to investigate whether cannabinoid administration is really 'blissful'. The neural substrate for the hedonic states related to drug abuse consists of the mesolimbic dopaminergic neurons projecting from the ventral tegmental area to the ventral nucleus accumbens and to the dorsal striatum, and glutamatergic inputs from the medial prefrontal cortex to these areas (Everitt & Robbins 2005; Di Chiara & Bassareo 2007; LeMoal & Koob 2007). Importantly, the endocannabinoid system is functional in all components of this circuitry (Maldonado 2002; Tanda & Goldberg 2003; Lupica, Riegel & Hoffman 2004).

If CB1 receptor-activation in this system were to be rewarding rather than aversive, some phenomena commonly seen with addictive substances would be expected to occur after cannabinoid administration. First, cannabinoid injections would increase dopamine release. Second, animals would undergo conditioned-place preference to cannabinoids, recognizing a context where they had received cannabinoid administration and exploring it, in detriment of others that are not associated with the drug. Third, animals would self-administer cannabinoids whenever they were available. Fourth, cannabinoids would reduce the electrical threshold that supports intracranial self-stimulation of the mesolimbic pathway. Finally, cannabinoids would induce tolerance and withdrawal effects after interruption of chronic treatment.

Increasing dopamine release in the shell of the nucleus accumbens is a common feature of most drugs of abuse (Di Chiara & Imperato 1988). Thus, Δ^9-THC is expected to have such an effect. Some studies showed that this natural cannabinoid may indeed increase dopamine release in freely moving rats (Chen *et al.* 1990; Tanda, Pontieri & Di Chiara 1997), although other investigations were not able to show this (Castaneda *et al.* 1991; Rodriguez de Fonseca *et al.* 1992). Dopamine release is also measurable in human subjects by imaging techniques, although such studies are scarce. One paper reported on a patient suffering from schizophrenia who smoked *Cannabis* during a break in the course of an imaging session (single photon emission computerized tomographic). The result was a worsening of psychotic reactions and a decrease in the binding of a radioactive tracer to dopamine D2 receptors in the striatum, suggesting that an increase of dopamine release occurred (Voruganti *et al.* 2001). However, more studies in humans are necessary to substantiate this hypothesis. Nevertheless, this neurochemical measure does not necessarily predict a potential for abuse. Behavioural models may be more informative in this respect, and a widely used test is the induction of conditioned-place preference in rodents. Contrary to what would be expected for a drug of abuse, some authors observed that rodents exhibit avoidance of, rather than preference for the context, where Δ^9-THC or a synthetic cannabinoid was administered (Parker & Gillies 1995; McGregor, Issakidis & Prior 1996). Another study did not find any effect of low dose (1.5 mg/kg), while a high dose (15 mg/kg) of Δ^9-THC induced place avoidance (Sañudo-Peña *et al.* 1997). It was the CB1 receptor antagonist rimonabant which induced conditioned-place preference in this study, indicating a tonic aversive role for the endocannabinoid system (Sañudo-Peña *et al.* 1997). Despite these inconsistencies, it is interesting to note that laboratory rodents do exhibit conditioned-place preference to Δ^9-THC under specific circumstances. In one study, it was hypothesized that the difficulty in finding place preference to this natural cannabinoid might be because of dysphoric consequences resulting from the first exposure to the drug, which would mask the rewarding effects. Thus, in a protocol in which mice had received a priming injection of Δ^9-THC, not paired with the context of the box, a second injection of a low dose of this drug (1 mg/kg) did induce conditioned-place preference in mice (Valjent & Maldonado 2000). Accordingly, while place avoidance occurred after injection of a high dose (5 mg/kg) in naive animals, no effect was found in mice which had received a priming injection in another context (Valjent & Maldonado 2000). In addition, another study revealed conditioned-place preference to the synthetic cannabinoid CP-55,940 (Braida *et al.* 2001a). Generally, the results obtained in these models are very much in line with those observed in models of anxiety-like behaviours, as the responses are bidirectional, low doses elicit rewarding and anxiolytic-like responses and high doses aversive and anxiogenic-like responses.

Considering that humans self-administer *Cannabis* for recreational purposes, one might conclude that CB1 receptor-activation may induce rewarding responses.

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However, even this view is not consistent for Δ^9-THC, as it may also induce emotional states reported as unpleasant (Zuardi *et al.* 1982; D'Souza *et al.* 2004). Along this line, it has been difficult to clarify whether cannabinoids work as positive reinforcers. A study in monkeys failed to show self-administration of either Δ^9-THC or CP-55,940 (Mansbach *et al.* 1994), while others showed self-administration of the synthetic cannabinoid WIN-55,212-2 in mice (Martellotta *et al.* 1998; Ledent *et al.* 1999). As it is the case with conditioned-place preference, previous experiences of the organism seem to be relevant. Thus, self-administration of Δ^9-THC was observed in monkeys that had previously self-administered other drugs of abuse (Tanda, Munzar & Goldberg 2000). However, intravenous self-administration of Δ^9-THC in naive monkeys was later found as well (Justinova *et al.* 2003). Self-administration was also described in rats receiving CP-55,940 via intracerebroventricular injections (Braida *et al.* 2001b). Thus, similar to conditioned-place preference, cannabinoid self-administration may occur when specific protocols are employed.

The effects of cannabinoids on intra-cranial self-stimulation in laboratory animals have also been inconsistent. This fascinating phenomenon was initially described by the studies of Olds and co-worker (Olds & Milner 1954, for a review see also Wise 2005), who showed that rodents would press a lever as an operant response to activate an electrode and thereby self-stimulate the medial forebrain bundle. Most addictive drugs reduce the threshold for intra-cranial self-stimulation. However, low doses of Δ^9-THC may reduce or does not modify the threshold in rats (Gardner *et al.* 1988; Lepore *et al.* 1996). Experiments with CP-55,940 have also failed to show any effect on intra-cranial self-stimulation (Arnold, Hunt & McGregor 2001). Furthermore, another study found that inhibition of endocannabinoid re-uptake or hydrolysis increased the threshold, suggesting that the endocannabinoid system has in fact inhibitory influence on this phenomenon (Vlachou, Nomikos & Panagis 2006).

Finally, as with other drugs of abuse, tolerance and withdrawal to cannabinoids may also occur. Studies with *Cannabis* consumers support the existence of a '*Cannabis* withdrawal syndrome'. Emotional symptoms occur in addition to appetite change, weight loss and physical discomfort (Budney & Hughes 2006). These features are also observed in laboratory animals. For instance, the CB1 receptor antagonist rimonabant precipitated signs of withdrawal in Δ^9-THC-treated rats, and abstinence signs were noted after an abrupt interruption of Δ^9-THC injections (Aceto *et al.* 1996). This natural cannabinoid also induced tolerance followed by withdrawal effects in mice, possibly because of a misbalancing in the adenylyl cyclase-mediated signal transduction coupled to CB1 receptors (Hutcheson *et al.* 1998). Withdrawal effects were also observed in rodents after treatment with the CB1 receptor agonist WIN-55,212-2 (Castañé, Maldonado & Valverde 2004) or exposure to marijuana smoke (Wilson *et al.* 2006). In humans, the '*Cannabis* withdrawal syndrome' is still a controversial issue, and its clinical relevance has remained unclear, although there are evidences that heavy consumers do develop tolerance to its subjective and cardiovascular effects and experience withdrawal effects (Hall & Solowij 1998; Budney & Hughes 2006).

In summary, cannabinoids may increase dopamine release, induce conditioned-place preference, support self-administration, lower intra-cranial self-stimulation threshold and provoke tolerance and withdrawal syndrome. Similar to models of anxiety- and depression-related behaviours, the responses can be bidirectional and, in this sense, can lead to opposing effects. As will be discussed below, understanding the brain regions and the neuronal population related to these effects is mandatory to clarify the complexity of action. Cannabinoids seem to interfere with several other neurotransmitter systems, not only the dopaminergic, but also glutamatergic, GABAergic and the endogenous opioid system. In addition, an obvious concern is whether the doses of Δ^9-THC or synthetic cannabinoids used in the experiments are representative of *Cannabis* smoking. High doses may induce not only aversion, but also motor impairments, confounding the interpretation of the observed behaviours. Furthermore, as discussed above, *Cannabis* does not only contain Δ^9-THC, and the contributions of other phytocannabinoids are not clear at the moment.

ATTEMPT TO RECONCILE THE BIDIRECTIONAL EFFECTS OF CANNABINOIDS ON EMOTIONS

Summarizing the data discussed above, the effects of cannabinoids on emotional responses are bidirectional, depending on various factors, such as the dose administered, paired context and previous experience of the organism. The following paragraphs will discuss the notion that these effects are not necessarily 'contradictory'. Rather, the unique temporal ('on-demand' activity), spatial (distinct neuro-anatomical distribution) and functional (regulation of distinct synaptic processes) properties of the endocannabinoid system may account for this diversity of effects.

'On-demand' functioning of the endocannabinoid system: effects of cannabinoids may be influenced by previous or current stressful events

One widely accepted feature of the endocannabinoid system is its 'on-demand' functioning. Briefly, this term

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refers to the fact that endocannabinoids are synthesized and released 'when they are needed', such as induced by increased neuronal activities. The synthesis occurs at post-synaptic sites in response to Ca^{2+} influx or after activation of metabotropic glutamate or acetylcholine receptors, from where endocannabinoids may reach the synaptic cleft, activate CB1 receptors located on the presynaptic terminal and finally, suppress neuronal activity (Chevaleyre, Takahashi & Castillo 2006; Marsicano & Lutz 2006). Several investigations addressed the possible behavioural consequences of this property, testing whether responses to cannabinoids vary as a function of stressful stimulus intensity, as discussed in the next paragraphs.

Endocannabinoids are released in several brain regions in response to both physical and psychological stress. Generally, their release activates protective mechanisms and counteracts aversive responses by activating CB1 receptor-mediated processes. For instance, anandamide is released in the periaqueductal gray after local electrical stimulation or after subcutaneous injection of a noxious chemical stimulus (Walker *et al.* 1999). Injection of a CB1 receptor antagonist prevented the effects of anandamide release, increasing the intensity of nociceptive responses.

In addition to protection after a physically harmful stimulus, psychological threats may also recruit the endocannabinoid system. Endocannabinoids are released in the amygdala of mice exposed to a tone that was previously paired with foot-shock (Marsicano *et al.* 2002). As commented in the previous section, fear extinction is impaired in CB1 receptor-deficient mice and in CB1 receptor antagonist-treated animals (Marsicano *et al.* 2002). Thus, the endocannabinoid system is presumably activated by threatening stimuli, aiming at reducing aversive memories, leading to fear extinction. 'On-demand' release of endocannabinoids after foot-shock also occurs in the periaqueductal gray, where both anandamide and 2-AG accounts for stress-induced analgesia (Hohmann *et al.* 2005).

Some studies have directly compared the consequences of interventions in the endocannabinoid system in stressed and non-stressed animals. One investigation found no changes in CB1 receptor-deficient mice exposed to the elevated-plus maze, although a phenotype of increased anxiety-like behaviour was revealed when the experiments were conducted under high light intensity, which represents a more stressful environment for mice (Haller *et al.* 2004). In addition, a study showed anxiolytic-like effect of URB597 in mice only when the animals were exposed to the apparatus under high light intensity (Naidu *et al.* 2007). Since stressors activate the endocannabinoid system as a protective mechanism, an elegant approach is the further enhancement of endocannabinoid levels instead of directly activating CB1 receptors with agonists. In line with this notion is the fact that the bidirectional responses often observed with CB1 agonists were not reported with inhibitors of endocannabinoid re-uptake or hydrolysis (Kathuria *et al.* 2003; Bortolato *et al.* 2006).

Direct CB1 receptor-activation by HU210, though, was also shown to be beneficial and was able to alleviate the consequences of chronic unpredictable stress in rats (Hill *et al.* 2005), suggesting that this might also be a promising target for novel antidepressant mechanisms (Hill & Gorzalka 2005a). In fact, apart from the behavioural effects in models predictive of antidepressant activity, endocannabinoids share other properties of widely used antidepressant drugs, such as promoting proliferation of neural progenitor cells in the hippocampus (Aguado *et al.* 2005; Galve-Roperh *et al.* 2007), a process required for the behavioural effects of CB1 receptor agonists in models of anxiety and depression (Jiang *et al.* 2005).

In addition to the nature and intensity of environmental stimuli, responses may also vary as a function of the developmental stage of the organism. The consequences of enhancing cannabinoid signalling may be different in adolescence, a critical developmental period, as compared with adulthood (Marco *et al.* 2007). In fact, conditioned-place aversion and the anxiogenic-like effect induced by Δ^9-THC are lower in adolescent rats than in adults, being in line with the widely observed feature that adolescents may be more prone to *Cannabis* abuse than adults (Quinn *et al.* in press; Schramm-Sapyta *et al.* 2007). To date, the underlying mechanisms for these differences are not known. However, the CB1 receptors display a distinct expression profile during the course of neural pre- and post-natal development, and are involved in several critical processes in the establishment of proper brain functions (Berghuis *et al.* 2007). Thus, disturbances in the CB1 receptor signalling might evoke different effects at the various stages in brain development.

In summary, in an adult, the diversity of effects of cannabinoids in relation to aversive or rewarding events may be because of different intensities of current or previous aversive stimuli. Endocannabinoids may prevent the consequences of stress when their 'on-demand' actions are enhanced by inhibitors of re-uptake or hydrolysis. However, further experiments have to investigate the effects of drugs that directly or indirectly modulate CB1 receptor signalling on experimental subjects under different levels of aversion.

Neuro-anatomical distribution of the endocannabinoid system: cannabinoids interfere with different processes in diverse brain regions

The previous section discussed the 'on-demand' function of the endocannabinoid system, which is activated

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'when' it is need. This paragraph likes to discuss the issue of the distinct spatial activation pattern, thus, 'where' it is activated. Although its precise role in each brain region remains to be established, several pieces of evidence support the view that this system is functional in various structures related to emotions and responses to stressful stimuli. Experiments with intra-cranial drug administration, neuro-anatomical lesions or immunohistochemistry revealed the existence of specific brain regions whose malfunction might lead to psychiatric disorders. The brain regions include the prefrontal cortex, hippocampus, amygdala, periaqueductal gray and some nuclei of the hypothalamus (Graeff 1994; Sewards & Sewards 2002; Dalgleish 2004; Morgane, Galler & Mokler 2005; Singewald 2007). The endocannabinoid system seems to be functional in all these structures (reviewed in Howlett *et al.* 2002; Mackie 2005; Pacher *et al.* 2006). The following paragraphs will discuss its possible role in each of these regions.

In order to address which of these structures are active after cannabinoid treatment, some investigations employed the quantification of c-Fos protein expression as a technique to evaluate neuronal activity (Morgan & Curran 1989). Thus, a single injection of the synthetic cannabinoid HU210 significantly increased the expression of this protein in several nuclei of the extended amygdala and the hypothalamus, in the hippocampus and periaqueductal gray (Rodriguez De Fonseca *et al.*, 1997). Intra-cerebroventricular injection of anandamide also increased the expression in these regions (Patel, Moldow, Patel *et al.* 1998). Among the most intensively activated regions were the dentate gyrus of the hippocampus, the central nucleus of the amygdala, several columns of the periaqueductal gray and the paraventricular nucleus of the hypothalamus (Patel, Moldow, Patel *et al.* 1998). A comparative investigation revealed some overlap between the effects of Δ^9-THC and anandamide, both of them activating the paraventricular nucleus of the hypothalamus and the central amygdala, the effect of Δ^9-THC being more significant (McGregor *et al.* 1998). In consonance with behavioural data reporting that high-dose of cannabinoids may aggravate the consequences of stress, there seems to be also a synergism between Δ^9-THC treatment and acute restraint stress in the activation of c-Fos protein in the central amygdala (Patel *et al.* 2005). Interestingly, this synergism was not observed after the injection of the FAAH inhibitor URB597, again revealing that increasing the levels of endocannabinoids is more successful in alleviating aversive emotions than direct CB1 receptor-activation (Patel *et al.* 2005). Finally, a low anxiolytic-like dose of Δ^9-THC was also able to prevent, rather than enhance, c-Fos protein expression in the amygdala and in the prefrontal cortex of rats exposed to the elevated-plus maze model (Rubino *et al.* 2007b). Thus, similar effects may be achieved by FAAH-blockade or by low dose of Δ^9-THC.

Overall, the brain regions activated by cannabinoids overlap with the neurocircuitries recruited after exposure to aversive stimuli. In addition, CB1 receptor-activation and stressful stimuli may interact to recruit different brain regions. It might be that the systemic administration of cannabinoids differentially modulates various sites in the brain, causing the inconsistent results often observed. In this regard, intra-cranial injections may be an interesting approach to unravel the role of the endocannabinoid system in specific structures and offer insights in their participation in emotional responses induced by cannabinoids.

Several groups have adopted this approach, and a summary from these studies employing intra-cranial injections of compounds that enhance CB1 receptor-mediated signalling is presented in Table 1. Although the picture is not entirely clear, the results suggest that important structures for the anti-aversive effects of cannabinoids are the prefrontal cortex and the periaqueductal gray, while it is possible that the amygdala mediates anxiogenic-like effects. An early study reported that Δ^9-THC failed to induce any behavioural change when injected into the nucleus accumbens of mice exposed to the elevated-plus maze, while an anxiogenic-like effect was detected after injection in central nucleus of the amygdala (Onaivi *et al.* 1995). Although this observation should be interpreted cautiously, since there was also a reduction in the exploration of the enclosed arms of the apparatus, possibly reflecting some motor impairments (Onaivi *et al.* 1995), a more recent publication confirmed this initial observation in rats (Rubino *et al.* 2008). This work also showed that anxiolytic-like effects occur after administration of Δ^9-THC in the prefrontal cortex and in the ventral hippocampus (Rubino *et al.* 2008). On the contrary, an anxiogenic effect was found after an injection of the synthetic cannabinoid WIN-55,212-2 into the CA1 region of the dorsal hippocampus of rats (Roohbakhsh *et al.* 2007). CB1 receptor-activation in the prefrontal cortex by methanandamide as well as by FAAH inhibition with URB597 also reduced anxiety-like behaviours in rats exposed to the elevated-plus maze (Rubino *et al.* in press).

Some investigations also detected anxiolytic-like or anti-aversive effects after local activation of CB1 receptors in the periaqueductal gray. Finn *et al.* (2003) studied the effects of the synthetic cannabinoid HU210 injected into the dorsal periaqueductal gray of rats on aversive responses (hyperlocomotion), provoked by an excitatory amino acid (D,L-homocysteine), a potential model of panic attacks (Beckett & Marsden 1995; Deakin & Graeff 1991). CB1 receptor-activation in this structure was able to inhibit panic-like behaviour (Finn *et al.* 2003). The

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Table 1 Effects of compounds that enhance the endocannabinoid system on reward- and aversion-related responses after injections into specific brain regions.

Substance injected (dose); mechanism	*Brain region (Species)*	*Model*	*Effect*	*Reference*
Δ^9-THC (50–150 μg); CB1/CB2 agonist	Nucleus accumbens (mouse)	Elevated-plus maze	No effect	Onaivi *et al.*, 1995
	Central nucleus of the amygdala		Anxiogenic-like	
HU210 (0.1–5 μg); CB1/CB2 agonist	Dorsal periaqueductal gray (rat)	Chemically-induced aversion	Anti-aversive	Finn *et al.*, 2003
HU210 (5 μg)	Dorsal periaqueductal gray (rat)	Ultra-sound induced aversion	Anti-aversive	Finn *et al.*, 2004
URB597 (0.1 nmol); FAAH inhibitor	Dorsolateral periaqueductal gray (rat)	Stress-induced analgesia	Enhanced	Hohmann *et al.*, 2005
URB602 (0.1 nmol); 2-AG hydrolysis inhibitor			Enhanced	
Δ^9-THC (200 pmol)	Posterior ventral tegmental area (rat)	Conditioned-place preference	Rewarding	Zangen *et al.*, 2006
	Posterior nucleus accumbens—shell		Rewarding	
Anandamide (0.5–50 pmol); CB1 and TRPV1 endogenous agonist	Dorsolateral periaqueductal gray (rat)	Elevated-plus maze	Anxiolytic-like	Moreira *et al.*, 2007
AM404 (0.5–50 pmol); anandamide re-uptake inhibitor			No effect; potentiated anandamide	
ACEA (0.05–5 pmol); anandamide analogue, CB1 agonist			Anxiolytic-like	
WIN-55,212-2 (1–5 μg); CB1/CB2 agonist	CA1 region of the dorsal hippocampus (rat)	Elevated-plus maze	Anxiogenic-like	Roohbakhsh *et al.* 2007
Methanandamide (0.1 μg); anandamide analogue, CB1 agonist	Prefrontal cortex (rat)	Elevated-plus maze	Anxiolytic-like	Rubino *et al.*, in press
Methanandamide (10 μg)			Anxiogenic-like	
URB597 (0.01 μg)			Anxiolytic-like	
URB597 (1 μg)			Trends towards anxiogenic-like	
Δ^9-THC (1–25 μg)	Basolateral amygdala (rat)	Elevated-plus maze	Anxiogenic-like	Rubino *et al.*, 2008
	Ventral hippocampus		Anxiolytic-like	
	Prefrontal cortex		Anxiolytic-like	
HU210 (1 and 2.5 μg)	Dentate gyrus of the dorsal hippocampus (rat)	Forced swim test	Antidepressant-like	McLughlin *et al.*, 2007
URB597 (0.5 and 1 μg)			No effect	
WIN-55,212-2 (1 and 5 μg)	Ventromedial prefrontal cortex (rat)	Forced swim test	Antidepressant-like	Bambico *et al.*, 2007

authors extended these results employing another model in which the aversive responses were induced, by exposing rats to ultrasonic emission (Finn *et al.* 2004). In addition, another work showed that endocannabinoids in the dorsolateral periaqueductal gray account for stress-induced analgesia in rats. This phenomenon was blocked by the CB1 receptor antagonist rimonabant and enhanced by the anandamide hydrolysis inhibitor URB597 or the 2-AG hydrolysis inhibitor URB602 (Hohmann *et al.* 2005). Further supporting a role for this structure in the anti-aversive effects of cannabinoids, local injections of anandamide or its potent analogue, arachidonoyl-2-chloro-ethylamide (ACEA), induced anxiolytic-like effects in rats exposed to the elevated-plus maze (Moreira, Aguiar & Guimaraes 2007). The effect of anandamide was prevented by the CB1 receptor antago-

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nist AM251 and potentiated by the endocannabinoid re-uptake inhibitor AM404, which was not active on its own (Moreira *et al*. 2007). These studies point to this midbrain structure as a major site of action for cannabinoids.

Although Onaivi *et al*. (1995) showed that injections of Δ^9-THC into the nucleus accumbens of mice did not modify anxiety-related behaviours, a more recent study showed that this natural cannabinoid was able to induce self-administration and conditioned-place preference when infused into this structure in rats, consistent with a role of the dopaminergic pathway (Zangen *et al*. 2006). Injection into the ventral tegmental area led to the same effect (Zangen *et al*. 2006), further consolidating the potential role of dopaminergic pathways in the reinforcing and addictive effects of cannabinoids.

Finally, some studies have also tried to identify the brain regions responsible for the antidepressant-like effects induced by CB1 receptor-activation or blockade of endocannabinoid hydrolysis. Injection of the cannabinoid HU210 into the dorsal hippocampus of rats enhanced stress-coping behaviour in the forced swim test, although URB597 was ineffective (McLughlin *et al*. 2007). Thus, different brain regions may account for the effects of CB1 agonists or FAAH inhibitors in models of responses to incontrollable stress. An antidepressant-like effect was observed in the same model after CB1 receptor-activation by WIN-55,212-2 injected into the ventromedial prefrontal cortex (Bambico *et al*. 2007).

As discussed in the paragraphs above, diverse brain regions may be responsible for the effects of systemically administered cannabinoids. It is possible that CB1 receptors have opposite contributions to the observed effects, depending on their locations of activation. Although these studies have provided a certain degree of anatomical resolution, some limitations have to be considered when evaluating the effects of intra-cranial injections of cannabinoids. Similar to the results obtained after systemic treatments, the effects may vary depending on the dose and the agonist, the previous experience of the subjects, the experimental model and the environmental influences. Despite these limitations, such studies may help to elucidate how the various brain structures may act in concert to mediate the complex and bidirectional effects of cannabinoids on emotional responses. The following paragraph addresses the neuronal subpopulations related to the effects of cannabinoids on each of these structures.

Neuromodulatory functions of the endocannabinoid system: cannabinoids interfere with neurotransmitters exerting opposite functions on emotional responses

In addition to the 'on-demand' feature (temporal course of action, contingency to stimuli) and the distinct neuro-anatomical distribution (spatial specificity), the third feature that determines the functional role of the endocannabinoid system in a particular behaviour is characterized by the distinct influence of CB1 receptor-activation on other neurotransmitter systems. Despite the insights gained from studies employing cannabinoid injections into particular brain structures, this technique does not allow us to identify the neuronal subpopulations mediating the effects of cannabinoids in a particular brain region. This is, however, an important issue, as CB1 receptors are located at pre-synaptic terminals with different neurochemical features. CB1 receptor-activation is able to regulate the release of diverse neurotransmitters, such as GABA and glutamate, and presumably also serotonin and dopamine (see e.g., Shen *et al*. 1996; Chevaleyre *et al*. 2006; Laviolette & Grace 2006; Marsicano & Lutz 2006; Bambico *et al*. 2007; Häring *et al*. 2007; Monory *et al*. 2007). Thus, studying neuronal subpopulations responsible for the effects of cannabinoids is a promising strategy in understanding the diversity of their effects, as detailed in the following paragraphs.

The CB1 receptor is one of the most densely expressed receptors in the brain (Herkenham *et al*. 1990). Immunocytochemical analyses in the rodent central nervous system revealed a wide distribution (Tsou *et al*. 1998). The CB1 receptor is predominantly expressed pre-synaptically (Egertova *et al*. 1998), although recently, CB1 receptors on the soma of particular cortical GABAergic interneurons were shown to be involved in endocannabinoid-mediated self-inhibitory processes (Bacci, Huguenard & Prince 2004). Furthermore, *in situ* hybridization techniques revealed forebrain neuronal populations expressing CB1 receptors at different levels. In cortical structures, high densities are present in cholecystokinin-positive GABAergic neurons and low densities in glutamatergic neurons (Marsicano & Lutz 1999). The histochemical data are in agreement with electrophysiological and neurochemical studies. CB1 receptor-activation constitutes an important mechanism to modulate the activity of both GABA- and glutamate-releasing synaptic terminals (Katona *et al*. 1999; Wilson & Nicoll 2002; Monory *et al*. 2006). GABA and glutamate are the two major systems acting in an opposite direction in the control of several neurophysiological processes related to memory and emotional responses, including anxiety, panic responses and depression (Millan 2003; 2006; Myhrer 2003; Charney 2004; Berton & Nestler 2006).

Using electrophysiological experiments, numerous publications addressed the involvement of endocannabinoids and CB1 receptors in the regulation of neurotransmission; in particular, of glutamate and GABA transmission. Endocannabinoids activating CB1 receptors are able to depress both GABA and glutamate transmission for short-term (called DSE and DSI,

respectively) and GABA for long-term (called I-LTD, LTDi; reviewed in Chevaleyre *et al.* 2006; Marsicano & Lutz 2006). It is interesting to note that CB1 receptor agonists such as Δ^9-THC evoke similar effects in these synaptic processes as a pharmacological blockade does (Straiker & Mackie 2005). CB1 receptor agonists apparently occlude the action of endocannabinoids by activating receptors without temporal specificity. Thus, when the endocannabinoid system is stimulated, CB1 receptors are already activated by exogenous cannabinoids and might be desensitized and internalized. This is not only observed in *in vitro* experiments, but also in a single Δ^9-THC injection that can lead to the same effects and inhibit endocannabinoid-mediate synaptic processes (Mato *et al.* 2004). Thus, Δ^9-THC might also prevent CB1 receptor-activation by endocannabinoids. This particular feature makes the interpretation of effects induced by THC rather complex.

Clear evidence for specific roles of CB1 receptors in GABAergic and glutamatergic neurons were provided by conditional deletion of this receptor in specific neuronal populations (Marsicano *et al.* 2003; Monory *et al.* 2006; 2007). For instance, mice lacking CB1 receptors in glutamatergic forebrain neurons are more susceptible to seizures and hippocampal neurotoxicity induced by kainic acid, suggesting an 'on-demand' protection by the endocannabinoid system. Increasing endocannabinoids may counteract excessive neuronal activation and control epileptic circuits by reducing glutamatergic activities (Marsicano *et al.* 2003; Monory *et al.* 2006). Considering that the hippocampus is also relevant for responses to stress and pathological processes underlying anxiety and depression, it is tempting to speculate that a similar mechanism would confer protection against stressful events that lead to these psychiatric disorders.

Distinct neuronal subpopulations possibly relevant to emotional responses and modulated by endocannabinoids are also located in the prefrontal cortex, where CB1 receptors may reduce the activity of both glutamatergic (Auclair *et al.* 2000) and GABAergic neurons (Ferraro *et al.* 2001). There is some evidence that these effects are behaviourally relevant. Cannabinoids may suppress inhibitory synapses in the ventromedial part of this structure, potentiating excitatory projections to serotonergic neurons in the dorsal raphe and thereby eliciting antidepressant-like behaviour in rats (Bambico *et al.* 2007). Endocannabinoids may also modulate dopamine transmission in the prefrontal cortex, a mechanism that might be relevant for the role of this system both in addiction and psychosis (Melis *et al.* 2004a; Laviolette & Grace 2006). A functional interaction of the endocannabinoid system with these other neurotransmitter systems may be particularly relevant for the disruptive effects of cannabinoids in processes of working memory, attention and cognition. The same may be true in the lateral amygdala, where CB1 receptor-activation may inhibit both GABA and glutamate release (Azad *et al.* 2003; 2004). These circuitries may participate in the CB1 receptor-mediated process of conditioned fear extinction (Lafenêtre *et al.* 2007). Furthermore, Laviolette and Grace (2006) proposed that endocannabinoids might work in concert with dopamine in the circuitry connecting the ventral tegmental area, amygdala and prefrontal cortex. Functional disruption in this system would result in emotional processing and sensory perception underlying addiction and schizophrenia. Finally, cannabinoids in the periaqueductal gray reduce electrically evoked inhibitory and excitatory postsynaptic currents via pre-synaptic mechanisms, reducing the probability of GABA or glutamate release, respectively (Vaughan *et al.* 2000). Inhibition of these neurons may activate output systems responsible for analgesic and anti-aversive action of cannabinoids in this structure. Nevertheless, in both the amygdala and the periaqueductal gray, it remains to be investigated how the endocannabinoid system orchestrates opposing mechanisms to mediate the analgesic and anti-aversive effects.

CB1 receptor-activation also modulates both inhibitory and excitatory neurotransmission in neuronal subpopulations relevant to responses to addiction, such as those located in the ventral tegmental area and the nucleus accumbens. In the ventral tegmental area, cannabinoids depress GABAergic input to dopaminergic neurons, thereby increasing the firing of the latter neurons (Szabo, Siemes & Wallmichrath 2002). This would be consistent with the notion that cannabinoids activate mesolimbic pathways and induce behavioural effects such as conditioned-place preference and self-administration. However, post-synaptic depolarization in dopamine-containing neurons of the ventral tegmental area may release endocannabinoids and reduce glutamatergic inputs via retrograde activation of pre-synaptic CB1 receptors. This means that cannabinoids would restrain, rather than excite, dopaminergic neurons (Melis *et al.* 2004b). The picture is not less complex in the nucleus accumbens, where CB1 activation reduces the activity of cortical afferent glutamatergic terminals. Since these neurons may synapse onto GABAergic cell bodies, local GABA release will be reduced. Therefore, the result of CB1 receptor-activation could be a disinhibition of dopaminergic neurons secondary to a reduction in GABAergic activity (Robbe *et al.* 2001). Cannabinoids may also directly inhibit GABAergic neurons in this structure and thereby, reduce the restrain on dopaminergic output from the ventral tegmental area (Manzoni & Bockaert 2001).

Thus, CB1 receptors may indirectly modulate dopaminergic projections from the ventral tegmental area to the

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nucleus accumbens via both excitatory and inhibitory mechanisms. Exogenously applied cannabinoids may misbalance this system and induce their complex effects, ranging from rewarding to aversive behaviours. However, addictive responses to cannabinoids are not only related to dopaminergic transmission. Other neurotransmitters may also contribute to cannabinoid addiction. For instance, several investigations have indicated that the endocannabinoid system seems to work in concert with the endogenous opioid system in relation to several physiological or pathological responses, such as pain and anxiety-related behaviours (Viganò, Rubino & Parolaro 2005). A cross-talk between opioid and cannabinoid systems in behavioural responses related to addiction was also reported (Navarro *et al.* 2001). In fact, the diversity of opioid receptors may contribute to the opposite effects of cannabinoids. One study showed that deletion of μ opioid receptors abolished conditioned-place preference induced by Δ^9-THC in mice, while absence of κ receptors ablated place aversion and unmasked Δ^9-THC-induced place preference (Ghozland *et al.* 2002). In addition to endogenous opioid mechanisms, serotonin-releasing neurons may be another neural subpopulation modulated by cannabinoids. There is neuro-anatomical evidence that CB1 receptors are located on serotonergic neurons (Häring *et al.* 2007), while electrophysiological experiments suggested that URB597 and WIN-55,212-2 increased the firing rate of these neurons at doses that induced an antidepressant activity (Gobbi *et al.* 2005; Bambico *et al.* 2007). Furthermore, the antidepressant-like effect of WIN-55,212-2 was prevented by inhibition of serotonin synthesis (Bambico *et al.* 2007).

Beyond the interactions of CB1 receptors with other neurotransmitters, cannabinoid actions mediated by non-CB1 receptor mechanisms might also be considered. CB2 receptors were also proposed to be relevant for emotional responses related to anxiety, depression and addiction (Onaivi 2006). Apart from CB1 and CB2 receptors, other subtypes of a cannabinoid receptor may exist (reviewed in Begg *et al.* 2005). Targets for endocannabinoids are the transient receptor potential vanilloid type 1 (TRPV1) ion channel (Starowicz, Nigam & Di Marzo 2007), and the peroxisome proliferator-activated nuclear receptor (O'Sulivan 2007). The G protein-coupled GPR55 receptor has recently also gained attention (Baker *et al.* 2006; Ryberg *et al.* 2007), although its functions has been scarcely defined to date. As for TRPV1, some of its functions may oppose those mediated by CB1 receptors. For instance, enhancing anandamide levels in the central nervous system induces biphasic responses on pain modulation, which are attributed to the activation of CB1 receptor and TRPV1 (Maione *et al.* 2006). Furthermore, TRPV1-deficient mice have phenotypes in emotional responses characterized by reduced anxiety and conditioned fear (Marsch *et al.* 2007), which is the opposite as compared with CB1 receptor-deficient mice (Martin *et al.* 2001; Haller *et al.* 2004).

In summary, considering the broad neuromodulatory nature of the endocannabinoid system, the effects of cannabinoids should be discussed in terms of their interactions with a neurotransmitter with opposite functions ('what is it modifying?'). One notion is that the neuronal subpopulations recruited might be a function of CB1 receptor activity. Low levels of ligands may inhibit glutamate transmission, while higher levels may inhibit GABA transmission. This might explain why low doses of cannabinoids tend to induce anxiolytic-like and rewarding effects, while high doses tend to increase anxiety-like behaviour and version. A viable approach to test this hypothesis is the behavioural characterization of conditional mutant mice lacking CB1 receptors in each of these neuronal subpopulations.

CONCLUSION—THE ENDOCANNABINOID SYSTEM AS A BALANCE FOR EMOTIONAL RESPONSES

Since the identification of the CB1 receptor 20 years ago, enormous advances have occurred towards a better understanding of the complex emotional responses to cannabinoids. Important key questions are regarding *when* the system is being activated, *where* it is working and *what* is being modified. The 'on-demand' function of the endocannabinoid system, its level of activity in various brain regions and the fine-tuning of inhibitory and excitatory neuronal activity may partially explain the apparent complexity of cannabinoid effects. Thus, the biphasic effects observed after CB1 receptor-activation are not necessarily contradictory, as the system works as a balance by modulation of excitatory and inhibitory neurotransmission. In fact, the biphasic effects observed after different doses of cannabinoids are not exclusive to emotions. For instance, anandamide, Δ^9-THC and synthetic cannabinoids may increase or decrease locomotion in laboratory rodents depending on the dose applied (McGregor *et al.* 1996; Sulcova, Mechoulam & Fride 1998; Sañudo-Peña *et al.* 2000).

A possible view of the problem may wish to differentiate the emotional responses related to reward and aversion. The idea may be put forward that a 'set point' exists for the endocannabinoid system. Thus, decrease of endocannabinoid activity may reduce reward and increase the aversive state. If this equilibrium is disturbed in psychiatric disorders, 'on-demand' enhancement of the endocannabinoid system (e.g., by FAAH inhibition) could re-establish this 'set point', alleviate the consequences of aversive encounters and thereby induce anxiolytic-like effects. However, at a higher level of

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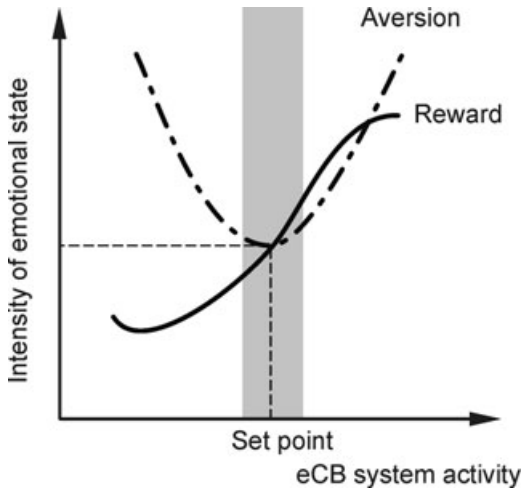

Figure 1 The 'set point hypothesis' for the modulation of reward versus aversion by the endocannabinoid system (eCB system). In this heuristic model, endocannabinoid signalling keeps emotional responses at a physiological range (gray shading). This could be disturbed in psychiatry disorders, such as anxiety and depression. Modulators of the eCB system (e.g., FAAH inhibitors) could restore the 'set point'. Impairment of the eCB system may reduce reward and increase aversive states. A discrete enhancement of the eCB system may favour reward, while aversive states would again prevail after a further increase in the activity of the eCB system

activation (e.g., high doses of CB1 receptor agonists) aversive states would again prevail, occluding the rewarding effects (Fig. 1).

The initial view of anandamide as an endogenous substance that creates a state of 'bliss' can be supported with some limitations, as the detailed temporal, spatial and functional (*when, where* and *what*) activities of the endocannabinoid system in particular behaviours have to be considered.

Acknowledgements

F.A.M. is a recipient of a fellowship from the Alexander von Humboldt Foundation (Germany). B.L. would like to acknowledge the generous support by the Hübner Stiftung.

References

Aceto MD, Scates SM, Lowe JA, Martin BR (1996) Dependence on delta 9-tetrahydrocannabinol: studies on precipitated and abrupt withdrawal. J Pharmacol Exp Ther 278:1290–1295.

Aguado T, Monory K, Palazuelos J, Stella N, Cravatt B, Lutz B, Marsicano G, Kokaia Z, Guzman M, Galve-Roperh I (2005) The endocannabinoid system drives neural progenitor proliferation. FASEB J 19:1704–1706.

Arnold JC, Hunt GE, McGregor IS (2001) Effects of the cannabinoid receptor agonist CP 55940 and the cannabinoid receptor antagonist SR 141716 on intracranial self-stimulation in Lewis rats. Life Sci 70:97–108.

Auclair N, Otani S, Soubrie P, Crepel F (2000) Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex pyramidal neurons. J Neurophysiol 83:3287–3293.

Azad SC, Eder M, Marsicano G, Lutz B, Zieglgansberger W, Rammes G (2003) Activation of the cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. Learn Mem 10:116–128.

Azad SC, Monory K, Marsicano G, Cravatt BF, Lutz B, Zieglgansberger W, Rammes G (2004) Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. J Neurosci 24:9953–9961.

Bacci A, Huguenard JR, Prince DA (2004) Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. Nature 431:312–316.

Baker D, Pryce G, Davies WL, Hiley CR (2006) In silico patent searching reveals a new cannabinoid receptor. Trends Pharmacol Sci 27:1–4.

Bambico FR, Katz N, Debonnel G, Gobby G (2007) Cannabinoids elicit antidepressant-like behaviour and activate serotonergic neurons through the medial prefrontal cortex. J Neurosci 27:11700–11711.

Beckett S, Marsden CA (1995) Computer analysis and quantification of periaqueductal grey-induced defence behaviour. J Neurosci Methods 58:157–161.

Begg M, Pacher P, Batkai S, Osei-Hyiaman D, Offertaler L, Mo FM, Liu J, Kunos G (2005) Evidence for novel cannabinoid receptors. Pharmacol Ther 106:133–145.

Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, Gabriella M, Urbán GM, Monory K, Marsicano G, Matteoli M, Canty A, Irving AJ, Katona I, Yanagawa Y, Rakic P, Lutz B, Mackie K, Harkany T (2007) Hardwiring the brain: endocannabinoids control axon guidance. Science 316:1212–1216.

Berrendero F, Maldonado R (2002) Involvement of the opioid system in the anxiolytic-like effects induced by Delta(9)-tetrahydrocannabinol. Psychopharmacology (Berl) 163:111–117.

Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 7:137–151.

Bortolato M, Campolongo P, Mangieri RA, Scattoni ML, Frau R, Trezza V, La Rana G, Russo R, Calignano A, Gessa GL, Cuomo V, Piomelli D (2006) Anxiolytic-like properties of the anandamide transport inhibitor AM404. Neuropsychopharmacology 31:2652–2659.

Bortolato M, Mangieri RA, Fu J, Kim JH, Arguello O, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D (2007) Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biol Psychiatry 62:1103–1110.

Braida D, Pozzi M, Cavallini R, Sala M (2001a) Conditioned place preference induced by the cannabinoid agonist CP 55,940: interaction with the opioid system. Neuroscience 104:923–926.

Braida D, Pozzi M, Parolaro D, Sala M (2001b) Intracerebral self-administration of the cannabinoid receptor agonist CP 55,940 in the rat: interaction with the opioid system. Eur J Pharmacol 413:227–234.

Braida D, Limonta V, Malabarba L, Zani A, Sala M (2007) 5-HT1A receptors are involved in the anxiolytic effect of Delta9-tetrahydrocannabinol and AM 404, the anandamide transport inhibitor, in Sprague-Dawley rats. Eur J Pharmacol 555:156–163.

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Budney AJ, Hughes JR (2006) The cannabis withdrawal syndrome. Curr Opin Psychiatry 19:233–238.

Castaneda E, Moss DE, Oddie SD, Whishaw IQ (1991) THC does not affect striatal dopamine release: microdialysis in freely moving rats. Pharmacol Biochem Behav 40:587–591.

Castañé A, Maldonado R, Valverde O (2004) Role of different brain structures in the behavioural expression of WIN 55,212-2 withdrawal in mice. Br J Pharmacol 142:1309–1317.

Castren E (2005) Is mood chemistry? Nat Rev Neurosci 6:241–246.

Charney DS (2004) Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. Am J Psychiatry 161:195–216.

Chen JP, Paredes W, Li J, Smith D, Lowinson J, Gardner EL (1990) Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. Psychopharmacology (Berl) 102:156–162.

Chevaleyre V, Takahashi KA, Castillo PE (2006) Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci 29:37–76.

Chhatwal JP, Davis M, Maguschak KA, Ressler KJ (2005) Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. Neuropsychopharmacology 30:516–524.

Compton DR, Johnson MR, Melvin LS, Martin BR (1992) Pharmacological profile of a series of bicyclic cannabinoid analogs: Classification as cannabimimetic agents. J Pharmacol Exp Ther 260:201–209.

Cota D, Steiner MA, Marsicano G, Cervino C, Herman JP, Grubler Y, Stalla J, Pasquali R, Lutz B, Stalla GK, Pagotto U (2007) Requirement of cannabinoid receptor type 1 for the basal modulation of hypothalamic-pituitary-adrenal axis function. Endocrinology 148:1574–1581.

D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu YT, Braley G, Gueorguieva R, Krystal JH (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology 29:1558–1572.

Dalgleish T (2004) The emotional brain. Nat Rev Neurosci 5:583–589.

Rodriguez De Fonseca F, Fernandez-Ruiz JJ, Murphy LL, Cebeira M, Steger RW, Bartke A, Ramos JA (1992) Acute effects of delta-9-tetrahydrocannabinol on dopaminergic activity in several rat brain areas. Pharmacol Biochem Behav 42:269–275.

Deakin JFW, Graeff FG (1991) 5-HT and mechanisms of defence. J Psychopharmacol 5:305–315.

Devane WA, Dysarz FA, 3rd, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34:605–613.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949.

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA 85:5274–5278.

Di Chiara G, Bassareo V (2007) Reward system and addiction: what dopamine does and does not do. Curr Opin Pharmacol 7:69–76.

Egertova M, Giang DK, Cravatt BF, Elphick MR (1998) A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. Proc Biol Sci 265:2081–2085.

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481–1489.

Ferraro L, Tomasini MC, Cassano T, Bebe BW, Siniscalchi A, O'Connor WT, Magee P, Tanganelli S, Cuomo V, Antonelli T (2001) Cannabinoid receptor agonist WIN 55,212-2 inhibits rat cortical dialysate gamma-aminobutyric acid levels. J Neurosci Res 66:298–302.

Finn DP, Jhaveri MD, Beckett SRG, Roe CH, Kendall DA, Marsden CA, Chapman V (2003) Effect of direct periaqueductal grey administration of a cannabinoid receptor agonist on nociceptive and aversive responses in rats. Neuropharmacology 45:594–604.

Finn DP, Jhaveri MD, Beckett SRG, Kendall DA, Marsden CA, Chapman V (2004) Cannabinoids modulate ultrasound-induced aversive responses in rats. Psychopharmacology 172:41–51.

Galve-Roperh I, Aguado T, Palazuelos J, Guzman M (2007) The endocannabinoid system and neurogenesis in health and disease. Neuroscientist 13:109–114.

Gardner EL, Paredes W, Smith D, Dorner A, Milling C, Cohen D Morrison D (1988) Facilitation of brain stimulation reward by delta-9-tetrahydrocannabinol. Psychopharmacology 96: 142–144.

Ghozland S, Matthes HW, Simonin F, Filliol D, Kieffer BL, Maldonado R (2002) Motivational effects of cannabinoids are mediated by mu-opioid and kappa-opioid receptors. J Neurosci 22:1146–1154.

Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D (2005) Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc Natl Acad Sci USA 102:18620–18625.

Graeff FG (1994) Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. Braz J Med Biol Res 27:811–829.

Green B, Kavanagh D, Young R (2003) Being stoned: a review of self-reported cannabis effects. Drug Alcohol Rev 22:453–460.

Griebel G, Stemmelin J, Scatton B (2005) Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. Biol Psychiatry 57:261–267.

Hall W, Solowij N (1998) Adverse effects of cannabis. Lancet 352:1611–1616.

Haller J, Varga B, Ledent C, Freund TF (2004) Context dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behaviour in mice. Eur J Neurosci 19:1906–1912.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 87:1932–1936.

Hill MN, Gorzalka BB (2005a) Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? Behav Pharmacol 16:333–352.

Hill MN, Gorzalka BB (2005b) Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test. Eur Neuropsychopharmacol 15:593–599.

Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, Hillard CJ, Gorzalka BB (2005) Downregulation of endocannabinoid

signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology 30:508–515.

Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Walker JM, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D (2005) An endocannabinoid mechanism for stress-induced analgesia. Nature 435:1108–1112.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54:161–202.

Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, Franck RA (2001) Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry 58:322–328.

Hutcheson DM, Tzavara ET, Smadja C, Valjent E, Roques BP, Hanoune J, Maldonado R (1998) Behavioural and biochemical evidence for signs of abstinence in mice chronically treated with delta-9-tetrahydrocannabinol. Br J Pharmacol 125: 1567–1577.

Häring M, Marsicano G, Lutz B, Monory K (2007) Identification of the cannabinoid receptor type 1 in serotonergic cells of raphe nuclei in mice. Neuroscience 146:1212–1219.

Jiang W, Zhang Y, Xiao L, Van Cleemput J, Ji SP, Bai G, Zhang X (2005) Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. J Clin Invest 115:3104–3116.

Johns A (2001) Psychiatric effects of cannabis. Br J Psychiatry 178:116–122.

Justinova Z, Tanda G, Redhi GH, Goldberg SR (2003) Self-administration of delta9-tetrahydrocannabinol (THC) by drug naive squirrel monkeys. Psychopharmacology (Berl) 169: 135–140.

Kamprath K, Marsicano G, Tang J, Monory K, Bisogno T, Di Marzo V, Lutz B, Wotjak CT (2006) Cannabinoid CB1 receptor mediates fear extinction via habituation-like processes. J Neurosci 26:6677–6686.

Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, *et al* (2003) Modulation of anxiety through blockade of anandamide hydrolysis. Nature Med 9:76–81.

Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K, Freund TF (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 19:4544–4558.

Lafenêtre P, Chaouloff F, Marsicano G (2007) The endocannabinoid system in the processing of anxiety and fear and how CB1 receptors may modulate fear extinction. Pharmacol Res 56:367–381.

Laviolette SR, Grace AA (2006) The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. Cell Mol Life Sci 63:1597–1613.

LeMoal M, Koob G (2007) Drug addiction: pathways to the disease and pathophysiological perspectives. Eur Neuropsychopharmacol 17:377–393.

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, Bohme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, Parmentier M (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 283:401–404.

Lepore M, Liu X, Savage V, Matalon D, Gardner EL (1996) Genetic differences in Δ9-tetrahydrocannabinol-induced facilitation of brain stimulation reward as measured by a rate-frequency curve-shift electrical brain stimulation paradigm in three different rat strains. Life Sci (Pharmacol Lett) 58:PL365–PL372.

Lupica CR, Riegel AC, Hoffman AF (2004) Marijuana and cannabinoid regulation of brain reward circuits. Br J Pharmacol 143:227–234.

Lutz B (2007) The endocannabinoid system and extinction learning. Mol Neurobiol 36:92–101.

McGregor IS, Arnold JC, Weber MF, Topple AN, Hunt GE (1998) A comparison of delta 9-THC and anandamide induced c-fos expression in the rat forebrain. Brain Res 802:19–26.

McGregor IS, Issakidis CN, Prior G (1996) Aversive effects of the synthetic cannabinoid CP 55,940 in rats. Pharmacol Biochem Behav 53:657–664.

Mackie K (2005) Distribution of cannabinoid receptors in the central and peripheral nervous system. Hand Exp Pharmacol 168:299–325.

McLughlin RJ, Hill MN, Morrish AC, Gorzalka BB (2007) Local enhancement of cannabinoid CB1 receptor signalling in the dorsal hippocampus elicits an antidepressant-like effect. Behav Pharmacol 18:431–438.

Maione S, Bisogno T, de Novellis V, Palazzo E, Cristino L, Valenti M, Petrosino S, Guglielmotti V, Rossi F, Di Marzo V (2006) Elevation of endocannabinoid levels in the ventrolateral periaqueductal grey through inhibition of fatty acid amide hydrolase affects descending nociceptive pathways via both cannabinoid receptor type 1 and transient receptor potential vanilloid type-1 receptors. J Pharmacol Exp Ther 316:969–982.

Maldonado R (2002) Study of cannabinoid dependence in animals. Pharmacol Ther 95:153–164.

Maldonado R, Valverde O, Berrendero F (2006) Involvement of the endocannabinoid system in drug addiction. Trends Neurosci 29:225–232.

Mansbach RS, Nicholson KL, Martin BR, Balster RL (1994) Failure of Delta(9)-tetrahydrocannabinol and CP 55,940 to maintain intravenous self-administration under a fixed-interval schedule in rhesus monkeys. Behav Pharmacol 5:219–225.

Manzoni OJ, Bockaert J (2001) Cannabinoids inhibit GABAergic synaptic transmission in mice nucleus accumbens. Eur J Pharmacol 412:R3–5.

Marco EM, Adriani W, Canese R, Podo F, Viveros MP, Laviola G (2007) Enhancement of endocannabinoid signalling during adolescence: modulation of impulsivity and long-term consequences on metabolic brain parameters in early maternally deprived rats. Pharmacol Biochem Behav 86:334–345.

Marsch R, Foeller E, Rammes G, Bunck M, Kossl M, Holsboer F, Zieglgansberger W, Landgraf R, Lutz B, Wotjak CT (2007) Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. J Neurosci 27:832–839.

Marsicano G, Lutz B (1999) Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci 11:4213–4225.

Marsicano G, Lutz B (2006) Neuromodulatory functions of the endocannabinoid system. J Endocrinol Invest 29(Suppl 3):27–46.

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgansberger W, Di Marzo V, Lutz B (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530–534.

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Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutierrez SO, van der Stelt M, Lopez-Rodriguez ML, Casanova E, Schutz G, Zieglgansberger W, Di Marzo V, Behl C, Lutz B (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 302:84–88.

Martellotta MC, Cossu G, Fattore L, Gessa GL, Fratta W (1998) Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. Neuroscience 85:327–330.

Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, Johnson MR, Melvin LS, Mechoulam R, Ward SJ (1991) Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. Pharmacol Biochem Behav 40:471–478.

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O (2001) Involvement of CB1 cannabinoid receptors in emotional behaviour. Psychopharmacology (Berl) 159:379–387.

Mato S, Chevaleyre V, Robbe D, Pazos A, Castillo PE, Manzoni OJ (2004) A single in-vivo exposure to delta 9THC blocks endocannabinoid-mediated synaptic plasticity. Nat Neurosci 7:585–586.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564.

Melis M, Perra S, Muntoni AL, Pillolla G, Lutz B, Marsicano G, Di Marzo V, Gessa GL, Pistis M (2004a) Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. J Neurosci 24:10707–10715.

Melis M, Pistis M, Perra S, Muntoni AL, Pillolla G, Gessa GL (2004b) Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. J Neurosci 24:53–62.

Millan MJ (2003) The neurobiology and control of anxious states. Prog Neurobiol 70:83–244.

Millan MJ (2006) Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. Pharmacol Ther 110:135–370.

Monory K, Massa F, Egertova M, Eder M, Blaudzun H, Westenbroek R, Kelsch W, Jacob W, Marsch R, Ekker M, Long J, Rubenstein JL, Goebbels S, Nave KA, During M, Klugmann M, Wolfel B, Dodt HU, Zieglgansberger W, Wotjak CT, Mackie K, Elphick MR, Marsicano G, Lutz B (2006) The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron 51:455–466.

Monory K, Blaudzun H, Massa F, Kaiser N, Lemberger T, Schutz G, Wotjak CT, Lutz B, Marsicano G (2007) Genetic dissection of behavioural and autonomic effects of Delta(9)-Tetrahydrocannabinol in Mice. PLoS Biol 5:e269.

Moreira FA, Aguiar DC, Guimaraes FS (2006) Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. Prog Neuropsychopharmacol Biol Psychiatry 30:1466–1471.

Moreira FA, Aguiar DC, Guimaraes FS (2007) Anxiolytic-like effect of cannabinoids injected into the rat dorsolateral periaqueductal gray. Neuropharmacology 52:958–965.

Moreira FA, Kaiser N, Monory K, Lutz B (2008) Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by CB1 receptors. Neuropharmacology 54:141–150.

Morgan JI, Curran T (1989) Stimulus transcription coupling in neurons: role of cellular immediate-early genes. Trends Neurosci 12:459–462.

Morgane PJ, Galler JR, Mokler DJ (2005) A review of systems and networks of the limbic forebrain/limbic midbrain. Prog Neurobiol 75:143–160.

Myhrer T (2003) Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based on studies of four behavioral tasks. Brain Res Brain Res Rev 41:268–287.

Nadulski T, Pragst F, Weinberg G, Roser P, Schnelle M, Fronk EM, Stadelmann AM (2005) Randomized, double-blind, placebo-controlled study about the effects of cannabidiol (CBD) on the pharmacokinetics of Delta9-tetrahydrocannabinol (THC) after oral application of THC verses standardized cannabis extract. Ther Drug Monit 27:799–810.

Naidu PS, Varvel SA, Ahn K, Cravatt BF, Martin BR, Lichtman AH (2007) Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. Psychopharmacology (Berl) 192:61–70.

Navarro M, Hernandez E, Munoz RM, del Arco I, Villanua MA, Carrera MR, Rodriguez de Fonseca F (1997) Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. Neuroreport 8:491–496.

Navarro M, Carrera MR, Fratta W, Valverde O, Cossu G, Fattore L, Chowen JA, Gomez R, del Arco I, Villanua MA, Maldonado R, Koob GF, Rodriguez de Fonseca F (2001) Functional interaction between opioid and cannabinoid receptors in drug self-administration. J Neurosci 21:5344–5350.

O'Sulivan SE (2007) Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptor. Br J Pharmacol 152:576–582.

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J Comp Physiol Psychol 47:419–427.

Onaivi ES (2006) Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. Neuropsychobiology 54:231–246.

Onaivi ES, Green MR, Martin BR (1990) Pharmacological characterization of cannabinoids in the elevated plus maze. J Pharmacol Exp Ther 253:1002–1009.

Onaivi ES, Chakrabarti A, Gwebu ET, Chaudhuri G (1995) Neurobehavioral effects of delta-9-THC and cannabinoid CB1 receptor gene expression in mice. Behav Brain Res 72:115–125.

Pacher P, Batkai S, Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 58:389–462.

Pamplona FA, Prediger RD, Pandolfo P, Takahashi RN (2006) The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. Psychopharmacology (Berl) 188:641–649.

Parker LA, Gillies T (1995) THC-induced place and taste aversions in Lewis and Sprague–Dawley rats. Behav Neurosci 109:71–78.

Patel NA, Moldow RL, Patel JA, Wu G, Chang SL (1998) Arachidonylethanolamide (AEA) activation of FOS proto-oncogene protein immunoreactivity in the rat brain. Brain Res 797:225–233.

Patel S, Hillard CJ (2006) Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. J Pharmacol Exp Ther 318:304–311.

Journal compilation © 2008 Society for the Study of Addiction. No claim to original US government works

Patel S, Cravat BF, Hillard CJ (2005) Synergistic interactions between cannabinoids and environmental stress in the activation of the central amygdala. Neuropsychopharmacology 30:497–507.

Pertwee RG, Thomas A, Stevenson LA, Ross RA, Varvel SA, Lichtman AH, Martin BR, Razdan RK (2007) The psychoactive plant cannabinoid, Delta9-tetrahydrocannabinol, is antagonized by Delta8- and Delta9-tetrahydrocannabivarin in mice in vivo. Br J Pharmacol 150:586–594.

Piomelli D (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4:873–884.

Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, Gunasekaran N, Thompson MR, Dawson B, Mallet PE, Kashem MA, Matsuda-Matsumoto H, Iwazaki T, McGregor IS (2007) Adolescent rats find repeated delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. Neuropsychopharmacology. Epub ahead of print.

Rademacher DJ, Hillard CJ (2007) Interactions between endocannabinoids and stress-induced decreased sensitivity to natural reward. Prog Neuropsychopharmacol Biol Psychiatry 31:633–641.

Robbe D, Alonso G, Duchamp F, Bockaert J, Manzoni OJ (2001) Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. J Neurosci 21:109–116.

Rodgers RJ, Haller J, Halasz J, Mikics E (2003) One-trial sensitization' to the anxiolytic-like effects of cannabinoid receptor antagonist SR141716A in the mouse elevated plus-maze. Eur J Neurosci 17:1279–1286.

Rodriguez De Fonseca F, Carrera MR, Navarro M, Koob GF, Weiss F (1997) Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. Science 276:2050–2054.

Roohbakhsh A, Moghaddam AH, Massoudi R, Zarrindast MR (2007) Role of dorsal hippocampal cannabinoid receptors and nitric oxide in anxiety like behaviours in rats using the elevated plus-maze test. Clin Exp Pharmacol Physiol 34:223–229.

Rubino T, Realini N, Castiglioni C, Guidali C, Vigano D, Marras E, Petrosino S, Perletti G, Maccarrone M, Di Marzo V, Parolaro D (2007a) Role in Anxiety behavior of the endocannabinoid system in the prefrontal cortex. Cerebral Cortex. Epub ahead of print.

Rubino T, Sala M, Vigano D, Braida D, Castiglioni C, Limonta V, Guidali C, Realini N, Parolaro D (2007b) Cellular mechanisms underlying the anxiolytic effect of low doses of peripheral delta(9)-tetrahydrocannabinol in rats. Neuropsychopharmacology 32:2036–2045.

Rubino T, Guidali C, Vigano D, Realini N, Valenti M, Massi P, Parolaro D (2008) CB1 receptor stimulation in specific brain areas differently modulate anxiety-related behaviour. Neuropharmacology 54:161–160.

Rutkowska M, Jamontt J, Gliniak H (2006) Effects of cannabinoids on the anxiety-like response in mice. Pharmacol Rep 58:200–206.

Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol 152:1092–1101.

Sanchis-Segura C, Spanagel R (2006) Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. Addict Biol 11:2–38.

Sañudo-Peña MC, Tsou K, Delay ER, Hohman AG, Force M, Walker JM (1997) Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. Neurosci Lett 223:125–128.

Sañudo-Peña MC, Romero J, Seale GE, Fernandez-Ruiz JJ, Walker JM (2000) Activational role of cannabinoids on movement. Eur J Pharmacol 391:269–274.

Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF (2006) RIO-Diabetes Study Group. Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. Lancet 368:1660–1672.

Schramm-Sapyta NL, Cha YM, Chaudhry S, Wilson WA, Swartzwelder HS, Kuhn CM (2007) Differential anxiogenic, aversive, and locomotor effects of THC in adolescent and adult rats. Psychopharmacology (Berl) 191:867–877.

Sewards TV, Sewards MA (2002) Fear and power-dominance drive motivation: neural representations and pathways mediating sensory and mnemonic inputs, and outputs to premotor structures. Neurosci Biobehav Rev 26:553–579.

Shearman LP, Rosko KM, Fleischer R, Wang J, Xu S, Tong XS, Rocha BA (2003) Antidepressant-like and anorectic effects of the cannabinoid CB1 receptor inverse agonist AM251 in mice. Behav Pharmacol 14:573–582.

Shen M, Piser TM, Seybold VS, Thayer SA (1996) Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci 16:4322–4334.

Singewald N (2007) Altered brain activity processing in high-anxiety rodents revealed by challenge paradigms and functional mapping. Neurosci Biobehav Rev 31:18–40.

Starowicz K, Nigam S, Di Marzo V (2007) Biochemistry and pharmacology of endovanilloids. Pharmacol Ther 114:13–33.

Steiner MA, Wanisch K, Monory K, Marsicano G, Borroni E, Bächli H, Holsboer F, Lutz B, Wotjak CT (2007) Impaired cannabinoid receptor type 1 signaling interferes with stress coping behavior in mice. Pharmacogenomics J. Epub ahead of print.

Steiner MA, Marsicano G, Nestler EJ, Holsboer F, Lutz B, Wotjak CT (2008) Antidepressant-like behavioural effects of impaired cannabinoid receptor type 1 signalling coincide with exaggerated corticosterone secretion in mice. Psychoneuroendocrionolgy 33:54–67.

Straiker A, Mackie K (2005) Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones. J Physiol 569(Part 2):501–517.

Sulcova E, Mechoulam R, Fride E (1998) Biphasic effects of anandamide. Pharmacol Biochem Behav 59:347–352.

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci 24:4787–4795.

Szabo B, Siemes S, Wallmichrath I (2002) Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. Eur J Neurosci 15:2057–2061.

Tanda G, Goldberg SR (2003) Cannabinoids: reward, dependence and underlying neurochemical mechanisms – a review of recent pre-clinical data. Psychopharmacology 169:115–134.

Tanda G, Pontieri FE, Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 276:2048–2050.

Tanda G, Munzar P, Goldberg SR (2000) Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat Neurosci 3:1073–1074.

Journal compilation © 2008 Society for the Study of Addiction. No claim to original US government works

Thomas H (1996) A community survey of adverse effects of cannabis use. Drug Alcohol Depend 42:201–207.

Tsou K, Brown S, Sañudo-Peña MC, Mackie K, Walker JM (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83:393–411.

Valjent E, Maldonado R (2000) A behavioural model to reveal conditioned place-preference to Δ9-tetrahydrocannabinol in mice. Psychopharmacology 147:436–438.

Valjent E, Mitchell JM, Besson MJ, Caboche J, Maldonado R (2002) Behavioural and biochemical evidence for interactions between Delta 9-tetrahydrocannabinol and nicotine. Br J Pharmacol 135:564–578.

Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S (2005) RIO-Europe Study Group. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. Lancet 365:1389–1397.

Vaughan CW, Connor M, Bagley EE, Christie MJ (2000) Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro. Mol Pharmacol 57:288–295.

Viganò D, Rubino T, Parolaro D (2005) Molecular and cellular basis of cannabinoid and opioid interactions. Pharmacol Biochem Behav 81:360–368.

Viveros MP, Marco EM, File SE (2005) Endocannabinoid system and stress and anxiety responses. Pharmacol Biochem Behav 81:331–342.

Vlachou S, Nomikos GG, Panagis G (2006) Effects of endocannabinoid neurotransmission modulators on brain stimulation reward. Psychopharmacology (Berl) 188:293–305.

Voruganti LN, Slomka P, Zabel P, Mattar A, Awad AG (2001) Cannabis induced dopamine release: an in-vivo SPECT study. Psychiatry Res 107:173–177.

Walker JM, Huang SM, Strangman NM, Tsou K, Sanudo-Pena MC (1999) Pain modulation by release of the endogenous cannabinoid anandamide. Proc Natl Acad of Sci USA 96:12198–12203.

Wilson DM, Varvel SA, Harloe JP, Martin BR, Lichtman AH (2006) SR 141716 (Rimonabant) precipitates withdrawal in marijuana-dependent mice. Pharmacol Biochem Behav 85:105–113.

Wilson RI, Nicoll RA (2002) Endocannabinoid signaling in the brain. Science 296:678–682.

Wise R (2005) Forebrain substrates of reward and motivation. J Comp Neurol 493:115–121.

Wotjak CT (2005) Role of endogenous cannabinoids in cognition and emotionality. Mini Rev Med Chem 5:659–670.

Zangen A, Solinas M, Ikemoto S, Goldberg SR, Wise RA (2006) Two brain sites for cannabinoid reward. J Neurosci 26:4901–4907.

Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG (1982) Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. Psychopharmacology 76:245–250.

Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimaraes FS (2006) Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. Braz J Med Biol Res 39:421–429.

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The award is for the best published paper on any topic related to addiction written by a young scholar from a low or middle income country. The successful applicant will receive a certificate and financial support to attend an international scientific or clinical meeting, to be chosen by the winner in consultation with ISAJE.

To be eligible, the paper must have been published either online or in print form in a peer-reviewed scholarly journal between 1 July 2007 and 30 June 2008. The research reported should have been carried out predominantly in a low or middle income country, as specified by the World Bank classification. Applicants must be less than 35 years old and should be the lead author in the paper being submitted for the award. They should hold a current academic or research position in a low or middle income country; or should have held such a position at the time the research for the paper was carried out.

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The award is sponsored by ISAJE, WHO and Virginia Commonwealth University.