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THE ENDOCANNABINOID SYSTEM: A KEY MODULATOR OF EMOTIONS AND COGNITION

Hosted by
Patrizia Campolongo and Viviana Trezza

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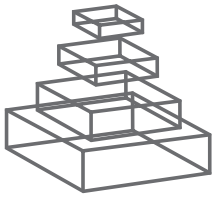

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THE ENDOCANNABINOID SYSTEM: A KEY MODULATOR OF EMOTIONS AND COGNITION

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The isolation and identification, in 1964, of delta-9-tetrahydrocannabinol (THC), the primary psychoactive compound in cannabis, opened the door to a whole new field of medical research.

The exploration of the therapeutic potential of THC and other natural and synthetic cannabinoid compounds was paralleled by the discovery of the endocannabinoid system, consisting of cannabinoid receptors (CB1 and CB2), their endogenous lipid ligands (endocannabinoids) and the enzymatic machinery for their synthesis and degradation. Cannabinoid receptors are highly expressed in the central nervous system, where endocannabinoids act as retrograde signaling messengers to exert a modulatory control of postsynaptic neurotransmission.

Endocannabinoid regulation of ion channel activity and neurotransmitter release in brain areas involved in the modulation of emotions and cognition has important functional consequences and provides unique therapeutic possibilities: thus, there is ample evidence that modulation of cannabinoid CB1 receptor signaling may affect emotional learning, executive functions, fear and stress responses, basal emotional states, gratification and perception of pleasure for both natural and drug rewards.

This special issue will bring together leading experts in the field to provide a deep overview of the physiological and pathophysiological role of the endocannabinoid system in the modulation of emotions and cognition, and will suggest the pathway of future research in this field.

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EDITORIAL
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The endocannabinoid system: a key modulator of emotions and cognition

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The endocannabinoid system is a unique neuromodulatory system in mammalian physiology. It consists of cannabinoid receptors (CB1 and CB2), their endogenous lipid ligands [endocannabinoids, including anandamide (AEA), and 2-arachidonoylglycerol (2-AG)] and the enzymes for ligand synthesis and degradation. In recent years, brain endocannabinoids have emerged as key modulators of affect, motivation and emotions, and the endocannabinoid system is nowadays considered an intriguing target for the development of selective and specific compounds able to treat several psychiatric disorders. This e-book brings together leading experts in the field to provide a deep overview of the physiological and pathophysiological role of the endocannabinoid system in the modulation of emotions and cognition.

The e-book opens with a review where Battista et al. provide a general overview on the endocannabinoid system and then focus on the metabolic and signal transduction pathways of the main endocannabinoids, AEA and 2-AG. At the end, the authors briefly discuss the therapeutic potential of new cannabinoid drugs (Battista et al., 2012). This issue is further elaborated in the following review, where Marco et al. provide both clinical and preclinical evidence supporting the involvement of the endocannabinoid system in several neuropsychiatric disorders (Marco et al., 2011).

The role of the endocannabinoid system in the modulation of emotions and cognition is widely underscored by several reviews of this e-book. Zanettini et al. broadly introduce this topic by discussing the results of studies performed in laboratory animals (Zanettini et al., 2011), while Rubino and Parolaro address this issue from a sexually-dimorphic perspective (Rubino and Parolaro, 2011).

The original research article by Terzian et al. investigated the potential cross-talk between dopaminergic and cannabinoid neurotransmission in the modulation of emotions and cognition (Terzian et al., 2011). The authors showed that conditional CB1 receptor knock-out animals lacking CB1 cannabinoid receptors in neurons expressing D1dopamine receptors exhibited significantly increased contextual and auditory-cued fear compared to wild-type animals, suggesting that a specific reduction of endocannabinoid signaling in neurons expressing dopamine D1 receptor is able to affect acute fear adaptation (Terzian et al., 2011). In their commentary on this research article, Akirav and Fattore discuss about the potential clinical implication of these findings, and indicate the future directions for research in this field (Akirav and Fattore, 2011).

The preclinical studies reviewed by Trezza and co-workers show that cannabinoid modulation of emotionality and cognitive performance appears since early developmental stages; indeed, evidence has been provided over the last few years that animals exposed to cannabinoid drugs during the perinatal, prenatal or adolescent period show long-lasting changes in emotional reactivity and cognitive processing (Campolongo et al., 2007, 2009, 2011; Trezza et al., 2012).

The effects of cannabinoid drugs on hippocampal memory and plasticity are discussed by Akirav (Akirav, 2011); on the basis of the existing literature, she concludes that these effects may vary depending on the route of drug administration, the nature of the task used, whether it involves emotional or non-emotional memory formation, and according to the memory stage under investigation (acquisition, consolidation, retrieval, and extinction) and the brain areas involved (Akirav, 2011).

To study the role of CB1 cannabinoid receptors in the medial prefrontal cortex on cognitive flexibility and emotional behavior in rats, Klugmann et al. upregulated CB1 cannabinoid receptors selectively in this brain area by using adeno-associated viral vector-mediated gene transfer (Klugmann et al., 2011). In their research article, these authors showed that upregulation of CB1 receptors specifically in the rat medial prefrontal cortex induces alterations in emotional reactivity, leads to inadequate social behavior, and impairs cognitive flexibility (Klugmann et al., 2011). In the following research article published on this e-book, Hernandez et al. shed more light on the role of CB1 cannabinoid receptors in mediating reward-seeking behaviors (Hernandez et al., 2011). In particular, the authors showed that, unlike lithium chloride, the CB1 receptor antagonist AM251 did not affect instrumental responding for brain stimulation reward. On the basis of these findings, the authors hypothesize that endocannabinoids are primarily involved with the motivational rather than the intrinsic aspects of reward processing (Hernandez et al., 2011).

The last three articles included in this e-book address the topic of cannabinoid modulation of emotions and cognition from a clinical perspective. The first of these studies is a research article where Spronk and colleagues showed that the active ingredient of Cannabis Δ9-tetrahydrocannabinol (THC) alters performance monitoring, that is a process that allows humans to respond actively and safely to changing environmental demands

(Spronk et al., 2011). This study supports the opinion that Cannabis use during performance of complex functions like driving, which require a high level of performance monitoring, might be particularly risky. Fattore and Fratta address a very hot and timely topic, that is the availability of a new generation of drugs that, although devoid of tobacco or Cannabis, when smoked produce effects similar to those induced by THC (Fattore and Fratta, 2011). The authors first outline the general characteristics of these drugs, such as their content and their effects, and then address the consequences that their use has for both health and society (Fattore and Fratta, 2011). The last contribution to this e-book is the opinion article by Bhattacharyya and Sendt, that provides evidence from neuroimaging studies that cannabinoid drugs affect brain areas involved in cognitive and emotional processes (Bhattacharyya and Sendt, 2012).

Altogether, the collection of articles included in this e-book demonstrates that endocannabinoids play a crucial role in the regulation of emotionality and cognitive performance, as outlined by both rodent and human studies. We hope that it will be apparent to the readers how far we have come in recent years in understanding the functions of brain endocannabinoids in both physiological and pathological conditions, and which are the current challenges for researchers working in this field.

REFERENCES

Akirav, I. (2011). The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus. *Front. Behav. Neurosci.* 5:34. doi: 10.3389/fnbeh.2011.00034

Akirav, I., and Fattore, L. (2011). Cannabinoid CB1 and dopamine D1 receptors partnership in the modulation of emotional neural processing. *Front. Behav. Neurosci.* 5:67. doi: 10.3389/fnbeh.2011.00067

Battista, N., Di Tommaso, M., Bari, M., and Maccarrone, M. (2012). The endocannabinoid system: an overview. *Front. Behav. Neurosci.* 6:9. doi: 10.3389/fnbeh.2012.00009

Bhattacharyya, S., and Sendt, K. V. (2012). Neuroimaging evidence for cannabinoid modulation of cognition and affect in man. *Front. Behav. Neurosci.* 6:22. doi: 10.3389/fnbeh.2012.00022

Campolongo, P., Trezza, V., Cassano, T., Gaetani, S., Morgese, M. G., Ubaldi, M., et al. (2007). Perinatal exposure to delta-9-tetrahydrocannabinol causes enduring cognitive deficits associated with alteration of cortical gene expression and neurotransmission in rats. *Addict. Biol.* 12, 485–495.

Campolongo, P., Trezza, V., Palmery, M., Trabace, L., and Cuomo, V. (2009). Developmental exposure to cannabinoids causes subtle and enduring neurofunctional alterations. *Int. Rev. Neurobiol.* 85, 117–133.

Campolongo, P., Trezza, V., Ratano, P., Palmery, M., and Cuomo, V. (2011). Developmental consequences of perinatal cannabis exposure: behavioral and neuroendocrine effects in adult rodents. *Psychopharmacology (Berl.)* 214, 5–15.

Fattore, L., and Fratta, W. (2011). Beyond THC: the new generation of cannabinoid designer drugs. *Front. Behav. Neurosci.* 5:60. doi: 10.3389/fnbeh.2011.00060

Hernandez, G., Bernstein, D., Schoenbaum, G., and Cheer, J. F. (2011). Contrasting effects of lithium chloride and CB1 receptor blockade on enduring changes in the valuation of reward. *Front. Behav. Neurosci.* 5:53. doi: 10.3389/fnbeh.2011.00053

Klugmann, M., Goepfrich, A., Friemel, C. M., and Schneider, M. (2011). AAV-Mediated overexpression of the CB1 receptor in the mPFC of adult rats alters cognitive flexibility, social behavior, and emotional reactivity. *Front. Behav. Neurosci.* 5:37. doi: 10.3389/fnbeh.2011.00037

Marco, E. M., Garcia-Gutierrez, M. S., Bermudez-Silva, F. J., Moreira, F. A., Guimaraes, F., Manzanares, J., et al. (2011). Endocannabinoid system and psychiatry: in search of a neurobiological basis for detrimental and potential therapeutic effects. *Front. Behav. Neurosci.* 5:63. doi: 10.3389/fnbeh.2011.00063

Rubino, T., and Parolaro, D. (2011). Sexually dimorphic effects of cannabinoid compounds on emotion and cognition. *Front. Behav. Neurosci.* 5:64. doi: 10.3389/fnbeh.2011.00064

Spronk, D., Dumont, G. J., Verkes, R. J., and De Bruijn, E. R. (2011). Acute effects of delta-9-tetrahydrocannabinol on performance monitoring in healthy volunteers. *Front. Behav. Neurosci.* 5:59. doi: 10.3389/fnbeh.2011.00059

Terzian, A. L., Drago, F., Wotjak, C. T., and Micale, V. (2011). The dopamine and cannabinoid interaction in the modulation of emotions and cognition: assessing the role of cannabinoid CB1 receptor in neurons expressing dopamine D1 receptors. *Front. Behav. Neurosci.* 5:49. doi: 10.3389/fnbeh.2011.00049

Trezza, V., Campolongo, P., Manduca, A., Morena, M., Palmery, M., Vanderschuren, L. J., et al. (2012). Altering endocannabinoid neurotransmission at critical developmental ages: impact on rodent emotionality and cognitive performance. *Front. Behav. Neurosci.* 6:2. doi: 10.3389/fnbeh.2012.00002

Zanettini, C., Panlilio, L. V., Alicki, M., Goldberg, S. R., Haller, J., and Yasar, S. (2011). Effects of endocannabinoid system modulation on cognitive and emotional behavior. *Front. Behav. Neurosci.* 5:57. doi: 10.3389/fnbeh.2011.00057

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REVIEW ARTICLE
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The endocannabinoid system: an overview

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Upon the identification of anandamide (AEA) in the porcine brain, numerous studies contributed to the current state of knowledge regarding all elements that form the "endocannabinoid system (ECS)." How this complex system of receptors, ligands, and enzymes is integrated in helping to regulate fundamental processes at level of central nervous and peripheral systems and how its regulation and dysregulation might counteract disturbances of such functions, is nowadays still under investigation. However, the most recent advances on the physiological distribution and functional role of ECS allowed the progress of various research tools aimed at the therapeutic exploitation of endocannabinoid (eCB) signaling, as well as the development of novel drugs with pharmacological advantages. Here, we shall briefly overview the metabolic and signal transduction pathways of the main eCBs representatives, AEA, and 2-arachidonoylglycerol (2-AG), and we will discuss the therapeutic potential of new ECS-oriented drugs.

Keywords: anandamide, 2-arachidonoylglycerol, endocannabinoids, metabolic pathways, signal transduction

ENDOCANNABINOID SYSTEM: METABOLISM AND TARGETS OF ENDOCANNABINOIDS

Starting from 1992, when anandamide (AEA) was identified for the first time in the porcine brain (Devane et al., 1992), numerous studies contributed to the current state of knowledge regarding all elements that form the "endocannabinoid system (ECS)" (Maccarrone et al., 2010). Endocannabinoids (eCBs) are lipid mediators, isolated from brain and peripheral tissues that include amides, esters, and ethers of long chain polyunsaturated fatty acids; they mimic the action of Δ^9-tetrahydrocannabinol (THC) in different biological processes. Until now, the most bioactive eCBs are anandamide (arachidonylethanolamide; AEA) and 2-arachidonoylglycerol (2-AG), yet the eCBs family includes also virodhamine, noladin ether, and *N*-arachidonoyldopamine (NADA), besides homo-linolenylethanolamide (HEA), docosatetraenylethanolamide (DEA), and other cognate compounds such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) (**Figure 1**).

eCBs are released "on demand" from membrane phospholipid precursors and, although AEA synthesis might be due to several metabolic routes (Muccioli, 2010), *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) is currently considered the major enzyme responsible for AEA production (Okamoto et al., 2009), whereas a specific phospholipase C followed by the activity of the *sn*-1-diacylglycerol lipase (DAGL) is responsible for 2-AG synthesis (Ueda et al., 2011). The cellular uptake from the extracellular to the intracellular space is ascribed to a purported "endocannabinoid membrane transporter (EMT)" that is likely to take up both AEA and 2-AG. However, while there is wide experimental evidence to support the concept that AEA transport across membranes is protein-mediated, conclusive evidence of its molecular identity is still lacking. Very recently, a partly truncated fatty acid amide hydrolase-1 (FAAH-1) termed FAAH-1 like anandamide transporter (FLAT) has been reported in neural cells (Fu et al., 2011). After re-uptake, the biological activity of eCBs is ended by a FAAH, for AEA (McKinney and Cravatt, 2005), and/or by a specific monoacylglycerol lipase (MAGL), for 2-AG (Dinh et al., 2002). Additionally, other enzymes showing "amidase signature," such as FAAH-2 (Wei et al., 2006) and the *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) (Tsuboi et al., 2005), which belongs to the choloylglycine hydrolase family, might bind with low affinity and hydrolyse AEA to release arachidonic acid and ethanolamine. Also cyclooxygenase-2 (COX-2), different lipoxygenase (LOX) isozymes and cytochrome P450 are able to accept AEA and 2-AG as a substrate, leading to the biosynthesis of prostaglandin-ethanolamides (Kozak et al., 2002) and -glyceryl esters (Kozak et al., 2001), hydroxy-anandamides, and hydroxyleicosatetraenoyl-glycerols (van der Stelt et al., 2002), respectively. For a comprehensive review on alternative pathways of eCBs see and Rouzer and Marnett (Rouzer and Marnett, 2011). eCBs act principally through cannabinoid receptors, that include type-1 and type-2 (CB_1 and CB_2) receptors; more recently, it has been highlighted the ability of some CB and non-CB ligands to bind also to GPR55 (Glucksmann and Weich, 1999; Wise and Brown, 2001; Drmota et al., 2004; Pertwee, 2007; Ryberg et al., 2007; Lauckner et al., 2008), thus suggesting that the latter protein might act as a novel "type-3 (CB_3)" cannabinoid receptor (Moriconi et al., 2010). CB receptors are members of the large family of heptahelical G protein coupled receptors (GPCRs), activate Gi/o proteins (Pertwee et al., 2010). Anatomical studies have revealed that these receptors display a highly divergent pattern of distribution throughout the organism: CB_1 mainly present in the central nervous system (Herkenham et al., 1991) and, on the other hand, CB_2 mainly distributed in peripheral and immune cells (Munro et al., 1993). This topographical dichotomy has been

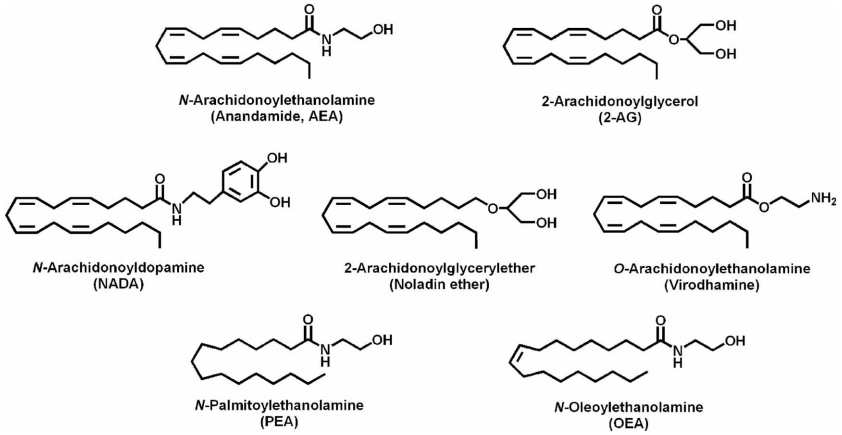


FIGURE 1 | Chemical structures of biologically active eCBs and of the eCB-like compounds.

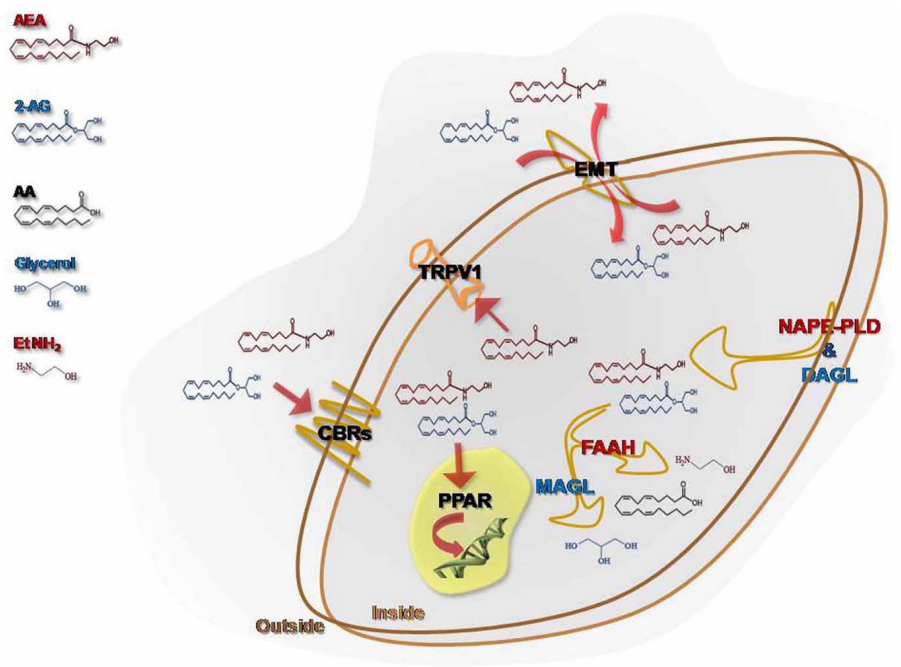


FIGURE 2 | Schematic representation of the main elements that constitute the endocannabinoid system. The synthesis of *N*-arachidonoyl-ethanolamine (AEA) is due to the activity of a NAPE-specific phospholipase D (NAPE-PLD), whereas a fatty acid amide hydrolase (FAAH) is responsible for its intracellular degradation to ethanolamine ($EtNH_2$) and arachidonic acid (AA). 2-Arachidonoylglycerol (2-AG) is released from membrane lipids through the activity of diacylglycerol lipase (DAGL), and it is hydrolyzed by a cytosolic monoacylglycerol lipase (MAGL) that releases glycerol and AA. A purported endocannabinoid membrane transporter (EMT) clears AEA and 2-AG from the extracellular space, and takes them up into the cell. Both AEA and 2-AG trigger several signal transduction pathways by acting at their targets, CB_1, CB_2, GPR55, and nuclear PPARs. AEA, but not 2-AG, binds intracellularly also TRPV1, and thus it is also designated as a true endovanilloid.

revised by a number of studies documenting the presence of CB_1 in several non-neuronal cells and tissues (Gong et al., 2006), and of CB_2 in the brain stem (van Sickle et al., 2005) and in neuronal cells upon exogenous insults (Viscomi et al., 2009). In addition, the non-selective cationic channel type-1 vanilloid receptor (transient receptor potential vanilloid 1, TRPV1), usually activated by capsaicin and by noxious stimuli-like heat and protons (Di Marzo and De Petrocellis, 2010), is an alternative target for AEA, but not for 2-AG. More recently, also nuclear receptors like the peroxisome proliferator-activated receptors (PPARs) have been added to the list of eCBs targets, activated under physiological and pathological conditions (Pistis and Melis, 2010). A schematic representation of eCBs, their receptors, biosynthetic and catabolic enzymes, as well as putative transporter, is depicted in **Figure 2**.

eCBS AND THEIR SIGNAL TRANSDUCTION PATHWAYS

The signal transduction pathways coupled to CB, TRPV1, and PPAR receptors are summarized in **Table 1**. Among the effects elicited by eCBs by binding to CB receptors, we should recall Ca^{2+} channels inhibition (including N-, P/Q-, and L-type channels), inhibition of adenylyl cyclase and subsequent decrease of cAMP-dependent protein kinase, which leads to decreased phosphorylation of the K^+ channels, regulation of ionic currents, activation of focal adhesion kinase, stimulation of mitogen-activated protein kinase (MAPK) cascades (Pertwee, 2006), and specifically ERK, p38 MAPK cascades (Derkinderen et al., 2001; Gertsch et al., 2004), and the stimulation of additional intracellular pathways including the phosphatidylinositol 3-kinase (PI3K)/Akt pathway through CB_2 (Molina-Holgado et al., 2002).

Unlike CB_2, CB_1 receptors are associated to special membrane microdomains, called "lipid rafts" (LR) that modulate CB_1-dependent signaling pathways. The functional relationship between CB_1 and LR is affected by cholesterol content; in particular, membrane cholesterol enrichment in both primary and immortalized cell lines reduces the binding to CB_1 and subsequent G-protein dependent signaling through adenylyl cyclase and MAPK (Bari et al., 2005). Moreover, the disruption of LRs by cholesterol depletion modifies AEA-induced endocytosis of CB_1, which apparently loses the capacity to be directed toward the lysosomal compartment. Therefore, LRs, besides representing a favorable platform to regulate CB_1 signaling, might also represent a cellular device for its intracellular trafficking (Sarnataro et al., 2005; Dainese et al., 2007). The general model to explain the neuromodulatory actions of AEA involves the release of eCBs from a postsynaptic neuron upon stimulation, then the back diffusion to presynaptic terminals, where AEA activates CB_1 receptors, thus modulating neuronal membrane permeability to Ca^{2+} e K^+ ions and the activity of adenylyl cyclase. The final outcome is a modified action of neurotransmitters (Di Marzo and De Petrocellis, 2010).

Table 1 | Signal transduction pathways triggered by eCBs at different target receptors.

Receptor	Effect
CB_1 and CB_2	↓ Adenylyl cyclase
	↑ Focal adhesion kinase (FAK) and mitogen-activated protein kinase (MAPK)
	↑ ERK, p38 through CB_1,and PI3K/Akt through CB_2
	↑ K^+ channels
	↓ Ca^{2+} channels
GPR55	↑ Intracellular [Ca^{2+}]
	↑ RhoA, Rac, and Cdc42
	↑ ERK phosphorylation
TRPV1	↑ Intracellular [Ca^{2+}]
	↑ Caspases
	↑ Cytochrome c release
	↑ Mitochondrial uncoupling
	↑ Pro-apoptotic kinases
PPARs	↑ ROS
	↑ Tyrosine kinases
	↑ Adiponectin and lipoprotein lipase

The activation of GPR55, the purported "CB_3" cannabinoid receptor, has been linked to (1) intracellular Ca^{2+} increase (Lauckner et al., 2008); (2) activation of the small GTPase proteins RhoA, Rac, and Cdc42 (Ryberg et al., 2007; Henstridge et al., 2009), and (3) ERK phosphorylation (Oka et al., 2007, 2009). Additionally, by triggering PPARs, eCBs exert a variety of long-term effects *via* genomic mechanisms and rapid non-genomic actions, which are opposite to those evoked by activation of "classical" surface cannabinoid receptors (Pistis and Melis, 2010). As a consequence, PPARs activation affects several physiological and pathological processes, such as lipid metabolism, energy balance, and feeding behavior, neuroprotection, epilepsy, circadian rhythms, inflammation, addiction, and cognitive functions (Pistis and Melis, 2010). However, AEA can also act as a modulator of other signaling pathways and, in fact, it has been observed that muscarinic and glutamate receptors have allosteric sites for AEA binding (Lanzafame et al., 2004). In this context, it should be underlined that there are several findings showing that eCBs modulate the signaling of several neuropeptides and hormones (Manzanares et al., 1999; Beinfeld and Connolly, 2001; Ghozland et al., 2002). This highly complex network of interactions is reflected in the multifaceted modulatory effects of eCBs on the regulation of brain and behavioral functions (López-Moreno et al., 2008).

PHYSIOLOGICAL ACTIONS OF ECS AND THERAPEUTIC PERSPECTIVES

The presence of ECS in vertebrates, mammals, and humans implies a role in several physiological processes, including appetite, cancer, cardiovascular diseases, fertility, immune functions, memory, neuroprotection, and pain modulation (Ligresti et al., 2009; Maccarrone et al., 2010) (**Figure 3**).

In the last 10 years, it has become clear that a dysregulation of ECS is connected to pathological conditions, and thus its modulation through inhibition of metabolic pathways and/or agonism or antagonism of its receptors has an enormous potential for research and intervention in multiple areas of human health.

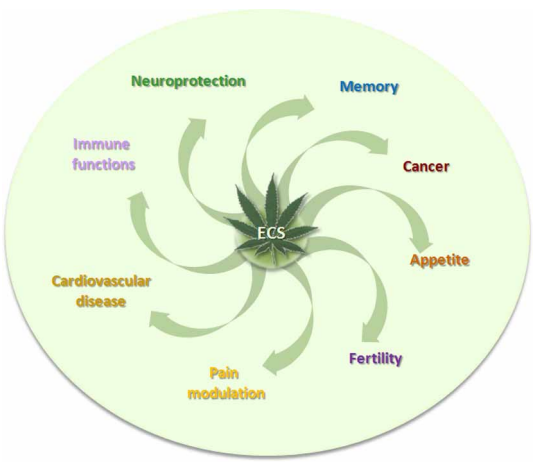


FIGURE 3 | The involvement of ECS in some pathophysiological conditions.

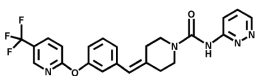
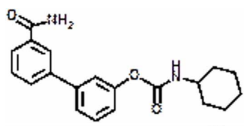
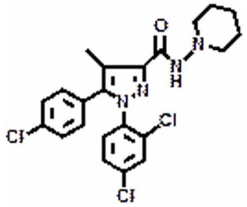
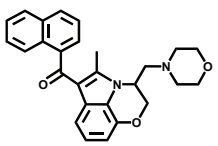
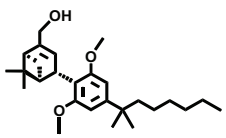
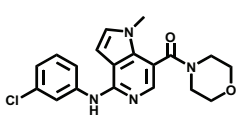
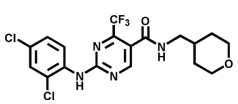
Therefore, based on the therapeutic potential of THC, known since centuries as medicine for its palliative effects in several pathologies, plant-derived cannabinoids, synthetic cannabinoids, and eCBs have been tested as novel therapeutics in a wide range of clinical trials.

The neuroprotective effect of eCBs might be mediated by either CB_1- or CB_2-dependent mechanisms. Research studies using $cb_1^{-/-}$ knock-out mice showed an increased mortality rate and an increased infarct area in cerebral ischemia models (Parmentier-Batteur et al., 2002). It has been reported that the administration of the CB_1 synthetic agonist WIN 55.212–2 attenuated the neurological damage and reduced infarct size in artery occlusion induced in rats (Nagayama et al., 1999), and additionally it reduced the glial damage after hypoxic-ischemic brain injury in preterm lambs (Alonso-Alconada et al., 2010). The presence of CB_2-positive cells in the brain during injury and in inflammatory neurodegenerative disorders might provide a novel strategy for cannabinoid-mediated intervention against stroke-induced neurodegeneration, without the unwanted psychoactive effects of CB_1 receptor stimulation (Cunha et al., 2011). O-3853 and O-1966, two selective CB_2 agonists, administrated 1 h before transient middle cerebral artery occlusion, significantly decreased the mobilization of white blood cells and their adherence to vascular endothelial cells, reduced the infarct size, and improved motor function after transient focal ischemia (Zhang et al., 2007, 2009).

According to these observations, pain management is preferably handled using CB_2 agonists, such as HU-308 and AM-1241, which display significant relief in inflammatory and neuropathic pain models, without exhibiting central nervous system side effects (Hanus et al., 1999; Yao et al., 2006). In this context, new selective CB_2 receptor modulators, designed by Glaxo Smith Kline as derivatives of pyrimidinecarboxamide, have been tested as good clinical candidates to treat inflammatory, acute, and chronic pain (Giblin et al., 2007, 2009).

In the past, several reports documented that the selective pharmacologic antagonism of the CB_1 receptor improves lipid abnormalities associated with obesity, as well as neurodegenerative diseases and nicotine or alcohol dependence (Centonze et al., 2007; Di Marzo, 2008). Following the good outcome obtained in

Table 2 | Chemical structures and therapeutic potential of some ECS-targeted molecules.

Chemical structure	Compound	ECS target	Diseases	References
	PF-04457845	FAAH	Pain, Osteoarthritis	Ahn et al., 2011
	URB 597		Anxiety, Cannabis dependence, Hyperalgesia	Bortolato et al., 2007
	SR141716A	CB_1	Eating disorder	Christopoulou and Kiortsis, 2011
	WIN 55.212–2		Ischemic stroke, Brain injury	Nagayama et al., 1999; Alonso-Alconada et al., 2010
	HU-308	CB_2	Neuropathic pain	Hanus et al., 1999
	GSK554418A		Acute/chronic pain	Giblin et al., 2009
	GW842166X		Inflammatory pain	Giblin et al., 2007

various clinical trials, the best known CB_1 blocker SR141617A, also called rimonabant (and commercially known as Acomplia®) was released on the worldwide market as anti-obesity drug, but only few months later it was withdrawn because of increased rates of depression, anxiety, and suicide among patients who received it (Christopoulou and Kiortsis, 2011). In addition, further concerns were raised considering the possible side effects of this weight loss pill on the reproductive functions and human infertility (Bari et al., 2011).

Alternative strategies to treat pain syndromes, such as neuropathic pain, fibromyalgia, but also spontaneous abortion, headache, psychiatric disorders, and neurodegenerative diseases, are based on the enhacement of the eCB tone, through the inhibition of eCBs-hydrolyzing enzymes (Lichtman and Chapman, 2001). The most promising FAAH inhibitor seems to be URB597 (also named KDS-4103), which has biochemical and behavioral effects during both sub-acute and chronic treatments. In rodents, once-daily dosing of URB597 for five weeks elicits antidepressant effects in chronically stressed animals, without altering CB_1 receptor mRNA levels (Bortolato et al., 2007). Pfizer and Vernalis pharmaceutical companies focused on FAAH as main target to design and develop new molecules (PF-04457845 and V158866, respectively), that are being tested in clinical studies as potential therapies for a range of pain disorders, including osteoarthritis (Ahn et al., 2011). It is noteworthy that FAAH inhibitors, because of their own pharmacological properties, are attractive remedial also for cannabis dependence; in fact, they do not appear to evoke tolerance following long-term administration, and they do not display significant abuse liability (Clapper et al., 2009). **Table 2** reports some agonists, antagonists, and/or inhibitors of ECS designed for the treatment of several pathological conditions.

CONCLUSION

Almost 20 years after the identification of AEA, all members of ECS are nowadays considered intriguing targets for the development of selective and specific compounds able to modulate human pathophysiology. A deeper and more detailed understanding of proteins involved in eCBs metabolism and signal-transduction pathways could help to design compounds that might prolong the activity of eCBs in a time- and site-dependent way, excluding undesired psychotropic effects, and to develop transgenic mice, where different ECS elements can be knocked down or knocked in, allowing innovative therapeutic strategies in a vast panorama of pathologies.

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REFERENCES

Ahn, K., Smith, S. E., Liimatta, M. B., Beidler, D., Sadagopan, N., Dudley, D. T., Young, T., Wren, P., Zhang, Y., Swaney, S., Van Becelaere, K., Blankman, J. L., Nomura, D. K., Bhattachar, S. N., Stiff, C., Nomanbhoy, T. K., Weerapana, E., Johnson, D. S., and Cravatt, B. F. (2011). Mechanistic and pharmacological characterization of PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces inflammatory and noninflammatory pain. *J. Pharmacol. Exp. Ther.* 338, 114–124.

Alonso-Alconada, D., Alvarez, F. J., Alvarez, A., Mielgo, V. E., Goñi-de-Cerio, F., Rey-Santano, M. C., Caballero, A., Martinez-Orgado, J., and Hilario, E. (2010). The cannabinoid receptor agonist WIN 55,212–2 reduces the initial cerebral damage after hypoxic-ischemic injury in fetal lambs. *Brain Res.* 1362, 150–159.

Bari, M., Battista, N., Fezza, F., Finazzi Agrò, A., and Maccarrone, M. (2005). Lipid rafts control signaling of type-1 cannabinoid receptors in neuronal cells. Implications for anandamide-induced apoptosis. *J. Biol. Chem.* 280, 12212–12220.

Bari, M., Battista, N., Pirazzi, V., and Maccarrone, M. (2011). The manifold actions of endocannabinoids on female and male reproductive events. *Front. Biosci.* 16, 498–516.

Beinfeld, M. C., and Connolly, K. (2001). Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids. *Neurosci. Lett.* 301, 69–71.

Bortolato, M., Mangieri, R. A., Fu, J., Kim, J. H., Arguello, O., Duranti, A., Tontini, A., Mor, M., Tarzia, G., and 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.

Centonze, D., Finazzi Agrò, A., Bernardi, G., and Maccarrone, M. (2007). The endocannabinoid system in targeting inflammatory neurodegenerative diseases. *Trends Pharmacol. Sci.* 28, 180–187.

Christopoulou, F. D., and Kiortsis, D. N. (2011). An overview of the metabolic effects of rimonabant in randomized controlled trials: potential for other cannabinoid 1 receptor blockers in obesity. *J. Clin. Pharm. Ther.* 36, 10–18.

Clapper, J. R., Mangieri, R. A., and Piomelli, D. (2009). The endocannabinoid system as a target for the treatment of cannabis dependence. *Neuropharmacology* 56, 235–243.

Cunha, P., Romão, A. M., Mascarenhas-Melo, F., Teixeira, H. M., and Reis, F. (2011). Endocannabinoid system in cardiovascular disorders – new pharmacotherapeutic opportunities. *J. Pharm. Bioallied. Sci.* 3, 350–360.

Dainese, E., Oddi, S., Bari, M., and Maccarrone, M. (2007). Modulation of the endocannabinoid system by lipid rafts. *Curr. Med. Chem.* 14, 2702–2715.

Derkinderen, P., Ledent, C., Parmentier, M., and Girault, J. A. (2001). Cannabinoids activate p38 mitogen-activated protein kinases through CB1 receptors in hippocampus. *J. Neurochem.* 77, 957–960.

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

Di Marzo, V. (2008). CB(1) receptor antagonism: biological basis for metabolic effects. *Drug Discov. Today* 13, 1026–1041.

Di Marzo, V., and De Petrocellis, L. (2010). Endocannabinoids as regulators of transient receptor potential (TRP) channels: a further opportunity to develop new endocannabinoid-based therapeutic drugs. *Curr. Med. Chem.* 17, 1430–1449.

Dinh, T. P., Freund, T. F., and Piomelli, D. (2002). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem. Phys. Lipids* 121, 149–158.

Drmota, T., Greasley, P., and Groblewski, T. (2004). AstraZeneca. WO04074844.

Fu, J., Bottegoni, G., Sasso, O., Bertorelli, R., Rocchia, W., Masetti, M., Guijarro, A., Lodola, A., Armirotti, A., Garau, G., Bandiera, T., Reggiani, A., Mor, M., Cavalli, A., and Piomelli, D. (2011). A catalytically silent FAAH-1 variant drives anandamide transport in neurons. *Nat. Neurosci.* 15, 64–69.

Gertsch, J., Schoop, R., Kuenzle, U., and Suter, A. (2004). Echinacea alkylamides modulate TNF-alpha gene expression via cannabinoid receptor CB2 and multiple signal transduction pathways. *FEBS Lett.* 577, 563–569.

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

Giblin, G. M. P., Billinton, A., Briggs, M., Brown, A. J., Chessell, I. P., Clayton, N. M., Eatherton, A. J., Goldsmith, P., Haslam, C., Johnson, M. R., Mitchell, W. L., Naylor, A., Perboni, A., Slingsby, B. P., and Wilson, A. W. (2009). Discovery

of 1-[4-(3-chlorophenylamino)-1-methyl-1H-pyrrolo [3, 2-c] pyridin-7-yl]- 1-morpholin-4-ylmethanone (GSK554418A), a brain penetrant 5-azaindole CB2 agonist for the treatment of chronic pain. *J. Med. Chem.* 52, 5785–5788.

Giblin, G. M. P., O'Shaughnessy, C. T., Naylor, A., Mitchell, W. L., Eatherton, A. J., Slingsby, B. P., Rawlings, D. A., Goldsmith, P., Brown, A. J., Haslam, C. P., Clayton, N. M., Wilson, A. W., Chessell, I. P., Wittington, A. R., and Green, R. (2007). Discovery of 2-[(2,4-dichlorophenyl)amino]-N-[(tetrahydro-2H-pyran-4-yl)methyl]-4-(trifluoromethyl)-5-pyrimidinecarboxamide, a selective CB2 receptor agonist for the treatment of inflammatory pain. *J. Med. Chem.* 50, 2597–2600.

Glucksmann, M. A., and Weich, N. (1999). Millennium Pharmaceuticals Inc. WO990023588.

Gong, J. P., Onaivi, E. S., Ishiguro, H., Liu, Q. R., Tagliaferro, P. A., Brusco, A., and Uhl, G. R. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res.* 1071, 10–23.

Hanus, L., Breuer, A., Tchillibon, S., Shiloah, S., Goldenberg, D., Horowitz, M., Pertwee, R. G., Ross, R. A., Mechoulam, R., and Fride, E. (1999). HU-308: a specific agonist for CB2, a peripheral cannabinoid receptor. *Proc. Natl. Acad. Sci. U.S.A.* 96, 14228–14233.

Henstridge, C. M., Balenga, N. A., Ford, L. A., Ross, R. A., Waldhoer, M., and Irving, A. J. (2009). The GPR55 ligand L-alphalysophosphatidylinositol promotes RhoA-dependent Ca2+ signaling and NFAT activation. *FASEB J.* 23, 183–193.

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., and Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J. Neurosci.* 11, 563–583.

Kozak, K. R., Crews, B. C., Morrow, J. D., Wang, L. H., Ma, H. Y., Weinander, R., Jakobsson, P. J., and Marnett, L. J. (2002). Metabolism of the endocannabinoids, 2-arachidonoylglkycerol and anandamide, into prostaglandin, tromboxane, and prostacyclin glycerol esters and ethanolamides. *J. Biol. Chem.* 277, 44877–44885.

Kozak, K. R., Crews, B. C., Ray, J. L., Tai, H. H., Morrow, J. D., and Marnett, L. J. (2001). Metabolism of prostaglandin glycerol esters and prostaglandin ethanolamides *in vitro* and *in vivo*. *J. Biol. Chem.* 276, 36993–36998.

Lanzafame, A. A., Guida, E., and Christopoulos, A. (2004). Effects of anandamide on the binding and signaling properties of M1 muscarinic acetylcholine receptors. *Biochem. Pharmacol.* 68, 2207–2219.

Lauckner, J. E., Jensen, J. B., Chen, H. Y., Lu, H. C., Hille, B., and Mackie, K. (2008). GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2699–2704.

Lichtman, A. H., and Chapman, V. (2001). A FAAH-fetched approach to treat osteoarthritis pain. *Pain* 152, 959–960.

Ligresti, A., Petrosino, S., and Di Marzo, V. (2009). From endocannabinoid profiling to "endocannabinoid therapeutics". *Curr. Opin. Chem. Biol.* 13, 21–31.

López-Moreno, J. A., González-Cuevas, G., Moreno, G., and Navarro, M. (2008). The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction. *Addict. Biol.* 13, 160–187.

Maccarrone, M., Dainese, E., and Oddi, S. (2010). Intracellular trafficking of anandamide: new concepts for signaling. *Trends Biochem. Sci.* 35, 601–608.

Manzanares, J., Corchero, J., Romero, J., Fernandez-Ruiz, J. J., Ramos, J. A., and Fuentes, J. A. (1999). Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends Pharmacol. Sci.* 20, 287–294.

McKinney, M. K., and Cravatt, B. F. (2005). Structure and function of fatty acid amide hydrolase. *Annu. Rev. Biochem.* 74, 411–432.

Molina-Holgado, E., Vela, J. M., Arévalo-Martín, A., Almazán, G., Molina-Holgado, F., Borrell, J., and Guaza, C. (2002). Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *J. Neurosci.* 22, 9742–9753.

Moriconi, A., Cerbara, I., Maccarrone, M., and Topai, A. (2010). GPR55: current knowledge and future perspectives of a purported "Type-3" cannabinoid receptor. *Curr. Med. Chem.* 17, 1411–1429.

Muccioli, G. G. (2010). Endocannabinoid biosynthesis and inactivation, from simple to complex. *Drug Discov. Today* 15, 474–483.

Munro, S., Munro, S., Thomas, K. L., and Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365, 61–65.

Nagayama, T., Sinor, A. D., Simon, R. P., Chen, J., Graham, S. H., Jin, K., and Greenberg, D. A. (1999). Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J. Neurosci.* 19, 2987–2995.

Oka, S., Nakajima, K., Yamashita, A., Kishimoto, S., and Sugiura, T. (2007). Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochem. Biophys. Res. Commun.* 362, 928–934.

Oka, S., Toshida, T., Maruyama, K., Nakajima, K., Yamashita, A., and Sugiura, T. (2009). 2-Arachidonoyl-sn-glycero-3-phosphoinositol: possible natural ligand for GPR55. *J. Biochem.* 145, 13–20.

Okamoto, Y., Tsuboi, K., and Ueda, N. (2009). Enzymatic formation of anandamide. *Vitam. Horm.* 81, 1–24.

Parmentier-Batteur, S., Jin, K., Mao, X. O., Xie, L., and Greenberg, D. A. (2002). Increased severity of stroke in CB1 cannabinoid receptor knock-out mice. *J. Neurosci.* 22, 9771–9775.

Pertwee, R. G. (2006). Cannabinoid pharmacology: the first 66 years. *Br. J. Pharmacol.* 147, S163–S171.

Pertwee, R. G. (2007). GPR55: a new member of the cannabinoid receptor clan? *Br. J. Pharmacol.* 152, 984–986.

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., Elphick, M. R., Greasley, P. J., Hansen, H. S., Kunos, G., Mackie, K., Mechoulam, R., and Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacol. Rev.* 62, 588–631.

Pistis, M., and Melis, M. (2010). From surface to nuclear receptors: the endocannabinoid family extends its assets. *Curr. Med. Chem.* 17, 1450–1467.

Rouzer, C. A., and Marnett, L. J. (2011). Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chem. Rev.* 111, 5899–5921.

Ryberg, E., Larsson, N., Sjögren, S., Hjorth, S., Hermansson, N. O., Leonova, J., Elebring, T., Nilsson, K., Drmota, T., and Greasley, P. J. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *Br. J. Pharmacol.* 152, 1092–1101.

Sarnataro, D., Grimaldi, C., Pisanti, S., Gazzerro, P., Laezza, C., Zurzolo, C., and Bifulco, M. (2005). Plasma membrane and lysosomal localization of CB1 cannabinoid receptor are dependent on lipid rafts and regulated by anandamide in human breast cancer cells. *FEBS Lett.* 579, 6343–6349.

Tsuboi, K., Sun, Y. X., Okamoto, Y., Araki, N., Tonai, T., and Ueda, N. (2005). Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. *J. Biol. Chem.* 280, 11082–11092.

Ueda, N., Tsuboi, K., Uyama, T., and Ohnishi, T. (2011). Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. *Biofactors* 37, 1–7.

van der Stelt, M., van Kuik, J. A., Bari, M., van Zadelhoff, G., Leeflang, B. R., Veldink, G. A., Finazzi Agro', A., Vliegenthart, J. F., and Maccarrone, M. (2002). Oxygenated metabolites of anandamide and 2-arachidonoyl-glycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter and fatty acid amide hydrolase. *J. Med. Chem.* 4, 3709–3720.

van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J. S., Marnett, L. J., Di Marzo, V., Pittman, Q. J., Patel, K. D., and Sharkey, K. A. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 310, 329–332.

Viscomi, M. T., Oddi, S., Latini, L., Pasquariello, N., Florenzano, F., Bernardi, G., Molinari, M., and Maccarrone, M. (2009). Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway. *J. Neurosci.* 29, 4564–4570.

Wei, B. Q., Mikkelsen, T. S., McKinney, M. K., Lander, E. S., and Cravatt, B. F. (2006). A second fatty acid amide hydrolase with variable distribution among placental mammals. *J. Biol. Chem.* 281, 36569–36578.

Wise, A., and Brown, A. J. (2001). Glaxosmithkline. WO0186305.

Yao, B. B., Mukherjee, S., Fan, Y., Garrison, T. R., Daza, A. V., Grayson, G. K., Hooker, B. A., Dart, M. J., Sullivan, J. P., and Meyer, M. D. (2006). *In vitro* pharmacological characterization of AM1241: a protean agonist at the cannabinoid CB2 receptor? *Br. J. Pharmacol.* 149, 145–154.

Zhang, M., Adler, M. W., Abood, M. E., Ganea, D., Jallo, J., and Tuma, R. F. (2009). CB2 receptor activation attenuates microcirculatory dysfunction during cerebral ischemic/reperfusion injury. *Microvasc. Res.* 78, 86–94.

Zhang, M., Martin, B. R., Adler, M. W., Razdan, R. K., Jallo, J. I., and Tuma, R. F. (2007). Cannabinoid CB(2) receptor activation decreases cerebral infarction in a mouse focal ischemia/reperfusion model. *J. Cereb. Blood Flow Metab.* 27, 1387–1396.

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Endocannabinoid system and psychiatry: in search of a neurobiological basis for detrimental and potential therapeutic effects

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Public concern on mental health has noticeably increased given the high prevalence of neuropsychiatric disorders. Cognition and emotionality are the most affected functions in neuropsychiatric disorders, i.e., anxiety disorders, depression, and schizophrenia. In this review, most relevant literature on the role of the endocannabinoid (eCB) system in neuropsychiatric disorders will be presented. Evidence from clinical and animal studies is provided for the participation of CB1 and CB2 receptors (CB1R and CB2R) in the above mentioned neuropsychiatric disorders. CBRs are crucial in some of the emotional and cognitive impairments reported, although more research is required to understand the specific role of the eCB system in neuropsychiatric disorders. Cannabidiol (CBD), the main non-psychotropic component of the *Cannabis sativa* plant, has shown therapeutic potential in several neuropsychiatric disorders. Although further studies are needed, recent studies indicate that CBD therapeutic effects may partially depend on facilitation of eCB-mediated neurotransmission. Last but not least, this review includes recent findings on the role of the eCB system in eating disorders. A deregulation of the eCB system has been proposed to be in the bases of several neuropsychiatric disorders, including eating disorders. Cannabis consumption has been related to the appearance of psychotic symptoms and schizophrenia. In contrast, the pharmacological manipulation of this eCB system has been proposed as a potential strategy for the treatment of anxiety disorders, depression, and anorexia nervosa. In conclusion, the eCB system plays a critical role in psychiatry; however, detrimental consequences of manipulating this endogenous system cannot be underestimated over the potential and promising perspectives of its therapeutic manipulation.

Keywords: cannabinoid receptor, cannabidiol, cognition, emotion, anxiety, depression, schizophrenia, eating disorders

Psychiatric disorders severely compromise the well-being of those affected causing serious psychological distress in the general population. These disorders have a relatively high prevalence [Kessler et al., 2005; Substance Abuse and Mental Health Services Administration, SAMHSA (2006)], can have an early onset (i.e., schizophrenia in young adulthood) or a relapsing-remitting course (as in mood and anxiety disorders), and frequently have disabling symptoms. Cognition and emotion regulation are the most affected functions in neuropsychiatric disorders. In fact, such functions have been reported to be critically impaired in patients suffering from anxiety disorders, schizophrenia, and major depression (Hyman, 2008; Dere et al., 2010).

Anxiety is an adaptive component of the acute stress response under circumstances that threaten the integrity of the individual, and thus can be regarded as a "normal" emotion. However, if anxiety is disproportional in intensity or chronicity, or is not associated with any actual risk, it constitutes a maladaptive response or even a neuropsychiatric disorder. Indeed, anxiety disorders are marked by excessive fear (and avoidance), often in response to specific objects or situations and in the absence of true danger. Anxiety disorders, such as panic disorder, social and specific phobia, generalized anxiety disorder and post-traumatic stress disorder (PTSD) are highly prevalent and strongly disabling class of neuropsychiatric disorders (Bekker and van Mens-Verhulst, 2007; Shin and Liberzon, 2010). Depression is characterized by abnormal representation and regulation of affect, mood and emotion. Anhedonia, that is, decreased levels of emotional activation after presentation of rewarding stimuli is generally considered as a core symptom of depressive patients (Davidson et al., 2002; Levens and Gotlib, 2009). Cognitive impairments are frequently observed

in patients with anxiety disorders and depression. Although mild anxiety seems to be associated with better cognitive performance, severe anxiety symptoms are negatively associated with cognitive functioning (Bierman et al., 2005; Gualtieri and Morgan, 2008). More particularly, PTSD, that develops after prolonged inescapable stress experience of exceptional severity (Rubin et al., 2008), has been associated with a number of cognitive impairments, including basic deficits in attention, concentration, and memory (Isaac et al., 2006). In contrast, depressive symptoms are always negatively associated with cognitive performance. Actually, low episodic memory performance has been proposed as a premorbid marker of depression (Airaksinen et al., 2007). Finally, schizophrenia is characterized by profound disruption in cognition and emotion, affecting the most fundamental human attributes. A wide diversity of symptoms is described in schizophrenic patients including hallucinations and delusions, together with remarkable cognitive deficits that critically influence the course of the disorder (Barch, 2005).

Cannabis is one of the illicit drugs more frequently abused in the western societies. A great variety of chemical compounds are present in the plan of *Cannabis sativa*, mainly delta-9-tetrahydrocannabinol (THC), responsible of the addictive and psychoactive properties of cannabis, and cannabidiol (CBD). Cannabis, as a drug of abuse, induces changes in the central nervous system (CNS) that may lead to dependence. Indeed, the development of a dependence syndrome is among the most probable adverse effects of cannabis consumption (Budney et al., 2004; Fattore et al., 2008). Cannabis consumption induces euphoria, and is frequently accompanied by decreases in anxiety, although acute aversive emotional reactions to cannabis have also been reported (consult Crippa et al., 2009 for review). Notable cognitive impairments have been observed following marijuana intake in humans, and a contribution neurochemical processes occurring in both prefrontal cortex and hippocampus have been proposed (Egerton et al., 2006; Cohen et al., 2008). Therefore, public concern is growing in relation to the adverse effects of regular use on adolescent psychosocial development and mental health (Jager and Ramsey, 2008; Hall and Degenhardt, 2009). There is increasing evidence indicating a close relationship between cannabis consumption and an increased risk for depression, anxiety disorders, psychotic symptoms, or even schizophrenia (Degenhardt et al., 2003; Manzanares et al., 2004; Sundram, 2006; Di Forti et al., 2007; Leweke and Koethe, 2008)).

Cannabis derivatives, also known as phytocannabinoids, influence the CNS through activation of the endocannabinoid (eCB) system, mainly composed by the endogenous ligands (endocannabinoids, eCBs) and their specific membrane receptors, together with the enzymatic machinery in charge of eCB synthesis and inactivation (Andre and Gonthier, 2010; Maccarrone et al., 2010). Endocannabinoids have been shown to modulate neurotransmission, mainly acting as retrograde transmitters (Marsicano and Lutz, 2006), and have been involved in a plethora of physiological functions. Data from human and animal studies have consistently demonstrated that the eCB system is pivotal for emotional homeostasis and cognitive function (Viveros et al., 2007; Moreira and Lutz, 2008; Solowij and Battisti, 2008; Marco and Viveros, 2009). In turn, deregulation of the eCB system has been associated with psychopathological conditions that compromise emotional and cognitive function, such as anxiety-related disorders, depression, and schizophrenia. Herein, we will review the latest breakthroughs on the role played by the eCB system in neuropsychiatric disorders, focusing in emotional, and cognitive impairments that critically affect individuals' well-being.

A BRIEF UPDATE ON THE ENDOCANNABINOID (eCB) SYSTEM

The eCB system system modulates the neurotransmission at inhibitory and excitatory synapses in brain regions relevant to the regulation of pain, emotion, motivation, and cognition (Viveros et al., 2005; Wotjak, 2005; Moreira and Lutz, 2008; Guindon and Hohmann, 2009; Finn, 2010; Moreira and Wotjak, 2010). In the last decades, investigation of the eCB system has considerably increased and our understanding of this system has achieved remarkable aims (see Andre and Gonthier, 2010; Maccarrone et al., 2010 for an updated review). Endocannabinoids, the endogenous ligands, are polyunsaturated fatty acid derivatives that bind to cannabinoid receptors. Two types of cannabinoid receptors have been characterized to date, CB1 (Herkenham et al., 1991) and CB2 receptors (Munro et al., 1993) both metabotropic receptors coupled to $G\alpha_{i/o}$ proteins. CB1Rs are expressed ubiquitously throughout the brain; they are found at highest concentrations in the hippocampus, neocortex, basal ganglia, and cerebellum; while a moderate presence is observed in the basolateral amygdala, hypothalamus, and midbrain (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992; Glass et al., 1997). Apart from neurons, CB1Rs have also been described in non-neuronal cells such as astrocytes (Bouaboula et al., 1995; Sanchez et al., 1998), microglia (Waksman et al., 1999; Walter et al., 2003), and oligodendrocytes (Molina-Holgado et al., 2002; for review consult Mackie, 2005). In contrast, discrepant opinions exist regarding the pattern of expression of CB2Rs. Initially, CB2R was identified at high levels in peripheral immune tissues, as rat spleen and immune cells in humans (Munro et al., 1993; Galiegue et al., 1995), in a lower extent, in the muscle, liver, intestine, and testis (Liu et al., 2009), as well as in the adipose tissue (Roche et al., 2006). Additionally, CB2R was also found in the brain under pathological conditions, i.e., in tumors (Joosten et al., 2002), glioma (Guzman et al., 2001), neuropathic pain (Ibrahim et al., 2003), senile plaques in Alzheimer's disease (Ehrhart et al., 2005), arteriosclerotic plaques (Steffens et al., 2005), while no CB2R expression was found in the brain under normal physiological conditions (Chakrabarti et al., 1995; Derocq et al., 1995; Schatz et al., 1997; Griffin et al., 1999; Carlisle et al., 2002). However, more recently CB2Rs have been identified in cerebellum and brainstem (Van Sickle et al., 2005). Indeed, further studies have now described the presence of CB2Rs, both gene and protein expression, in different brain regions under normal physiological conditions, including cerebral cortex, striatum, hippocampus, amygdala, periaqueductal gray (PAG), and several hypothalamic nuclei (Gong et al., 2006; Onaivi, 2006; García-Gutiérrez et al., 2010). Although CB1Rs and CB2Rs are well-known and characterized, numerous pharmacological studies have suggested the existence of additional cannabinoid receptors. In this regard, eCB ligands have been reported to bind to the transient receptor potential vanilloid type 1 (TRPV1) ion

channel (Starowicz et al., 2007), and two G protein-coupled receptors, GPR55 and GPR119, have been proposed as novel potential cannabinoid receptors (Baker et al., 2006). Moreover, increasing evidence now suggests that eCBs are also natural activators of the peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors (O'Sullivan and Kendall, 2010).

Endocannabinoids, due to their lipophilic nature, are synthesized and released "on demand" by the cleavage of membrane phospholipid precursors in response to diverse physiological and pathological stimuli. The two most widely studied eCBs are *N*-arachidonoyl-ethanolamide (AEA), also called anandamide, and 2-arachidonoylglycerol (2-AG). The biological actions of these polyunsaturated lipids are controlled by key agents responsible for their synthesis, transport, and degradation. eCBs can passively diffuse through lipid membranes, but a high affinity transporter, not yet identified, seems to accelerate this process. A fatty acid amide hydrolase (FAAH) is the main AEA hydrolase, whereas 2-AG inactivation is mainly afforded by the enzyme monoacylglycerol lipase (MGL), and by novel 2-AG-hydrolyzing lipases recently identified (consult Ahn et al., 2008; Andre and Gonthier, 2010; Maccarrone et al., 2010; Pamplona and Takahashi, 2011; Ueda et al., 2011; for more detailed information and/or an updated review).

INVOLVEMENT OF CB1Rs IN NEUROPSYCHIATRIC DISORDERS

CB1Rs IN ANXIETY DISORDERS

There is substantial evidence from both human and animal studies for a role of the eCB system in the control of emotional states. CB1Rs, as mentioned above (A Brief Update on the Endocannabinoid (eCB) System), is widely distributed in brain areas associated with emotional regulation and stress responsiveness such as prefrontal cortex, hippocampus, amygdala, and hypothalamus (Mackie, 2005), thus a role for eCB signaling in anxiety-related disorders might be suggested. Genetic and pharmacological blockade of CB1Rs further support a role for the eCB system in emotional homeostasis, and thus in anxiety-related disorders. Mutant mice lacking CB1Rs (CB1R knock-out mice, CB1KO) display increased anxiety levels compared to control animals (wild-type) in a variety of behavioral paradigms, i.e., the light–dark box, the elevated plus-maze test, and the social interaction test, as well as increased aggressiveness as measured in the resident–intruder test (Haller et al., 2002; Martin et al., 2002b; Urigüen et al., 2004; but see also Marsicano et al., 2002; Haller et al., 2004). Notwithstanding, baseline trait levels of emotionality critically affect animals' performance in theses tests, and it is in turn notably influenced by both genetic strain and environmental testing conditions (Clement et al., 2002; Yilmazer-Hanke, 2008). In this regard, mutant CB1R mice exclusively exhibited an anxious phenotype under aversive conditions, i.e., high illumination and first exposure in the elevated plus-maze and the social interaction test (Haller et al., 2002, 2004; Marsicano et al., 2002; Martin et al., 2002a; Urigüen et al., 2004). Similarly, systemic administration of rimonabant (SR141716A), a cannabinoid antagonist, induces an anxiogenic profile in rats, i.e., elevated plus-maze and defensive withdrawal test (Navarro et al., 1997; Arevalo et al., 2001) in particular if the animals are tested under highly aversive conditions, i.e., brightly lit environments (Haller et al., 2004), although contradictory results have been obtained in mice, i.e., elevated plus-maze (Akinshola et al., 1999; Haller et al., 2002; Rodgers et al., 2003). It is worth noting that clinical data resemble rat literature, and in humans rimonabant has been associated with increased anxiety and depressed mood (Doggrell, 2008; Rosenstock et al., 2008; Scheen, 2008; Van Gaal et al., 2008). Indeed, these adverse psychiatric effects of rimonabant led to the withdrawal of this anti-obesity drug from the European market[1] (Doc. Ref. EMEA/CHMP/537777/2008). In addition, CB1KO seem not to respond to the anxiolytic actions of benzodiazepines (Urigüen et al., 2004) and further studies demonstrated that CB1R is critically involved in the control of GABAergic neurotransmission, and so in the anxiolytic actions of benzodiazepines (García-Gutiérrez and Manzanares, 2010; Urigüen et al., 2011). Given that benzodiazepines are one of the most prescribed anxiolytic drugs, the participation of CB1R in their pharmacological action additionally supports the eCB system as a fundamental piece in anxiety disorders (see also Viveros et al., 2005; Wotjak, 2005; Marco and Viveros, 2009; Finn, 2010; Moreira and Wotjak, 2010 for review).

The role of CB1R in learning and memory is well documented (for review, Wotjak, 2005; Lutz, 2007). CB1R has been specifically involved in the facilitation of behavioral adaptation after the acquisition of aversive memories. Marsicano et al. (2002) demonstrated that the eCB system has a central function in extinction of aversive memories. Genetic disruption of CB1Rs strongly impaired short-term and long-term extinction in auditory fear-conditioning tests, in the absence of changes in memory acquisition and consolidation processes (Marsicano et al., 2002). Interestingly, eCBs seem to be specifically involved in extinction of aversive memories since extinction of appetitive memories were not affected in CB1KO mice (Holter et al., 2005). Similarly, the pharmacological blockade of CB1Rs led to a significant impairment in extinction, when rimonabant was administered prior to extinction training in the fear-potentiated startle test (Chhatwal et al., 2005). Recent evidence suggests that eCBs may primarily affect habituation-like processing, thought to be more related to acute fear relief (Kamprath et al., 2006). In this context, it has been postulated that only if a certain threshold of averseness is exceeded by a stimulus and/or test situation is the eCB system activated to exert fear alleviating effects (Kamprath et al., 2009). Consequently, Moreira and Wotjak (2010) hypothesized that the eCB system may have a prevailing protective role to prevent exaggerated fear responses. If this hypothesis is confirmed, then the eCB system may underlie the aberrant memory processing and impaired adaptation to changed environmental conditions that has been described in several human neuropsychiatric disorders, such as PTSD (Isaac et al., 2006), and new therapeutic opportunities could be offered for the management of PTSD. In fact, a clinical trial (phase IV) on the efficacy of THC treatment for the management of PTSD is going on. Adult subjects of both genders are being currently recruited and first results will be soon available[2].

In humans, cannabis is mainly consumed due to its euphoriant properties, which are usually accompanied by decreases in anxiety. However, dysphoric reactions, feelings of anxiety, panic,

[1] www.emea.europa.eu
[2] http://ClinicalTrials.gov/show/NCT00965809

paranoia, and psychosis are also frequently reported (see Crippa et al., 2009 for review). Similarly, in rodents a bidirectional profile regarding anxiety-like responses has been reported with low doses of cannabinoid compounds exerting anxiolytic-like effects while the opposite is observed following the administration of high doses (consult for review, Viveros et al., 2005; Moreira and Lutz, 2008). Despite basal emotional state as well as contextual testing conditions are critical in this respect, putative neural mechanisms underlying this biphasic profile have been thoroughly investigated. Anxiolytic- and anxiogenic-like effects of cannabinoid agonists appear to be mediated through the same neurotransmitter systems although by activating different receptors. The endogenous opioid system is involved in the regulation of cannabinoid-induced anxiety-like responses; pharmacological studies indicate that anxiolytic-like responses are mediated by μ- and δ-opioid receptors (Berrendero and Maldonado, 2002) while κ-opioid receptors might be involved in the anxiogenic-like responses (Marin et al., 2003).The serotonergic system, particularly 5-HT1A receptors (5-HT1ARs), participate in the anxiety-related effects of cannabinoid compounds although controversial results have been reported (Marco et al., 2004; Braida et al., 2007). GABAergic and glutamatergic neurotransmissions have also been involved in the anxiety-like responses to cannabinoid compounds. CB1Rs have been localized on both glutamatergic (Domenici et al., 2006; Kawamura et al., 2006; Monory et al., 2006) and GABAergic (Katona et al., 1999, 2001) neurons but such receptors may differ in their sensitivity to cannabinoid compounds. Actually, differences in the cannabinoid sensitivity of glutamatergic and GABAergic neurotransmission between mice and rats was suggested to underlie the differences in cannabinoid-induced anxiety-related responses previously described (Haller et al., 2007). More recently, the contribution of GABAergic and glutamatergic neurotransmission in the biphasic emotional effects of cannabinoids has been analyzed by using KO mice specifically lacking CB1R in GABAergic neurons (GABA-CB1KO) or glutamatergic forebrain neurons (Glu-CB1-KO). The presence of CB1Rs on the glutamatergic terminals may be considered as a requirement for the anxiolytic-like responses elicited following the administration of low doses of a cannabinoid agonist. In contrast, CB1Rs on GABAergic terminals seem to be involved in the anxiogenic-like effects associated to high doses of cannabinoid compounds (Lutz et al., 2010). Taken together, cannabinoid agonists, depending upon their chemical structure and dosage, may act on a diversity of cannabinoid and non-cannabinoid receptors [see above, A Brief Update on the Endocannabinoid (eCB) System] present in distinct neuroanatomical regions and differing in their binding properties. Despite the great efforts devoted to understand the biphasic profile of cannabinoid-induced effects, not exclusive of emotional-related responses, a consensus on the underlying mechanisms has not yet been reached.

Our knowledge on the role of the eCB system in emotion and anxiety disorders has notably increased in the last decade. Data presently available provide evidence for an intrinsic eCB tone that may control emotional homeostasis, mainly acting through CB1R activation. Equilibrium in eCB signaling is pivotal not only to maintain adequate baseline anxiety levels, but also to promote recovery and/or adaptation to stressful and aversive situations. Disequilibrium or malfunctioning of the eCB system might contribute to the etiology of anxiety-related disorders (Sundram, 2006; Marco and Viveros, 2009; Finn, 2010; Parolaro et al., 2010), whereas the pharmacological enhancement of eCB activation may provide a promising therapeutic tool for the management of such disorders (Pacher et al., 2006; Piomelli et al., 2006). Given the successful results accomplished in animal studies, great expectations exist for the future clinical exploitation of this system.

CB1Rs IN DEPRESSION

Several hypotheses for the neurobiological basis of depression have been formulated (Nestler et al., 2002), and, in the last years, a deregulation of the eCB system has been proposed (for review consult Vinod and Hungund, 2006; Parolaro et al., 2010; Gorzalka and Hill, 2011). Evidence for a relationship between the eCB system and human depression has arisen. Clinical populations diagnosed with depression are found to have reduced levels of circulating eCBs (Hill et al., 2009) and an up-regulation of CB1R was observed in the prefrontal cortex of subjects with major depression who died by suicide (Hungund et al., 2004). Furthermore, a genetic risk factor for depression in Parkinson's disease was found to be associated with polymorphisms of human gene for CB1R (CNR1), mapped to chromosome 6q14–15 (Barrero et al., 2005). More recent studies have confirmed that polymorphisms in the CNR1 gene are a risk factor for depression, and have suggested that the CNR1 gene influences vulnerability to psychosocial adversity to later develop depressive symptoms (Juhasz et al., 2009).

Evidence from animal models further support the participation of CB1R in depression. Genetic deletion of CB1R has been reported to induce a behavioral state analogous to depression in experimental animals. CB1KO mice became anhedonic before than wild-type mice when exposed to chronic mild stress (CMS), so lack of CB1R may render animals more vulnerable to the anhedonic effect of chronic stress (Martin et al., 2002a). CB1KO mice have been reported to exhibit a decreased sensitivity to rewarding stimuli (Sanchis-Segura et al., 2004) and a depressive-like phenotype in both the forced–swim test (FST; Steiner et al., 2008) and the test suspension test (TST; Aso et al., 2008). A deficit in extinction of aversive memories has also been reported (Marsicano et al., 2002). Impairments in working memory, measured as spontaneous alternation, have been described in CB1KO mice (Ledent et al., 1999), although performance in other cognitive tasks, i.e., object recognition and active avoidance, was not found to be affected (Reibaud et al., 1999; Maccarrone et al., 2002; Martin et al., 2002a). In addition, anomalies in the hypothalamus–pituitary–adrenal (HPA) axis have been described in CB1KO mice. In particular, an hyperactivity of the HPA axis as suggested by the higher corticosterone levels registered in CB1KO mice after exposure to stress compared to control wild-type animals (Urigüen et al., 2004). Taken together, genetic depletion of the CB1R resulted in a "depressive-like" phenotype at the preclinical level; CB1KO mice displayed an anhedonic state, emotional changes, cognitive deficits, an increased HPA axis activity as well as impairments in stress adaptation (reviewed by Vinod and Hungund, 2006; Parolaro et al., 2010; Gorzalka and Hill, 2011). Brain derived neurotrophic factor (BDNF) is considered a biochemical marker of depression. Actually, depression has been associated to a reduced

expression of BDNF in the hippocampus (Yu and Chen, 2010). Accordingly, decreased BDNF levels have been observed in the hippocampus of CB1KO mice (Aso et al., 2008; Steiner et al., 2008). In a more recent study, gene expression of CB1KO *versus* control wild-type mice has been analyzed by using microarrays technology (Aso et al., 2011). The study revealed an altered gene expression pattern in CB1KO mice (at basal conditions) that may contribute to the depressive-like phenotype and to the increased reactivity to stress previously described in these mutant animals (Aso et al., 2011). A comparative study following repeated exposure to stress was also performed, and most differences in stress reactivity were observed in the raphe nucleus, a brain region closely related to depression (Aso et al., 2011).

Apart from evidences from the genetic and pharmacological blockade of CB1R, changes in receptor expression have also been described in diverse animal models of depression (**Table 1**). A consistent increase of CB1R expression in the prefrontal cortex has been reported in different animal models of depression, i.e., CMS (Bortolato et al., 2007), chronic unpredictable stress (Hill et al., 2008), and bilateral olfactory bulbectomy (Rodriguez-Gaztelumendi et al., 2009). Noticeably, a similar effect was found for cortical CB1R expression in a population of depressed suicides (Hungund et al., 2004). In contrast, decreases in CB1R expression have been reported in hippocampus (Hill et al., 2005, 2008; Reich et al., 2009), hypothalamus, ventral striatum (nucleus accumbens; Hill et al., 2008), and midbrain (Bortolato et al., 2007). However, discrepancies regarding changes in CB1R expression in animal models of depression have been found, and may probably be due to differences in the animal model employed and/or in the technique used. Sex differences have been reported for several aspects of pathologies, however, male and female animals are hardly considered in preclinical studies. In the CMS model of depression a down-regulation of hippocampal CB1R has been observed among adult male animals (Hill et al., 2005, 2008; Reich et al., 2009), whereas a CB1R up-regulation was found exclusively in the dorsal hippocampus of females (Reich et al., 2009). In accordance with these findings, sex differences in the eCB system might be hypothesized, at least in relation to stress-responding circuitries. However, more research is still needed to better understand the behavioral implications of the regional and sexual specific changes in brain CB1R expression.

As for anxiety disorders, a dysfunction of the eCB system has been proposed to be in the bases of depression (Vinod and Hungund, 2006; Parolaro et al., 2010; Gorzalka and Hill, 2011). Enhancing the levels of eCBs by inhibiting their deactivation has become a promising antidepressant strategy (Pacher et al., 2006; Bambico and Gobbi, 2008). In contrast, inactivation of CB1Rs can have detrimental consequences provoking depressive-like symptoms. In fact, rimonabant adverse effects included not only increased anxiety, but also depression and suicidal ideations (Doggrell, 2008; Rosenstock et al., 2008; Scheen, 2008; Van Gaal et al., 2008). In this line, an association between depression and prolonged cannabis consumption and its withdrawal have also been reported (Degenhardt et al., 2003). Despite appealing, existing literature suggests caution in the pharmacological exploitation of the eCB system. Indeed, further investigation is necessary to understand the clinical limits of such manipulation that may differ among sexes, age, and individuals.

Table 1 | Changes in CB1R expression in depressed patients and animal models of depression.

Model/diagnosis	Species	Brain region	CB1R expression	References
Depressed suicide victims	Human	Dorsolateral prefrontal cortex	↑	Hungund et al. (2004)[1]
CMS	Rat, Wistar	Prefrontal cortex	↑	Bortolato et al. (2007)[3]
		Hippocampus	–	
		Midbrain	↓	
	Rat, Sprague-Dawley	Hippocampus – males	↓	Reich et al. (2009)[1]
		Hippocampus (dorsal) – females	↑	
CUS	Rat, Long-Evans	Hippocampus	↓	Hill et al. (2005)[1]
		Limbic forebrain	–	
	Rat, Long-Evans	Prefrontal cortex	↑	Hill et al. (2008)[2]
		Hippocampus	↓	
		Hypothalamus	↓	
		Amygdala	–	
		Midbrain	–	
		Ventral striatum	↓	
OBX	Rat, Sprague-Dawley	Prefrontal cortex	↑	Rodriguez-Gaztelumendi et al. (2009)[2]
		Caudate–putamen	–	
		Hippocampus	–	
		Amygdala	↑	
		Dorsal raphe nucleus	–	

Animal models of depression: CMS, chronic mild stress; CUS, chronic unpredictable stress; OBX, bilateral olfactory bulbectomy. Symbols, ↑ increased, – not modified, or ↓ decreased receptor expression. Receptor protein expression was evaluated by Western blotting[1], or binding assays[2]; and gene expression by real-time PCR[3]. Modified from Parolaro et al. (2010) and extended.

CB1Rs IN SCHIZOPHRENIA

The association between cannabis use and psychosis has long been recognized (see for review, D'Souza et al., 2009), and recent advances in the neurobiology of cannabinoids have renewed interest in the association between cannabis and schizophrenia (see Muller-Vahl and Emrich, 2008; Fernandez-Espejo et al., 2009; Parolaro et al., 2010 for review). There are several lines of evidence that support an association between an altered eCB system and the pathogenesis of schizophrenia. In clinical studies, up-regulation of CB1R has been described in cortical brain regions such as the dorsolateral prefrontal cortex (Dean et al., 2001) and in cingulate cortex (Zavitsanou et al., 2004; Newell et al., 2006) of schizophrenic patients. However, investigation in post mortem schizophrenic brains have yielded contrasting results, and a both no changes or even decreases in CB1R expression have also been described. No changes in the superior temporal gyrus (Deng et al., 2007), and the anterior cingulate cortex (Koethe et al., 2007) have been found as well as decreases in CB1R expression in the prefrontal cortex (Eggan et al., 2008). Unfortunately, most of the studies did not consider the pharmacological treatment given to patients, a confounding factor that may have altered the results achieved. Indeed, antipsychotics have been reported to decrease prefrontal cortex CB1R expression in schizophrenic patients in the absence of changes in drug-free schizophrenics (Uriguen et al., 2009).

In addition, genetic studies have indicated that variants within the CNR1 gene are directly associated with schizophrenia. Individuals with a 9-repeat allele of an AAT-repeat polymorphism of the CNR1 gene showed a 2.3-fold higher susceptibility to the hebephrenic form of schizophrenia in a Japanese population (Ujike and Morita, 2004), that was further confirmed in a population of the Central Valley of Costa Rica (Chavarria-Siles et al., 2008). However, such an association was no longer present if more general types of schizophrenia were considered. In addition, the presence of a polymorphism (G allele) of CNR1 has been associated with a better therapeutic effect of antipsychotics (Hamdani et al., 2008). Despite numerous data that support this association, negative data have also been found (Tsai et al., 2000; Seifert et al., 2007); therefore, the debate about the existence of a real relationship between CNR1 mutations and schizophrenia is still open and deserves further investigations.

Contribution of CB1Rs to schizophrenia has also been investigated in animal models that mimic some of the symptoms of the disease (**Table 2**). The schizophrenic-like effects induced by the administration of phencyclidine (PCP), a *N*-methyl-D-aspartate (NMDA) antagonist, in wild-type mice (e.g., increased locomotion, stereotyped behaviors, and decreased social interactions) were not observed in CB1KO mice. In contrast, PCP administration in CB1KO mice decreased locomotion, notably enhanced ataxia and stereotypy but induced no changes in social interaction. Since genetic CB1R blockade dramatically alters the behavioral consequences of PCP, this receptor may play a critical role in schizophrenia, although a differential participation in the negative (e.g., social disruption) and positive symptoms (e.g., stereotypy) of schizophrenia was hypothesized (Haller et al., 2005). Repeated PCP injections have been extensively used to induce enduring cognitive deficits with particular relevance to schizophrenia (consult Amitai et al., 2007; Grayson et al., 2007 as examples), and the participation of the eCB system in this PCP model of cognitive dysfunction have been analyzed (Vigano et al., 2009). Chronic-intermittent PCP administration induced an enhancement in CB1R density in the amygdala and in the ventral tegmental area when compared to the control group. Similarly, CB1R functionality was also altered in several brain areas implicated in schizophrenia; in particular, it was reduced in the prefrontal cortex, hippocampus, substantia nigra, and cerebellum, and increased in the globus pallidus. Alterations in endocannabinoid levels mainly in the prefrontal cortex, i.e., an increase in the levels of 2-AG in PCP-treated rats, were also found. These findings allowed authors to suggest that a maladaptation of the endocannabinoid system might contribute to the glutamatergic-related cognitive symptoms encountered in schizophrenia disorders (Vigano et al., 2009). Furthermore, chronic THC administration worsened cognitive performance in this animal model (Vigano et al., 2009), providing evidence for the hypothesis that cannabis consumption may be a risk factor for development or worsening of the schizophrenia disorder.

Increasing evidence gives support to the fact that schizophrenia is a subtle disorder of brain development and plasticity (Lewis and Levitt, 2002; Tyrka et al., 2008), thus reinforcing the neurodevelopment hypothesis of schizophrenia. Brain developmental abnormalities, often related to early traumatic experiences, have been extensively associated to schizophrenia (Lewis and Levitt, 2002; Tyrka et al., 2008). Therefore, changes in CB1R expression have been analyzed in schizophrenia animal models with a base in neurodevelopment, i.e., early maternal deprivation (Ellenbroek and Riva, 2003; Marco et al., 2009). At adulthood, maternally deprived animals (24 h at postnatal day 9) show behavioral abnormalities that resemble psychotic-like symptoms (Ellenbroek and Riva, 2003) including notable cognitive impairments (Llorente et al., 2011; Llorente-Berzal et al., 2011). Notably, a long-lasting decrease in CB1R expression has been found in maternally deprived animals within the hippocampus. Such a reduction in CB1R expression was observed in the short-term, at postnatal day 13, in both male and female rat pups (Suarez et al., 2009), as well as in the long-term, at adulthood (Llorente-Berzal et al., 2011), thus suggesting a endurable impact of early maternal deprivation in the eCB system that may contribute to some of the behavioral anomalies observed in these animals. Rearing rats in isolation has also been used as a model for the investigation of schizophrenia (consult Fone and Porkess, 2008 for review). Following isolation rearing, rats display social, and cognitive impairments. In particular, hyperlocomotion and increased aggressiveness have been reported, together with deficits in memory recognition and a reduce PPI response (Sciolino et al., 2010; Zamberletti et al., 2010). In relation to changes in the eCB system, raising rats in isolation led to a significant decrease in CB1R expression in caudate–putamen and amygdala (Malone et al., 2008). However, discrepant data have been achieved in more recent studies using this animal model of schizophrenia. Increases in CB1R expression has been described in the caudate–putamen of isolated-reared rats (Sciolino et al., 2010) as well as in prefrontal cortex, certain thalamic nuclei and the posterior area of the hypothalamus (Robinson et al., 2010). In contrast, decreases in receptor expression have been reported in the supraoptic nucleus of the hypothalamus and in the ventrolateral thalamic nucleus

Table 2 | Changes in CB1R expression in schizophrenia and animal models of neuropsychiatric disorders.

Model/diagnosis	Species	Brain region	CB1R expression	References
Schizophrenia	Human, Victorian Institute of Forensic Medicine, Victoria (Australia)	Dorsolateral prefrontal cortex	↑	Dean et al. (2001)
		Caudate–putamen	–	
		Areas within the temporal lobe	–	
	Human, New South Wales Tissue Resource Centre, University of Sydney (Australia)	Anterior cingulate cortex	↑	Zavitsanou et al. (2004)
	Human, New South Wales Tissue Resource Center, University of Sydney (Australia)	Posterior cingulate cortex	↑	Newell et al. (2006)
	Human, NSW Tissue Resource Centre (Australia)	Superior temporal gyrus	–	Deng et al. (2007)
	Human, Stanley Neuropathology Consortium Collection, Bethesda, MD (USA)	Anterior cingulate cortex	–	Koethe et al. (2007)
	Human, mainly died by suicide, Basque Institute of Legal Medicine, Bilbao (Spain), Institute of Forensic Medicine, Geneva (Switzerland)	Dorsolateral prefrontal cortex	–	Uriguen et al. (2009)
	Human, Allegheny County Medical Examiner's Office, Pittsburgh, Pennsylvania	Dorsolateral prefrontal cortex	↓	Eggan et al. (2008)
C–PCP	Rats, Lister-Hooded	Amygdala	↑	Vigano et al. (2009)
		Ventral tegmental area	↑	
MD	Rats, Wistar	Hippocampus	↓	Suarez et al. (2009)
	Rats, Wistar	Hippocampus	↓	Llorente-Berzal et al. (2011)
IR	Rats, Sprague-Dawley	Caudate–putamen	↓	Malone et al. (2008)
		Amygdala	↓	
	Rats, Sprague-Dawley	Diverse brain regions	–	Zamberletti et al. (2010)
	Rats, Sprague-Dawley	Caudate–putamen (rostral)	↑	Sciolino et al. (2010)
		Hypothamalus (superoptic nucleus)	↓	
		Thalamus nuclei (ventrolateral)	↓	
	Rats, Sprague-Dawley	Prefrontal cortex	↑	Robinson et al. (2010)
		Thalamic nuclei	↑	
		Hypothalamus (posterior area)	↑	

Animal models of schizophrenia: C–PCP, chronic and intermittent PCP administration; MD, maternal deprivation (24 h on postnatal day 9); IR, rearing in social isolation. Symbols, ↑ increased or ↓ decreased receptor expression. In animal models, only changes in affected brain regions are described.

(Sciolino et al., 2010). In another study, in the absence of changes in CB1R expression, isolated-reared rats presented a consistent decrease in CB1R functionality in most of the regions analyzed, i.e., prefrontal cortex, nucleus accumbens, caudate–putamen, hippocampus, and ventral tegmental area (Zamberletti et al., 2010). Discrepancies may be due to the different techniques employed to evaluate CB1R density, to differences in the rat strains used, as well as in the duration of the isolation rearing protocol. Even though, taken together, these results indicate that the eCB system is altered in this animal model of schizophrenia, i.e., rearing in social isolation. In spite of the current discrepancies regarding CB1R changes in animal models of schizophrenia, present findings point to the eCB system as a pivotal neuromodulatory pathway that may have a critical relevance in the psychotic-related behaviors observed in these animals, i.e., altered emotionality and social and cognitive deficits. However, further research is needed to better understand the region-specific CB1R changes here described, and to establish a direct correlation between such changes and the behavioral anomalies reported.

There is now evidence demonstrating an association between increased rates of cannabis use and new cases of schizophrenia (see for review, Di Forti et al., 2007; Cohen et al., 2008; Leweke and Koethe, 2008). Epidemiological studies suggest a high incidence of schizophrenia within marijuana smokers (Moore et al., 2007) and

long-term users of cannabis exhibit similar cognitive deficits to those seen in schizophrenia (Solowij and Michie, 2007). Cannabis has been considered a risk factor for development or worsening of the schizophrenia disorder, and evidence indicating that young people at genetic high risk for schizophrenia are particularly vulnerable to mental health problems associated with cannabis use is now available (Hollis et al., 2008). Moreover, cannabis use has been associated with a decrease in age of onset of schizophrenia, frequently related with a poorer outcome (Sugranyes et al., 2009). Literature from animal models further support adolescence as a highly vulnerable age for the consequences of cannabis exposure (Schneider, 2008). Adolescent chronic cannabinoid treatment leads to long-lasting behavioral deficits. Decreased emotionality (Biscaia et al., 2003; Wegener and Koch, 2009), although no changes in locomotor activity nor in object recognition memory have been reported (Schneider et al., 2005). Lasting disruption of pre-pulse inhibition (Schneider et al., 2005; Wegener and Koch, 2009) as well as persistent deficits in social recognition and impaired social interaction have been described following adolescent cannabinoid administration (Leweke and Schneider, 2011). Such behavioral anomalies were restored by antipsychotic treatment, further confirming the suitability of chronic pubertal cannabinoid administration as an animal model for diverse aspect of schizophrenia (Wegener and Koch, 2009; Leweke and Schneider, 2011). Despite more research is needed, reducing and/or limiting cannabis consumption in our society, especially among vulnerable populations (adolescents and people at risk for psychopathologies) might be convenient in order to reduce dependence and mental health risks in society.

A ROLE FOR CB2Rs IN NEUROPSYCHIATRIC DISORDERS

Despite CB2R was initially claimed as a peripheral cannabinoid receptor, its presence in CNS is still controversial [see A Brief Update on the Endocannabinoid (eCB) System]. As previously mentioned, CB2R has been detected in a diversity of brain regions including cerebral cortex, hippocampus, amygdala, hypothalamus, and cerebellum (Van Sickle et al., 2005; Gong et al., 2006; Onaivi, 2006; García-Gutiérrez et al., 2010), thus suggesting a role for CB2Rs in emotional and cognitive function.

CB2Rs IN MOOD DISORDERS: ANXIETY AND DEPRESSION

Recent results from mice with genetically modified CB2R suggest that CB2R-signaling is clearly involved in the regulation of emotional behavior (**Table 3**). Mice lacking CB2R (CB2R knockout mice, CB2KO) presented increased vulnerability to stressful stimuli in the light–dark box, the elevated plus-maze, and the TST (Ortega-Alvaro et al., 2011). In contrast, transgenic mice over-expressing CB2R in the CNS (CB2xP; Racz et al., 2008a,b) presented a clear endophenotype resistant to stressful stimuli in the light–dark box and elevated plus-maze tests (Ortega-Alvaro et al., 2011). These results are not consistent with those reported by Onaivi et al. (2008b) showing that intracerebroventricular administration of an antisense oligonucleotide directed against CB2R mRNA resulted in anxiolytic-like effects in mice. Discrepancies between these two studies may be due to the fact that (1) the effects of intracerebroventricular administration of CB2 antisense oligonucleotide could act on different brain regions that may differ from those where CB2R are over-expressed in CB2xP mice, and (2) the different genetic background used in both studies (DBA/2, C57BL/6, BALB/c, and Swiss ICR). Similarly, CB2xP mice exhibited an endophenotype resistant to acute depressogenic-like stimuli (novelty-suppressed feeding test, NSFT, and TST) and CMS. Indeed, 6 weeks after CMS, CB2xP mice presented reduced passive coping behavior in the TST and did not experience anhedonia (García-Gutiérrez et al., 2010). The marked behavioral alterations occurring in CB2xP mice were associated with changes in BDNF, a well-known biochemical marker of depression (see also section "CB1RS in depression"). BDNF plays an important role in adult neurogenesis by modulating survival and plasticity of adult neurons and glia cells (Huang and Reichardt, 2001). As previously mentioned, depression has been associated to a decrease in hippocampal BDNF expression (Yu and Chen, 2010), probably related to the reported reduction in hippocampal neurogenesis previously described among patients with mood disorders (Sheline, 2000). In accordance, a similar decrease in hippocampal BDNF has been reported in animals exposed to CMS, probably indicating diminished hippocampal neurogenesis (Manji et al., 2001; Nestler et al., 2002). Interestingly, CMS failed to produce any modification in BDNF protein and gene expressions in the hippocampus of CB2xP mice (García-Gutiérrez et al., 2010). All these data support the role of CB2R in the normalization of reduced BDNF expression of mice exposed to CMS and suggests the involvement of CB2R in the regulation of mood disorders. In line with these findings, an association between cannabinoid CB2R polymorphism Q63R has been detected in Japanese depressed subjects (Onaivi et al., 2008a).

Taken together, these results allow us to hypothesize that the overexpression of CB2R decreases the vulnerability to depressogenic stimuli. The idea to induce CB2R overexpression by pharmacological manipulation was carried out by treating chronically wild-type mice with the cannabinoid CB2R antagonist, AM630. Indeed, 4 weeks of administration with AM630 increased CB2R gene expression (thus mimicking the phenotype of CB2xP mice), reversed the CMS-induced reduction of immobility evaluated in the TST, the diminished sucrose solution intake, and the diminished CB2R and BDNF gene and protein expression in the hippocampus (García-Gutiérrez et al., 2010). However, previous studies reported a lack of effects after the administration of AM630 on the intake of sucrose solution in CMS (Onaivi et al., 2008a). These discrepancies may be due to: (1) individual and species differences between the strains used and (2) different dosage or pattern of administration of AM630. Onaivi and colleagues used doses of 1 or 3 mg/kg once a day. In contrast, García-Gutiérrez et al. (2010) studied the effects of AM630 (1 mg/kg) administered twice a day.

In addition, the behavioral picture of CB2xP mice was paralleled with alterations in the HPA axis. Changes in the HPA axis have been associated with anxiety-related disorders in rodents and humans (see also previous section). The reduced secretion of HPA axis hormones was also detected in patients with stress-related disorders. Indeed, hypocortisolism was observed in patients with PTSD, fatigue syndrome, fibromyalgia, and other somatoform disorders (Heim et al., 2000). Restraint stress slightly increased pro-opiomelanocortin (POMC) gene expression (22%) in the arcuate nucleus of hypothalamus in CB2xP mice whereas failed to

Table 3 | Evidences for a role of CB2R in emotional behavior and neuropsychiatric disorders.

Genetic manipulation of CB2Rs

Mutation	Species	Paradigm	Behavioral phenotype	References
Lack of CB2R, knock-out (CB2KO)	Mouse, Swiss ICR	OF	↓ Spontaneous motor activity	Ortega-Alvaro et al. (2011)
		OF	↑ Sensitivity to the motor stimulant effects of cocaine	
		LD, EPM, TST	↑ Vulnerability to anxiogenic and depressogenic-like stimuli	
		SDIA	Disrupted short- and long-term memory consolidation	
		PPI	↑ PPI response	
Overexpression of CB2R (CB2xP)	Mouse, Swiss ICR	TST, NSFT, CUMS	↓ Vulnerability to depressogenic-like stimuli	García-Gutiérrez et al. (2010)
		LD, EPM	↓ Vulnerability to anxiogenic-like stimuli	García-Gutiérrez and Manzanares (2011)
		LD	Lack of anxiolytic effects of benzodiazepines (alprazolam)	

Pharmacological manipulation of CB2Rs

Drug	Species	Paradigm	Treatment, dose	Response	References
ACUTE TREATMENT					
GW405833 (CB2R agonist)	Rats, Sprague-Dawley	MB	10 and 30 mg/kg	–	Valenzano et al. (2005)
		Rotarod	100 mg/kg	↓ Anxiety-like responses and ataxia	
JWH015 (CB2R agonist)	Mouse, C57BL/6	EPM	1–20 mg/kg	↓ Anxiety-like responses	Onaivi (2006)
	Mouse, C57BL/6	LD	1–20 mg/kg	↑ Anxiety-like responses	Onaivi et al. (2008b)
JWH133 (CB2R agonist)	Mouse, Swiss ICR	LD	0.5, 1, and 2 mg/kg	–	García-Gutiérrez et al. (2011)
SR144528 (CB2R antagonist)	Mouse, BALBc	LD	1–20 mg/kg	–	Onaivi et al. (2008b)
AM630 (CB2R antagonist)	Mouse, Swiss ICR	LD	1, 2, or 3 mg/kg	↑ Anxiety-like responses	García-Gutiérrez et al. (2011)
CHRONIC TREATMENT					
JWH133 (CB2R agonist)	Mouse, Swiss ICR	LD	0.5, 1, or 2 mg/kg	↑ Anxiety-like responses	García-Gutiérrez et al. (2011)
		EPM	1 week (twice a day)		
JWH015 (CB2R agonist)	Mouse, BALBc	AT	20 mg/kg	Enhanced sucrose consumption	Onaivi et al. (2008b)
			4 weeks (once a day)		
AM630 (CB2R antagonist)	Mouse, BALBc	AT	1 and 3 mg/kg	–	Onaivi et al. (2008b)
			4 weeks (once a day)		
	Mouse, Swiss ICR	CUMS	1, 2, and 3 mg/kg	Antidepressant-like	García-Gutiérrez et al. (2010)
			4 weeks (twice a day)		
	Mouse, Swiss ICR	LD	1, 2, and 3 mg/kg	↓ Anxiety-like responses	García-Gutiérrez et al. (2011)
		EPM	1 week (twice a day)		

All drugs were administered intraperitoneally (ip).Behavioral paradigms: OF, Open field; LD, light–dark box; EPM, elevated plus-maze; TST, tail suspension test; SDIA, step down inhibitory avoidance; PPI, pre-pulse inhibition of the acoustic startle response; NSFT, novelty-suppressed feeding test; CUMS: chronic unpredictable mild stress; MB, marble burying test; AT, anhedonia test: sucrose consumption. Symbols, ↑ increased (anxiogenic-like), ↓ decreased (anxiolytic-like), or – no changes in behaviour.

produce any modification in corticotropin releasing factor (CRF) gene expression of the paraventricular nucleus (García-Gutiérrez and Manzanares, 2011). These results suggest that CB2R may be contributing to the maintenance of the steady state control of the HPA axis. The fact that the overexpression of CB2R blocked the effects of stress on CRF gene expression points to the idea that the mechanism controlling the HPA axis in CB2xP mice may be acting at the level of synthesis or release of CRF. The overexpression of CB2R was also accompanied by changes in the GABAergic system, more particularly by alterations of GABA-A receptor subunits. Benzodiazepines are anxiolytic drugs often used in the treatment of certain anxiety or mood related disorders. Benzodiazepines, acting through their binding on the interface of α and γ subunits of the GABA-A receptor complex are known to act as anxiolytics promoting the inhibitory actions of the GABA neurotransmitter in the CNS (Da Settimo et al., 2007). Recent studies suggested that GABA-A receptors containing α2 and γ2, enriched in corticolimbic structures mediate the anxiolytic effect of benzodiazepines (Low et al., 2000). The administration of alprazolam, a well-known anxiolytic benzodiazepine, failed to produce any effect in CB2xP mice at either of the doses used (45 and 70 μg/kg; García-Gutiérrez and Manzanares, 2011). This behavioral scenario was associated to changes in the expression of α2 and γ2 subunits of GABA-A receptors in specific brain areas. Increased GABA-A α2 and γ2 subunits receptor gene expression was found in the amygdala and hippocampus of CB2xP mice (García-Gutiérrez and Manzanares, 2011). The increased gene expression of both GABAergic subunits may be related, at least in part, with the lack of the anxiolytic effect of benzodiazepines in CB2xP mice. Moreover, these results support the potential implication of CB2R in the regulation of GABAergic system. In this respect, recent studies revealed a suppression of GABAergic inhibitory signaling in the entorhinal cortex–hippocampal slices following the administration of the cannabinoid CB2R agonist, JWH133 (50 nM). Interestingly, these effects could be blocked by prior administration of AM630 (50 nM) supporting the involvement of CB2R in the effects of JWH133 on GABAergic signaling (Morgan et al., 2009). These results further strengthen the involvement of CB2R in the regulation of GABAergic release from neuronal terminals.

Pharmacological manipulation of CB2R may alter the response to anxiogenic or depressogenic-like stimuli (see **Table 3** for details). In rodents, the pharmacological manipulation of CB2R by the administration of agonists or antagonists resulted in controversial reported effects on emotional behavior. Acute administration of GW405833 (100 mg/kg), a CB2R agonist, induced anxiolytic effects in the marble burying test (Valenzano et al., 2005). Indeed, an anxiolytic effect in the elevated plus-maze test was reported after the acute administration of the CB2R agonist, JWH015 (Onaivi, 2006). In contrast, the same group reported anxiogenic effects of JWH015 in the light–dark box (Onaivi et al., 2006). The present discrepancies may be due to: (1) the drugs used, (2) the route of drug administration, (3) the doses used, and (4) the strain of mice studied. Indeed, the fact that the doses of the CB2R agonist used resulted in motor alterations may have masked the interpretation of these behavioral effects. A recent publication evaluated the effects of acute and chronic administration of JWH133, a CB2R agonist, and AM630, a CB2R antagonist, on emotional behavior at doses that did not modify motor activity. Acute administration of JWH133 failed to produce any modification in the response to acute stimuli in the light–dark test. In contrast, the acute blockade of CB2R by AM630 resulted in anxiogenic effects. The fact that the anxiogenic effects of AM630 were blocked by the previous administration of JWH133 supported the involvement of CB2R in the acute effects of AM630 on emotional behavior (García-Gutiérrez et al., 2011). However, chronic blockade of CB2R by the antagonist AM630 resulted in anxiolytic effect associated with increased CB2R gene and reduced CB2R protein expression in cortex and amygdala. In contrast, chronic activation of CB2Rs by JWH133 resulted in an opposite behavioral and molecular alterations. Mice chronically treated with JWH133 presented an anxiogenic effect associated with reduced CB2R gene and increased CB2R protein expression in cortex and amygdala. Indeed, the administration of JWH133 and AM630 are associated with alterations in GABAergic system. Chronic blockade of CB2R by AM630 increased GABA-Aα2 and GABA-Aγ2 gene expressions in the cortex and amygdala. In contrast, the protein expression of these genes was reduced by chronic treatment with AM630 in the brain regions mentioned. Interestingly, activation of CB2R by JWH133 reduced the GABA-Aα2 and GABA-Aγ2 gene expression and increased its protein expression in the cortex and amygdala. The opposite behavioral and molecular changes observed between chronic CB2R blockade (AM630) or activation (JWH133) gives support to the key role of these targets (CB2R, GABA-Aα2, and GABA-Aγ2) in the behavioral effects of AM630 or JWH133. These results provide new insights into the different molecular events related to GABA-A receptor gene and protein expression produced by chronic manipulation of CB2R with CB2R agonism or antagonism.

In summary, the data presented here provide evidence for the interesting putative role of CB2R in anxiety and depressive-like disorders and as a possible target for the development of a novel class of anxiolytic or antidepressant drug. In this respect, the administration of AM630 clearly decreased the anxious state of DBA/2 mice (García-Gutiérrez et al., 2011). Further pharmacological studies are necessary to explore the potential therapeutic uses of cannabinoid CB2R in humans and the precise mechanisms underlying these effects.

CB2Rs IN SCHIZOPHRENIA

Different evidences support the involvement of CB2R in schizophrenia disorders. Clinical remission of schizophrenia has been reported to be accompanied by significant decreases in AEA and CB2R mRNA levels in peripheral blood mononuclear cells (Giuffrida et al., 2004). In addition, a recent publication revealed a close relation between diminished CB2R function (polymorphism Q63R) and increased susceptibility to schizophrenia in the presence of other risk factors (Ishiguro et al., 2010b).

The use of genetically modified mice led to further investigate the potential role of CB2R in schizophrenic-related disorders. Similarly to the alterations observed in schizophrenic patients (Braff et al., 2001; Iyer et al., 2008; Peralta et al., 2010), the lack of CB2Rs resulted in alterations of motor activity, anxiety and depressive disorders, and cognitive deficits including impaired

sensoriomotor gating (Ortega-Alvaro et al., 2011). CB2KO mice exhibited decreased spontaneous motor activity and increased sensitivity to the motor stimulant effects of acute cocaine administration in the open field test. Indeed, as mentioned before, CB2KO mice presented increased vulnerability to anxiogenic (light–dark box and elevated plus-maze) and depressogenic-like stimuli (TST). Furthermore, CB2KO mice showed disrupted short- and long-term memory consolidation in the step down inhibitory avoidance paradigm. Indeed, CB2KO mice presented a significantly reduced pre-pulse inhibition of the acoustic startle response (PPI), alteration observed in rodent models of schizophrenia and schizophrenic patients (Braff et al., 2001). Interestingly, the PPI deficit observed in CB2KO mice was markedly enhanced after chronic oral treatment with risperidone, an antipsychotic drug (see **Table 3** for details).

It is known that schizophrenia is associated with brain abnormalities induced during the development of the CNS (Rapoport et al., 2005; Ross et al., 2006). A number of findings suggest a pro-neurogenic role of CB2R in the control of fundamental neural cell processes (Harkany et al., 2007; Galve-Roperh et al., 2008; Katona and Freund, 2008). Therefore, it can be hypothesized that the lack of CB2R might impair neural development, thus inducing relevant alterations in several brain areas. In this respect, CB2KO mice presented increased dopamine D2 receptor (D2R) and adrenergic α2C receptor (α2CR) gene expression in prefrontal cortex and locus coeruleus, and decreased serotoninergic 5-HT2C (5-HT2CR) and 5-HT2A receptors (5-HT2AR) gene expression in the dorsal raphe and the prefrontal cortex, respectively. Interestingly, risperidone treatment to CB2KO mice induced a reduction in the gene expression of D2R, 5-HT2CR, and 5-HT2AR in the prefrontal cortex, and α2CR in the locus coeruleus; but increased 5-HT2CR and 5-HT2AR gene expression in the dorsal raphe. Despite additional targets may be involved in the behavioral alterations observed in CB2KO mice, the fact that the risperidone tended to "normalize" the molecular alterations observed in these mice supports the involvement of dopaminergic, serotoninergic, and adrenergic alterations in the PPI deficit of CB2KO mice (Ortega-Alvaro et al., 2011). These results suggest that CB2R deletion was related to the observed schizophrenia-like behaviors. Pharmacological manipulation of CB2R may be further explored as a potential therapeutic target for the treatment of schizophrenia-related disorders.

A DISTINCTIVE ROLE OF CANNABIDIOL (CBD) IN NEUROPSYCHIATRIC DISORDERS

Cannabidiol (CBD) is the main non-psychotropic phytocannabinoid found in the *C. sativa* plant, constituting up to 40% of its extract. Recent comprehensive reviews indicate that CBD is one of the most promising candidates for therapeutic use in a wide range of disorders, including neuropsychiatric (Mechoulam et al., 2007; Zuardi, 2008; Izzo et al., 2009). As discussed bellow, part of its effects seems to depend on facilitation of eCB-mediated neurotransmission.

CBD IN ANXIETY AND DEPRESSION

Initial studies in laboratory animals produced contradictory results. Whereas Zuardi and Karniol (1983) showed that low doses (10 mg/kg) of CBD attenuated conditioned emotional responses in rats, Silveira Filho and Tufik (1981) failed to find any effect of a much higher dose (100 mg/kg) in a conflict paradigm. These apparently opposite results were subsequently explained by Guimarães et al. (1990), who verified that CBD causes an inverted U-shaped dose-related anxiolytic response curve in the elevated plus-maze, with the anxiolytic doses ranging from 2.5 to 20 mg/kg. Subsequent studies employing diverse animal models, including the elevated plus-maze, Vogel conflict test, contextual fear conditioning, marble burying test, and attenuation of stress responses, confirmed that systemically injected CBD decreases anxiety-like behaviors in rodents (Onaivi et al., 1990; Guimarães et al., 1994; Moreira et al., 2006; Resstel et al., 2006; Casarotto et al., 2010).

More recently the brain sites responsible of these effects have been investigated by direct brain administration of CBD. The drug caused anxiolytic-like effects after microinjection into the dorsolateral periaqueductal gray (dlPAG), bed nucleus of the *stria terminalis* (BNST), and prelimbic medial prefrontal cortex (Campos and Guimarães, 2008; Moreira et al., 2009; Alves et al., 2010; Lemos et al., 2010; Soares et al., 2010; Gomes et al., 2011). It also facilitated extinction in a contextual aversive conditioning model after intracerebral ventricular administration (Bitencourt et al., 2008). Interestingly, in the infralimbic prefrontal cortex CBD induced an anxiogenic effect, facilitating conditioned emotional responses (Lemos et al., 2010). Moreover, no consistent effect was found in the amygdala (Lisboa and Guimarães, unpublished results). Taken together, these results indicate that the anxiolytic effects of CBD depend on drug action in specific brain areas related to defensive responses.

Clinical studies have confirmed that CBD possess anxiolytic properties. In addition to prevent the anxiogenic effects of high doses of THC (Zuardi et al., 1982), CBD was able to decrease anxiety in healthy subjects submitted to a simulated public speaking paradigm (Zuardi et al., 1993a). Using a similar paradigm, Bergamaschi et al. (2011) have recently shown that the drug reduces public speaking anxiety in treatment-naïve social phobic patients. In agreement with these findings, neuroimaging studies show that CBD can change brain activity in regions related to emotional responses. It impairs connectivity between the prefrontal and subcortical regions (Fusar-Poli et al., 2010), attenuates blood oxygenation level-dependent responses to fearful faces in the amygdala and cingulate cortex (Fusar-Poli et al., 2009) and decreases activation in the left amygdala–hippocampal complex and left posterior cingulate gyrus (Crippa et al., 2004). In relation to depressive-related disorders, CBD has been reported to prevent the physiological and delayed anxiogenic consequences of restraint stress in animal models (Resstel et al., 2009), an effect that has been related to depressive responses (Guimarães et al., 1993). More recently, CBD was shown, similarly to the prototype antidepressant imipramine, to decrease immobility time in the forced swimming test (Zanelati et al., 2010). Although more studies are clearly needed, these initial results suggest that CBD does possess antidepressive properties.

CBD AND PSYCHOSIS/SCHIZOPHRENIA

Several studies performed in laboratory animals during the 1970s indicated that CBD could interact with the main cannabinoid

present in the *C. sativa* plant, delta-9-tetrahydrocannabinol (THC, for review see Zuardi et al., 2006a; Zuardi, 2008). In a seminal study published in 1982, Zuardi et al. (1982) observed that CBD could block the psychotomimetic and anxiogenic effects of THC in healthy volunteers. This observation led to the hypothesis that CBD could have antipsychotic and/or anxiolytic properties. The former proposal was supported by preclinical results indicating that the drug is able to reduce the stereotypies and hyperlocomotion caused by apomorphine and amphetamine, respectively, without causing catalepsy or increasing prolactin levels (Zuardi et al., 1991, 1993b; Moreira and Guimarães, 2005). This "atypical" antipsychotic profile was further confirmed in a c-Fos study where CBD, similarly to clozapine, increased c-Fos expression in limbic areas and prefrontal cortex, but not in the dorsal striatum (Guimarães et al., 2004).

In addition to dopamine-based models predictive of antipsychotic activity, CBD effects have also been tested in glutamate-based models. CBD blocked the hyperlocomotion induced by ketamine (Moreira and Guimarães, 2005) as well as the disruption of pre-pulse inhibition induced by MK-801 (Long et al., 2006). CBD was also able to restore the deficit in social interactions induced by this drug (Gururajan et al., 2011).

The possible antipsychotic profile of CBD indicated by these preclinical results is further supported by clinical studies (for review see Zuardi, 2008). In agreement with the initial report by Zuardi et al. (1982), showing that CBD is able to antagonize the psychotomimetic effects of THC in healthy volunteers, the presence of this compound in *Cannabis* strains seems to be protective against the occurrence of psychotic reactions (Morgan and Curran, 2008) as well as Cannabis-associated decrease in hippocampal volume (Demirakca et al., 2011). CBD is also able to attenuate psychosis symptoms induced by ketamine or L-DOPA in healthy volunteers and Parkinson's patients, respectively (Zuardi et al., 2009). In accordance with these results, preliminary studies in schizophrenic patients showed positive therapeutic effects of CBD (Zuardi et al., 1995, 2006b).

CBD MECHANISMS AND THE eCB SYSTEM

Results of experiments aimed at elucidating CBD mechanisms are usually complicated by the common bell-shaped dose–response curves produced by this drug (Campos and Guimarães, 2008; Izzo et al., 2009). Even so, it is now clear that multiple pharmacological actions are involved in the wide range biological effects induced by CBD (Izzo et al., 2009). These actions include complex interactions with the eCB system. CBD was initially described as possessing little affinity for cannabinoid receptors (Petitet et al., 1998; Thomas et al., 1998). A more recent study, nonetheless, has suggested that CBD can antagonize CB1Rs and CB2Rs at relatively low concentrations (Thomas et al., 2007). This antagonism, however, seems to be non-competitive in nature, with evidence suggesting that CBD could act as a CB1R or CB2R inverse agonist (Pertwee, 2008). Contrasting with these findings, CBD could also facilitate eCB-mediated neurotransmission by decreasing AEA hydrolysis or reuptake (Bisogno et al., 2001).

The findings regarding eCBs involvement in CBD effects, however, have been mainly obtained in studies performed *in vitro* and little is known about the involvement of these mechanisms in the central effects of the drug. To investigate this issue, we initially tested if facilitation of eCB-mediated neurotransmission could explain the anxiolytic effects of CBD in the dlPAG. This hypothesis was based on our previous study showing that direct injection of AEA into this region induces anxiolytic-like effects that were prevented by prior administration of AM251, a CB1R antagonist (Moreira et al., 2007). Surprisingly, the same dose of AM251 that had antagonized AEA failed to prevent CBD anxiolytic effects in the dlPAG (Campos and Guimarães, 2008). Considering that CBD could also act, at μM concentration range, as an agonist of 5-HT1ARs *in vitro* (Russo et al., 2005) and *in vivo* (Mishima et al., 2005), we decided to test if CBD effects would be prevented by local pre-treatment with WAY100635, a selective 5-HT1AR antagonist. Supporting this hypothesis, this drug completely blocked the anxiolytic effects of CBD (Campos and Guimarães, 2008). Following this initial result, we have now confirmed that WAY100635 is able to prevent CBD anxiolytic effects after intracerebral injections into the BNST (Gomes et al., 2011) or dlPAG (Soares et al., 2010) as well as following CBD systemic administration (Resstel et al., 2009). 5-HT1A mechanisms are also responsible for the antidepressive-like effects of CBD in the forced swimming test (Zanelati et al., 2010). Despite these findings, which clearly related 5-HT1A-mediated neurotransmission with CBD anxiolytic and antidepressive effects, more recent results showed that the eCB system is also involved in at least some of the central effects of CBD. For example, the facilitatory effect of intracerebroventricular (i.c.v.) administration of CBD in contextual fear-conditioning extinction was prevented by Rimonabant (Bitencourt et al., 2008). Also, the plastic effects of repeated CBD administration seem to involve eCB mechanisms. For example, chronic CBD treatment was shown to increase adult neurogenesis in the dentate gyrus, an effect that was absent in CB1KO mice (Wolf et al., 2010). In line with these results, the *in vitro* proliferative effects of CBD on embryonic hippocampal cells were prevented by CB1 or CB2 receptor antagonists (Campos et al., 2010).

We have also recently found that a CB1R antagonist, but not a 5-HT1AR, is able to prevent the effects of CBD in the marble burying test (Casarotto et al., 2010). Although initially proposed as an animal model aimed at detecting possible anxiolytic drug effects, it is now thought to evaluate a natural, repetitive behavior that can become compulsive. This test, therefore, has been proposed to model aspects of obsessive–compulsive disorder (OCD; Thomas et al., 2009). CBD effects in this model, depending on CB1R rather than on 5-HT1AR, gives support to the interpretation that the marble burying test (and OCD) engages brain mechanisms somehow different from those of related to classical animal models of anxiety (Witkin, 2008).

Both CBD and AEA can activate TRPV1Rs (Bisogno et al., 2001). These receptors are expressed in several brain areas related to anxiety such as the amygdala, hippocampus, prefrontal cortex, and PAG (Cristino et al., 2006). Activation of TRPV1 receptors can facilitate glutamate release (Marsch et al., 2007; Xing and Li, 2007), the main excitatory neurotransmitter in the CNS. Since antagonism of glutamate and TRPV1Rs in the dlPAG induce anxiolytic-like effects (Aguiar and Guimarães, 2009; Terzian et al., 2009), we hypothesized that at higher doses CBD could also be activating local TRPV1Rs (directly and maybe by inhibiting

AEA metabolism/uptake), facilitating glutamate neurotransmission and increasing anxiety. Corroborating this proposal, we found that a low dose of capsazepine, a TRPV1R antagonist, was able to turn the higher but ineffective dose of CBD into an anxiolytic one (Campos and Guimarães, 2009). Interaction with TRPV1Rs has also been suggested to explain the antipsychotic-like effects of CBD on MK-801 induced disruption of PPI (Long et al., 2006). Other mechanisms have also been proposed to account for the effects of CBD, for example blockade of adenosine uptake (Carrier et al., 2006) and antagonism of the putative cannabinoid receptor GPR55 (Mechoulam et al., 2007). Although the former has been related to CBD anxiolytic properties in a preliminary study (Carrier et al., 2007), the involvement of these mechanisms in the central effects of this drug remains to be further investigated. In summary, preclinical and clinical studies indicate that CBD has therapeutic potential in several neuropsychiatric disorders that depend on multiple mechanisms, including interaction with the eCB system. In addition, considering its safety profile (Mechoulam et al., 2007; Zuardi, 2008; Izzo et al., 2009), CBD could be a useful pharmacological tool to modulate this system.

THE eCB SYSTEM AND EATING DISORDERS: TOWARD A NEW THERAPEUTICALLY VALID APPROACH?

PATHOPHYSIOLOGY OF EATING DISORDERS

Food intake or eating is the process by which edible substances are consumed in order to balance the energy expenditure in living creatures. This process relies in physiologic mechanisms regulating appetite and the natural drive to eat. In some conditions human feeding behavior is altered leading to diseases, collectively known as eating disorders. These are a group of disorders characterized by physiological and psychological disturbances in appetite or food intake. They can be divided into three main pathologies, i.e., binge eating, bulimia nervosa (BN), and anorexia nervosa (AN). Binge-eating disorder is associated with three or more of the following: eating until feeling uncomfortably full; eating large amounts of food when not physically hungry; eating much more rapidly than normal; eating alone due to embarrassment; feeling of disgust, depression, or guilt after overeating. Criteria includes occurrence on average, at least 2 days a week for 6 months. Binge eating is not associated with compensatory behavior (i.e., purging, excessive exercise, etc.) and does not co-occur exclusively with BN or AN (From DSM-IV, 1994). BN is characterized by a cycle of binge-eating followed by purging to avert weight gain. Purging methods often include self-induced vomiting, use of laxatives or diuretics, excessive exercise, and fasting. AN is characterized by the loss of appetite and is associated with other features including an excessive fear of becoming overweight, body image disturbances, significant weight loss, refusal to maintain minimal normal weight, excessive exercise, and amenorrhea (Walker, 1994). In this review we will not include obesity because actually it is not formally considered an eating disorder. However, we would like to underline the increasing evidence dealing with specific changes in the CNS of obese people, including those occurring in brain areas involved in the rewarding aspects of food (reviewed in (Volkow and Wise, 2005). Likewise, and maybe reflecting direct central consequences of obesity, it is noteworthy the high incidence of anxiety and depression (also present in classical eating disorders) in obese people, affecting around 50% of this population. Also deserving greater consideration are the striking similarities in the pathophysiologic sequel occurring with obesity and addiction, also suggesting a re-evaluation of how these diseases are classified (Volkow and Wise, 2005).

Eating disorders can be chronic and disabling conditions characterized by aberrant patterns of feeding behavior and weight regulation, including abnormal attitudes and perceptions toward body weight and shape (Kaye, 2008). Indeed, AN has the highest mortality rate among neuropsychiatric diseases (Lowe et al., 2001). The etiologies of these diseases are at present poorly understood, but both AN and BN occur most frequently in adolescent females. This increased incidence and prevalence may very well be a direct reflection of cultural pressures for thinness (Strober et al., 1995). However, the discrete occurrence and heritability suggest there are some biological vulnerabilities involved in these diseases (Kaye, 2008). In fact, twin studies on AN and BN suggest there is a 50–80% genetic contribution to these diseases (Bulik et al., 1998; Klump et al., 2001). However, there is little knowledge about the connection between psychological symptoms and the neuropathophysiology associated with these diseases and on how such genetic vulnerabilities impact on brain pathways and what systems are primarily involved. Because of the neuropsychiatric nature of these diseases, the monoamine systems (i.e., serotonin, dopamine, and norepinephrine pathways) have been explored in greater detail. Among these, the serotoninergic system may be the more adversely affected and its deregulation is present in AN patients. However, the response to selective serotonin reuptake inhibitors is variable among patients suffering different subtypes of the illness, and the efficacy of such medication has been also questioned due to the common occurrence of relapse (Kaye et al., 2001; Walsh et al., 2006). Current research on eating disorders also points to a deregulation of neuronal circuits involved in food intake, including those related to emotional and reward pathways linked to feeding behavior (Stoving et al., 2009). In particular, a deranged leptin signaling system has been found in AN and BN (Monteleone et al., 2004; Holtkamp et al., 2006) and it has been hypothesized that the reward systems could be compromised leading to food intake-related dysphoria that would promote a vicious cycle of decreasing eating in order to avoid the dysphoric consequences of food consumption (Kaye, 2008). In this context, the reward system could have an important role since it integrates "liking" (pleasure/palatability) and "wanting" (appetite/incentive motivation) perceptions associated with food and, thus, AN and BN could be considered as dependency syndromes.

THE ROLE OF THE eCB SYSTEM IN ENERGY HOMEOSTASIS

The eCB system is strategically located in all the key points involved in food intake and energy expenditure, both at the central and the peripheral level. Thus, it is perhaps one of the few that can coordinate all the players involved in energy balance (reviewed in Pagotto et al., 2006; Matias and Di Marzo, 2007). Together with its action on peripheral tissues, the eCB system influences feeding behavior at the CNS by acting on circuits located in the hypothalamus, the reward system and the brain stem, with the overall net effect being anabolic (reviewed in Di Marzo et al., 2009).

Briefly, the hypothalamus is a key brain structure involved in energy balance homeostasis. Despite the low expression of CB1R in the hypothalamus, a number of studies demonstrate that eCBs through CB1Rs exerts a profound influence on the hypothalamic regulation of food intake (reviewed in Bermudez-Silva et al., 2011). CB1Rs are also important in the hypothalamic leptin-mediated anorectic effects (Di Marzo et al., 2001). Leptin inhibits eCB production in the hypothalamus and, conversely, hypothalamic eCBs are increased in genetically obese rodents lacking leptin or its receptor (Di Marzo et al., 2001). The reward system is a group of brain structures which regulate and control behavior by inducing pleasurable effects. The major rewarding pathway in the brain is the mesolimbic pathway that goes from the ventral tegmental area via the medial forebrain bundle to nucleus accumbens, which is the primary release site for the main brain's pleasure chemical, i.e., the neurotransmitter dopamine. CB1Rs are expressed in presynaptic glutamatergic and GABAergic nerve terminals in the ventral tegmental area, and eCBs are synthesized by ventral tegmental area dopamine neurons, having a role in the fine-tuned regulation of these cells (Maldonado et al., 2006). While it is still unclear exactly what cell populations express CB1Rs in the nucleus accumbens, it seems that eCBs within this area are able of increasing food intake in a CB1-dependent manner (Kirkham et al., 2002). Additional studies have also reported that eCBs acting in the nucleus accumbens modulate the palatability of food (Mahler et al., 2007). The brainstem is also a relevant player in food intake regulation: satiety signals from the stomach and duodenum reach the brainstem through sensory and vagal fibers. Among these, cholecystokinin (CCK) and peptide YY have been related with the eCB system (reviewed in Di Marzo et al., 2009; Bermudez-Silva et al., 2010). CB1Rs are expressed in the brainstem and in vagal afferent neurons modulating these signals (Burdyga et al., 2004; DiPatrizio and Simansky, 2008). Furthermore, eCB tone changes in the brainstem during the different phases of eating (reviewed in Di Marzo et al., 2009).

THE eCB SYSTEM IN EATING DISORDERS

The widespread role of the eCB system in regulating energy balance has spawned investigations into putative defects in eCB signaling that may underlie eating disorders. Increased blood levels of AEA have been found in both AN and binge-eating disorder patients, but not in BN patients (Monteleone et al., 2005). Indeed, AEA levels were significantly and inversely correlated with plasma leptin concentrations in both healthy controls and anorexic women. Interestingly, there is evidence to suggest that hypoleptinemia in AN patients may be an important factor underlying the excessive physical activity (Holtkamp et al., 2006), one of the hallmarks in AN. Thus, these results suggest that alterations in the eCB system associated with deregulated leptin signaling could be involved in the pathophysiology of AN. It is well-known that the eCB system and leptin interact functionally at the molecular level (reviewed in Bermudez-Silva et al., 2011), and thus it is easy to draw a theoretical frame in support of the important role played by both systems in AN and the therapeutic potential of leptin and cannabinoids in this disease (Stoving et al., 2009). Furthermore, elevated levels of CB1R but not CB2R mRNA have been found in the blood of females with AN and BN, further supporting the hypothesis of deregulated eCB signaling in eating disorders (Frieling et al., 2009). Paradoxically, these authors found an association between lower CB1R expression and more severe forms of the disorders.

AEA belongs to the lipid family of acylethanolamides. Another member of this group of lipids, named oleoylethanolamide, has also an important role on energy balance by promoting satiety and lipolysis through the activation of the PPARα (Fu et al., 2003). This molecule has an anorexigenic action by inducing oxytocin expression in the paraventricular nucleus of the hypothalamus and, interestingly, preliminary clinical results have shown altered levels of oleoylethanolamide in the cerebrospinal fluid and plasma of subjects recovered from eating disorders (Gaetani et al., 2008). These preliminary observations could extend the findings of altered levels of eCBs in eating disorders to a more general involvement of acylethanolamides.

Given the important contribution of genetics to AN and BN (in fact, the heritability estimates are similar to disorders typically viewed as biological like schizophrenia and bipolar disorder) human genetic association studies have been performed in order to identify genes involved in these pathologies, including genes belonging to the eCB system. Among these, CNR1 and CNR2 (the genes encoding cannabinoid CB1Rs and CB2Rs, respectively), as well as the genes encoding the main enzyme responsible in the degradation of AEA (FAAH), NAAA (*N*-Acylethanolamine-hydrolyzing acid amidase, which functions similar to FAAH but has a different optimal pH), and MAGL have been studied. The first family based study involved 52 families (parents with one or two affected siblings) that were genotyped for the (AAT) trinucleotide repeat of CNR1 gene. The distribution of alleles transmitted to the patients was not found to be significantly different from the non-transmitted parental alleles. However, upon dividing the samples to restricting and binging/purging subtypes of AN, the data analysis revealed a preferential transmission of different alleles in each of the subtypes, suggesting restricting AN and binging/purging AN may be associated with different alleles of the CNR1 gene (Siegfried et al., 2004). However, a subsequent study involving up to 91 German AN trios (patient with AN and both biological parents) was unable to confirm these results, nor did it show an association for any of 15 single nucleotide polymorphisms representative of regions with restricted haplotype diversity in FAAH, NAAA, and MAGL genes (Muller et al., 2008). Another study in 115 overweight/obese subjects with binge-eating disorder, 74 non-binge-eating disorder patients with obesity and 110 normal weight healthy controls investigated one of these FAAH polymorphisms, previously implicated in obesity in binge-eating disorder, and reporting a lack of association (Monteleone et al., 2008) and in a more recent article these authors studied the association of this FAAH polymorphism and the CNR1 polymorphism in both AN and BN, in 134 patients with AN, 180 patients with BN and 148 normal weight healthy controls (Monteleone et al., 2009). The authors found a significant increase in the frequency of both polymorphisms in AN and BN patients, a result in sharp contrast with the previous findings by Muller et al. (2008) that showed a lack of association of these polymorphisms with AN. Additionally, Monteleone et al. (2009) found a synergistic effect of the two polymorphisms in AN but not in BN. Finally, a recent article has detected an association of

a CNR2 polymorphism with both AN and BN (Ishiguro et al., 2010a) in a study comprising in 204 subjects with eating disorders and 1876 healthy volunteers in Japanese population. Taken together, the human genetic association studies show evidence of association between eCB system genes and eating disorders, but further studies are necessary to definitively confirm these findings.

THERAPEUTIC USE OF CANNABINOID DRUGS IN EATING DISORDERS

Cannabis preparations have been used for both medicinal and recreational purposes for centuries. Its ancient medicinal use has been primarily related to ameliorate pain and increase appetite in disease states. However, because of their psychostimulant properties and the lack of an adequate body of knowledge, their use in western medicine has been excluded until recently. During the last 20 years this picture has dramatically changed. There has been an exponential increase in the knowledge of the molecular mechanisms underlying cannabinoid effects, and morphological, physiological and pathophysiological studies have shown that the molecular system supporting these effects (i.e., the eCB system), is ubiquitous and has a highly relevant role in maintaining whole body homeostasis and, especially, energy homeostasis (Matias and Di Marzo, 2007). This fact has led to an increased interest in the medical use of cannabinoid-related drugs. Thus, in 1985 the Food and Drug Administration approved Marinol® (dronabinol), a synthetically derived THC preparation, to relieve nausea, and vomiting associated with chemotherapy in cancer patients who have failed to respond adequately to other antiemetics, and in 1992 this compound was also approved for inducing appetite in AIDS patients suffering from cachexia (Nelson et al., 1994; Beal et al., 1995). Similarly, Nabilone® (a synthetic cannabinoid that mimics THC) was also approved in 1985 for ameliorating the nausea of cancer chemotherapy. A more controversial step forward was the use of a cannabinoid CB1R antagonist/inverse agonist (rimonabant) for management of complicated obesity. Although the Food and Drug Administration never approved this drug, the European Medicine Agency did and Acomplia® (the commercial name of rimonabant) was in the market for approximately 2 years. Despite the weight loss and improved cardiometabolic profile observed in obese patients, the drug had to be removed from the market due to its undesirable central side effects (see previous sections, but also Bermudez-Silva et al., 2010 for review). More recently, Sativex® (the combination of THC and CBD) has been marketed in Canada and European countries like the United Kingdom and Spain for the treatment of spasticity due to multiple sclerosis, and it is currently in phase III clinical development for the treatment of cancer pain.

Taken into account the good therapeutic management of cannabinoids in cachexia and malnutrition associated with cancer and AIDS, it looks feasible that this kind of pharmacotherapy could be also useful in the treatment of eating disorders. Unfortunately, there are only two small trials assessing cannabinoid treatment in AN (reviewed in Stoving et al., 2009). The former involved 11 AN patients in a 4-week crossover trial and THC treatment resulted in increased sleep disturbances and interpersonal sensitivity, whereas there was no significant effect on weight gain (Gross et al., 1983). Unfortunately, this study raised several concerns given it was an in-patient study and the occasional tube feeding was used. In addition, THC was compared to diazepam instead of placebo, which could be a confounding factor given diazepam has also been reported to increase food intake *per se* (Naruse et al., 1991). The latter involved nine AN out-patients treated with THC. The results showed a significant improvement of depression and perfectionism scores without improving weight gain (Berry, 2006).

Currently, there is an ongoing phase III clinical trial involving 22 subjects to reveal if severe chronic AN patients treated with Marinol® have significant improvement on weight, with secondary objectives of the study being evaluation of eating disorder inventory scale, motor and inner restlessness and endocrine parameters[3] (EudraCT Number: 2007-005631-29). With this very limited number of performed trials (the last one being still not finished) it seems clear that no conclusions can be drawn out regarding the therapeutic validity of a cannabinoid-based approach in eating disorders. However, the satisfactory clinical use of cannabinoid agonists in other pathologies demands and encourages the development of further clinical trials on eating disorders patients. Interestingly, a very recent preclinical study in rodent have shown that the main active constituent of cannabis, THC, is able of reducing the weight loss associated with the development of AN via a mechanism involving reduced energy expenditure (Verty et al., 2011), thus providing encouraging preclinical data on the validity of a eCB-based therapy in AN.

CONCLUDING REMARKS

Evidence for a critical role of the eCB system in neuropsychiatric disorders has been provided, and special attention has been paid to its contribution to the emotional and cognitive deficits compromised in these disorders. Not only CB1Rs, but also CB2Rs and CBD, through facilitation of eCB-mediated neurotransmission, have been involved in the emotional and cognitive deficits reported in anxiety disorders, depression, and schizophrenia. Indeed, a deregulation of the eCB system seems to be in the bases of several neuropsychiatric disorders, including eating disorders. The pharmacological enhancement of eCB signaling has yield promising results in rodents, particularly as an anxiolytic and antidepressant therapy. Eating disorders may also benefit of this therapeutic approach, and a clinical trial with synthetic THC is ongoing for the management of severe AN. However, in spite of these potential benefits, further research is needed to prevent undesirable side effects. In fact, the prolonged and continuous activation of the eCB system, e.g., by chronic cannabis consumption, has been associated with an increased risk for schizophrenia. Alternatively, CBD may arise as an optimal candidate to modulate the eCB system. CBD has consistently demonstrated an anti-anxiety and antidepressant profile, and its potential as an antipsychotic drug is gaining relevance in preclinical and clinical studies. In conclusion, the eCB system is seriously involved in neuropsychiatric disorders. In spite of the promising results achieved in animal studies, detrimental consequences of manipulating this endogenous system cannot be underestimated over the potential and promising perspectives of its therapeutic manipulation.

[3]https://www.clinicaltrials register.eu

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REFERENCES

Aguiar, D. C., and Guimarães, F. S. (2009). Blockade of NMDA receptors and nitric oxide synthesis in the dorsolateral periaqueductal gray attenuates behavioral and cellular responses of rats exposed to a live predator. *J. Neurosci. Res.* 87, 2418–2429.

Ahn, K., Mckinney, M. K., and Cravatt, B. F. (2008). Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chem. Rev.* 108, 1687–1707.

Airaksinen, E., Wahlin, A., Forsell, Y., and Larsson, M. (2007). Low episodic memory performance as a premorbid marker of depression: evidence from a 3-year follow-up. *Acta Psychiatr. Scand.* 115, 458–465.

Akinshola, B. E., Chakrabarti, A., and Onaivi, E. S. (1999). In-vitro and in-vivo action of cannabinoids. *Neurochem. Res.* 24, 1233–1240.

Alves, F. H., Crestani, C. C., Gomes, F. V., Guimaraes, F. S., Correa, F. M., and Resstel, L. B. (2010). Cannabidiol injected into the bed nucleus of the stria terminalis modulates baroreflex activity through 5-HT1A receptors. *Pharmacol. Res.* 62, 228–236.

Amitai, N., Semenova, S., and Markou, A. (2007). Cognitive-disruptive effects of the psychotomimetic phencyclidine and attenuation by atypical antipsychotic medications in rats. *Psychopharmacology (Berl.)* 193, 521–537.

Andre, A., and Gonthier, M. P. (2010). The endocannabinoid system: its roles in energy balance and potential as a target for obesity treatment. *Int. J. Biochem. Cell Biol.* 42, 1788–1801.

Arevalo, C., De Miguel, R., and Hernandez-Tristan, R. (2001). Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. *Pharmacol. Biochem. Behav.* 70, 123–131.

Aso, E., Ozaita, A., Serra, M. A., and Maldonado, R. (2011). Genes differentially expressed in CB1 knockout mice: involvement in the depressive-like phenotype. *Eur. Neuropsychopharmacol.* 21, 11–22.

Aso, E., Ozaita, A., Valdizan, E. M., Ledent, C., Pazos, A., Maldonado, R., and Valverde, O. (2008). BDNF impairment in the hippocampus is related to enhanced despair behavior in CB1 knockout mice. *J. Neurochem.* 105, 565–572.

Baker, D., Pryce, G., Davies, W. L., and Hiley, C. R. (2006). In silico patent searching reveals a new cannabinoid receptor. *Trends Pharmacol. Sci.* 27, 1–4.

Bambico, F. R., and Gobbi, G. (2008). The cannabinoid CB1 receptor and the endocannabinoid anandamide: possible antidepressant targets. *Expert Opin. Ther. Targets* 12, 1347–1366.

Barch, D. M. (2005). The cognitive neuroscience of schizophrenia. *Annu. Rev. Clin. Psychol.* 1, 321–353.

Barrero, F. J., Ampuero, I., Morales, B., Vives, F., De Dios Luna Del Castillo, J., Hoenicka, J., and Garcia Yebenes, J. (2005). Depression in Parkinson's disease is related to a genetic polymorphism of the cannabinoid receptor gene (CNR1). *Pharmacogenomics J.* 5, 135–141.

Beal, J. E., Olson, R., Laubenstein, L., Morales, J. O., Bellman, P., Yangco, B., Lefkowitz, L., Plasse, T. F., and Shepard, K. V. (1995). Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *J. Pain Symptom Manage.* 10, 89–97.

Bekker, M. H., and van Mens-Verhulst, J. (2007). Anxiety disorders: sex differences in prevalence, degree, and background, but gender-neutral treatment. *Gend. Med.* 4(Suppl. B), S178–S193.

Bergamaschi, M. M., Queiroz, R. H., Chagas, M. H., De Oliveira, D. C., De Martinis, B. S., Kapczinski, F., Quevedo, J., Roesler, R., Schroder, N., Nardi, A. E., Martin-Santos, R., Hallak, J. E., Zuardi, A. W., and Crippa, J. A. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. *Neuropsychopharmacology* 36, 1219–1226.

Bermudez-Silva, F. J., Cardinal, P., and Cota, D. (2011). The role of the endocannabinoid system in the neuroendocrine regulation of energy balance. *J. Psychopharmacol.* PMID: 21824982. [Epub ahead of print].

Bermudez-Silva, F. J., Viveros, M. P., Mcpartland, J. M., and Rodriguez De Fonseca, F. (2010). The endocannabinoid system, eating behavior and energy homeostasis: the end or a new beginning? *Pharmacol. Biochem. Behav.* 95, 375–382.

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

Berry, E. M. (2006). "Pilot study of THC (2.5mgx2) in 9 ambulatory AN patients," in *The sixth Nordic Congress on Eating Disorders*, Aarhus, Denmark.

Bierman, E. J., Comijs, H. C., Jonker, C., and Beekman, A. T. (2005). Effects of anxiety versus depression on cognition in later life. *Am. J. Geriatr. Psychiatry* 13, 686–693.

Biscaia, M., Marin, S., Fernandez, B., Marco, E. M., Rubio, M., Guaza, C., Ambrosio, E., and Viveros, M. P. (2003). Chronic treatment with CP 55,940 during the peri-adolescent period differentially affects the behavioural responses of male and female rats in adulthood. *Psychopharmacology (Berl.)* 170, 301–308.

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D. E., Brandi, I., Moriello, A. S., Davis, J. B., Mechoulam, R., and Di Marzo, V. (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br. J. Pharmacol.* 134, 845–852.

Bitencourt, R. M., Pamplona, F. A., and Takahashi, R. N. (2008). Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *Eur. Neuropsychopharmacol.* 18, 849–859.

Bortolato, M., Mangieri, R. A., Fu, J., Kim, J. H., Arguello, O., Duranti, A., Tontini, A., Mor, M., Tarzia, G., and 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.

Bouaboula, M., Bourrie, B., Rinaldi-Carmona, M., Shire, D., Le Fur, G., and Casellas, P. (1995). Stimulation of cannabinoid receptor CB1 induces krox-24 expression in human astrocytoma cells. *J. Biol. Chem.* 270, 13973–13980.

Braff, D. L., Geyer, M. A., and Swerdlow, N. R. (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl.)* 156, 234–258.

Braida, D., Limonta, V., Malabarba, L., Zani, A., and 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.

Budney, A. J., Hughes, J. R., Moore, B. A., and Vandrey, R. (2004). Review of the validity and significance of cannabis withdrawal syndrome. *Am. J. Psychiatry* 161, 1967–1977.

Bulik, C. M., Sullivan, P. F., and Kendler, K. S. (1998). Heritability of binge-eating and broadly defined bulimia nervosa. *Biol. Psychiatry* 44, 1210–1218.

Burdyga, G., Lal, S., Varro, A., Dimaline, R., Thompson, D. G., and Dockray, G. J. (2004). Expression of cannabinoid CB1 receptors by vagal afferent neurons is inhibited by cholecystokinin. *J. Neurosci.* 24, 2708–2715.

Campos, A. C., Aguado, T., Palazuellos, J., Guzman, M., Guimarães, F. S., and Roperh, I. G. (2010). Cannabidiol increases cell-proliferation by activating ERK1/2 MAP Kinase signalling. *Can. Neurosci.* [Abstract].

Campos, A. C., and Guimarães, F. S. (2008). Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacology (Berl.)* 199, 223–230.

Campos, A. C., and Guimarães, F. S. (2009). Evidence for a potential role for TRPV1 receptors in the dorsolateral periaqueductal gray in the attenuation of the anxiolytic effects of cannabinoids. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 1517–1521.

Carlisle, S. J., Marciano-Cabral, F., Staab, A., Ludwick, C., and Cabral, G. A. (2002). Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. *Int. Immunopharmacol.* 2, 69–82.

Carrier, E. J., Auchampach, J. A., and Hillard, C. J. (2006). Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7895–7900.

Carrier, E. J., Patel, S., Roelke, C. T., Shrestha, L., Meier, S., Gizewski, E., Fredholm, F. B., Auchampach, J. A., and Hillard, C. J. (2007). "The anxiolytic response to cannabidiol is modulated by the adenosine A1 receptor but not by the cannabinoid CB1 or CB2 receptors," in *International Cannabinoid Research Society (ICRS) Meeting*, Saint-Sauveur, Québec, CA, 147.

Casarotto, P. C., Gomes, F. V., Resstel, L. B., and Guimaraes, F. S. (2010). Cannabidiol inhibitory effect on marble-burying behaviour: involvement of CB1 receptors. *Behav. Pharmacol.* 21, 353–358.

Chakrabarti, A., Onaivi, E. S., and Chaudhuri, G. (1995). Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. *DNA Seq.* 5, 385–388.

Chavarria-Siles, I., Contreras-Rojas, J., Hare, E., Walss-Bass, C., Quezada, P., Dassori, A., Contreras, S., Medina, R., Ramirez, M., Salazar, R., Raventos, H., and Escamilla, M. A. (2008). Cannabinoid receptor 1 gene (CNR1) and susceptibility to a quantitative phenotype for hebephrenic schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 147, 279–284.

Chhatwal, J. P., Davis, M., Maguschak, K. A., and Ressler, K. J. (2005). Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology* 30, 516–524.

Clement, Y., Calatayud, F., and Belzung, C. (2002). Genetic basis of anxiety-like behaviour: a critical review. *Brain Res. Bull.* 57, 57–71.

Cohen, M., Solowij, N., and Carr, V. (2008). Cannabis, cannabinoids and schizophrenia: integration of the evidence. *Aust. N. Z. J. Psychiatry* 42, 357–368.

Crippa, J. A., Zuardi, A. W., Garrido, G. E., Wichert-Ana, L., Guarnieri, R., Ferrari, L., Azevedo-Marques, P. M., Hallak, J. E., Mcguire, P. K., and Filho Busatto, G. (2004). Effects of cannabidiol (CBD) on regional cerebral blood flow. *Neuropsychopharmacology* 29, 417–426.

Crippa, J. A., Zuardi, A. W., Martin-Santos, R., Bhattacharyya, S., Atakan, Z., Mcguire, P., and Fusar-Poli, P. (2009). Cannabis and anxiety: a critical review of the evidence. *Hum. Psychopharmacol.* 24, 515–523.

Cristino, L., De Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., and Di Marzo, V. (2006). Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience* 139, 1405–1415.

Da Settimo, F., Taliani, S., Trincavelli, M. L., Montali, M., and Martini, C. (2007). GABA A/Bz receptor subtypes as targets for selective drugs. *Curr. Med. Chem.* 14, 2680–2701.

Davidson, R. J., Lewis, D. A., Alloy, L. B., Amaral, D. G., Bush, G., Cohen, J. D., Drevets, W. C., Farah, M. J., Kagan, J., Mcclelland, J. L., Nolen-Hoeksema, S., and Peterson, B. S. (2002). Neural and behavioral substrates of mood and mood regulation. *Biol. Psychiatry* 52, 478–502.

Dean, B., Sundram, S., Bradbury, R., Scarr, E., and Copolov, D. (2001). Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience* 103, 9–15.

Degenhardt, L., Hall, W., and Lynskey, M. (2003). Exploring the association between cannabis use and depression. *Addiction* 98, 1493–1504.

Demirakca, T., Sartorius, A., Ende, G., Meyer, N., Welzel, H., Skopp, G., Mann, K., and Hermann, D. (2011). Diminished gray matter in the hippocampus of cannabis users: possible protective effects of cannabidiol. *Drug Alcohol Depend.* 114, 242–245.

Deng, C., Han, M., and Huang, X. F. (2007). No changes in densities of cannabinoid receptors in the superior temporal gyrus in schizophrenia. *Neurosci. Bull.* 23, 341–347.

Dere, E., Pause, B. M., and Pietrowsky, R. (2010). Emotion and episodic memory in neuropsychiatric disorders. *Behav. Brain Res.* 215, 162–171.

Derocq, J. M., Segui, M., Marchand, J., Lefur, G., and Casellas, P. (1995). Cannabinoids enhance human B-cell growth at low nanomolar concentrations. *FEBS Lett.* 369, 177–182.

Di Forti, M., Morrison, P. D., Butt, A., and Murray, R. M. (2007). Cannabis use and psychiatric and cogitive disorders: the chicken or the egg? *Curr. Opin. Psychiatry* 20, 228–234.

Di Marzo, V., Goparaju, S. K., Wang, L., Liu, J., Batkai, S., Jarai, Z., Fezza, F., Miura, G. I., Palmiter, R. D., Sugiura, T., and Kunos, G. (2001). Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 410, 822–825.

Di Marzo, V., Ligresti, A., and Cristino, L. (2009). The endocannabinoid system as a link between homoeostatic and hedonic pathways involved in energy balance regulation. *Int. J. Obes. (Lond.)* 33(Suppl. 2), S18–S24.

DiPatrizio, N. V., and Simansky, K. J. (2008). Activating parabrachial cannabinoid CB1 receptors selectively stimulates feeding of palatable foods in rats. *J. Neurosci.* 28, 9702–9709.

Doggrell, S. A. (2008). Is rimonabant efficacious and safe in the treatment of obesity? *Expert Opin. Pharmacother.* 9, 2727–2731.

Domenici, M. R., Azad, S. C., Marsicano, G., Schierloh, A., Wotjak, C. T., Dodt, H. U., Zieglgansberger, W., Lutz, B., and Rammes, G. (2006). Cannabinoid receptor type 1 located on presynaptic terminals of principal neurons in the forebrain controls glutamatergic synaptic transmission. *J. Neurosci.* 26, 5794–5799.

D'Souza, D. C., Sewell, R. A., and Ranganathan, M. (2009). Cannabis and psychosis/schizophrenia: human studies. *Eur. Arch. Psychiatry Clin. Neurosci.* 259, 413–431.

Egerton, A., Allison, C., Brett, R. R., and Pratt, J. A. (2006). Cannabinoids and prefrontal cortical function: insights from preclinical studies. *Neurosci. Biobehav. Rev.* 30, 680–695.

Eggan, S. M., Hashimoto, T., and Lewis, D. A. (2008). Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. *Arch. Gen. Psychiatry* 65, 772–784.

Ehrhart, J., Obregon, D., Mori, T., Hou, H., Sun, N., Bai, Y., Klein, T., Fernandez, F., Tan, J., and Shytle, R. D. (2005). Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J. Neuroinflammation* 2, 29.

Ellenbroek, B. A., and Riva, M. A. (2003). Early maternal deprivation as an animal model for schizophrenia. *Clin. Neurosci. Res.* 3, 297–302.

Fattore, L., Fadda, P., Spano, M. S., Pistis, M., and Fratta, W. (2008). Neurobiological mechanisms of cannabinoid addiction. *Mol. Cell. Endocrinol.* 286, S97–S107.

Fernandez-Espejo, E., Viveros, M. P., Nunez, L., Ellenbroek, B. A., and Rodriguez De Fonseca, F. (2009). Role of cannabis and endocannabinoids in the genesis of schizophrenia. *Psychopharmacology (Berl.)* 206, 531–549.

Finn, D. P. (2010). Endocannabinoid-mediated modulation of stress responses: physiological and pathophysiological significance. *Immunobiology* 215, 629–646.

Fone, K. C., and Porkess, M. V. (2008). Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. *Neurosci. Biobehav. Rev.* 32, 1087–1102.

Frieling, H., Albrecht, H., Jedtberg, S., Gozner, A., Lenz, B., Wilhelm, J., Hillemacher, T., De Zwaan, M., Kornhuber, J., and Bleich, S. (2009). Elevated cannabinoid 1 receptor mRNA is linked to eating disorder related behavior and attitudes in females with eating disorders. *Psychoneuroendocrinology* 34, 620–624.

Fu, J., Gaetani, S., Oveisi, F., Lo Verme, J., Serrano, A., Rodriguez De Fonseca, F., Rosengarth, A., Luecke, H., Di Giacomo, B., Tarzia, G., and Piomelli, D. (2003). Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. *Nature* 425, 90–93.

Fusar-Poli, P., Allen, P., Bhattacharyya, S., Crippa, J. A., Mechelli, A., Borgwardt, S., Martin-Santos, R., Seal, M. L., O'carrol, C., Atakan, Z., Zuardi, A. W., and Mcguire, P. (2010). Modulation of effective connectivity during emotional processing by Delta 9-tetrahydrocannabinol and cannabidiol. *Int. J. Neuropsychopharmacol.* 13, 421–432.

Fusar-Poli, P., Crippa, J. A., Bhattacharyya, S., Borgwardt, S. J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S. A., O'carrol, C., Atakan, Z., Zuardi, A. W., and Mcguire, P. K. (2009). Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Arch. Gen. Psychiatry* 66, 95–105.

Gaetani, S., Kaye, W. H., Cuomo, V., and Piomelli, D. (2008). Role of endocannabinoids and their analogues in obesity and eating disorders. *Eat. Weight Disord.* 13, e42–e48.

Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., and Casellas, P. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* 232, 54–61.

Galve-Roperh, I., Aguado, T., Palazuelos, J., and Guzman, M. (2008). Mechanisms of control of neuron survival by the endocannabinoid system. *Curr. Pharm. Des.* 14, 2279–2288.

García-Gutiérrez, M. S., and Manzanares, J. (2010). The cannabinoid CB1 receptor is involved in the anxiolytic, sedative and amnesic actions of benzodiazepines. *J. Psychopharmacol. (Oxford)* 24, 757–765.

García-Gutiérrez, M. S., and Manzanares, J. (2011). Overexpression of CB2 cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. *J. Psychopharmacol. (Oxford)* 25, 111–120.

García-Gutiérrez, M. S., Garcia-Bueno, B., Zoppi, S., Leza, J. C., and Manzanares, J. (2011). Chronic blockade of cannabinoid CB(2) receptors induces anxiolytic-like actions associated to alterations in GABA(A) receptors. *Br. J. Pharmacol.* doi: 10.1111/j.1476-5381.2011.01625.x. [Epub ahead of print].

García-Gutiérrez, M. S., Perez-Ortiz, J. M., Gutierrez-Adan, A., and Manzanares, J. (2010). Depression-resistant endophenotype in mice overexpressing cannabinoid CB(2) receptors. *Br. J. Pharmacol.* 160, 1773–1784.

Giuffrida, A., Leweke, F. M., Gerth, C. W., Schreiber, D., Koethe, D., Faulhaber, J., Klosterkotter, J., and Piomelli, D. (2004). Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology* 29, 2108–2114.

Glass, M., Dragunow, M., and Faull, R. L. (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 77, 299–318.

Gomes, F. V., Resstel, L. B., and Guimaraes, F. S. (2011). The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. *Psychopharmacology (Berl.)* 213, 465–473.

Gong, J. P., Onaivi, E. S., Ishiguro, H., Liu, Q. R., Tagliaferro, P. A., Brusco, A., and Uhl, G. R. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res.* 1071, 10–23.

Gorzalka, B. B., and Hill, M. N. (2011). Putative role of endocannabinoid signaling in the etiology of depression and actions of antidepressants. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 1575–1585.

Grayson, B., Idris, N. F., and Neill, J. C. (2007). Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. *Behav. Brain Res.* 184, 31–38.

Griffin, G., Wray, E. J., Tao, Q., Mcallister, S. D., Rorrer, W. K., Aung, M. M., Martin, B. R., and Abood, M. E. (1999). Evaluation of the cannabinoid CB2 receptor-selective antagonist, SR144528: further evidence for cannabinoid CB2 receptor absence in the rat central nervous system. *Eur. J. Pharmacol.* 377, 117–125.

Gross, H., Ebert, M. H., Faden, V. B., Goldberg, S. C., Kaye, W. H., Caine, E. D., Hawks, R., and Zinberg, N. (1983). A double-blind trial of delta 9-tetrahydrocannabinol in primary anorexia nervosa. *J. Clin. Psychopharmacol.* 3, 165–171.

Gualtieri, C. T., and Morgan, D. W. (2008). The frequency of cognitive impairment in patients with anxiety, depression, and bipolar disorder: an unaccounted source of variance in clinical trials. *J. Clin. Psychiatry* 69, 1122–1130.

Guimarães, F. S., Chiaretti, T. M., Graeff, F. G., and Zuardi, A. W. (1990). Antianxiety effect of cannabidiol in the elevated plus-maze. *Psychopharmacology (Berl.)* 100, 558–559.

Guimarães, F. S., De Aguiar, J. C., Mechoulam, R., and Breuer, A. (1994). Anxiolytic effect of cannabidiol derivatives in the elevated plus-maze. *Gen. Pharmacol.* 25, 161–164.

Guimarães, F. S., Del Bel, E. A., Padovan, C. M., Netto, S. M., and De Almeida, R. T. (1993). Hippocampal 5-HT receptors and consolidation of stressful memories. *Behav. Brain Res.* 58, 133–139.

Guimarães, V. M., Zuardi, A. W., Del Bel, E. A., and Guimaraes, F. S. (2004). Cannabidiol increases Fos expression in the nucleus accumbens but not in the dorsal striatum. *Life Sci.* 75, 633–638.

Guindon, J., and Hohmann, A. G. (2009). The endocannabinoid system and pain. *CNS Neurol. Disord. Drug Targets* 8, 403–421.

Gururajan, A., Taylor, D. A., and Malone, D. T. (2011). Effect of cannabidiol in a MK-801-rodent model of aspects of schizophrenia. *Behav. Brain Res.* 222, 299–308.

Guzman, M., Sanchez, C., and Galve-Roperh, I. (2001). Control of the cell survival/death decision by cannabinoids. *J. Mol. Med.* 78, 613–625.

Hall, W., and Degenhardt, L. (2009). Adverse health effects of non-medical cannabis use. *Lancet* 374, 1383–1391.

Haller, J., Bakos, N., Szirmay, M., Ledent, C., and Freund, T. F. (2002). The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur. J. Neurosci.* 16, 1395–1398.

Haller, J., Matyas, F., Soproni, K., Varga, B., Barsy, B., Nemeth, B., Mikics, E., Freund, T. F., and Hajos, N. (2007). Correlated species differences in the effects of cannabinoid ligands on anxiety and on GABAergic and glutamatergic synaptic transmission. *Eur. J. Neurosci.* 25, 2445–2456.

Haller, J., Szirmai, M., Varga, B., Ledent, C., and Freund, T. F. (2005). Cannabinoid CB1 receptor dependent effects of the NMDA antagonist phencyclidine in the social withdrawal model of schizophrenia. *Behav. Pharmacol.* 16, 415–422.

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

Hamdani, N., Tabeze, J. P., Ramoz, N., Ades, J., Hamon, M., Sarfati, Y., Boni, C., and Gorwood, P. (2008). The CNR1 gene as a pharmacogenetic factor for antipsychotics rather than a susceptibility gene for schizophrenia. *Eur. Neuropsychopharmacol.* 18, 34–40.

Harkany, T., Guzman, M., Galve-Roperh, I., Berghuis, P., Devi, L. A., and Mackie, K. (2007). The emerging functions of endocannabinoid signaling during CNS development. *Trends Pharmacol. Sci.* 28, 83–92.

Heim, C., Ehlert, U., and Hellhammer, D. H. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology* 25, 1–35.

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., De Costa, B. R., and Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J. Neurosci.* 11, 563–583.

Hill, M. N., Carrier, E. J., Mclaughlin, R. J., Morrish, A. C., Meier, S. E., Hillard, C. J., and Gorzalka, B. B. (2008). Regional alterations in the endocannabinoid system in an animal model of depression: effects of concurrent antidepressant treatment. *J. Neurochem.* 106, 2322–2336.

Hill, M. N., Miller, G. E., Carrier, E. J., Gorzalka, B. B., and Hillard, C. J. (2009). Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. *Psychoneuroendocrinology* 34, 1257–1262.

Hill, M. N., Patel, S., Carrier, E. J., Rademacher, D. J., Ormerod, B. K., Hillard, C. J., and Gorzalka, B. B. (2005). Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. *Neuropsychopharmacology* 30, 508–515.

Hollis, C., Groom, M. J., Das, D., Calton, T., Bates, A. T., Andrews, H. K., Jackson, G. M., and Liddle, P. F. (2008). Different psychological effects of cannabis use in adolescents at genetic high risk for schizophrenia and with attention deficit/hyperactivity disorder (ADHD). *Schizophr. Res.* 105, 216–223.

Holter, S. M., Kallnik, M., Wurst, W., Marsicano, G., Lutz, B., and Wotjak, C. T. (2005). Cannabinoid CB1 receptor is dispensable for memory extinction in an appetitively-motivated learning task. *Eur. J. Pharmacol.* 510, 69–74.

Holtkamp, K., Herpertz-Dahlmann, B., Hebebrand, K., Mika, C., Kratzsch, J., and Hebebrand, J. (2006). Physical activity and restlessness correlate with leptin levels in patients with adolescent anorexia nervosa. *Biol. Psychiatry* 60, 311–313.

Huang, E. J., and Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. *Annu. Rev. Neurosci.* 24, 677–736.

Hungund, B. L., Vinod, K. Y., Kassir, S. A., Basavarajappa, B. S., Yalamanchili, R., Cooper, T. B., Mann, J. J., and Arango, V. (2004). Upregulation of CB1 receptors and agonist-stimulated [35S]GTPgammaS binding in the prefrontal cortex of depressed suicide victims. *Mol. Psychiatry* 9, 184–190.

Hyman, S. E. (2008). A glimmer of light for neuropsychiatric disorders. *Nature* 455, 890–893.

Ibrahim, M. M., Deng, H., Zvonok, A., Cockayne, D. A., Kwan, J., Mata, H. P., Vanderah, T. W., Lai, J., Porreca, F., Makriyannis, A., and Malan, T. P. Jr. (2003). Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10529–10533.

Isaac, C. L., Cushway, D., and Jones, G. V. (2006). Is posttraumatic stress disorder associated with specific deficits in episodic memory? *Clin. Psychol. Rev.* 26, 939–955.

Ishiguro, H., Carpio, O., Horiuchi, Y., Shu, A., Higuchi, S., Schanz, N., Benno, R., Arinami, T., and Onaivi, E. S. (2010a). A nonsynonymous polymorphism in cannabinoid CB2 receptor gene is associated with eating disorders in humans and food intake is modified in mice by its ligands. *Synapse* 64, 92–96.

Ishiguro, H., Horiuchi, Y., Ishikawa, M., Koga, M., Imai, K., Suzuki, Y., Morikawa, M., Inada, T., Watanabe, Y., Takahashi, M., Someya, T., Ujike, H., Iwata, N., Ozaki, N., Onaivi, E. S., Kunugi, H., Sasaki, T., Itokawa, M., Arai, M., Niizato, K., Iritani, S., Naka, I., Ohashi, J., Kakita, A., Takahashi, H., Nawa, H., and Arinami, T. (2010b). Brain cannabinoid CB2 receptor in schizophrenia. *Biol. Psychiatry* 67, 974–982.

Iyer, S. N., Boekestyn, L., Cassidy, C. M., King, S., Joober, R., and Malla, A. K. (2008). Signs and symptoms in the pre-psychotic phase: description and implications for diagnostic trajectories. *Psychol. Med.* 38, 1147–1156.

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., and Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol. Sci.* 30, 515–527.

Jager, G., and Ramsey, N. F. (2008). Long-term consequences of adolescent cannabis exposure on the development of cognition, brain structure and function: an overview of animal and human research. *Curr. Drug Abuse Rev.* 1, 114–123.

Joosten, M., Valk, P. J., Jorda, M. A., Vankan-Berkhoudt, Y., Verbakel, S., Van Den Broek, M., Beijen, A., Lowenberg, B., and Delwel, R. (2002). Leukemic predisposition of pSca-1/Cb2 transgenic mice. *Exp. Hematol.* 30, 142–149.

Juhasz, G., Chase, D., Pegg, E., Downey, D., Toth, Z. G., Stones, K., Platt, H., Mekli, K., Payton, A., Elliott, R., Anderson, I. M., and Deakin, J. F. (2009). CNR1 gene is associated with high neuroticism and low agreeableness and interacts with recent negative life events to predict current depressive symptoms. *Neuropsychopharmacology* 34, 2019–2027.

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

Kamprath, K., Plendl, W., Marsicano, G., Deussing, J. M., Wurst, W., Lutz, B., and Wotjak, C. T. (2009). Endocannabinoids mediate acute fear adaptation via glutamatergic neurons independently of corticotropin-releasing hormone signaling. *Genes Brain Behav.* 8, 203–211.

Katona, I., and Freund, T. F. (2008). Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat. Med.* 14, 923–930.

Katona, I., Rancz, E. A., Acsady, L., Ledent, C., Mackie, K., Hajos, N., and Freund, T. F. (2001). Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.* 21, 9506–9518.

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

Kawamura, Y., Fukaya, M., Maejima, T., Yoshida, T., Miura, E., Watanabe, M., Ohno-Shosaku, T., and Kano, M. (2006). The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J. Neurosci.* 26, 2991–3001.

Kaye, W. (2008). Neurobiology of anorexia and bulimia nervosa. *Physiol. Behav.* 94, 121–135.

Kaye, W. H., Nagata, T., Weltzin, T. E., Hsu, L. K., Sokol, M. S., Mcconaha, C., Plotnicov, K. H., Weise, J., and Deep, D. (2001). Double-blind placebo-controlled administration of fluoxetine in restricting- and restricting-purging-type anorexia nervosa. *Biol. Psychiatry* 49, 644–652.

Kessler, R. C., Demler, O., Frank, R. G., Olfson, M., Pincus, H. A., Walters, E. E., Wang, P., Wells, K. B., and Zaslavsky, A. M. (2005). Prevalence and treatment of mental disorders, 1990 to 2003. *N. Engl. J. Med.* 352, 2515–2523.

Kirkham, T. C., Williams, C. M., Fezza, F., and Di Marzo, V. (2002). Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br. J. Pharmacol.* 136, 550–557.

Klump, K. L., Miller, K. B., Keel, P. K., Mcgue, M., and Iacono, W. G. (2001). Genetic and environmental influences on anorexia nervosa syndromes in a population-based twin sample. *Psychol. Med.* 31, 737–740.

Koethe, D., Llenos, I. C., Dulay, J. R., Hoyer, C., Torrey, E. F., Leweke, F. M., and Weis, S. (2007). Expression of CB1 cannabinoid receptor in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression. *J. Neural Transm.* 114, 1055–1063.

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

Lemos, J. I., Resstel, L. B., and Guimaraes, F. S. (2010). Involvement of the prelimbic prefrontal cortex on cannabidiol-induced attenuation of contextual conditioned fear in rats. *Behav. Brain Res.* 207, 105–111.

Levens, S. M., and Gotlib, I. H. (2009). Impaired selection of relevant positive information in depression. *Depress. Anxiety* 26, 403–410.

Leweke, F. M., and Koethe, D. (2008). Cannabis and psychiatric disorders: it is not only addiction. *Addict. Biol.* 13, 264–275.

Leweke, F. M., and Schneider, M. (2011). Chronic pubertal cannabinoid treatment as a behavioural model for aspects of schizophrenia: effects of the atypical antipsychotic quetiapine. *Int. J. Neuropsychopharmacol.* 14, 43–51.

Lewis, D. A., and Levitt, P. (2002). Schizophrenia as a disorder of neurodevelopment. *Annu. Rev. Neurosci.* 25, 409–432.

Liu, Q. R., Pan, C. H., Hishimoto, A., Li, C. Y., Xi, Z. X., Llorente-Berzal, A., Viveros, M. P., Ishiguro, H., Arinami, T., Onaivi, E. S., and Uhl, G. R. (2009). Species differences in cannabinoid receptor 2 (CNR2 gene): identification of novel human and rodent CB2 isoforms, differential tissue expression and regulation by cannabinoid receptor ligands. *Genes Brain Behav.* 8, 519–530.

Llorente, R., Miguel-Blanco, C., Aisa, B., Lachize, S., Borcel, E., Meijer, O. C., Ramirez, M. J., De Kloet, E. R., and Viveros, M. P. (2011). Long term sex-dependent psychoneuroendocrine effects of maternal deprivation and juvenile unpredictable stress in rats. *J. Neuroendocrinol.* 23, 329–344.

Llorente-Berzal, A., Mela, V., Borcel, E., Valero, M., Lopez-Gallardo, M., Viveros, M. P., and Marco, E. M. (2011). Neurobehavioral and metabolic long-term consequences of neonatal maternal deprivation stress and adolescent olanzapine treatment in male and female rats. *Neuropharmacology*. PMID: 21819999. [Epub ahead of print].

Long, L. E., Malone, D. T., and Taylor, D. A. (2006). Cannabidiol reverses MK-801-induced disruption of prepulse inhibition in mice. *Neuropsychopharmacology* 31, 795–803.

Low, K., Crestani, F., Keist, R., Benke, D., Brunig, I., Benson, J. A., Fritschy, J. M., Rulicke, T., Bluethmann, H., Mohler, H., and Rudolph, U. (2000). Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* 290, 131–134.

Lowe, B., Zipfel, S., Buchholz, C., Dupont, Y., Reas, D. L., and Herzog, W. (2001). Long-term outcome of anorexia nervosa in a prospective 21-year follow-up study. *Psychol. Med.* 31, 881–890.

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

Lutz, B., Aparisi, A., and Viveros, M. P. (2010). "The dual role of the endocannabinoid system as a regulator of anxiety responses," in *FENS Forum 2010*, Amsterdam, The Netherlands. FENS Abstr., 088.035.

Maccarrone, M., Gasperi, V., Catani, M. V., Diep, T. A., Dainese, E., Hansen, H. S., and Avigliano, L. (2010). The endocannabinoid system and its relevance for nutrition. *Annu. Rev. Nutr.* 30, 423–440.

Maccarrone, M., Valverde, O., Barbaccia, M. L., Castane, A., Maldonado, R., Ledent, C., Parmentier, M., and Finazzi-Agro, A. (2002). Age-related changes of anandamide metabolism in CB1 cannabinoid receptor knockout mice: correlation with behaviour. *Eur. J. Neurosci.* 15, 1178–1186.

Mackie, K. (2005). "Distribution of cannabinoid receptors in the central and peripheral nervous system," in *Cannabinoids. Handbook of Experimental Pharmacology*, ed. R. G. Pertwee (Berlin: Springer), 299–323.

Mahler, S. V., Smith, K. S., and Berridge, K. C. (2007). Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances "liking" of a sweet reward. *Neuropsychopharmacology* 32, 2267–2278.

Mailleux, P., and Vanderhaeghen, J. J. (1992). Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. *Neuroscience* 48, 655–668.

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

Malone, D. T., Kearn, C. S., Chongue, L., Mackie, K., and Taylor, D. A. (2008). Effect of social isolation on CB1 and D2 receptor and fatty acid amide hydrolase expression in rats. *Neuroscience* 152, 265–272.

Manji, H. K., Drevets, W. C., and Charney, D. S. (2001). The cellular neurobiology of depression. *Nat. Med.* 7, 541–547.

Manzanares, J., Uriguen, L., Rubio, G., and Palomo, T. (2004). Role of endocannabinoid system in mental diseases. *Neurotox. Res.* 6, 213–224.

Marco, E. M., Adriani, W., Llorente, R., Laviola, G., and Viveros, M. P. (2009). Detrimental psychophysiological effects of early maternal deprivation in adolescent and adult rodents: altered responses to cannabinoid exposure. *Neurosci. Biobehav. Rev.* 33, 498–507.

Marco, E. M., Perez-Alvarez, L., Borcel, E., Rubio, M., Guaza, C., Ambrosio, E., File, S. E., and Viveros, M. P. (2004). Involvement of 5-HT1A receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. *Behav. Pharmacol.* 15, 21–27.

Marco, E. M., and Viveros, M. P. (2009). The critical role of the endocannabinoid system in emotional homeostasis: avoiding excess and deficiencies. *Mini Rev. Med. Chem.* 9, 1407–1415.

Marin, S., Marco, E., Biscaia, M., Fernandez, B., Rubio, M., Guaza, C., Schmidhammer, H., and Viveros, M. P. (2003). Involvement of the kappa-opioid receptor in the anxiogenic-like effect of CP 55,940 in male rats. *Pharmacol. Biochem. Behav.* 74, 649–656.

Marsch, R., Foeller, E., Rammes, G., Bunck, M., Kossl, M., Holsboer, F., Zieglgansberger, W., Landgraf, R., Lutz, B., and Wotjak, C. T. (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., and Lutz, B. (2006). Neuromodulatory functions of the endocannabinoid system. *J. Endocrinol. Invest.* 29, 27–46.

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

Martin, M., Ledent, C., Parmentier, M., Maldonado, R., and Valverde, O. (2002a). Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl.)* 159, 379–387.

Martin, M., Ledent, C., Parmentier, M., Maldonado, R., and Valverde, O. (2002b). Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl.)* 159, 379–387.

Matias, I., and Di Marzo, V. (2007). Endocannabinoids and the control of energy balance. *Trends Endocrinol. Metab.* 18, 27–37.

Mechoulam, R., Peters, M., Murillo-Rodriguez, E., and Hanus, L. O. (2007). Cannabidiol – recent advances. *Chem. Biodivers.* 4, 1678–1692.

Mishima, K., Hayakawa, K., Abe, K., Ikeda, T., Egashira, N., Iwasaki, K., and Fujiwara, M. (2005). Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. *Stroke* 36, 1077–1082.

Molina-Holgado, E., Vela, J. M., Arevalo-Martin, A., Almazan, G., Molina-Holgado, F., Borrell, J., and Guaza, C. (2002). Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *J. Neurosci.* 22, 9742–9753.

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

Monteleone, P., Bifulco, M., Di Filippo, C., Gazzerro, P., Canestrelli, B., Monteleone, F., Proto, M. C., Di Genio, M., Grimaldi, C., and Maj, M. (2009). Association of CNR1 and FAAH endocannabinoid gene polymorphisms with anorexia nervosa and bulimia nervosa: evidence for synergistic effects. *Genes Brain Behav.* 8, 728–732.

Monteleone, P., Dilieto, A., Castaldo, E., and Maj, M. (2004). Leptin functioning in eating disorders. *CNS Spectr.* 9, 523–529.

Monteleone, P., Matias, I., Martiadis, V., De Petrocellis, L., Maj, M., and Di Marzo, V. (2005). Blood levels of the endocannabinoid anandamide are increased in anorexia nervosa and in binge-eating disorder, but not in bulimia nervosa. *Neuropsychopharmacology* 30, 1216–1221.

Monteleone, P., Tortorella, A., Martiadis, V., Di Filippo, C., Canestrelli, B., and Maj, M. (2008). The cDNA 385C to A missense polymorphism of the endocannabinoid degrading enzyme fatty acid amide hydrolase (FAAH) is associated with overweight/obesity but not with binge eating disorder in overweight/obese women. *Psychoneuroendocrinology* 33, 546–550.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., and Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet* 370, 319–328.

Moreira, F. A., Aguiar, D. C., Campos, A. C., Lisboa, S. F., Terzian, A. L., Resstel, L. B., and Guimaraes, F. S. (2009). Antiaversive effects of cannabinoids: is the periaqueductal gray involved? *Neural Plast.* 2009, 625469.

Moreira, F. A., Aguiar, D. C., and Guimaraes, F. S. (2006). Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 30, 1466–1471.

Moreira, F. A., Aguiar, D. C., and Guimaraes, F. S. (2007). Anxiolytic-like effect of cannabinoids injected into the rat dorsolateral periaqueductal gray. *Neuropharmacology* 52, 958–965.

Moreira, F. A., and Guimarães, F. S. (2005). Cannabidiol inhibits the hyperlocomotion induced by psychotomimetic drugs in mice. *Eur. J. Pharmacol.* 512, 199–205.

Moreira, F. A., and Lutz, B. (2008). The endocannabinoid system: emotion, learning and addiction. *Addict. Biol.* 13, 196–212.

Moreira, F. A., and Wotjak, C. T. (2010). Cannabinoids and anxiety. *Curr. Top. Behav. Neurosci.* 2, 429–450.

Morgan, C. J., and Curran, H. V. (2008). Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *Br. J. Psychiatry* 192, 306–307.

Morgan, N. H., Stanford, I. M., and Woodhall, G. L. (2009). Functional CB2 type cannabinoid receptors at CNS synapses. *Neuropharmacology* 57, 356–368.

Muller, T. D., Reichwald, K., Bronner, G., Kirschner, J., Nguyen, T. T., Scherag, A., Herzog, W., Herpertz-Dahlmann, B., Lichtner, P., Meitinger, T., Platzer, M., Schafer, H., Hebebrand, J., and Hinney, A. (2008). Lack of association of genetic variants in genes of the endocannabinoid system with anorexia nervosa. *Child Adolesc. Psychiatry Ment. Health* 2, 33.

Muller-Vahl, K. R., and Emrich, H. M. (2008). Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. *Expert Rev. Neurother.* 8, 1037–1048.

Munro, S., Thomas, K. L., and Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365, 61–65.

Naruse, T., Amano, H., and Koizumi, Y. (1991). Possible involvement of dopamine D-1 and D-2 receptors in diazepam-induced hyperphagia in rats. *Fundam. Clin. Pharmacol.* 5, 677–693.

Navarro, M., Hernandez, E., Munoz, R. M., Del Arco, I., Villanua, M. A., Carrera, M. R., and 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.

Nelson, K., Walsh, D., Deeter, P., and Sheehan, F. (1994). A phase II study of delta-9-tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia. *J. Palliat. Care* 10, 14–18.

Nestler, E. J., Barrot, M., Dileone, R. J., Eisch, A. J., Gold, S. J., and Monteggia, L. M. (2002). Neurobiology of depression. *Neuron* 34, 13–25.

Newell, K. A., Deng, C., and Huang, X. F. (2006). Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. *Exp. Brain Res.* 172, 556–560.

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

Onaivi, E. S., Green, M. R., and Martin, B. R. (1990). Pharmacological characterization of cannabinoids in the elevated plus maze. *J. Pharmacol. Exp. Ther.* 253, 1002–1009.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Meozzi, P. A., Myers, L., Perchuk, A., Mora, Z., Tagliaferro, P. A., Gardner, E., Brusco, A., Akinshola, B. E., Hope, B., Lujilde, J., Inada, T., Iwasaki, S., Macharia, D., Teasenfitz, L., Arinami, T., and Uhl, G. R. (2008a). Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. *PLoS ONE* 3, e1640. doi:10.1371/journal.pone.0001640

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Meozzi, P. A., Myers, L., Perchuk, A., Mora, Z., Tagliaferro, P. A., Gardner, E., Brusco, A., Akinshola, B. E., Liu, Q. R., Chirwa, S. S., Hope, B., Lujilde, J., Inada, T., Iwasaki, S., Macharia, D., Teasenfitz, L., Arinami, T., and Uhl, G. R. (2008b). Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. *Ann. N. Y. Acad. Sci.* 1139, 434–449.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Perchuk, A., Meozzi, P. A., Myers, L., Mora, Z., Tagliaferro, P., Gardner, E., Brusco, A., Akinshola, B. E., Liu, Q. R., Hope, B., Iwasaki, S., Arinami, T., Teasenfitz, L., and Uhl, G. R. (2006). Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann. N. Y. Acad. Sci.* 1074, 514–536.

Ortega-Alvaro, A., Aracil-Fernandez, A., García-Gutiérrez, M. S., Navarrete, F., and Manzanares, J. (2011). Deletion of CB2 cannabinoid receptor induces schizophrenia-related behaviors in mice. *Neuropsychopharmacology* 36, 1489–1504.

O'Sullivan, S. E., and Kendall, D. A. (2010). Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory disease. *Immunobiology* 215, 611–616.

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

Pagotto, U., Marsicano, G., Cota, D., Lutz, B., and Pasquali, R. (2006). The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr. Rev.* 27, 73–100.

Pamplona, F. A., and Takahashi, R. N. (2011). Psychopharmacology of the endocannabinoids: far beyond anandamide. *J. Psychopharmacol.* PMID: 21652605. [Epub ahead of print].

Parolaro, D., Realini, N., Vigano, D., Guidali, C., and Rubino, T. (2010). The endocannabinoid system and psychiatric disorders. *Exp. Neurol.* 224, 3–14.

Peralta, V., Campos, M. S., De Jalon, E. G., and Cuesta, M. J. (2010). Motor behavior abnormalities in drug-naive patients with schizophrenia spectrum disorders. *Mov. Disord.* 25, 1068–1076.

Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br. J. Pharmacol.* 153, 199–215.

Petitet, F., Jeantaud, B., Reibaud, M., Imperato, A., and Dubroeucq, M. C. (1998). Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of delta9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. *Life Sci.* 63, PL1–PL6.

Piomelli, D., Tarzia, G., Duranti, A., Tontini, A., Mor, M., Compton, T. R., Dasse, O., Monaghan, E. P., Parrott, J. A., and Putman, D. (2006). Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). *CNS Drug Rev.* 12, 21–38.

Racz, I., Nadal, X., Alferink, J., Banos, J. E., Rehnelt, J., Martin, M., Pintado, B., Gutierrez-Adan, A., Sanguino, E., Bellora, N., Manzanares, J., Zimmer, A., and Maldonado, R. (2008a). Interferon-gamma is a critical modulator of CB(2) cannabinoid receptor signaling during neuropathic pain. *J. Neurosci.* 28, 12136–12145.

Racz, I., Nadal, X., Alferink, J., Banos, J. E., Rehnelt, J., Martin, M., Pintado, B., Gutierrez-Adan, A., Sanguino, E., Manzanares, J., Zimmer, A., and Maldonado, R. (2008b). Crucial role of CB(2) cannabinoid receptor in the regulation of central immune responses during neuropathic pain. *J. Neurosci.* 28, 12125–12135.

Rapoport, J. L., Addington, A. M., Frangou, S., and Psych, M. R. (2005). The neurodevelopmental model of schizophrenia: update 2005. *Mol. Psychiatry* 10, 434–449.

Reibaud, M., Obinu, M. C., Ledent, C., Parmentier, M., Bohme, G. A., and Imperato, A. (1999). Enhancement of memory in cannabinoid CB1 receptor knockout mice. *Eur. J. Pharmacol.* 379, R1–R2.

Reich, C. G., Taylor, M. E., and Mccarthy, M. M. (2009). Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats. *Behav. Brain Res.* 203, 264–269.

Resstel, L. B., Joca, S. R., Moreira, F. A., Correa, F. M., and Guimaraes, F. S. (2006). Effects of cannabidiol and diazepam on behavioral and cardiovascular responses induced by contextual conditioned fear in rats. *Behav. Brain Res.* 172, 294–298.

Resstel, L. B., Tavares, R. F., Lisboa, S. F., Joca, S. R., Correa, F. M., and Guimaraes, F. S. (2009). 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *Br. J. Pharmacol.* 156, 181–188.

Robinson, S. A., Loiacono, R. E., Christopoulos, A., Sexton, P. M., and Malone, D. T. (2010). The effect of social isolation on rat brain expression of genes associated with endocannabinoid signaling. *Brain Res.* 1343, 153–167.

Roche, R., Hoareau, L., Bes-Houtmann, S., Gonthier, M. P., Laborde, C., Baron, J. F., Haffaf, Y., Cesari, M., and Festy, F. (2006). Presence of the cannabinoid receptors, CB1 and CB2, in human omental and subcutaneous adipocytes. *Histochem. Cell Biol.* 126, 177–187.

Rodgers, R. J., Haller, J., Halasz, J., and 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-Gaztelumendi, A., Rojo, M. L., Pazos, A., and Diaz, A. (2009). Altered CB receptor-signaling in prefrontal cortex from an animal model of depression is reversed by chronic fluoxetine. *J. Neurochem.* 108, 1423–1433.

Rosenstock, J., Hollander, P., Chevalier, S., and Iranmanesh, A. (2008). SERENADE: the Study Evaluating Rimonabant Efficacy in Drug-naive Diabetic Patients: effects of monotherapy with rimonabant, the first selective CB1 receptor antagonist, on glycemic control, body weight, and lipid profile in drug-naive type 2 diabetes. *Diabetes Care* 31, 2169–2176.

Ross, C. A., Margolis, R. L., Reading, S. A., Pletnikov, M., and Coyle, J. T. (2006). Neurobiology of schizophrenia. *Neuron* 52, 139–153.

Rubin, D. C., Berntsen, D., and Bohni, M. K. (2008). A memory-based model of posttraumatic stress disorder: evaluating basic assumptions underlying the PTSD diagnosis. *Psychol. Rev.* 115, 985–1011.

Russo, E. B., Burnett, A., Hall, B., and Parker, K. K. (2005). Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem. Res.* 30, 1037–1043.

Sanchez, C., Galve-Roperh, I., Canova, C., Brachet, P., and Guzman, M. (1998). Delta9-tetrahydrocannabinol induces apoptosis in C6 glioma cells. *FEBS Lett.* 436, 6–10.

Sanchis-Segura, C., Cline, B. H., Marsicano, G., Lutz, B., and Spanagel, R. (2004). Reduced sensitivity to reward in CB1 knockout mice. *Psychopharmacology (Berl.)* 176, 223–232.

Schatz, A. R., Lee, M., Condie, R. B., Pulaski, J. T., and Kaminski, N. E. (1997). Cannabinoid receptors CB1 and CB2: a characterization of expression and adenylate cyclase modulation within the immune system. *Toxicol. Appl. Pharmacol.* 142, 278–287.

Scheen, A. J. (2008). CB1 receptor blockade and its impact on cardiometabolic risk factors: overview of the RIO programme with rimonabant. *J. Neuroendocrinol.* 20(Suppl. 1), 139–146.

Schneider, M. (2008). Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addict. Biol.* 13, 253–263.

Schneider, M., Drews, E., and Koch, M. (2005). Behavioral effects in adult rats of chronic prepubertal treatment with the cannabinoid receptor agonist WIN 55,212-2. *Behav. Pharmacol.* 16, 447–454.

Sciolino, N. R., Bortolato, M., Eisenstein, S. A., Fu, J., Oveisi, F., Hohmann, A. G., and Piomelli, D. (2010). Social isolation and chronic handling alter endocannabinoid signaling and behavioral reactivity to context in adult rats. *Neuroscience* 168, 371–386.

Seifert, J., Ossege, S., Emrich, H. M., Schneider, U., and Stuhrmann, M. (2007). No association of CNR1 gene variations with susceptibility to schizophrenia. *Neurosci. Lett.* 426, 29–33.

Sheline, Y. I. (2000). 3D MRI studies of neuroanatomic changes in unipolar major depression: the role of stress and medical comorbidity. *Biol. Psychiatry* 48, 791–800.

Shin, L. M., and Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology* 35, 169–191.

Siegfried, Z., Kanyas, K., Latzer, Y., Karni, O., Bloch, M., Lerer, B., and Berry, E. M. (2004). Association study of cannabinoid receptor gene (CNR1) alleles and anorexia nervosa: differences between restricting and binging/purging subtypes. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 125B, 126–130.

Silveira Filho, N. G., and Tufik, S. (1981). Comparative effects between cannabidiol and diazepam on neophobia, food intake and conflict behavior. *Res. Commun. Psychol. Psychiatr. Behav.* 6, 251–266.

Soares, P., Campos, A. C., Bortoli, V. C., Zangrossi, H. Jr., Guimaraes, F. S., and Zuardi, A. W. (2010). Intradorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. *Behav. Brain Res.* 213, 225–229.

Solowij, N., and Battisti, R. (2008). The chronic effects of cannabis on memory in humans: a review. *Curr. Drug Abuse Rev.* 1, 81–98.

Solowij, N., and Michie, P. T. (2007). Cannabis and cognitive dysfunction: parallels with endophenotypes of schizophrenia? *J. Psychiatry Neurosci.* 32, 30–52.

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

Steffens, S., Veillard, N. R., Arnaud, C., Pelli, G., Burger, F., Staub, C., Karsak, M., Zimmer, A., Frossard, J. L., and Mach, F. (2005). Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature* 434, 782–786.

Steiner, M. A., Wanisch, K., Monory, K., Marsicano, G., Borroni, E., Bachli, H., Holsboer, F., Lutz, B., and Wotjak, C. T. (2008). Impaired cannabinoid receptor type 1 signaling interferes with stress-coping behavior in mice. *Pharmacogenomics J.* 8, 196–208.

Stoving, R. K., Andries, A., Brixen, K., Flyvbjerg, A., Horder, K., and Frystyk, J. (2009). Leptin, ghrelin, and endocannabinoids: potential therapeutic targets in anorexia nervosa. *J. Psychiatr. Res.* 43, 671–679.

Strober, M., Schmidt-Lackner, S., Freeman, R., Bower, S., Lampert, C., and Deantonio, M. (1995). Recovery and relapse in adolescents with bipolar affective illness: a five-year naturalistic, prospective follow-up. *J. Am. Acad. Child Adolesc. Psychiatry* 34, 724–731.

Substance Abuse and Mental Health Services Administration, SAMHSA. (2006). *Results from the 2005 National Survey on Drug Use and Health: National Finding. Office of Applied Studies, NSDUH Series H-30, DHHS (Publication No. SMA 06-4194)*, Rockville, MD.

Suarez, J., Llorente, R., Romero-Zerbo, S. Y., Mateos, B., Bermudez-Silva, F. J., De Fonseca, F. R., and Viveros, M. P. (2009). Early maternal deprivation induces gender-dependent changes on the expression of hippocampal CB(1) and CB(2) cannabinoid receptors of neonatal rats. *Hippocampus* 19, 623–632.

Sugranyes, G., Flamarique, I., Parellada, E., Baeza, I., Goti, J., Fernandez-Egea, E., and Bernardo, M. (2009). Cannabis use and age of diagnosis of schizophrenia. *Eur. Psychiatry* 24, 282–286.

Sundram, S. (2006). Cannabis and neurodevelopment: implications for psychiatric disorders. *Hum Psychopharmacol* 21, 245–254.

Terzian, A. L., Aguiar, D. C., Guimaraes, F. S., and Moreira, F. A. (2009). Modulation of anxiety-like behaviour by Transient Receptor Potential Vanilloid Type 1 (TRPV1) channels located in the dorsolateral periaqueductal gray. *Eur. Neuropsychopharmacol.* 19, 188–195.

Thomas, A., Baillie, G. L., Phillips, A. M., Razdan, R. K., Ross, R. A., and Pertwee, R. G. (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br. J. Pharmacol.* 150, 613–623.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., and Paylor, R. (2009). Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology (Berl.)* 204, 361–373.

Thomas, B. F., Gilliam, A. F., Burch, D. F., Roche, M. J., and Seltzman, H. H. (1998). Comparative receptor binding analyses of cannabinoid agonists and antagonists. *J. Pharmacol. Exp. Ther.* 285, 285–292.

Tsai, S. J., Wang, Y. C., and Hong, C. J. (2000). Association study of a cannabinoid receptor gene (CNR1) polymorphism and schizophrenia. *Psychiatr. Genet.* 10, 149–151.

Tyrka, A. R., Wier, L., Price, L. H., Ross, N., Anderson, G. M., Wilkinson, C. W., and Carpenter, L. L. (2008). Childhood parental loss and adult hypothalamic-pituitary-adrenal function. *Biol. Psychiatry* 63, 1147–1154.

Ueda, N., Tsuboi, K., Uyama, T., and Ohnishi, T. (2011). Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. *Biofactors* 37, 1–7.

Ujike, H., and Morita, Y. (2004). New perspectives in the studies on endocannabinoid and cannabis: cannabinoid receptors and schizophrenia. *J. Pharmacol. Sci.* 96, 376–381.

Uriguen, L., Garcia-Fuster, M. J., Callado, L. F., Morentin, B., La Harpe, R., Casado, V., Lluis, C., Franco, R., Garcia-Sevilla, J. A., and Meana, J. J. (2009). Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: effect of antipsychotic treatment. *Psychopharmacology (Berl.)* 206, 313–324.

Urigüen, L., García-Gutiérrez, M. S., and Manzanares, J. (2011). Decreased GABAA and GABAB receptor functional activity in cannabinoid CB1 receptor knockout mice. *J. Psychopharmacol. (Oxford)* 25, 105–110.

Urigüen, L., Perez-Rial, S., Ledent, C., Palomo, T., and Manzanares, J. (2004). Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. *Neuropharmacology* 46, 966–973.

Valenzano, K. J., Tafesse, L., Lee, G., Harrison, J. E., Boulet, J. M., Gottshall, S. L., Mark, L., Pearson, M. S., Miller, W., Shan, S., Rabadi, L., Rotshteyn, Y., Chaffer, S. M., Turchin, P. I., Elsemore, D. A., Toth, M., Koetzner, L., and Whiteside, G. T. (2005). Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. *Neuropharmacology* 48, 658–672.

Van Gaal, L., Pi-Sunyer, X., Despres, J. P., Mccarthy, C., and Scheen, A. (2008). Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: pooled 1-year data from the rimonabant in obesity (RIO) program. *Diabetes Care* 31(Suppl. 2), S229–S240.

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J. S., Marnett, L. J., Di Marzo, V., Pittman, Q. J., Patel, K. D., and Sharkey, K. A. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 310, 329–332.

Verty, A. N., Evetts, M. J., Crouch, G. J., Mcgregor, I. S., Stefanidis, A., and Oldfield, B. J. (2011). The cannabinoid receptor agonist THC attenuates weight loss in a rodent model of activity-based anorexia. *Neuropsychopharmacology* 36, 1349–1358.

Vigano, D., Guidali, C., Petrosino, S., Realini, N., Rubino, T., Di Marzo, V., and Parolaro, D. (2009). Involvement of the endocannabinoid system in phencyclidine-induced cognitive deficits modelling schizophrenia. *Int. J. Neuropsychopharmacol.* 12, 599–614.

Vinod, K. Y., and Hungund, B. L. (2006). Role of the endocannabinoid system in depression and suicide. *Trends Pharmacol. Sci.* 27, 539–545.

Viveros, M. P., Marco, E. M., and File, S. E. (2005). Endocannabinoid system and stress and anxiety responses. *Pharmacol. Biochem. Behav.* 81, 331–342.

Viveros, M. P., Marco, E. M., Llorente, R., and Lopez-Gallardo, M. (2007). Endocannabinoid system and synaptic plasticity: implications for emotional responses. *Neural Plast.* 2007, 52908.

Volkow, N. D., and Wise, R. A. (2005). How can drug addiction help us understand obesity? *Nat. Neurosci.* 8, 555–560.

Waksman, Y., Olson, J. M., Carlisle, S. J., and Cabral, G. A. (1999). The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. *J. Pharmacol. Exp. Ther.* 288, 1357–1366.

Walker, A. (1994). *Thesaurus of Psychological Index Terms*. Washington, DC: American psychological Association.

Walsh, B. T., Kaplan, A. S., Attia, E., Olmsted, M., Parides, M., Carter, J. C., Pike, K. M., Devlin, M. J., Woodside, B., Roberto, C. A., and Rockert, W. (2006). Fluoxetine after weight restoration in anorexia nervosa: a randomized controlled trial. *JAMA* 295, 2605–2612.

Walter, L., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., Mackie, K., and Stella, N. (2003). Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J. Neurosci.* 23, 1398–1405.

Wegener, N., and Koch, M. (2009). Behavioural disturbances and altered Fos protein expression in adult rats after chronic pubertal cannabinoid treatment. *Brain Res.* 1253, 81–91.

Witkin, J. M. (2008). Animal models of obsessive-compulsive disorder. *Curr. Protoc. Neurosci.* 45, 9.30.1–9.30.9.

Wolf, S. A., Bick-Sander, A., Fabel, K., Leal-Galicia, P., Tauber, S., Ramirez-Rodriguez, G., Muller, A., Melnik, A., Waltinger, T. P., Ullrich, O., and Kempermann, G. (2010). Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. *Cell Commun. Signal.* 8, 12.

Wotjak, C. T. (2005). Role of endogenous cannabinoids in cognition and emotionality. *Mini Rev. Med. Chem.* 5, 659–670.

Xing, J., and Li, J. (2007). TRPV1 receptor mediates glutamatergic synaptic input to dorsolateral periaqueductal gray (dl-PAG) neurons. *J. Neurophysiol.* 97, 503–511.

Yilmazer-Hanke, D. M. (2008). Morphological correlates of emotional and cognitive behaviour: insights from studies on inbred and outbred rodent strains and their crosses. *Behav. Pharmacol.* 19, 403–434.

Yu, H., and Chen, Z. Y. (2010). The role of BDNF in depression on the basis of its location in the neural circuitry. *Acta Pharmacol. Sin.* 32, 3–11.

Zamberletti, E., Vigano, D., Guidali, C., Rubino, T., and Parolaro, D. (2010). Long-lasting recovery of psychotic-like symptoms in isolation-reared rats after chronic but not acute treatment with the cannabinoid antagonist AM251. *Int. J. Neuropsychopharmacol.* 1–14.

Zanelati, T. V., Biojone, C., Moreira, F. A., Guimaraes, F. S., and Joca, S. R.

(2010). Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. *Br. J. Pharmacol.* 159, 122–128.

Zavitsanou, K., Garrick, T., and Huang, X. F. (2004). Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 28, 355–360.

Zuardi, A. W. (2008). Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Rev. Bras. Psiquiatr.* 30, 271–280.

Zuardi, A. W., Cosme, R. A., Graeff, F. G., and Guimarães, F. S. (1993a). Effects of ipsapirone and cannabidiol on human experimental anxiety. *J. Psychopharmacol.* 7, 82–88.

Zuardi, A. W., Guimaraes, F. S., and Moreira, A. C. (1993b). Effect of cannabidiol on plasma prolactin, growth hormone and cortisol in human volunteers. *Braz. J. Med. Biol. Res.* 26, 213–217.

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Moreira, F. A., and Guimaraes, F. S. (2006a). Cannabidiol, a *Cannabis sativa* constituent, as an antipsychotic drug. *Braz. J. Med. Biol. Res.* 39, 421–429.

Zuardi, A. W., Hallak, J. E., Dursun, S. M., Morais, S. L., Sanches, R. F., Musty, R. E., and Crippa, J. A. (2006b). Cannabidiol monotherapy for treatment-resistant schizophrenia. *J. Psychopharmacol. (Oxford)* 20, 683–686.

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Pinto, J. P., Chagas, M. H., Rodrigues, G. G., Dursun, S. M., and Tumas, V. (2009). Cannabidiol for the treatment of psychosis in Parkinson's disease. *J. Psychopharmacol. (Oxford)* 23, 979–983.

Zuardi, A. W., and Karniol, I. G. (1983). Changes in the conditioned emotional response of rats induced by Δ9-THC, CBD and mixture of the two cannabinoids. *Braz. Arch. Biol. Technol.* 26, 391–397.

Zuardi, A. W., Morais, S. L., Guimaraes, F. S., and Mechoulam, R. (1995). Antipsychotic effect of cannabidiol. *J. Clin. Psychiatry* 56, 485–486.

Zuardi, A. W., Rodrigues, J. A., and Cunha, J. M. (1991). Effects of cannabidiol in animal models predictive of antipsychotic activity. *Psychopharmacology (Berl.)* 104, 260–264.

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

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Effects of endocannabinoid system modulation on cognitive and emotional behavior

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Cannabis has long been known to produce cognitive and emotional effects. Research has shown that cannabinoid drugs produce these effects by driving the brain's endogenous cannabinoid system and that this system plays a modulatory role in many cognitive and emotional processes. This review focuses on the effects of endocannabinoid system modulation in animal models of cognition (learning and memory) and emotion (anxiety and depression). We review studies in which natural or synthetic cannabinoid agonists were administered to directly stimulate cannabinoid receptors or, conversely, where cannabinoid antagonists were administered to inhibit the activity of cannabinoid receptors. In addition, studies are reviewed that involved genetic disruption of cannabinoid receptors or genetic or pharmacological manipulation of the endocannabinoid-degrading enzyme, fatty acid amide hydrolase (FAAH). Endocannabinoids affect the function of many neurotransmitter systems, some of which play opposing roles. The diversity of cannabinoid roles and the complexity of task-dependent activation of neuronal circuits may lead to the effects of endocannabinoid system modulation being strongly dependent on environmental conditions. Recent findings are reviewed that raise the possibility that endocannabinoid signaling may change the impact of environmental influences on emotional and cognitive behavior rather than selectively affecting any specific behavior.

Keywords: endocannabinoids, cognition, anxiety, depression, learning, memory, animal models

Cannabis has been used by humans for millennia and has long been known to produce cognitive and emotional effects. Research over the past two decades has shown that cannabinoid drugs produce these effects by driving the brain's endogenous cannabinoid system, and that this system plays a modulatory role in many cognitive and emotional processes. This review will focus on the effects of endocannabinoid system modulation in animal models of cognition (learning and memory) and emotion (anxiety and depression).

This research has been facilitated by the availability of pharmacological tools that are used in four general ways:

(1) An exogenous cannabinoid agonist can be administered to directly stimulate cannabinoid receptors. Administering cannabinoid receptor agonists such as Δ^9-THC (the main active constituent of cannabis) or WIN55212 (a synthetic agonist) can provide information about the effects of illicit cannabinoid use and also about the potential therapeutic or adverse effects of cannabinoid-related medications. Cannabinoid substances that occur endogenously, such as anandamide, can also be synthesized and administered exogenously to gain insight into their function.
(2) A cannabinoid receptor antagonist can be administered along with another treatment to determine whether effects of the treatment depend on its actions at cannabinoid receptors. For example, if blocking cannabinoid CB_1 receptors with an antagonist such as rimonabant prevents the treatment from having a certain effect, that effect of the treatment is said to be mediated by CB_1 receptors.
(3) Ongoing endocannabinoid signaling can be blocked by administering a cannabinoid receptor antagonist alone. When endocannabinoid signaling is blocked, behaviors that are modulated by this signaling should increases or decrease, depending on whether the modulation is negative or positive. This approach assumes that the antagonist blocks endogenously released endocannabinoids, but does not otherwise affect signaling. Unfortunately, the antagonists that have been used most frequently for this purpose (rimonabant and AM251) also function as inverse agonists and may affect neuronal functions even in the absence of the release of endocannabinoid agonists.
(4) Endocannabinoid signaling can be enhanced by administering an enzyme inhibitor that prevents the breakdown of endocannabinoids that have been released. Endocannabinoids are synthesized "on demand" when synaptic neurotransmission surpasses a certain threshold. Treatments that prevent the breakdown of endocannabinoids should mainly affect cells in the immediate areas where an endocannabinoid is being released. In contrast, exogenous agonists affect synapses wherever the receptors are expressed. Thus, treatments that prevent

endocannabinoid breakdown should magnify the ongoing effects of endocannabinoids and might provide better insight into normal function. Anandamide, the most frequently studied endocannabinoid, is degraded by the enzyme fatty acid amid hydrolase (FAAH). Inhibitors of FAAH first became available about 7 years ago (e.g., Tarzia et al., 2003; Mor et al., 2004), and there is now a large amount of information concerning the effects of FAAH inhibitors on cognitive and emotional behavior. Inhibitors of the degradation of 2-AG, the other major endocannabinoid that has been identified, have also been recently developed (e.g., Long et al., 2009), but information on their cognitive or emotional impact is too scarce to be reviewed at this time.

In addition to these four pharmacological approaches, the role of endocannabinoids in cognitive and emotional processes can be investigated with genetically modified strains of rodents. This has been accomplished in two general ways: deleting a specific cannabinoid receptor subtype (i.e., CB_1), which excludes cannabinoid signaling; and deleting a metabolizing enzyme (i.e., FAAH), which enhances endogenous endocannabinoid signaling.

Each of these pharmacological and genetic approaches has advantages and disadvantages. For example, manipulating FAAH affects not only endocannabinoids but related fatty acids that bind at non-cannabinoid sites, such as peroxisome proliferator-activated receptors and transient receptor-potential vanilloid receptors. When a receptor or enzyme is genetically deleted, other mechanisms may be affected by their absence. Therefore, the best understanding is gained through convergence, comparing the results obtained with different approaches.

EFFECTS OF ENDOCANNABINOID SYSTEM MODULATION ON LEARNING AND MEMORY

The memory-impairing effects of marijuana in humans have been widely recognized since at least the 1970s (Tart, 1970). Interest in the role played by endocannabinoids in cognitive processes has been stimulated by evidence that CB_1 receptors are highly expressed (Herkenham et al., 1991) – and endocannabinoids (anandamide and 2-AG) occur in high concentrations (Di Marzo et al., 2000) – in the hippocampus, a brain area that plays a critical role in learning and memory. Animal models have been used extensively to assess the effects of cannabinoid manipulations on various stages of learning and memory, including acquisition, consolidation, and retrieval (Riedel and Davies, 2005; Varvel et al., 2009). Most of these studies have involved spatial learning. In general, the findings are that exogenous and endogenous cannabinoid agonists impair working memory and the acquisition of long-term memory, while cannabinoid antagonists/inverse agonists or genetic deletion of cannabinoid receptors are sometimes found to enhance learning and memory.

Endocannabinoid signaling can affect many behavioral and physiological processes, including locomotion, feeding, anxiety, reward, and nociception. Therefore, to confidently attribute the effects of cannabinoid manipulations to learning and memory processes *per se*, as opposed to motivational, emotional, or motor processes, it is important to consider complementary models. For example, some memory models involve aversive motivation (e.g., escape from a water-filled pool), while others involve appetitive motivation (e.g., food-reinforced behavior in delayed matching tasks); finding similar effects of a drug in both aversive and appetitive models would suggest an effect on memory rather than motivation. It can also be informative to test the effects of a treatment in both a memory model and a more general, non-cognitive behavioral assay, such as spontaneous locomotor activity in an open field. In the following sections, we will consider the findings obtained with specific models of long-term memory (see Effects of Endocannabinoid System Modulation on Learning and Memory) and working memory (see Working Memory).

EFFECTS OF CANNABINOID CB_1 RECEPTOR AGONISTS AND ANTAGONISTS ON MEMORY ACQUISITION AND LONG-TERM MEMORY

Water maze

Much of the evidence that activating cannabinoid receptors can impair learning comes from studies using water maze procedures, which focus on spatial memory. In these tests the animals are trained to find a submerged platform in a tank filled with opaque water. Memory acquisition becomes evident over trials as successive reductions in the path length or the latency to reach the platform. In mice, acute systemic administration of Δ^9-THC (8 mg/kg, IP) before the training session disrupts acquisition in the water maze test without affecting locomotion; this effect is prevented by the CB_1 antagonist/inverse agonist rimonabant (DaSilva and Takahashi, 2002). Deficits in place-learning have also been reported in rats treated repeatedly with Δ^9-THC (Moore et al., 2010) or acutely with Δ^8-THC (Diana et al., 2003) or synthetic CB_1 agonists such as HU-210 (Ferrari et al., 1999), but not with the synthetic agonist nabilone (Diana et al., 2003). However, in these experiments, the effects of CB_1-receptor blockade were not tested. Another synthetic cannabinoid, WIN55212-2 (1 and 3 mg/kg), has been found to impair acquisition in the water maze, but his effect was not blocked by CB_1 antagonists, suggesting WIN55212-2 may impair learning by more than one mechanism (Robinson et al., 2010).

Water maze procedures have also been used to study the effects of Δ^9-THC on memory retrieval. For this purpose, rats that have already reached a criterion level of performance in the task are injected with the drug prior to a test session. Two laboratories have reported that – at doses known to impair memory acquisition – Δ^9-THC did not impair memory retrieval in the water maze (Mishima et al., 2001; Varvel et al., 2001, 2007). These findings suggest that, once established, reference memory is not susceptible to modulation by cannabinoid compounds.

Contextual fear conditioning

CB_1 agonists can also impair acquisition in another model of spatial memory, contextual fear conditioning. In this test, rodents are briefly exposed to footshock in a distinctive context, then tested by re-exposing them to the context. Immobility (freezing) during the test provides a measure of memory. The synthetic CB_1 agonist WIN55212-2 (2.5 and 5.0 mg/kg), given 30 min before the conditioning phase, impaired acquisition of contextual fear conditioning, but not conditioning to a discrete auditory cue (tone), which unlike contextual conditioning is believed to be independent of hippocampal function (Pamplona and Takahashi, 2006).

This finding is consistent with an impairment of hippocampal functioning, since the hippocampus mediates acquisition of fear conditioning involving contextual cues but not discrete cues (Phillips and LeDoux, 1992). Rimonabant (1 mg/kg) blocked the impairing effects of WIN55212-2, demonstrating the involvement of CB_1 receptors (Pamplona and Takahashi, 2006). Sink et al. (2010) showed that administration of CB_1 inverse agonists during the acquisition phase improves the retention of the contextual fear, consistent with endogenous cannabinoids having a negative modulatory effect on memory acquisition.

Object recognition and social recognition

In a typical object recognition task, animals are exposed to an object during one session, and then exposed to the same object plus a novel object in a subsequent test session. The relative amount of time spent exploring the novel object provides an index of memory. Systemic or intra-hippocampal administration of Δ^9-THC or WIN55212-2, either acute or repeated, impaired object recognition in rats (Barna et al., 2007; Quinn et al., 2008; Schneider et al., 2008). This impairment is associated with differential expression of proteins in the hippocampus (Quinn et al., 2008). However, in another study acute systemic administration of Δ^9-THC before the task failed to affect object recognition in adult rats (Ciccocioppo et al., 2002). Enhanced memory performance was observed in CB_1-knockout in this task (Maccarrone et al., 2002).

The roles of hippocampal functioning and spatial learning in the conventional object recognition procedure are still controversial (Ainge et al., 2006; Heuer and Bachevalier, 2011). It is possible to modify the procedure to focus on spatial memory by presenting objects during the exposure phase, then presenting the same objects during the test but with one placed in a different position. Suenaga and Ichitani (2008) found that microinjection of WIN55212-2 (1–2 μg/side in the hippocampus 10 min before the initial exposure to the objects) did not affect memory in the conventional procedure but impaired in a CB_1 dependent fashion the ability to recognize a new spatial configuration of objects.

The social recognition test is similar to the object recognition test but uses conspecifics instead of objects as the stimuli. Using long delays (15–30 min) it has been shown that the administration of WIN55212-2 impairs the performance of rats in a CB_1 dependent fashion (Schneider and Koch, 2002; Schneider et al., 2008). Rimonabant has been found to enhance recognition memory in this test (Terranova et al., 1996).

Radial maze

The effects of CB_1 compounds on the acquisition and recall of spatial memory in rodents have also been studied using a modified version of the radial maze test. In the conventional version of the test a food pellet is available at the end of each of the eight arms of the maze, and re-entering the same arm more than once indicates a working-memory error. In the modified version, to manipulate the mnemonic demand of the test, the rat is removed from the maze after it enters the seventh arm of the maze, and then it is placed back in the maze after a delay (Lichtman, 2000). With long delays, this test provides a test of long-term memory. Rimonabant (3 mg/kg), given to rats before the first placement in the maze, reduces the number of errors after a 6-h delay (Lichtman, 2000). Rimonabant had no effect when administered immediately after the first placement (Wise et al., 2007) or before the test placement (Lichtman, 2000), suggesting rimonabant enhances memory acquisition but not consolidation or retrieval. However, in other studies, the facilitating effects of CB_1 antagonism have been observed not only for acquisition, but also for consolidation (Wolff and Leander, 2003; Wise et al., 2008).

Passive avoidance

Data obtained with passive-avoidance procedures suggest a modulatory action of the endocannabinoids system on all phases of memory. In a widely used, hippocampal-dependent version of this test, rodents are allowed to explore a apparatus with two compartments, one lighted and one dark (Isaacson and Wickelgren, 1962). Entrance into the dark compartment is paired with a foot shock during a training session, and increased latency to enter the dark compartment during a subsequent test session is used as an index of conditioning. Systemic injections of Δ^9-THC or anandamide or intra-hippocampal injections of WIN55212-2 impair memory acquisition, consolidation, and recall in rats and mice (Castellano et al., 1997; Mishima et al., 2001; Costanzi et al., 2004; Nasehi et al., 2010). However it has been shown that the effects of anandamide on passive-avoidance performance can vary depending on the strain of the animals and on the protocol used (e.g., whether subjects are pre-exposed to the testing apparatus; Castellano et al., 1999; Costanzi et al., 2004).

Caveats

Taken together, the findings with these various animal models of long-term memory suggest a modulatory role of the endocannabinoid system during the acquisition phase of a place memory (see **Table 1**). Generally, CB agonists have been found to impair acquisition, and antagonism or deletion of CB receptors has been found to enhance it. However, there are some caveats to this conclusion. For example, neither the CB_1 inverse agonist/antagonist rimonabant at different doses (1, 3 mg/kg) nor the genetic disruption of CB_1 receptors facilitated acquisition in the water maze (DaSilva and Takahashi, 2002; Varvel and Lichtman, 2002; Varvel et al., 2007). Several reports have indicated that the effects of CB_1 agonists are not limited to acquisition in passive avoidance and delayed radial maze procedures.

In some cases, discrepant results in models of memory may be attributable to cannabinoid effects on other processes. For example, Mikics et al. (2006) reported an enhancement of fear conditioning, rather than an impairment, after administration of WIN55212-2, and tests employing genetic disruption or pharmacological blockade of CB_1 receptors indicated that this enhancement of fear conditioning was due to actions of WIN55212-2 at CB_1 receptors. Although this finding is inconsistent with the more common finding that CB_1 activation impairs memory acquisition, in this case it is possible that WIN55212-2 may have increased anxiety. It is possible that some of the effects of CB_1 agonists on water maze behavior are due to thigmotaxis, an anxiety-related tendency to maintain close proximity to the wall of the maze. When Acheson et al. (2011) controlled for thigmotaxis, the impairing effects of WIN55212-2 were no longer detectable.

Another issue to consider is that endocannabinoid receptors localized in different brain structures may modulate distinct

Table 1 | Summary of studies investigating the effects of cannabinoid receptor agonists, cannabinoid receptor antagonists, FAAH inhibitors, or genetic deletion of cannabinoid receptors on learning and memory in rodents.

Authors	Animals	Drug	Doses and route	Test	Administered before	Effects on memory
Harloe et al. (2008)	C57BL/6J	Rimonabant	3 mg/kg, IP	Appetitive Barnes maze tasks	Extinction	=
Harloe et al. (2008)	C57BL/6J	Rimonabant	3 mg/kg, IP	Aversive Barnes maze tasks	Extinction	↓
Pamplona and Takahashi (2006)	Wistar rat	AM404	10 mg/kg, IP	Contextual fear conditioning	Extinction	↑
Pamplona and Takahashi (2006)	Wistar rat	WIN55,212-2	0.25 mg/kg, IP	Contextual fear conditioning	Extinction	↑
Bitencourt et al. (2008)	Wistar rats	AM404	1.0 μg/μL, i.c.v.	Contextual fear conditioning	Extinction	↑
Suzuki et al. (2004)	C57BL/6	Rimonabant	1–3–10 mg/kg, IP	Contextual fear conditioning	Extinction	↓
Niyuhire et al. (2007)	C57BL/6J	Rimonabant	3 mg/kg, IP	Contextual fear conditioning	Extinction	↓
Pamplona and Takahashi (2006)	Wistar rat	Rimonabant	1 mg/kg, IP	Contextual fear conditioning	Extinction	↓
Ganon-Elazar and Akirav (2009)	Sprague-Dawley rats	WIN55,212-2	2.5 μg/0.5 μL, IC (basolateral amygdala)	Contextual fear conditioning after stress	Extinction	↑
Mikics et al. (2006)	CD1 mice	WIN55,212-2	3 mg/kg, IP	Contextual fear conditioning	Recall	↑
Mikics et al. (2006)	CD1 mice	AM251	3 mg/kg, IP	Contextual fear conditioning	Recall	↓
Mikics et al. (2006)	CB_1 KO	N/A	N/A	Contextual fear conditioning		↓
Pamplona and Takahashi (2006)	Wistar rats	WIN55,212-2	2.5 and, IP 5.0 mg/kg	Contextual fear conditioning	Acquisition	↓
Sink et al. (2010)	Sprague-Dawley rats	AM251	4.0 or 8.0 mg/kg, IP	Contextual fear conditioning	Acquisition	↑
Pamplona and Takahashi (2006)	Wistar rats	Rimonabant	1 mg/kg, IP	Contextual fear conditioning	Acquisition	=
Pamplona and Takahashi (2006)	Wistar rats	WIN55,212-2	2.5 and 5.0 mg/kg, IP	Cue fear conditioning	Acquisition	=
Marsicano et al. (2002)	CB_1 KO	N/A	N/A	Cue fear conditioning	Extinction	↓
Kamprath et al. (2006)	CB_1 KO	N/A	N/A	Cue fear conditioning	Extinction	↓
Wise et al. (2008)	Sprague-Dawley rats	CE	0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg, IP	Delay radial maze	Acquisition	↑
Wise et al. (2008)	Sprague-Dawley rats	CE	0.1 mg/kg, IP	Delay radial maze	Consolidation	↑
Wise et al. (2008)	Sprague-Dawley rats	CE	0.1 mg/kg, IP	Delay radial maze	Recall	=

(Continued)

Table 1 | Continued

Authors	Animals	Drug	Doses and route	Test	Administered before	Effects on memory
Nakamura et al. (1991)	Wistar rats	Δ 9-THC	1.25 mg/kg, IP	Delay radial maze	Recall	=
Lichtman (2000)	Sprague-Dawley rats	Rimonabant	3 mg/kg, IP	Delay radial maze	Acquisition	↑
Wise et al. (2007)	Sprague-Dawley rats	Rimonabant	1 mg/kg, IP	Delay radial maze	Acquisition	↑
Wise et al. (2007)	Sprague-Dawley rats	Rimonabant	1 mg/kg, IP	Delay radial maze	Consolidation	=
Wolff and Leander (2003)	Sprague-Dawley rats	Rimonabant	1 mg/kg, IP	Delay radial maze	Consolidation	↑
Lichtman (2000)	Sprague-Dawley rats	Rimonabant	3 mg/kg, IP	Delay radial maze	Recall	=
Wise et al. (2007)	Sprague-Dawley rats	Rimonabant	1 mg/kg, IP	Delay radial maze	Recall	=
Hampson and Deadwyler (2000)	Long–Evans rats	WIN55,212-2	0.25–0.75 mg/kg, IP	DNMTP	Working-memory test	↓
Deadwyler et al. (2007)	Long–Evans rats	WIN55,212-2	0,35 mg/kg, IP	DNMTP	Working-memory test	↓
Hampson and Deadwyler (2000)	Long–Evans rats	Δ 9-THC	0.5, 1.0, 1.5, and 2.0 mg/kg, IP	DNMTP	Working-memory test	↓
Heyser et al. (1993)	Sprague-Dawley rats	Cannabidiol	2 mg/kg, IP	DNMTP	Working-memory test	↓
Heyser et al. (1993)	Sprague-Dawley rats	Δ 9-THC	2 mg/kg, IP	DNMTP	Working-memory test	↓
Panlilio et al. (2011)	Sprague-Dawley and Long–Evans hooded rats	Δ 9-THC	1–5.6 mg/kg, IP	DNMTP	Working-memory test	
Mallet and Beninger (1998)	Wistar rats	Anandamide	2 mg/kg, IP	DNMTP	Working-memory test	↓
Deadwyler et al. (2007)	Long–Evans rats	Rimonabant	2 mg/kg, IP	DNMTP	Working-memory test	↑
Mallet and Beninger (1998)	Wistar rats	Rimonabant	2 mg/kg, IP	DNMTP	Working-memory test	=
Chhatwal et al. (2005)	Sprague-Dawley rats	WIN 55,212-2	5 mg/kg, IP	Fear potentiated startle response	Extinction	=
Chhatwal et al. (2005)	Sprague-Dawley rats	AM404	10 mg/kg, IP	Fear potentiated startle response	Extinction	↑

(Continued)

Table 1 | Continued

Authors	Animals	Drug	Doses and route	Test	Administered before	Effects on memory
Chhatwal et al. (2005)	Sprague-Dawley rats	Rimonabant	1.5 and 5 mg/kg, IP	Fear potentiated startle response	Extinction	↓
Niyuhire et al. (2007)	C57BL/6J	Rimonabant	3 mg/kg	Food Self administration	Extinction	=
Hölter et al. (2005)	CB_1 KO	N/A	N/A	Food Self administration	Extinction	=
Varvel et al. (2007)	C57BL/6 mice	OL-135	30 mg/kg, IP	Modified water maze	Acquisition	↑
Varvel et al. (2001)	C57BL/6 mice	Δ 9-THC	3 mg/kg, IP	Modified water maze	Recall	↓
Varvel et al. (2005b)	C57BL/6 mice	Δ 9-THC	10 mg/kg, IP	Modified water maze	Recall	↓
Varvel et al. (2007)	FAAH KO	N/A	N/A	Modified water maze		↑
Varvel et al. (2006)	FAAH KO	N/A	N/A	Modified water maze		↑
Schneider et al. (2008)	Sprague-Dawley rats	WIN55,212-2	1.2 mg/kg, IP	Object recognition test	Acquisition	↓
Ciccocioppo et al. (2002)	Wistar rats	Δ 9-THC	2 or 5 mg/kg, IP	Object recognition test	Acquisition	=
Barna et al. (2007)	Wistar rats	WIN55,212-2	Osmotic pump 0.13TBq/mmol, IC (hippocampus)	Object recognition test	Acquisition	↓
Quinn et al. (2008)	Wistar rats	Δ 9-THC	5 mg/kg, IP	Object recognition test	Acquisition	↓
Suenaga and Ichitani (2008)	Wistar-Imamichi rats	WIN55,212-2	1–2 μg/side, IC (hippocampus)	Object recognition test	Acquisition	=
Maccarrone et al. (2002)	CB_1 KO	N/A	N/A	Object recognition test		↑
Costanzi et al. (2004)	CD1 mice	Anandamide	0.3 and 0.5 mg/kg, IP	Passive avoidance	Consolidation	↓
Castellano et al. (1999)	CD1 mice	Anandamide	1.5, 3, 6 mg/kg, IP	Passive avoidance	Consolidation	↓
Nasehi et al. (2010)	NMRI mice	WIN55,212-2	0.25, 0.5, and 1 μg/mouse, IC (hippocampus)	Passive avoidance	Recall	↓
Mazzola et al. (2009)	Sprague-Dawley	URB597	0.1–0.3–1 mg/kg, IP	Passive avoidance	Acquisition	↑

(Continued)

Table 1 | Continued

Authors	Animals	Drug	Doses and route	Test	Administered before	Effects on memory
Mazzola et al. (2009)	Sprague-Dawley	WY14643	10 20 40 mg/kg, IP	Passive avoidance	Acquisition	↑
Mazzola et al. (2009)	Sprague-Dawley	URB597	0.1–0.3–1 mg/kg, IP	Passive avoidance	Consolidation	=
Mazzola et al. (2009)	Sprague-Dawley	URB597	0.1–0.3–1 mg/kg, IP	Passive avoidance	Recall	=
Mishima et al. (2001)	Wistar rats	Δ 9-THC	10 mg/kg, IP	Passive avoidance	Acquisition	↓
Murillo-Rodríguez et al. (2001)	Wistar rats	OEA	30 mg/kg	Passive avoidance	Extinction	↑
Mishima et al. (2001)	Wistar rats	Δ 9-THC	6 mg/kg, IP	Passive avoidance	Recall	↓
Campolongo et al. (2009b)	Sprague-Dawley	WIN55,212-2	50 ng, intra BLA	Passive avoidance	Recall	↑
Campolongo et al. (2009b)	Sprague-Dawley	AM251	0.28 ng, IC (basolateral amygdala)	Passive avoidance	Recall	↓
Niyuhire et al. (2007)	C57BL/6J	Rimonabant	3 mg/kg, IP	Passive avoidance	Extinction	↓
Suenaga and Ichitani (2008)	Wistar–Imamichi rats	WIN55,212-2	1–2 μg/side, IC (hippocampus)	Place recognition test	Acquisition	↓
Inui et al. (2004)	Wistar rats	Δ 9-THC	6 mg/kg, IP	Radial maze	Working-memory test	↓
Lichtman et al. (1995)	Sprague-Dawley	CP-55,940	0.13 mg/kg, IP	Radial maze	Working-memory test	↓
Lichtman et al. (1995)	Sprague-Dawley	CP-55,940	8 μg/rat, IC (hippo)	Radial maze	Working-memory test	↓
Lichtman et al. (1995)	Sprague-Dawley	WIN55,212-2	2.1 and 2.2 mg/kg, IP	Radial maze	Working-memory test	↓
Lichtman et al. (1995)	Sprague-Dawley	Δ 9-THC	2.1 and 2.2 mg/kg, IP	Radial maze	Working-memory test	↓
Lichtman and Martin (1996)	Sprague-Dawley	Δ 9-THC	3 mg/kg, IP	Radial maze	Working-memory test	↓
Wise et al. (2009b)	Sprague-Dawley rats	CP-55,940	10 μg/rat, IC (hippocampus)	Radial maze	Working-memory test	↓
Wise et al. (2009b)	Sprague-Dawley rats	Δ 9-THC	5.6 mg/kg, IP	Radial maze	Working-memory test	↓
Rubino et al. (2009)	Sprague-Dawley rats	Δ 9-THC	2.5 to 10 mg/kg in 10 days, IP	Radial maze	Working-memory test	↓

(Continued)

Table 1 | Continued

Authors	Animals	Drug	Doses and route	Test	Administered before	Effects on memory
Egashira et al. (2002)	Wistar rats	Δ 9-THC	20 μg/side, IC (hippocampus)	Radial maze	Working-memory test	↓
Egashira et al. (2008)	Wistar rats	Δ 9-THC	6 mg/kg, IP	Radial maze	Working-memory test	↓
Molina-Holgado et al. (1993)	Wistar rats	Δ 9-THC	5 mg/kg, PO	Radial maze	Working-memory test	↓
Nakamura et al. (1991)	Wistar rats	Δ 9-THC	1.25 mg/kg, IP	Radial maze	Working-memory test	↓
Rodrigues et al. (2011)	Wistar rats	Δ 9-THC	0.5 μL, IC (medial prefrontal cortex)	Radial maze	Working-memory test	↓
Mishima et al. (2001)		Δ 9-THC	4–6 mg/kg, IP	Radial maze	Working-memory test	↓
Varvel et al. (2005b)	C57BL/6 mice	Δ 9-THC	10 mg/kg	T-maze	Working-memory test	↓
Nava et al. (2001)	Sprague-Dawley	Δ 9-THC	2.5 and 5 mg/kg, IP	T-maze	Working-memory test	↓
Jentsch et al. (1998)	Sprague-Dawley	Δ 9-THC	5 mg/kg, IP	T-maze	Working-memory test	↓
Varvel et al. (2007)	C57BL/6 mice	Δ 9-THC	0.1, 0.3, 1, or 10 mg/kg, IP	Water maze	Extinction	=
Varvel et al. (2001)	C57Bl/6 mice	Δ 9-THC	3, 10, and 30 mg/kg, IP	Water maze	Recall	=
Varvel et al. (2007)	C57BL/6J	OL-135	30 mg/kg, IP	Water maze	Extinction	↑
Robinson et al. (2010)	Lister Hooded rats	WIN55,212-2	1 and 3 mg/kg, IP	Water maze	Acquisition	↓
Moore et al. (2010)	Sprague-Dawley CD rats	Δ 9-THC	10 mg/kg, IP	Water maze	Acquisition	↓
Diana et al. (2003)	Sprague-Dawley rats	Nabilone	0.1, 0.5, and 1.0 mg/kg, IP	Water maze	Acquisition	=
Acheson et al. (2011)	Sprague-Dawley rats	WIN55,212-2	1 mg/kg, IP	Water maze	Acquisition	=
Diana et al. (2003)	Sprague-Dawley rats	Δ 8-THC	5 mg/kg, IP	Water maze	Acquisition	↓
DaSilva and Takahashi (2002)	Swiss albino	Δ 9-THC	8 mg/kg, IP	Water maze	Acquisition	↓

(Continued)

Table 1 | Continued

Authors	Animals	Drug	Doses and route	Test	Administered before	Effects on memory
Ferrari et al. (1999)	Wistar Hannover rat	HU-210	50 and 100 μg/kg, IP	Water maze	Acquisition	↓
Mishima et al. (2001)	Wistar rats	Δ 9-THC	6 and 10 mg/kg, IP	Water maze	Recall	=
Varvel et al. (2007)	C57BL/6 mice	Rimonabant	3 mg/kg, IP	Water maze	Acquisition	=
Varvel et al. (2007)	C57BL/6 mice	Rimonabant	3 mg/kg, IP	Water maze	Acquisition	=
Varvel et al. (2005a)	C57BL/6J	Rimonabant	3 mg/kg, IP	Water maze	Extinction	↓
DaSilva and Takahashi (2002)	Swiss albino mice	Rimonabant	1 mg/kg, IP	Water maze	Acquisition	=
Varvel et al. (2005a)	CB_1 KO	N/A	N/A	Water maze	(Extinction)	↓
Varvel et al. (2007)	FAAH KO	N/A	N/A	Water maze	(Extinction)	↑
Varvel et al. (2006)	FAAH KO	N/A	N/A	Water maze	Working-memory test	↑
Varvel et al. (2007)	FAAH KO	N/A	N/A	Water maze		↑
Varvel et al. (2006)	FAAH KO	N/A	N/A	Water maze reversal learning		↑
Varvel and Lichtman (2002)	CB_1 KO	N/A	N/A	Water maze		=

DNMTP, delayed non-matching to position; KO, knockout; WT, wild type. For effects on memory, ↓ indicates impairment; ↑ indicates enhancement; = indicates no effect.

memory process. This may explain cases where microinfusion of cannabinoid compounds into specific areas produces effects opposite to those usually seen with systemic administration. For example, Campolongo et al. (2009b) found that micro-injections of WIN55212-2 into the basolateral amygdala enhanced memory retention and the CB_1 antagonist AM251 caused impairments in a passive-avoidance test.

WORKING MEMORY

Working memory involves the temporary storage and manipulation of information. The memory impairments induced by cannabis and Δ^9-THC in humans are most robust in tests of short-term episodic and working memory (Ranganathan and D'Souza, 2006). In animal models, the effects of cannabinoids on working memory have received much attention (see **Table 1**), and the data appear more congruent than in the models of long-term reference memory discussed above. Some of the procedures used to study working memory are adapted from procedures used to study acquisition of long-term memory.

Water maze

The basic water maze procedure can be modified to test working memory by changing the location of the platform each day and testing with only a brief delay between acquisition and a test trial. Varvel et al. (2001) have demonstrated that Δ^9-THC administered before the testing session impairs memory in a CB_1 dependent manner without affecting locomotion.

Radial maze

The findings obtained with the working-memory version of the water maze procedure agree with those obtained with the conventional version of the radial maze, which focuses on working memory. In rodents, systemic administration of Δ^9-THC or CB_1 agonists like WIN55212 or CP-55,940 increase the number of errors (Molina-Holgado et al., 1993; Lichtman et al., 1995; Lichtman and Martin, 1996; Mishima et al., 2001). Interestingly, Nakamura et al. (1991) found that Δ^9-THC (given 30 min before the task) impaired performance in the test when a short delay of 5 s was introduced between entering the fourth and fifth arms, but not when the delay was longer (1 h); this suggests a more prominent effect of Δ^9-THC on working memory than on long-term reference memory. However, under a similar task Silva de Melo et al. (2005) obtained opposite results, with systemic or intra medial prefrontal cortex administration of THC selectively impairing memory in the long-delay condition.

A series of experiments exploring the brain structure involved in cannabinoid-induced impairments of working memory in the radial maze have shown that both the hippocampus and prefrontal cortex are involved (Egashira et al., 2002; Silva de Melo et al., 2005; Suenaga et al., 2008; Rubino et al., 2009; Wise et al., 2009b; Rodrigues et al., 2011) and that CB_1 and D_{1-2} receptors play critical roles (Wise et al., 2009b; Rodrigues et al., 2011).

T-maze

T-maze procedures also provide a test of spatial working memory. There are two goal arms, and rodents obtain food by entering

the goal arm that was not entered on the previous trial. Systemic administration of Δ^9-THC (Jentsch et al., 1998; Nava et al., 2001; Varvel et al., 2005b) or intra-hippocampal administration of WIN55212 (Suenaga et al., 2008) impairs the performance of and rats, and CB_1 antagonists reverse these effects. Several lines of evidence indicate the involvement of acetylcholine systems in the effects of Δ^9-THC on working memory in task such as the T-maze and radial maze. Extracellular levels of hippocampal acetylcholine have been shown to decrease after Δ^9-THC administration (Mishima et al., 2002), and drugs that reestablish levels of this neurotransmitter can reverse the impairing effects of Δ^9-THC (Nava et al., 2000, 2001; Mishima et al., 2002; Inui et al., 2004; Wise et al., 2007; Egashira et al., 2008).

Delayed spatial matching

Extensive studies of working memory have been performed by Hampton, Deadwyler, and associates, using the delayed nonmatching to position task in rats. In this task, one of two retractable levers is extended as a sample. After the rat presses the sample lever, the lever is retracted. After a delay period, both levers are extended and the rat receives food or water if it presses the nonmatching lever (i.e., the one that was not presented as a sample; Deadwyler et al., 1996; Mallet and Beninger, 1998). Many such trials can be conducted during a daily session, with the length of the delay varied across trials. Administration of Δ^9-THC, anandamide, or WIN55212-2 before the session impairs performance (Heyser et al., 1993; Mallet and Beninger, 1998; Hampson and Deadwyler, 2000; Deadwyler et al., 2007; Goonawardena et al., 2010; Panlilio et al., 2011). This effect is associated with a drug-induced decrease in the firing rate of hippocampal pyramidal neurons during the initiation of the trial; preadministration of rimonabant (IP 1.5 mg/kg) reestablishes a normal level of hippocampal neuronal activity and blocks the memory-impairing effects of Δ^9-THC and WIN55,212-2 (Hampson and Deadwyler, 2000; Goonawardena et al., 2010). Under some conditions, the administration of a higher concentration (IP, 2 mg/mL) of rimonabant alone can enhance performance in this working-memory task (Deadwyler and Hampson, 2008). However, this result has not been reported consistently by the Deadwyler lab and was not obtained with the same dose of rimonabant in a study by Mallet and Beninger (1998). Possibly, the enhancing effect is sometimes prevented by ceiling effects and requires modifications of the procedure (e.g., longer delay periods) to be observed.

ENHANCED ANANDAMIDE SIGNALING AND PPAR-α ACTIVATION

Compounds that inhibit the activity of the fatty acid amide hydrolase enzyme (FAAH) prevent the degradation of endocannabinoid anandamide and thereby magnify and prolong anandamide's actions (Kathuria et al., 2003). FAAH inhibition also increases levels of several other fatty acids – oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) – that constitute endocannabinoid-like systems in the brain (Fegley et al., 2005). OEA and PEA do not bind to cannabinoid receptors, but are ligands for alpha-type peroxisome proliferator-activated nuclear receptors (PPAR-α). PPAR-α is well known to be involved in a number of physiological processes, but is just beginning to received attention for having cognitive and other behavioral effects (Mazzola et al., 2009; Melis et al., 2010; Mascia et al., 2011).

Surprisingly, given the preponderance of findings that cannabinoid agonists impair memory and the fact that FAAH inhibitors increase levels of the endogenous cannabinoid agonist anandamide, FAAH inhibitors have been found to enhance learning in several procedures. The FAAH inhibitor OL-135 (30 mg/kg) enhanced the acquisition rate in a working-memory version of the water maze test or in the conventional fixed-platform test in (Varvel et al., 2007); however, the same dose of OL-135 did not have such effects in an earlier study (Varvel et al., 2006). Another FAAH inhibitor, URB597 (0.1–1 mg/kg), enhanced the acquisition of passive-avoidance learning, but was not found to affect consolidation or retrieval (Mazzola et al., 2009). In genetically modified FAAH-compromised mice, acquisition was enhanced in the working-memory water maze test, but not in the conventional, fixed-platform of test (Varvel et al., 2007; Wise et al., 2009a).

Although both Varvel et al. (2007) and Mazzola et al. (2009) found that rimonabant (1 mg/kg in rats; 3 mg/kg in mice) was able to block the facilitating effects of FAAH inhibition on memory acquisition, suggesting mediation by CB_1 receptors, there is evidence that non-cannabinoid effects of FAAH inhibition also can enhance learning and memory. Mazzola et al. (2009) found that the enhancing effects of FAAH inhibition on passive-avoidance learning could be blocked not only by rimonabant but by the PPAR-α antagonist MK886. This suggests that FAAH inhibition might enhance memory by increasing the levels of the endogenous PPAR-α ligands OEA and PEA. Consistent with this hypothesis, the PPAR-α agonist WY14643 produced effects similar to those of the FAAH inhibitor URB597 on acquisition of passive-avoidance test, and this effect of WY14643 was also blocked by the PPAR-α antagonist MK886. In both a passive avoidance and a fixed-platform water maze procedure in rats, administration of exogenous OEA enhanced memory (Campolongo et al., 2009a). However, it should be noted that in the study where the FAAH inhibitor OL-135 did not enhance water maze learning (Varvel et al., 2006), OEA (50 mg/kg) and PEA (50 mg/kg) also failed to affect working memory in the water maze. In addition, while Mazzola et al. (2009) found that FAAH inhibition or administration of the PPAR-α agonist WY14643 specifically affected acquisition of passive avoidance, Campolongo et al. (2009a) found that OEA had effects when given immediately post-training, indicating an effect on consolidation.

The finding that FAAH inhibition has memory effects opposite to those of cannabinoid agonists might be at least partially explained by differences in the brain areas affected by these two kinds of treatment. As mentioned above, systemic injection of a drug such as Δ^9-THC affects CB_1 receptors throughout the brain. Systemic injection of a FAAH inhibitor selectively increasing anandamide levels in areas where it is being released. It is likely that different brain areas subserve different mnemonic processes; for example, endocannabinoid signaling in the hippocampus might be more involved in acquisition, while endocannabinoid signaling in the amygdala might be more involved in consolidation and forgetting (Riedel and Davies, 2005).

EXTINCTION AND FORGETTING

While the fact that exogenous cannabinoids impair memory has been studied in humans and animals for decades, it has only

recently been recognized that endocannabinoid systems might be involved in extinction learning. Extinction learning refers to the cessation of a learned response when the conditions that induced the learning no longer hold. For example, after the initial exposure to shock in contextual fear conditioning, the conditioned freezing response will gradually decrease if the subject is repeatedly exposed to the context but no longer shocked. This loss of the learned response might be described as forgetting, or as the establishment of new learning appropriate to the current situation.

Using fear conditioning with a discrete cue, Marsicano et al. (2002) were the first to report compromised extinction learning in CB_1-knockout mice and in wild type mice given rimonabant (3 mg/kg). Interestingly, the behavioral patterns observed in CB_1-knockout and rimonabant-treated mice were associated with decreased long-term depression of neurons in the amygdala, a structure known to play a critical role in extinction learning (Quirk and Mueller, 2008). Moreover, presentation of the shock-associated tone during extinction was followed by increased release of anandamide in the basolateral amygdala of wild type mice, suggesting the involvement of endocannabinoid neurotransmission in extinction learning (Marsicano et al., 2002). It has been proposed that endocannabinoids modulate fear-related extinction learning by regulating the activity of kinases and phosphatases in regions involved in fear and memory processing (Cannich et al., 2004).

The impairing effects of genetic and pharmacological blockade of CB_1 receptors on extinction learning, but not on the acquisition of long-term and short-term fear-related memory, has been replicated in many laboratories with rats and mice (Suzuki et al., 2004; Chhatwal et al., 2005; Kamprath et al., 2006; Niyuhire et al., 2007; Pamplona et al., 2008). Also consistent with the hypothesis that CB_1 dependent mechanisms modulate extinction learning, activation of CB_1 receptors has been shown to facilitate fear conditioning, producing effects opposite to those of CB_1 antagonists. Administration of the anandamide uptake inhibitor AM404 (IP: 10 mg/kg; 1.0 μg/μL, i.c.v.) during extinction training facilitated the extinction of startle or freezing elicited by a shock-associated context (Chhatwal et al., 2005; Bitencourt et al., 2008; Pamplona et al., 2008); this effect was CB_1 dependent, since it is blocked by a dose of rimonabant that was ineffective by itself (Bitencourt et al., 2008). Low doses (0.25 mg/kg, IP) but not a high dose (5 mg/kg) of the CB_1 agonist WIN55,212-2 impaired contextual fear conditioning under the same conditions where rimonabant enhanced it (Chhatwal et al., 2005; Pamplona and Takahashi, 2006). Moreover Ganon-Elazar and Akirav (2009) have shown that micro-injection of a low dose of WIN55,212-2 in the basolateral amygdala has no effect by itself but can reverse the disrupting effect of a stressor on extinction of passive avoidance.

The effects of cannabinoid compounds on extinction learning have also been confirmed with another aversively motivated test, the water maze. In this test, Varvel et al. (2005b, 2007) found that rimonabant (3 mg/kg) treatment or genetic CB_1 disruption impaired extinction learning, but THC did not affect extinction (Varvel et al., 2007). Surprisingly, pharmacological and genetic manipulations of CB_1 have not been found to affect extinction learning in tasks based on appetitive conditioning (Hölter et al., 2005; Niyuhire et al., 2007; Harloe et al., 2008).

It has been suggested that the effects of CB_1 antagonism in extinction procedures may depend on perseverance. For example, rimonabant-treated or CB_1-knockout mice show deficits in learning when the platform is moved to a new location in the water maze test (Varvel and Lichtman, 2002; Pamplona et al., 2006). However this view is not supported by another study in which certain doses of CB_1 agonists and antagonists facilitated or impaired, respectively, flexibility between different strategies (Hill et al., 2006). In this experiment, separate groups of rats were trained to use either a visual cue or a spatial (left vs. right) strategy to locate food in one arm of a plus-maze. Flexibility was then measured as the number of perseverative errors when the opposite strategy was required. Administration of the CB_1 antagonist AM251 (2 mg/kg) 20 min before testing reduced perseverative errors, whereas the CB_1 agonist HU-210 (20 μg/kg, IP) increased them.

The effects of FAAH inhibition on extinction learning have also been studied. FAAH null mice and mice treated with the FAAH inhibitor OL-135 show enhanced extinction learning in the water maze test (Varvel et al., 2007). Therefore FAAH inhibitors have unique effects among the endocannabinoid-related compounds, facilitating both acquisition and extinction processes. This characteristic may be due, as previously mentioned, to the ability of FAAH inhibitors to increase not only brain levels of anandamide but also of PEA and OEA. Indeed it has been shown that OEA administration (30 mg/kg) can facilitate extinction of passive avoidance in rats (Murillo-Rodríguez et al., 2001).

CONCLUSION – ENDOCANNABINOID SIGNALING AND COGNITION

Most of the evidence indicates that activating the endocannabinoid system interferes with situation-dependent working memory and the acquisition of long-term memory (see **Table 1**). Inhibiting the endocannabinoid system, on the other hand, can enhance learning and memory. Surprisingly, increasing endogenous levels of anandamide and facilitating endocannabinoid signaling with a FAAH inhibitor can enhance learning; but, this probably occurs through the endocannabinoid-related PPAR-α system and the fatty acids OEA and PEA. There is accumulating evidence that the endocannabinoid system plays a special role in extinction learning related to aversive conditioning. This role, along with its role in emotion, suggests cannabinoid-related medications might be developed for treating phobias.

EFFECTS OF ENDOCANNABINOID SYSTEM MODULATION ON EMOTIONAL BEHAVIOR

The effects of cannabinoid agonists and antagonists on emotional behavior have recently been reviewed elsewhere (Bambico et al., 2009; Moreira and Wotjak, 2010) and will only be discussed briefly here. Instead, we will focus on studies that involve genetic disruption of CB_1 receptors or genetic or pharmacological manipulation of the anandamide-degrading enzyme FAAH. These methods generally provide more direct information about endocannabinoid function because they exclude or enhance cannabinoid signaling, rather than directly stimulating cannabinoid receptors.

The animal models of anxiety that have been used with these endocannabinoid manipulations generally measure changes in rodents' tendency to avoid certain inherently aversive situations;

increased avoidance implies increased anxiety (an anxiogenic effect), and decreased avoidance indicates decreased anxiety (an anxiolytic effect). The avoided situations include brightly lit areas (light/dark test), open areas (open field test), open elevated areas (elevated plus-maze and O-maze tests), social interaction with unfamiliar conspecifics (social interaction test), and pain-associated stimuli (Vogel conflict test, shock prod burying test). In most of these tests, locomotor activity can also be monitored to assess the possibility that a drug or dose is causing non-specific sedation or motor depression, rather than affecting emotional behavior. Animal tests of depression generally model specific depression-like symptoms. For example, some tests measure changes in rodents' tendency to eventually becoming immobile when it is not possible to escape from water (forced swim test) or being suspended by the tail (tail suspension test). A decrease in the duration of immobility is considered an antidepressant-like effect and an increase in duration a depressant-like effect. Increased immobility is believed to be a sign of "behavioral despair" that putatively models the depression symptoms "loss of energy" and/or "feelings of hopelessness." A depression-like state can be induced in laboratory rodents by exposing them to mild but recurrent and unpredictable stressors (chronic mild stress model). In this model, a decrease in consumption of sucrose is believed to model anhedonia (loss of pleasure) another important symptom of depression in humans.

EFFECTS OF CB_1 RECEPTOR AGONISTS AND ANTAGONISTS ON EMOTIONAL BEHAVIOR

Cannabinoid receptor agonists decrease depression-like behaviors in a variety of species and models (Bambico et al., 2009). For example, in the forced swim test the CB_1 agonists anandamide, Δ9-THC, CP-55,940, HU-210, and WIN55,212-2 decrease immobility in the forced swim paradigm in BALB/C and CD1 mice and in Long–Evans, Sprague-Dawley, and Wistar rats, effects that are blocked by the CB_1 antagonists rimonabant and AM251. Although these findings suggest that cannabinoid receptor agonists hold promise as targets for the treatment of depression, these drugs have significant side effects (e.g., psychosis and panic) that preclude their clinical use (Moreira et al., 2009). Similarly, CB_1 antagonists also been found to have both therapeutic potential and unacceptable side effects; the antagonist rimonabant, which showed promise as a treatment for obesity, was recalled from the market because of emotional, depression-like side effects.

The effects of cannabinoid agonists are somewhat more complex in animal models of anxiety than in animal models of depression. High and low doses of cannabinoid agonists often have opposite effects (Moreira and Wotjak, 2010), with low doses inducing anxiolytic effects, while high doses induce anxiogenic effects. Both effects can be inhibited by CB_1 antagonists, although paradoxical agonist/antagonist interactions have also been reported (Haller et al., 2007).

Caveats

Discrepant findings with CB_1 receptor ligands are usually attributed to differences in dosage and treatment duration, experimental conditions, and species (Bambico et al., 2009; Moreira and Wotjak, 2010). However, these factors have rarely been studied systematically, and the reasons for discrepant findings are actually poorly understood. One possible explanation lies in the fact that CB_1 receptors are expressed on both glutamatergic and GABAergic synapses and these neurotransmitter systems often have opposite effects on emotions, especially on anxiety. We have shown that the relative cannabinoid sensitivity of GABA and glutamate neurotransmission differs between CD1 and Wistar rats and that these differences are likely responsible for the differential effects of cannabinoids on anxiety in these two species (Haller et al., 2007). Similar differences in cannabinoid function might be present in different strains of the same species, or even individual subjects. Thus, discrepant findings could be due to differences in the expression, distribution, and functional characteristics of CB_1 receptors.

GENETIC DELETION OF CB_1 RECEPTORS

The impact of genetic deletion of CB_1 in animal models of anxiety and depression was demonstrated in three studies published in 2002 (see **Table 2**). Maccarrone et al. (2002) showed that CB_1-knockout mice were more anxious than wild type in the open field and light/dark tests; however, this effect was present in young mice but not in 4-month-old mice. Martin et al. (2002) reported that deletion of the CB_1 gene induced signs of anxiety in the light/dark test and depression-like symptoms in the sucrose consumption test after chronic mild stress. Finally, Haller et al. (2002) showed that CB_1-knockout mice robustly express anxiety in the elevated plus-maze, but this kind of effect was not induced by the CB_1 antagonist rimonabant in wildtype mice. A later study by the same group found that, unlike rimonabant but like CB_1 deletion, the CB_1 antagonist AM251 did increase anxiety (Haller et al., 2004b). Subsequent studies have also replicated the depression-like phenotype of CB_1-knockout mice in the forced swim test (Fride et al., 2005), but others have not (Steiner et al., 2008a,b). Conditional mutants lacking CB_1 receptors at their cortical glutamatergic neurons showed decreased immobility in the forced swim test, suggesting an antidepressant effect of this more targeted genetic manipulation (Steiner et al., 2008b).

Like the depressant effects, the anxiogenic effects of CB_1 deletion have been replicated in a number of studies using a variety of procedures (see **Table 2**). These include the elevated plus-maze, social interaction, and light/dark tests (Urigüen et al., 2004; Mikics et al., 2009; Hill et al., 2011). In other cases, however, the effect was weak. For example, risk assessment was decreased in the elevated plus-maze, but open arm exploration (the main measure of anxiety in this test) was not affected (Jacob et al., 2009). Also, mutant mice lacking the CB_1 receptor at their glutamatergic synapses showed no changes in anxiety (Jacob et al., 2009). The effects of gene disruption were also weak in the mouse defense test battery, a less commonly used but behaviorally valid model of anxiety that measures responses to an unconditioned predator-related stimulus (Griebel et al., 2005). In one study, the anxiolytic effects of ethanol were not diminished in CB_1-knockout in the elevated plus-maze (Houchi et al., 2005). In another experiment that used the shock prod burying test, CB_1 deletion itself had anxiolytic effects (Degroot and Nomikos, 2004).

Some of the inconsistency in the effects of CB_1 on anxiety- and depression-like behavior might be due to changes in

Table 2 | Summary of studies investigating anxiety-like and depression-like behavior in knockout with cannabinoid CB_1 with deleted.

Authors	Animals	Test	Anxiety	Depression
Maccarrone et al. (2002)	CB_1 KO adolescents (CD1)	Open field (bright light)	↑	
Maccarrone et al. (2002)	CB_1 KO adults (CD1)	Open field (bright light)	=	
Maccarrone et al. (2002)	CB_1 KO adolescents (CD1)	Light–dark test	↑	
Maccarrone et al. (2002)	CB_1 KO adults (CD1)	Light–dark test		
Martin et al. (2002)	CB_1 KO (CD1)	Light–dark test	↑	
Martin et al. (2002)	CB_1 KO (CD1)	Active avoidance	↑	
Haller et al. (2002)	CB_1 KO (CD1)	EPM	↑	
Fride et al. (2005)	CB_1 KO (C57BL/6J)	Forced swim test		↑
Steiner et al. (2008a)	CB_1 KO (C57BL)	Forced swim test		=
Steiner et al. (2008b)	Glu-CB_1 KO (C57BL/6N)	Forced swim test		↓
Steiner et al. (2008b)	CaMK-CB_1 KO (C57BL/6N)	Forced swim test		=
Steiner et al. (2008b)	GABA-CB_1 KO (C57BL/6N)	Forced swim test		=
Jacob et al. (2009)	CB_1 KO (C57BL/6N)	Light–dark test (high illumination)	↑	
Jacob et al. (2009)	CB_1 KO (C57BL/6N)	EPM	↑	
Jacob et al. (2009)	Glu-CB_1 KO (C57BL/6N)	Light–dark test	=	
Jacob et al. (2009)	Glu-CB_1 KO (C57BL/6N)	EPM	=	
Griebel et al. (2005)	CB_1 KO (C57BL)	Mouse defense test battery	=	
Houchi et al. (2005)	CB_1 KO (CD1)	EPM	=	
Houchi et al. (2005)	CB_1 KO (CD1) treated with 1.5 mg/kg ethanol, IP	EPM	KO = WT	
Degroot and Nomikos (2004)	CB_1 KO (C57BL/6J)	Shock-probe burying test	↑(under some parameters)	
Haller et al. (2004a)	CB_1 KO (CD1)	EPM (high illumination)	↑	
Hill et al. (2011)	CB_1 KO (ICR)	EPM	↑	

EPM, elevated plus-maze; KO, knockout; WT, wild type. For effects in models of anxiety and depression, ↓ indicates impairment; ↑ indicates enhancement; = indicates no effect.

responsiveness to environmental stimuli. In a recent study, Jacob et al. (2009) showed that behavioral differences between wild type and CB_1-knockout mice were strongly influenced by the level of illumination under which the test was performed; in models of anxiety, such as the open field and the elevated plus-maze the behavior of CB_1-knockout differed markedly depending on light intensity. The impact of light intensity was also studied by Haller et al. (2004a), who reported that the anxiogenic effects of CB_1 gene disruption are evident when mice are tested in light, but not when they are tested in darkness. The same study suggested that the impact of CB_1 gene deletion on social behaviors depends on the level of familiarity with the testing environment; opposite effects were obtained in the home-cage and in an unfamiliar cage. Even the study where CB_1 deletion had an anxiolytic effect (Degroot and Nomikos, 2004) can be perceived as a particular case of the interaction between environmental stimuli and CB_1-knockout behavior, as the shock prod burying test examines the immediate behavioral response to electric shocks.

Taken together, these findings suggest that deletion of endogenous CB_1 signaling generally produces an anxious phenotype, but this effect is strongly dependent on environmental conditions. Intriguingly, Hill et al. (2011) recently demonstrated that the behavioral and neural changes associated with CB_1 gene disruption are very similar to those seen in chronically stressed wild type mice. This suggests that CB_1 deletion produces a chronic stress state that might contribute to altered responsiveness to environmental stimuli.

ENHANCEMENT OF ANANDAMIDE SIGNALING THROUGH INHIBITION OF FAAH

The first study demonstrating the impact of FAAH inhibitors on emotional behavior was published by Kathuria et al. (2003). They showed that the FAAH inhibitor URB597 robustly increases brain levels of anandamide but not 2-AG, and it has the anxiolytic effects of decreasing pup ultrasonic vocalizations and promoting exploration of the open section of the elevated O-maze. The authors concluded that their "results indicate that anandamide participates in the modulation of emotional states and point to fatty acid amide hydrolase inhibition as an innovative approach to anti-anxiety therapy." In a later publication, an overlapping group of authors demonstrated that URB597 decreases depression-like behaviors in both the forced swim and tail suspension models of depression, findings that "support a role for anandamide in mood regulation and point to fatty acid amide hydrolase as a previously uncharacterized target for antidepressant drugs" (Gobbi et al., 2005). Piomelli et al. (2006) concluded that URB597 does not evoke classical cannabinoid-like effects, but enhances the tonic actions of anandamide on a subset of CB_1 receptors that are normally engaged in controlling emotion and pain. As such, FAAH inhibition in general and URB597 in particular show promise as treatments for anxiety and depression.

Effects of FAAH inhibition in models of depression

These early publications on the antidepressant- and anxiolytic-like effects of FAAH inhibition were supported by a series of

Table 3 | Summary of studies investigating the effects of the FAAH inhibitor URB597 or genetic deletion of FAAH on anxiety-like and depression-like behavior in rodents.

Authors	Animals	Drug	Doses and route	Test	Anxiety	Depression
Kathuria et al. (2003)	Sprague-Dawley rats	URB597	0.1 mg/kg, IP	Elevated 0 maze	↓	
Kathuria et al. (2003)	Sprague-Dawley rats (pups)	URB597	0.1 mg/kg, IP	Isolation induced USVs	↓	
Gobbi et al. (2005)	Sprague-Dawley rats	URB597	0.1 mg/kg, IP	Tail suspension test		↓
Gobbi et al. (2005)	Sprague-Dawley rats	URB597	0.1 mg/kg, IP	Forced swim test		↓
Gobbi et al. (2005)	Sprague-Dawley rats	URB597	0.1 mg/kg, IP (repeated 4 days)	Forced swim test		↓
Adamczyk et al. (2008)	Wistar rats	URB597	0.1–0.3 mg/kg, IP	Forced swim test		↓
Bambico et al. (2010)	FAAH KO mice (C57BL6/6J)	N/A	N/A	Tail suspension test		↓
Bambico et al. (2010)	Mice, FAAH KO (C57BL6/6J)	N/A	N/A	Forced swim test		↓
Bortolato et al. (2007)	Wistar rats	URB597	0.3 mg/kg IP (repeated 5 weeks)	Sucrose consumption after chronic mild stress		↓
Hill et al. (2007)	Long–Evans rats (ovariectomized female + estradiol treatment)	URB597	0.1 mg/kg, IP	Forced swim test		↓
Realini et al. (2011)	Sprague-Dawley rats (females + 10 days THC treatment)	URB597	0.3 mg/kg IP (repeated 30 days)	Forced swim test		↓
Realini et al. (2011)	Sprague-Dawley rats (females + 10 days THC treatment)	URB597	0.3 mg/kg, IP (repeated 30 days)	Sucrose consumption		↓
Wright et al. (2010)	Sprague-Dawley rats (DFP treated)	URB597	3 mg/kg, IP	Forced swim test	=	
McLaughlin et al. (2007)	Sprague-Dawley rats	URB597	0.5 and 1 μg (hippo campus)	Forced swim test	=	
Manna and Jain (2011)	Swiss mice	URB597	0.05–10 μg/mouse, ICV	Forced swim test		↓
Moise et al. (2008)	Syrian hamsters	URB597	0.1–0.3 mg/kg, IP	EPM	↓	
Moise et al. (2008)	Syrian hamsters	URB597	0.3–3 mg/kg, IP	Conditioned and unconditioned social defeat test	=	
Moreira et al. (2008)	C57BL/6N mice	URB597	10 mg/kg, IP	EPM	↓	
Patel and Hillard (2006)	ICR mice	URB597	0.1–0.3 mg/kg, IP	EPM	↓	
Lisboa et al. (2008)	Wistar rats	URB597	0.01 nmol, IC (dorsal periaqueductal gray)	Vogel conflict test	↓	
Rubino et al. (2008)	Sprague-Dawley rats	URB597	0.1 μg/rat	EPM	↓	
Scherma et al. (2008)	Sprague-Dawley rats	URB597	0.1–0.3 mg/kg, IP	Light–dark test	↓	
Naderi et al. (2008)	NMRI mice	AM404	1–2 mg/kg, IP	EPM	↓	
Naderi et al. (2008)	NMRI mice	URB597	0.03–0.3 mg/kg, IP	EPM	=	
Micale et al. (2009)	C57BL/6J mice	URB597	1 mg/kg, IP	EPM	↓	
Micale et al. (2009)	C57BL/6J mice	URB597	0.1–0.5 mg/kg, IP	EPM	=	
Naidu et al. (2007)	C57BL/6J-ICR mice	URB597	0.3–1–3 mg/kg, IP	EPM	=	
Naidu et al. (2007)	C57BL/6J-ICR mice	URB597	10 mg/kg, IP	EPM	=	
Naidu et al. (2007)	C57BL/6J-ICR mice	URB597	0.1 mg/kg, IP	Modified EPM	↓	
Naidu et al. (2007)	FAAH KO mice (C57BL/6J)	N/A	N/A	EPM	=	
Naidu et al. (2007)	FAAH KO mice (C57BL/6J)	N/A	N/A	Tail suspension test		=
Naidu et al. (2007)	C57BL/6J mice	URB597	0.1–10 mg/kg, IP	Tail suspension test		=

(Continued)

Table 3 | Continued

Authors	Animals	Drug	Doses and route	Test	Anxiety	Depression
Naidu et al. (2007)	FAAH KO mice (C57BL/6J)	N/A	N/A	Modified tail suspension test		↓
Naidu et al. (2007)	C57BL/6J mice	URB597	0.1 mg/kg, IP	Modified tail suspension test		↓
Seillier and Giuffrida (2011)	Wistar rats	URB597	0.1, 0.3, 1 mg/kg, IP	EPM	=	
Haller et al. (2009)	Sprague-Dawley rats	URB597	0.1–0.3 mg/kg, IP	EPM (low aversiveness)	=	
Haller et al. (2009)	Sprague-Dawley rats	URB597	0.1–0.3 mg/kg, IP	EPM (high aversiveness)	↓	

EPM, elevated plus-maze. For effects in models of anxiety and depression, ↓ indicates impairment; ↑ indicates enhancement; = indicates no effect.

subsequent findings (see **Table 3**). In models of depression, systemic, and i.c.v. treatments with URB597, as well as genetic deletion of FAAH, decreased immobility in the forced swim, and tail suspension tests (Adamczyk et al., 2008; Bambico et al., 2010; Manna and Jain, 2011; Umathe et al., 2011), while systemic URB597 administration counteracted the deleterious effects of chronic mild stress (Bortolato et al., 2007), abolished estrogen deficiency-induced depression in female rats (Hill et al., 2007), and reversed depression-like symptoms induced by THC in adolescent female rats (Realini et al., 2011). In the forced swim test, URB597 reversed depression-like effects in rats 29 days (but not 8 days) after exposure to diisopropylfluorophosphate (Wright et al., 2010). The CB_1 dependence of these effects was verified in most of the cited studies, confirming that they were due to FAAH-induced enhancement of anandamide signaling at CB_1 receptors. The role of anandamide in these antidepressant effects is further supported by the finding that the anandamide-transport inhibitor AM404 exerted similar effects in some studies (Adamczyk et al., 2008; Umathe et al., 2011).

However, conflicting findings also exist. URB597 had no effect when infused into the dentate gyrus of the hippocampus, despite the fact that the direct CB_1 agonist HU-210 administered in the same way produced antidepressant effects in the forced swim test (McLaughlin et al., 2007). This finding suggests that depression-like behavior is affected by anandamide-independent cannabinoid mechanisms in certain cases and in certain brain areas. Naidu et al. (2007) found that the FAAH inhibitors URB597 and OL-135 only affected depression-like behavior in the forced swim and tail suspension tests when the tests were performed under modified lighting conditions and when large sample sizes were used.

Effects of FAAH inhibition in models of anxiety

URB597 decreased anxiety in the elevated plus-maze when given systemically (Patel and Hillard, 2006; Moise et al., 2008; Moreira et al., 2008) or when injected into the medial prefrontal cortex or dorsolateral periaqueductal gray, two regions that play important roles in the control of anxiety (Lisboa et al., 2008; Rubino et al., 2008).URB597 also abolished the anxiogenic response measured in the elevated plus-maze during withdrawal after an acute administration of alcohol (Cippitelli et al., 2008). Anxiolytic effects of URB597 were also shown in the Vogel conflict test (injected into dorsolateral periaqueductal gray; Lisboa et al., 2008) and light–dark test (injected systemically; Scherma et al., 2008). Like FAAH inhibition, anandamide-transport inhibition decreased anxiety (Lisboa et al., 2008; Naderi et al., 2008), suggesting that the enhancement of endogenous anandamide release decreases anxiety irrespective of the method by which it was achieved. Mice with FAAH genetically deleted showed reduced emotionality in both the social interaction test and the open field test, and these differences were abolished by treatment with rimonabant (Cassano et al., 2011).

However, there are also a number of conflicting findings regarding the effects of FAAH inhibition on anxiety (see **Table 3**). Some of these contradictions can be considered negligible. For example, acute or chronic treatment with URB597 doses that were very effective at producing anxiolytic effects in other studies (0.1, and 0.5 mg/kg) did not affect anxiety in the elevated plus-maze in the study by Micale et al. (2009), but a higher dose (1 mg/kg) did. In another study, URB597 had no effect on anxiety in the mouse defense test battery, but had an anxiolytic effect in a more conventional model, the elevated plus-maze (Moise et al., 2008). To a certain extent, the findings by Scherma et al. (2008) are also at variance with the assumption that enhanced anandamide signaling decreases anxiety. Although these authors did show an anxiolytic effect with URB597, co-administration of anandamide reversed this effect. This finding might be explained by the fact that FAAH inhibition selectively affects areas where endogenous anandamide is being released, while exogenous administration of anandamide (the effects of which are prolonged by FAAH inhibition) would affect cannabinoid receptors throughout the brain.

Harder to explain are the findings of Naderi et al. (2008), Naidu et al. (2007), and Seillier and Giuffrida (2011), who failed to detect any anxiolytic effect of URB597 in the elevated plus-maze (i.e., the test in which FAAH inhibition was first found to be anxiolytic). Haller et al. (2009) reported that URB597 did not decrease anxiety when the elevated plus-maze test was performed under mildly aversive conditions (e.g., in a familiar room or under low light). In contrast, the benzodiazepine anxiolytic chlordiazepoxide decreased anxiety under all conditions. In the case of genetic deletion of FAAH, mutant mice showed evidence of decreased anxiety relative to wild type mice under both bright and dim lighting conditions in the social interaction and open field tests; but, when the mutant mice received rimonabant under dim lighting conditions in the open field test (i.e., under less aversive conditions), their behavior suggested hypersensitivity to anxiogenic effects of CB_1 blockade (Cassano et al., 2011). After carefully reviewing published

methodological details and personally interviewing the authors of earlier publications, Haller et al. (2009) suggested that success or failure in detecting anxiolytic effects with URB597 was largely explained by the degree of aversiveness of the testing environment in particular studies. Since various testing conditions can differentially model specific forms of anxiety, these findings suggest that FAAH inhibition (and its functional equivalent, anandamide-transport inhibition) might blunt the anxiogenic effects of stressful environmental stimuli rather than producing an overall reduction in anxiety.

CONCLUSION – ENDOCANNABINOID SIGNALING AND EMOTIONAL BEHAVIOR

Cannabinoid signaling appears to decrease depression-like and anxiety-like behaviors in laboratory models. These effects were observed using a variety of means to affect cannabinoid signaling, a variety of animal models, and a variety of species. The reasons for discrepancies are multiple, but an increasing number of publications suggest that the emotional effects of enhanced endocannabinoid signaling largely depend on environmental influences. These findings suggest that the anxiolytic effects, and possibly the antidepressant effects, of endocannabinoid signaling are enhanced under aversive conditions, which strengthens, rather than weakens, the putative usefulness of medications that enhance endocannabinoid signaling in the treatment of emotional disorders.

CONTEXT DEPENDENCE OF ENDOCANNABINOID MODULATION OF COGNITIVE AND EMOTIONAL BEHAVIOR

BRAIN FUNCTIONS AND ASSUMPTIONS FOR BEHAVIOR

Uniquely, endocannabinoids signal "backward": they are released from the post-synaptic membrane and inhibit the synaptic neurotransmission that triggered their release (Wilson and Nicoll, 2001). Although a certain, probably low, level of tonic activation cannot be excluded, the endocannabinoid signal occurs phasically on demand, i.e., when the intensity of anterograde synaptic communication reaches certain levels (Di Marzo et al., 1999; Marsicano et al., 2003; Lutz, 2004; Adermark and Lovinger, 2007). As such, the main role of endocannabinoid signaling appears to be the blockade of excessive neuronal activation. The CB_1 receptor is strongly expressed in limbic structures (Herkenham et al., 1991), suggesting that cannabinoid signaling has a particularly important role in the control of neuronal responses induced by environmental challenges that often involve an emotional dimension. As brain anandamide levels are strongly increased by aversive stimuli (Walker et al., 1999; Kirkham et al., 2002; Marsicano et al., 2002; Hohmann et al., 2005), one can hypothesize that the activity dependent release of endocannabinoids serves as a feedback mechanism that reduces the amplitude of challenge-induced neuronal excitations (Gerdeman and Lovinger, 2001; Adermark and Lovinger, 2007; Straiker and Mackie, 2009). This mechanism may be one that explains the strong impact of environmental conditions on the behavioral consequences of FAAH inhibition. Particularly, enhanced dampening of aversion-induced neuronal activations may lessen the behavioral impact of aversive stimuli.

In most cases the cognitive and emotional consequences of FAAH inhibition have been demonstrated to be CB_1-mediated. The broad effects of anandamide signaling may offer an alternative explanation for the impact of environmental conditions on the behavioral consequences of FAAH inhibition. CB_1 receptors occur on GABAergic and glutamatergic synapses, and activation of these receptors can inhibit the release of several neurotransmitters, including glycine, acetylcholine, norepinephrine, dopamine, serotonin, and cholecystokinin (Gifford and Ashby, 1996; Ishac et al., 1996; Cadogan et al., 1997; Katona et al., 1999, 2001; Nakazi et al., 2000; Beinfeld and Connolly, 2001; Hájos and Freund, 2002; Fernández-Ruiz et al., 2010). Thus, endocannabinoids affect the function of many neurotransmitter systems, some of which play opposing roles. For example, glutamatergic mechanisms appear to promote anxiety while GABAergic mechanisms appear to inhibit it (Millan, 2003). This diversity of cannabinoid roles and the complexity of task-dependent activation of neuronal circuits may inherently lead to the effects of endocannabinoid activation being strongly dependent on environmental conditions.

Presumably, each environmental challenge and behavioral response is bound to the activation of particular neuronal circuits. The effects of cannabinoid signaling probably depend on the ratio, brain location, and neurochemical nature of those neurons that express cannabinoid receptors *and* are activated in the particular situation. A small change in the environment might recruit new neurons in the situation-dependent circuit, changing the share, location, and neurochemical nature of the cannabinoid-controlled synapses that were activated. Thus, each effect of cannabinoids would be specific to the situation.

The hypothesis presented here has two parts: that cannabinoid signaling has an important role in dampening excessive neuronal responses induced by environmental challenges that often involve an emotional dimension, and that the function of endocannabinoid neuronal circuits is situation-dependent. Endocannabinoid signaling is activated when there is a relatively high level of synaptic activity, as would be triggered by environmental challenges that require prompt behavioral responses. Retrograde signaling by cannabinoids would affect only those neurons that: (1) are highly activated by the perception or interpretation of the challenging information and by the behavioral response; and (2) also express CB_1 receptors on their axon terminals. These conditions are likely to be met by neurons that have opposing roles overall (e.g., glutamatergic and GABAergic neurons) or have wide ranging behavioral effects (e.g., monoaminergic neurotransmission). As a result, cannabinoids selectively affect a mosaic of widely heterogeneous neurons that may have convergent, divergent, or independent effects on the development of the behavioral response, and leave many neurons unaffected, or affected only indirectly. Interfering with such a complex regulatory process naturally leads to complex and situation-dependent effects. Under such conditions, the relative consistency of available findings may be due to the fact that scientific studies are highly standardized. Even small deviations from experimental protocols (e.g., directing the light on the tail of rats in the tail suspension test; Naidu et al., 2007) may bring about surprising findings. More surprising findings can be expected after more dramatic changes in experimental conditions, for example by varying the aversiveness of environmental conditions (Haller et al., 2009).

One possible argument against this hypothesis is that anandamide may not be directly involved in CB_1-mediated retrograde endocannabinoid signaling, because the post-synaptic localization of its synthesizing enzymes is at variance with the pre-synaptic localization of the CB_1 receptor (Katona and Freund, 2008). One has to note, however, that cannabinoids were shown to affect extra-synaptic (volumetric) neurotransmission (Lau and Schloss, 2008; Morgese et al., 2009), and endocannabinoids, especially anandamide, are able to exert effects *via* the putative CB_3 (non-CB_1/non-CB_2) cannabinoid receptor (De Petrocellis and Di Marzo, 2010). One also has to note that discrepancies between functional and morphological findings may be fairly common in the case of cannabinoid signaling (see e.g., Kawamura et al., 2006).

CONCLUSION AND PRACTICAL IMPLICATIONS

Conflicting findings are not rare in behavioral pharmacology. Yet, the enhancement or blockade of endocannabinoid signaling has provided inconsistent findings even within the same laboratory; moreover, deliberate changes in environmental conditions have resulted in marked changes in the effects of the same manipulations within the same series of experiments. Taken together, the findings reviewed here raise the possibility that endocannabinoid signaling may change the impact of environmental influences on behavior rather than affecting one or another specific behavior. This assumption may be especially valid for emotional behaviors, but it may indirectly affect findings obtained in tests where emotions are not the focus, such as learning and memory. Further research in this respect appears warranted.

From a practical point of view, the assumption formulated above may not necessarily invalidate cannabinoid neurotransmission as a pharmaceutical target. Altered responses to environmental stimuli are at the core of emotional disorders, and also appertain to disorders related to learning and memory. Thus, the ability of cannabinoid-related treatments to modulate the impact of challenging environmental conditions on emotional and cognitive behavior could be a productive focus for medications development.

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REFERENCES

Acheson, S. K., Moore, N. L. T., Kuhn, C. M., Wilson, W. A., and Swartzwelder, H. S. (2011). The synthetic cannabinoid WIN 55212-2 differentially modulates thigmotaxis but not spatial learning in adolescent and adult animals. *Neurosci. Lett.* 487, 411–414.

Adamczyk, P., Golda, A., McCreary, A. C., Filip, M., and Przegalinski, E. (2008). Activation of endocannabinoid transmission induces antidepressant-like effects in rats. *J. Physiol. Pharmacol.* 59, 217–228.

Adermark, L., and Lovinger, D. M. (2007). Retrograde endocannabinoid signaling at striatal synapses requires a regulated postsynaptic release step. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20564–20569.

Ainge, J. A., Heron-Maxwell, C., Theofilas, P., Wright, P., de Hoz, L., and Wood, E. R. (2006). The role of the hippocampus in object recognition in rats: examination of the influence of task parameters and lesion size. *Behav. Brain Res.* 167, 183–195.

Bambico, F. R., Cassano, T., Dominguez-Lopez, S., Katz, N., Walker, C. D., Piomelli, D., and Gobbi, G. (2010). Genetic deletion of fatty acid amide hydrolase alters emotional behavior and serotonergic transmission in the dorsal raphe, prefrontal cortex, and hippocampus. *Neuropsychopharmacology* 35, 2083–2100.

Bambico, F. R., Duranti, A., Tontini, A., Tarzia, G., and Gobbi, G. (2009). Endocannabinoids in the treatment of mood disorders: evidence from animal models. *Curr. Pharm. Des.* 15, 1623–1646.

Barna, I., Soproni, K., Arszovszki, A., Csabai, K., and Haller, J. (2007). WIN-55,212-2 chronically implanted into the CA3 region of the dorsal hippocampus impairs learning: a novel method for studying chronic, brain-area-specific effects of cannabinoids. *Behav. Pharmacol.* 18, 515–520.

Beinfeld, M. C., and Connolly, K. (2001). Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids. *Neurosci. Lett.* 301, 69–71.

Bitencourt, R. M., Pamplona, F. A., and Takahashi, R. N. (2008). Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *Eur. Neuropsychopharmacol.* 18, 849–859.

Bortolato, M., Mangieri, R. A., Fu, J., Kim, J. H., Arguello, O., Duranti, A., Tontini, A., Mor, M., Tarzia, G., and 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.

Cadogan, A. K., Alexander, S. P., Boyd, E. A., and Kendall, D. A. (1997). Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. *J. Neurochem.* 69, 1131–1137.

Campolongo, P., Roozendaal, B., Trezza, V., Cuomo, V., Astarita, G., Fu, J., McGaugh, J. L., and Piomelli, D. (2009a). Fat-induced satiety factor oleoylethanolamide enhances memory consolidation. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8027–8031.

Campolongo, P., Roozendaal, B., Trezza, V., Hauer, D., Schelling, G., McGaugh, J. L., and Cuomo, V. (2009b). Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4888–4893.

Cannich, A., Wotjak, C. T., Kamprath, K., Hermann, H., Lutz, B., and Marsicano, G. (2004). CB1 cannabinoid receptors modulate kinase and phosphatase activity during extinction of conditioned fear in mice. *Learn. Mem.* 11, 625–632.

Cassano, T., Gaetani, S., Macheda, T., Laconca, L., Romano, A., Morgese, M. G., Cimmino, C. S., Chiarotti, F., Bambico, F. R., Gobbi, G., Cuomo, V., and Piomelli, D. (2011). Evaluation of the emotional phenotype and serotonergic neurotransmission of fatty acid amide hydrolase-deficient mice. *Psychopharmacology (Berl.)* 214, 465–476.

Castellano, C., Cabib, S., Palmisano, A., Di Marzo, V., and Puglisi-Allegra, S. (1997). The effects of anandamide on memory consolidation in involve both D1 and D2 dopamine receptors. *Behav. Pharmacol.* 8, 707–712.

Castellano, C., Ventura, R., Cabib, S., and Puglisi-Allegra, S. (1999). Strain-dependent effects of anandamide on memory consolidation in are antagonized by naltrexone. *Behav. Pharmacol.* 10, 453–457.

Chhatwal, J. P., Davis, M., Maguschak, K. A., and Ressler, K. J. (2005). Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology* 30, 516–524.

Ciccocioppo, R., Antonelli, L., Biondini, M., Perfumi, M., Pompei, P., and Massi, M. (2002). Memory impairment following combined exposure to delta-tetrahydrocannabinol and ethanol in rats. *Eur. J. Pharmacol.* 449, 245–252.

Cippitelli, A., Cannella, N., Braconi, S., Duranti, A., Tontini, A., Bilbao, A., Defonseca, F. R., Piomelli, D., and Ciccocioppo, R. (2008). Increase of brain endocannabinoid anandamide levels by FAAH inhibition and alcohol abuse behaviours in the rat. *Psychopharmacology (Berl.)* 198, 449–460.

Costanzi, M., Battaglia, M., Rossi-Arnaud, C., Cestari, V., and Castellano, C. (2004). Effects of anandamide and morphine combinations on memory consolidation in cd1 mice: involvement of dopaminergic mechanisms. *Neurobiol. Learn. Mem.* 81, 144–149.

DaSilva, G. E., and Takahashi, R. N. (2002). SR 141716A prevents delta 9-tetrahydrocannabinol-induced spatial learning deficit in a Morris-type water maze in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 26, 321–325.

De Petrocellis, L., and Di Marzo, V. (2010). Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: focus on G-protein-coupled receptors and transient receptor potential channels. *J. Neuroimmune Pharmacol.* 5, 103–121.

Deadwyler, S. A., Bunn, T., and Hampson, R. E. (1996). Hippocampal ensemble activity during spatial delayed-nonmatch-to-sample performance in rats. *J. Neurosci.*16, 354–372.

Deadwyler, S. A., Goonawardena, A. V., and Hampson, R. E. (2007). Short-term memory is modulated by the spontaneous release of endocannabinoids: evidence from hippocampal population codes. *Behav. Pharmacol.* 18, 571–580.

Deadwyler, S. A., and Hampson, R. E. (2008). Endocannabinoids modulate encoding of sequential memory in the rat hippocampus. *Psychopharmacology (Berl.)* 198, 577–586.

Degroot, A., and Nomikos, G. G. (2004). Genetic deletion and pharmacological blockade of CB1 receptors modulates anxiety in the shock-probe burying test. *Eur. J. Neurosci.* 20, 1059–1064.

Di Marzo, V., Breivogel, C. S., Tao, Q., Bridgen, D. T., Razdan, R. K., Zimmer, A. M., Zimmer, A., and Martin, B. R. (2000). Levels, metabolism, and pharmacological activity of anandamide in CB1 cannabinoid receptor knockout mice: evidence for non-CB1, non-CB receptor-mediated actions of anandamide in mouse brain. *J. Neurochem.* 75, 2434–2444.

Di Marzo, V., De Petrocellis, L., Bisogno, T., and Melck, D. (1999). Metabolism of anandamide and 2-arachidonoylglycerol: an historical overview and some recent developments. *Lipids* 34(Suppl.), S319–S325.

Diana, G., Malloni, M., and Pieri, M. (2003). Effects of the synthetic cannabinoid nabilone on spatial learning and hippocampal neurotransmission. *Pharmacol. Biochem. Behav.* 75, 585–591.

Egashira, N., Ishigami, N., Mishima, K., Iwasaki, K., Oishi, R., and Fujiwara, M. (2008). Delta9-tetrahydrocannabinol-induced cognitive deficits are reversed by olanzapine but not haloperidol in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32, 499–506.

Egashira, N., Mishima, K., Iwasaki, K., and Fujiwara, M. (2002). Intracerebral microinjections of delta 9-tetrahydrocannabinol: search for the impairment of spatial memory in the eight-arm radial maze in rats. *Brain Res.* 952, 239–245.

Fegley, D., Gaetani, S., Duranti, A., Tontini, A., Mor, M., Tarzia, G., and Piomelli, D. (2005). Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleoylethanolamide deactivation. *J. Pharmacol. Exp. Ther.* 313, 352–358.

Fernández-Ruiz, J., Hernández, M., and Ramos, J. A. (2010). Cannabinoid-dopamine interaction in the pathophysiology and treatment of CNS disorders. *CNS Neurosci. Ther.* 16, e72–e91.

Ferrari, F., Ottani, A., Vivoli, R., and Giuliani, D. (1999). Learning impairment produced in rats by the cannabinoid agonist HU 210 in a water-maze task. *Pharmacol. Biochem. Behav.* 64, 555–561.

Fride, E., Suris, R., Weidenfeld, J., and Mechoulam, R. (2005). Differential response to acute and repeated stress in cannabinoid CB1 receptor knockout newborn and adult mice. *Behav. Pharmacol.* 16, 431–440.

Ganon-Elazar, E., and Akirav, I. (2009). Cannabinoid receptor activation in the basolateral amygdala blocks the effects of stress on the conditioning and extinction of inhibitory avoidance. *J. Neurosci.* 29, 11078–11088.

Gerdeman, G., and Lovinger, D. M. (2001). CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J. Neurophysiol.* 85, 468–471.

Gifford, A. N., and Ashby, C. R. Jr. (1996). Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. *J. Pharmacol. Exp. Ther.* 277, 1431–1436.

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

Goonawardena, A. V., Robinson, L., Hampson, R. E., and Riedel, G. (2010). Cannabinoid and cholinergic systems interact during performance of a short-term memory task in the rat. *Learn. Mem.* 28, 502–211.

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

Hájos, N., and Freund, T. F. (2002). Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. *Neuropharmacology* 43, 503–510.

Haller, J., Bakos, N., Szirmay, M., Ledent, C., and Freund, T. F. (2002). The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur. J. Neurosci.* 16, 1395–1398.

Haller, J., Barna, I., Barsvari, B., Gyimesi Pelczer, K., Yasar, S., Panlilio, L. V., and Goldberg, S. (2009). Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats. *Psychopharmacology (Berl.)* 204, 607–616.

Haller, J., Mátyás, F., Soproni, K., Varga, B., Barsy, B., Németh, B., Mikics, E., Freund, T. F., and Hájos, N. (2007). Correlated species differences in the effects of cannabinoid ligands on anxiety and on GABAergic and glutamatergic synaptic transmission. *Eur. J. Neurosci.* 25, 2445–2456.

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

Haller, J., Varga, B., Ledent, C., and Freund, T. F. (2004b). CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. *Behav. Pharmacol.* 15, 299–304.

Hampson, R. E., and Deadwyler, S. A. (2000). Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *J. Neurosci.* 20, 8932–8942.

Harloe, J. P., Thorpe, A. J., and Lichtman, A. H. (2008). Differential endocannabinoid regulation of extinction in appetitive and aversive Barnes maze tasks. *Learn. Mem.* 15, 806–809.

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., and Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J. Neurosci.* 11, 563–583.

Heuer, E., and Bachevalier, J. (2011). Effects of selective neonatal hippocampal lesions on tests of object and spatial recognition memory in monkeys. *Behav. Neurosci.* 125, 137–149.

Heyser, C. J., Hampson, R. E., and Deadwyler, S. A. (1993). Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. *J. Pharmacol. Exp. Ther.* 264, 294–307.

Hill, M. N., Froese, L. M., Morrish, A. C., Sun, J. C., and Floresco, S. B. (2006). Alterations in behavioral flexibility by cannabinoid CB1 receptor agonists and antagonists. *Psychopharmacology (Berl.)* 187, 245–259.

Hill, M. N., Hillard, C. J., and McEwen, B. S. (2011). Alterations in corticolimbic dendritic morphology and emotional behavior in cannabinoid CB1 receptor-deficient parallel the effects of chronic stress. *Cereb. Cortex* 21, 2056–2064.

Hill, M. N., Karacabeyli, E. S., and Gorzalka, B. B. (2007). Estrogen recruits the endocannabinoid system to modulate emotionality. *Psychoneuroendocrinology* 32, 350–357.

Hohmann, A. G., Suplita, R. L., Bolton, N. M., Neely, M. H., Fegley, D., Mangieri, R., Krey, J. F., Walker, J. M., Holmes, P. V., Crystal, J. D., Duranti, A., Tontini, A., Mor, M., Tarzia, G., and Piomelli, D. (2005). An endocannabinoid mechanism for stress-induced analgesia. *Nature* 435, 1108–1112.

Hölter, S. M., Kallnik, M., Wurst, W., Marsicano, G., Lutz, B., and Wotjak, C. T. (2005). Cannabinoid CB1 receptor is dispensable for memory extinction in an appetitively-motivated learning task. *Eur. J. Pharmacol.* 510, 69–74.

Houchi, H., Babovic, D., Pierrefiche, O., Ledent, C., Daoust, M., and Naassila, M. (2005). CB1 receptor knockout display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. *Neuropsychopharmacology* 30, 339–349.

Inui, K., Egashira, N., Mishima, K., Yano, A., Matsumoto, Y., Hasebe, N., Abe, K., Hayakawa, K., Ikeda, T., Iwasaki, K., and Fujiwara, M. (2004). The serotonin1A receptor agonist 8-OHDPAT reverses delta 9-tetrahydrocannabinol-induced impairment of spatial memory and reduction of acetylcholine release in the dorsal hippocampus in rats. *Neurotox. Res.* 6, 153–158.

Isaacson, R. L., and Wickelgren, W. O. (1962). Hippocampal ablation and passive avoidance. *Science* 138, 1104–1106.

Ishac, E. J., Jiang, L., Lake, K. D., Varga, K., Abood, M. E., and Kunos, G. (1996). Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. *Br. J. Pharmacol.* 118, 2023–2028.

Jacob, W., Yassouridis, A., Marsicano, G., Monory, K., Lutz, B., and Wotjak, C. T. (2009). Endocannabinoids render exploratory behaviour largely independent of the test aversiveness: role of glutamatergic transmission. *Genes Brain Behav.* 8, 685–698.

Jentsch, J. D., Verrico, C. D., Le, D., and Roth, R. H. (1998). Repeated exposure to delta-9-tetrahydrocannabinol reduces prefrontal cortical dopamine metabolism in the rat. *Neurosci. Lett.* 246, 169–172.

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

Kathuria, S., Gaetani, S., Fegley, D., Valiño, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V., and Piomelli, D. (2003). Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* 9, 76–81.

Katona, I., and Freund, T. F. (2008). Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat. Med.* 14, 923–930.

Katona, I., Rancz, E. A., Acsady, L., Ledent, C., Mackie, K., Hajos, N., and Freund, T. F. (2001). Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.* 21, 9506–9518.

Katona, I., Sperlágh, B., Sík, A., Käfalvi, A., Vizi, E. S., Mackie, K., and Freund, T. F. (1999). Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* 19, 4544–4558.

Kawamura, Y., Fukaya, M., Maejima, T., Yoshida, T., Miura, E., Watanabe, M., Ohno-Shosaku, T., and Kano, M. (2006). The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J. Neurosci.* 26, 2991–3001.

Kirkham, T. C., Williams, C. M., Fezza, F., and Di Marzo, V. (2002). Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br. J. Pharmacol.* 136, 550–557.

Lau, T., and Schloss, P. (2008). The cannabinoid CB1 receptor is expressed on serotonergic and dopaminergic neurons. *Eur. J. Pharmacol.* 578, 137–141.

Lichtman, A. H. (2000). SR 141716A enhances spatial memory as assessed in a radial-arm maze task in rats. *Eur. J. Pharmacol.* 404, 175–179.

Lichtman, A. H., Dimen, K. R., and Martin, B. R. (1995). Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology (Berl.)* 119, 282–290.

Lichtman, A. H., and Martin, B. R. (1996). Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. *Psychopharmacology (Berl.)* 126, 125–131.

Lisboa, S. F., Resstel, L. B. M., Aguiar, D. C., and Guimarães, F. S. (2008). Activation of cannabinoid CB1 receptors in the dorsolateral periaqueductal gray induces anxiolytic effects in rats submitted to the Vogel conflict test. *Eur. J. Pharmacol.* 593, 73–78.

Long, J. Z., Li, W., Booker, L., Burston, J. J., Kinsey, S. G., Schlosburg, J. E., Pavón, F. J., Serrano, A. M., Selley, D. E., Parsons, L. H., Lichtman, A. H., and Cravatt, B. F. (2009). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat. Chem. Biol.* 5, 37–44.

Lutz, B. (2004). On-demand activation of the endocannabinoid system in the control of neuronal excitability and epileptiform seizures. *Biochem. Pharmacol.* 68, 1691–1698.

Maccarrone, M., Valverde, O., Barbaccia, M. L., Castañé, A., Maldonado, R., Ledent, C., Parmentier, M., and Finazzi-Agrò, A. (2002). Age-related changes of anandamide metabolism in CB1 cannabinoid receptor knockout mice: correlation with behaviour. *Eur. J. Neurosci.* 15, 1178–1186.

Mallet, P. E., and Beninger, R. J. (1998). The cannabinoid CB1 receptor antagonist SR141716A attenuates the memory impairment produced by delta9-tetrahydrocannabinol or anandamide. *Psychopharmacology (Berl.)* 140, 11–19.

Manna, S. S. S., and Jain, N. S. (2011). Involvement of endocannabinoids in antidepressant and anti-compulsive effect of fluoxetine in mice. *Behav. Brain Res.* 223, 125–134.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S. C., Cascio, M. G., Gutiérrez, S. O., van der Stelt, M., López-Rodriguez, M. L., Casanova, E., Schütz, G., Zieglgänsberger, W., Di Marzo, V., Behl, C., and Lutz, B. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302, 84–88.

Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., Hermann, H., Tang, J., Hofmann, C., Zieglgänsberger, W., Di Marzo, V., and Lutz, B. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418, 530–534.

Martin, M., Ledent, C., Parmentier, M., Maldonado, R., and Valverde, O. (2002). Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl.)* 159, 379–387.

Mascia, P., Pistis, M., Justinova, Z., Panlilio, L. V., Luchicchi, A., Lecca, S., Scherma, M., Fratta, W., Fadda, P., Barnes, C., Redhi, G. H., Yasar, S., Le Foll, B., Tanda, G., Piomelli, D., and Goldberg, S. R. (2011). Blockade of nicotine reward and reinstatement by activation of alpha-type peroxisome proliferator-activated receptors. *Biol. Psychiatry* 69, 633–641.

Mazzola, C., Medalie, J., Scherma, M., Panlilio, L. V., Solinas, M., Tanda, G., Drago, F., Cadet, J. L., Goldberg, S. R., and Yasar, S. (2009). Fatty acid amide hydrolase (FAAH) inhibition enhances memory acquisition through activation of PPAR-alpha nuclear receptors. *Learn. Mem.* 16, 332–337.

McLaughlin, R. J., Hill, M. N., Morrish, A. C., and Gorzalka, B. B. (2007). Local enhancement of cannabinoid CB1 receptor signalling in the dorsal hippocampus elicits an antidepressant-like effect. *Behav. Pharmacol.* 18, 431–438.

Melis, M., Carta, S., Fattore, L., Tolu, S., Yasar, S., Goldberg, S. R., Fratta, W., Maskos, U., and Pistis, M. (2010). Peroxisome proliferator-activated receptors-alpha modulate dopamine cell activity through nicotinic receptors. *Biol. Psychiatry* 68, 256–264.

Micale, V., Cristino, L., Tamburella, A., Petrosino, S., Leggio, G. M., Drago, F., and Di Marzo, V. (2009). Anxiolytic effects in of a dual blocker of fatty acid amide hydrolase and transient receptor potential vanilloid type-1 channels. *Neuropsychopharmacology* 34, 593–606.

Mikics, E., Dombi, T., Barsvári, B., Varga, B., Ledent, C., Freund, T. F., and Haller, J. (2006). The effects of cannabinoids on contextual conditioned fear in CB1 knockout and CD1 mice. *Behav. Pharmacol.* 17, 223–230.

Mikics, E., Vas, J., Aliczki, M., Halasz, J., and Haller, J. (2009). Interactions between the anxiogenic effects of CB1 gene disruption and 5-HT3 neurotransmission. *Behav. Pharmacol.* 20, 265–272.

Millan, M. J. (2003). The neurobiology and control of anxious states. *Prog. Neurobiol.* 70, 83–244.

Mishima, K., Egashira, N., Hirosawa, N., Fujii, M., Matsumoto, Y., Iwasaki, K., and Fujiwara, M. (2001). Characteristics of learning and memory impairment induced by delta9-tetrahydrocannabinol in rats. *Jpn. J. Pharmacol.* 87, 297–308.

Mishima, K., Egashira, N., Matsumoto, Y., Iwasaki, K., and Fujiwara, M. (2002). Involvement of reduced acetylcholine release in delta9-tetrahydrocannabinol-induced impairment of spatial memory in the 8-arm radial maze. *Life Sci.* 72, 397–407.

Moise, A. M., Eisenstein, S. A., Astarita, G., Piomelli, D., and Hohmann, A. G. (2008). An endocannabinoid signaling system modulates anxiety-like behavior in male Syrian hamsters. *Psychopharmacology (Berl.)* 200, 333–346.

Molina-Holgado, F., Molina-Holgado, E., Leret, M. L., González, M. I., and Reader, T. A. (1993). Distribution of indoleamines and [3H]paroxetine binding in rat brain regions following acute or perinatal delta 9-tetrahydrocannabinol treatments. *Neurochem. Res.* 18, 1183–1191.

Moore, N. L. T., Greenleaf, A. L. R., Acheson, S. K., Wilson, W. A., Swartzwelder, H. S., and Kuhn, C. M. (2010). Role of cannabinoid receptor type 1 desensitization in greater tetrahydrocannabinol impairment of memory in adolescent rats. *J. Pharmacol. Exp. Ther.* 335, 294–301.

Mor, M., Rivara, S., Lodola, A., Plazzi, P. V., Tarzia, G., Duranti, A., Tontini, A., Piersanti, G., Kathuria, S., and

Piomelli, D. (2004). Cyclohexylcarbamic acid 3′- or 4′-substituted biphenyl-3-yl esters as fatty acid amide hydrolase inhibitors: synthesis, quantitative structure-activity relationships, and molecular modeling studies. *J. Med. Chem.* 47, 4998–5008.

Moreira, F. A., Grieb, M., and Lutz, B. (2009). Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. *Best Prac. Res. Clin. Endocrinol. Metab.* 23, 133–144.

Moreira, F. A., Kaiser, N., Monory, K., and 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.

Moreira, F. A., and Wotjak, C. T. (2010). Cannabinoids and anxiety. *Curr. Top. Behav. Neurosci.* 2, 429–450.

Morgese, M. G., Cassano, T., Gaetani, S., Macheda, T., Laconca, L., Dipasquale, P., Ferraro, L., Antonelli, T., Cuomo, V., and Giuffrida, A. (2009). Neurochemical changes in the striatum of dyskinetic rats after administration of the cannabinoid agonist WIN55,212-2. *Neurochem. Int.* 54, 56–64.

Murillo-Rodríguez, E., Giordano, M., Cabeza, R., Henriksen, S. J., Méndez Díaz, M., Navarro, L., and Prospéro-García, O. (2001). Oleamide modulates memory in rats. *Neurosci. Lett.* 313, 61–64.

Naderi, N., Haghparast, A., Saber-Tehrani, A., Rezaii, N., Alizadeh, A.-M., Khani, A., and Motamedi, F. (2008). Interaction between cannabinoid compounds and diazepam on anxiety-like behaviour of mice. *Pharmacol. Biochem. Behav.* 89, 64–75.

Naidu, P. S., Varvel, S. A., Ahn, K., Cravatt, B. F., Martin, B. R., and Lichtman, A. H. (2007). Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. *Psychopharmacology (Berl.)* 192, 61–70.

Nakamura, E. M., da Silva, E. A., Concilio, G. V., Wilkinson, D. A., and Masur, J. (1991). Reversible effects of acute and long-term administration of delta-9-tetrahydrocannabinol (THC) on memory in the rat. *Drug Alcohol Depend.* 28, 167–175.

Nakazi, M., Bauer, U., Nickel, T., Kathmann, M., and Schlicker, E. (2000). Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB1 receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* 361, 19–24.

Nasehi, M., Piri, M., Jamali-Raeufy, N., and Zarrindast, M. R. (2010). Influence of intracerebral administration of NO agents in dorsal hippocampus (CA1) on cannabinoid state-dependent memory in the step-down passive avoidance test. *Physiol. Behav.* 100, 297–304.

Nava, F., Carta, G., Battasi, A. M., and Gessa, G. L. (2000). D dopamine receptors enable delta-tetrahydrocannabinol induced memory impairment and reduction of hippocampal extracellular acetylcholine concentration. *Br. J. Pharmacol.* 130, 1201–1210.

Nava, F., Carta, G., Colombo, G., and Gessa, G. L. (2001). Effects of chronic delta-tetrahydrocannabinol treatment on hippocampal extracellular acetylcholine concentration and alternation performance in the T-maze. *Neuropharmacology* 41, 392–399.

Niyuhire, F., Varvel, S. A., Thorpe, A. J., Stokes, R. J., Wiley, J. L., and Lichtman, A. H. (2007). The disruptive effects of the CB1 receptor antagonist rimonabant on extinction learning in are task-specific. *Psychopharmacology (Berl.)* 191, 223–231.

Pamplona, F. A., Bitencourt, R. M., and Takahashi, R. N. (2008). Short- and long-term effects of cannabinoids on the extinction of contextual fear memory in rats. *Neurobiol. Learn. Mem.* 90, 290–293.

Pamplona, F. A., Prediger, R. D., Pandolfo, P., and Takahashi R. N. (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.

Pamplona, F. A., and Takahashi, R. N. (2006). WIN 55212-2 impairs contextual fear conditioning through the activation of CB1 cannabinoid receptors. *Neurosci. Lett.* 397, 88–92.

Panlilio, L. V., Yasar, S., Thorndike, E. B., Goldberg, S. R., and Schindler, C. W. (2011). Automatic recording of mediating behavior in delayed matching- and nonmatching-to-position procedures in rats. *Psychopharmacology (Berl.)* 214, 495–504.

Patel, S., and Hillard, C. J. (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.

Phillips, R. G., and LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* 106, 274–285.

Piomelli, D., Tarzia, G., Duranti, A., Tontini, A., Mor, M., Compton, T. R., Dasse, O., Monaghan, E. P., Parrott, J. A., and Putman, D. (2006). Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). *CNS Drug Rev.* 12, 21–38.

Quinn, H. R., Matsumoto, I., Callaghan, P. D., Long, L. E., Arnold, J. C., Gunasekaran, N., Thompson, M. R., Dawson, B., Mallet, P. E., Kashem, M. A., Matsuda-Matsumoto, H., Iwazaki, T., and McGregor, I. S. (2008). Adolescent rats find repeated delta-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology* 33, 1113–1126.

Quirk, G. J., and Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology* 33, 56–72.

Ranganathan, M., and D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl.)* 188, 425–444.

Realini, N., Vigano, D., Guidali, C., Zamberletti, E., Rubino, T., and Parolaro, D. (2011). Chronic URB597 treatment at adulthood reverted most depressive-like symptoms induced by adolescent exposure to THC in female rats. *Neuropharmacology* 60, 235–243.

Riedel, G., and Davies, S. N. (2005). Cannabinoid function in learning, memory and plasticity. *Handb. Exp. Pharmacol.* 168, 445–477.

Robinson, L., Goonawardena, A. V., Pertwee, R., Hampson, R. E., Platt, B., and Riedel, G. (2010). WIN55,212-2 induced deficits in spatial learning are mediated by cholinergic hypofunction. *Behav. Brain Res.* 208, 584–592.

Rodrigues, L. C., de, M., Conti, C. L., and Nakamura-Palacios, E. M. (2011). Clozapine and SCH 23390 prevent the spatial working memory disruption induced by (Δ-THC administration into the medial prefrontal cortex. *Brain Res.* 1382, 230–237.

Rubino, T., Realini, N., Braida, D., Guidi, S., Capurro, V., Viganò, D., Guidali, C., Pinter, M., Sala, M., Bartesaghi, R., and Parolaro, D. (2009). Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus* 19, 763–772.

Rubino, T., Realini, N., Castiglioni, C., Guidali, C., Viganó, D., Marras, E., Petrosino, S., Perletti, G., Maccarrone, M., Di Marzo, V., and Parolaro, D. (2008). Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. *Cereb. Cortex* 18, 1292–1301.

Scherma, M., Medalie, J., Fratta, W., Vadivel, S. K., Makriyannis, A., Piomelli, D., Mikics, E., Haller, J., Yasar, S., Tanda, G., and Goldberg, S. R. (2008). The endogenous cannabinoid anandamide has effects on motivation and anxiety that are revealed by fatty acid amide hydrolase (FAAH) inhibition. *Neuropharmacology* 54, 129–140.

Schneider, M., and Koch, M. (2002). The cannabinoid agonist WIN 55,212-2 reduces sensorimotor gating and recognition memory in rats. *Behav. Pharmacol.* 13, 29–37.

Schneider, M., Schömig, E., and Leweke, F. M. (2008). PRECLINICAL STUDY: acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addict. Biol.* 13, 345–357.

Seillier, A., and Giuffrida, A. (2011). Inhibition of fatty acid amide hydrolase modulates anxiety-like behavior in PCP-treated rats. *Pharmacol. Biochem. Behav.* 98, 583–586.

Silva de Melo, L. C., Cruz, A. P., Rios Valentim, S. J. Jr., Marinho, A. R., Mendonça, J. B., and Nakamura-Palacios, E. M. (2005). Delta-THC administered into the medial prefrontal cortex disrupts the spatial working memory. *Psychopharmacology (Berl.)* 183, 54–64.

Sink, K. S., Segovia, K. N., Collins, L. E., Markus, E. J., Vemuri, V. K., Makriyannis, A., and Salamone, J. D. (2010). The CB1 inverse agonist AM251, but not the CB1 antagonist AM4113, enhances retention of contextual fear conditioning in rats. *Pharmacol. Biochem. Behav.* 95, 479–484.

Steiner, M. A., Marsicano, G., Nestler, E. J., Holsboer, F., Lutz, B., and Wotjak, C. T. (2008a). Antidepressant-like behavioral effects of impaired cannabinoid receptor type 1 signaling coincide with exaggerated corticosterone secretion in mice. *Psychoneuroendocrinology* 33, 54–67.

Steiner, M. A., Marsicano, G., Wotjak, C. T., and Lutz, B. (2008b). Conditional cannabinoid receptor type 1 mutants reveal neuron subpopulation-specific effects on behavioral and neuroendocrine stress responses. *Psychoneuroendocrinology* 33, 1165–1170.

Straiker, A., and Mackie, K. (2009). Cannabinoid signaling in inhibitory autaptic hippocampal neurons. *Neuroscience* 163, 190–201.

Suenaga, T., and Ichitani, Y. (2008). Effects of hippocampal administration of a cannabinoid receptor agonist WIN 55,212-2 on spontaneous object and place recognition in rats. *Behav. Brain Res.* 190, 248–252.

Suenaga, T., Kaku, M., and Ichitani, Y. (2008). Effects of intrahippocampal cannabinoid receptor agonist and antagonist on radial maze and T-maze delayed alternation performance in rats. *Pharmacol. Biochem. Behav.* 91, 91–96.

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

Tart, C. T. (1970). Marijuana intoxication common experiences. *Nature* 226, 701–704.

Tarzia, G., Duranti, A., Tontini, A., Piersanti, G., Mor, M., Rivara, S., Plazzi, P. V., Park, C., Kathuria, S., and Piomelli, D. (2003). Design, synthesis, and structure-activity relationships of alkylcarbamic acid aryl esters, a new class of fatty acid amide hydrolase inhibitors. *J. Med. Chem.* 46, 2352–2360.

Terranova, J. P., Storme, J. J., Lafon, N., Pério, A., Rinaldi-Carmona, M., Le Fur, G., Soubrié, P., and Umathe, S. N. (1996). Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716. *Psychopharmacology (Berl.)* 126, 165–172.

Umathe, S. N., Manna, S. S., and Jain, N. S. (2011). Involvement of endocannabinoids in antidepressant and anti-compulsive effect of fluoxetine in mice. *Behav. Brain Res.* 223, 125–134.

Urigüen, L., Pérez-Rial, S., Ledent, C., Palomo, T., and Manzanares, J. (2004). Impaired action of anxiolytic drugs in deficient in cannabinoid CB1 receptors. *Neuropharmacology* 46, 966–973.

Varvel, S. A., Anum, E. A., and Lichtman, A. H. (2005a). Disruption of CB1 receptor signaling impairs extinction of spatial memory in mice. *Psychopharmacology (Berl.)* 179, 863–872.

Varvel, S. A., Anum, E., Niyuhire, F., Wise, L. E., and Lichtman, A. H. (2005b). Delta-THC-induced cognitive deficits in are reversed by the GABA(A) antagonist bicuculline. *Psychopharmacology (Berl.)* 178, 317–327.

Varvel, S. A., Cravatt, B. F., Engram, A. E., and Lichtman, A. H. (2006). Fatty acid amide hydrolase (-/-) exhibit an increased sensitivity to the disruptive effects of anandamide or oleamide in a working memory water maze task. *J. Pharmacol. Exp. Ther.* 317, 251–257.

Varvel, S. A., Hamm, R. J., Martin, B. R., and Lichtman, A. H. (2001). Differential effects of delta 9-THC on spatial reference and working memory in mice. *Psychopharmacology (Berl.)* 157, 142–150.

Varvel, S. A., and Lichtman, A. H. (2002). Evaluation of CB1 receptor knockout in the Morris water maze. *J. Pharmacol. Exp. Ther.* 301, 915–924.

Varvel, S. A., Wise, L. E., and Lichtman, A. H. (2009). Are CB1 receptor antagonists nootropic or cognitive impairing agents? *Drug Dev. Res.* 70, 555–565.

Varvel, S. A., Wise, L. E., Niyuhire, F., Cravatt, B. F., and Lichtman, A. H. (2007). Inhibition of fatty-acid amide hydrolase accelerates acquisition and extinction rates in a spatial memory task. *Neuropsychopharmacology* 32, 1032–1041.

Walker, J. M., Huang, S. M., Strangman, N. M., Tsou, K., and Sañudo-Peña, M. C. (1999). Pain modulation by release of the endogenous cannabinoid anandamide. *Proc. Natl. Acad. Sci. U.S.A.* 96, 12198–12203.

Wilson, R. I., and Nicoll, R. A. (2001). Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 410, 588–592.

Wise, L. E., Harloe, J. P., and Lichtman, A. H. (2009a). Fatty acid amide hydrolase (FAAH) knockout exhibit enhanced acquisition of an aversive, but not of an appetitive, Barnes maze task. *Neurobiol. Learn. Mem.* 92, 597–601.

Wise, L. E., Thorpe, A. J., and Lichtman, A. H. (2009b). Hippocampal CB1 receptors mediate the memory impairing effects of delta-tetrahydrocannabinol. *Neuropsychopharmacology* 34,2072–2080.

Wise, L. E., Iredale, P. A., and Lichtman, A. H. (2008). The cannabinoid CB1 receptor antagonist CE prolongs spatial memory duration in a rat delayed radial arm maze memory task. *Eur. J. Pharmacol.* 590, 246–249.

Wise, L. E., Iredale, P. A., Stokes, R. J., and Lichtman, A. H. (2007). Combination of rimonabant and donepezil prolongs spatial memory duration. *Neuropsychopharmacology* 32, 1805–1812.

Wolff, M. C., and Leander, J. D. (2003). SR141716A, a cannabinoid CB1 receptor antagonist, improves memory in a delayed radial maze task. *Eur. J. Pharmacol.* 477, 213–217.

Wright, L. K. M., Liu, J., Nallapaneni, A., and Pope, C. N. (2010). Behavioral sequelae following acute diisopropylfluorophosphate intoxication in rats: comparative effects of atropine and cannabinomimetics. *Neurotoxicol. Teratol.* 32, 329–335.

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Sexually dimorphic effects of cannabinoid compounds on emotion and cognition

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This review addresses the issue of sex differences in the response to cannabinoid compounds focusing mainly on behaviors belonging to the cognitive and emotional sphere. Sexual dimorphism exists in the different components of the endocannabinoid system. Males seem to have higher CB1 receptor binding sites than females, but females seem to possess more efficient CB1 receptors. Differences between sexes have been also observed in the metabolic processing of THC, the main psychoactive ingredient of marijuana. The consistent dimorphism in the endocannabinoid system and THC metabolism may justify at least in part the different sensitivity observed between male and female animals in different behavioral paradigms concerning emotion and cognition after treatment with cannabinoid compounds. On the basis of these observations, we would like to emphasize the need of including females in basic research and to analyze results for sex differences in epidemiological studies.

Keywords: cannabinoid, sex differences, emotionality, cognition

Although the notion of sex differences in brain functionality was already present at the end of the nineteenth century (Andreano and Cahill, 2009), it is only in the last decade that increasing literature has supported and documented it. In fact for long, females were under-represented or even excluded in both clinical and preclinical studies. Indeed, until recently, the prevalent strategy in animal studies was to use males only, ironically to avoid likely sex effects.

The most characterized brain regions where functional and structural dimorphism have been studied are the hippocampus, amygdala, hypothalamus, and cortex, cerebral areas associated with cognition and emotion. Besides the anatomy, also the neurochemistry and physiology could differ in these areas between males and females. For example, dopamine, serotonin, and GABA, among others, have been shown to exhibit significant sex differences in their metabolism (Andreano and Cahill, 2009), as well as various neuropeptidergic systems (Bielsky et al., 2005; Kauffman, 2010). It is not surprising then that compounds acting in these areas and through mechanisms involving these neurotransmitters could trigger different responses in males and females.

This review addresses the issue of sex influences on the endocannabinoid system both in term of differences in the components of the system between sexes and differences in the response to cannabinoid compounds, focusing on behaviors belonging to the cognitive and emotional sphere.

SEX DIFFERENCES IN THE ENDOCANNABINOID SYSTEM

Very few data are available regarding sex differences in cannabinoid CB1 receptor density and coupling to G proteins, and fewer ones are available on the endocannabinoid levels.

Despite this limitation, a rather clear picture arises for CB1 receptor: in all the papers where CB1 receptor levels were measured in both male and female animals, a higher density was observed in males in almost all the cerebral regions analyzed (Rubino et al., 2008; Burston et al., 2010; Mateos et al., 2010; Riebe et al., 2010). The increase in CB1 receptor density was observed in both adolescent and adult animals, however it was stronger and wider in younger rats. For example in the adult amygdala, CB1 receptor binding site density was higher in females than males, a difference that appears to be dependent upon the presence of estradiol, since in ovariectomized female rats it was no longer seen (Riebe et al., 2010). Despite the lower receptor density, however, adolescent females showed the higher G protein activation after CB1 receptor stimulation in several brain areas (Rubino et al., 2008; Burston et al., 2010), thus suggesting the presence of more efficient receptors. At adulthood, higher CB1 receptor/G protein coupling was still present in the prefrontal cortex of female rats (Burston et al., 2010; Mateos et al., 2010), whereas it was no longer evident in the amygdala (Mateos et al., 2010), hypothalamus, periaqueductal gray, ventral midbrain, and cerebellum (Burston et al., 2010). Contrasting data have been reported for the hippocampus: Burston et al. (2010) described higher CP-55,940-stimulated G protein activation in male rats whereas Mateos et al. (2010), reported it in females. Different hypotheses can be put forward to explain this discrepancy: first of all, different rat strains have been used, Long Evans vs. Wistar rats. A different approach was employed to assess CB1 receptor/G protein coupling, namely autoradiographic analysis on brain sections in Mateos' study and binding studies on membrane samples from brain tissue in the Burston's one. Most importantly, in the study by Mateos et al. (2010), rats underwent intense behavioral analysis before the biochemical studies whereas in that of Burston they didn't.

Sex differences in CB1 receptor density were also reported in humans, again with men showing higher binding levels in

early adulthood (age 18–45; Van Laere et al., 2008). Sex differences were still evident later in life (age 45–70), but while men maintained or lost some CB1 binding sites, women increased them throughout the brain, thus presenting higher CB1 receptor levels at this specific interval of age (Van Laere et al., 2008).

Only one paper dealt with endocannabinoid levels in adult male and female animals (Bradshaw et al., 2006). Among the seven different brain areas analyzed, the authors found no significant differences in anandamide levels between male and female rats, whereas 2-arachidonoylglycerol (2-AG) was higher in the female pituitary gland and hypothalamus, but lower in the cerebellum. When the different phases of the estrous cycle were taken into account the picture became more complex, with fluctuation of the endocannabinoid levels among them and therefore much more diversity between male and female rats. In neonatal rats, females had lower amounts of the endocannabinoids 2-AG and anandamide in the amygdala and, accordingly, higher content of the endocannabinoid degradation enzymes, fatty acid amid hydrolase and monoacylglycerol lipase than males in this cerebral area (Krebs-Kraft et al., 2010).

SEX DIFFERENCES IN THE PHARMACOKINETICS OF CANNABINOID COMPOUNDS

Animal studies have shown sex differences in the metabolic processing of delta 9-tetrahydrocannabinol (THC). For example THC was oxidized selectively to 11-OH-delta 9-THC by liver microsomes of female rats, a form that retains the potency of THC, while in male rats, besides 11-OH-delta 9-THC, it was biotransformed to at least three different less active metabolites (Narimatsu et al., 1991). Accordingly, after intraperitoneal injections of THC, levels of its metabolites in brain tissue, including 11-OH-delta 9-THC, the major active metabolite, were higher in females than in males (Tseng et al., 2004). Moreover cannabinoids are lipophilic and are sequestered in fat tissue. Adult male rats have a greater percentage of body fat than adult females and therefore their fat cells may retain more THC allowing a smaller amount to reach the brain.

SEX DIFFERENCES IN THE RESPONSE TO CANNABINOID COMPOUNDS

In view of this consistent dimorphism in the endocannabinoid system and THC metabolism, it is not surprising that cannabinoid compounds, and particularly THC, might have different effects when administered in male or female animals. Despite this obvious observation, very few studies have taken into account this possibility, performing the same experiments in both males and females. Curiously enough, most of them regarded the long-term effects of adolescent exposure to cannabinoids with particular emphasis on cognition and emotionality.

When the object recognition test was used, adolescent exposure to increasing doses of the synthetic cannabinoid agonist CP-55,940 for 21 days (post-natal days 30–50) induced impaired working memory checked following a long drug-free period in both female (O'Shea et al., 2004) and male rats (O'Shea et al., 2006). However, the same treatment at adulthood led to long-term memory impairments in male but not female rats (O'Shea et al., 2004, 2006). In contrast, when the spatial memory was assessed through the Morris water maze, THC significantly disrupted learning in the adolescent males and females and also in adult females, whereas it did not affect learning in adult males (Cha et al., 2007). However chronic THC during either adolescence or adulthood had no effect on spatial learning in animals of both sexes tested after a long drug-free period (Cha et al., 2007). Accordingly, Higuera-Matas et al. (2009) reported that also the cannabinoid agonist CP-55,940 administered during adolescence did not affect adult performance of animals of both sexes in the water maze. In our work, both male and female rats showed spatial working memory deficits tested in the radial maze long after adolescent exposure to THC (Rubino et al., 2009a,b).

As a whole, this behavioral picture seems to suggest that whenever the exposure to cannabinoid agonists occurs during adolescence, it disrupts cognitive behaviors in both sexes if animals were tested immediately after, whilst the presence of long-term effects might depend upon the specific type of memory assessed and the sex of the animals.

Besides the behavioral picture, also the molecular underpinnings of the cognitive impairments induced by cannabinoids might present sexual dimorphism. For example we showed that THC, although inducing the same behavioral deficit in the radial maze in both male and female rats, triggered a different cellular alteration at the level of brain circuitries (Rubino et al., 2009a,b). In adult female rats exposed to THC in adolescence the spatial working memory impairment was correlated to a significant decrease in synaptophysin and PSD95 proteins in the prefrontal cortex. Moreover, proteomic analysis of the synaptosomes from this brain area, demonstrated the presence of less active synapses characterized by reduced ability in maintaining normal synaptic efficiency (Rubino et al., 2009a), thus suggesting the occurrence of altered synaptic plasticity throughout the prefrontal cortex in THC-pre-exposed female rats. In adult male rats chronically treated with THC during adolescence, the spatial working memory deficit was instead related to a significant decrease in the astroglial marker GFAP as well as in pre- and post-synaptic protein expression (VAMP2, PSD95) and NMDA receptor levels in the hippocampus. These animals also exhibited lower total dendritic length and number as well as reduced spine density in the hippocampal dentate gyrus (Rubino et al., 2009b), suggesting that male THC pre-treated rats may establish less synaptic contacts and/or less efficient synaptic connections throughout the hippocampus.

These data support the notion that males and females may use differing neural paths to reach the same behavioral end point (see for review Andreano and Cahill, 2009) and that the same THC exposure may have different neuronal consequences in the brain of male or female rats.

At the human level, besides the well-known notion that acute cannabis intoxication has been associated with transient and reversible decrements in attention, memory, and executive functions (see for review Solowij and Pesa, 2010), no evidence exists about sexual dimorphism in this dimension. Females are still too under-represented in epidemiological studies to gain a picture of different cognitive effects after THC exposure in men and women.

Moreover, although few papers addressed this issue, they seem to support the notion that adolescent female rats appear to be more sensitive to the long-lasting effects triggered by chronic cannabinoid consumption on emotional responses than males; on the contrary, at adulthood, no sex differences are evident, or even males appear to be the more affected in the emotional domain. In fact, chronic CP-55,940 in adolescence impaired social interaction in both male and female rats, however when the same treatment was performed in adult animals, only males were affected (O'Shea et al., 2004, 2006). Moreover, when HU210 was chronically administered in adult male and female rats, a significant antidepressant response was observed in both sexes (Morrish et al., 2009). In the hole board test, which measures the propensity for novelty and uncertainty, adolescent CP-55,940 treatment increased general motor activity and inspective exploration in female rats, whereas decreased explorative behavior without affecting general motor activity in males (Biscaia et al., 2003). Finally we observed that adolescent exposure to THC triggered the development of a complex depressive-like phenotype at adulthood only in female rats, male rats not presenting both behavioral and biochemical parameters of depression (Rubino et al., 2008). Among the biochemical parameters, the transcription factor CREB seems to be involved in both the mechanism of action of antidepressants as well as the disease itself (Blendy, 2006). Accordingly, adolescent THC significantly reduced pCREB in the prefrontal cortex and hippocampus of female rats but not in males. Conversely, elevated CREB activity in the NAc produces various depressive-like effects in rodents (see for review Carlezon et al., 2005), and THC significantly increased it in the nucleus accumbens of female rats. Again, male rats showed no changes in pCREB levels in this cerebral region (Rubino et al., 2008).

As a whole these data seem to suggest that the adolescent female brain is more vulnerable to the adverse effects of chronic cannabinoid administration on emotional behavior than the adult brain. In support of this, when the same chronic THC treatment performed in adolescent female rats was administered in adult females, it did not induce long-lasting impairment in the emotional domain (Realini et al., 2011). The reason for this vulnerability is still unknown, however, possible sex steroid-dependent differences in the sensitivity of certain neuronal processes to cannabinoid treatment could be put forward. Accordingly, it was reported the existence of fluctuations along the ovarian cycle and sex steroid replacement in CB1 receptor density and affinity in certain brain areas (Rodríguez de Fonseca et al., 1994; Riebe et al., 2010), suggesting that estradiol may affect it. Estradiol elicits anxiolytic and antidepressant effects when injected in female rats (Fink et al., 1998; Bodo and Rissman, 2006; Walf and Frye, 2009; Romano-Torres and Fernández-Guasti, 2010). Estradiol-induced changes in emotionality are sensitive to the blockade of CB1 receptors, thus suggesting that alterations in endocannabinoid activity may contribute to estradiol's ability to modulate mood and affect (Hill et al., 2007). Therefore it could be speculated that the disruption of the endocannabinoid system homeostasis by exogenous administration of cannabinoid compounds in adolescence, a period where hormonal changes leading to sexual maturation occur, might impact emotionality in developing females.

As already observed for cognitive studies, no clear evidence exists about sexual dimorphism in emotional responses to cannabinoids at human levels. However in some epidemiological studies, although not clear stated, a gender difference might be found. For example, in a study where withdrawal symptoms after cessation of cannabis use was assessed, the symptoms formed two factors, one characterized by weakness, hypersomnia, and psychomotor retardation, and the second by anxiety, restlessness, depression, and insomnia (Hasin et al., 2008). When the authors examined the relationship of demographic characteristics to cannabis withdrawal symptoms in the full sample of frequent cannabis users, gender was associated with both the weakness symptoms and the anxiety/depression symptoms. Moreover, in an Indigenous Arnhem Land community sample, a strong association between heavy cannabis use in young people and moderate–severe depressive symptoms was found, and the rates of depression were nearly a third of females and one in six males reporting moderate–severe symptoms (Lee et al., 2008).

On the other hand, clinical data seem to indicate that the endocannabinoid system may be disturbed in affective disease, especially in females (Hill et al., 2008). Serum 2-AG content was significantly decreased in female patients diagnosed with major depression, and this decrease was correlated significantly and negatively with duration of the depressive episode (Hill et al., 2008).

Together these observations suggest the potential utility of targeting the endocannabinoid system for the treatment of affective disorders in females.

Finally, in addition to cognitive and emotional ones, other cannabinoid effects have been also shown to be sexually dimorphic. Cannabinoids are more potent and in some cases more efficacious in females than males in producing antinociception and altering movement (Craft, 2005). In Long–Evans and Lister Hooded rats, females showed a significant faster acquisition of WIN 55212-2 self-administration and maintained higher levels of responding than males, suggesting that cannabinoids might be more reinforcing for females than males (Fattore et al., 2007). Ovarian hormones might be involved in the modulation of the reinforcing effect of cannabinoids, in fact, when compared to intact females, a lower percentage of ovariectomized females acquired and maintained stable drug intake (Fattore et al., 2007).

CONCLUSION

The data here reported clearly suggest the presence of sex differences in behavioral and neurochemical responses to cannabinoid compounds. The involvement of sex steroid hormones in most of the sex differences in cannabinoid-induced behavioral effects has been already put forward and appears to be the more likely explanation (González et al., 2000; Viveros et al., 2010).

Intriguingly, a very recent work even suggested the involvement of cannabinoid signaling in the establishment of normal sex differences in the brain (Krebs-Kraft et al., 2010). The authors demonstrated that early exposure to cannabinoids masculinizes social play in females without altering this behavior in males. The likely cellular mechanism for this sexual differentiation of

the developing brain and behavior might be the regulation of cell proliferation and cell type in the developing amygdala.

On the basis of these observations, we would like to emphasize the need of including females in basic research and to analyze results for sex differences in epidemiological studies. Moreover, when acute cannabinoid effects are taken into account it would be very useful also to discriminate among the different female hormonal status. As a whole these data will help to better understand the therapeutic possibilities of the endocannabinoid system and to better exploit them, perhaps in a sex-dependent manner.

REFERENCES

Andreano, J. M., and Cahill, L. (2009). Sex influences on the neurobiology of learning and memory. *Learn. Mem.* 16, 248–266.

Bielsky, I. F., Hu, S. B., and Young, L. J. (2005). Sexual dimorphism in the vasopressin system: lack of an altered behavioral phenotype in female V1a receptor knockout mice. *Behav. Brain Res.* 164, 132–136.

Biscaia, M., Marín, S., Fernández, B., Marco, E. M., Rubio, M., Guaza, C., Ambrosio, E., and Viveros, M. P. (2003). Chronic treatment with CP 55,940 during the peri-adolescent period differentially affects the behavioural responses of male and female rats in adulthood. *Psychopharmacology (Berl.)* 170, 301–308.

Blendy, J. A. (2006). The role of CREB in depression and antidepressant treatment. *Biol. Psychiatry* 59, 1144–1150.

Bodo, C., and Rissman, E. F. (2006). New roles for estrogen receptor beta in behavior and neuroendocrinology. *Front. Neuroendocrinol.* 27, 217–232.

Bradshaw, H. B., Rimmerman, N., Krey, J. F., and Walker, J. M. (2006). Sex and hormonal cycle differences in rat brain levels of pain-related cannabimimetic lipid mediators. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 291, R349–R358.

Burston, J. J., Wiley, J. L., Craig, A. A., Selley, D. E., and Sim-Selley, L. J. (2010). Regional enhancement of cannabinoid CB1 receptor desensitization in female adolescent rats following repeated delta-tetrahydrocannabinol exposure. *Br. J. Pharmacol.* 161, 103–112.

Carlezon, W. A. Jr., Duman, R. S., and Nestler, E. J. (2005). The many faces of CREB. *Trends Neurosci.* 28, 436–445.

Cha, Y. M., Jones, K. H., Kuhn, C. M., Wilson, W. A., and Swartzwelder, H. S. (2007). Sex differences in the effects of delta9-tetrahydrocannabinol on spatial learning in adolescent and adult rats. *Behav. Pharmacol.* 18, 563–569.

Craft, R. M. (2005). Sex differences in behavioral effects of cannabinoids. *Life Sci.* 77, 2471–2478.

Fattore, L., Spano, M. S., Altea, S., Angius, F., Fadda, P., and Fratta, W. (2007). Cannabinoid self-administration in rats: sex differences and the influence of ovarian function. *Br. J. Pharmacol.* 152, 795–804.

Fink, G., Sumner, B. E., McQueen, J. K., Wilson, H., and Rosie, R. (1998). Sex steroid control of mood, mental state and memory. *Clin. Exp. Pharmacol. Physiol.* 25, 764–775.

González, S., Bisogno, T., Wenger, T., Manzanares, J., Milone, A., Berrendero, F., Di Marzo, V., Ramos, J. A., and Fernández-Ruiz, J. J. (2000). Sex steroid influence on cannabinoid CB(1) receptor mRNA and endocannabinoid levels in the anterior pituitary gland. *Biochem. Biophys. Res. Commun.* 270, 260–266.

Hasin, D. S., Keyes, K. M., Alderson, D., Wang, S., Aharonovich, E., and Grant, B. F. (2008). Cannabis withdrawal in the United States: results from NESARC. *J. Clin. Psychiatry* 69, 1354–1363.

Higuera-Matas, A., Botreau, F., Miguéns, M., Del Olmo, N., Borcel, E., Pérez-Alvarez, L., García-Lecumberri, C., and Ambrosio, E. (2009). Chronic periadolescent cannabinoid treatment enhances adult hippocampal PSA-NCAM expression in male Wistar rats but only has marginal effects on anxiety, learning and memory. *Pharmacol. Biochem. Behav.* 93, 482–490.

Hill, M. N., Karacabeyli, E. S., and Gorzalka, B. B. (2007). Estrogen recruits the endocannabinoid system to modulate emotionality. *Psychoneuroendocrinology* 32, 350–357.

Hill, M. N., Miller, G. E., Ho, W. S., Gorzalka, B. B., and Hillard, C. J. (2008). Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. *Pharmacopsychiatry* 41, 48–53.

Kauffman, A. S. (2010). Gonadal and nongonadal regulation of sex differences in hypothalamic Kiss1 neurons. *J. Neuroendocrinol.* 22, 682–691.

Krebs-Kraft, D. L., Hill, M. N., Hillard, C. J., and McCarthy, M. M. (2010). Sex difference in cell proliferation in developing rat amygdala mediated by endocannabinoids has implications for social behavior. *Proc. Natl. Acad. Sci. U.S.A.* 107, 20535–20540.

Lee, K. S., Clough, A. R., Jaragba, M. J., Conigrave, K. M., and Patton, G. C. (2008). Heavy cannabis use and depressive symptoms in three aboriginal communities in Arnhem Land, Northern Territory. *Med. J. Aust.* 188, 605–608.

Mateos, B., Borcel, E., Loriga, R., Luesu, W., Bini, V., Llorente, R., Castelli, M., and Viveros, M. P. (2010). Adolescent exposure to nicotine and/or the cannabinoid agonist CP 55,940 induces gender-dependent long-lasting memory impairments and changes in brain nicotinic and CB1 cannabinoid receptors. *J. Psychopharmacol. (Oxford).* doi: 10.1177/0269881110370503. [Epub ahead of print].

Morrish, A. C., Hill, M. N., Riebe, C. J. N., and Gorzalka, B. B. (2009). Protracted cannabinoid administration elicits antidepressant behavioral responses in rats: role of gender and noradrenergic transmission. *Physiol. Behav.* 98, 118–124.

Narimatsu, S., Watanabe, K., Yamamoto, I., and Yoshimura, H. (1991). Sex difference in the oxidative metabolism of delta 9-tetrahydrocannabinol in the rat. *Biochem. Pharmacol.* 41, 1187–1194.

O'Shea, M., McGregor, I. S., and Mallet, P. E. (2006). Repeated cannabinoid exposure during perinatal, adolescent or early adult ages produces similar long lasting deficits in object recognition and reduced social interaction in rats. *J. Psychopharmacol. (Oxford)* 20, 611–621.

O'Shea, M., Singh, M. E., McGregor, I. S., and Mallet, P. E. (2004). Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. *J. Psychopharmacol. (Oxford)* 18, 502–508.

Realini, N., Vigano, D., Guidali, C., Zamberletti, E., Rubino, T., and Parolaro, D. (2011). Chronic URB597 treatment at adulthood reverted most depressive-like symptoms induced by adolescent exposure to THC in female rats. *Neuropharmacology* 60, 235–243.

Riebe, C. J., Hill, M. N., Lee, T. T., Hillard, C. J., and Gorzalka, B. B. (2010). Estrogenic regulation of limbic cannabinoid receptor binding. *Psychoneuroendocrinology* 35, 1265–1269.

Rodríguez de Fonseca, F., Cebeira, M., Ramos, J. A., Martín, M., and Fernández-Ruiz, J. J. (1994). Cannabinoid receptors in rat brain areas: sexual differences, fluctuations during estrous cycle and changes after gonadectomy and sex steroid replacement. *Life Sci.* 54, 159–170.

Romano-Torres, M., and Fernández-Guasti, A. (2010). Estradiol valerate elicits antidepressant-like effects in middle-aged female rats under chronic mild stress. *Behav. Pharmacol.* 21, 104–111.

Rubino, T., Realini, N., Braida, D., Alberio, T., Capurro, V., Viganò, D., Guidali, C., Sala, M., Fasano, M., and Parolaro, D. (2009a). The depressive phenotype induced in adult female rats by adolescent exposure to THC is associated with cognitive impairment and altered neuroplasticity in the prefrontal cortex. *Neurotox. Res.* 15, 291–302.

Rubino, T., Realini, N., Braida, D., Guidi, S., Capurro, V., Viganò, D., Guidali, C., Pinter, M., Sala, M., Bartesaghi, R., and Parolaro, D. (2009b). Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus* 19, 763–772.

Rubino, T., Vigano, D., Realini, N., Guidali, C., Braida, D., Capurro, V., Castiglioni, C., Cherubino, F., Romualdi, P., Candeletti, S., Sala, M., and Parolaro, D. (2008). Chronic delta 9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. *Neuropsychopharmacology* 33, 2760–2771.

Solowij, N., and Pesa, N. (2010). Cognitive abnormalities and cannabis use. *Rev. Bras. Psiquiatr.* 32(Suppl. 1), S31–S40.

Tseng, A. H., Harding, J. W., and Craft, R. M. (2004). Pharmacokinetic factors in sex differences in delta 9-tetrahydrocannabinol-induced behavioral effects in rats. *Behav. Brain Res.* 154, 77–83.

Van Laere, K., Goffin, K., Casteels, C., Dupont, P., Mortelmans, L.,

de Hoon, J., and Bormans, G. (2008). Gender-dependent increases with healthy aging of the human cerebral cannabinoid-type 1 receptor binding using [(18)F]MK-9470 PET. *Neuroimage* 39, 1533–1541.

Viveros, M. P., Marco, E. M., López-Gallardo, M., Garcia-Segura, L. M., and Wagner, E. J. (2010). Framework for sex differences in adolescent neurobiology: a focus on cannabinoids. *Neurosci. Biobehav. Rev.* 35, 1740–1751.

Walf, A. A., and Frye, C. A. (2009). Effects of two estradiol regimens on anxiety and depressive behaviors and trophic effects in peripheral tissues in a rodent model. *Gend. Med.* 6, 300–311.

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The dopamine and cannabinoid interaction in the modulation of emotions and cognition: assessing the role of cannabinoid CB1 receptor in neurons expressing dopamine D1 receptors

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Although cannabinoid CB1 receptors (CB1Rs) are densely expressed in neurons expressing dopamine D1 receptors (D1Rs), it is not fully understood to what extent they modulate emotional behaviors. We used conditional CB1R knock-out animals lacking CB1Rs in neurons expressing D1R (D1–CB1$^{-/-}$) in order to answer this question. To elucidate the behavioral effects of CB1R deficiency in this specific neuronal subpopulation, we subjected D1–CB1$^{-/-}$ mice to a battery of behavioral tests which included exploration-based tests, depressive-like behavioral tests, social behavior, and fear-related memory paradigms. D1–CB1$^{-/-}$ did not show any difference in the exploration-based paradigms such as open field, elevated plus maze, or novel object investigation test, except for an increase in novelty-induced grooming. By contrast, they showed a mild anhedonia-like state as described by the slightly decreased preference for sweet solution, as compared to wild-type control group. This decrease, however, could be observed only during the first day of exposure, thus suggesting increased neophobia as an alternative explanation. Accordingly, mutant mice performed normally in the forced swim test, a procedure widely used for evaluating behavioral despair in rodents. However, weak- to moderate anxiety-like phenotypes were evident when D1–CB1$^{-/-}$ mice were tested for social behavior. Most strikingly, D1–CB1$^{-/-}$ mice exhibited significantly increased contextual and auditory-cued fear, with attenuated within session extinction, suggesting that a specific reduction of endocannabinoid signaling in neurons expressing dopamine D1Rs is able to affect acute fear adaptation. These results provided first direct evidence for a cross-talk between dopaminergic D1Rs and endocannabinoid system in terms of controlling negative affect.

Keywords: cannabinoids, CB1R, dopamine, D1R, social behavior, aversive memories, anxiety, fear extinction

INTRODUCTION

In the central nervous system (CNS), endogenous cannabinoids compounds activate cannabinoid CB1 receptors (CB1Rs), which are located pre-synaptically in several brain regions such as prefrontal cortex, hippocampus, amygdala, and basal ganglia. They act as inhibitory retrograde signaling messengers at glutamatergic and GABAergic synapses, modulating the release of several neurotransmitters such as acetylcholine or dopamine (DA) (Marsicano and Lutz, 1999; Piomelli, 2003). Thus, the endocannabinoid system (ECS), through its neuromodulating activity, could be involved in several physiological functions as memory processing, pain perception, locomotion, and inflammation; additionally, its dysregulation could underlie several pathological conditions known to accompanying psychiatric disorders (Di Marzo, 2008).

The role of the dopaminergic (DAergic) neurotransmitter system in the processing of emotional behavior is well established and supported by several preclinical and clinical data showing that DA, acting on D1- or D2-like receptors, is one of the most important neuromodulators of fear and anxiety (LeDoux, 2000). A DAergic and EC interaction at different anatomical levels (i.e., amygdala, nucleus accumbens and striatum) seems to be involved in several neurophysiological responses. More specifically, it has been suggested that CB1R signaling modulates DAergic pathways by influencing directly or indirectly the activity of DAergic neurons through either post- or pre-synaptic mechanisms (Laviolette and Grace, 2006). However, both the mechanisms through which DAergic and EC signaling cross-talk and the role played by the dopamine D1 receptor positive neurons still remain unclear. The dopamine D1 receptors (D1Rs), which belong to the "D1-like" group, are expressed in brain regions involved in aversive learning and memory such as nucleus accumbens, hippocampus, and amygdala. (Kamei et al., 1995; Bernabeu et al., 1997; El-Ghundi et al., 2001; Nagai et al., 2007). Interestingly, the colocalization of CB1Rs with D1Rs indicates that these receptors may interact by potentially modifying their respective functions with important behavioral and pharmacological consequences (Hermann et al., 2002).

Although the use of complete CB1 knock-out mice together with pharmacological approaches suggest that ECS controls fear and anxiety primarily under highly aversive situations (Moreira and Wotjak, 2010), the cellular substrates of these effects with regard to specific neuronal subpopulation involved (i.e., dopamine receptor D1-expressing neurons) is still largely unexplored, except

for a specific contribution of principal neurons of the forebrain (Kamprath et al., 2009). Thus, conditional CB1 knock-out animals, lacking CB1Rs specifically in D1R positive neurons provide an important tool to answer these questions.

Based on the above premises, this study was undertaken to investigate the role of CB1R signaling in the dopamine receptor D1-expressing neurons in affecting emotional behavior. For this purpose, conditional CB1 mutant mice, lacking CB1Rs expression in neurons containing dopamine D1Rs (D1–CB1$^{-/-}$; Monory et al., 2007), were submitted to a battery of behavioral tests, which included exploration-based tests, depressive-like behavioral tests, and fear-related memory paradigms. Since it has been hypothesized that ECS is a relevant modulator of dopamine D1Rs-mediated behaviors including social activity (Martín et al., 2008; Zenko et al., 2011), we also evaluated the phenotype of these mice in social approach tests.

MATERIALS AND METHODS

ANIMALS

Male mice at the age of 8–16 weeks were used throughout the experiments. Conditional D1–CB1 knock-out mice (D1–CB1$^{-/-}$ or KO) and their respective wild-type (WT) littermate controls (D1–CB1$^{+/+}$ or WT) were generated and genotyped as previously described (Monory et al., 2007). Animals (n = 6–10 per group) were single housed and maintained in standard conditions with food and water *ad libitum* under a 12-h inverse light–dark (LD) cycle (lights off at 9 a.m.) for at least 14 days before starting the experiments. All behavioral experiments were performed during the active (dark) phase of mice between 9:30 a.m. and 5 p.m. Experimenters were always blind to the genotype. All behavioral tests took place in an experimental room with the same LD cycle and environmental conditions (i.e., humidity, temperature) as in the housing facility. All experiments were carried out according to the European Community Council Directive 86/609/EEC and efforts have been made to minimize animal suffering and reduce the number of animals used.

BEHAVIORAL TESTING

Novelty-induced grooming test

Grooming behavior was observed under the same environmental conditions as previously described (Kalueff and Tuohimaa, 2005). The mice were placed individually into a clean unfamiliar Plexiglass box (27 cm × 16 cm × 12 cm) without bedding for 10 min. Three ethological measures of grooming activity were scored: latency to start grooming, grooming episodes (washing, general grooming, scratching, licking of paws, or genital grooming), and total time spent grooming. All trials were recorded for subsequent video analysis.

Open field test

Exploratory activity of D1–CB1$^{-/-}$ and WT mice was evaluated in the open field (OF) test, as previously described (Jacob et al., 2009). The experiment was performed in a squared box (26 cm × 26 cm), in which the animal was placed in the central zone of the apparatus equipped with infrared beams (TruScan; Coulbourn Instruments, Allentown, PA, USA) and allowed to explore for 30 min at 300 lux. All sensor rings were connected via interface to a computer equipped with TruScan Software Version 99 (Coulbourn Instruments). Boxes and sensor rings were surrounded by an additional box made of opaque Plexiglas side walls (47 cm × 47 cm × 38 cm) without roof and floor. Horizontal locomotion (total, margin, or central distance moved) vertical movements (exploratory rearing) and time spent at rest were analyzed during the 30-min monitoring period with a sampling rate of 4 Hz. After each session, the apparatus was cleaned with a solution containing neutral soap.

Elevated plus maze test

The apparatus consisted of two opposite open arms, (30 cm × 5 cm) and two arms with walls (30 cm × 5 cm × 14 cm) that were attached to a central platform (5 cm × 5 cm) to form a cross. The maze was elevated 50 cm from the floor (Pellow et al., 1985). Illumination measured at the center of the maze was 300 lux. The animal was placed in the center of the maze facing one of the closed arms, and observed for 5 min, according to the following parameters: number of entries in the open or closed arms and time of permanence in each arm (i.e., the time spent by the animal in the open or closed arms). An entry was defined as all four paws having crossed the line between an arm and the central area. It is accepted that the anxiolytic effect of a drug treatment is illustrated by increased parameters in open arms (time and/or number of entries; Pamplona et al., 2011: for pharmacological validation of our current set-up). The augmented percentage of entries in open arms over the total entries in both arms is a good indicator of reduced anxious-like phenotype as well. Entries in closed arms and total entries reflect the motor component of the exploratory activity. On removal of each mouse, the maze floor was carefully wiped with a wet towel. All trials were recorded on a HDD using a video-camera and then scored off-line by an experienced observer by means of a video/computer system ANY-MAZE (Stoelting).

Light/dark test

Set-up and test procedure were essentially the same as previously described (Jacob et al., 2009). The LD box was divided in two compartments: (1) one dark compartment (15 cm × 20 cm × 38 cm) with black walls and (2) one lit compartment (30 cm × 20 cm × 38 cm) with white plastic walls. Both compartments were connected by a 4-cm long tunnel. Light intensity was 600 lux in the light compartment and 15 lux in the dark compartment measured at floor level. Mice were placed into the corner of the dark compartment at the start of the experiment which lasted for 5 min. After each test, the LD box was thoroughly cleaned with soap and water. Entries and time spent in the light compartment were assessed by video analysis by a trained observer. These two variables were expressed as percentage of the total observation period and the total number of LD transitions, respectively.

Novel object investigation test

The novel object investigation (NOI) test was performed at 30 lux (which still allowed the assessment of exploration of the objects) for 10 min. Experimental subjects were habituated to the test arena (36 cm × 22 cm × 14 cm, with sawdust bedding material and transparent walls) for 2 days for 10 min (one cage per mouse without cleaning or changing of bedding). On the third day, mice were transferred into the same test cages and two identical objects (cone

made of aluminum: ∅ 6 cm + H 13 cm) were placed in a symmetrical position at the short walls of the cages. Between animals, objects were thoroughly cleaned with water containing detergent to eliminate olfactory cues. Objects were heavy enough that a mouse could not displace them. Every trial was video recorded and analyzed using ANY-MAZE (Stoelting). Investigation was defined as follows: directing the nose toward the object at a distance of not more than 2 cm and/or touching the object with the nose and paws (Jacob et al., 2009).

Sucrose consumption test

During this test, mice are given a free choice between two bottles for 10 h – one filled with 2.5% sucrose solution and the other with tap water – for two consecutive days (Strekalova and Steinbusch, 2010). To prevent possible effects of side preference in drinking behavior, the bottles position was switched in the mid-point of testing. Animals were not food or water-deprived before the test. For habituation, 1 day prior to the first testing day, animals were allowed to drink a 2.5% sucrose solution for 2 h. The consumption in water, sucrose solution, and total intake of liquids were estimated simultaneously in the both groups by weighing the bottles before and after each trial. The preference for sucrose was calculated as a percentage of the consumed sucrose solution from the total amount of liquid drunk, by the formula: Sucrose Preference = V(Sucrose solution)/[V(Sucrose solution)+V(Water)]×100%.

Forced swim test

The forced swim test (FST) employed here was essentially similar to that described elsewhere (Porsolt et al., 1978). Mice were individually placed into transparent cylinders (height 23.5 cm; diameter 16.5 cm) containing 15 cm water at 25 ± 1°C for 6 min. The water was changed after each trial. After vigorous activity, swimming attempts cease and the animal adopts a characteristic immobile posture. A mouse is judged to be immobile when it floats in upright position and makes only small movements to keep its head above water. The duration of mobility was recorded during the last 4-min of the 6-min testing period. All trials were recorded for subsequent off-line analysis.

Social interaction test

The procedure was adopted from (Smit-Rigter et al., 2010). Experiments were performed in a new cage (27 cm × 16 cm × 12 cm) with fresh bedding at 5 lux (i.e., red light) or 700 lux (light intensity measured at the level of test cages). The lid of the new cage was removed and the walls elongated by 12.5 cm of semi transparent plastic. Briefly, pairs of unfamiliar mice of the same genotype ($n = 7$ pairs of D1–CB1$^{-/-}$ and WT) were placed into the cage for 5 min. The time spent in social interactions (SI; active contact such as sniffing, licking, close following, and grooming) was recorded for each pair of mice. Each session was video recorded and analyzed off-line using ANY-MAZE (Stoelting).

Social investigation test

Social investigation (SInv) task was conducted as previously described with slight modifications (Crawley et al., 2007). It took place in a rectangular box made of white PVC walls and with a dark gray PVC floor. The box was divided into three equal compartments (30 cm × 30 cm × 30 cm) that were interconnected by small opening (6 cm × 5 cm) with guillotine doors. Each animal was allowed to free exploration of the apparatus for 10 min (habituation). An empty perforated 50 ml falcon tube was placed in each side of the box. This 10 min exposure was designed to familiarize the subject mouse with the testing environment. After habituation session, the animal was kept in the center compartment and one of the tubes was replaced by a tube containing an ovariectomized female. For the next 10 min session, the mouse was allowed to explore all three compartments and the time spent in the SInv (active contact such as sniffing) was recorded.

Fear conditioning task

The set-up has been described and displayed in detail before (Kamprath and Wotjak, 2004; Plendl and Wotjak, 2010). Two different protocols were programmed and carried out. The first experiment was performed in two contexts: (1) the neutral test context – a cylinder made of transparent Plexiglas, lined with wood shavings – and (2) the shock context – a cubic-shaped box with a metal grid for shock application. For conditioning (d0), mice were placed in the conditioning context. Three minutes later, a tone (80 dB, 9 kHz sine-wave, 10 ms rising, and falling time) was presented to the animals for 20 s that coterminated with a 2-s scrambled electric footshock of 0.7 mA. Mice were returned to their home cages 60 s later. On day 1 (d1), mice were exposed to the neutral context and on day 2 (d2) to the grid context for 7 and 3 min, respectively. Briefly, mice were placed in the test context, which differed from the conditioning context in material, shape, surface texture, and odor of the cleaning solution. After an initial 3 min of habituation, a 180-s permanent tone [9 kHz, 80 dB, sine-wave] was delivered. To test the contextual freezing, animals were re-exposed to the shock chamber for 3 min without tone presentation and without further shock application, and immediately returned to their home cages afterward.

In the second experiment, mice were conditioned as described for the first experiment. On day 1 (d1) and on day 7 (d7), mice were exposed to the 180-s tone in the neutral test context. Animals' behavior was video recorded by small CCD cameras (Conrad Electronics, Hirschau, Germany) and rated off-line by a trained observer (EVENTLOG, designed by Robert Henderson, 1986). Freezing behavior was defined as immobility except for respiration movements.

EXPERIMENTAL DESIGN

Behavioral experiments were conducted in two screens to reduce the number of animals used for the study with separate cohorts of animals for every screen (**Table 1**). If not stated otherwise, the different screens were accomplished with 4–5 days in between two consecutive tests. Animals were submitted to a battery of behavioral tests, which was divided in three main categories, in the following order: (1) low- or mild-stress situation (a) exploratory-based approach avoidance conflict tests: open field, elevated plus maze, light/dark, novel object investigation, and novelty-induced grooming (b) depressive-like behavior paradigms: sucrose consumption and forced swim test (2) social approach: social interaction and social investigation test (3) high-stress situation: fear conditioning (FC) tests. The order of tests within the battery was designed in such manner that mice would be evaluated on what were thought to be least invasive tests before

Table 1 | Comprehensive behavioral test battery of D1–CB1 knock-out mice.

Test	Age (weeks)	Days	*n*	Results
FIRST GROUP				
OF	8–10	1	6–9	**Figures 1A–F**
EPM	8–10	5	7–8	**Figures 2A, B**
LD	9–11	9	8–9	**Figures 2C,D**
SI	9–11	13	6–8	**Figures 6A,B**
FST	10–12	20	7–9	**Figure 5B**
FC	11–13	27	8–9	**Figures 7A,B**
SECOND GROUP				
NGT	8–10	2	8–9	**Figures 4A–C**
NOI	8–10	1	8–10	**Figure 3**
SC	8–10	5	10	**Figure 5A**
SInv	9–11	9	9–10	**Figures 6C,D**
FC	11–13	27	9–10	**Figures 7C,D**

n, Animal number; OF, open field; EPM, elevated plus maze; LD, light/dark; SI, social interaction; FST, forced swim test; FC, fear conditioning; NOI, novel object investigation; SC, sucrose consumption; SInv, social investigation; NGT, novelty-induced grooming test.

being tested on more invasive assays. This design was developed with the assumptions that testing from least to most invasive would allow for recovery time between tests and would reduce the likelihood that behavioral responses would be influenced by previous testing experience.

STATISTICAL ANALYSIS

Data were analyzed using unpaired *t*-test or two-factor ANOVA by means of Sigma Stat 3.5 (Systat Software Inc., San Jose, CA, USA). Newman–Keuls test was used as *post hoc* test, if appropriate. Data are presented as mean ± SEM. Statistical significance was accepted if $p < 0.05$.

RESULTS

EXPLORATORY AVOIDANCE CONFLICT TESTS

Open field test

In the OF test, there was no difference in the exploratory activity between D1–CB1$^{-/-}$ and WT mice (**Figures 1A–F**). Both groups showed the same horizontal activity (total distance: $t = 1.246$; $p = 0.2348$; central distance: $t = 1.501$; $p = 0.1574$, margin distance: $t = 0.2401$; $p = 0.8140$), total duration of movement ($t = 1.217$; $p = 0.2452$), rearing ($t = 1.715$; $p = 0.1101$), and jumping episodes ($t = 1.344$; $p = 0.2021$). This response indicates that in our test conditions, genetic deletion of CB1 in neurons expressing D1Rs did not alter basal locomotor activity of mice.

Elevated plus maze and light/dark test

As described in **Figures 2A–D**, statistical analysis did not reveal any significant difference between D1–CB1$^{-/-}$ and WT mice both in the time spent ($t = 0.5568$; df = 14; $p = 0.5871$) or in the number of entries ($t = 0.6133$; df = 14; $p = 0.5502$) into open arms of the EPM test. Also, there was no difference in the time spent ($t = 0.2827$; df = 15; $p = 0.7813$) or in number of entries ($t = 0.9739$; df = 15; $p = 0.3430$) into light compartment of the LD test. No locomotion difference was found, considering the total arm entries ($t = 0.7276$; df = 14; $p = 0.4798$) and the total LD transitions ($t = 0.8154$; df = 15; $p = 0.4255$) as index.

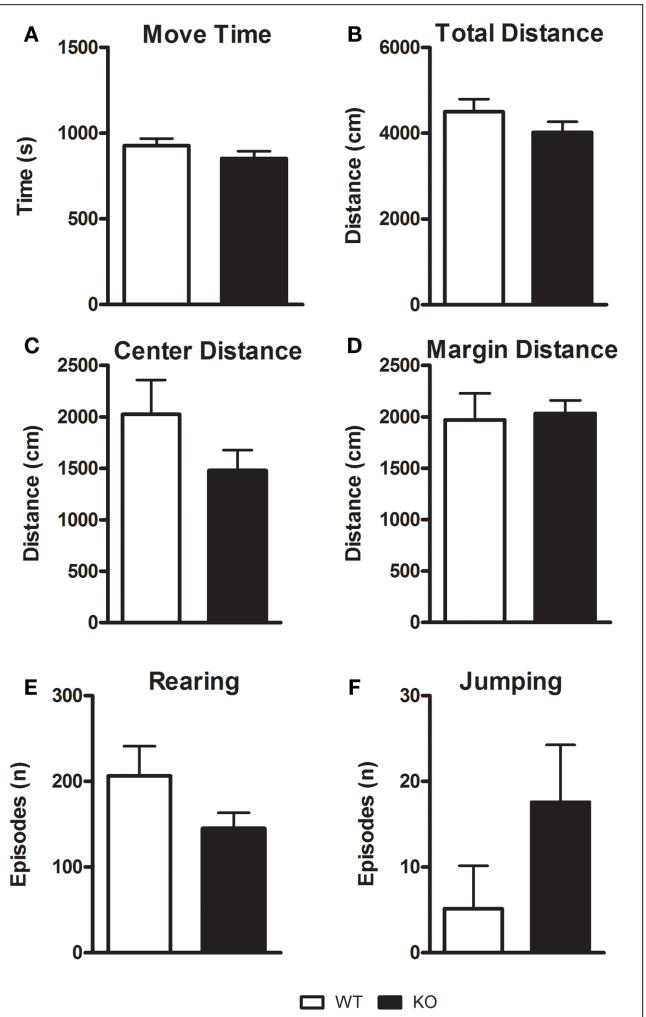

FIGURE 1 | Assessment of general locomotor activity of D1–CB1$^{-/-}$ mice in the open field (OF) test. Conditional D1–CB1$^{-/-}$ mutant mice (KO) and their respective wild-type (WT) littermates were tested in an open field for 30 min. Values are mean ± SEM in terms of locomotor activity **(A–D)** rearing **(E)** and jumping episodes **(F)**.

Novel object investigation

Unpaired *t*-test showed that D1–CB1$^{-/-}$ and WT mice, during the 10-min test, spent the same amount of time investigating the pair of novel objects ($t = 0.5887$; $p = 0.5643$), as well as they approached them with the same frequency ($t = 0.5705$; $p = 0.5762$; **Figure 3**).

Novelty-induced grooming activity test

As described in **Figures 4A–C**, D1–CB1$^{-/-}$ mice performed more grooming episodes ($t = 2.240$; $p < 0.05$; df = 15) as well as they spent more time grooming as compared to WT animals ($t = 2.568$; $p < 0.05$; df = 15). However, the latency to start grooming was not significantly different between the two groups ($t = 1.170$; $p = 0.2603$; df = 15).

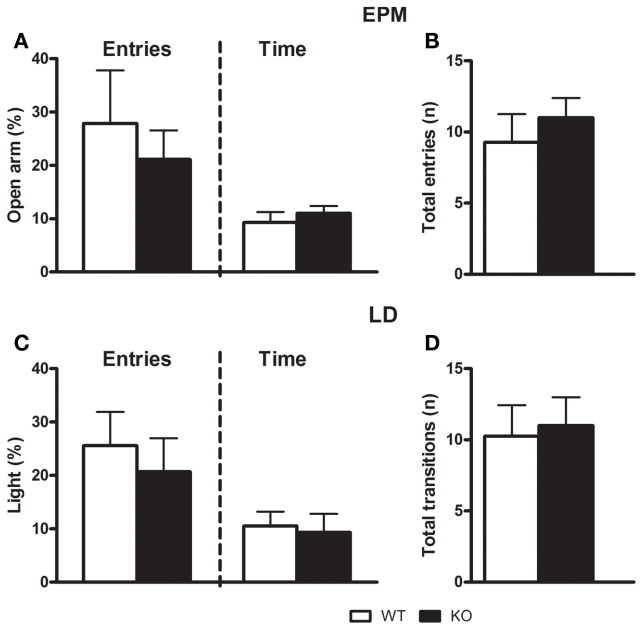

FIGURE 2 | Anxiety-like behaviors of D1–CB1$^{-/-}$ mice. Conditional D1–CB1$^{-/-}$ mutant mice (KO) and their respective wild-type (WT) littermates were tested for 5 min in the elevated plus maze (upper panel) or in the light/dark box (lower panel). Data are presented as mean ± SEM regarding open arm entries and open arm time **(A)** the total arm entries **(B)**, light compartment entries and light compartment time **(C)**, and total compartment transitions **(D)**.

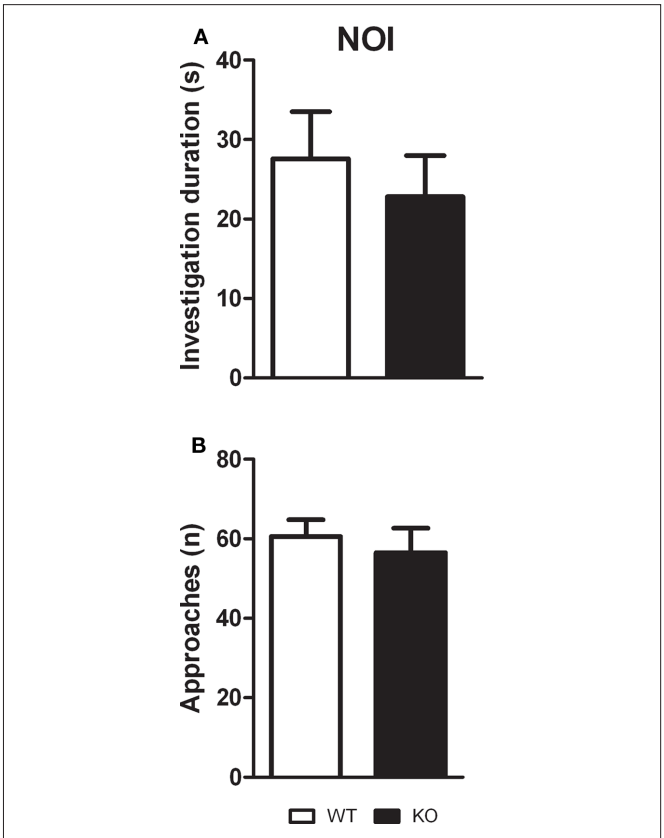

FIGURE 3 | Novel object investigation test. Conditional D1–CB1$^{-/-}$ mutant mice (KO) and their respective wild-type (WT) littermates were exposed to two novel objects for 10 min. Data are presented as mean ± SEM regarding total investigation duration **(A)** and number of approaches **(B)**.

DEPRESSIVE-LIKE BEHAVIOR

Forced swim test and sucrose consumption

As described in **Figure 5**, D1–CB1$^{-/-}$ mice showed a significant lower SC as compared to WT mice on the first ($t = 2.868$; $p < 0.05$), but not on the second testing day ($t = 0.3575$; $p = 0.7249$). However, WT and D1–CB1$^{-/-}$ mice showed a high percentage of SC as compared to the total amount of liquid consumed. In the FST, although D1–CB1$^{-/-}$ mice showed a decrease in the mobility as compared to WT animals, the difference between the two genotypes did not reach statistical significance ($t = 1.904$; $p = 0.0777$).

SOCIAL ACTIVITY TESTS

Social interaction

As described in **Figures 6A,B**, two-way ANOVA (factor 1: light intensity, factor 2: genotype) revealed a main effect of light intensity ($F_{1,13} = 14.656$; $p < 0.01$) genotype ($F_{1,13} = 6.366$; $p < 0.05$) and a light intensity × genotype interaction ($F_{1,13} = 10.904$; $p < 0.01$) for time of interaction. There were also a main effect of light intensity ($F_{1,13} = 18.472$; $p < 0.01$) genotype ($F_{1,13} = 5.285$; $p < 0.05$) and a light intensity × genotype interaction ($F_{1,13} = 12.947$; $p < 0.01$) for the frequency of interaction. *Post hoc* analysis showed that in the less aversive environment (0 lux), D1–CB1$^{-/-}$ expressed a lower SI during the 5-min test than WT mice as described by the decreased number and time of interactions ($p < 0.05$). WT approached the low level of performance seen in D1–CB1$^{-/-}$ under aversive conditions (700 lux).

Social investigation

Figures 6C,D describe the time and the number of active approaches toward the empty falcon tube vs. the tube containing the ovariectomized female. Two-way ANOVA analyses (factor 1: object, factor 2: genotype) revealed a main effect of object (number of interaction: $F_{1,34} = 35.588$; $p < 0.001$; time of interaction: $F_{1,34} = 25.023$; $p < 0.001$), but no main effect of genotype (number of interaction: $F_{1,34} = 0.0182$; $p = 0.893$; time of interaction: $F_{1,34} = 1.402$; $p = 0.245$) or a object × genotype interaction (number of interaction: $F_{1,34} = 0.839$; $p = 0.366$; time of interaction: $F_{1,34} = 1.780$; $p = 0.191$), indicating that mice of both genotype display a preference for the ovariectomized female. Additional *t*-test was performed separately for each genotype. D1–CB1$^{-/-}$ and WT mice showed higher interest for the tube containing the female, as described by the significant increase of time of investigation (WT: $t = 3.782$; $p < 0.01$; D1–CB1$^{-/-}$: $t = 3.489$; $p < 0.01$) and by number of interactions (WT: $t = 3.904$; $p < 0.01$; D1–CB1$^{-/-}$: $t = 4.459$; $p < 0.001$).

Fear conditioning

As shown in **Figure 7A**, unpaired *t*-test revealed that D1–CB1$^{-/-}$ showed a significant increase on freezing response to the tone at day 1 ($t = 2.497$; $p < 0.05$) and to the context at day 2 ($t = 3.210$; $p < 0.01$) as index of increased auditory-cued and contextual fear responses, respectively. When analyzed in 20-s intervals, all mice

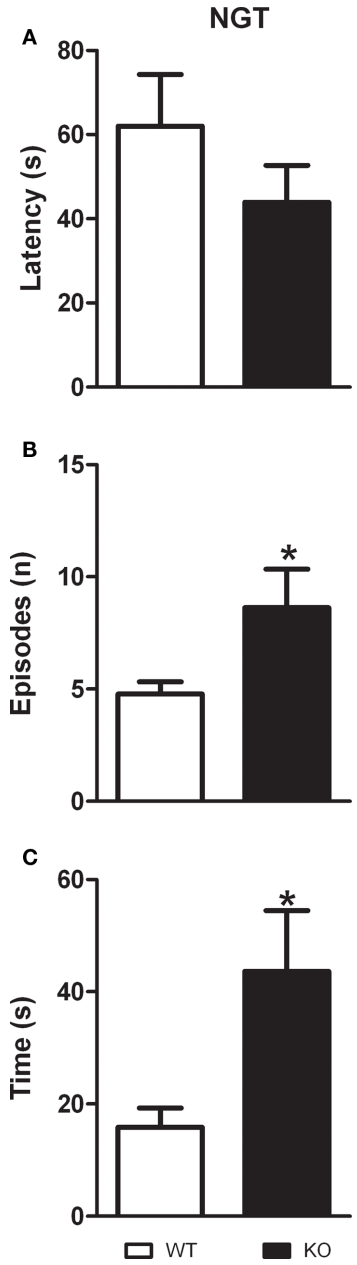

FIGURE 4 | Grooming behavior in D1–CB1$^{-/-}$ mice. Conditional D1–CB1$^{-/-}$ mutant mice (KO) and their respective wild-type (WT) littermates were tested for the grooming activity measure. Data are presented as mean ± SEM regarding latency to start grooming **(A)**, number of grooming episodes **(B)** and total grooming duration **(C)**. *$p < 0.05$ as compared to WT mice (unpaired *t*-test).

showed the same initial freezing response on day 1. However, whereas WT mice showed a rapidly waning freezing response during the tone presentation, D1–CB1$^{-/-}$ mice showed a deficit in acute fear adaptation (**Figure 6B**). The second experiment, largely confirmed their phenotype (**Figures 6C,D**): D1–CB1$^{-/-}$ mice showed a significant increase on freezing response to the tone on day 1 ($t = 4.234$; $p < 0.001$) and on day 7 ($t = 2.923$; $p < 0.01$), which again results from impaired acute fear adaptation over the course of tone presentation (**Figure 6D**). Freezing before tone presentation on day 1 was low and indistinguishable between the two groups.

DISCUSSION

In the present study we provide first evidence that the genetic deletion of cannabinoids CB1Rs in dopamine D1Rs-expressing neurons is able to affect the emotional behavior in mice in highly selective manner. Several studies reported increased anxiety-related behaviors after impaired CB1R signaling only when aversive stimulus cannot be avoided (Haller et al., 2004, 2009; Thiemann et al., 2007; Kamprath et al., 2009). However, little is known about how ECS modulation of the DAergic system could be involved in this effect.

It is accepted that ECs modulate several neurotransmitter systems (glutamatergic, GABAergic, and DAergic) at multiple levels (Piomelli, 2003). In the brain, where exogenously administered (Δ^9-tetrahydrocannabinol [THC]) and endogenously released cannabinoids exert most of their behavioral effects, the CB1Rs are expressed at different levels at different neuronal subpopulations. More specifically, they are present at very high levels in GABAergic interneurons, where they mediate cannabinoid-dependent inhibition of GABA release, and to a minor extent, in glutamatergic terminals (Marsicano and Lutz, 1999). In the glutamatergic neuronal subpopulation, they play a pivotal role in both neuroprotection and fear extinction in highly aversive situations, through the modulation of glutamate release, further confirming that the fear-alleviating effects of CB1 became evident primarily under highly aversive conditions (Monory et al., 2006; Kamprath et al., 2009; Moreira and Wotjak, 2010).

Several lines of evidence suggest that DA is released in several brain regions such as the amygdala and prefrontal cortex under stress conditions. By acting on D1- or D2-like receptors, DA is involved in physiological processes subserving affective behaviors and emotional learning (LeDoux, 2000). Although, coexpression of the cannabinoid CB1Rs and D1Rs supports an ECS–DAergic system cross-talk, as in forebrain basal ganglia and piriform cortex, the exact role of D1Rs is not fully understood. Thus, the development of conditional CB1 mutant mice, in which the CB1Rs are specifically deleted in neurons expressing D1Rs (Monory et al., 2007) has been an useful tool to understand their role in the emotional behavior.

It should be recalled that growing evidence indicates that measures of anxiety from different tests could reflect different states of anxiety. This prompted us to use different behavioral paradigms such as exploration-based tests and social paradigms, that primarily focus on reciprocal SI and on the preference for social novelty, respectively, as well as tasks involving a strong mnemonic component, such as fear based tests, to assess different aspects that could mimic symptoms of human anxiety disorders as agoraphobia, social phobia or post traumatic stress disorder (Lister, 1990; File, 1992; Cryan and Holmes, 2005).

The first novel result of the present study was that D1–CB1$^{-/-}$ mice did not show any anxiety-like phenotype when tested in exploratory behavioral paradigms such as EPM, LD, or NOI. These procedures mostly reflect the conflict between exploration and avoidance of a novel environment; thus, the inhibition of exploratory behavior given by the reduced open arms or light compartment entries and novel object exploration is commonly associated with high emotionality or anxiety. D1–CB1$^{-/-}$ mice also failed to show alteration in spontaneous

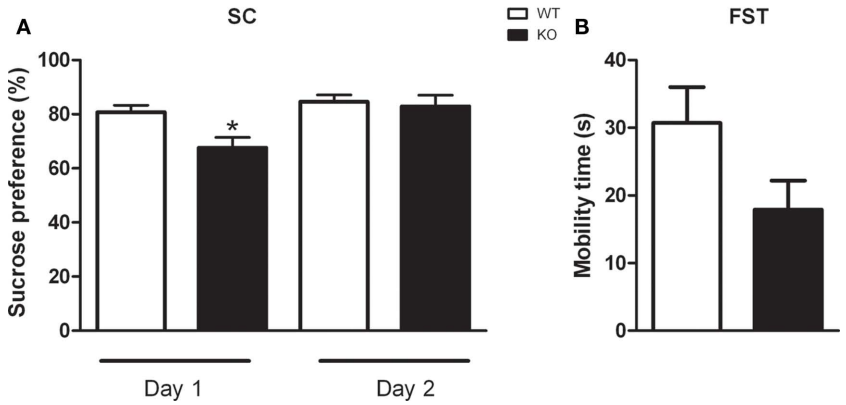

FIGURE 5 | Depressive-like behaviors of D1–CB1$^{-/-}$ mice. Conditional D1–CB1$^{-/-}$ mutant mice (KO) and their respective wild-type (WT) littermates were tested in the sucrose consumption test **(A)** or in the forced swim test (FST) paradigm **(B)**. Data are presented as mean ± SEM regarding percentage of sucrose consumption or mobility time expressed in seconds. *$p < 0.05$ as compared to WT mice (unpaired *t*-test).

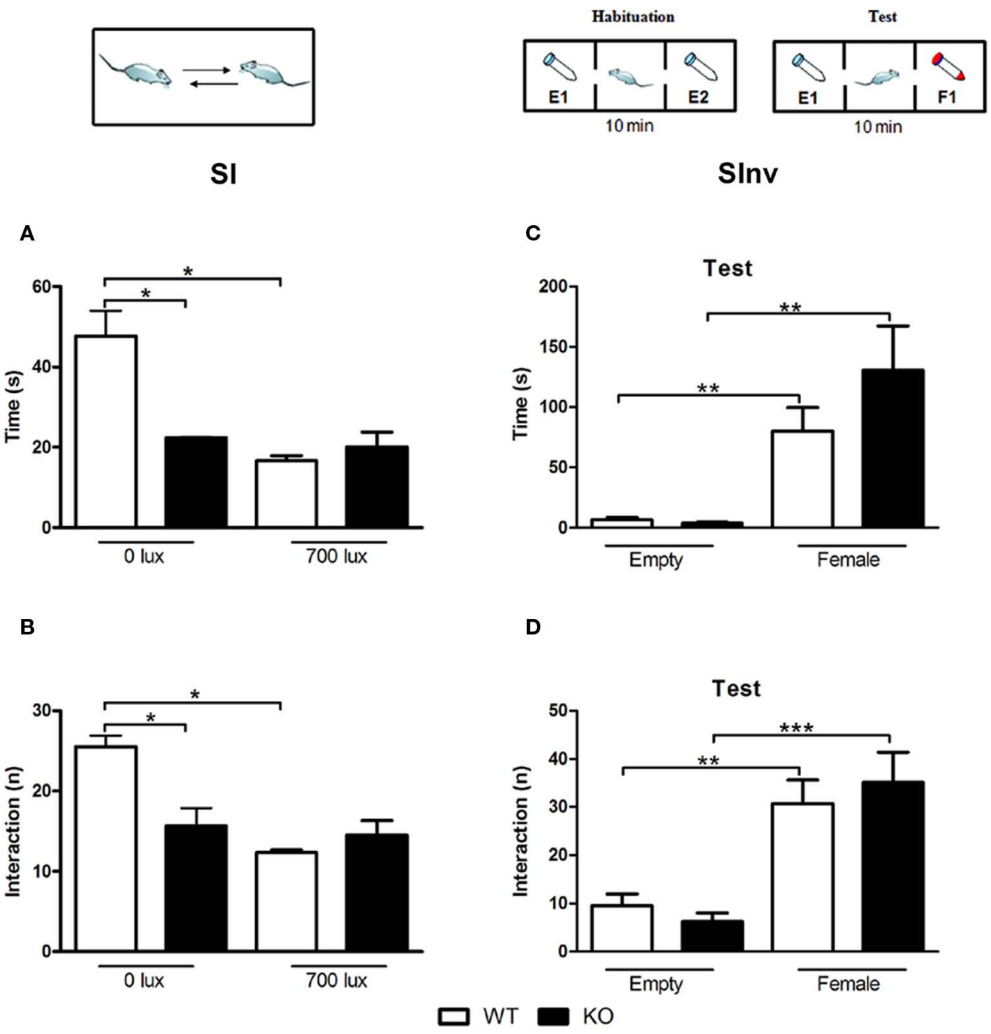

FIGURE 6 | Social behaviors in D1–CB1$^{-/-}$ mice. Conditional D1–CB1$^{-/-}$ mutant mice (KO) and their respective wild-type (WT) littermates were tested in the social interaction **(A,B)** or in the social investigation **(C,D)** test. Data are presented as mean ± SEM regarding time in interaction and number of interactions. E1: empty tube 1; E2: empty tube 2; F1: tube with female. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ (Newman–Keuls *post hoc* test or unpaired *t*-test).

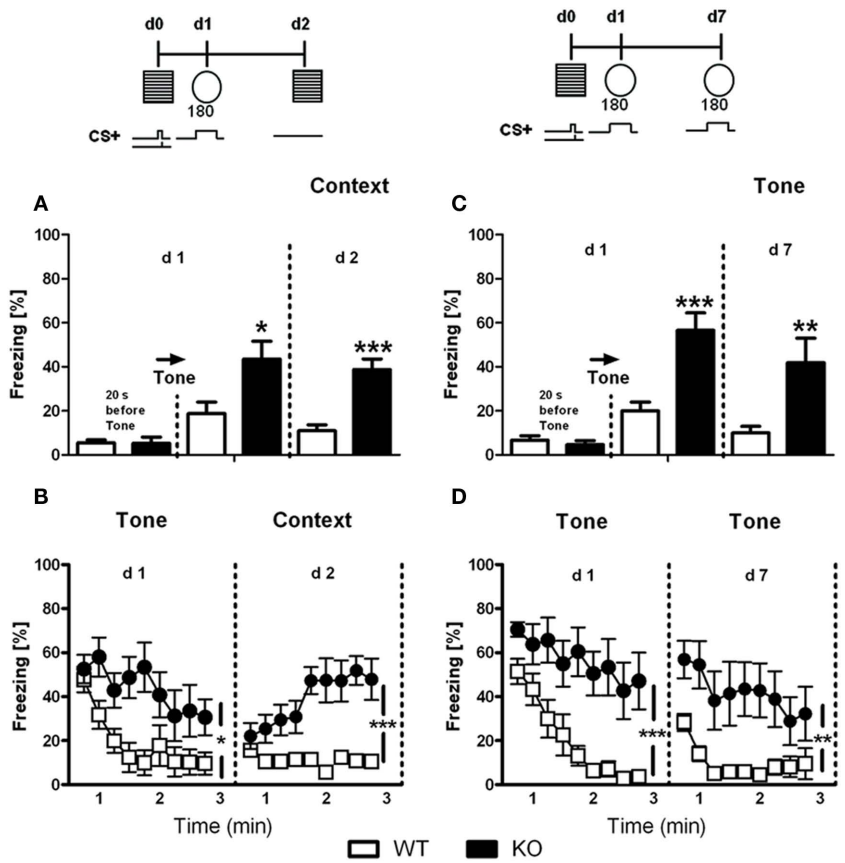

FIGURE 7 | Fear memory in D1–CB1$^{-/-}$ mice. Auditory-cued (Tone) and contextual (Context) fear memory assessed by freezing responses (mean ± SEM) of conditional D1–CB1$^{-/-}$ mutant mice (KO) and their respective wild-type (WT) littermates in two independent sets of experiments **(A/B, C/D)**. If not stated otherwise, freezing was averaged over the entire 180 s observation periods **(A,C)** or analyzed in 20 s intervals **(B,D)**. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ as compared to WT mice (unpaired *t*-test).

exploration and locomotor behavior. These findings are in line with previous data showing no anxiety-like phenotype in mice with total CB1Rs deletion and with specific CB1Rs deletion on glutamatergic neurons (Marsicano et al., 2002; Jacob et al., 2009). However, the D1–CB1$^{-/-}$ mice showed increased grooming activity. Grooming is considered a "maintenance" behavior, a common species-characteristic movement pattern with readily definable components (Bolles, 1960) that serves a range of adaptive functions, including stress reduction and social interplay (Kalueff and Tuohimaa, 2005). In rodents, spontaneous grooming behavior may occupy as much as 25–40% of the wakeful time, but it is specifically elicited in situations (i.e., NGT) in which an animal is in stress-induced conflict or frustration, as well as being reduced following anxiolytic treatment (Dunn et al., 1981; Gispen and Isaacson, 1981; van Erp et al., 1994; Micale et al., 2008). Thus, our results suggest that if the exposure to novelty cannot be controlled by the animals, the novel environment is able to influence the emotionality of D1–CB1$^{-/-}$ mice.

The D1–CB1$^{-/-}$ mice exhibited a decreased preference for sweet solutions on the first but not on the second day of the SC test under basal conditions, indicating a mild anhedonia-like state. Although anhedonia is commonly associated with depression-like behavior phenotype, mutant mice performed normally in the FST, a procedure widely used for evaluating behavioral despair in rodents (Cryan and Holmes, 2005). Thus, the modulation of depressive-like behaviors in D1–CB1$^{-/-}$ mice evaluated in different tasks may be mediated by distinct neuronal circuits. On the other hand, the lower SC was only evident upon the first confrontation with the novel taste (day 1) and disappeared on the next day, suggesting a significant contribution of neophobia. In fact, a weak- to moderate anxiety-like phenotype of D1–CB1$^{-/-}$ mice became evident when the animals were tested under low (0 lux) aversive conditions in an unavoidable situation (i.e. SI test), where the WT control mice demonstrated social approach (intense interaction). These findings suggest that the deletion of CB1Rs specifically in D1Rs-expressing neurons elicited SI impairments, similarly to those observed in mice lacking CB1 in cortical glutamatergic neurons (Jacob et al., 2009). By contrast, it did not affect the preference for social novelty with female stimulus.

Interestingly, D1–CB1$^{-/-}$ mice showed sustained auditory-cued and contextual fear responses, thus resembling the phenotype of impaired fear adaptation observed in mice with complete deletion of CB1Rs (Marsicano et al., 2002; Kamprath et al., 2006) or selective deletion from principal neurons of the forebrain (Kamprath et al., 2009). Since Monory et al. (2007) showed that the deletion of

CB1 in dopamine D1-expressing neurons did not alter the analgesic effects of THC, we can exclude the possibility that the phenotype of D1–CB1$^{-/-}$ mice in the FC paradigms could be due to different nociceptive thresholds.

Currently, we do not know exactly how the CB1Rs modulate D1Rs-mediated emotional behavior. However, due to their coexpression, it is tempting to assume that a direct or indirect receptor–receptor interaction, via intracellular signaling pathways, might be involved (Glass et al., 1997; Gangarossa et al., 2011). In support of this hypothesis, Martín et al. (2008) showed in rats that pharmacological CB1R blockade or activation could facilitate or inhibit animal behavior, respectively; and this latter effect was absent in D1Rs knock-out mice, demonstrating a DR1s dependence on CB1-mediated actions. Thus, in the highly aversive situations of FC paradigms, where a strong stimulus as a footshock was delivered, the EC signaling failed. This happens due to the CB1 deletion, to negatively modulate the D1Rs-emotional behavior, leading to an impaired fear adaptation. However, we cannot rule out the involvement of different pathways as well as the potential compensatory mechanisms occurring during development, which represents a limitation of experiments with mutant mice in general. Nevertheless, the present data add a new facet to the cross-talk between DAergic and the EC systems, within the framework of fear adaptation.

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REFERENCES

Bernabeu, R., Bevilaqua, L., Ardenghi, P., Bromberg, E., Schmitz, P., Bianchin, M., Izquierdo, I., and Medina, J. H. (1997). Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. *Proc. Natl. Acad. Sci. U.S.A.* 94, 7041–7046.

Bolles, R. C. (1960). Grooming behavior in the rat. *J. Comp. Physiol. Psychol.* 53, 306–310.

Crawley, J. N., Chen, T., Puri, A., Washburn, R., Sullivan, T. L., Hill, J. M., Young, N. B., Nadler, J. J., Moy, S. S., Young, L. J., Caldwell, H. K., and Young, W. S. (2007). Social approach behaviors in oxytocin knockout mice: comparison of two independent lines tested in different laboratory environments. *Neuropeptides* 41, 145–163.

Cryan, J. F., and Holmes, A. (2005). The ascent of mouse: advances in modeling human depression and anxiety. *Nat. Rev. Drug Discov.* 4, 775–790.

Di Marzo, V. (2008). Targeting the endocannabinoid system: to enhance or reduce? *Nat. Rev. Drug Discov.* 7, 438–455.

Dunn, A. J., Guild, A. L., Kramarcy, N. R., and Ware, M. D. (1981). Benzodiazepines decrease grooming in response to novelty but not ACTH or beta-endorphin. *Pharmacol. Biochem. Behav.* 15, 605–608.

El-Ghundi, M., O'Dowd, B. F., and George, S. R. (2001). Prolonged fear responses in mice lacking dopamine D1 receptor. *Brain Res.* 892, 86–93.

File, S. E. (1992). Usefulness of animal models with newer anxiolytics. *Clin. Neuropharmacol.* 15, 525A–526A.

Gangarossa, G., Di Benedetto, M., O'Sullivan, G. J., Dunleavy, M., Alcacer, C., Bonito-Oliva, A., Henshall, D. C., Waddington, J. L., Valjent, E., and Fisone, G. (2011). Convulsant doses of a dopamine D1 receptor agonist result in Erk-dependent increases in Zif268 and Arc/Arg3.1 expression in mouse dentate gyrus. *PLoS ONE* 6, e19415. doi: 10.1371/journal.pone.0019415

Gispen, W. H., and Isaacson, R. L. (1981). ACTH-induced excessive grooming in the rat. *Pharmacol. Ther.* 12, 209–246.

Glass, M., Brodie, J. M., and Maneuf, Y. P. (1997). Modulation of neurotransmission by cannabinoids in the basal ganglia. *Eur. J. Neurosci.* 9, 199–203.

Haller, J., Barna, I., Barsvari, B., Gyimesi Pelczer, K., Yasar, S., Panlilio, L. V., and Goldberg, S. (2009). Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats. *Psychopharmacology (Berl.)* 204, 607–616.

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

Hermann, H., Marsicano, G., and Lutz, B. (2002). Coexpression of the cannabinoid receptor type 1 with dopamine and serotonin receptors in distinct neuronal subpopulations of the adult mouse forebrain. *Neuroscience* 109, 451–460.

Jacob, W., Yassouridis, A., Marsicano, G., Monory, K., Lutz, B., and Wotjak, C. T. (2009). Endocannabinoids render exploratory behaviour largely independent of the test aversiveness: role of glutamatergic transmission. *Genes Brain Behav.* 8, 685–698.

Kalueff, A. V., and Tuohimaa, P. (2005). Contrasting grooming phenotypes in three mouse strains markedly different in anxiety and activity (129S1, BALB/c and NMRI). *Behav. Brain Res.* 160, 1–10.

Kamei, H., Kameyama, T., and Nabeshima, T. (1995). Activation of both dopamine D1 and D2 receptors necessary for amelioration of conditioned fear stress. *Eur. J. Pharmacol.* 273, 229–233.

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

Kamprath, K., Plendl, W., Marsicano, G., Deussing, J. M., Wurst, W., Lutz, B., and Wotjak, C. T. (2009). Endocannabinoids mediate acute fear adaptation via glutamatergic neurons independently of corticotropin-releasing hormone signaling. *Genes Brain Behav.* 8, 203–211.

Kamprath, K., and Wotjak, C. T. (2004). Nonassociative learning processes determine expression and extinction of conditioned fear in mice. *Learn. Mem.* 11, 770–786.

Laviolette, S. R., and Grace, A. A. (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.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annu. Rev. Neurosci.* 23, 155–184.

Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. *Pharmacol. Ther.* 46, 321–340.

Marsicano, G., and 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., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., Hermann, H., Tang, J., Hofmann, C., Zieglgänsberger, W., Di Marzo, V., and Lutz, B. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418, 530–534.

Martín, A. B., Fernandez-Espejo, E., Ferrer, B., Gorriti, M. A., Bilbao, A., Navarro, M., Rodriguez de Fonseca, F., and Moratalla, R. (2008). Expression and function of CB1 receptor in the rat striatum: localization and effects on D1 and D2 dopamine receptor-mediated motor behaviors. *Neuropsychopharmacology* 33, 1667–1679.

Micale, V., Tamburella, A., Leggio, G. M., Mazzola, C., Li Volsi, V., and Drago, F. (2008). Behavioral effects of saredutant, a tachykinin NK2 receptor antagonist, in experimental models of mood disorders under basal and stress-related conditions. *Pharmacol. Biochem. Behav.* 90, 463–469.

Monory, K., Blaudzun, H., Massa, F., Kaiser, N., Lemberger, T., Schütz, G., Wotjak, C. T., Lutz, B., and Marsicano, G. (2007). Genetic dissection of behavioural and autonomic effects of delta (9)-tetrahydrocannabinol in mice. *PLoS Biol.* 5, e269. doi: 10.1371/journal.pbio.0050269

Monory, K., Massa, F., Egertová, M., Eder, M., Blaudzun, H., Westenbroek, R., Kelsch, W., Jacob, W., Marsch, R., Ekker, M., Long, J., Rubenstein, J. L., Goebbels, S., Nave, K. A., During, M., Klugmann, M., Wölfel, B., Dodt, H. U., Zieglgänsberger, W., Wotjak, C. T., Mackie, K., Elphick, M. R., Marsicano, G., and Lutz, B. (2006). The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* 51, 455–466.

Moreira, F. A., and Wotjak, C. T. (2010). Cannabinoids and anxiety. *Curr. Top. Behav. Neurosci.* 2, 429–450.

Nagai, T., Takuma, K., Kamei, H., Ito, Y., Nakamichi, N., Ibi, D., Nakanishi, Y., Murai, M., Mizoguchi, H., Nabeshima, T., and Yamada, K. (2007). Dopamine D1 receptors regulate protein synthesis-dependent long-term recognition memory via extracellular signal-regulated kinase 1/2 in the prefrontal cortex. *Learn. Mem.* 14, 117–125.

Pamplona, F. A., Henes, K., Micale, V., Mauch, C. P., Takahashi, R. N., and Wotjak, C. T. (2011). Prolonged fear incubation leads to generalized avoidance behavior in mice. *J. Psychiatr. Res.* 45, 354–360.

Pellow, S., Chopin, P., and File, S. E. (1985). Are the anxiogenic effects of yohimbine mediated by its action at benzodiazepine receptors? *Neurosci. Lett.* 55, 5–9.

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

Plendl, W., and Wotjak, C. T. (2010). Dissociation of within- and between-session extinction of conditioned fear. *J. Neurosci.* 30, 4990–4998.

Porsolt, R. D., Bertin, A., and Jalfre, M. (1978). "Behavioural despair" in rats and mice: strain differences and the effects of imipramine. *Eur. J. Pharmacol.* 51, 291–294.

Smit-Rigter, L. A, Wadman, W. J., and van Hooft, J. A. (2010). Impaired social behavior in 5-HT(3A) receptor knockout mice. *Front. Behav. Neurosci.* 4:169. doi: 10.3389/fnbeh.2010.00169

Strekalova, T., and Steinbusch, H. W. (2010). Measuring behavior in mice with chronic stress depression paradigm. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 348–361.

Thiemann, G., Fletcher, B. C., Ledent, C., Molleman, A., and Hasenöhrl, R. U. (2007). The genetic versus pharmacological invalidation of the cannabinoid CB(1) receptor results in differential effects on "non-associative" memory and forebrain monoamine concentrations in mice. *Neurobiol. Learn. Mem.* 88, 416–423.

van Erp, A. M., Kruk, M. R., Meelis, W., and Willekens-Bramer, D. C. (1994). Effect of environmental stressors on time course, variability and form of self-grooming in the rat: handling, social contact, defeat, novelty, restraint, and fur moistening. *Behav. Brain Res.* 65, 47–55.

Zenko, M., Zhu, Y., Dremencov, E., Ren, W., Xu, L., and Zhang, X. (2011). Requirement for the endocannabinoid system in social interaction impairment induced by coactivation of dopamine D1 and D2 receptors in the piriform cortex. *J. Neurosci. Res.* 89, 1245–1258.

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Cannabinoid CB1 and dopamine D1 receptors partnership in the modulation of emotional neural processing

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A commentary on

The dopamine and cannabinoid interaction in the modulation of emotions and cognition: assessing the role of cannabinoid CB1 receptor in neurons expressing dopamine D1 receptors
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The contribution of dopamine and cannabinoid neurotransmission in emotional brain circuits regulating motivational and emotional neural processing has been well acknowledged by both animal and clinical studies (LeDoux, 2000; Laviolette and Grace, 2006). The endocannabinoid system has arisen as part of the complex circuitry that regulates stress and as a crucial mediator of emotional learning (for a comprehensive review see Viveros et al., 2005). The idea that this system is involved in the control of emotions is rooted in the fact that *Cannabis sativa* is used recreationally, mainly for its euphoric effects. The dopamine (DA) neurotransmitter system is significantly crucial for the neural processing of motivational and emotionally salient information (Wise, 2004). Yet, less is known about the functional interaction between cannabinoid and dopaminergic receptors in the control of emotional behavior. Much of our understanding has been improved only recently by studies using knockout (KO) animals (Micale et al., 2009; Ortega-Alvaro et al., 2011; Thanos et al., 2011), behavioral approaches (Ramiro-Fuentes and Fernandez-Espejo, 2011; Zarrindast et al., 2011), or neuroanatomical and electrophysiological techniques (Chiu et al., 2010).

In an intriguing article of the Special Issue "The endocannabinoid system: a key modulator of emotions and cognition" published in Frontiers in Behavioral Neuroscience, Terzian and colleagues provide the first evidence for a physiological cross-talk between the cannabinoid CB1 receptors (CB1Rs) and the dopamine D1 receptors (D1Rs) in the modulation of depression-like behavior, social skills, and fear conditioning. Specifically, these authors examined the responses of conditional CB1 mutant mice genetically selected for the absence of CB1Rs exclusively in neurons containing D1Rs receptors (D1–CB1 KO mice), in a battery of behavioral tests, and reported the following interesting results. First, when compared to their WT counterparts, D1–CB1 KO mice displayed similar performance in the social novelty, the elevated plus maze, and the light/dark test (all paradigms evaluating different aspects of unconditioned anxiety), but spent more time on grooming activity and showed less social interaction when tested under low aversive conditions. By measuring the anxiety-like profile of these animals using different tests, authors thus disentangled different components of the anxiety state, bringing to light the specific role of CB1–D1 receptors in modulating emotional states under conditions of stress-induced conflict/frustration (grooming behavior) or inescapable situations (social interactions). Secondly, D1–CB1 KO mice showed lower sucrose consumption than WT mice, a behavior reminiscent of a mild anhedonia-like state (commonly associated with depression). However, the finding that such a difference disappears on the second day of testing does not support a depressive-like behavioral phenotype, as also suggested by the observation that KO mice performed as WTs in the forced swim test (commonly used to evaluate animals' behavioral despair). Finally, in the fear conditioning task (a fear-related memory test involving a strong mnemonic component), D1–CB1 KO mice showed significantly increased auditory-cued and contextual fear responses, which is not surprising when considering the important role of DA receptors in fear adaptation processes (El-Ghundi et al., 2001; de la Mora et al., 2010) and that of CB1 receptors in fear alleviation (Marsicano et al., 2002; Kamprath et al., 2006).

The main outcome of this study is the demonstration that CB1Rs and D1Rs cooperate for the control of a negative affect. This implies that D1R and CB1R signaling systems may mediate overlapping pharmacological responses in clinically important brain areas that mediate diseases, such as Parkinson and epilepsy, in which these two classes of receptors have been reported to strictly interact (Ferrer et al., 2003; Gangarossa et al., 2011).

Although limited by potential compensatory mechanisms that may take place during development, these conditional animals represent a precious tool for investigating whether CB1 and D1 receptors also interact in modulating other brain circuits (motivation, reward) and behavioral traits (impulsivity/compulsivity), which may contribute to the development of several disorders (i.e., binge eating disorder). The involvement of the two receptor systems in motivational and emotional neural processing phenomena also suggests that their interaction might be implicated in other neuropathologies such as schizophrenia and addiction (Grace, 1995, 2000; Zavitsanou et al., 2004; Semple et al., 2005).

Perhaps the most robust effect demonstrated by Terzian and colleagues involves the enhancement of conditioned fear and the possible attenuation of extinction learning. This is an important step in advancing our knowledge on the functional interaction between D1Rs and CB1Rs in emotional neural processing and specifically fear adaptation. However, further studies are required to clarify the mechanisms underlying cannabinoids modulation of the DAergic system. For example, it needs to be determined what are the specific neuronal circuits mediating the effects on fear retrieval and fear adaptation in the D1–CB1 KO mice. Another issue to focus on is short-

and long-term extinction in these mutant mice. The ability to extinguish emotional responses in the face of a no-longer relevant conditioned cue is an essential part of a healthy emotional memory system (Charney et al., 1993) and deficits in fear extinction are thought to contribute to anxiety disorders such as post-traumatic stress disorder (PTSD). It has been consistently demonstrated that CB1R-deficient mice and CB1R antagonists block fear extinction. The results by Terzian et al. (2011) suggest that this may also be the case with the D1–CB1 KO mice. This should be carefully examined as it is a clinically relevant issue to ask whether or not a stimulation of D1R–CB1R signaling might accelerate extinction of fear and hence, might be therapeutically effective in the treatment of anxiety disorders, particularly PTSD.

In conclusion, findings here reported by Terzian and colleagues represent an appealing topic of investigation from both pharmacological and pharmaceutical points of view in that it provides a rationale of developing in the future chemical compounds that, by manipulating simultaneously both the D1Rs and CB1Rs, may ameliorate negative and aversive emotional states.

REFERENCES

Charney, D. S., Davis, M., Deutch, A. Y., Krystal, J. H., and Southwick, S. M. (1993). Psychobiologic mechanisms of posttraumatic stress disorder. *Arch. Gen. Psychiatry* 50, 295–305.

Chiu, C. Q., Puente, N., Grandes, P., and Castillo, P. E. (2010). Dopaminergic modulation of endocannabinoid-mediated plasticity at GABAergic synapses in the prefrontal cortex. *J. Neurosci.* 30, 7236–7248.

de la Mora, M. P., Gallegos-Cari, A., Arizmendi-García, Y., Marcellino, D., and Fuxe, K. (2010). Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: structural and functional analysis. *Prog. Neurobiol.* 90, 198–216.

El-Ghundi, M., O'Dowd, B. F., and George, S. R. (2001). Prolonged fear responses in mice lacking dopamine D1 receptor. *Brain Res.* 892, 86–93.

Ferrer, B., Asbrock, N., Kathuria, S., Piomelli, D., and Giuffrida, A. (2003). Effects of levodopa on endocannabinoid levels in rat basal ganglia: implications for the treatment of levodopa-induced dyskinesias. *Eur. J. Neurosci.* 18, 1607–1614.

Gangarossa, G., Di Benedetto, M., O'Sullivan, G. J., Dunleavy, M., Alcacer, C., Bonito-Oliva, A., Henshall, D. C., Waddington, J. L., Valjent, E., and Fisone, G. (2011). Convulsant doses of a dopamine D1 receptor agonist result in Erk-dependent increases in Zif268 and Arc/Arg3.1 expression in mouse dentate gyrus. *PLoS ONE* 6, e19415. doi: 10.1371/journal.pone.0019415

Grace, A. A. (1995). The tonic/phasic model of dopamine system regulation: its relevance for understanding how stimulant abuse can alter basal ganglia function. *Drug Alcohol Depend.* 37, 111–129.

Grace, A. A. (2000). Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Res. Brain Res. Rev.* 31, 330–341.

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

Laviolette, S. R., and Grace, A. A. (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.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annu. Rev. Neurosci.* 23, 155–184.

Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., Hermann, H., Tang, J., Hofmann, C., Zieglgänsberger, W., Di Marzo, V., and Lutz, B. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418, 530–534.

Micale, V., Cristino, L., Tamburella, A., Petrosino, S., Leggio, G. M., Drago, F., and Di Marzo, V. (2009). Altered responses of dopamine D3 receptor null mice to excitotoxic or anxiogenic stimuli: possible involvement of the endocannabinoid and endovanilloid systems. *Neurobiol. Dis.* 36, 70–80.

Ortega-Alvaro, A., Aracil-Fernández, A., García-Gutiérrez, M. S., Navarrete, F., and Manzanares, J. (2011). Deletion of CB2 cannabinoid receptor induces schizophrenia-related behaviors in mice. *Neuropsychopharmacology* 36, 1489–1504.

Ramiro-Fuentes, S., and Fernandez-Espejo, E. (2011). Sensitization to cocaine is inhibited after intra-accumbal GR103691 or rimonabant, but it is enhanced after co-infusion indicating functional interaction between accumbens D(3) and CB1 receptors. *Psychopharmacology (Berl.)* 214, 949–959.

Semple, D. M., McIntosh, A. M., and Lawrie, S. M. (2005). Cannabis use as a risk factor for psychosis: systematic review. *J. Psychopharmacol.* 19, 187–194.

Terzian, A. L., Drago, F., Wotjak, C. T., and Micale, V. (2011). The dopamine and cannabinoid interaction in the modulation of emotions and cognition: assessing the role of cannabinoid CB1 receptor in neurons expressing dopamine D1 receptors. *Front. Behav. Neurosci.* 5:49. doi: 10.3389/fnbeh.2011.00049

Thanos, P. K., Gopez, V., Delis, F., Michaelides, M., Grandy, D. K., Wang, G. J., Kunos, G., and Volkow, N. D. (2011). Upregulation of cannabinoid type 1 receptors in dopamine D2 receptor knockout mice is reversed by chronic forced ethanol consumption. *Alcohol. Clin. Exp. Res.* 35, 19–27.

Viveros, M. P., Marco, E. M., and File, S. E. (2005). Endocannabinoid system and stress and anxiety responses. *Pharmacol. Biochem. Behav.* 81, 331–342.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nat. Rev. Neurosci.* 5, 483–494.

Zarrindast, M. R., Mahboobi, S., Sadat-Shirazi, M. S., and Ahmadi, S. (2011). Anxiolytic-like effect induced by the cannabinoid CB1 receptor agonist, arachydonilcyclopropylamide (ACPA), in the rat amygdala is mediated through the D1 and D2 dopaminergic systems. *J. Psychopharmacol.* 25, 131–140.

Zavitsanou, K., Garrick, T., and Huang, X. F. (2004). Selective antagonist SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulated cortex in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 28, 355–360.

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Altering endocannabinoid neurotransmission at critical developmental ages: impact on rodent emotionality and cognitive performance

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The endocannabinoid system shows functional activity from early stages of brain development: it plays an important role in fundamental developmental processes such as cell proliferation, migration, and differentiation, thus shaping brain organization during pre- and postnatal life. *Cannabis sativa* preparations are among the illicit drugs most commonly used by young people, including pregnant women. The developing brain can be therefore exposed to cannabis preparations during two critical periods: first, in offspring of cannabis-using mothers through perinatal and/or prenatal exposure; second, in adolescent cannabis users during neural maturation. In the last decade, it has become clear that the endocannabinoid system critically modulates memory processing and emotional responses. Therefore, it is well possible that developmental exposure to cannabinoid compounds induces enduring changes in behaviors and neural processes belonging to the cognitive and emotional domains. We address this issue by focusing on rodent studies, in order to provide a framework for understanding the impact of cannabinoid exposure on the developing brain.

Keywords: endocannabinoid system, behavior, development, emotion, cognition, pregnancy, adolescence

INTRODUCTION

The endocannabinoid system consists of two types of G-protein-coupled receptors (CB_1, highly expressed in the brain, and CB_2, more abundant in immune cells), their endogenous lipid ligands, and the enzymatic machinery for their synthesis and degradation (Piomelli, 2003; Di Marzo et al., 2005; De Petrocellis and Di Marzo, 2009). Endogenous ligands for cannabinoid receptors, i.e., endocannabinoids [mainly anandamide and 2-arachidonoylglycerol (2-AG)], are synthesized on demand in an activity-dependent manner and released from postsynaptic neurons (Freund et al., 2003; Piomelli, 2003). Once released into the synaptic cleft, the newly synthesized endocannabinoids travel in retrograde direction and bind to cannabinoid receptors on presynaptic terminals (Freund et al., 2003; Piomelli, 2003). The primary consequences of activation of cannabinoid receptors are regulation of ion channel activity and neurotransmitter release (Szabo and Schlicker, 2005). Thus, by acting on cannabinoid receptors on both excitatory and inhibitory terminals, endocannabinoids play a major role in several forms of short- and long-term synaptic plasticity (Freund et al., 2003; Piomelli, 2003; Chevaleyre et al., 2006). Endocannabinoid modulation of synaptic activity affects several biological functions, including regulation of emotionality and cognitive performance (Wotjak, 2005; Moreira and Lutz, 2008; Campolongo et al., 2009a,b; Lutz, 2009; Akirav, 2011; Marco et al., 2011; Rubino and Parolaro, 2011; Terzian et al., 2011; Zanettini et al., 2011). It has indeed repeatedly been shown that cannabis exposure produces a wide range of subjective emotional effects in humans (Tunving, 1985; Williamson and Evans, 2000; Degenhardt et al., 2003; Di Forti et al., 2007; Murray et al., 2007; Fattore and Fratta, 2011). Furthermore, many clinical studies have reported that acute challenges with or prolonged use of cannabis and its products may impair attentional processing and working memory in humans (Iversen, 2003; Ranganathan and D'Souza, 2006; Pattij et al., 2008; Solowij and Pesa, 2010; Fattore and Fratta, 2011). These observations have their counterpart in animal studies, showing that cannabinoid compounds elicit dose-dependent and environment-dependent anxiolytic and anxiogenic effects in rodent models of anxiety (Onaivi et al., 1990; Rodriguez de Fonseca et al., 1996, 1997; Haller et al., 2002, 2004a,b; Martin et al., 2002; Kathuria et al., 2003; Wotjak, 2005; Bortolato et al., 2006; Moreira et al., 2008; Marco et al., 2011), and affect learning and memory in rodents (Castellano et al., 2003; Riedel and Davies, 2005; Wotjak, 2005; Schneider et al., 2008; Suenaga and Ichitani, 2008; Baek et al., 2009; Marsicano and Lafenetre, 2009; Akirav, 2011).

In both humans and rodents, the endocannabinoid system is present and active in the central nervous system (CNS)

from early developmental ages (Berrendero et al., 1998; Fernandez-Ruiz et al., 2000; Mato et al., 2003; Fride, 2004; Galve-Roperh et al., 2006; Harkany et al., 2007) and continues to develop throughout adolescence (Rodriguez de Fonseca et al., 1993; Belue et al., 1995; Romero et al., 1997; Berrendero et al., 1999). Therefore, developmental exposure to cannabinoid compounds can have profound effects on brain architecture, chemistry and neurobehavioral function, by changing for instance neurotransmitter levels, and by modulating expression of their receptors, transporters, and degrading enzymes.

Developmental studies on the effects of cannabinoid drugs are of special relevance for two main reasons. First, cannabis preparations are the illicit drugs most widely used by young people, peaking between 15 and 30 years of age (NIDA, 2005; Hall and Degenhardt, 2009; SAMHSA, 2009). Importantly, there is an emerging trend for continued cannabis use in people aged 30–40 (NIDA, 2005; SAMHSA, 2009). This pattern of use potentially exposes the developing brain to cannabis at two periods: first, in offspring of cannabis-using mothers during the perinatal and/or prenatal period; second, in adolescent cannabis users during neural maturation. Therefore, a better understanding of the mechanisms by which exposure to cannabinoid drugs during development leads to neurobehavioral alterations or induces neuropsychiatric disorders later in life is an important issue. Furthermore, in addition to the well-known therapeutic effects of drugs directly acting at cannabinoid receptors (e.g., as appetite stimulants, anti-emetics, analgesics in neuropathic pain; Pacher et al., 2006; Di Marzo, 2009; Bermudez-Silva et al., 2010), the endocannabinoid system is now emerging as a novel therapeutic target for the treatment of the emotional and cognitive disturbances that characterize some neuropsychiatric disorders (Piomelli et al., 2006; Vinod and Hungund, 2006; Petrosino and Di Marzo, 2010; Marco et al., 2011), including neurodevelopmental disorders. However, the potential therapeutic application of cannabinoid drugs in young populations requires a profound investigation of possible adverse effects of such compounds, particularly on the CNS of immature individuals.

In order to provide a deeper understanding of the long-lasting, subtle neurobehavioral effects of developmental exposure to cannabinoid drugs, and to adopt effective public health strategies, it is critical to stimulate a dialog between human and animal studies. While studies in humans are, of course, most relevant for understanding the human situation, they can only provide limited information about the specific molecular and cellular consequences that underlie drug-induced behavioral and neural changes. The important advantage of animal studies is that they allow for exquisite control over the possible confounding factors that characterize human studies, and for examination of the independent contribution of a certain drug to adverse neurodevelopmental consequences.

Here, we examine and discuss preclinical evidence for how cannabinoid exposure during critical developmental ages, such as the perinatal, prenatal, and adolescent periods, affects emotionality and cognitive performance in rodents, thus providing a framework for understanding the impact of cannabinoid exposure on the developing brain.

EFFECTS OF DEVELOPMENTAL EXPOSURE TO CANNABINOIDS ON COGNITIVE PERFORMANCE IN RODENTS

PRENATAL AND PERINATAL CANNABINOID EXPOSURE

First, we will briefly summarize the results of human studies that investigated the consequences of developmental exposure to cannabinoids on cognitive performance, and then we will focus on rodent studies.

Since the late 1970s, two extended longitudinal cohort studies, the Ottawa Prenatal Prospective Study (OPPS) and the Maternal Health Practices and Child Development Study (MHPCD), have been measuring the cognitive functions of children born from mothers who consumed *Cannabis sativa* preparations during pregnancy (Day et al., 1992; Fried, 2002b; Trezza et al., 2008b; Campolongo et al., 2009c, 2010). These studies showed that the consequences of prenatal exposure to cannabis are rather subtle. Immediately after birth, there is little evidence for a prenatal cannabis effect either upon growth or behavior (Fried and Watkinson, 1988). However, beyond the age of 3, there are findings suggesting an association between prenatal cannabis exposure and aspects of cognitive behavior that fall in the domain of executive functions (Fried and Watkinson, 1990; Day et al., 1992, 1994; Fried et al., 1998; Fried and Smith, 2001; Fried, 2002b; Trezza et al., 2008b). Executive functions refer to higher-order cognitive functions such as cognitive flexibility, sustained and focused attention, planning and working memory. These functions enable us to organize and manage the many tasks in our daily life; for instance, to account for short- and long-term consequences of our actions, to make real time evaluations of our actions, and make necessary adjustments if these actions are not achieving the desired results. Impairments in executive functions have a major impact on our ability to perform tasks as planning, prioritizing, organizing, paying attention to and remembering details, and controlling our emotional reactions. In particular, the facets of executive functions which appear to be affected by cannabis exposure are the domains of attention/impulsivity and problem solving situations requiring integration and manipulation of basic visuoperceptual skills (Fried and Watkinson, 1990; Day et al., 1992, 1994; Fried et al., 1998; Fried and Smith, 2001; Fried, 2002b; Trezza et al., 2008b). The deficits in executive functions induced by prenatal cannabis exposure seem to be long-lasting, since 18- to 22-year-old young adults exposed to cannabis during pregnancy showed altered neuronal functioning during visuospatial working memory processing (Smith et al., 2006).

Although there is a convergence of evidence in human studies, the very limited number of studies which have followed children beyond the age of 3 emphasizes the need for further, well-controlled investigations in this area. Furthermore, it cannot be excluded from human studies that genetic and environmental variables also contribute to the relationship between maternal cannabis use and long-term cognitive deficits in the offspring. Therefore, the long-term effects of prenatal exposure to cannabinoid drugs on cognitive functions in rodents have received a great deal of attention. Prenatal exposure to a moderate dose of the synthetic CB_1 cannabinoid receptor agonist WIN55,212-2 (0.5 mg/kg from GD 5 to GD 20) has been shown to induce

a disruption of memory retention in 40- and 80-day-old rat offspring tested in the inhibitory avoidance task (Mereu et al., 2003). This cognitive impairment was not due to alterations of non-associative nature, since the approach latency during the acquisition trials of the task was unaffected. The memory impairment in WIN55,212-2-exposed offspring was associated with alterations in hippocampal long-term potentiation (Mereu et al., 2003). *In vivo* microdialysis experiments also showed a significant decrease in basal and K^+-evoked extracellular glutamate levels in the hippocampus of juvenile and adult rats born from WIN55,212-2-treated dams (Mereu et al., 2003). The decrease in hippocampal glutamate overflow was suggested to be the cause of disrupted long-term potentiation, which could, in turn, underlie the long-lasting memory impairment caused by gestational exposure to the cannabinoid receptor agonist (Mereu et al., 2003). To further support the hypothesis that changes in glutamatergic neurotransmission might be responsible of the cognitive impairment observed in WIN55,212-2-exposed offspring, *in vivo* microdialysis experiments showed that basal and K^+-evoked glutamate levels were significantly lower in the cerebral cortex of both adult (90-day-old) and adolescent (40-day-old) rats exposed to WIN55,212-2 during gestation than in those born from vehicle-treated mothers (Antonelli et al., 2004; Castaldo et al., 2007; Ferraro et al., 2009). Interestingly, the cognitive deficits induced by prenatal exposure to WIN55,212-2 appeared already at early developmental ages. Thus, 10- to 12-day-old WIN55,212-2-exposed pups showed a poorer performance in homing behavior, a simple form of learning occurring during the early phases of postnatal life (Antonelli et al., 2005). At the neurochemical level, basal and K^+-evoked glutamate levels were significantly lower in primary cell cultures of hippocampus (Mereu et al., 2003) and cerebral cortex (Antonelli et al., 2005, 2006) obtained from pups exposed to WIN55,212-2 compared to pups from the control group. The alteration of cortical glutamate transmission induced by prenatal WIN55,212-2 exposure was also associated with a significant reduction of NMDA receptor-mediated regulation of glutamate levels (Ferraro et al., 2009). In fact, the NMDA-induced concentration-dependent increase of glutamate levels observed in cortical cell cultures obtained from neonates born from vehicle-treated dams was completely lost in cell cultures obtained from pups prenatally exposed to WIN55,212-2 (Antonelli et al., 2005). These results suggest that chronic prenatal treatment with WIN55,212-2 induces a loss of NMDA receptor activity in the exposed offspring (Antonelli et al., 2005; Ferraro et al., 2009).

Morphological experiments have shown that prenatal exposure to WIN55,212-2 also affects neuronal proliferation: a different neurite growth pattern was observed in cortical cell cultures obtained from pups born from mothers exposed to WIN55,212-2 during pregnancy (Antonelli et al., 2005; Ferraro et al., 2009). Cortical cell cultures from vehicle-exposed pups showed a high number of healthy neurons, which developed in a monolayer to form a complex network of neurites. On the contrary, cortical cultures obtained from pups exposed to WIN55,212-2 during pregnancy showed a minor population of neurons and abnormal neurite outgrowth, characterized by impairments of neurite branching (Antonelli et al., 2005; Ferraro et al., 2009).

Exposure to cannabinoid agonists during critical periods of brain development is known to cause long-term changes in the functionality of several neurotransmitter systems in adulthood, such as alterations in dopaminergic (Rodriguez de Fonseca et al., 1991; Bonnin et al., 1994, 1995), opioidergic (Vela et al., 1995, 1998), serotonergic (Molina-Holgado et al., 1996), and GABAergic (Garcia-Gil et al., 1999a) systems. In addition, prenatal exposure to WIN55,212-2 has been found to induce long-term changes in the activity of the endocannabinoid system: in particular, the functionality of CB_1 receptors in the hippocampus differed between adult WIN55,212-2- and vehicle-exposed offspring (Castelli et al., 2007). Thus, it can be speculated on basis of the *in vitro* and *in vivo* results that gestational WIN55,212-2-exposure produces enduring alterations of the endocannabinoid system in the developing brain, which may lead to a long-lasting and irreversible disruption of glutamate cortical and hippocampal function (Castelli et al., 2007; Ferraro et al., 2009).

As for the clinical relevance of these preclinical studies, it is important to estimate, by extrapolation, whether the dose of the synthetic cannabinoid agonist WIN55,212-2 is comparable to that of the main active ingredient of cannabis, Δ9-tetrahydrocannabinol (THC), absorbed by cannabis users. It has been estimated that a dose of 5 mg/kg of THC in rats corresponds to a moderate exposure to the drug in humans, correcting for the differences in route of administration and body weight surface area (Garcia-Gil et al., 1997, 1999a,b). WIN55,212-2 has been found to be 3–10 times more potent than THC, depending on the administration route and the behavioral endpoints considered (Compton et al., 1992; French et al., 1997; Hampson et al., 2000). This mirrors the CB_1 receptor affinity rank order for the two drugs (Matsuda, 1997; Pertwee, 1997). Based on these considerations, the dose of WIN55,212-2 used in the studies described above corresponds to a moderate, or even to a low, exposure to cannabis in humans (Mereu et al., 2003). Furthermore, in line with studies that used a protocol of prenatal WIN55,212-2 exposure, it has been demonstrated that the active ingredient of cannabis, THC, administered during the perinatal period at a dose (5 mg/kg, per os, from GD 15 to PND 9) that is not associated with gross malformations and/or overt signs of toxicity, induces cognitive impairments in the adult offspring (Campolongo et al., 2007). Importantly, perinatal exposure to THC not only induced a long-term memory impairment in the adult offspring, as revealed by the inhibitory avoidance test, but also a disruption in short-term olfactory memory, as assessed in the social discrimination test (Campolongo et al., 2007). This form of memory, that plays a crucial role in the processing of social information, requires integral glutamatergic projections from the hippocampal formation to prefrontal areas (Steckler et al., 1998; McGaugh, 2002), and then back from the prefrontal cortex to the hippocampus. Interestingly, the cognitive impairments observed in THC-exposed adult offspring were associated with long-lasting alterations in the cortical expression of genes related to glutamatergic neurotransmission, together with a decrease in the cortical extracellular levels of this neurotransmitter (Campolongo et al., 2007). Furthermore, in line with studies that used a protocol of prenatal WIN55,212-2 exposure, the neurochemical changes induced by prenatal THC exposure appeared early in development, as altered regulation of glutamate release and

decreased functional activity and expression of GLT1 and GLAST glutamate transporters in the hippocampus of adolescent rats perinatally exposed to THC have been reported (Castaldo et al., 2010). Again, these studies strongly suggest that changes in glutamatergic neurotransmission might be responsible for the cognitive deficits induced by prenatal cannabinoid exposure.

ADOLESCENT CANNABINOID EXPOSURE

In most Western Countries, the first episodes of cannabis use often occur during adolescence (NIDA, 2005; Hall and Degenhardt, 2009; SAMHSA, 2009). Adolescence is a critical phase for CNS development during the transition from childhood to adulthood (Spear, 2000; Andersen, 2003). It is a period characterized by widespread neuronal plasticity and maturation at the neural and network level, when the brain undergoes both progressive and regressive changes including extensive synaptic remodeling and pruning and alterations in neurotransmitter levels and their receptors in cortical and limbic brain regions across different species (Spear, 2000; Andersen, 2003), processes in which the endocannabinoid system plays a major role (Spear, 2000; Andersen, 2003; Freund et al., 2003; Bossong and Niesink, 2011; Rubino et al., 2011).

Both neuropsychological and functional imaging studies indicate that the detrimental effects of cannabis on cognitive performance may be more pronounced when cannabis is used during adolescence (Ehrenreich et al., 1999; Jager and Ramsey, 2008; Schweinsburg et al., 2008; Bossong and Niesink, 2011). Most imaging studies in adolescent subjects reported cannabis-induced alterations in working memory (Jacobsen et al., 2004, 2007; Schweinsburg et al., 2008). Studies making a distinction between the initiation of cannabis use in adolescence and in adult life showed attention deficits and poor cognitive performance in early-onset cannabis users (onset before age 17), but not in late-onset users or control subjects (Ehrenreich et al., 1999; Pope Jr. et al., 2003).

Despite the increasing use of cannabis among adolescents and the sometimes conflicting results provided by clinical studies, it is only in recent years that the short- and long-term behavioral effects of acute and chronic adolescent exposure to cannabinoid compounds in rodents have been investigated in more detail (Rubino and Parolaro, 2008; Trezza et al., 2008b; Realini et al., 2009; Rubino et al., 2011).

Quinn et al. (2008) showed that adolescent but not adult rats displayed significantly impaired object recognition memory and altered protein expression profiles in the hippocampus following repeated THC exposure. Similarly, Schneider and Koch (2003) showed that chronic pubertal treatment with WIN55,212-2 resulted in impaired object recognition memory in adulthood, associated with disrupted prepulse inhibition of the acoustic startle response and lower break points in a progressive-ratio operant behavioral task (Schneider and Koch, 2003). Again, it is worth noting that if the chronic cannabinoid treatment was administered during adulthood, none of the tested behaviors was affected (Schneider and Koch, 2003). Gender-specific effects of chronic adolescent cannabinoid exposure have also been reported (O'Shea et al., 2004, 2006). In these studies, the cannabinoid receptor agonist CP-55,940 was administered daily for 21 consecutive days to either adolescent or adult male and female rats. Following a long drug-free period, working memory was assessed in the object recognition task (O'Shea et al., 2004, 2006). In females, cannabinoid-treated adolescent, but not adult rats demonstrated impaired working memory compared to vehicle-treated controls (O'Shea et al., 2004, 2006). Interestingly, in males, cannabinoid treatment during adolescence and adulthood produced similar working memory deficits (O'Shea et al., 2004). Thus, in females, adolescents may be more susceptible and adults more resilient to long-lasting cannabinoid-induced cognitive deficits, whereas in males, both adolescents and adults are equally vulnerable. Deficits in object recognition memory have also been reported in adult female rats treated chronically with THC during adolescence (Realini et al., 2011).

Developmental and gender sensitivity to cannabinoid compounds has been further investigated by Cha et al. (2006, 2007), who assessed spatial memory in the Morris water maze task following acute and chronic THC exposure in male and female adolescent and adult rats. Acute THC exposure led to greater learning impairments in adolescent than in adult male and female rats tested in both the spatial and non-spatial versions of the water maze tasks (Cha et al., 2006, 2007). Conversely, chronic THC administration during either adolescence or adulthood had no effect on spatial learning in animals of both sexes tested after a long drug-free period (Cha et al., 2006, 2007). Thus, while adolescents may be more sensitive to the acute effects of cannabinoids, both adolescents and adults demonstrated similar recovery of cognitive performance following discontinuation of chronic treatment (Cha et al., 2006, 2007). In line with these findings, it has been reported that adolescent exposure to the cannabinoid receptor agonist CP-55,940 did not affect adult performance of animals of both sexes in the water maze task (Higuera-Matas et al., 2009). However, following adolescent exposure to THC, spatial working memory in the radial maze task was impaired in both male and female adult rats, while aversive memory in the inhibitory avoidance task was unaffected (Rubino et al., 2009a,b). The neural underpinnings of the spatial working memory impairments observed in the latter studies may differ between males and females (Rubino and Parolaro, 2011). Indeed, adult female rats showed reduced levels of proteins involved in synaptic plasticity and altered pattern of protein expression in synaptosomes from prefrontal cortex, with no alterations in the hippocampus (Rubino et al., 2009a). Conversely, in adult male rats pre-exposed to THC during adolescence, the spatial working memory deficit was related to reduced levels of markers of neuroplasticity and morphological alterations in the hippocampus (Rubino et al., 2009b). These results suggest that the same protocol of adolescent THC exposure, although resulting in similar behavioral endpoints, may have different neuronal consequences in the brain of male or female rats.

Long-term sexually dimorphic effects induced by adolescent THC exposure on cognitive performance have also been described by Harte and Dow-Edwards (2010), who examined the effects of adolescent THC exposure on visual spatial learning in adulthood using the active place avoidance test. This cognitive task allows to simultaneously assess the ability to learn and retrieve spatial information, as well as flexibility of learning, by training animals to actively move over a slowly rotating arena and avoid an unmarked

sector, entering which is punished by a mild footshock. The shock sector is defined in a stable position with respect to the experimental room. Animals must thus localize the shock sector exclusively by its spatial relations to distal orienting cues located in the room and walk into the safe part of the arena in a direction opposite to arena rotation (Cimadevilla et al., 2000). By using this task, Harte and Dow-Edwards (2010) showed that THC administration during early adolescence had no effect on the acquisition of the task. However, male and female animals treated with THC during early adolescence made more errors on the reversal trial requiring flexibility in learning. Conversely, THC administration during late adolescence had no effect in both sexes. Therefore, early adolescence appeared to be more sensitive to the cognitive effects of THC than late adolescence (Harte and Dow-Edwards, 2010). These findings indicate that the time window during adolescence in which THC is administered can have a profound influence on its long-lasting cognitive outcomes.

SUMMARY

Taken together, the preclinical studies outlined here show that maternal and adolescent exposure to either natural or synthetic cannabinoid agonists alters cognitive performance in the offspring. The cognitive alterations displayed by cannabinoid-treated rats are long-lasting, since they persist into adulthood. Furthermore, in line with clinical observations, it appears from preclinical studies that adolescent rats may be more susceptible than adults to the cognitive effects induced by chronic exposure to cannabinoid compounds.

EFFECTS OF DEVELOPMENTAL EXPOSURE TO CANNABINOIDS ON EMOTIONALITY IN RODENTS

PRENATAL AND PERINATAL CANNABINOID EXPOSURE

Although *C. sativa* preparations have long been known to produce a wide range of subjective emotional effects, it is only in recent years that the crucial role of the endocannabinoid system in the modulation of emotional states has been underscored (Haller et al., 2002; Millan, 2003; Witkin et al., 2005; Mangieri and Piomelli, 2007; Trezza et al., 2008a; Bambico et al., 2009; Marco and Viveros, 2009; Marco et al., 2011; Zanettini et al., 2011). It has, indeed, been shown that CB_1 cannabinoid receptors are highly expressed in brain areas involved in the modulation of emotions (Tsou et al., 1998; Ameri, 1999; Davies et al., 2002). In these regions, endocannabinoids modulate the release of neurotransmitters and neuropeptides that play a key role in the control of emotionality, such as serotonin, dopamine (Tsou et al., 1998; Katona et al., 2001; Schlicker and Kathmann, 2001; Hermann et al., 2002) and the anxiogenic neuropeptides, CCK and CRF (Rodriguez de Fonseca et al., 1997; Ameri, 1999). Therefore, it is well conceivable that *in utero* cannabis exposure might produce changes in the emotional reactivity of the exposed offspring. Human studies support this hypothesis, by showing that prenatal exposure to cannabis in the first and third trimesters of pregnancy predicts levels of self-reported anxiety and depressive symptoms in children (Goldschmidt et al., 2004; Gray et al., 2005; Leech et al., 2006). Again, however, only few clinical studies followed the exposed children past the age of 10 (Fried, 2002a,b; Fried et al., 2003; Goldschmidt et al., 2004; Gray et al., 2005; Leech et al., 2006), so that most of the available information about the long-term consequences of *in utero* cannabis exposure on the emotional reactivity of the offspring comes from preclinical studies.

Concerning the neonatal age, we found that 12-day-old pups exposed to THC during the perinatal period displayed an increased rate of ultrasonic vocalizations (USVs) when separated from the mother and siblings compared to the control group (Trezza et al., 2008a). The USV test has been extensively validated and widely used to investigate the ontogeny of emotionality (Insel et al., 1986; Cuomo et al., 1987; Branchi et al., 2001, 2004). USVs are emitted by rodent pups in response to separation from the mother and the nest and play an important communicative role in mother–offspring interaction. They are, indeed, a potent stimulus for maternal retrieval and elicit caregiving behaviors in the dam (Farrell and Alberts, 2002; Trezza et al., 2011). As high rates of USVs are generally indicative of an anxiety-like state, the present results show that perinatal exposure to THC induces an increased emotional reactivity of the offspring (Trezza et al., 2008a). Conversely, a reduction of separation-induced USVs in rat pups either prenatally exposed to the synthetic cannabinoid agonist WIN55,212-2 (Antonelli et al., 2005) or acutely treated with the synthetic cannabinoid agonist CP-55,940 (McGregor et al., 1996) has also been reported, highlighting how different time windows of exposure to cannabinoids can induce opposite neurofunctional effects (Costa et al., 2004). However, differences in the cannabinoid agonist used, tested dose, and treatment schedule (acute vs. chronic treatment) could also account for the apparent discrepancies between these preclinical findings. Interestingly, the alterations we observed in the emotional reactivity of THC-exposed pups were long-lasting (Trezza et al., 2008a). Indeed, at adolescence, THC-exposed rats displayed lower social activity than controls in the social interaction test (Trezza et al., 2008a). These results are in agreement with findings showing that the synthetic cannabinoid agonist CP-55,940, repeatedly administered from PND 4 to PND 25, reduced social interaction in 60-day-old rats (O'Shea et al., 2006). In adulthood, THC-exposed rats showed increased anxiety in the elevated plus-maze: they spent more time in the closed arms of the maze, exhibited a significantly lower number of head dippings and a higher number of stretched-attend postures than vehicle-exposed rats (Trezza et al., 2008a). The number of total entries, however, was unaffected, indicating that perinatal THC treatment did not alter locomotor activity in the offspring. To further support an altered emotional reactivity induced by perinatal THC exposure, Newsom and Kelly reported that adult rats perinatally exposed to THC spent less time in the central part of an open field arena compared to vehicle-exposed animals, with no changes in general locomotor activity (Newsom and Kelly, 2008).

ADOLESCENT CANNABINOID EXPOSURE

The possible causal relation between cannabis use during adolescence and psychotic and affective neuropsychiatric diseases later in life is widely debated. While some clinical studies indicate that exposure to cannabis preparations during adolescence may be a risk factor for neuropsychiatric disorders such as schizophrenia, depression, and other mood pathologies (Arseneault et al., 2002; Fergusson et al., 2002, 2003; Patton et al., 2002; Degenhardt et al.,

2003; Stefanis et al., 2004; Hayatbakhsh et al., 2007; Moore et al., 2007; Lee et al., 2008), other authors have found no strong evidence that cannabis use by young people induces deleterious mental health outcomes (Iversen, 2003; Macleod et al., 2004, 2007; de Graaf et al., 2010). Therefore, human studies are still inconclusive as to whether cannabis use during adolescence has a direct causal influence on psychotic, depressive, and/or anxiety disorders later in life, whether cannabis exposure and subsequent psychopathology are related by a common liability, or if the association results from a combination of correlated and causal processes.

ANXIETY-RELATED BEHAVIORS

Despite the fact that the majority of preclinical studies supports the hypothesis that adolescent exposure to cannabinoid drugs alters emotional reactivity in adulthood, inconsistent and sometimes sex-dependent effects have also been reported (Rubino et al., 2011). For instance, some authors reported no changes in emotional reactivity in animals pretreated with cannabinoid drugs during adolescence and tested in the elevated plus-maze test after a washout period (Rubino et al., 2008; Higuera-Matas et al., 2009; Bambico et al., 2010), while others described cannabinoid-induced anxiolytic-like effects in the same behavioral test (Biscaia et al., 2003; Wegener and Koch, 2009). Contrasting results also emerged from other behavioral tests commonly used to assess emotional reactivity in rodents. For instance, cannabinoid exposure during adolescence induced anxiety-like behaviors in the novelty-suppressed feeding test (Bambico et al., 2010), which assesses anxiety-induced hyponeophagia by measuring the inhibition of ingestion and approach to food when animals are exposed to an anxiety-provoking novel environment. Conversely, no evidence of increased anxiety induced by adolescent cannabinoid exposure was found in adult rats tested in the emergence test (O'Shea et al., 2006), that measures the animal's conflict between exploring a novel environment, and avoiding an open area. When emotionality was assessed by measuring exploratory behavior and the time spent in the central and peripheral parts of an open field arena, some authors reported no effects of adolescent cannabinoid exposure (Rubino et al., 2008; Bambico et al., 2010), while others reported anxiolytic-like responses (Biscaia et al., 2003; Wegener and Koch, 2009).

SOCIAL BEHAVIOR

More consistent results have been obtained when the social interaction test was used to assess the emotional reactivity of adult rats exposed to cannabinoid drugs during adolescence. The synthetic cannabinoid agonist CP-55,940, administered for 21 days to adolescent rats, reduced social interaction at adulthood, both in male (O'Shea et al., 2006) and female (O'Shea et al., 2004) subjects. Similar results have been reported following chronic adolescent treatment with THC (Realini et al., 2011) or the synthetic cannabinoid receptor agonist WIN55,212-2 (Leweke and Schneider, 2011). There are many internal and external factors that influence an animal's sociability, and anxiety has been identified as one of them (File and Seth, 2003). Therefore, reduced social interaction is widely interpreted as reflecting increased anxiety. However, it can not be excluded from social interaction experiments that changes in sociability reflect other aspects of social behavior, such as social reward, or the subjective interpretation of social signals, that might also be affected by adolescent cannabinoid exposure. For instance, we have recently shown that the endocannabinoid system modulates the most abundant and rewarding form of social interaction displayed by adolescent mammals, that is social play behavior (Trezza et al., 2010). In particular, we found that systemic administration of indirect cannabinoid receptor agonists, i.e., drugs that increase endocannabinoid signaling by interfering with endocannabinoid deactivation, enhances social play, through interaction with opioid and dopaminergic neurotransmission (Trezza and Vanderschuren, 2008a,b, 2009). This suggests that during social play, endocannabinoids are released in brain areas mediating this behavior. Increased endocannabinoid activity might facilitate social play, so that drugs that prevent endocannabinoid deactivation likely enhance social play by magnifying the ongoing endocannabinoid tone. In contrast, we have also previously shown that stimulation of CB_1 cannabinoid receptors throughout the brain using the cannabinoid receptor agonist WIN55,212-2 or the stable analog of anandamide, (R)-methanandamide reduced social play (Trezza and Vanderschuren, 2008a,b, 2009). Therefore, it appears from these studies that the effects of cannabinoid drugs on social behavior differ according to the way the endocannabinoid system is targeted: drugs that prevent endocannabinoid deactivation enhance rewarding aspects of social interactions by magnifying ongoing endocannabinoid tone; conversely, drugs that directly activate cannabinoid receptors in multiple brain areas reduce sociability, perhaps by disrupting cognitive functions necessary to perform adequate social interactions (Egerton et al., 2006).

DEPRESSIVE-LIKE BEHAVIORS

Alongside changes in anxiety-related and social behaviors, other facets of emotionality are also affected by adolescent cannabinoid exposure. Thus, chronic treatment with both synthetic and natural cannabinoid agonists resulted in behavioral despair in adulthood, assessed as increased immobility in the forced swimming test, and anhedonia, measured as decreased sucrose preference in the sucrose-preference test (Rubino et al., 2008; Bambico et al., 2010; Realini et al., 2011). Other measures of anhedonia, such as impairment of progressive-ratio instrumental responding for food rewards and changes in sleep–wake cycle have also been reported following adolescent cannabinoid exposure (Schneider and Koch, 2003, 2005). Interestingly, as already reported for cannabis-induced cognitive impairments, the depression-like phenotype did not develop when the chronic cannabinoid administration was performed in older animals (Schneider and Koch, 2003, 2005; Bambico et al., 2010; Realini et al., 2011), confirming that the adult brain is less susceptible to the deleterious impact of chronic cannabinoid exposure.

As for the neural substrates underlying the depressive-like behaviors induced by adolescent cannabinoid exposure, electrophysiological recordings revealed that adolescent but not adult chronic cannabinoid treatment attenuated serotonergic neurotransmission in the dorsal raphe nucleus, while it induced a significant increase in noradrenergic neurotransmission in the locus coeruleus (Bambico et al., 2010). It has recently been

proposed that the endocannabinoid system regulates affective homeostasis by interacting with monoaminergic neurotransmission (for review, see Bambico et al., 2009). Thus, activation of cannabinoid receptors by cannabinoid receptor agonists modulates serotonin (Gobbi et al., 2005; Palazzo et al., 2006; Bambico et al., 2007) and noradrenaline (Gobbi et al., 2005; Oropeza et al., 2005, 2007) activity. CB_1 receptors are expressed on serotonergic neurons in the dorsal raphe nucleus (Elphick and Egetova, 2000; Haring et al., 2007) as well as on noradrenergic neurons in the locus coeruleus (Oropeza et al., 2007). Furthermore, they are highly expressed in limbic mood-regulatory brain areas innervated by these nuclei, such as the amygdala (for review, see Bambico and Gobbi, 2008; Bambico et al., 2009). During adolescence, serotonergic, noradrenergic, and cannabinoid neurotransmission undergo critical changes (Spear, 2000; Schneider, 2008). Thus, chronic cannabinoid exposure during adolescence may interfere with the cross-talk between these neural systems, eventually leading to persistent affective dysfunctions.

Interestingly, it has been shown that the depression-like phenotype displayed by adult rats treated with cannabinoid drugs during adolescence was paralleled by changes in other biochemical parameters linked to depression, such as decreased CREB activation in the prefrontal cortex and hippocampus, increased CREB activation and dynorphin levels in the nucleus accumbens, decreased neurogenesis in the dentate gyrus of the hippocampus, likely triggered by a long-lasting impairment of CB_1 receptor signaling in the ventral tegmental area, amygdala, and nucleus accumbens (Rubino et al., 2008; Realini et al., 2011; Rubino and Parolaro, 2011). Since endocannabinoid neurotransmission in these brain areas is fundamental for normal emotional behavior and stress responses (Viveros et al., 2005; Laviolette and Grace, 2006; Zanettini et al., 2011), then changes in cannabinoid receptor function induced by adolescent cannabinoid exposure might underlie the altered emotional responses in adulthood.

SUMMARY

Altogether, the preclinical studies currently available show that prenatal and adolescent cannabinoid exposure affects different aspects of emotional reactivity, from early developmental ages till adulthood. In particular, it appears from preclinical studies that the outcome of developmental cannabinoid exposure on emotional reactivity later in life might depend on the specific component of emotionality taxed in the different behavioral tests. For instance, anxiety-related behaviors in tests that depend on spontaneous, exploratory behavior, such as the elevated plus-maze and open field tests, appear be more resistant to the long-term consequences of cannabinoid exposure. On the other hand, the anxiety-related measures in the novelty-suppressed feeding test, that depends on appetitive drive, and the reduction in social behavior observed in the social interaction test appear to be particularly sensitive to developmental cannabinoid exposure. The differences observed at the behavioral level might also be the result of the different neuroanatomical and molecular correlates involved in each behavioral test. The changes in anxiety- and depressive-like behaviors and the altered sociability induced by developmental cannabinoid exposure might, in turn, affect the ability of the subject to cope with every day challenges and with fellow group members. This hypothesis, however, needs to be further investigated.

CONCLUSIONS

The endocannabinoid system plays a relevant role in brain organization during pre- and post-natal life. In Western countries, *C. sativa* preparations are among the illicit drugs most commonly used by young people, including pregnant women. Therefore, understanding the long-lasting consequences of cannabis exposure on the developing brain is an important issue. The clinical findings currently available suggest an association between developmental cannabis exposure and executive dysfunctions. Furthermore, cannabis exposure during the prenatal/perinatal and adolescent periods has been shown to induce subtle changes in emotionality that may persist into adulthood. Although there is some consistency in the clinical literature, the very limited number of findings emphasizes the need for further, well-controlled follow-up studies in this area. Relevant information is available from preclinical studies, demonstrating that even low to moderate doses of cannabinoids, when administered during particular periods of brain development, can have profound consequences for brain maturation, leading to long-lasting alterations of cognitive functions and emotional behaviors. Although there is still scarce information about the neurobiological substrates of the observed behavioral alterations, it appears that developmental cannabinoid exposure induces changes in the endocannabinoid system and in other neurotransmitter systems that are already functional at early developmental ages. These alterations may disrupt the integrity of mood- and cognition-regulating brain circuits, thus inducing long-lasting emotional and cognitive disturbances.

Multiple experimental approaches, including genetics, molecular biology, pharmacology, neuroanatomy, and neurophysiology, in both preclinical and clinical settings should be encouraged in the near future to further clarify the potential relationship between developmental cannabis exposure and long-lasting neurofunctional outcomes.

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REFERENCES

Akirav, I. (2011). The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus. *Front. Behav. Neurosci.* 5:34. doi: 10.3389/fnbeh.2011.00034

Ameri, A. (1999). The effects of cannabinoids on the brain. *Prog. Neurobiol.* 58, 315–348.

Andersen, S. L. (2003). Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci. Biobehav. Rev.* 27, 3–18.

Antonelli, T., Tanganelli, S., Tomasini, M. C., Finetti, S., Trabace, L., Steardo, L., Sabino, V., Carratu, M. R., Cuomo, V., and Ferraro, L. (2004). Long-term effects on cortical glutamate release

induced by prenatal exposure to the cannabinoid receptor agonist (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinyl-methyl)pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone: an in vivo microdialysis study in the awake rat. *Neuroscience* 124, 367–375.

Antonelli, T., Tomasini, M. C., Tattoli, M., Cassano, T., Finetti, S., Mazzoni, E., Trabace, L., Carratu, M. R., Cuomo, V., Tanganelli, S., and Ferraro, L. (2006). Prenatal exposure to the cannabinoid receptor agonist WIN 55,212-2 and carbon monoxide reduces extracellular glutamate levels in primary rat cerebral cortex cell cultures. *Neurochem. Int.* 49, 568–576.

Antonelli, T., Tomasini, M. C., Tattoli, M., Cassano, T., Tanganelli, S., Finetti, S., Mazzoni, E., Trabace, L., Steardo, L., Cuomo, V., and Ferraro, L. (2005). Prenatal exposure to the CB1 receptor agonist WIN 55,212-2 causes learning disruption associated with impaired cortical NMDA receptor function and emotional reactivity changes in rat offspring. *Cereb. Cortex* 15, 2013–2020.

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A., and Moffitt, T. E. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *BMJ* 325, 1212–1213.

Baek, J., Zheng, Y., Darlington, C. L., and Smith, P. F. (2009). The CB1 receptor agonist, WIN 55,212-2, dose-dependently disrupts object recognition memory in adult rats. *Neurosci. Lett.* 464, 71–73.

Bambico, F. R., Duranti, A., Tontini, A., Tarzia, G., and Gobbi, G. (2009). Endocannabinoids in the treatment of mood disorders: evidence from animal models. *Curr. Pharm. Des.* 15, 1623–1646.

Bambico, F. R., and Gobbi, G. (2008). The cannabinoid CB1 receptor and the endocannabinoid anandamide: possible antidepressant targets. *Expert Opin. Ther. Targets* 12, 1347–1366.

Bambico, F. R., Katz, N., Debonnel, G., and Gobbi, G. (2007). Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. *J. Neurosci.* 27, 11700–11711.

Bambico, F. R., Nguyen, N. T., Katz, N., and Gobbi, G. (2010). Chronic exposure to cannabinoids during adolescence but not during adulthood impairs emotional behaviour and monoaminergic neurotransmission. *Neurobiol. Dis.* 37, 641–655.

Belue, R. C., Howlett, A. C., Westlake, T. M., and Hutchings, D. E. (1995). The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. *Neurotoxicol. Teratol.* 17, 25–30.

Bermudez-Silva, F. J., Viveros, M. P., Mcpartland, J. M., and Rodriguez de Fonseca, F. (2010). The endocannabinoid system, eating behavior and energy homeostasis: the end or a new beginning? *Pharmacol. Biochem. Behav.* 95, 375–382.

Berrendero, F., Garcia-Gil, L., Hernandez, M. L., Romero, J., Cebeira, M., De Miguel, R., Ramos, J. A., and Fernandez-Ruiz, J. J. (1998). Localization of mRNA expression and activation of signal transduction mechanisms for cannabinoid receptor in rat brain during fetal development. *Development* 125, 3179–3188.

Berrendero, F., Sepe, N., Ramos, J. A., Di Marzo, V., and Fernandez-Ruiz, J. J. (1999). Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestation and early postnatal period. *Synapse* 33, 181–191.

Biscaia, M., Marin, S., Fernandez, B., Marco, E. M., Rubio, M., Guaza, C., Ambrosio, E., and Viveros, M. P. (2003). Chronic treatment with CP 55,940 during the peri-adolescent period differentially affects the behavioural responses of male and female rats in adulthood. *Psychopharmacology (Berl.)* 170, 301–308.

Bonnin, A., De Miguel, R., Hernandez, M. L., Ramos, J. A., and Fernandez-Ruiz, J. J. (1995). The prenatal exposure to delta 9-tetrahydrocannabinol affects the gene expression and the activity of tyrosine hydroxylase during early brain development. *Life Sci.* 56, 2177–2184.

Bonnin, A., De Miguel, R., Rodriguez-Manzaneque, J. C., Fernandez-Ruiz, J. J., Santos, A., and Ramos, J. A. (1994). Changes in tyrosine hydroxylase gene expression in mesencephalic catecholaminergic neurons of immature and adult male rats perinatally exposed to cannabinoids. *Brain Res. Dev. Brain Res.* 81, 147–150.

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

Bossong, M. G., and Niesink, R. J. (2011). Adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia. *Prog. Neurobiol.* 92, 370–385.

Branchi, I., Campolongo, P., and Alleva, E. (2004). Scopolamine effects on ultrasonic vocalization emission and behavior in the neonatal mouse. *Behav. Brain Res.* 151, 9–16.

Branchi, I., Santucci, D., and Alleva, E. (2001). Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. *Behav. Brain Res.* 125, 49–56.

Campolongo, P., Roozendaal, B., Trezza, V., Cuomo, V., Astarita, G., Fu, J., McGaugh, J. L., and Piomelli, D. (2009a). Fat-induced satiety factor oleoylethanolamide enhances memory consolidation. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8027–8031.

Campolongo, P., Roozendaal, B., Trezza, V., Hauer, D., Schelling, G., Mcgaugh, J. L., and Cuomo, V. (2009b). Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4888–4893.

Campolongo, P., Trezza, V., Palmery, M., Trabace, L., and Cuomo, V. (2009c). Developmental exposure to cannabinoids causes subtle and enduring neurofunctional alterations. *Int. Rev. Neurobiol.* 85, 117–133.

Campolongo, P., Trezza, V., Cassano, T., Gaetani, S., Morgese, M. G., Ubaldi, M., Soverchia, L., Antonelli, T., Ferraro, L., Massi, M., Ciccocioppo, R., and Cuomo, V. (2007). Perinatal exposure to delta-9-tetrahydrocannabinol causes enduring cognitive deficits associated with alteration of cortical gene expression and neurotransmission in rats. *Addict. Biol.* 12, 485–495.

Campolongo, P., Trezza, V., Ratano, P., Palmery, M., and Cuomo, V. (2010). Developmental consequences of perinatal cannabis exposure: behavioral and neuroendocrine effects in adult rodents. *Psychopharmacology (Berl.)* 214, 5–15.

Castaldo, P., Magi, S., Cataldi, M., Arcangeli, S., Lariccia, V., Nasti, A. A., Ferraro, L., Tomasini, M. C., Antonelli, T., Cassano, T., Cuomo, V., and Amoroso, S. (2010). Altered regulation of glutamate release and decreased functional activity and expression of GLT1 and GLAST glutamate transporters in the hippocampus of adolescent rats perinatally exposed to Delta(9)-THC. *Pharmacol. Res.* 61, 334–341.

Castaldo, P., Magi, S., Gaetani, S., Cassano, T., Ferraro, L., Antonelli, T., Amoroso, S., and Cuomo, V. (2007). Prenatal exposure to the cannabinoid receptor agonist WIN 55,212-2 increases glutamate uptake through overexpression of GLT1 and EAAC1 glutamate transporter subtypes in rat frontal cerebral cortex. *Neuropharmacology* 53, 369–378.

Castellano, C., Rossi-Arnaud, C., Cestari, V., and Costanzi, M. (2003). Cannabinoids and memory: animal studies. *Curr. Drug Targets CNS Neurol. Disord.* 2, 389–402.

Castelli, M. P., Paola Piras, A., D'agostino, A., Pibiri, F., Perra, S., Gessa, G. L., Maccarrone, M., and Pistis, M. (2007). Dysregulation of the endogenous cannabinoid system in adult rats prenatally treated with the cannabinoid agonist WIN 55,212-2. *Eur. J. Pharmacol.* 573, 11–19.

Cha, Y. M., Jones, K. H., Kuhn, C. M., Wilson, W. A., and Swartzwelder, H. S. (2007). Sex differences in the effects of delta9-tetrahydrocannabinol on spatial learning in adolescent and adult rats. *Behav. Pharmacol.* 18, 563–569.

Cha, Y. M., White, A. M., Kuhn, C. M., Wilson, W. A., and Swartzwelder, H. S. (2006). Differential effects of delta9-THC on learning in adolescent and adult rats. *Pharmacol. Biochem. Behav.* 83, 448–455.

Chevaleyre, V., Takahashi, K. A., and Castillo, P. E. (2006). Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu. Rev. Neurosci.* 29, 37–76.

Cimadevilla, J. M., Kaminsky, Y., Fenton, A., and Bures, J. (2000). Passive and active place avoidance as a tool of spatial memory research in rats. *J. Neurosci. Methods* 102, 155–164.

Compton, D. R., Gold, L. H., Ward, S. J., Balster, R. L., and Martin, B. R. (1992). Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from delta 9-tetrahydrocannabinol. *J. Pharmacol. Exp. Ther.* 263, 1118–1126.

Costa, L. G., Steardo, L., and Cuomo, V. (2004). Structural effects and neurofunctional sequelae of developmental exposure to psychotherapeutic drugs: experimental and clinical aspects. *Pharmacol. Rev.* 56, 103–147.

Cuomo, V., De Salvia, M. A., Maselli, M. A., Santo, L., and Cagiano, R. (1987). Ultrasonic calling in rodents: a new experimental approach in behavioural toxicology. *Neurotoxicol. Teratol.* 9, 157–160.

Davies, S. N., Pertwee, R. G., and Riedel, G. (2002). Functions of cannabinoid

receptors in the hippocampus. *Neuropharmacology* 42, 993–1007.

Day, N., Cornelius, M., Goldschmidt, L., Richardson, G., Robles, N., and Taylor, P. (1992). The effects of prenatal tobacco and marijuana use on offspring growth from birth through 3 years of age. *Neurotoxicol. Teratol.* 14, 407–414.

Day, N. L., Richardson, G. A., Goldschmidt, L., Robles, N., Taylor, P. M., Stoffer, D. S., Cornelius, M. D., and Geva, D. (1994). Effect of prenatal marijuana exposure on the cognitive development of offspring at age three. *Neurotoxicol. Teratol.* 16, 169–175.

Degenhardt, L., Hall, W., and Lynskey, M. (2003). Exploring the association between cannabis use and depression. *Addiction* 98, 1493–1504.

de Graaf, R., Radovanovic, M., Van Laar, M., Fairman, B., Degenhardt, L., Aguilar-Gaxiola, S., Bruffaerts, R., De Girolamo, G., Fayyad, J., Gureje, O., Haro, J. M., Huang, Y., Kostychenko, S., Lepine, J. P., Matschinger, H., Mora, M. E., Neumark, Y., Ormel, J., Posada-Villa, J., Stein, D. J., Tachimori, H., Wells, J. E., and Anthony, J. C. (2010). Early cannabis use and estimated risk of later onset of depression spells: epidemiologic evidence from the population-based World Health Organization World Mental Health Survey Initiative. *Am. J. Epidemiol.* 172, 149–159.

De Petrocellis, L., and Di Marzo, V. (2009). An introduction to the endocannabinoid system: from the early to the latest concepts. *Best Pract. Res. Clin. Endocrinol. Metab.* 23, 1–15.

Di Forti, M., Morrison, P. D., Butt, A., and Murray, R. M. (2007). Cannabis use and psychiatric and cognitive disorders: the chicken or the egg? *Curr. Opin. Psychiatry* 20, 228–234.

Di Marzo, V. (2009). The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. *Pharmacol. Res.* 60, 77–84.

Di Marzo, V., De Petrocellis, L., and Bisogno, T. (2005). The biosynthesis, fate and pharmacological properties of endocannabinoids. *Handb. Exp. Pharmacol.* 168, 147–185.

Egerton, A., Allison, C., Brett, R. R., and Pratt, J. A. (2006). Cannabinoids and prefrontal cortical function: insights from preclinical studies. *Neurosci. Biobehav. Rev.* 30, 680–695.

Ehrenreich, H., Rinn, T., Kunert, H. J., Moeller, M. R., Poser, W., Schilling, L., Gigerenzer, G., and Hoehe, M. R. (1999). Specific attentional dysfunction in adults following early start of cannabis use. *Psychopharmacology (Berl.)* 142, 295–301.

Elphick, M. R., and Egetova, M. (2000). Cannabinoid signalling in the nervous system: comparative localization of CB1 receptors and fatty acid amide hydrolase in rat and mouse brain. *Eur. J. Neurosci.* 12, 68.

Farrell, W. J., and Alberts, J. R. (2002). Stimulus control of maternal responsiveness to Norway rat (*Rattus norvegicus*) pup ultrasonic vocalizations. *J. Comp. Psychol.* 116, 297–307.

Fattore, L., and Fratta, W. (2011). Beyond THC: the new generation of cannabinoid designer drugs. *Front. Behav. Neurosci.* 5:60. doi: 10.3389/fnbeh.2011.00060

Fergusson, D. M., Horwood, L. J., and Swain-Campbell, N. (2002). Cannabis use and psychosocial adjustment in adolescence and young adulthood. *Addiction* 97, 1123–1135.

Fergusson, D. M., Horwood, L. J., and Swain-Campbell, N. R. (2003). Cannabis dependence and psychotic symptoms in young people. *Psychol. Med.* 33, 15–21.

Fernandez-Ruiz, J., Berrendero, F., Hernandez, M. L., and Ramos, J. A. (2000). The endogenous cannabinoid system and brain development. *Trends Neurosci.* 23, 14–20.

Ferraro, L., Tomasini, M. C., Beggiato, S., Gaetani, S., Cassano, T., Cuomo, V., Amoroso, S., Tanganelli, S., and Antonelli, T. (2009). Short- and long-term consequences of prenatal exposure to the cannabinoid agonist WIN55,212-2 on rat glutamate transmission and cognitive functions. *J. Neural Transm.* 116, 1017–1027.

File, S. E., and Seth, P. (2003). A review of 25 years of the social interaction test. *Eur. J. Pharmacol.* 463, 35–53.

French, E. D., Dillon, K., and Wu, X. (1997). Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *Neuroreport* 8, 649–652.

Freund, T. F., Katona, I., and Piomelli, D. (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev* 83, 1017–1066.

Fride, E. (2004). The endocannabinoid-CB(1) receptor system in pre- and postnatal life. *Eur. J. Pharmacol.* 500, 289–297.

Fried, P. A. (2002a). Adolescents prenatally exposed to marijuana: examination of facets of complex behaviors and comparisons with the influence of in utero cigarettes. *J. Clin. Pharmacol.* 42, 97S–102S.

Fried, P. A. (2002b). Conceptual issues in behavioral teratology and their application in determining long-term sequelae of prenatal marihuana exposure. *J. Child Psychol. Psychiatry* 43, 81–102.

Fried, P. A., and Smith, A. M. (2001). A literature review of the consequences of prenatal marihuana exposure. An emerging theme of a deficiency in aspects of executive function. *Neurotoxicol. Teratol.* 23, 1–11.

Fried, P. A., and Watkinson, B. (1988). 12- and 24-month neurobehavioural follow-up of children prenatally exposed to marihuana, cigarettes and alcohol. *Neurotoxicol. Teratol.* 10, 305–313.

Fried, P. A., and Watkinson, B. (1990). 36- and 48-month neurobehavioral follow-up of children prenatally exposed to marijuana, cigarettes, and alcohol. *J. Dev. Behav. Pediatr.* 11, 49–58.

Fried, P. A., Watkinson, B., and Gray, R. (1998). Differential effects on cognitive functioning in 9- to 12-year olds prenatally exposed to cigarettes and marihuana. *Neurotoxicol. Teratol.* 20, 293–306.

Fried, P. A., Watkinson, B., and Gray, R. (2003). Differential effects on cognitive functioning in 13- to 16-year-olds prenatally exposed to cigarettes and marihuana. *Neurotoxicol. Teratol.* 25, 427–436.

Galve-Roperh, I., Aguado, T., Rueda, D., Velasco, G., and Guzman, M. (2006). Endocannabinoids: a new family of lipid mediators involved in the regulation of neural cell development. *Curr. Pharm. Des.* 12, 2319–2325.

Garcia-Gil, L., De Miguel, R., Munoz, R. M., Cebeira, M., Villanua, M. A., Ramos, J. A., and Fernandez-Ruiz, J. J. (1997). Perinatal delta(9)-tetrahydrocannabinol exposure alters the responsiveness of hypothalamic dopaminergic neurons to dopamine-acting drugs in adult rats. *Neurotoxicol. Teratol.* 19, 477–487.

Garcia-Gil, L., De Miguel, R., Romero, J., Perez, A., Ramos, J. A., and Fernandez-Ruiz, J. J. (1999a). Perinatal delta9-tetrahydrocannabinol exposure augmented the magnitude of motor inhibition caused by GABA(B), but not GABA(A), receptor agonists in adult rats. *Neurotoxicol. Teratol.* 21, 277–283.

Garcia-Gil, L., Romero, J., Ramos, J. A., and Fernandez-Ruiz, J. J. (1999b). Cannabinoid receptor binding and mRNA levels in several brain regions of adult male and female rats perinatally exposed to delta9-tetrahydrocannabinol. *Drug Alcohol Depend.* 55, 127–136.

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

Goldschmidt, L., Richardson, G. A., Cornelius, M. D., and Day, N. L. (2004). Prenatal marijuana and alcohol exposure and academic achievement at age 10. *Neurotoxicol. Teratol.* 26, 521–532.

Gray, K. A., Day, N. L., Leech, S., and Richardson, G. A. (2005). Prenatal marijuana exposure: effect on child depressive symptoms at ten years of age. *Neurotoxicol. Teratol.* 27, 439–448.

Hall, W., and Degenhardt, L. (2009). Adverse health effects of non-medical cannabis use. *Lancet* 374, 1383–1391.

Haller, J., Bakos, N., Szirmay, M., Ledent, C., and Freund, T. F. (2002). The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur. J. Neurosci.* 16, 1395–1398.

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

Haller, J., Varga, B., Ledent, C., and Freund, T. F. (2004b). CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. *Behav. Pharmacol.* 15, 299–304.

Hampson, R. E., Mu, J., and Deadwyler, S. A. (2000). Cannabinoid and kappa opioid receptors reduce potassium K current via activation of G(s) proteins in cultured hippocampal neurons. *J. Neurophysiol.* 84, 2356–2364.

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

Harkany, T., Guzman, M., Galve-Roperh, I., Berghuis, P., Devi, L. A., and Mackie, K. (2007). The emerging functions of endocannabinoid signaling during CNS development. *Trends Pharmacol. Sci.* 28, 83–92.

Harte, L. C., and Dow-Edwards, D. (2010). Sexually dimorphic alterations in locomotion and reversal learning after adolescent tetrahydrocannabinol exposure in the rat. *Neurotoxicol. Teratol.* 32, 515–524.

Hayatbakhsh, M. R., Najman, J. M., Jamrozik, K., Mamun, A. A., Alati, R., and Bor, W. (2007). Cannabis

and anxiety and depression in young adults: a large prospective study. *J. Am. Acad. Child Adolesc. Psychiatry* 46, 408–417.

Hermann, H., Marsicano, G., and Lutz, B. (2002). Coexpression of the cannabinoid receptor type 1 with dopamine and serotonin receptors in distinct neuronal subpopulations of the adult mouse forebrain. *Neuroscience* 109, 451–460.

Higuera-Matas, A., Botreau, F., Miguens, M., Del Olmo, N., Borcel, E., Perez-Alvarez, L., Garcia-Lecumberri, C., and Ambrosio, E. (2009). Chronic periadolescent cannabinoid treatment enhances adult hippocampal PSA-NCAM expression in male Wistar rats but only has marginal effects on anxiety, learning and memory. *Pharmacol. Biochem. Behav.* 93, 482–490.

Insel, T. R., Hill, J. L., and Mayor, R. B. (1986). Rat pup ultrasonic isolation calls: possible mediation by the benzodiazepine receptor complex. *Pharmacol. Biochem. Behav.* 24, 1263–1267.

Iversen, L. (2003). Cannabis and the brain. *Brain* 126, 1252–1270.

Jacobsen, L. K., Mencl, W. E., Westerveld, M., and Pugh, K. R. (2004). Impact of cannabis use on brain function in adolescents. *Ann. N.Y. Acad. Sci.* 1021, 384–390.

Jacobsen, L. K., Pugh, K. R., Constable, R. T., Westerveld, M., and Mencl, W. E. (2007). Functional correlates of verbal memory deficits emerging during nicotine withdrawal in abstinent adolescent cannabis users. *Biol. Psychiatry* 61, 31–40.

Jager, G., and Ramsey, N. F. (2008). Long-term consequences of adolescent cannabis exposure on the development of cognition, brain structure and function: an overview of animal and human research. *Curr. Drug Abuse Rev.* 1, 114–123.

Kathuria, S., Gaetani, S., Fegley, D., Valino, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V., and Piomelli, D. (2003). Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* 9, 76–81.

Katona, I., Rancz, E. A., Acsady, L., Ledent, C., Mackie, K., Hajos, N., and Freund, T. F. (2001). Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.* 21, 9506–9518.

Laviolette, S. R., and Grace, A. A. (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.

Lee, K. S., Clough, A. R., Jaragba, M. J., Conigrave, K. M., and Patton, G. C. (2008). Heavy cannabis use and depressive symptoms in three Aboriginal communities in Arnhem Land, Northern Territory. *Med. J. Aust.* 188, 605–608.

Leech, S. L., Larkby, C. A., Day, R., and Day, N. L. (2006). Predictors and correlates of high levels of depression and anxiety symptoms among children at age 10. *J. Am. Acad. Child Adolesc. Psychiatry* 45, 223–230.

Leweke, F. M., and Schneider, M. (2011). Chronic pubertal cannabinoid treatment as a behavioural model for aspects of schizophrenia: effects of the atypical antipsychotic quetiapine. *Int. J. Neuropsychopharmacol.* 14, 43–51.

Lutz, B. (2009). Endocannabinoid signals in the control of emotion. *Curr. Opin. Pharmacol.* 9, 46–52.

Macleod, J., Davey Smith, G., Hickman, M., and Egger, M. (2007). Cannabis and psychosis. *Lancet* 370, 1539; author reply 1539–1540.

Macleod, J., Oakes, R., Copello, A., Crome, I., Egger, M., Hickman, M., Oppenkowski, T., Stokes-Lampard, H., and Davey Smith, G. (2004). Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. *Lancet* 363, 1579–1588.

Mangieri, R. A., and Piomelli, D. (2007). Enhancement of endocannabinoid signaling and the pharmacotherapy of depression. *Pharmacol. Res.* 56, 360–366.

Marco, E. M., Garcia-Gutierrez, M. S., Bermudez-Silva, F. J., Moreira, F. A., Guimaraes, F., Manzanares, J., and Viveros, M. P. (2011). Endocannabinoid system and psychiatry: in search of a neurobiological basis for detrimental and potential therapeutic effects. *Front. Behav. Neurosci.* 5:63. doi: 10.3389/fnbeh.2011.00063

Marco, E. M., and Viveros, M. P. (2009). The critical role of the endocannabinoid system in emotional homeostasis: avoiding excess and deficiencies. *Mini Rev. Med. Chem.* 9, 1407–1415.

Marsicano, G., and Lafenetre, P. (2009). Roles of the endocannabinoid system in learning and memory. *Curr. Top. Behav. Neurosci.* 1, 201–230.

Martin, M., Ledent, C., Parmentier, M., Maldonado, R., and Valverde, O. (2002). Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl.)* 159, 379–387.

Mato, S., Del Olmo, E., and Pazos, A. (2003). Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain. *Eur. J. Neurosci.* 17, 1747–1754.

Matsuda, L. A. (1997). Molecular aspects of cannabinoid receptors. *Crit. Rev. Neurobiol.* 11, 143–166.

McGaugh, J. L. (2002). Memory consolidation and the amygdala: a systems perspective. *Trends Neurosci.* 25, 456.

McGregor, I. S., Dastur, F. N., Mclellan, R. A., and Brown, R. E. (1996). Cannabinoid modulation of rat pup ultrasonic vocalizations. *Eur. J. Pharmacol.* 313, 43–49.

Mereu, G., Fa, M., Ferraro, L., Cagiano, R., Antonelli, T., Tattoli, M., Ghiglieri, V., Tanganelli, S., Gessa, G. L., and Cuomo, V. (2003). Prenatal exposure to a cannabinoid agonist produces memory deficits linked to dysfunction in hippocampal long-term potentiation and glutamate release. *Proc. Natl. Acad. Sci. U.S.A.* 100, 4915–4920.

Millan, M. J. (2003). The neurobiology and control of anxious states. *Prog. Neurobiol.* 70, 83–244.

Molina-Holgado, F., Amaro, A., Gonzalez, M. I., Alvarez, F. J., and Leret, M. L. (1996). Effect of maternal delta 9-tetrahydrocannabinol on developing serotonergic system. *Eur. J. Pharmacol.* 316, 39–42.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., and Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet* 370, 319–328.

Moreira, F. A., Kaiser, N., Monory, K., and 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.

Moreira, F. A., and Lutz, B. (2008). The endocannabinoid system: emotion, learning and addiction. *Addict. Biol.* 13, 196–212.

Murray, R. M., Morrison, P. D., Henquet, C., and Di Forti, M. (2007). Cannabis, the mind and society: the hash realities. *Nat. Rev. Neurosci.* 8, 885–895.

Newsom, R. J., and Kelly, S. J. (2008). Perinatal delta-9-tetrahydrocannabinol exposure disrupts social and open field behavior in adult male rats. *Neurotoxicol. Teratol.* 30, 213–219.

NIDA. (2005). *NIDA Research Monograph Series: Marijuana Abuse*. Washington, DC: NIH.

Onaivi, E. S., Green, M. R., and Martin, B. R. (1990). Pharmacological characterization of cannabinoids in the elevated plus maze. *J. Pharmacol. Exp. Ther.* 253, 1002–1009.

Oropeza, V. C., Mackie, K., and Van Bockstaele, E. J. (2007). Cannabinoid receptors are localized to noradrenergic axon terminals in the rat frontal cortex. *Brain Res.* 1127, 36–44.

Oropeza, V. C., Page, M. E., and Van Bockstaele, E. J. (2005). Systemic administration of WIN 55,212-2 increases norepinephrine release in the rat frontal cortex. *Brain Res.* 1046, 45–54.

O'Shea, M., McGregor, I. S., and Mallet, P. E. (2006). Repeated cannabinoid exposure during perinatal, adolescent or early adult ages produces similar longlasting deficits in object recognition and reduced social interaction in rats. *J. Psychopharmacol.* 20, 611–621.

O'Shea, M., Singh, M. E., McGregor, I. S., and Mallet, P. E. (2004). Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. *J. Psychopharmacol.* 18, 502–508.

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

Palazzo, E., De Novellis, V., Petrosino, S., Marabese, I., Vita, D., Giordano, C., Di Marzo, V., Mangoni, G. S., Rossi, F., and Maione, S. (2006). Neuropathic pain and the endocannabinoid system in the dorsal raphe: pharmacological treatment and interactions with the serotonergic system. *Eur. J. Neurosci.* 24, 2011–2020.

Pattij, T., Wiskerke, J., and Schoffelmeer, A. N. (2008). Cannabinoid modulation of executive functions. *Eur. J. Pharmacol.* 585, 458–463.

Patton, G. C., Coffey, C., Carlin, J. B., Degenhardt, L., Lynskey, M., and Hall, W. (2002). Cannabis use and mental health in young people: cohort study. *BMJ* 325, 1195–1198.

Pertwee, R. G. (1997). Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol. Ther.* 74, 129–180.

Petrosino, S., and Di Marzo, V. (2010). FAAH and MAGL inhibitors: therapeutic opportunities from regulating endocannabinoid levels. *Curr. Opin. Investig. Drugs* 11, 51–62.

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

Piomelli, D., Tarzia, G., Duranti, A., Tontini, A., Mor, M., Compton, T. R., Dasse, O., Monaghan, E. P., Parrott, J. A., and Putman, D. (2006). Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). *CNS Drug Rev.* 12, 21–38.

Pope, H. G. Jr., Gruber, A. J., Hudson, J. I., Cohane, G., Huestis, M. A., and Yurgelun-Todd, D. (2003). Early-onset cannabis use and cognitive deficits: what is the nature of the association? *Drug Alcohol Depend.* 69, 303–310.

Quinn, H. R., Matsumoto, I., Callaghan, P. D., Long, L. E., Arnold, J. C., Gunasekaran, N., Thompson, M. R., Dawson, B., Mallet, P. E., Kashem, M. A., Matsuda-Matsumoto, H., Iwazaki, T., and McGregor, I. S. (2008). 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* 33, 1113–1126.

Ranganathan, M., and D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl.)* 188, 425–444.

Realini, N., Rubino, T., and Parolaro, D. (2009). Neurobiological alterations at adult age triggered by adolescent exposure to cannabinoids. *Pharmacol. Res.* 60, 132–138.

Realini, N., Vigano, D., Guidali, C., Zamberletti, E., Rubino, T., and Parolaro, D. (2011). Chronic URB597 treatment at adulthood reverted most depressive-like symptoms induced by adolescent exposure to THC in female rats. *Neuropharmacology* 60, 235–243.

Riedel, G., and Davies, S. N. (2005). Cannabinoid function in learning, memory and plasticity. *Handb. Exp. Pharmacol.* 168, 445–477.

Rodriguez de Fonseca, F., Carrera, M. R., Navarro, M., Koob, G. F., and Weiss, F. (1997). Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* 276, 2050–2054.

Rodriguez de Fonseca, F., Cebeira, M., Fernandez-Ruiz, J. J., Navarro, M., and Ramos, J. A. (1991). Effects of pre- and perinatal exposure to hashish extracts on the ontogeny of brain dopaminergic neurons. *Neuroscience* 43, 713–723.

Rodriguez de Fonseca, F., Ramos, J. A., Bonnin, A., and Fernandez-Ruiz, J. J. (1993). Presence of cannabinoid binding sites in the brain from early postnatal ages. *Neuroreport* 4, 135–138.

Rodriguez de Fonseca, F., Rubio, P., Menzaghi, F., Merlo-Pich, E., Rivier, J., Koob, G. F., and Navarro, M. (1996). Corticotropin-releasing factor (CRF) antagonist [D-Phe12,Nle21,38,C alpha MeLeu37] CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *J. Pharmacol. Exp. Ther.* 276, 56–64.

Romero, J., Garcia-Palomero, E., Berrendero, F., Garcia-Gil, L., Hernandez, M. L., Ramos, J. A., and Fernandez-Ruiz, J. J. (1997). Atypical location of cannabinoid receptors in white matter areas during rat brain development. *Synapse* 26, 317–323.

Rubino, T., and Parolaro, D. (2008). Long lasting consequences of cannabis exposure in adolescence. *Mol. Cell. Endocrinol.* 286, S108–S113.

Rubino, T., and Parolaro, D. (2011). Sexually dimorphic effects of cannabinoid compounds on emotion and cognition. *Front. Behav. Neurosci.* 5:64. doi: 10.3389/fnbeh.2011.00064

Rubino, T., Realini, N., Braida, D., Alberio, T., Capurro, V., Vigano, D., Guidali, C., Sala, M., Fasano, M., and Parolaro, D. (2009a). The depressive phenotype induced in adult female rats by adolescent exposure to THC is associated with cognitive impairment and altered neuroplasticity in the prefrontal cortex. *Neurotox. Res.* 15, 291–302.

Rubino, T., Realini, N., Braida, D., Guidi, S., Capurro, V., Vigano, D., Guidali, C., Pinter, M., Sala, M., Bartesaghi, R., and Parolaro, D. (2009b). Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus* 19, 763–772.

Rubino, T., Vigano, D., Realini, N., Guidali, C., Braida, D., Capurro, V., Castiglioni, C., Cherubino, F., Romualdi, P., Candeletti, S., Sala, M., and Parolaro, D. (2008). Chronic delta 9-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. *Neuropsychopharmacology* 33, 2760–2771.

Rubino, T., Zamberletti, E., and Parolaro, D. (2011). Adolescent exposure to cannabis as a risk factor for psychiatric disorders. *J Psychopharmacol.* doi: 10.1177/0269881111405362

Schlicker, E., and Kathmann, M. (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.* 22, 565–572.

Schneider, M. (2008). Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addict. Biol.* 13, 253–263.

Schneider, M., and Koch, M. (2003). Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology* 28, 1760–1769.

Schneider, M., and Koch, M. (2005). Deficient social and play behavior in juvenile and adult rats after neonatal cortical lesion: effects of chronic pubertal cannabinoid treatment. *Neuropsychopharmacology* 30, 944–957.

Schneider, M., Schomig, E., and Leweke, F. M. (2008). Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addict. Biol.* 13, 345–357.

Schweinsburg, A. D., Brown, S. A., and Tapert, S. F. (2008). The influence of marijuana use on neurocognitive functioning in adolescents. *Curr. Drug Abuse Rev.* 1, 99–111.

Smith, A. M., Fried, P. A., Hogan, M. J., and Cameron, I. (2006). Effects of prenatal marijuana on visuospatial working memory: an fMRI study in young adults. *Neurotoxicol. Teratol.* 28, 286–295.

Solowij, N., and Pesa, N. (2010). Cognitive abnormalities and cannabis use. *Rev. Bras. Psiquiatr.* 32(Suppl. 1), S31–S40.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* 24, 417–463.

Steckler, T., Drinkenburg, W. H., Sahgal, A., and Aggleton, J. P. (1998). Recognition memory in rats – II. Neuroanatomical substrates. *Prog. Neurobiol.* 54, 313–332.

Stefanis, N. C., Delespaul, P., Henquet, C., Bakoula, C., Stefanis, C. N., and Van Os, J. (2004). Early adolescent cannabis exposure and positive and negative dimensions of psychosis. *Addiction* 99, 1333–1341.

Substance Abuse and Mental Health Services Administration (SAMHSA). (2009). *Results from the 2009 National Survey on Drug Use and Health*, Vol. I, *Summary of National Findings*. Rockville, MD: SAMHSA.

Suenaga, T., and Ichitani, Y. (2008). Effects of hippocampal administration of a cannabinoid receptor agonist WIN 55,212-2 on spontaneous object and place recognition in rats. *Behav. Brain Res.* 190, 248–252.

Szabo, B., and Schlicker, E. (2005). Effects of cannabinoids on neurotransmission. *Handb. Exp. Pharmacol.* 168, 327–365.

Terzian, A. L., Drago, F., Wotjak, C. T., and Micale, V. (2011). The dopamine and cannabinoid interaction in the modulation of emotions and cognition: assessing the role of cannabinoid CB1 receptor in neurons expressing dopamine D1 receptors. *Front. Behav. Neurosci.* 5:49. doi: 10.3389/fnbeh.2011.00049

Trezza, V., Baarendse, P. J., and Vanderschuren, L. J. M. J. (2010). The pleasures of play: pharmacological insights into social reward mechanisms. *Trends Pharmacol. Sci.* 31, 463–469.

Trezza, V., Campolongo, P., Cassano, T., Macheda, T., Dipasquale, P., Carratu, M. R., Gaetani, S., and Cuomo, V. (2008a). Effects of perinatal exposure to delta-9-tetrahydrocannabinol on the emotional reactivity of the offspring: a longitudinal behavioral study in Wistar rats. *Psychopharmacology (Berl.)* 198, 529–537.

Trezza, V., Cuomo, V., and Vanderschuren, L. J. M. J. (2008b). Cannabis and the developing brain: insights from behavior. *Eur. J. Pharmacol.* 585, 441–452.

Trezza, V., Campolongo, P., and Vanderschuren, L. J. M. J. (2011). Evaluating the rewarding nature of social interactions in laboratory animals. *Dev. Cogn. Neurosci.* 1, 444–458.

Trezza, V., and Vanderschuren, L. J. M. J. (2008a). Bidirectional cannabinoid modulation of social behavior in adolescent rats. *Psychopharmacology (Berl.)* 197, 217–227.

Trezza, V., and Vanderschuren, L. J. M. J. (2008b). Cannabinoid and opioid modulation of social play behavior in adolescent rats: differential behavioral mechanisms. *Eur. Neuropsychopharmacol.* 18, 519–530.

Trezza, V., and Vanderschuren, L. J. M. J. (2009). Divergent effects of anandamide transporter inhibitors with different target selectivity on social play behavior in adolescent rats. *J. Pharmacol. Exp. Ther.* 328, 343–350.

Tsou, K., Brown, S., Sanudo-Pena, M. C., Mackie, K., and Walker, J. M. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 83, 393–411.

Tunving, K. (1985). Psychiatric effects of cannabis use. *Acta Psychiatr. Scand.* 72, 209–217.

Vela, G., Fuentes, J. A., Bonnin, A., Fernandez-Ruiz, J., and Ruiz-Gayo, M. (1995). Perinatal exposure to delta 9-tetrahydrocannabinol (delta 9-THC) leads to changes in opioid-related behavioral patterns in rats. *Brain Res.* 680, 142–147.

Vela, G., Martin, S., Garcia-Gil, L., Crespo, J. A., Ruiz-Gayo, M., Javier Fernandez-Ruiz, J., Garcia-Lecumberri, C., Pelaprat, D., Fuentes, J. A., Ramos, J. A., and Ambrosio, E. (1998). Maternal exposure to delta9-tetrahydrocannabinol facilitates morphine self-administration behavior and changes regional binding to central mu opioid receptors in adult offspring female rats. *Brain Res.* 807, 101–109.

Vinod, K. Y., and Hungund, B. L. (2006). Cannabinoid-1 receptor: a novel target for the treatment of neuropsychiatric disorders. *Expert Opin. Ther. Targets* 10, 203–210.

Viveros, M. P., Marco, E. M., and File, S. E. (2005). Endocannabinoid system and stress and anxiety responses. *Pharmacol. Biochem. Behav.* 81, 331–342.

Wegener, N., and Koch, M. (2009). Behavioural disturbances and altered Fos protein expression in adult rats after chronic pubertal cannabinoid treatment. *Brain Res.* 1253, 81–91.

Williamson, E. M., and Evans, F. J. (2000). Cannabinoids in clinical practice. *Drugs* 60, 1303–1314.

Witkin, J. M., Tzavara, E. T., and Nomikos, G. G. (2005). A role for cannabinoid CB1 receptors in mood and anxiety disorders. *Behav. Pharmacol.* 16, 315–331.

Wotjak, C. T. (2005). Role of endogenous cannabinoids in cognition and emotionality. *Mini Rev. Med. Chem.* 5, 659–670.

Zanettini, C., Panlilio, L. V., Alicki, M., Goldberg, S. R., Haller, J., and Yasar, S. (2011). Effects of endocannabinoid system modulation on cognitive and emotional behavior. *Front. Behav. Neurosci.* 5:57. doi: 10.3389/fnbeh.2011.00057

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The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus

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Cannabinoid agonists generally have a disruptive effect on memory, learning, and operant behavior that is considered to be hippocampus-dependent. Nevertheless, under certain conditions, cannabinoid receptor activation may facilitate neuronal learning processes. For example, CB_1 receptors are essential for the extinction of conditioned fear associations, indicating an important role for this receptor in neuronal emotional learning and memory. This review examines the diverse effects of cannabinoids on hippocampal memory and plasticity. It shows how the effects of cannabinoid receptor activation may vary depending on the route of administration, the nature of the task (aversive or not), and whether it involves emotional memory formation (e.g., conditioned fear and extinction learning) or non-emotional memory formation (e.g., spatial learning). It also examines the memory stage under investigation (acquisition, consolidation, retrieval, extinction), and the brain areas involved. Differences between the effects of exogenous and endogenous agonists are also discussed. The apparently biphasic effects of cannabinoids on anxiety is noted as this implies that the effects of cannabinoid receptor agonists on hippocampal learning and memory may be attributable to a general modulation of anxiety or stress levels and not to memory *per se*. The review concludes that cannabinoids have diverse effects on hippocampal memory and plasticity that cannot be categorized simply into an impairing or an enhancing effect. A better understanding of the involvement of cannabinoids in memory processes will help determine whether the benefits of the clinical use of cannabinoids outweigh the risks of possible memory impairments.

Keywords: cannabinoids, CB_1 receptors, hippocampus, LTP, stress, emotional memory, anxiety, extinction

INTRODUCTION

Considerable evidence suggests that cannabinoids impair hippocampal-dependent learning and memory processes, such as spatial learning and context-related memory tasks (Sullivan, 2000; Riedel and Davies, 2005). In this review, I will provide evidence that suggests that the effects of cannabinoids on memory and plasticity are complex and depend on several factors, such as the nature of the task (emotional or non-emotional), the memory stage investigated (acquisition, retrieval, and extinction), and the experimental model used. Naturally, the behavioral effects of cannabinoids on memory may vary as a function of dose, route of administration, and the specific drug used.

CANNABINOID RECEPTORS IN THE HIPPOCAMPUS

Cannabis has a long history of consumption both for recreational and medicinal uses. The main psychoactive constituent of marijuana, delta-9-tetrahydrocannabinol (THC), was identified in 1964 (Gaoni and Mechoulam, 1964) and this discovery led to the identification of the endogenous endocannabinoid (eCB) system. This system includes cannabinoid receptors (CB_1 and CB_2), eCBs [anandamide and 2-arachidonoyl-glycerol (2-AG)], enzymes involved in their synthesis and metabolism [fatty acid amide hydrolase (FAAH) for anandamide and the monoacylglycerol lipase (MAGL) for 2-AG], and an eCB transporter (Devane et al., 1992; Freund et al., 2003; Kogan and Mechoulam, 2006). Recent cDNA cloning of the key enzymes such as *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL) accelerated molecular biological studies on the eCB biosyntheses (Bisogno et al., 2003; Okamoto et al., 2004). eCBs are synthesized "on demand" at the post-synaptic sites of neurons after an increase in neural activity and calcium ion influx, and are then released into the synaptic cleft. Their main function appears to be the suppression of neurotransmitter release from the presynapse. Thus, eCBs act as retrograde neurotransmitters, modulating other neurotransmitter systems.

CB_1 and CB_2 are metabotropic receptors coupled to G-proteins of the Gi/o type. CB_1 receptors are localized mainly in the central nervous system, but are also present in a variety of peripheral tissues; they are among the most abundant and widely distributed G-protein coupled receptors in the brain. CB_1 receptors are expressed in multiple brain areas, including the olfactory bulb, neocortex, pyriform cortex, hippocampus, amygdala, basal ganglia, thalamic and hypothalamic nuclei, cerebellar cortex, and brainstem nuclei (Herkenham et al., 1990, 1991; Katona et al., 2001). CB_2 receptors are mostly peripherally located on immunological tissues, but they have also been found within the central nervous system on neurons and glial cells with their expression mainly related to conditions of inflammation (Galiegue et al., 1995; Schat et al., 1997; Begg et al., 2005). More recent immunohistochemical analyses have revealed the presence of CB_2 receptors in apparently neuronal and glial processes in diverse rat brain areas, including the cerebellum and hippocampus (Van Sickle et al., 2005; Onaivi et al., 2006).

In the hippocampus, CB_1 receptors are expressed at an especially high density in the dentate gyrus, CA1, and CA3 regions (Herkenham et al., 1990, 1991; Matsuda et al., 1990; Tsou et al., 1998). CB_1 receptors are predominantly localized on the axon terminals and preterminal segments of cholecystokinin (CCK)-expressing GABAergic interneurons (Nyíri et al., 2005); however, they have also been demonstrated to inhibit glutamatergic transmission in cultured hippocampal cells (Shen, et al., 1996). CB_1 receptors located on GABAergic axon terminals are activated by lower concentrations of cannabinoid receptor agonists than CB_1 receptors located on glutamatergic terminals (Ohno-Shosaku et al., 2001; Hoffman et al., 2007) and CB_1 receptor expression is significantly lower on glutamatergic terminals than on GABA axon terminals in the hippocampus (Katona et al., 2006; Kawamura et al., 2006). Specifically, activation of hippocampal CB_1 receptors decreases GABA release (Katona et al., 1999; Hajos et al., 2000; Hoffman and Lupica, 2000; Hoffman et al., 2003). The CB_1-containing GABergic interneurons are thought to control oscillatory electrical activity in the hippocampus in the theta and gamma frequencies, which plays a role in synchronizing pyramidal cell activity (Hoffman and Lupica, 2000).

Overall, the evidence favors a predominant role for GABAergic pathways in the effects of cannabinoids on hippocampal-dependent memory processes.

CANNABINOID AGONISTS IMPAIR HIPPOCAMPAL-DEPENDENT LEARNING AND MEMORY

In humans, non-human primates, and rodents, cannabinoids impair the performance of a wide variety of memory tasks that share the common feature of requiring the hippocampus for normal performance (Sullivan, 2000; Davies et al., 2002; Riedel and Davies, 2005). In laboratory rodents, activation of cannabinoid receptors via THC or synthetic analogues such as WIN 55,212-2, CP55940, HU-210 or the endogenous agonist anandamide impairs learning (Davies et al., 2002). Administration of THC disrupts hippocampal-dependent learned behavior in operant and spatial maze models of memory (Nakamura et al., 1991; Heyser et al., 1993; Lichtman et al., 1995; Brodkin and Moerschbaecher, 1997; Mallet and Beninger, 1998; Ferrari et al., 1999; Varvel et al., 2001). For example, systemic THC administration (2–6 mg/kg i.p.) impairs working memory tested in the radial-arm spatial task and the cannabinoid antagonist SR141716A (1–10 mg/kg) prevents these deficits in a dose-dependent manner (Lichtman and Martin, 1996). Similarly, THC (8 mg/kg) impairs the acquisition of spatial learning in the water maze and the performance of mice in a working memory task, while consolidation and retrieval of a previously learned task are not affected. Pre-treatment with the antagonist SR 141716A (1 mg/kg i.p.) prevents these learning deficits (Da and Takahashi, 2002). Additionally, systemic administration of THC or the synthetic cannabinoid receptor agonist WIN 55,212-2 reliably impairs performance in delayed-match-to-sample and delayed-non-match-to-sample tasks, and this is accompanied by decreases in hippocampal cell firing during the sample phases of the task (Heyser et al., 1993; Hampson and Deadwyler, 1999, 2000).

Overall, the literature discussed above suggests that activation of cannabinoid receptors impairs learning. However, since the agonists were systemically infused, most of these experiments do not specifically show that cannabinoids impair learning and memory via action on the hippocampus. Rather, the involvement of the hippocampus is assumed because it is an important target for systemically administered cannabinoids and because most of the paradigms described are spatial tasks known to be hippocampus-dependent.

More recent research has directly tested whether specific administration of cannabinoids into the hippocampus would have similar effects (summarized in **Table 1**). Intrahippocampal infusions of the agonists CP55940, THC, or WIN 55,212-2 were found to disrupt performance in the radial-arm maze, and in T-maze delayed alternation, passive avoidance, spatial learning, and place recognition memory tasks (Lichtman et al., 1995; Mishima et al., 2001; Egashira et al., 2002; Suenaga and Ichitani, 2008; Suenaga et al., 2008; Wegener et al., 2008; Abush and Akirav, 2010). For example, activation of hippocampal cannabinoid receptors by the agonist WIN 55,212-2 (1–2 μg) dose-dependently decreases the exploration of an object in a new place, and this effect is antagonized by pre-treatment with the cannabinoid receptor antagonist AM 281 (2 mg/kg, i.p.; Suenaga and Ichitani, 2008). WIN 55,212-2 (5 μg) injected into the dorsal hippocampus increases the number of reference memory errors in the eight-arm radial-maze task, suggesting impairment of memory retrieval (Wegener et al., 2008). Additionally, post-training intrahippocampal administration of WIN 55,212-2 (2.5 and 5 μg) disrupts long-term spatial memory, but not acquisition or short-term memory, in a rat reference memory task in the water maze (Yim et al., 2008). We have recently found that WIN 55,212-2 administered systemically (0.5 mg/kg) or specifically into the hippocampal CA1 area (5 μg/side) before massed training in the Morris water maze impairs spatial learning (Abush and Akirav, 2010). Thus experiments that specifically targeted the hippocampus confirm the implications of the earlier systemic research as to the impairing effect of cannabinoids on hippocampal-dependent learning and memory.

CANNABINOID AGONISTS IMPAIR HIPPOCAMPAL SYNAPTIC PLASTICITY

In neuronal circuits, memory storage depends on activity-dependent modifications in synaptic efficacy, such as long-term potentiation (LTP) and long-term depression (LTD), which are

Table 1 | Effects of intra-dorsal hippocampal WIN 55,212-2 on learning and memory.

Doses (μg)	Task	Memory stage	Effects	References
1–2	Place recognition	Short-term retrieval	Impair	Suenaga and Ichitani (2008)
5	Radial-maze	Long-term retrieval	Impair	Wegener et al. (2008)
2.5 and 5	Spatial (water maze)	Long-term retrieval	Impair	Yim et al. (2008)
5	Spatial (water maze)	Acquisition	Impair	Abush and Akirav (2010)

the two main forms of synaptic plasticity in the brain. A key feature of LTP and LTD is that a short period of synaptic activity (either high- or low-frequency stimulation) can trigger persistent changes in synaptic transmission lasting at least several hours and often longer. This single property initially led investigators to suggest that these forms of plasticity are the cellular correlate of learning (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973). Indeed, efforts to understand synaptic plasticity are driven by the belief that such synaptic modifications might occur during learning and memory. However, it is extremely difficult to demonstrate directly that learning-induced synaptic changes occur following experience.

The mechanisms underlying synaptic plasticity have been studied more intensely in the hippocampus than in any other brain region. Both forms of synaptic plasticity have been studied most intensively at the Schaffer collateral–CA1 synapses of the hippocampus because of the established role of the CA1 area in spatial memory (Behr et al., 2009). LTP and LTD are thought to be involved in memory formation at glutamatergic synapses in the hippocampus. Cannabinoids appear to work by reducing glutamate release below the level needed to activate *N*-Methyl-D-aspartate (NMDA) receptors that are required for LTP and LTD (Shen et al., 1996; Misner and Sullivan, 1999). CB_1 receptors are capable of regulating both inhibitory and excitatory neurotransmitter release in the hippocampus and are thus capable of exerting subtle control over synaptic plasticity.

Most of our knowledge about cannabinoids and activity-dependent changes in synaptic strength comes from studies performed at excitatory synapses, largely using acute hippocampal slices as the experimental model (Chevaleyre et al., 2006). Cannabinoid receptor activation inhibits both LTP and LTD induction in the hippocampal slice. The inhibition of LTP in field potentials in the CA1 region has been demonstrated using THC, HU-210, WIN 55,212-2, 2-AG, and anandamide (Nowicky et al., 1987; Collins et al., 1994, 1995; Terranova et al., 1995; Misner and Sullivan, 1999) and has been found recently to inhibit hippocampal LTD of CA1 field potentials as well (Misner and Sullivan, 1999). The impairment in the induction of LTP in the CA1 is blocked by cannabinoid antagonists such as SR141716A.

We have recently examined cannabinoid modulation of LTP and LTD in a different experimental model: acute anesthetized rats. Using this experimental condition, we found that i.p. administration of WIN 55,212-2 or the CB_1 receptor antagonist AM251 at the doses tested impairs LTP in the Schaffer collateral–CA1 projection, with no effect on LTD (Abush and Akirav, 2010; see **Figure 1**).

de Oliveira Alvares et al. (2006) have also demonstrated impairment of LTP in a CA1 slice preparation following AM251 administration. Sokal et al. (2008) found that the CB_1 receptor antagonist SR141716A blocked the potentiation of the fEPSP slope observed following HFS to the perforant path. However, other studies conducted on hippocampal slices of the Schaffer collateral–CA1 synapses have shown that CB_1 blockade favors LTP in the hippocampus (Slanina et al., 2005) and that mice lacking CB_1 receptors show enhanced LTP (Bohme et al., 2000). However, in the study by Slanina et al. (2005), the drug was present throughout the experiment and LTP was elicited by moderate stimulations (20 or 50 pulses). Thus, the discrepancies with our findings could result from the examination of field potential in an intact rat model versus slices, or from various methodological issues, such as different stimulation protocols, different drug doses, etc.

EFFECTS OF CANNABINOID AGONISTS ON EMOTIONAL AND NON-EMOTIONAL MEMORY

Although considerable evidence suggests that activation of CB_1 receptors can induce learning and memory impairments (Sullivan, 2000; Robinson et al., 2003; O'Shea et al., 2004; Varvel et al., 2005), CB_1 receptors are essential for the extinction of conditioned fear associations (Marsicano et al., 2002), indicating an important role for this receptor in neuronal emotional learning and memory.

ROLE OF THE CANNABINOID SYSTEM IN EXTINCTION

Extinction was established as a tool to treat conditioned fear by Freud in the 1920s. It has become widely accepted that a deficit in the capacity to extinguish memories of fear is at the root of fear disorders as a result of the distinction between those who do and do not develop serious symptoms after fearsome experiences, and the fact that fear disorders are treated with therapy based on extinction procedures. Moreover, panic attacks, phobias, and particularly post-traumatic stress disorder (PTSD) are viewed by many as a deficit of extinction that should therefore be treated by an intensification of extinction (Charney et al., 1993; Wessa and Flor, 2007; Milad et al., 2008).

Conditioned fear is induced by pairing a neutral, conditioned stimulus (CS; e.g., a light, a tone, or a context) with an aversive stimulus (unconditioned stimulus, US; e.g., a mild footshock) that evokes a measurable fear response. Experimental extinction learning occurs when a CS that previously predicted a US no longer does so, and over time, the conditioned response (e.g., freezing or elevated skin conductance responses) decreases. Extinction learning involves the ventromedial prefrontal cortex (PFC), amygdala, and hippocampus (Milad and Quirk, 2002; Phelps et al., 2004; Bouton et al., 2006). PTSD patients continue to re-experience the traumatic event over a long timeframe and avoid trauma-related stimuli, even though they recognize that the traumatic event is no longer occurring. It has been suggested that dysfunctional fear extinction plays an important role in the development of clinical symptoms, such as reexperiencing trauma in PTSD (Rothbaum and Davis, 2003; Milad et al., 2006; Quirk et al., 2006; Rauch et al., 2006). PTSD patients also demonstrate impaired extinction in the aftermath of new trauma. For example, Milad et al. (2008) have shown deficient extinction recall as measured in skin conductance response in a 2-day fear conditioning and extinction procedure in PTSD patients.

Clearly, animal models do not entirely mimic the complex features of psychiatric disorders. However, they can predict the clinical effects of substances and provide insights into the biological mechanisms of these diseases. Marsicano et al. (2002) found that CB_1 receptor-deficient mice show normal acquisition and consolidation in a fear conditioning task, but fear extinction is strongly impaired. Impaired extinction is also observed when the antagonist SR141716 is injected systemically into wild-type mice before the extinction trial, indicating that CB_1 receptors are required at the moment of the extinction training. The findings that CB_1 knockout mice exhibit impaired short- and long-term extinction

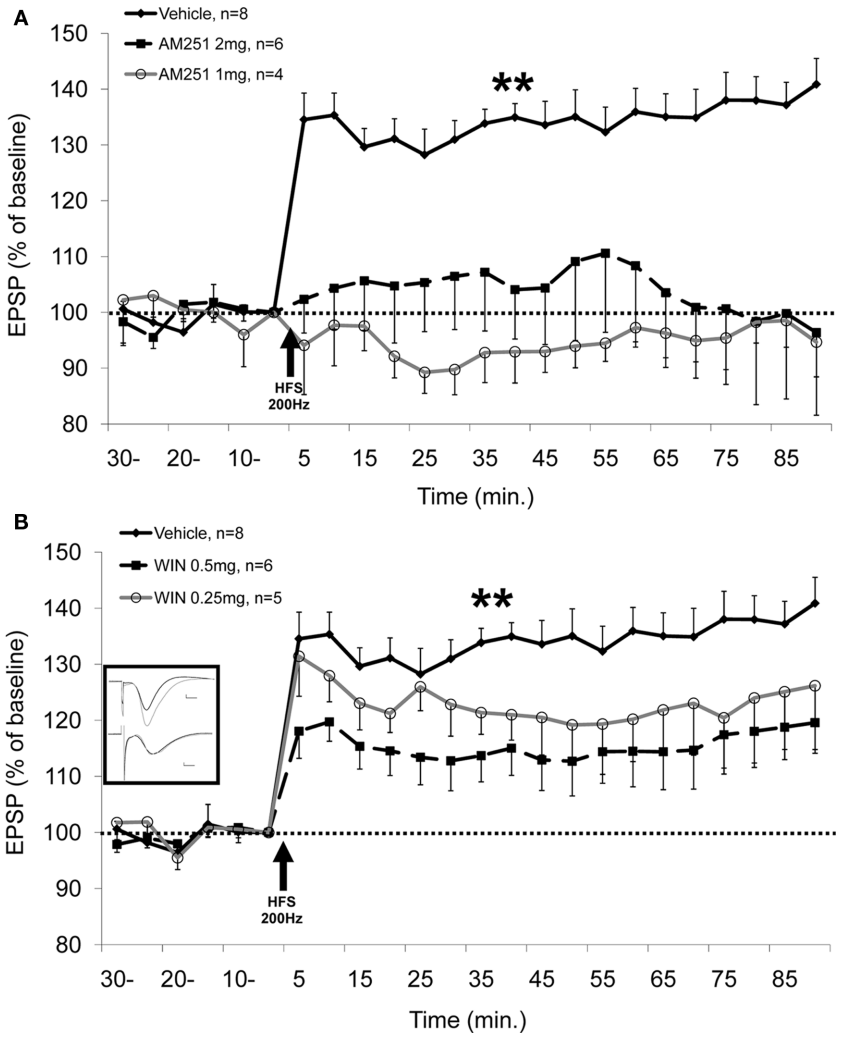

FIGURE 1 | CB_1 receptor antagonist and agonist impair the induction of LTP. (A) AM251 injected i.p. (1 or 2 mg/kg) 30 min before application of high frequency stimulation (HFS; 200 Hz) to the Schaffer collateral significantly impairs the induction of LTP in the CA1 compared with the vehicle group ($P < 0.01$, vehicle differs from all the groups). No significant difference is observed between the groups before HFS. **(B)** WIN 55,212-2 (0.5 mg/kg) injected i.p. 20 min before application of HFS (200 Hz) to the Schaffer collateral significantly impairs the induction of LTP in the CA1 compared with the vehicle group ($P < 0.01$). No significant difference is observed between the groups before HFS. Inset: representative traces in the CA1 for vehicle (upper traces) and WIN 0.5 mg (lower traces) groups taken before (black) and 90 min after (gray) HFS to the Schaffer collateral (calibration: 0.2 mV, 10 μs). Data published by Abush and Akirav (2010) in *Hippocampus*.

of cue-induced conditioned fear responses have been replicated by other groups for the extinction of both cue- and context-induced fear responses (Finn et al., 2004; Suzuki et al., 2004; Chhatwal et al., 2005; Lafenêtre et al., 2007; Lutz, 2007; Niyuhire et al., 2007). We have recently shown that microinjecting the antagonist AM251 (6 ng) into the BLA or the CA1 significantly impairs extinction of inhibitory avoidance (Ganon-Elazar and Akirav, 2009; Abush and Akirav, 2010). Several studies suggest that the eCB system is not involved in the extinction of non-aversive memories (Hölter et al., 2005; Niyuhire et al., 2007).

On the other hand, studies have demonstrated that pharmacological activation of eCB signaling promotes extinction of fear memories. For example, Chhatwal et al. (2005) found that systemic administration of the eCB transporter AM404 (10 mg/kg) promotes extinction of fear that was conditioned using fear-potentiated startle. This was replicated using systemic (Pamplona et al., 2008) and intracerebroventricular (Bitencourt et al., 2008) injections. In another study (Varvel et al., 2007), OL-135 (30 mg/kg), an inhibitor of FAAH, enhanced the rate of extinction in a water maze task. Pamplona et al. (2006) showed that WIN 55,212-2 (0.25 mg/kg) facilitates the extinction of contextual fear in the fear conditioning task and of spatial memory in the water maze reversal task. We have used the light–dark inhibitory avoidance procedure to demonstrate the effects of WIN 55,212-2 administered into the CA1 or the BLA on extinction. This procedure is dependent on both the amygdala and hippocampus as a single CS–US (context–footshock) pairing establishes a robust long-term memory, expressed as an increase in latency to enter the dark chamber at testing. Repeated retrieval of the avoidance response in the absence of the US induces extinction of inhibitory avoidance memory, meaning that the animal learns

that the context no longer predicts the footshock. We found that WIN 55,212-2 administered into the CA1 facilitates the extinction of inhibitory avoidance, with no effect on extinction kinetics when microinjected into the BLA (Ganon-Elazar and Akirav, 2009; Abush and Akirav, 2010).

Hence, the results of Marsicano et al. (2002) and subsequent investigations demonstrate that inhibition of eCB transmission robustly inhibits (or prolongs) fear extinction (Suzuki et al., 2004; Pamplona et al., 2006; Ganon-Elazar and Akirav, 2009; Abush and Akirav, 2010). Conversely, stimulation of eCB transmission accelerates fear extinction (Suzuki et al., 2004; Chhatwal et al., 2005; Barad et al., 2006; Abush and Akirav, 2010).

COMPARING THE EFFECTS OF CANNABINOID AGONISTS ON AVERSIVE AND NON-AVERSIVE TASKS

It has been suggested that the neural processes underlying emotional memory formation (such as extinction learning) and non-emotional memories (such as spatial learning) are differentially sensitive to cannabinoid receptor activation (Chhatwal and Ressler, 2007). An intriguing question is whether cannabinoids have a similar effect on other types of emotional memories that do not involve fear and extinction learning.

We have recent findings suggesting that cannabinoid receptor activation has differential effects on learning and memory that are task-, brain region-, and memory stage-dependent (Segev and Akirav, 2011). We examined the effects of WIN 55,212-2 microinjected into the amygdala and the subiculum on the acquisition and retrieval of a neutral learning task (i.e., social discrimination) and an aversive learning task (i.e., contextual fear conditioning). The subiculum is the principal target of CA1 pyramidal cells. It functions as a mediator of hippocampal–cortical interaction and has been proposed to play an important role in the encoding and retrieval of long-term memory. In fear conditioning paradigms, the BLA plays a central role in the formation and consolidation of fear-related memory traces (LeDoux, 2003; Maren and Quirk, 2004), whereas the hippocampus's role is to integrate the features of the context and not to form a context–shock association (Fanselow, 1998). Unlike the aversive fear conditioning task, social discrimination is considered neutral or even rewarding. This finding was established using both conditioned place preference paradigms and T-maze learning rewarded by social interaction (Van den Berg et al., 1999). Social recognition processes depend on brain regions such as the medial amygdala, which modulates the initial social encounter and formation of social memory (Ferguson et al., 2001; Bielsky and Young, 2004) and the ventral hippocampus (Van Wimersma Greidanus and Maigret, 1996; Kogan et al., 2000).

We found that in the aversive contextual fear task, WIN 55,212-2 administered into the BLA impairs fear acquisition/consolidation, but not retrieval, whereas in the ventral subiculum (vSub), WIN 55,212-2 impairs fear retrieval. In the non-aversive or rewarding social discrimination task, WIN 55,212-2 into the vSub impairs acquisition/consolidation and retrieval, whereas in the medial amygdala, WIN 55,212-2 impairs acquisition (Segev and Akirav, 2011). These findings suggest that cannabinoid agonists can impair emotional (or aversive) as well as neutral (or rewarding) memory-related processes in a task-, region-, and memory stage-dependent manner. This is consistent with other studies suggesting that exogenous acute cannabinoid treatment may have different outcomes depending on task aversiveness and the brain region involved (Suzuki et al., 2004; de Oliveira Alvares et al., 2005; Varvel et al., 2005; Ganon-Elazar and Akirav, 2009; Abush and Akirav, 2010).

EFFECTS OF CANNABINOIDS ON STRESS AND ANXIETY

Considerable evidence suggests that cannabinoids are anxiolytics and modulate the behavioral and physiological response to stressful events (Viveros et al., 2007; Hill et al., 2010). Consequently, the effects of CB_1 agonists on learning and memory may be attributable to a general modulation of anxiety or stress levels and not to memory *per se*.

Stress is most readily defined as any stimulus that presents a challenge to homeostasis including any actual or potential disturbance of an individual's environment. The stress response enables the animal to adapt to the changing environment (Joëls and Baram, 2009). Fear is an adaptive component of the acute stress response to potentially dangerous stimuli that threaten the integrity of the individual. However, when disproportionate in its intensity, chronic, irreversible, and/or not associated with any actual risk, it constitutes a maladaptive response and may be symptomatic of anxiety-related neuropsychiatric disorders (Taber and Hurley, 2009).

Anxiety disorders are marked by excessive fear (and avoidance), often in response to specific objects or situations, in the absence of true danger, and they are common in the general population (Shin and Liberzon, 2010). As excessive fear is a key component of anxiety disorders, the search for the neurocircuitry of anxiety disorders has focused extensively on studies of fear circuits in animal models. These studies examined the neurocircuitry associated with fear responses in rats and mice using fear conditioning paradigms, inhibitory avoidance, and fear-potentiated startle models. The amygdala, PFC, and hippocampus have arisen as clear regions of interest in studies of anxiety disorders and are implicated in PTSD (Shin and Liberzon, 2010).

The hippocampus is often implicated in the neurobiology of stress. Mineralocorticoid and glucocorticoid receptors are expressed in high numbers within the hippocampus. Although stress-induced corticosteroid signaling in the hippocampus has a beneficial role in regulating the time course of the hypothalamic–pituitary–adrenal (HPA) axis stress response (De Kloet et al., 2005), prolonged glucocorticoid signaling can damage the hippocampus as measured by dendritic atrophy, decreased neurogenesis, and deficits in synaptic plasticity (McEwen and Gould, 1990; Sapolsky, 1996; McEwen, 1999; Meaney, 2001). In PTSD and major depression patients, hippocampus volumes are reduced (Bremner et al., 1995; Sheline et al., 1999; Woon and Hedges, 2008), and smaller hippocampal volumes are predictive of vulnerability to developing stress-related disorders (Pitman et al., 2006).

ROLE OF THE ENDOCANNABINOID SYSTEM IN UNCONDITIONED STRESS AND ANXIETY

Results from many studies indicate that the eCB system modulates unconditioned stress- and anxiety-like responses (Viveros et al., 2005; Gorzalka et al., 2008; Lutz, 2009). A general conclusion that can be tentatively derived from the complicated and often contradictory literature is that inhibition of eCB signaling increases stress

and anxiety, while moderate increases in eCB signaling decrease stress and anxiety (Lutz, 2009; summarized in **Table 2**). The term "moderate" is used because strong stimulation of eCB signaling by high doses of CB_1 receptor agonists potentiates stress- and anxiety-like responses (Rodriguez de Fonseca et al., 1996; Scherma et al., 2008; Lutz, 2009). This biphasic effect has been demonstrated in animal models of anxiety (Lafenêtre et al., 2007; Hill and Gorzalka, 2009), and also in humans. Cannabis may induce aversive states in some smokers, precipitating anxiety and panic attacks (Hall and Solowij, 1998). Furthermore, THC administration may result in psychotic-like states (Linszen and van Amelsvoort, 2007). These bidirectional effects of cannabinoids observed in humans can be mimicked in laboratory animals. Hence, in models predictive of anxiolytic-like activity, low doses of CB_1 agonists tend to be anxiolytic and high doses tend to increase aversion and anxiety-related behaviors (Viveros et al., 2005).

Procedures used in studies on the role of eCBs in stress and anxiety evaluate the anxiolytic/anxiogenic effects of drugs by using standard tasks such as the elevated plus maze (EPM), social interaction, and defensive burying (Viveros et al., 2005; Lutz, 2009). Using the EPM, Patel and Hillard (2006) found that cannabinoid receptor agonists WIN 55212-2 (0.3–10 mg/kg) and CP55940 (0.001–0.3 mg/kg) administered systemically increase the time mice spend on the open arms (i.e., elicit an anxiolytic response) only at low doses. At the highest doses, both compounds alter overall locomotor activity. In contrast, THC (0.25–10 mg/kg) produces a dose-dependent reduction in time spent on open arms. The eCB uptake/catabolism inhibitor AM404 (0.3–10 mg/kg) produces an increase in time spent on the open arms at low doses and has no effect at the highest dose tested. The FAAH inhibitor URB597 (0.03–0.3 mg/kg) produces a monophasic, dose-dependent increase in time spent on the open arms. Systemic administration of the CB_1 receptor antagonists SR141716 (1–10 mg/kg) and AM251 (1–10 mg/kg) produce dose-related decreases in time spent on open arms. Onaivi et al. (1990) have shown that THC induces increased aversion to the open arms of the EPM in both rats and mice that is similar to the aversion produced by anxiogenic agents. In contrast, mice treated with the agonists cannabidiol and nabilone spend a greater amount of time in the open arms of the maze, an effect similar to that produced by diazepam, the reference anxiolytic agent.

In the light–dark box, Berrendero and Maldonado (2002) have shown that the systemic administration of a low dose of THC (0.3 mg/kg) produces clear anxiolytic-like responses. The CB_1 cannabinoid receptor antagonist SR 141716A (0.5 mg/kg) completely blocks the anxiolytic-like response induced by THC, suggesting that this effect is mediated by CB_1 cannabinoid receptors. In another study, systemic administration of the FAAH inhibitors URB597 and URB532 reduces anxiety-related behavior in the rat elevated zero-maze and in isolation-induced ultrasonic vocalization tests (Kathuria et al., 2003). These effects are dose-dependent and blocked by the antagonist rimonabant. The FAAH inhibitor and eCB re-uptake inhibitor AM404 also exhibit a dose-dependent anxiolytic profile in the EPM, defensive withdrawal test, and ultrasonic vocalization test (Bortolato et al., 2006). URB597 has also been shown to be anxiolytic in the rat EPM and open-field tests (Hill et al., 2007) and has recently been shown to reduce anxiety-related behavior in the EPM in Syrian hamsters (Moise et al., 2008).

Ribeiro et al. (2009) examined the dose-response effects of exogenous anandamide at doses of 0.01, 0.1, and 1.0 mg/kg in mice sequentially submitted to the open field and EPM. Systemically administered at 0.1 mg/kg (but not at 0.01 or 1 mg/kg), anandamide increases the time spent and the distance covered in the central zone of the open field, as well as exploration of the open arms of the EPM. Recently, Rubino et al. (2008b) demonstrated that the anxiolytic-like effect of a low anandamide dose is reversed by administration of the antagonist AM251, whereas the anxiogenic-like effect is

Table 2 | Effects of cannabinoids on anxiety-related responses.

Agonist	Species	Doses	Apparatus	Effects	References
WIN 55,212-2	Mice	0.3–10 mg/kg	EPM	+	Patel and Hillard (2006)
CP55940	Mice	0.001–0.3 mg/kg	EPM	+	Patel and Hillard (2006)
THC	Mice	0.25–10 mg/kg	EPM	–	Patel and Hillard (2006)
	Rats	1–10 mg/kg	EPM	–	Onaivi et al. (1990)
	Mice	10–20 mg/kg	EPM	–	Onaivi et al. (1990)
	Mice	0.3 mg/kg	Light–dark box	+	Berrendero and Maldonado (2002)
AM404	Mice	0.3–10 mg/kg	EPM	+	Patel and Hillard (2006)
URB597	Mice	0.03–0.3 mg/kg	EPM	+	Patel and Hillard (2006)
	Rats	0.05–0.1 mg/kg	Zero-maze	+	Kathuria et al. (2003)
			Ultrasonic test	+	Kathuria et al. (2003)
URB532	Rats	0.1–10 mg/kg	Zero-maze	+	Kathuria et al. (2003)
			Ultrasonic test	+	
Nabilone	Mice	10–100 μg/kg	EPM	+	Onaivi et al. (1990)
Cannabidiol	Mice	1–10 mg/kg	EPM	+	Onaivi et al. (1990)
Anandamide	Mice	0.1 mg/kg	EPM	+	Ribeiro et al. (2009)
			Open field	+	

Effects: –, anxiogenic effect; +, anxiolytic effect. EPM, elevated plus maze.

inhibited by pre-treatment with capsazepine, a transient receptor potential vanilloid type 1 (TRPV1) receptor antagonist. The authors suggested that the anxiolytic effect evoked by anandamide might be due to the interaction with the CB_1 cannabinoid receptor, whereas vanilloid receptors seem to be involved in the anxiogenic action of anandamide (Rubino et al., 2008b). Marsch et al. (2007) reported that TRPV1 "null" mice exhibit a significantly reduced response to anxiogenic stimuli. Therefore, the anandamide-induced inverted U-shape pattern might be based on the fact that the intrinsic efficacy of anandamide on TRPV1 is relatively low compared to that observed on the CB_1 receptor (Ross, 2003).

Transgenic mice deficient for FAAH, the enzyme that degrades anandamide, demonstrate reduced anxiety-like behavior in the EPM and light–dark box compared with wild-type mice and these effects are prevented by systemic administration of the antagonist rimonabant (Moreira et al., 2008). By contrast, transgenic mice lacking expression of the CB_1 receptor demonstrate an anxiogenic profile in the EPM, the light–dark box, open-field arena, and social interaction test (Haller et al., 2002, 2004; Maccarrone et al., 2002; Martin et al., 2002; Urigüen et al., 2004) and demonstrate impaired stress coping behavior in the forced swim test (Steiner et al., 2008). Similarly, CB_1 receptor antagonists increase anxiety-related behaviors in the EPM (Patel and Hillard, 2006). Taken together, these studies suggest that eCBs act at CB_1 receptors to reduce anxiety.

ROLE OF THE ENDOCANNABINOID SYSTEM IN CONDITIONED FEAR AND ANXIETY

Understanding the role of the eCB system in conditioned fear and aversive memories is important because a number of anxiety disorders, including PTSD and phobias, are thought to result from dysregulated fear neurocircuitry (Rauch et al., 2006). Investigators have examined the effect of CB_1 receptor agonists and antagonists on contextual and cue fear conditioning. Results from these studies were somewhat mixed. In rats, systemic injections of the CB_1 receptor antagonist AM251 enhance both the acquisition and expression of cue fear conditioning (Arenos et al., 2006; Reich et al., 2008). Administering AM251 (5 mg/kg, i.p) during tone–footshock conditioning enhances acquisition of freezing behavior for both trace fear conditioning (hippocampal-dependent) and delay fear conditioning (amygdala-dependent; Reich et al., 2008). Recently, we used an inhibitory avoidance task and found that microinjecting AM251 (6 ng) into the BLA significantly enhances conditioned avoidance but has no effect on conditioning when microinjected into the hippocampal CA1 area (Ganon-Elazar and Akirav, 2009; Abush and Akirav, 2010). However, others have shown that mice lacking the CB_1 receptor or systemically administered with the CB_1 receptor antagonist AM251 (0.3–3 mg/kg) 30 min before behavioral testing show no contextually induced fear response (Mikics et al., 2006). Furthermore, the CB_1 receptor antagonist rimonabant or genetic deletion of the CB_1 receptor has no effect on the acquisition of cue and context fear conditioning in mice (Marsicano et al., 2002; Suzuki et al., 2004). On the other hand, cue-fear-potentiated startle is decreased by medial PFC injections of the CB_1 receptor agonist WIN 55212-2 or the FAAH inhibitor URB597 (Lin et al., 2008, 2009) and contextual fear conditioning is decreased by dorsolateral periaqueductal gray injections of either anandamide or the anandamide transport inhibitor AM404 (Resstel et al., 2008). Overall it appears that, as in the case of unconditioned fear, inhibition of eCB transmission increases fear while moderate stimulation of eCB transmission decreases fear.

THE INVOLVEMENT OF THE HIPPOCAMPUS IN ENDOCANNABINOID MODULATION OF STRESS AND ANXIETY

Techniques based on intracranial injections of cannabinoids in rats revealed that activation of CB_1 receptors is involved in inducing anxiolytic- or antidepressant-like effects (Bambico et al., 2007; Moreira et al., 2007; Rubino et al., 2008a,b). For example, Rubino et al. (2008a) found that low doses of THC microinjected into the PFC (10 μg) or ventral hippocampus (5 μg) in rats induces an anxiolytic-like response during tests in the EPM, while higher doses do not show an anxiolytic effect and even seem to switch into an anxiogenic profile. Nevertheless, other studies demonstrated that eCB activation in the amygdala and dorsal hippocampus results in an anxiogenic-like response. Low THC doses (1 μg) in the BLA produce an anxiogenic-like response whereas higher doses are ineffective (Rubino et al., 2008a). WIN-55212-2 in the dorsal hippocampus (2.5 and 5 μg) produces a significant anxiogenic-like effect in rats that is reversed by AM251 (Roohbakhsh et al., 2007).

Local infusion of cannabinoid compounds into specific brain areas might be instrumental in identifying neural pathways and neuroanatomically separated CB_1 receptor subpopulations that may play distinct roles in and mediate the opposing actions of cannabinoids, notably, anxiolytic versus anxiogenic effects (Moreira et al., 2007; Viveros et al., 2007). We examined the role of cannabinoids in modulating aversive and non-aversive learning paradigms in the hippocampus and amygdala (Ganon-Elazar and Akirav, 2009; Abush and Akirav, 2010; Segev and Akirav, 2011). Microinjecting the antagonist AM251 (6 ng) or the agonist WIN-55212-2 (5 μg) into the BLA, CA1, or vSub had no effect on anxiety levels as measured in the open-field, pain sensitivity (Ganon-Elazar and Akirav, 2009; Abush and Akirav, 2010; Segev and Akirav, 2011), or EPM tests (Abush and Akirav, 2010). However, both agonist and antagonist had profound effects on aversive and non-aversive learning tasks. These findings suggest that in these studies the impairing and facilitating effects of local infusions of WIN-55212-2 on learning and memory are probably not attributable to a general modulation of anxiety. Nevertheless, the effects of cannabinoids on the interplay between anxiety and memory processes are difficult to separate and further examination of the effects of different cannabinoids is required.

To summarize the role of the eCB system in stress, anxiety, and conditioned fear, there is a general consensus that the effects of cannabinoid agonists on anxiety seem to be biphasic, with low doses being anxiolytic and high doses being ineffective or possibly anxiogenic. There are several important characteristics of the eCB system that might explain these different effects of eCB modulation. First, in a physiological situation, eCB synthesis, and thus CB_1 receptor activation, occurs in particular activated neuronal circuits. This is a notable difference from the situation following pharmacological treatment with receptor agonists, when the agent activates all CB_1 receptors in the brain regardless of their specific involvement in a particular physiological process. Second, the CB_1

receptor is expressed in diverse brain structures of relevance to psychiatric disorders and is mainly located presynaptically where it can suppress the release of other neurotransmitters (Marsicano and Lutz, 1999, 2006; Mackie, 2005). These neurotransmitters include the main inhibitory neurotransmitter GABA, the main excitatory neurotransmitter glutamate, as well as acetylcholine, noradrenaline, and serotonin (Katona et al., 1999; Harkany et al., 2005; Monory et al., 2006; Häring et al., 2007; Oropeza et al., 2007). Thus, synthetic compounds delivered systemically lack both the spatial and temporal specificity of endogenous compounds (Lafenêtre et al., 2007; Viveros et al., 2007; Moreira and Lutz, 2008). This may explain not only the bell-shaped relationship between dose and effect that some studies have observed, but also why elevation of eCB levels sometimes has effects that are different from those observed with exogenous cannabinoids. Finally, the diversity of eCB ligands with their multiple synthetic and degradation pathways adds a further level of complexity to the eCB system (Di Marzo, 2008).

SUMMARY

The findings demonstrate that the cannabinoid system has diverse effects on hippocampal memory and plasticity that cannot be categorized simply into an impairing or an enhancing effect, but are rather dependent on important variables such as the nature of the task (i.e., aversive, emotional or not), the memory stage under investigation (acquisition, consolidation, retrieval, extinction), and the brain areas involved.

The involvement of the eCB system in multiple aspects of brain function provides new targets for the development of novel therapeutic agents for a wide range of psychiatric disorders, including the treatment of anxiety disorders. Studies examining the involvement of cannabinoids in memory processes advance our understanding of the potential harmful consequences of cannabis use and the mechanisms underlying the close relationship between cannabinoids and cognition. This will help in determining whether the clinical benefits of using cannabinoids outweigh the risks, and to better cope with the deficits induced by cannabinoids.

REFERENCES

Abush, H., and Akirav, I. (2010). Cannabinoids modulate hippocampal memory and plasticity. *Hippocampus* 20, 1126–1138.

Arenos, J. D., Musty, R. E., and Bucci, D. J. (2006). Blockade of cannabinoid CB1 receptors alters contextual learning and memory. *Eur. J. Pharmacol.* 539, 177–183.

Bambico, F. R., Katz, N., Debonnel, G., and Gobbi, G. (2007). Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. *J. Neurosci.* 27, 11700–11711.

Barad, M., Gean, P. W., and Lutz, B. (2006). The role of the amygdala in the extinction of conditioned fear. *Biol. Psychiatry* 60, 322–328.

Begg, M., Pacher, P., Bátkai, S., Osei-Hyiaman, D., Offertáler, L., Mo, F. M., Liu, J., and Kunos, G. (2005). Evidence for novel cannabinoid receptors. *Pharmacol. Ther.* 106, 133–145.

Behr, J., Wozny, C., Fidzinski, P., and Schmitz, D. (2009). Synaptic plasticity in the subiculum. *Prog. Neurobiol.* 89, 334–342.

Berrendero, F., and Maldonado, R. (2002). Involvement of the opioid system in the anxiolytic-like effects induced by G9-tetrahydrocannabinol. *Psychopharmacology (Berl.)* 163, 111–117.

Bielsky, I. F., and Young, L. J. (2004). Oxytocin, vasopressin, and social recognition in mammals. *Peptides* 25, 1565–1574.

Bisogno, T., Howell, F., Williams, G., Minassi, A., Cascio, M. G., Ligresti, A., Matias, I., Schiano-Moriello, A., Paul, P., Williams, E. J., Gangadharan, U., Hobbs, C., Di Marzo, V., and Doherty, P. (2003). Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* 163, 463–468.

Bitencourt, R. M., Pamplona, F. A., and Takahashi, R. N. (2008). Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *Eur. Neuropsychopharmacol.* 18, 849–859.

Bliss, T. V., and Gardner-Medwin, A. R. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the unanaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* 232, 357–374.

Bliss, T. V., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* 232, 331–356.

Bohme, G. A., Laville, M., Ledent, C., Parmentier, M., and Imperato, A. (2000). Enhanced long-term potentiation in mice lacking cannabinoid CB1 receptors. *Neuroscience* 95, 5–7.

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

Bouton, M. E., Westbrook, R. F., Corcoran, K. A., and Maren, S. (2006). Contextual and temporal modulation of extinction: behavioral and biological mechanisms. *Biol. Psychiatry* 60, 352–360.

Bremner, J. D., Randall, P., Scott, T. M., Bronen, R. A., Seibyl, J. P., Southwick, S. M., Delaney, R. C., McCarthy, G., Charney, D. S., and Innis, R. B. (1995). MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. *Am. J. Psychiatry* 152, 973–981.

Brodkin, J., and Moerschbaecher, J. M. (1997). SR141716A antagonizes the disruptive effects of cannabinoid ligands on learning in rats. *J. Pharmacol. Exp. Ther.* 282, 1526–1532.

Charney, D. S., Deutch, A. Y., Krystal, J. H., Southwick, S. M., and Davis, M. (1993). Psychobiologic mechanisms of posttraumatic stress disorder. *Arch. Gen. Psychiatry* 50, 294–305.

Chevaleyre, V., Takahashi, K. A., and Castillo, P. E. (2006). Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu. Rev. Neurosci.* 29, 37–76.

Chhatwal, J. P., Myers, K. M., Ressler, K. J., and Davis, M. (2005). Regulation of gephyrin and GABAA receptor binding within the amygdala after fear acquisition and extinction. *J. Neurosci.* 25, 502–506.

Chhatwal, J. P., and Ressler, K. J. (2007). Modulation of fear and anxiety by the endogenous cannabinoid system. *CNS Spectr.* 12, 211–220.

Collins, D. R., Pertwee, R. G., and Davies, S. N. (1994). The action of synthetic cannabinoids on the induction of long-term potentiation in the rat hippocampal slice. *Eur. J. Pharmacol.* 11, R7–R8.

Collins, D. R., Pertwee, R. G., and Davies, S. N. (1995). Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. *Br. J. Pharmacol.* 115, 869–870.

Da, S., and Takahashi, R. N. (2002). SR 141716A prevents delta 9-tetrahydrocannabinol-induced spatial learning deficit in a Morris-type water maze in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 26, 321–325.

Davies, S. N., Pertwee, R. G., and Riedel, G. (2002). Functions of cannabinoid receptors in the hippocampus. *Neuropharmacology* 42, 993–1007.

De Kloet, E. R., Joëls, M., and Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nat. Rev. Neurosci.* 6, 463–475.

de Oliveira Alvares, L., de Oliveira, L. F., Camboim, C., Diehl, F., Genro, B. P., Lanziotti, V. B., and Quillfeldt, J. A. (2005). Amnestic effect of intrahippocampal AM251, a CB1-selective blocker, in the inhibitory avoidance, but not in the open field habituation task, in rats. *Neurobiol. Learn. Mem.* 83, 119–124.

de Oliveira Alvares, L., Genro, B. P., Vaz Breda, R., Pedroso, M. F., Da Costa, J. C., and Quillfeldt, J. A. (2006). AM251, a selective antagonist of the CB1 receptor, inhibits the induction of long-term potentiation and induces retrograde amnesia in rats. *Brain Res.* 1075, 60–67.

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

Di Marzo, V. (2008). Endocannabinoids: synthesis and degradation. *Rev. Physiol. Biochem. Pharmacol.* 160, 1–24.

Egashira, N., Mishima, K., Iwasaki, K., and Fujiwara, M. (2002). Intracerebral microinjections of delta 9-tetrahydrocannabinol: search for the impairment of spatial memory in the eight-arm radial maze in rats. *Brain Res.* 952, 239–245.

Fanselow, M. S. (1998). Pavlovian conditioning, negative feedback, and blocking: mechanisms that regulate association formation. *Neuron* 20, 625–627.

Ferguson, J. N., Aldag, J. M., Insel, T. R., and Young, L. J. (2001). Oxytocin in the medial amygdala is essential for social recognition in the mouse. *J. Neurosci.* 21, 278–8285.

Ferrari, F., Ottani, A., Vivoli, R., and Giuliani, D. (1999). Learning impairment produced in rats by the cannabinoid agonist HU 210 in a water-maze task. *Pharmacol. Biochem. Behav.* 64, 555–561.

Finn, D., Beckett, S., Richardson, D., Kendall, D., Marsden, C., and Chapman, V. (2004). Evidence for differential modulation of conditioned aversion and fear-conditioned analgesia by CB1 receptors. *Eur. J. Neurosci.* 20, 848–852.

Freund, T. F., Katona, I., and Piomelli, D. (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* 83, 1017–1066.

Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carrière, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., and Casellas, P. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* 232, 54–61.

Ganon-Elazar, E., and Akirav, I. (2009). Cannabinoid receptor activation in the basolateral amygdala blocks the effects of stress on the conditioning and extinction of inhibitory avoidance. *J. Neurosci.* 29, 11078–11088.

Gaoni, Y., and Mechoulam, R. (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* 86, 1646–1647.

Gorzalka, B. B., Hill, M. N., and Hillard, C. J. (2008). Regulation of endocannabinoid signaling by stress: implications for stress-related affective disorders. *Neurosci. Biobehav. Rev.* 32, 1152–1160.

Hajos, N., Katona, I., Naiem, S., MacKie, K., Ledent, C., Mody, I., and Freund, T. (2000). Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur. J. Neurosci.* 12, 3239–1349.

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

Haller, J., Bakos, N., Szirmay, M., Ledent, C., and Freund, T. (2002). The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur. J. Neurosci.* 16, 1395–1398.

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

Hampson, R. E., and Deadwyler, S. A. (1999). Cannabinoids, hippocampal function and memory. *Life Sci.* 65, 715–723.

Hampson, R. E., and Deadwyler, S. A. (2000). Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *J. Neurosci.* 20, 8932–8942.

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

Harkany, T., Dobszay, M., Cayetanot, F., Härtig, W., Siegemund, T., Aujard, F., and Mackie, K. (2005). Redistribution of CB1 cannabinoid receptors during evolution of cholinergic basal forebrain territories and their cortical projection areas: A comparison between the gray mouse lemur (microcebus murinus, primates) and rat. *Neuroscience* 135, 595–609.

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., and Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J. Neurosci.* 11, 563–583.

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., de Costa, B. R., and Rice, K. C. (1990). Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. U.S.A.* 87, 1932–1936.

Heyser, C. J., Hampson, R. E., and Deadwyler, S. A. (1993). Effects of Δ9-tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. *J. Pharmacol. Exp. Ther.* 264, 294–307.

Hill, M. N., and Gorzalka, B. B. (2009). The endocannabinoid system and the treatment of mood and anxiety disorders. *CNS Neurol. Disord. Drug Targets* 8, 451–458.

Hill, M. N., Karacabeyli, E. S., and Gorzalka, B. B. (2007). Estrogen recruits the endocannabinoid system to modulate emotionality. *Psychoneuroendocrinology* 32, 350–357.

Hill, M. N., Patel, S., Campolongo, P., Tasker, J. G., Wotjak, C. T., and Bains, J. S. (2010). Functional interactions between stress and the endocannabinoid system: from synaptic signaling to behavioral output. *J. Neurosci.* 10, 14980–14986.

Hoffman, A. F., and Lupica, C. R. (2000). Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *J. Neurosci.* 20, 2470–2479.

Hoffman, A. F., Oz, M., Caulder, T., and Lupica, C. R. (2003). Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. *J. Neurosci.* 23, 4815–4820.

Hoffman, A. F., Oz, M., Yang, R., Lichtman, A. H., and Lupica, C. R. (2007). Opposing actions of chronic {Delta} 9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. *Learn. Mem.* 14, 63–74.

Hölter, S. M., Kallnik, M., Wurst, W., Marsicano, G., Lutz, B., and Wotjak, C. T. (2005). Cannabinoid CB1 receptor is dispensable for memory extinction in an appetitively-motivated learning task. *Eur. J. Pharmacol.* 510, 69–74.

Joëls, M., and Baram, T. Z. (2009). The neuro-symphony of stress. *Nat. Rev. Neurosci.* 10, 459–466.

Kathuria, S., Gaetani, S., Fegley, D., Valiño, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V., and Piomelli, D. (2003). Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* 9, 76–81.

Katona, I., Rancz, E. A., Acsády, L., Ledent, C., Mackie, K., Hajos, N., and Freund, T. F. (2001). Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.* 21, 9506–9518.

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

Katona, I., Urban, G. M., Wallace, M., Ledent, C., Jung, K. M., Piomelli, D., Mackie, K., and Freund, T. F. (2006). Molecular composition of the endocannabinoid system at glutamatergic synapses. *J. Neurosci.* 26, 5628–5637.

Kawamura, Y., Fukaya, M., Maejima, T., Yoshida, T., Miura, E., Watanabe, M., Ohno-Shosaku, T., and Kano, M. (2006). The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J. Neurosci.* 26, 2991–3001.

Kogan, J. H., Frankland, P. W., and Silva, A. J. (2000). Long-term memory underlying hippocampus-dependent social recognition in mice. *Hippocampus* 10, 47–56.

Kogan, N., and Mechoulam, R. (2006). The chemistry of endocannabinoids. *J. Endocrinol. Invest.* 29, 3–14.

Lafenêtre, P., Chaouloff, F., and 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.

LeDoux, J. (2003). The emotional brain, fear, and the amygdala. *Cell. Mol. Neurobiol.* 23, 727–738.

Lichtman, A. H., Dimen, K. R., and Martin, B. R. (1995). Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology* 119, 282–290.

Lichtman, A. H., and Martin, B. R. (1996). Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. *Psychopharmacology (Berl.)* 126, 125–131.

Lin, H. C., Mao, S. C., Chen, P. S., and Gean, P. (2008). Chronic cannabinoid administration in vivo compromises extinction of fear memory. *Learn. Mem.* 15, 876–884.

Lin, H. C., Mao, S. C., Su, C. L., and Gean, P. W. (2009). The role of prefrontal cortex CB1 receptors in the modulation of fear memory. *Cereb. Cortex* 19, 165–175.

Linszen, D., and van Amelsvoort, T. (2007). Cannabis and psychosis: an update on course and biological plausible mechanisms. *Curr. Opin. Psychiatry* 20, 116–120.

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

Lutz, B. (2009). Endocannabinoid signals in the control of emotion. *Curr. Opin. Pharmacol.* 9, 46–52.

Maccarrone, M., Valverde, O., Barbaccia, M. L., Castañé, A., Maldonado, R., Ledent, C., Parmentier, M., and Finazzi-Agrò, A. (2002). Age-related changes of anandamide metabolism in CB1 cannabinoid receptor knockout mice: correlation with behaviour. *Eur. J. Neurosci.* 15, 1178–1186.

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

Mallet, P. E., and Beninger, R. J. (1998). The cannabinoid CB1 receptor antagonist SR141716A attenuates the memory impairment produced by delta-9-tetrahydrocannabinol or

anandamide. *Psychopharmacology* 140, 11–19.

Maren, S., and Quirk, G. J. (2004). Neuronal signalling of fear memory. *Nat. Rev. Neurosci.* 5, 844–852.

Marsch, R., Foeller, E., Rammes, G., Bunck, M., Kossl, M., Holsboer, F., Zieglgänsberger, W., Landgraf, R., Lutz, B., and Wotjak, C. T. (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., and 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., and Lutz, B. (2006). Neuromodulatory functions of the endocannabinoid system. *J. Endocrinol. Invest.* 29, 27–46.

Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., Hermann, H., Tang, J., Hofmann, G., Zieglansberger, W., Di Marzo, V., and Lutz, B. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418, 530–534.

Martin, M., Ledent, C., Parmentier, M., Maldonado, R., and Valverde, O. (2002). Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl.)* 159, 379–387.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., and Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346, 561–564.

McEwen, B. C., and Gould, E. (1990). Adrenal steroid influences on the survival of hippocampal neurons. *Biochem. Pharmacol.* 40, 2393–2402.

McEwen, B. S. (1999). Stress and hippocampal plasticity. *Annu. Rev. Neurosci.* 22, 105–122.

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Neuroscience* 24, 1161–1192.

Mikics, É., Dombi, T., Barsvári, B., Varga, B., Ledent, C., Freund, T. F., and Haller, J. (2006). The effects of cannabinoids on contextual conditioned fear in CB1 knockout and CD1 mice. *Behav. Pharmacol.* 17, 223–230.

Milad, M. R., Orr, S. P., Lasko, N. B., Chang, Y., Rauch, S. L., and Pitman, R. K. (2008). Presence and acquired origin of reduced recall for fear extinction in PTSD: results of a twin study. *J. Psychiatr. Res.* 42, 515–520.

Milad, M. R., and Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature* 420, 70–74.

Milad, M. R., Rauch, S. L., Pitman, R. K., and Quirk, G. J. (2006). Fear extinction in rats: implications for human brain imaging and anxiety disorders. *Biol. Psychol.* 73, 61–71.

Mishima, K., Egashira, N., Hirosawa, N., Fujii, M., Matsumoto, Y., Iwasaki, K., and Fujiwara, M. (2001). Characteristics of learning and memory impairment induced by delta9-tetrahydrocannabinol in rats. *Jpn. J. Pharmacol.* 87, 297–308.

Misner, D. L., and Sullivan, J. M. (1999). Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. *J. Neurosci.* 19, 6795–6805.

Moise, A. M., Eisenstein, S. A., Astarita, G., Piomelli, D., and Hohmann, A. G. (2008). An endocannabinoid signaling system modulates anxiety-like behavior in male syrian hamsters. *Psychopharmacology (Berl.)* 200, 333–346.

Monory, K., Massa, F., Egertová, M., Eder, M., Blaudzun, H., Westenbroek, R., Kelsch, W., Jacob, W., Marsch, R., Ekker, M., Long, J., Rubenstein, J. L., Goebbels, S., Nave, K. A., During, M., Klugmann, M., Wölfel, B., Dodt, H. U., Zieglgänsberger, W., Wotjak, C. T., Mackie, K., Elphick, M. R., Marsicano, G., and Lutz, B. (2006). The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* 51, 455–466.

Moreira, F. A., Aguiar, D. C., and Guimarães, F. S. (2007). Anxiolytic-like effect of cannabinoids injected into the rat dorsolateral periaqueductal gray. *Neuropharmacology* 52, 958–965.

Moreira, F. A., Kaiser, N., Monory, K., and 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.

Moreira, F. A., and Lutz, B. (2008). The endocannabinoid system: emotion, learning and addiction. *Addict. Biol.* 13, 196–212.

Nakamura, E. M., da Silva, E. A., Concilio, G. V., Wilkinson, D. A., and Masur, J. (1991). Reversible effects of acute and long-term administration of Δ9-tetrahydrocannabinol (THC) on memory in the rat. *Drug Alcohol Depend.* 28, 167–175.

Niyuhire, F., Varvel, S. A., Thorpe, A. J., Stokes, R. J., Wiley, J. L., and Lichtman, A. H. (2007). The disruptive effects of the CB 1 receptor antagonist rimonabant on extinction learning in mice are task-specific. *Psychopharmacology (Berl.)* 191, 223–231.

Nowicky, A. V., Teyler, T. J., and Vardaris, R. M. (1987). The modulation of long term potentiation by delta-9-tetrahydrocannabinol in the rat hippocampus, in vitro. *Brain Res. Bull.* 19, 663–672.

Nyíri, G., Cserép, C., Szabadits, E., Mackie, K., and Freund, T. F. (2005). CB1 cannabinoid receptors are enriched in the perisynaptic annulus and on preterminal segments of hippocampal GABAergic axons. *Neuroscience* 136, 811–822.

Ohno-Shosaku, T., Maejima, T., and Kano, M. (2001). Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 29, 729–738.

Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T., and Ueda, N. (2004). Molecular characterization of a phospholipase D generating anandamide and its congeners. *J. Biol. Chem.* 279, 5298–5305.

Onaivi, E., Green, M., and Martin, B. (1990). Pharmacological characterization of cannabinoids in the elevated plus maze. *J. Pharmacol. Exp. Ther.* 253, 1002–1009.

Onaivi, E. S., Ishiguro, H., Gong, J.-P., Patel, S., Perchuk, A., Meozzi, P. A., Myers, L., Mora, Z., Tagliaferro, P., Gardner, E., Brusco, A., Akinshola, B. E., Liu, Q. R., Hope, B., Iwasaki, S., Arinami, T., Teasenfitz, L., and Uhl, G. R. (2006). Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann. N. Y. Acad. Sci.* 1074, 514–536.

Oropeza, V. C., Mackie, K., and Van Bockstaele, E. J. (2007). Cannabinoid receptors are localized to noradrenergic axon terminals in the rat frontal cortex. *Brain Res.* 1127, 36–44.

O'Shea, M., Singh, M. E., McGregor, I. S., and Mallet, P. E. (2004). Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. *J. Psychopharmacol.* 18, 502–508.

Pamplona, F. A., Bitencourt, R. M., and Takahashi, R. N. (2008). Short-and long-term effects of cannabinoids on the extinction of contextual fear memory in rats. *Neurobiol. Learn. Mem.* 90, 290–293.

Pamplona, F. A., Prediger, R. D. S., Pandolfo, P., and Takahashi, R. N. (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.

Patel, S., and Hillard, C. J. (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.

Phelps, E. A., Delgado, M. R., Nearing, K. I., and LeDoux, J. E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron* 43, 897–905.

Pitman, R. K., Gilbertson, M. W., Gurvits, T. V., May, F. S., Lasko, N. B., Metzger, L. J., Shenton, M. E., Yehuda, R., Orr, S. P., and Harvard/VA PTSD Twin Study Investigators. (2006). Clarifying the origin of biological abnormalities in PTSD through the study of identical twins discordant for combat exposure. *Ann. N. Y. Acad. Sci.* 1071, 242–254.

Quirk, G. J., Garcia, R., and González-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biol. Psychiatry* 60, 337–343.

Rauch, S. L., Shin, L. M., and Phelps, E. A. (2006). Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research – past, present, and future. *Biol. Psychiatry* 60, 376–382.

Reich, C. G., Mohammadi, M. H., and Alger, B. E. (2008). Endocannabinoid modulation of fear responses: learning and state-dependent performance effects. *J. Psychopharmacol.* 22, 769–777.

Resstel, L. B. M., Lisboa, S., Aguiar, D., Corrêa, F. M. A., and Guimaraes, F. (2008). Activation of CB1 cannabinoid receptors in the dorsolateral periaqueductal gray reduces the expression of contextual fear conditioning in rats. *Psychopharmacology (Berl.)* 198, 405–411.

Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M., and Palermo-Neto, J. (2009). Dose-response effects of systemic anandamide administration in mice sequentially submitted to the open field and elevated plus-maze tests. *Braz. J. Med. Biol. Res.* 42, 556–560.

Riedel, G., and Davies, S. N. (2005). Cannabinoid function in learning, memory and plasticity. *Handb. Exp. Pharmacol.* 168, 445–477.

Robinson, L., Hinder, L., Pertwee, R. G., and Riedel, G. (2003). Effects of delta-9-THC and WIN 55,212-2 on place preference in the water maze in rats. *Psychopharmacology (Berl.)* 166, 40–50.

Rodriguez de Fonseca, F., Rubio, P., Menzaghi, F., Merlo-Pich, E., Rivier, J., Koob, G. F., and Navarro, M. (1996). Corticotropin-releasing factor (CRF) antagonist [D-Phe12, Nle21, 38, C alpha MeLeu37] CRF attenuates the acute actions of the highly potent

cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *J. Pharmacol. Exp. Ther.* 276, 56–64.

Roohbakhsh, A., Moghaddam, A. H., Massoudi, R., and Zarrindast, M. R. (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.

Ross, R. A. (2003). Anandamide and vanilloid TRPV1 receptors. *Br. J. Pharmacol.* 140, 790–801.

Rothbaum, B. O., and Davis, M. (2003). Applying learning principles to the treatment of post-trauma reactions. *Ann. N. Y. Acad. Sci.* 1008, 112–121.

Rubino, T., Guidali, C., Vigano, D., Realini, N., Valenti, M., Massi, P., and Parolaro, D. (2008a). CB1 receptor stimulation in specific brain areas differently modulate anxiety-related behaviour. *Neuropharmacology* 54, 151–160.

Rubino, T., Realini, N., Castiglioni, C., Guidali, C., Viganó, D., Marras, E., Petrosino, S., Perletti, G., Maccarrone, M., Di Marzo, V., and Parolaro, D. (2008b). Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. *Cereb. Cortex* 18, 1292–1301.

Sapolsky, R. M. (1996). Why stress is bad for your brain. *Science* 273, 749–750.

Schat, A. R., Lee, M., Condie, R. B., Pulaski, J. T., and Kaminski, N. E. (1997). Cannabinoid receptors CB1 and CB2: a characterization of expression and adenylate cyclase modulation within the immune system. *Toxicol. Appl. Pharmacol.* 142, 278–287.

Scherma, M., Medalie, J., Fratta, W., Vadivel, S. K., Makriyannis, A., Piomelli, D., Mikics, E., Haller, J., Yasar, S., Tanda, G., and Goldberg, S. R. (2008). The endogenous cannabinoid anandamide has effects on motivation and anxiety that are revealed by fatty acid amide hydrolase (FAAH) inhibition. *Neuropharmacology* 54, 129–140.

Segev, A., and Akirav, I. (2011). Differential effects of cannabinoid receptor agonist on social discrimination and contextual fear in amygdala and hippocampus. *Learn. Mem.* 18, 254–259.

Sheline, Y. I., Sanghavi, M., Mintun, M. A., and Gado, M. H. (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J. Neurosci.* 19, 5034–5043.

Shen, M., Piser, T. M., Seybold, V. S., and Thayer, S. A. (1996). Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J. Neurosci.* 16, 4322–4334.

Shin, L. M., and Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology* 35, 169–191.

Slanina, K. A., Roberto, M., and Schweitzer, P. (2005). Endocannabinoids restrict hippocampal long term potentiation via CB1. *Neuropharmacology* 49, 660–668.

Sokal, D. M., Benetti, C., Girlanda, E., and Large, C. H. (2008). The CB1 receptor antagonist, SR141716A, prevents high-frequency stimulation-induced reduction of feedback inhibition in the rat dentate gyrus following perforant path stimulation in vivo. *Brain Res.* 1223, 50–58.

Steiner, M. A., Marsicano, G., Nestler, E. J., Holsboer, F., Lutz, B., and Wotjak, C. T. (2008). Antidepressant-like behavioral effects of impaired cannabinoid receptor type 1 signaling coincide with exaggerated corticosterone secretion in mice. *Psychoneuroendocrinology* 33, 54–67.

Suenaga, T., and Ichitani, Y. (2008). Effects of hippocampal administration of a cannabinoid receptor agonist WIN 55,212-2 on spontaneous object and place recognition in rats. *Behav. Brain Res.* 190, 248–252.

Suenaga, T., Kaku, M., and Ichitani, Y. (2008). Effects of intrahippocampal cannabinoid receptor agonist and antagonist on radial maze and T-maze delayed alternation performance in rats. *Pharmacol. Biochem. Behav.* 91, 91–96.

Sullivan, J. M. (2000). Cellular and molecular mechanisms underlying learning and memory impairments produced by cannabinoids. *Learn. Mem.* 7, 132–139.

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

Taber, K. H., and Hurley, R. A. (2009). Endocannabinoids: stress, anxiety, and fear. *J. Neuropsychiatry Clin. Neurosci.* 21, 108–113.

Terranova, J. P., Michaud, J. C., Le Fur, G., and Soubrie, P. (1995). Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212–2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* 352, 576–579.

Tsou, K., Brown, S., Sanudo-Pena, M. C., Mackie, K., and Walker, J. M. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 83, 393–411.

Urigüen, L., Pérez-Rial, S., Ledent, C., Palomo, T., and Manzanares, J. (2004). Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. *Neuropharmacology* 46, 966–973.

Van den Berg, C. L., Hol, T., Van Ree, J. M., Spruijt, B. M., Everts, H., and Koolhaas, J. M. (1999). Play is indispensable for an adequate development of coping with social challenges in the rat. *Dev. Psychobiol.* 34, 129–138.

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J. S., Marnett, L. J., Di Marzo, V., Pittman, Q. J., Patel, K. D., and Sharkey, K. A. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 310, 329–332.

Van Wimersma Greidanus, T. B., and Maigret, C. (1996). The role of limbic vasopressin and oxytocin in social recognition. *Brain Res.* 713, 153–159.

Varvel, S. A., Anum, E. A., and Lichtman, A. H. (2005). Disruption of CB1 receptor signaling impairs extinction of spatial memory in mice. *Psychopharmacology (Berl.)* 179, 863–872.

Varvel, S. A., Hamm, R. J., Martin, B. R., and Lichtman, A. H. (2001). Differential effects of delta9-THC on spatial reference and working memory in mice. *Psychopharmacology (Berl.)* 157, 142–150.

Varvel, S. A., Wise, L. E., Niyuhire, F., Cravatt, B. F., and Lichtman, A. H. (2007). Inhibition of fatty-acid amide hydrolase accelerates acquisition and extinction rates in a spatial memory task. *Neuropsychopharmacology* 32, 1032–1041.

Viveros, M., Marco, E., Llorente, R., and Lopez-Gallardo, M. (2007). Endocannabinoid system and synaptic plasticity: implications for emotional responses. *Neural Plast.* 2007, 52908.

Viveros, M., Marco, E. M., and File, S. E. (2005). Endocannabinoid system and stress and anxiety responses. *Pharmacol. Biochem. Behav.* 81, 331–342.

Wegener, N., Kuhnert, S., Thuns, A., Roese, R., and Koch, M. (2008). Effects of acute systemic and intracerebral stimulation of cannabinoid receptors on sensorimotor gating, locomotion and spatial memory in rats. *Psychopharmacology (Berl.)* 198, 375–385.

Wessa, M., and Flor, H. (2007). Failure of extinction of fear responses in posttraumatic stress disorder: evidence from second-order conditioning. *Am. J. Psychiatry* 164, 1684–1692.

Woon, F. L., and Hedges, D. W. (2008). Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: a meta-analysis. *Hippocampus* 18, 729–736.

Yim, T. T., Hong, N. S., Ejaredar, M., McKenna, J. E., and McDonald, R. J. (2008). Post-training CB1 cannabinoid receptor agonist activation disrupts long-term consolidation of spatial memories in the hippocampus. *Neuroscience* 151, 929–936.

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AAV-mediated overexpression of the CB1 receptor in the mPFC of adult rats alters cognitive flexibility, social behavior, and emotional reactivity

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The endocannabinoid (ECB) system is strongly involved in the regulation of cognitive processing and emotional behavior and evidence indicates that ECB signaling might affect these behavioral abilities by modulations of prefrontal cortical functions. The aim of the present study was to examine the role of the CB1 receptor in the medial prefrontal cortex (mPFC) on cognitive flexibility and emotional behavior. Therefore, the CB1 receptor was overexpressed by adeno-associated virus vector-mediated gene transfer specifically in the mPFC of adult Wistar rats. Animals were then tested in different anxiety-related paradigms for emotional reactivity [e.g., elevated plus maze (EPM), light/dark emergence test (EMT), social interaction] and the attentional set shift task (ASST) – an adaptation of the human Wisconsin card sorting test – for cognitive abilities and behavioral flexibility. A subtle increase in exploratory behavior was found in CB1 receptor overexpressing animals (CB1-R) compared to Empty vector injected controls (Empty) in the EMT and EPM, although general locomotor activity did not differ between the groups. During social interaction testing, social contact behavior toward the unknown conspecific was found to be decreased, whereas social withdrawal was increased in CB1-R animals and they showed an inadequate increase in exploratory behavior compared to control animals. In the ASST, impaired reversal learning abilities were detected in CB1-R animals compared to controls, indicating reduced behavioral flexibility. In conclusion, upregulation of the CB1 receptor specifically in the rat mPFC induces alterations in emotional reactivity, leads to inadequate social behavior, and impairs cognitive flexibility. These findings might be relevant for neuropsychiatric disorders, since higher cortical CB1 receptor expression levels as well as similar behavioral impairments as observed in the present study have been described in schizophrenic patients.

Keywords: CB1 receptor, mPFC, cognitive flexibility, emotional behavior, social interaction, attentional set shift task

INTRODUCTION

The endocannabinoid (ECB) system has emerged in recent years as a key modulator of neuronal activity of various neurotransmitter systems and appears to be involved in synaptic plasticity in diverse brain structures. Accordingly, the ECB system and ECB signaling have been implicated in a variety of behavioral functions, including among others the regulation of emotional states, affective, and cognitive processes (for review see Viveros et al., 2005; Egerton et al., 2006; Pattij et al., 2008; Moreira et al., 2009).

One important brain region through which cannabinoids might exert their modulatory effects on cognition and emotional behavior is the prefrontal cortex (PFC). An abundant expression of CB1 receptors in this brain area (Herkenham et al., 1990; Mailleux and Vanderhaeghen, 1992; Marsicano and Lutz, 1999) indicates the significance of ECB signaling for the modulation of prefrontocortical neurotransmission (Egerton et al., 2006). It has been reported that systemic activation or blockade of cannabinoid CB1 receptors in the rat medial prefrontal cortex (mPFC) modulates emotional associative learning and memory formation, mainly through functional inputs from the basolateral amygdala (Laviolette and Grace, 2006). Additionally, the importance of the ECB system for cognitive flexibility – a behavior that is highly dependent on prefrontocortical functions (Owen et al., 1991; Birrell and Brown, 2000; Egerton et al., 2005) – has been indicated in various studies in humans and rodents (for review see Egerton et al., 2006; Pattij et al., 2008). In humans, heavy marijuana use was shown to be associated with deficits in behavioral flexibility measured in a Wisconsin card sorting test (WCST; Bolla et al., 2002; Lane et al., 2007). Likewise, administration of cannabinoid agonists in laboratory rodents has also been found to impair cognitive flexibility in attentional set shifting paradigms – developed as an equivalent to the human WCST – (Egerton et al., 2005; Hill et al., 2006) and in an olfactory go/no-go discrimination task (Sokolic et al., 2011). It has been suggested that these cannabinoid effects might be related to the modulatory influence of ECB signaling on PFC neurotransmission (e.g., dopamine, GABA, and glutamate; Egerton et al., 2006; Pattij et al., 2008).

Beside this strong connection between prefrontocortical ECB signaling and cognitive functioning, the cortical ECB system appears to be also important for emotional reactivity (Valverde,

2005; Viveros et al., 2005; Holmes and Wellman, 2009; Moreira et al., 2009). Stress- and anxiety-inducing stimuli consistently activate the PFC in rats (Singewald et al., 2003; Rubino et al., 2007), and in particular the mPFC appears to be an important region for anxiety-related behaviors (Holmes and Wellman, 2009). Lesions of the mPFC in rats have been found to induce anxiolytic-like effects in the elevated plus maze (EPM), social interaction and the shock-probe test (Shah and Treit, 2003). Additionally, an excitatory influence of corticotropin-releasing hormone in the mPFC has been suggested to modulate stress-induced HPA activity and anxiety-related behavior (Jaferi and Bhatnagar, 2007). With respect to ECB signaling in the PFC, experimental modulations of levels of the endocannabinoid anandamide (AEA), mainly by inhibition of the AEA degrading enzyme fatty acid amide hydrolase (FAAH) in the PFC, have been found to alter emotional behavior in rats (Rubino et al., 2008). While a strong decrease of AEA levels in the PFC by lentivirus-mediated overexpression of FAAH, was found to induce anxiogenic behavior, microinjections of the selective FAAH inhibitor, URB597, were found to induce anxiolytic responses at low doses and no effect or even an anxiogenic profile at higher doses (Rubino et al., 2008).

For the present study we were aiming to further examine the role of prefrontocortical ECB signaling on behavioral flexibility and emotional reactivity by region-specific overexpression of the CB1 receptor gene. Adeno-associated virus (AAV) gene transfer into a distinct brain region serves as an outstanding tool for studying gene function in complex behaviors of rodents (Klugmann and Szumlinski, 2008). We therefore employed the AAV-technology to overexpress the CB1 receptor gene in neurons of the mPFC of adult rats. The consequences of this manipulation on emotional behavior and cognition were investigated by a series of classical behavioral paradigms for emotional reactivity, including EPM, light/dark emergence test (EMT), and the social interaction test, and additionally, cognitive functions and behavioral flexibility were examined by the attentional set shift task (ASST).

MATERIALS AND METHODS

SUBJECTS

Twenty-eight male Wistar™ Han rcc (Wistar) rats weighing 200–250 g were purchased from Harlan Laboratories (AN Venray, Netherlands). They were housed in groups of six in standard Makrolon™ cages (Eurostandard type IV) under a 12/12-h light–dark cycle with the light phase starting at 8 am. During the light phase, a radio provided background noise. Animals had *ad libitum* access to tap water and standard lab chow if not indicated otherwise.

All experiments were conducted in accordance with the ethical guidelines for the care and use of laboratory animals, and were approved by local animal care committees (Sydney, Australia and Karlsruhe, Germany).

AAV VECTOR PRODUCTION AND STEREOTAXIC DELIVERY

The cDNA encoding the rat CB1 receptor was cloned into an AAV expression cassette containing the 1.1-kb CMV immediate early enhancer/chicken β-actin hybrid promoter (CBA), the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), and the bovine growth hormone polyadenylation sequence flanked by AAV2 inverted terminal repeats (pAAV-CB1). The same plasmid backbone with no cDNA was used as a control construct (pAAV-Empty). Of note, the CB1 cDNA (but not the AAV expression cassette) used in this study was identical to the one used in our previous work when we employed AAV-mediated overexpression of CB1 specifically in glutamatergic cells of the hippocampus and showed by GTPgammaS the biological functionality of the transgenic CB1 receptor (Guggenhuber et al., 2010). Packaging of AAV1/2 mosaic vectors with equal ratios of AAV1 and AAV2 capsid proteins was performed as described (Klugmann et al., 2005b). Briefly, using the standard $CaPO_4$ precipitation method, HEK293 cells were transfected with the AAV plasmid, two helper plasmids encoding AAV1 and AAV2 rep and cap genes, and the adenoviral helper plasmid pF Δ6. Cells were harvested 60 h after transfection, pellets lysed and vectors purified by heparin affinity chromatography. Genomic titers were determined by quantitative real-time PCR of vector genomes using primers against WPRE (During et al., 2003). For stereotaxic delivery of the AAV vector, adult rats were randomly assigned to treatment groups ($n = 14$) and control groups ($n = 14$). Animals were anesthetized with isoflurane (4% for induction and 1.5–2.5% for maintenance), administered via inhalation. The rats were then injected using 1.5 µl of either AAV-Empty or AAV-CB1 (6×10^{11} viral genomes/ml) bilaterally into the mPFC (+2.7 mm AP, ±0.5 mm ML, 4.5 mm DV from bregma), of adult rats based on established coordinates (Paxinos and Watson, 1998). Vector delivery was performed at a rate of 200 nl/min using a microprocessor-controlled mini-pump (World Precision Instruments, Sarasota, FL, USA) with 33 × G beveled needles (World Precision Instruments) in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). After the injection, the needle remained in place for two more minutes and was then carefully retracted in order to avoid vector backflow. Subsequently, the scalp was sutured and the rat was kept singly in a Makrolon™ cage (Eurostandard type III) until full recovery from anesthesia.

IMMUNOHISTOCHEMISTRY

The rostral–caudal extent of transgene expression of all animals used in behavioral experiments was assessed by CB1 immunohistochemistry. Animals not showing a robust pattern of transgene expression in the mPFC were excluded from the study. Rats were shortly anesthetized with a mixture of air and carbon dioxide (CO_2) and sacrificed by decapitation. Brains were quickly dissected, frozen on dry ice and stored at −80 until histological processing. Cryostat-cut 14 µm coronal sections were collected on Superfrost microscopic slides (Menzel GmbH & Co KG, Braunschweig, Germany) before postfixing in 10% buffered neutral formalin (SIGMA, Castle Hill, NSW, Australia). Then sections were rinsed with PBS containing 0.2% Triton X-100 (PBS-Triton), blocked in immunobuffer, (4% horse serum in PBS, pH 7.4, with 0.4% Triton X-100) for 30 min, and incubated overnight with a polyclonal anti-CB1 antiserum (1:2000; Cayman, Ann Arbor, USA). After washes, sections were incubated with anti-rabbit-Alexa488 antibody (1:1000, Molecular Probes, OR, USA). After two washes, the nuclear stain DAPI (Roche, Castle Hill, NSW, Australia) was administered for 5 min, and sections coverslipped in Mowiol. Immunostaining was visualized using a BX51U epifluorescent microscope (Olympus, Tokyo, Japan).

BEHAVIORAL TESTING

Behavioral testing began 3 weeks after vector infusion when AAV1/2-mediated transgene protein expression had peaked to remain at stable levels (Klugmann et al., 2005a). Behavioral paradigms were conducted in the order listed below and animals were left undisturbed for at least 3 days between the different test sessions. The experimenter was blind to the treatment of the test subjects.

Open field

Locomotor activity was measured in an open field. The open field consisted of four equal arenas (51 cm × 51 cm × 50 cm) made of dark PVC. Distance traveled (cm) was recorded for 30 min at a light intensity of 50 lx. For the analysis of locomotor activity the observation program Viewer[2] (Biobserve GmbH, Bonn, Germany) was used. Animals were habituated 1 day before testing to the new environment for 10 min.

Light/dark emergence test

The EMT took place in a light/dark box which consisted of two different compartments, separated by a dividing wall with a 10-cm × 15-cm wide opening which enabled the test subjects to move freely between the compartments. The first compartment, with black walls (25 cm × 25 cm × 40 cm) could be closed by a lid and was used as start box. The second compartment had gray walls (25 cm × 50 cm × 40 cm) and was brightly illuminated (90 lx). Rats were initially placed for 1 min in the dark, closed compartment and their behavior was recorded for 5 min after the start box was opened. Subsequent video analysis by a trained experimenter scored the latency of the animals to emerge from the dark compartment into the light compartment (s) (an entry was defined when the animal entered the compartment with all four limbs), the emergence frequency, the duration of time spent in the light compartment (s), the amount of rearings and risk assessment behavior (only head or forepaws are placed in the open compartment without concomitant movement of the hindlimbs, even if the rat subsequently entered the area). The apparatus was thoroughly cleaned with 70% ethanol between the sessions.

Elevated plus maze

The EPM consisted of a plus-shaped apparatus made of dark gray PVC elevated 50 cm above the floor with two open arms (12 cm × 50 cm × 50 cm) which were illuminated by 80 lx and two enclosed arms (12 cm × 50 cm × 50 cm). All arms extended from a central square (10 cm × 10 cm). At the beginning of each trial, rats were placed in a closed arm of the EPM. Each rat was videotaped for 5 min and the following behaviors were analyzed: number of entries into open or closed arms (an entry was defined if all four paws were placed on that arm), time spent in open and closed arms (s), head dips (the whole head is lowered beneath the edge of an open arm), risk assessment (only head or forepaws are placed in an open arm without concomitant movement of the hindlimbs, even if the rat subsequently entered the arm), self grooming and self-grooming time (s). Percentage of open arm entries [open arm entries/(open + closed arm entries) × 100] and percentage of time spent in open arms [open arm time/(open + closed arm time) × 100] were calculated as well. The apparatus was thoroughly cleaned with 70% ethanol between the sessions.

Social interaction test

Social interaction with an unfamiliar social partner (7- to 8-week-old male Wistar rat) was assessed in an open field for 5 min as described before (Schneider et al., 2008; Waltereit et al., 2011). The following behavioral elements were quantified only for the experimental rat: (1) Social behavior, including contact behavior (grooming and crawling over), social exploration (anogenital and non-anogenital investigation), and approach/following; (2) social evade was scored as an active withdrawal from social contact; and (3) self-grooming behavior (for detailed description see Schneider et al., 2008).

Attentional set shift test

Apparatus. The test apparatus was made of dark gray PVC consisting of a small start compartment (20 cm × 20 cm × 40 cm) adjacent to the test compartment (40 cm × 50 cm × 40 cm). The two compartments were separated by a sliding door of 20 cm width. Two small ceramic pots (diameter 7 cm, depth 4 cm) were positioned into the test compartment 16 cm apart from each other and separated by a solid divider (20 cm length). One of the cups was baited with a casein pellet (Bio Serve Dustless Precision Pellets®, Bilaney, Kent, UK). The bowls were filled with different digging materials that were scented and the food reward was deeply buried into one of the pots. Rats were trained to dig in the bowls to retrieve the rewards. The presence or absence of the reward pellet in the digging bowl was targeted by either an olfactory (odor of digging medium) or a visual–tactile cue (shape and tactile quality of digging medium).

Habituation. Animals were familiarized with the food reward, the ceramic pots, and different digging materials prior to testing. During 1–2 nights, the pots were filled with homecage bedding and casein pellets were placed on top and buried in the bowls. The pots were rebaited several times and left in the homecage overnight (not more than three pots per cage). The following nights, some of the digging materials were introduced in the same manner. On the second day of habituation, two familiar animals were placed into the test apparatus and allowed to freely explore the entire test box for 15 min. Afterward, the rats were returned to the homecage. At the next day, each rat was placed into the apparatus individually for a 15-min habituation period. **Figure 1** illustrates the timeline of habituation and testing procedure. During the complete period of ASST testing (including the habituation period) all animals were maintained on approximately 90% of their free-feeding bodyweight by applying a mild food restriction schedule (12 g chow/rat/day).

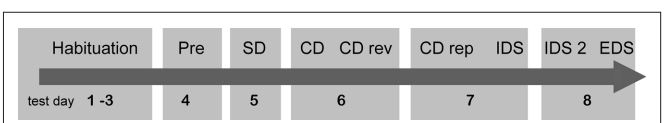

FIGURE 1 |Timeline of the ASST paradigm. Pre: pretraining; SD: simple discrimination; CD: compound discrimination; CDrev: compound discrimination reversal; CDrep: compound discrimination repetition; IDS; intradimensional shift; EDS: extradimensional shift.

Testing procedure. The testing procedure was adapted from Birrell and Brown (2000; see also Colacicco et al., 2002; Egerton et al., 2005; **Figure 1**). After habituation, rats were subjected to a pretraining schedule. Therefore, animals had to retrieve the reward from Empty pots in the apparatus and subsequently from pots filled with digging medium. First, the reward was placed on the top of the digging medium and in following trials the pellet was gradually buried deeper in the digging material. The rats had to retrieve the reward five times within 2 min trials and then four times within 1 min trials. As soon as the rat retrieved the pellet or the trial time expired, the animal was gently pushed by the experimenter into the waiting compartment. The pots were rebaited during an intertrial interval of 30 s during which the rat waited in the start compartment until the sliding door was lifted again for the next trial. After the test session the rat was returned to its homecage. Material from the pretraining was not used again in later testing stages.

For the training sessions, eight common spices: capsicum, cumin, basil, thyme, rosemary, nutmeg, dill, and cardamom were used as odor stimuli. The digging media were colored and black silica sand, beech chipping, pine bark, cork granules, hamster bedding, straw pellets, and rough stones (see **Table 1**). The digging media were mixed with the spices and additional casein pellet powder was intermixed in order to exclude the possibility of olfactory reward detection.

In all training sessions, a criterion of six consecutive correct trials was used for successful learning (trials to criterion). This method was applied for all subsequent training trials. For the simple discrimination (SD) task, each rat was presented two bowls containing scented digging medium with the same odor but different media. The visual/tactile stimulus dimension indicated the position of the reward during SD testing and therefore, rats had to learn that only the bowl with a certain medium contained the food pellet. For the compound discrimination (CD), which was tested 1 day later, an additional odor was introduced and used together with the two familiar digging media and the previous odor. In this training stage, the digging media could be paired with one of the two odors respectively. However, still the previously baited digging medium – used during SD – indicated the location of the reward during this stage, independent from the two odors. In the next session (on the same day), the previously learned rule was reversed (CD reversal, CDrev). The medium that had previously been incorrect was now associated with the food reward and accordingly, the unrewarded sets became baited. On the following day, a repetition test (CD repetition, CDrep) ensured that the animals had not forgotten the rules of the CDrev. In the following test session, the intradimensional shift (IDS), a set of new complex stimuli was introduced, and the rat had to discriminate the baited from the unbaited cup by attending at the same perceptual dimension (digging material) as in the previous training. The subsequent day, a new set of complex stimuli was introduced and the rat had to apply the same rule (IDS2). In the last test session, again a new set of complex stimuli was presented but this time a cue of the previously irrelevant perceptual dimension predicted the reward (extradimensional shift, EDS). Therefore, not the type of digging material predicted the reward any longer, but the odor was relevant to obtain the reward.

If an animal stopped responding for several trials during a test session it was returned to the homecage for up to 1 h before resuming the test again. In this case, the sum of the number of trials was taken.

Table 1 | Examples of odor-medium pairs employed in the ASST.

Digging medium	Digging medium	Odor	Odor
Seramis®			
Colored silica sand (3–4 mm)	Hamster bedding	Cumin	Capsicum
Beech chipping	Rough stones	Nutmeg	Basil
Straw pellets	Pine bark	Thyme	Dill
Cork granules	Black silica sand (1–2 mm)	Rosemary	Cardamom

STATISTICAL ANALYSIS

Differences between CB1-R and Empty vector expressing animals for locomotor activity, EPM and EMT performance as well as social interaction testing were analyzed by Student's *t*-tests. Performance in the ASST was analyzed by MANOVA. The overall performance in the ASST between the groups was compared and specified by Wilk's λ, whereas learning differences at each ASST stage were calculated with multiple ANOVAs.

All data are expressed as mean ± SEM. The overall level of statistical significance was defined as $p < 0.05$.

RESULTS

HISTOLOGICAL ANALYSIS

After completion of behavioral testing (4 month after infusion), the gene transfer efficacy was determined by immunohistochemical analysis using an antibody against CB1 receptors. Abundant ectopic CB1 receptor immunoreactivity could be detected specifically in the mPFC of AAV-CB1-injected animals including prelimbic, infralimbic, and cingulate cortical areas (**Figures 2A–C**). Consistent with our previous studies using the same AAV serotype but different transgenes (Lominac et al., 2005), the rostro-caudal extension of the vector spread was observed 1 mm around the injection site. In comparison, immunoreactivity of endogenous CB1 in AAV-Empty treated brains revealed by increased exposure time was more homogenous and less abundant (**Figures 2D–F**) indicating anatomically correct and efficient gene delivery. At higher magnification, ectopic CB1 receptor expression was visualized in neuronal soma and the neuropil (**Figures 2A′–C′**). Inconsistent or low CB1 receptor expression was detected in the mPFC of two animals and these animals were therefore excluded from further analysis.

LOCOMOTOR ACTIVITY

No significant differences were detected between CB1-R rats and Empty animals for locomotor activity in an open field (**Figure 3**). Both groups did not differ in distance traveled (cm) during the 30-min test period (Student's *t*-test, $p > 0.05$; CB1-R: $n = 12$; Empty: $n = 14$).

LIGHT/DARK EMT

No significant differences were detected in the light/dark EMT between CB1-R and Empty animals for emergence latency, risk assessment, time spent in the lit compartment and rearing (Student's *t*-test, $p > 0.05$). However, statistical analysis revealed a strong trend ($p = 0.056$) for a higher emergence frequency of CB1-R rats compared to controls (**Table 2**; CB1-R: $n = 12$; Empty: $n = 14$).

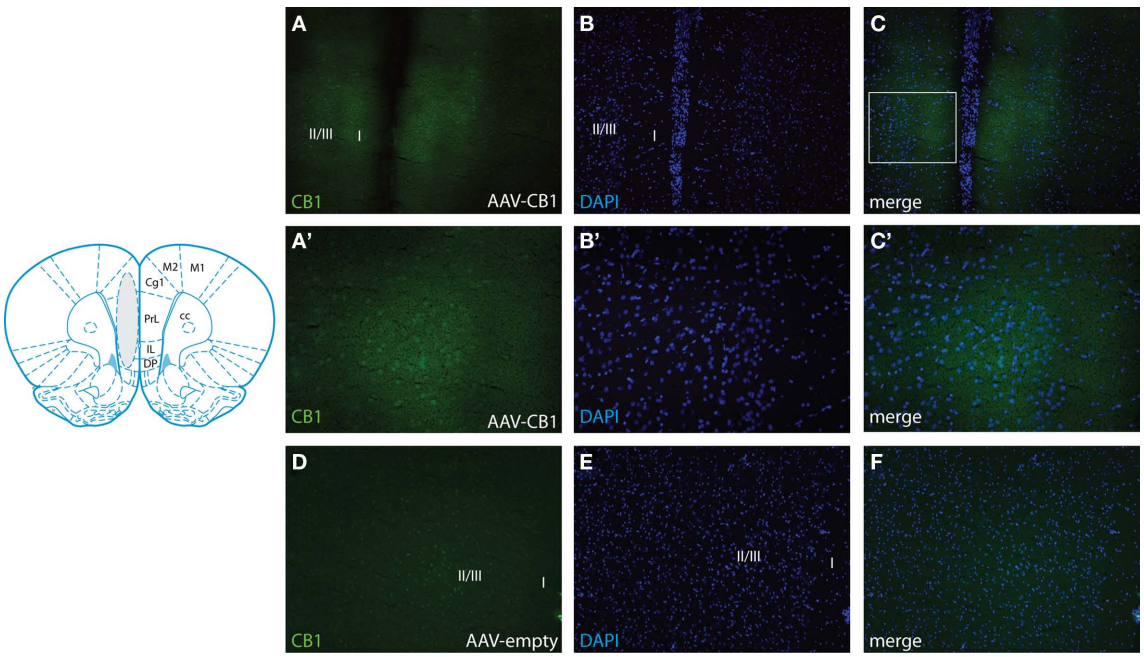

FIGURE 2 | Adeno-associated virus-mediated CB1 expression in the mPFC. The cartoon (adapted from Paxinos and Watson, 1998) shows the representative transduced area (gray) in animals injected with AAV-CB1. Representative immunostaining for CB1 in the mPFC of AAV-CB1-injected rats at low (10× for **A–C**) and high (20× for **A′–C′**) magnification. **(A)** Extent of CB1 immunoreactivity showing robust transduction in the target area. **(B)** Same section stained for the nuclear stain DAPI. **(C)** Overlay of **(A)** and **(B)**. The box in **(C)** indicates the area magnified in **(A′–C′)**. Note that adjustment of the exposure time for visualization of ectopic CB1 precludes visualization of endogenous CB1. The counterstaining with DAPI delineates the tissue and shows correct targeting of ectopic CB1 expression **(C)**. (**C′**) High power micrograph showing CB1 immunoreactivity in the neuronal soma and neuropil of the mPFC. **(D)** Representative brain section (left hemisphere) of an AAV-Empty-injected animal showing endogenous CB1 immunoreactivity at low power (10×). Note that the exposure time was 2.5× more than for the visualization of transgenic CB1 shown in **(A)**. **(E)** DAPI stain. **(F)** Merger of **(D)** and **(E)**. Prl, prelimbic cortex; Cg1, area 1 of the cingulate cortex; IL, infralimbic cortex; DP, dorsal peduncular cortex; M1/2, motor cortex; cc, corpus callosum; I, II, III, cortical layers.

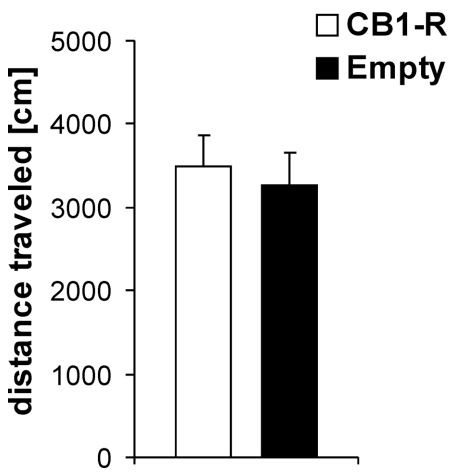

FIGURE 3 | Locomotor activity in an open field. No significant differences were found between CB1 receptor overexpressing animals (CB1-R) and Empty vector injected controls (Empty). Values are expressed as mean ± SEM.

Table 2 | Light/dark EMT performance in CB1-R rats and Empty control animals.

EMT	Empty	CB1-R
Emergence frequency	3.0 (±0.5)	4.83 (±0.7)#
Emergence latency (s)	126.6 (±22.4)	81.3 (±16.3)
Rearing	5.8 (±1.7)	8.9 (±1.2)
Risk assessment	9.0 (±1.2)	10.4 (±0.8)
Time in lit compartment (s)	47.8 (±13.1)	70.1 (±9.2)

Values are expressed as mean ± SEM ($p < 0.1$ is indicated by #).

ELEVATED PLUS MAZE

Similar as for the EMT, only subtle differences were detected between CB1-R rats and Empty controls in the EPM (**Table 3**). The two groups did not differ for time in open and closed arms, percentage of time spent in open arms, open arm entries, percentage of open arm entries, head dips, risk assessment, and self grooming (Student's *t*-test, $p > 0.05$). However, a strong increase in closed arm entries was detected in rats overexpressing the CB1 receptor ($p = 0.009$; CB1-R: $n = 12$; Empty: $n = 14$).

SOCIAL INTERACTION TEST

Several behavioral differences between CB1-R rats and controls were observed during social interaction testing (**Figure 4**). CB1-R animals engaged significantly more in anogenital exploration (Student's *t*-test, $p = 0.009$) and approach/following ($p = 0.032$) during interaction with the unfamiliar social partner compared to Empty animals. Additionally, CB1-R rats showed significant lower

social contact behavior compared with the Empty group ($p = 0.013$). Finally, CB1-R animals were found to withdraw significantly more often from social contact if initiated by the social partner (social evade; $p = 0.024$). No significant differences between the groups were detected for non-anogenital exploration and self-grooming behavior (Student's t-test, $p > 0.05$; CB1-R: $n = 12$; Empty: $n = 14$).

ATTENTIONAL SET SHIFT TASK

All animals learned to perform a series of six consecutive correct trials at each stage of the set shifting paradigm. No differences in initial reward consumption were detected during habituation between CB1-R and Empty animals (data not shown). Over the course of the whole experiment animals needed gradually less trials to reach the learning criterion for pretraining, SD and CD as well as for IDS and IDS2. All rats needed more trials to criterion to successfully complete the CD reversal and the EDS. The higher number of trials to criterion indicates that (a) CDrev and EDS were more challenging than the others stages (see also Barense et al., 2002; Colacicco et al., 2002) since subjects had formed attentional sets, and (b) that the set shift test worked properly and rats learned the cognitive task (**Figure 5**). MANOVA analysis revealed an overall significant difference between CB1-R and Empty control animals for the ASST (Wilk's $\lambda = 0.14$ $F_{8,16} = 2.9$ $p = 0.035$). Further analysis by multiple ANOVAs indicated a significant difference between CB1-R and Empty control animals only for the CDrev stage ($F_{1,23} = 7.4$ $p = 0.012$), where CB1-R animals required more trials to criterion than Empty animals. No statistical differences between CB1-R and Empty control rats were found in the performance at all other stages ($p > 0.05$; CB1-R: $n = 12$; Empty: $n = 14$).

Table 3 | Elevated plus maze performance in CB1-R rats and Empty control animals.

EPM	Empty	CB1-R
Open arm time (s)	25.6 (±8.8)	35.2 (±8.9)
Closed arm time (s)	250.6 (±15.4)	231.4 (±16.7)
Open arm time (%)	10.3 (±3.7)	14.1 (±3.7)
Open arm entries	2.1 (±0.7)	2.3 (±0.5)
Closed arm entries	6.4 (±0.8)	9.5 (±0.7)*
Open arm entries (%)	18.4 (±5.7)	16.8 (±3.1)
Head dips	7.4 (±1.7)	6.6 (±1.0)
Risk assessment	7.7 (±1.0)	9.4 (±0.7)
Self grooming	0.6 (±0.2)	0.4 (±0.1)

Values are expressed as mean ± SEM ($p < 0.05$ is indicated by asterisks).

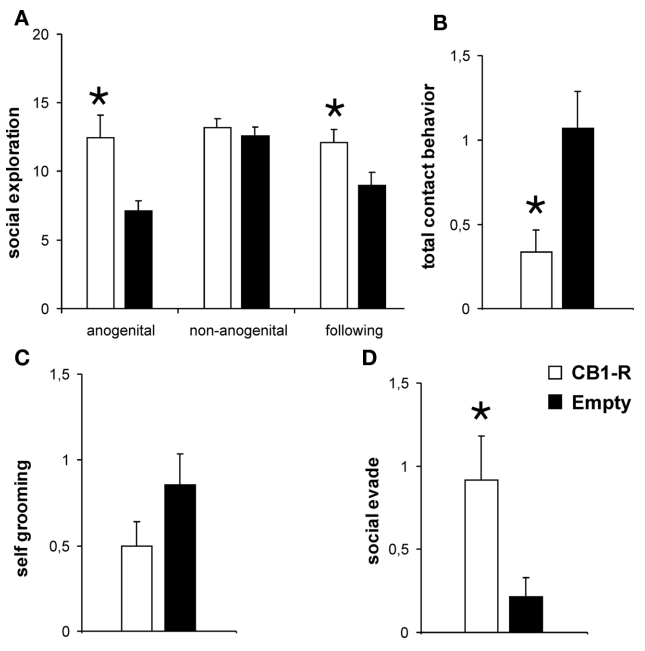

FIGURE 4 | Behavioral performance during social interaction with an unknown social partner. Significant differences between CB1-R and Empty animals were detected for anogenital exploration, approach/following **(A)**, social contact behavior **(B)** and evade upon social contact **(D)**. No differences were observed for non-anogenital exploration **(A)** and self-grooming behavior **(C)**. Values are expressed as mean ± SEM ($p < 0.05$ is indicated by asterisks).

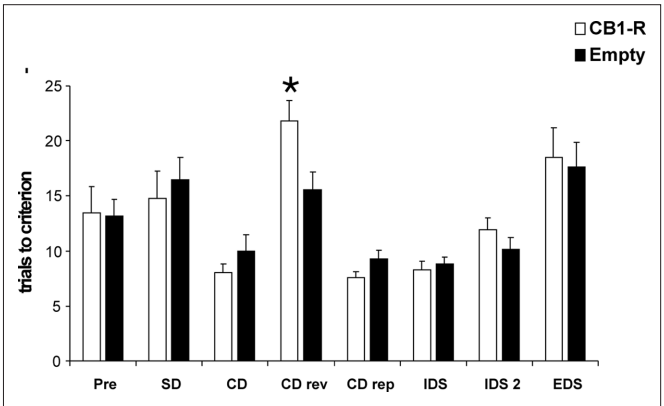

FIGURE 5 | Behavioral performance in the ASST. CB1-R rats differed significantly from Empty animals in the CDrev stage. Values are expressed as mean ± SEM ($p < 0.05$ is indicated by asterisks).

DISCUSSION

Local overexpression of the CB1 receptor by AAV-mediated gene transfer in the mPFC (including prelimbic, infralimbic, and anterior cingulate regions) of adult rats was found to alter emotional reactivity and social behavior and induce a deficit in cognitive flexibility. We detected subtle differences during testing of classical anxiety-related behavioral paradigms – EPM and light/dark EMT – between CB-R rats and Empty controls, which were mainly related to activity levels in these anxiety-inducing environments. Additionally, inadequate social behavior and social withdrawal were observed after cortical CB1 receptor overexpression during interaction with an unfamiliar conspecific. Finally, CB1-R rats showed a deficit specifically in reversal learning during the ASST.

The PFC is an essential brain region for higher-order cognitive functions and emotional processing, mainly due to its important integral role for the selection and processing of information necessary to plan, control and direct behavior according to changing environmental demands (Holmes and Wellman, 2009). CB1 receptors are abundantly expressed in the PFC (Herkenham et al., 1990; Mailleux and Vanderhaeghen, 1992; Marsicano and Lutz, 1999), strongly indicating an involvement of ECB signaling for

the modulation of prefrontocortical-mediated behaviors (Egerton et al., 2006). CB1 receptors are expressed by GABAergic interneurons and by pyramidal neurons in the PFC (Marsicano and Lutz, 1999) and ECBs appear to play a key role in the induction of LTD at PFC synapses of evoked and spontaneous excitatory postsynaptic currents recorded in layer V/VI pyramidal neurons (Lafourcade et al., 2007). A plethora of data indicates differences in the function of the CB1 receptor in glutamatergic versus GABAergic cells with respect to the brain region and experimental paradigms. These data are obtained mainly from pharmacological studies or genetic studies using conditional mouse mutants with cell-type-specific CB1 receptor ablation (e.g., Azad et al., 2008; Steiner et al., 2008; Massa et al., 2010). Only recently, we could achieve AAV-mediated conditional overexpression of CB1 receptors in glutamatergic hippocampal neurons (Guggenhuber et al., 2010). However, this approach depends on the availability of transgenic animals expressing cre-recombinase under tissue specific promoters and is therefore limited so far to mice. For the present study we have employed a neurotropic AAV vector system known to transduce all types of neurons at similar efficiency (Guggenhuber et al., 2010). The major difference to the genetic studies using germ line transgenics is that animals subjected to AAV-mediated CB1 receptor delivery in this study had a normal development into adulthood and the somatic gene transfer was highly specific to a confined brain area (mPFC). A single administration of AAV-CB1 results in long-term and stable transgene expression (Klugmann et al., 2005a) and to our knowledge this is the first study to demonstrate behavioral effects after persistent overexpression of the CB1 receptor in the adult rat PFC. However, despite the extraordinary spatio-temporal control that is achieved by employing the AAV-technology, one must be cautious to what extent conclusions can made about the role of endogenous CB1 receptors in physiological/behavioral processes based on an approach where protein levels are artificially elevated. In fact, since the tropism of the AAV1/2 serotype used in this study is pan-neuronal (Richichi et al., 2004; Guggenhuber et al., 2010), it is likely that GABAergic and glutamatergic neurons in the targeted PFC were transduced with equal efficacy. By elevating CB1 expression in the mPFC, neural activity in this brain region is likely to be affected in various ways, perhaps by reducing glutamatergic transmission or GABAergic transmission, most likely both. Importantly, the protein levels of endogenous CB1 are 20–30-fold enriched in GABAergic over glutamatergic neurons, so it is conceivable that the introduction of comparable absolute amounts of ectopic CB1 will yield very different levels of relative overexpression in these different types of neurons. This consideration suggests that overexpression may be supraphysiological in glutamatergic but not in GABAergic cells.

EMOTIONAL BEHAVIOR

Although, the ECB system has been shown to act as an important modulator of emotional behavior and emotional reactivity (Valverde, 2005; Viveros et al., 2005; Moreira et al., 2009) and especially ECB signaling in the PFC has been implicated in these behaviors (Rubino et al., 2007, 2008), we did not observe profound alterations in anxiety-related behavior after overexpression of the CB1 receptor in the mPFC in the light/dark EMT and the EPM. Statistical differences compared to Empty controls were only detected in emergence frequency into the lit compartment (EMT) and the number of closed arm entries (EPM), which were both increased in CB1-R animals. Therefore, overexpression of the CB1 receptor in the mPFC appears to stimulate locomotor activity in anxiogenic environments. Since we did not observe any differences between the testing groups for normal activity in an open field under non-anxiogenic conditions (low lux and familiar environment), this hyperlocomotion and increased exploratory behavior seems to be linked exclusively to emotionally challenging environments, without affecting anxiety-related behaviors *per se*.

The involvement of ECB signaling in the mediation of anxiety-related behaviors is very complex and only partially understood. In animal studies cannabinoids have been shown to induce anxiogenic as well as anxiolytic-like responses, depending upon dosage, behavioral tests used, the context, species, or genetic strain (Valverde, 2005; Viveros et al., 2005). CB1 receptor deficient mice have been shown to display anxiogenic-like responses in different behavioral paradigms, such as EPM, light/dark box, and open field (Haller et al., 2002; Viveros et al., 2005). Accordingly, in FAAH knockout mice, where AEA levels are increased, reduced anxiety-related behaviors have been reported both in the EMP and in the light/dark EMT test. These genotype-related differences were prevented by the CB1 receptor antagonist SR141716 (Moreira et al., 2008).

With respect to prefrontocortical effects of cannabinoids on emotional behavior, it has been demonstrated recently that the endocannabinoid AEA in the PFC appears to be an important modulator of anxiety-related behaviors (Rubino et al., 2008). Administration of methanandamide (a metabolically stable analog of AEA) directly into the PFC revealed anxiolytic-like responses in rats in the EPM test for low doses, whereas high doses induced anxiogenic effects. In line with this, a marked decrease of AEA levels in the PFC, achieved by lentivirus-mediated local overexpression of FAAH, produced an anxiogenic response, supporting an anxiolytic role for a physiological increases in AEA in the PFC (Rubino et al., 2008).

In the present study we did not observe a clear anxiolytic response. Our data indicate that increased expression of the CB1 receptor in the mPFC does not affect overall anxiety-related behaviors, but increases arousal and locomotor response in anxiogenic environments, probably through interaction with the amygdala. An important role for CB1 receptors within the amygdala–prefrontal cortical circuit has been suggested for heightened emotional processing since CB1 receptor activation was found to potentiate the encoding of emotional associative learning at the level of single mPFC neurons (Laviolette and Grace, 2006). Further studies will have to examine how AAV-mediated CB1 receptor overexpression in the mPFC affects AEA levels or FAAH activity within the mPFC and other prefrontal or subcortical regions.

SOCIAL INTERACTION

The social interaction test is an ethologically based test that measures explorative and social behavior between two rodents meeting for the first time in an open field and has been suggested as a measure for anxiety-related behaviors (File and Hyde, 1978). For the present study the test was used to assess social behaviors in CB1 receptor overexpressing animals and controls in an emotionally arousing context. Various differences between CB1-R rats and

Empty controls were detected during social interaction testing. A higher number of anogenital exploration and approach/following behavior was observed in CB1-R animals. Additionally, prefrontocortical overexpression of the CB1 receptor increased the number of active social evade upon contact of the social partner and decreased social contact behaviors (grooming and crawling over/under). Similar, as observed during the EPM and EMT, CB1-R animals appeared to be more active and showed increased exploratory behavior toward the unfamiliar conspecific during social interaction testing, compared to control animals. Despite this high and inadequate increase in social exploration, CB1-R animals were found to avoid normal social contact, especially when the contact was initiated by the social partner.

It is well known that the PFC is involved in the modulation of social behaviors and social skills (De Bruin, 1990; Wood, 2003) and we could show in a previous study that neonatal mPFC lesions in rats decreased social contact behavior persistently in adulthood (Schneider and Koch, 2005). A clear involvement of the ECB system and cannabinoids in social behaviors during development and adulthood has been demonstrated before for social interaction, social recognition, homecage social behavior, and social play behavior (e.g., Sieber, 1982; Schneider and Koch, 2002, 2005; Schneider et al., 2008; Trezza and Vanderschuren, 2008). With respect to social interaction testing it has been shown that administration of Δ9-tetrahydrocannabinol (THC; Sieber et al., 1980; Van Ree et al., 1984), as well as adolescent cannabinoid exposure (O'Shea et al., 2004) reduces social interaction in rodents. Additionally, reduced social interaction has also been described in CB1 receptor knockout mice (Haller et al., 2004). However, in most of these studies all social behaviors during social interaction testing were summed up to a single social interaction score and therefore no information is given on possible changes in particular behaviors (e.g., social exploration versus social contact behavior). We have found recently that acute treatment with the cannabinoid agonist WIN 55,212-2 (WIN) attenuates social exploratory behavior in adult rats. However, chronic pubertal WIN treatment was found to persistently decrease social contact behavior and to increase anogenital exploration and social withdrawal during social interaction testing and homecage recording (Schneider et al., 2008). Cannabinoid effects on active evade from social contact were also described in baboons, where THC was found to induce social withdrawal and isolation (Sieber, 1982). This is in line with our present observation on increased social evade upon social contact in CB1-R animals, although behavioral effects of cannabinoid pharmacology (in particular if administered systemically) can not be considered equivalent with the persistent and region-specific overexpression of the CB1 receptor in the mPFC.

BEHAVIORAL FLEXIBILITY IN THE ASST

Behavioral flexibility is an important cognitive skill for survival of an individual, since it enables an organism to successfully adapt to changing environments and circumstances, and requires the capacity to adjust behavioral strategies and to suppress "previous" whilst initiating "new" response patterns (Pattij et al., 2008). For the present study behavioral flexibility was assessed with the ASST – developed as an equivalent to the human WCST (Birrell and Brown, 2000). The ASST involves a series of compound perceptual discriminations that require subjects either to maintain attention and discriminate between two stimuli within one modality or dimension (IDS), or shift the attention between two stimuli from two different modalities or dimensions (EDS). CB1-R animals were found to show impaired learning in the CD reversal stage compared to Empty controls, whereas no significant differences were observed for other training stages. Our data indicate that overexpression of CB1 receptors in the mPFC does neither affect intradimensional or extradimensional set shifting abilities but impairs reversal learning of a previous rule. While extradimensional (attentional) set shifting ability serves as a measure of the capacity to shift attentional bias between different perceptual features of complex stimuli, reversal learning requires the capacity to update associations (to form new associations and at the same time inhibit those previously learned) between exteroceptive stimuli and reinforcement presentation when the contingencies between stimuli and reward presentation are reversed (Egerton et al., 2005).

An important role of the ECB system in these attentional and adaptational cognitive functions has been suggested by various studies in humans and rodents (for review see Egerton et al., 2006; Pattij et al., 2008). In humans, heavy marijuana use was shown to be associated with deficits in behavioral flexibility measured with the WCST (Bolla et al., 2002; Lane et al., 2007). Likewise, administration of cannabinoid agonists in laboratory rodents has also been found to impair cognitive flexibility in the ASST paradigm (Egerton et al., 2005), a cross maze task (Hill et al., 2006) and in an olfactory go/no-go discrimination task (Sokolic et al., 2011). Acute administration of THC impaired performance on the ASST when rats were required to reverse stimulus reward associations or shift cognitive set between stimuli belonging to the same perceptual dimension (IDS). In contrast, the ability to shift attentional set between perceptual dimensions (EDS) was unaffected by THC administration (Egerton et al., 2005). These results are partially in line with our present findings where persistent CB1 receptor overexpression impaired reversal learning without affecting EDS, although we did not detect additional effects on IDS in our CB1-R animals. Egerton et al. (2005) concluded from their findings that acute THC administration might selectively increase rigidity in the processes required to update responses based on affective associations between stimuli and reward presentation, but does not affect higher-order attentional flexibility. By testing attentional set shift abilities in a cross maze task, Hill et al. (2006) detected that administration of a high dose of the CB1 receptor agonist HU-210 consistently increased the tendency for rats to perseverate when shifting from a response to a visual-cue-based discrimination and vice versa, whereas a low dose of HU-210 elicited an opposite behavioral profile, with reliable reductions in perseverative errors. Additionally, systemic administration of a low dose of the CB1 receptor antagonist AM251 facilitated set shifting by reducing the number of perseverative errors. The cross maze task differs in many aspects from the ASST paradigm applied by Egerton et al. (2005) and therefore the outcome of both studies is difficult to compare. While deficits observed after CB1 receptor activation in the Hill et al. (2006) study in the cross maze paradigm could be interpreted as an impairment in extradimensional set shifting, it is also possible however that the effects observed are related to impairments in cognitive processes related to reversal learning (Hill et al., 2006), which

would be consistent with our present findings. Concerning reversal learning abilities in other behavioral tasks, a clear involvement of the ECB system has been demonstrated. Acute THC treatment was found to impair performance in rats during the reversal phase of spatial learning in the Morris water maze (Boucher et al., 2009). Additionally, it has been shown that low doses of THC and URB597 impaired reversal learning, but not the acquisition or performance, of a two-odor discrimination task (Sokolic et al., 2011). In contrast with these findings, it has been reported that CB1 receptor knockout mice also displayed impaired reversal learning in a water maze task (Varvel and Lichtman, 2002). Therefore, the ECB system indeed appears to be important in adjusting behavioral strategies, however, the detailed direction of its modulations has not been completely clarified so far, although our present data demonstrate that increased availability of CB1 receptors in the mPFC impairs reversal learning abilities.

Notably, our present findings indicate an important role of CB1 receptors in the rat mPFC in reversal learning, but not in set shifting abilities. This finding is quite surprising since lesion studies reported that reversal learning strategies in the ASST depend mainly on the orbitofrontal cortex (McAlonan and Brown, 2003), whereas set shifting is mediated by the mPFC (Birrell and Brown, 2000). However, in contrast to these findings, other studies indicated that the rodent mPFC is indeed important for reversal learning abilities in different behavioral paradigms (Wolf et al., 1987; Joel et al., 1997; Li and Shao, 1998). In particular restricted lesions to either the prelimbic or the infralimbic area of the mPFC, but not damage to the anterior cingulate area, was found to strongly inhibit reversal learning in a t-maze task (Li and Shao, 1998).

CONCLUSION

The present data indicate an important modulatory role for prefrontocortical CB1 receptors in emotional reactivity and arousal, social exploration and social withdrawal, as well as in cognitive flexibility. These findings might be relevant for neuropsychiatric disorders, since higher CB1 receptor expression levels in the PFC have been described post-mortem in schizophrenic patients (Dean et al., 2001; Zavitsanou et al., 2004). Schizophrenia is a chronic and severe brain disorder that has its symptomatic onset in early adulthood and affects multiple cognitive and behavioral functions, including behavioral flexibility and social skills (American Psychiatric Association, 1994). The AAV-technology applied in the present study therefore serves as an excellent tool to examine region-specific effects of alterations in CB1 receptor availability in the brain.

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REFERENCES

American Psychiatric Association. (1994). *Diagnostic and Statistical Manual of Mental Health Disorders (DSM IV)*. Washington, DC: American Psychiatric Press.

Azad, S. C., Kurz, J., Marsicano, G., Lutz, B., Zieglgansberger, W., and Rammes, G. (2008). Activation of CB1 specifically located on GABAergic interneurons inhibits LTD in the lateral amygdala. *Learn. Mem.* 15, 143–152.

Barense, M. D., Fox, M. T., and Baxter, M. G. (2002). Aged rats are impaired on an attentional set-shifting task sensitive to medial frontal cortex damage in young rats. *Learn. Mem.* 9, 191–201.

Birrell, J. M., and Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *J. Neurosci.* 20, 4320–4324.

Bolla, K. I., Brown, K., Eldreth, D., Tate, K., and Cadet, J. L. (2002). Dose-related neurocognitive effects of marijuana use. *Neurology* 59, 1337–1343.

Boucher, A. A., Vivier, L., Metna-Laurent, M., Brayda-Bruno, L., Mons, N., Arnold, J. C., and Micheau, J. (2009). Chronic treatment with [delta]9-tetrahydrocannabinol impairs spatial memory and reduces zif268 expression in the mouse forebrain. *Behav. Pharmacol.* 20, 45–55.

Colacicco, G., Welzl, H., Lipp, H. P., and Würbel, H. (2002). Attentional set-shifting in mice: modification of a rat paradigm, and evidence for strain-dependent variation. *Behav. Brain Res.* 132, 95–102.

De Bruin, J. P. C. (1990). Social behaviour and the prefrontal cortex. *Prog. Brain Res.* 85, 485–497.

Dean, B., Sundram, S., Bradbury, R., Scarr, E., and Copolov, D. D. (2001). Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience* 103, 9–15.

During, M. J., Young, D., Baer, K., Lawlor, P., and Klugmann, M. (2003). Development and optimization of adeno-associated virus vector transfer into the central nervous system. *Methods Mol. Med.* 76, 221–236.

Egerton, A., Allison, C., Brett, R. R., and Pratt, J. A. (2006). Cannabinoids and prefrontal cortical function: insights from preclinical studies. *Neurosci. Biobehav. Rev.* 30, 680–695.

Egerton, A., Brett, R. R., and Pratt, J. A. (2005). Acute delta9-tetrahydrocannabinol-induced deficits in reversal learning: neural correlates of affective inflexibility. *Neuropsychopharmacology* 30, 1895–1905.

File, S. E., and Hyde, J. R. G. (1978). Can social interaction be used to measure anxiety? *Br. J. Pharmacol.* 62, 19–24.

Guggenhuber, S., Monory, K., Lutz, B., and Klugmann, M. (2010). AAV vector-mediated overexpression of CB1 cannabinoid receptor in pyramidal neurons of the hippocampus protects against seizure-induced excitoxicity. *PLoS ONE* 5, e15707. doi: 10.1371/journal.pone.0015707

Haller, J., Bakos, N., Szirmay, M., Ledent, C., and Freund, T. F. (2002). The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur. J. Neurosci.* 16, 1395–1398.

Haller, J., Varga, B., Ledent, C., Barna, I., and Freund, T. F. (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, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., De Costa, B. R., and Rice, K. C. (1990). Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. U.S.A* 87, 1932–1936.

Hill, M. N., Froese, L. M., Morrish, A. C., Sun, J. C., and Floresco, S. B. (2006). Alterations in behavioral flexibility by cannabinoid CB1 receptor agonists and antagonists. *Psychopharmacology (Berl.)* 187, 245–259.

Holmes, A., and Wellman, C. L. (2009). Stress-induced prefrontal reorganization and executive dysfunction in rodents. *Neurosci. Biobehav. Rev.* 33, 773–783.

Jaferi, A., and Bhatnagar, S. (2007). Corticotropin-releasing hormone receptors in the medial prefrontal cortex regulate hypothalamic-pituitary-adrenal activity and anxiety-related behavior regardless of prior stress experience. *Brain Res.* 1186, 212–223.

Joel, D., Weiner, I., and Feldon, J. (1997). Electrolytic lesions of the medial prefrontal cortex in rats disrupt performance on an analog of the Wisconsin card sorting test, but do not disrupt latent inhibition: implications for animal models of schizophrenia. *Behav. Brain Res.* 85, 187–201.

Klugmann, M., Leichtlein, C. B., Symes, C. W., Serikawa, T., Young, D., and During, M. J. (2005a). Restoration of aspartoacylase activity in CNS neurons does not ameliorate motor deficits and demyelination in a model of Canavan disease. *Mol. Ther.* 11, 745–753.

Klugmann, M., Symes, C. W., Leichtlein, C. B., Klaussner, B. K., Dunning, J., Fong, D., Young, D., and During, M. J. (2005b). AAV-mediated hippocampal expression of short and long Homer 1 proteins differentially affect cognition and seizure activity in adult rats. *Mol. Cell. Neurosci.* 28, 347–360.

Klugmann, M., and Szumlinski, K. K. (2008). Targeting Homer genes using adeno-associated viral vector: lessons

learned from behavioural and neurochemical studies. *Behav. Pharmacol.* 19, 485–500.

Lafourcade, M., Elezgarai, I., Mato, S., Bakiri, Y., Grandes, P., and Manzoni, O. J. (2007). Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex. *PLoS ONE* 2, e709. doi: 10.1371/journal.pone.0000709

Lane, S. D., Cherek, D. R., Tcheremissine, O. V., Steinberg, J. L., and Sharon, J. L. (2007). Response perseveration and adaptation in heavy marijuana-smoking adolescents. *Addict. Behav.* 32, 977–990.

Laviolette, S. R., and Grace, A. A. (2006). Cannabinoids potentiate emotional learning plasticity in neurons of the medial prefrontal cortex through basolateral amygdala inputs. *J. Neurosci.* 26, 6458–6468.

Li, L., and Shao, J. (1998). Restricted lesions to ventral prefrontal subareas block reversal learning but not visual discrimination learning in rats. *Physiol. Behav.* 65, 371–379.

Lominac, K. D., Oleson, E. B., Pava, M., Klugmann, M., Schwarz, M. K., Seeburg, P. H., During, M. J., Worley, P. F., Kalivas, P. W., and Szumlinski, K. K. (2005). Distinct roles for different Homer1 isoforms in behaviors and associated prefrontal cortex function. *J. Neurosci.* 25, 11586–11594.

Mailleux, P., and Vanderhaeghen, J. J. (1992). Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. *Neuroscience* 48, 655–668.

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

Massa, F., Mancini, G., Schmidt, H., Steindel, F., Mackie, K., Angioni, C., Oliet, S. H., Geisslinger, G., and Lutz, B. (2010). Alterations in the hippocampal endocannabinoid system in diet-induced obese mice. *J. Neurosci.* 30, 6273–6281.

McAlonan, K., and Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behav. Brain Res.* 146, 97–103.

Moreira, F. A., Grieb, M., and Lutz, B. (2009). Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. *Best Pract. Res. Clin. Endocrinol. Metab.* 23, 133–144.

Moreira, F. A., Kaiser, N., Monory, K., and 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.

O'Shea, M., Singh, M. E., McGregor, I. S., and Mallet, P. E. (2004). Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. *J. Psychopharmacol.* 18, 502–508.

Owen, A. M., Roberts, A. C., Polkey, C. E., Sahakian, B. J., and Robbins, T. W. (1991). Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. *Neuropsychologia* 29, 993–1006.

Pattij, T., Wiskerke, J., and Schoffelmeer, A. N. M. (2008). Cannabinoid modulation of executive functions. *Eur. J. Pharmacol.* 585, 458–463.

Paxinos, G., and Watson, C. (1998). *The Rat Brain in Stereotaxic Coordinates.* San Diego, CA: Academic Press.

Richichi, C., Lin, E. J., Stefanin, D., Colella, D., Ravizza, T., Grignaschi, G., Veglianese, P., Sperk, G., During, M. J., and Vezzani, A. (2004). Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. *J. Neurosci.* 24, 3051–3059.

Rubino, T., Realini, N., Castiglioni, C., Guidali, C., Vigano, D., Marras, E., Petrosino, S., Perletti, G., Maccarrone, M., Di Marzo, V., and Parolaro, D. (2008). Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. *Cereb. Cortex* 18, 1292–1301.

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

Schneider, M., and Koch, M. (2002). The cannabinoid agonist WIN 55,212-2 reduces sensorimotor gating and recognition memory in rats. *Behav. Pharmacol.* 13, 29–37.

Schneider, M., and Koch, M. (2005). Deficient social and play behavior in juvenile and adult rats after neonatal cortical lesion: effects of chronic pubertal cannabinoid treatment. *Neuropsychopharmacology* 30, 944–957.

Schneider, M., Schömig, E., and Leweke, F. M. (2008). Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addict. Biol.* 13, 345–357.

Shah, A. A., and Treit, D. (2003). Excitotoxic lesions of the medial prefrontal cortex attenuate fear responses in the elevated-plus maze, social interaction and shock probe burying tests. *Brain Res.* 969, 183–194.

Sieber, B. (1982). Influence of hashish extract on the social behaviour of encountering male baboons (*Papio c. anubis*). *Pharmacol. Biochem. Behav.* 17, 209–216.

Sieber, B., Frischknecht, H. R., and Waser, P. G. (1980). Behavioral effects of hashish in mice. I. Social interactions and nest-building behavior of mice. *Psychopharmacology* 70, 149–154.

Singewald, N., Salchner, P., and Sharp, T. (2003). Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. *Biol. Psychiatry* 53, 275–283.

Sokolic, L., Long, L. E., Hunt, G. E., Arnold, J. C., and McGregor, I. S. (2011). Disruptive effects of the prototypical cannabinoid delta9-tetrahydrocannabinol and the fatty acid amide inhibitor URB-597 on go/no-go auditory discrimination performance and olfactory reversal learning in rats. *Behav. Pharmacol.* 22, 191–202.

Steiner, M. A., Marsicano, G., Wotjak, C. T., and Lutz, B. (2008). Conditional cannabinoid receptor type 1 mutants reveal neuron subpopulation-specific effects on behavioral and neuroendocrine stress responses. *Psychoneuroendocrinology* 33, 1165–1170.

Trezza, V., and Vanderschuren, L. J. M. J. (2008). Cannabinoid and opioid modulation of social play behavior in adolescent rats: differential behavioral mechanisms. *Eur. Neuropsychopharmacol.* 18, 519–530.

Valverde, O. (2005). Participation of the cannabinoid system in the regulation of emotional-like behaviour. *Curr. Pharm. Des.* 11, 3421–3429.

Van Ree, J. M., Niesink, R. J. M., and Nir, I. (1984). [delta]1-Tetrahydrocannabinol but not cannabidiol reduces contact and aggressive behavior of rats tested in dyadic encounters. *Psychopharmacology (Berl.)* 84, 561–565.

Varvel, S. A., and Lichtman, A. H. (2002). Evaluation of CB1 receptor knockout mice in the Morris water maze. *J. Pharmacol. Exp. Ther.* 301, 915–924.

Viveros, M. P., Marco, E. M., and File, S. E. (2005). Endocannabinoid system and stress and anxiety responses. *Pharmacol. Biochem. Behav.* 81, 331–342.

Waltereit, R., Japs, B., Schneider, M., de Vries, P., and Bartsch, D. (2011). Epilepsy and Tsc2 haploinsufficiency lead to autistic-like social deficit behaviors in rats. *Behav. Genet.* 41, 364–372.

Wolf, C., Waksman, D., Finger, S., and Almli, C. R. (1987). Large and small medial frontal cortex lesions and spatial performance of the rat. *Brain Res. Bull.* 18, 1–5.

Wood, J. N. (2003). Social cognition and the prefrontal cortex. *Behav. Cogn. Neurosci. Rev.* 2, 97–114.

Zavitsanou, K., Garrick, T., and Huang, X. F. (2004). Selective antagonist [3H] SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 28, 355–360.

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Contrasting effects of lithium chloride and CB1 receptor blockade on enduring changes in the valuation of reward

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When an organism responds for a reward, its learned behavior can be characterized as goal-directed or habitual based on whether or not it is susceptible to reward devaluation. Here, we evaluated whether instrumental responding for brain stimulation reward (BSR) can be devalued using a paradigm traditionally used for natural rewards. Rats were trained to lever press for BSR; afterward, BSR was paired with either lithium chloride (LiCl, 5 mg/kg, i.p.), a pro-emetic, or AM251, a CB1 receptor antagonist (3 mg/kg, i.p.) or the vehicle of these compounds. Pairings of BSR with these compounds and their vehicles were performed in a novel environment so that only unconditional effects of BSR would be affected by the pharmacological manipulations. Subsequently, in a probe test, all rats were returned in the drug-free state to the boxes where they had received training and instrumental responding was reassessed in the absence of BSR delivery. When compared to control, LiCl produced a significant decrease in the number of responses during the test session, whereas AM251 did not. These results show that instrumental responding for BSR is susceptible to devaluation, in accord with the proposal that this behavior is supported at least in part by associations between the response and the rewarding outcome. Further, they suggest that reward modulation observed in studies involving the use of CB1 receptor antagonists arises from changes in the organism's motivation rather than drug-induced changes in the intrinsic value of reward.

Keywords: brain stimulation reward, reward devaluation, endocannabinoids, AM251, lithium chloride

INTRODUCTION

Goal-directed behavior, unlike habits, is adjusted immediately and appropriately to changes in the value of the expected outcome. This reflects the finding that such behavior is based on associations between the response and the outcome or goal of the action, so that organisms may continuously re-evaluate their goal objects and dynamically change their actions in order to effectively produce adaptive behaviors (Dickinson, 1985). A rewarding goal's value can be diminished by selective satiety and by induction of taste aversion (Colwill and Rescorla, 1986; Yin and Knowlton, 2002). Such manipulations do not produce a significant change in habitual behaviors; habits persist even if the reward becomes less attractive or if the action is not necessary to earn the reward (Adams and Dickinson, 1981; Adams, 1982). Thus, once lever pressing for a reward becomes habitual in this sense, induced taste aversion or unlimited exposure to the reward prior to a probe test have very little consequences on subsequent lever pressing behavior.

Since the discovery that organisms will seek and reinitiate electrical stimulation to certain brain areas (Olds and Milner, 1954; Olds, 1962), brain stimulation reward (BSR) has become the paradigm of choice for studying the neural reward circuitry. Some of the reasons for this are that the electrical stimulation can be precisely manipulated and that its parameters have neurophysiological meaning. The current passed through the electrode tip depolarizes nearby neurons thereby triggering action potentials. If the train and pulse duration are held constant, the number of action potentials elicited in the neurons close to the electrode tip is determined by the pulse frequency, whereas the stimulation current or pulse amplitude determines the radius of effective stimulation, and thus the number of cells excited by the electrode (Gallistel et al., 1981).

The behavior elicited and controlled by the electrical stimulation, unlike the behavior controlled by natural rewards (McSweeney and Roll, 1993), is stable both between and within sessions. The electrical signal is delivered directly into the brain, bypassing sensory inputs, and physiological feedback mechanisms that discount natural rewards over the length of the experimental session. Moreover, it is delivered with a minimal delay after the behavior that procures the reward has occurred; therefore response–reward delays that degrade natural rewards are avoided. The behavior controlled by the rewarding signal that arises as a result of the delivery of electrical pulses is very sensitive to changes in the stimulation parameters and therefore the rewarding efficacy.

Even though BSR has very peculiar characteristics, the rewarding signal delivered by the electrode and that of natural rewards are evaluated and compared on a similar scale. The rewarding signal produced by the stimulation can compete with, summate with (Conover and Shizgal, 1994; Conover et al., 1994), and substitute for (Green and Rachlin, 1991) natural rewards. Drugs that are used to devaluate natural rewards like lithium chloride (LiCl) decrease the rewarding effect of electrical brain stimulation. Specifically, when the curve shift paradigm is used it has been reported that injecting LiCl at relatively high doses (100 or 200 mg/kg, i.p.) produces an increase in self-stimulation threshold, meaning that

higher stimulation is required to produce a response similar to that observed during vehicle conditions (Tomasiewicz et al., 2006; Mavrikaki et al., 2009). Thus, a rightward shift of the curve that relates operant performance to stimulation frequency occurs, without significantly disrupting performance capacity (Miliaressis et al., 1986).

A similar increase in reinforcement threshold is observed when the post-reinforcement pause method is used (Cassens and Mills, 1973). In this method the experimental subjects are trained under a concurrent fixed ratio (FR)–continuous reinforcement (CRF) schedule of reinforcement, in which the stimulation for the FR schedule is kept at maximal intensity whereas for the CRF stimulation is varied between zero and maximal. Increasing and decreasing stimulus intensity on the CRF schedule leads to the switching in schedule control over the behavior and a gradual disappearance and reappearance, of post-reinforcement pauses (PRPs) on the concurrent FR schedule. These PRPs are critical for providing a criterion for changeover in schedule control, and constitute a measure for reinforcement threshold (Buscher et al., 1990). The threshold obtained through this method, like the one obtained with the curve shift method, is then used as a baseline against which the effect of various experimental manipulations are expressed quantitatively in psychophysical units therefore avoiding the confounds effects of drugs on response rate (Bozarth, 1987).

These studies suggest that LiCl produces a hypofunction of brain reward systems and immediate effects on reward. One of the goals of the present study was to further characterize reward devaluation of BSR by providing evidence of long-lasting effects of LiCl when non-contingent reward delivery is paired with this drug, using a paradigm commonly used with natural rewards (Holland and Rescorla, 1975; Adams and Dickinson, 1981; Schoenbaum and Setlow, 2005; Nelson and Killcross, 2006). An advantage of using this approach is that BSR will be given in a different context than where the rats will be trained or tested (instead of performance under the effects of the drug), therefore minimizing associations between training context and reward that could counteract the effects of LiCl.

Additionally we also evaluated the effects of AM251, a cannabinoid receptor (CB1) antagonist. Behavioral output during the pursuit of reward can be potently modulated by activation of CB1 receptors, which are ubiquitous in brain circuitry associated with reward (Solinas et al., 2008). For example, injection of a CB1 agonist can reinstate drug-seeking behavior (De Vries et al., 2001). Similarly CB1 receptor agonists can potentiate the rewarding effect of drugs of abuse and natural rewards (Gallate et al., 1999; Valjent et al., 2002; Solinas et al., 2005); whereas antagonists have the opposite effect (Fattore et al., 2003, 2007; Cippitelli et al., 2005; Economidou et al., 2006). When the role of CB1 receptors is evaluated in the context of BSR the results are contradictory. Some studies using CB1 receptor agonists show small or no decreases in self-stimulation threshold (Lepore et al., 1996; Arnold et al., 2001); whereas other experiments report pronounced decreases in self-stimulation thresholds (Vlachou et al., 2005, 2006). When CB1 receptor antagonists are used, similar contradictory results are observed; some studies report no effects (Vlachou et al., 2005; Xi et al., 2008) whereas other show significant increases (Deroche-Gamonet et al., 2001; De Vry et al., 2004). The contrast between the robust effects of CB1 receptor manipulations on the reinforcing effects of natural rewards and drugs of abuse with those obtained with BSR could be an indirect indication of what factors are affected by CB1 receptor activation. It is possible that these receptors elicit a change in reinforcement by affecting the organism's motivational state and not the reward's intrinsic value. Indeed, it has been recently reported that CB1 receptors produce their effects on BSR by altering factors others than reward sensitivity (Trujillo-Pisanty et al., 2011). Therefore we hypothesized that the effects of pairing AM251 with non-contingent rewarding stimulation should not produce enduring effects on the valuation of reward.

MATERIALS AND METHODS

SUBJECTS

Forty male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) Weighting between 350 and 400 g at the moment of the surgery were used ($n = 24$ for LiCl experiments and $n = 16$ for AM251 experiments). The subjects were individually housed on a 12-h normal cycle (lights on from 0700 to 1900), with *ad libitum* access to water and food (Purina Rat Chow).

SURGERY

Animals were anesthetized with isoflurane, and implanted with a bipolar stimulating electrode (Plastics One, Roanoke, VA, USA) with prongs spaced 0.5 mm apart. The electrode was stereotaxically aimed at the ventral tegmental area (VTA; −0.5 mm ML, 5.4 mm AP, −8.7 mm DV) relative to bregma and secured with dental acrylic and skull-screw anchors. At the end of the surgery, the rats were injected with carprofen (5 mg/kg; s.c.) to reduce the pain and with sterile saline solution (1 ml/kg; s.c.) as post surgery fluid therapy. The rats were allowed to recuperate for 5–7 days post surgery before any experimental manipulation.

SELF-STIMULATION TRAINING

Each of the rats implanted with stimulating electrodes was shaped to press a lever for 24 biphasic square pulses (2 ms per phase) delivered at 60 Hz. The current varied across animals between 100 and 150 μA and it was delivered using a constant current isolator (A-M Systems, Sequim, WA, USA) controlled by a PC running custom-written LabVIEW software (National Instruments, Austin, TX, USA). Shaping took place in an operant conditioning chamber (12.5″ L × 13.5″ W × 13.5″ H; Med Associates, Georgia, VT, USA) located within ventilated sound attenuation chambers. Control of operant boxes and response acquisition was achieved with Med-PC IV software (Med Associates, Georgia, VT, USA).

The operant boxes were equipped with a house light, two cue lights above two retractable levers, a sonalert module (2900 Hz tone delivery), and a white noise amplifier. Rats were shaped to press a lever to obtain electrical stimulation delivery at the VTA. Once they pressed the lever on their own they were trained under a fixed ratio 1 schedule with an inter-trial interval of 10 s. Both retractable levers were present during the experiment, but only one was associated with an illuminated cue light and reward delivery (active lever). Responses on the other lever (inactive lever) did not have any scheduled consequences. A trial began with the cue light on top of the active lever and the house light on and the extension of the active and inactive levers. Once the rat pressed down

the active lever, both levers retracted and the electrical stimulation train was delivered, the cue and house lights were turned off, and the 2900-Hz tone started. At the end of the 10-s inter-trial interval, the tone was muted and the houselight was turned off for 1 s and a new trial began. White noise and fans were on throughout the experimental session. Animals were considered to be at criterion once they pressed 100 consecutive times for stimulation. Those rats that showed motor or aversive effects to the stimulation were removed from the experiment.

DEVALUATION PROCEDURE

Experiment 1

Twenty-four hours after training, rats were randomly divided into two groups. The first group ($n = 12$) was injected with 5 mg/kg i.p. of LiCl (Sigma Aldrich) dissolved in 0.9% saline; the second group ($n = 12$) was injected with saline. Injections took place in the home cage 30 min prior to the delivery of non-contingent stimulation. The non-contingent stimulation was carried out in similar operant boxes as the ones the rats were trained; but no levers, stimuli, houselights, or white noise were present and the doors of the isolation cubicles were left open. When the rats were inside the boxes they received the stimulation according to a variable time 80 s schedule of reinforcement (VT 80″). The non-contingent stimulation ended when the rats received 50 stimulations in a 60-min period. This procedure was carried out approximately at the same time for three consecutive days. Twenty-four hours after the last non-contingent stimulation experiment, rats were returned to the operant chambers where training had taken place. For this test session, all stimuli associated with lever presentation and reward delivery were presented as during self-stimulation responding; but the electrical stimulation was withheld. The session ended after an hour had elapsed.

Experiment 2

Twenty-four hours after training, rats were randomly divided into two groups. The first group ($n = 8$) was injected with 3 mg/kg, i.p. of AM251 dissolved in a solution of (1:1:18) ethanol, emulphor (Rhodia, Cranbury, NJ, USA), and saline (0.9%). The second group ($n = 8$) was injected with the vehicle. Drug delivery and experimental design were identical to experiment 1. This dose was chosen in accordance with previous studies (Xi et al., 2008; Trujillo-Pisanty et al., 2011).

HISTOLOGY

After completion of the experiment, a lethal dose of urethane (5 g/kg, i.p.) was administered and a 1-mA anodal current was passed through the stimulating electrode for 15 s to deposit iron ions at the site of the electrode tip. Rats were then perfused intracardially with 0.9% sodium chloride and a solution of potassium ferrocyanide (3%), potassium ferricyanide (3%), and trichloroacetic acid (0.5%) in 10% formalin. The brains were removed from the skulls and fixed with 10% formalin solution for at least 7 days. Coronal sections of 40 μm thickness were cut with a cryostat (Thermo Scientific). The stimulating electrode location was determined microscopically at low magnification with reference to the stereotaxic atlas of Paxinos and Watson (2007). The histological reconstructions of the electrode placement show that the tips of the electrodes were located within the VTA (see **Figures 1A,B**).

STATISTICS

The number of lever presses as well as the latency to press during the extinction session were analyzed for each pair of groups using independent groups t-test. A level of $p < 0.05$ for a two-tailed test was the criterion for statistical significance. The analysis was carried out using Statistica (Statsoft, Inc., Tulsa, OK, USA).

RESULTS

During the test session the group of subjects that received the pretreatment with LiCl pressed the lever an average of 8.41 ± 0.98 times with an average latency of 135 ± 6.17 s, whereas animals that received the pretreatment with saline pressed the lever on average 29 ± 5.86 times with an average latency of 135 ± 9.31 s (**Figures 2A,B**). The difference in the total number of lever presses between these two groups is statistically reliable [$t_{(22)} = 3.45$; $p = 0.002$]. There was not a statistically significant difference in the observed latency to press between these two groups [$t_{(22)} = -0.04$; $p = 0.498$].

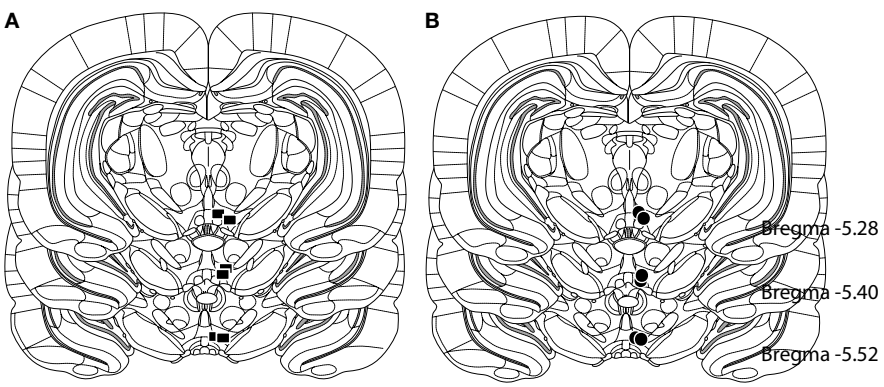

FIGURE 1 | (A) Location of electrodes tips (squares) for selected rats in the LiCl and saline groups. **(B)** Location of electrodes tips (circles) for selected rats in the AM251 and vehicle groups. All stimulation sites lay within the ventral tegmental area. The coronal drawings are from the Paxinos and Watson (2007) atlas, plates 87–89.

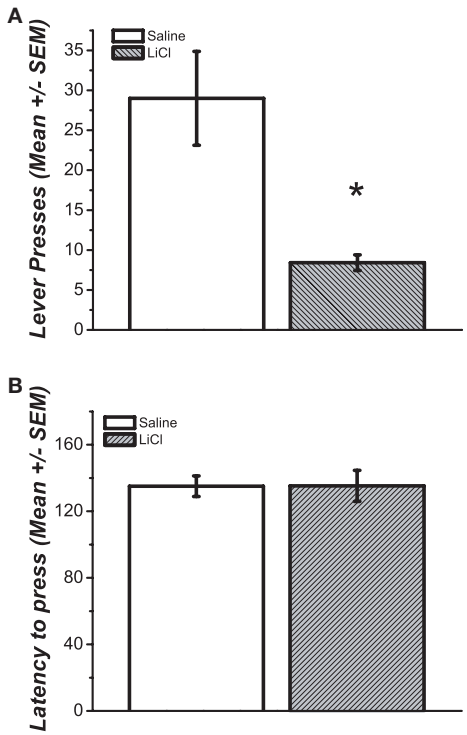

FIGURE 2 | (A) Average number of responses during extinction session after three sessions of non-contingent reward delivery. The group pretreated with LiCl showed significantly fewer responses than the group pretreated with vehicle ($p < 0.05$). **(B)** The latency to press for both groups was statistically similar.

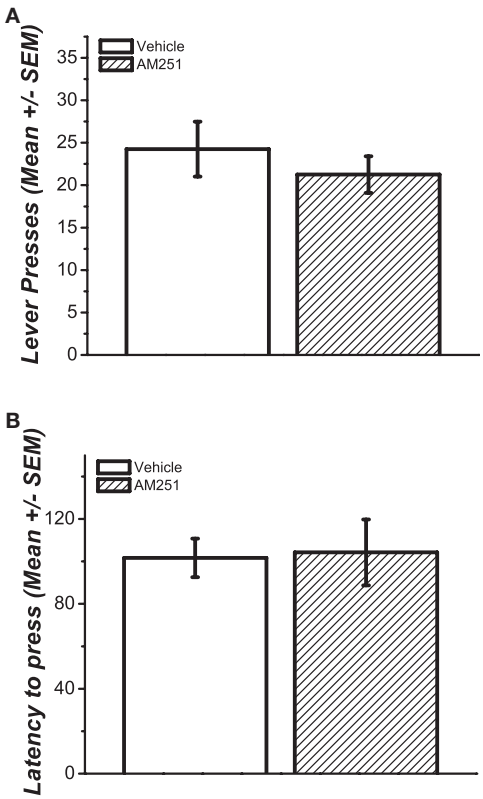

FIGURE 3 | (A) Average number of responses during extinction session after three sessions of non-contingent reward delivery. The group pretreated with AM251 showed fewer responses than the group pretreated with vehicle but this difference was not statistically significant ($p > 0.05$). **(B)** The latency to press for both groups was statistically similar.

The rats that received the pretreatment with AM251 pressed an average of 24.25 ± 3.22 times whereas the rats that were pretreated with vehicle pressed in average 21.25 ± 2.16 times. The average latency to press for these groups was 101.66 ± 9.07 and 104.25 ± 15.52 s, respectively (**Figures 3A,B**). There were no statistically significant differences between the groups for neither the total number of lever press [$t_{(14)} = 0.58$; $p = 0.282$] nor the latency to press [$t_{(14)} = -0.14$; $p = 0.443$].

DISCUSSION

The present results show that instrumental responding for BSR is susceptible to reinforcer devaluation effects, when devaluation is conducted according to classically established procedures. Specifically the current study is unique from prior attempts in that BSR was devalued independently of the learned instrumental behavior, and the instrumental behavior was assessed without re-exposure to the now-devalued BSR. Thus the demonstrated change in responding in the rats that received the LiCl–BSR pairings must reflect an underlying associative structure in which the instrumental response (or perhaps associated cues) drives responding in part by activating a cognitive representation of BSR and its current value. The finding that responding for BSR is sensitive to LiCl devaluation draws an important parallel between responding for BSR and natural rewards, and adds to evidence supporting the use of BSR as a model to examine the brain circuits mediating reward.

Other studies have shown that BSR and natural rewards share a common circuitry; BSR can be modulated by factors that have been shown to modulate the behavior controlled by natural rewards. Food restriction can potentiate BSR at certain brain sites (Blundell and Herberg, 1968; Carr and Wolinsky, 1993; Fulton et al., 2002). Furthermore, leptin, a hormone secreted by fat cells that suppresses food intake and promotes weight loss, has modulatory effects on BSR. Intracerebroventricular infusion of leptin attenuates the effectiveness of BSR in those brain sites in which BSR is susceptible to food restriction, whereas this hormone has the opposite effect when the electrode is located in sites that are not sensitive to food restriction manipulations (Fulton et al., 2000). Not only manipulations that alter natural rewards can potentially alter the behavior controlled by BSR, but BSR can also exert effects on behaviors typically elicited by natural rewards. For example, BSR can induce feeding (Valenstein et al., 1970; Berridge and Valenstein, 1991) and hoarding (Blundell and Herberg, 1973). The effect of BSR on these behaviors is probably due to potentiated salience of external stimuli rather than increased hedonic value (Berridge and Valenstein, 1991). At the electrophysiological level, conduction velocities and refractory period between the neurons that mediate BSR and stimulation-induced feeding are indistinguishable (Gratton and Wise, 1988).

Our results also have important implications for understanding the role of CB1 receptors in mediating reward-seeking behaviors. CB1 receptors have been identified in reward pathways (Robbe et al., 2002; Cota et al., 2003; Melis et al., 2004; Le Foll and Goldberg, 2005) and play an important role in the behavioral expression of the rewarding effects of drugs of abuse, as well of natural rewards. CB1 receptor agonists increase operant responses for natural rewards and drugs of abuse (Gallate et al., 1999; Valjent et al., 2002; Solinas and Goldberg, 2005; Solinas et al., 2005). In an opposite fashion, CB1 receptor antagonists blunt operant performance for natural rewards and drugs of abuse (De Vry et al., 2004). The malleability of behavior elicited by these manipulations suggests that these receptors play a crucial role in changing the attractiveness of reward. However, in the present task pairing AM251 with BSR did not affect subsequent instrumental responding. This suggests that, unlike LiCl, CB1 antagonism does not induce a lasting shift in the value of BSR. It could be argued that the dose of AM251 used in the present study was too low to produce any significant effect. However this possibility can be discarded since this dose given when the experimental subjects are performing for BSR produces significant effects on the mountain model testing paradigm (Trujillo-Pisanty et al., 2011) and produces significant changes in reward-seeking behavior when drugs of abuse are used (Xi et al., 2006, 2008) as well as natural rewards (Droste et al., 2010).

The contrast in reward devaluation obtained with LiCl and AM251 could arise because antagonism of CB1 receptors does not affect the intrinsic value of reward, but the organism's motivational state. This would explain why AM251 administered during instrumental responding decreases progressive ratio breakpoints for a diversity of rewards (Ward and Dykstra, 2005; Droste et al., 2010), whereas AM251 administered separately with BSR does not. Also, the inconsistent effects of cannabinoid antagonists on BSR (Solinas et al., 2008) may be a product of the lack of dimensionality of the traditional curve shift method. When operant performance for BSR is measured as a joint product of its stimulation strength and opportunity cost (Hernandez et al., 2010), AM251 produces consistent leftward shifts of the function that relates operant performance to the opportunity cost of the reward, whereas the function that relates operant performance to stimulation strength was conserved (Trujillo-Pisanty et al., 2011). Such shift is believed to be a product of factors that could include a decrease in the reward signal gain, or an increase in the subjective reward cost and the value of competing activities such as grooming, resting, and exploring (Herrnstein, 1970, 1974; Killeen, 1972; Heyman, 1988). This result strongly suggests that CB1 receptors play their principal role in other parts of the reward circuit that that are not involved in the determination of reward sensitivity.

In summary, the present results show that LiCl has long-term effects on the valuation of BSR, which suggests that this compound is effective in reducing its intrinsic value and that the BSR task utilized in this study and others (Cheer et al., 2005, 2007) is indeed goal-directed. In contrast, treatment with the CB1 receptor antagonist AM251 did not produce such a change, suggesting that endocannabinoids preferentially engage the circuitry involved with motivation. The present results clarify that BSR is a goal-directed behavior and reinforce the notion that endocannabinoids are primarily involved with motivational rather than intrinsic aspects of reward.

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REFERENCES

Adams, C. D. (1982). Variations in the sensitivity of instrumental responding to reinforcer devaluation. *Q. J. Exp. Psychol. B* 34B, 77–98.

Adams, C. D., and Dickinson, A. (1981). Instrumental responding following reinforcer devaluation. *Q. J. Exp. Psychol. B* 33, 109–121.

Arnold, J. C., Hunt, G. E., and Mcgregor, I. S. (2001). Effects of the cannabinoid receptor agonist CP 55,940 and the cannabinoid receptor antagonist SR 141716 on intracranial self-stimulation in Lewis rats. *Life Sci.* 70, 97–108.

Berridge, K. C., and Valenstein, E. S. (1991). What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? *Behav. Neurosci.* 105, 3–14.

Blundell, J. E., and Herberg, L. J. (1968). Relative effects of nutritional deficit and deprivation period on rate of electrical self-stimulation of lateral hypothalamus. *Nature* 219, 627–628.

Blundell, J. E., and Herberg, L. J. (1973). Effectiveness of lateral hypothalamic stimulation, arousal, and food deprivation in the initiation of hoarding behaviour in naive rats. *Physiol. Behav.* 10, 763–767.

Bozarth, M. A. (1987). "Intracranial self-administration procedures for the assessment of drug reinforcement," in *Methods of Assessing the Reinforcing Properties of Abused Drugs*, ed. M. A. Bozarth (New York: Springer-Verlag), 173–187.

Buscher, W., Wagner, U., and Huston, J. P. (1990). Measuring threshold of reinforcing brain stimulation by the method of constant stimuli. *J. Neurosci. Methods* 31, 225–234.

Carr, K. D., and Wolinsky, T. D. (1993). Chronic food restriction and weight loss produce opioid facilitation of perifornical hypothalamic self-stimulation. *Brain Res.* 607, 141–148.

Cassens, G. P., and Mills, A. W. (1973). Lithium and amphetamine: opposite effects on threshold of intracranial reinforcement. *Psychopharmacology (Berl.)* 30, 283–290.

Cheer, J. F., Aragona, B. J., Heien, M. L., Seipel, A. T., Carelli, R. M., and Wightman, R. M. (2007). Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. *Neuron* 54, 237–244.

Cheer, J. F., Heien, M. L., Garris, P. A., Carelli, R. M., and Wightman, R. M. (2005). Simultaneous dopamine and single-unit recordings reveal accumbens GABAergic responses: implications for intracranial self-stimulation. *Proc. Natl. Acad. Sci. U.S.A.* 102, 19150–19155.

Cippitelli, A., Bilbao, A., Hansson, A. C., Del Arco, I., Sommer, W., Heilig, M., Massi, M., Bermúdez-Silva, F. J., Navarro, M., Ciccocioppo, R., and De Fonseca, F. R. (2005). Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. *Eur. J. Neurosci.* 21, 2243–2251.

Colwill, R. M., and Rescorla, R. A. (1986). "Associative structures in instrumental learning," in *Psychology of Learning and Motivation*, ed. Gordon H. Bower (Orlando, FL: Academic Press), 55–104.

Conover, K. L., and Shizgal, P. (1994). Competition and summation between rewarding effects of sucrose and lateral hypothalamic stimulation in the rat. *Behav. Neurosci.* 108, 537–548.

Conover, K. L., Woodside, B., and Shizgal, P. (1994). Effects of sodium depletion on competition and summation between rewarding effects of salt and lateral hypothalamic stimulation in the rat. *Behav. Neurosci.* 108, 549–558.

Cota, D., Marsicano, G., Lutz, B., Vicennati, V., Stalla, G. K., Pasquali, R.,

and Pagotto, U. (2003). Endogenous cannabinoid system as a modulator of food intake. *Int. J. Obes. Relat. Metab. Disord.* 27, 289–301.

De Vries, T. J., Shaham, Y., Homberg, J. R., Crombag, H., Schuurman, K., Dieben, J., Vanderschuren, L. J., and Schoffelmeer, A. N. (2001). A cannabinoid mechanism in relapse to cocaine seeking. *Nat. Med.* 7, 1151–1154.

De Vry, J., Schreiber, R., Eckel, G., and Jentzsch, K. R. (2004). Behavioral mechanisms underlying inhibition of food-maintained responding by the cannabinoid receptor antagonist/inverse agonist SR141716A. *Eur. J. Pharmacol.* 483, 55–63.

Deroche-Gamonet, V., Le Moal, M., Piazza, P. V., and Soubrie, P. (2001). SR141716, a CB1 receptor antagonist, decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. *Psychopharmacology (Berl.)* 157, 254–259.

Dickinson, A. (1985). Actions and habits: the development of behavioural autonomy. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 308, 67–78.

Droste, S. M., Saland, S. K., Schlitter, E. K., and Rodefer, J. S. (2010). AM 251 differentially effects food-maintained responding depending on food palatability. *Pharmacol. Biochem. Behav.* 95, 443–448.

Economidou, D., Mattioli, L., Cifani, C., Perfumi, M., Massi, M., Cuomo, V., Trabace, L., and Ciccocioppo, R. (2006). Effect of the cannabinoid CB1 receptor antagonist SR-141716A on ethanol self-administration and ethanol-seeking behaviour in rats. *Psychopharmacology (Berl.)* 183, 394–403.

Fattore, L., Spano, M. S., Cossu, G., Deiana, S., and Fratta, W. (2003). Cannabinoid mechanism in reinstatement of heroin-seeking after a long period of abstinence in rats. *Eur. J. Neurosci.* 17, 1723–1726.

Fattore, L., Spano, M. S., Deiana, S., Melis, V., Cossu, G., Fadda, P., and Fratta, W. (2007). An endocannabinoid mechanism in relapse to drug seeking: a review of animal studies and clinical perspectives. *Brain Res. Rev.* 53, 1–16.

Fulton, S., Woodside, B., and Shizgal, P. (2000). Modulation of brain reward circuitry by leptin. *Science* 287, 125–128.

Fulton, S., Woodside, B., and Shizgal, P. (2002). Does neuropeptide Y contribute to the modulation of brain stimulation reward by chronic food restriction? *Behav. Brain Res.* 134, 157–164.

Gallate, J. E., Saharov, T., Mallet, P. E., and Mcgregor, I. S. (1999). Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur. J. Pharmacol.* 370, 233–240.

Gallistel, C. R., Shizgal, P., and Yeomans, J. S. (1981). A portrait of the substrate for self-stimulation. *Psychol. Rev.* 88, 228–273.

Gratton, A., and Wise, R. A. (1988). Comparisons of connectivity and conduction velocities for medial forebrain bundle fibers subserving stimulation-induced feeding and brain stimulation reward. *Brain Res.* 438, 264–270.

Green, L., and Rachlin, H. (1991). Economic substitutability of electrical brain stimulation, food, and water. *J. Exp. Anal. Behav.* 55, 133–143.

Hernandez, G., Breton, Y. A., Conover, K., and Shizgal, P. (2010). At what stage of neural processing does cocaine act to boost pursuit of rewards? *PLoS ONE* 5, e15081. doi: 10.1371/journal.pone.0015081

Herrnstein, R. J. (1970). On the law of effect. *J. Exp. Anal. Behav.* 13, 243–266.

Herrnstein, R. J. (1974). Formal properties of the matching law. *J. Exp. Anal. Behav.* 21, 159–164.

Heyman, G. (1988). "How drugs affect cells and reinforcement affects behavior: formal analogies," in *Quantitative Analyses of Behavior. Biological Determinants of Behavior*, eds M. Commons, R. Church, J. Stellar, and A. Wagner (Hillsdale, NJ: Lawrence Erlbaum), 157–182.

Holland, P. C., and Rescorla, R. A. (1975). The effect of two ways of devaluing the unconditioned stimulus after first- and second-order appetitive conditioning. *J. Exp. Psychol. Anim. Behav. Process.* 1, 355–363.

Killeen, P. (1972). The matching law. *J. Exp. Anal. Behav.* 17, 489–495.

Le Foll, B., and Goldberg, S. R. (2005). Cannabinoid CB1 receptor antagonists as promising new medications for drug dependence. *J. Pharmacol. Exp. Ther.* 312, 875–883.

Lepore, M., Liu, X., Savage, V., Matalon, D., and Gardner, E. L. (1996). Genetic differences in [Delta]9-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.* 58, PL365–PL372.

Mavrikaki, M., Nomikos, G. G., and Panagis, G. (2009). Effects of mood stabilizers on brain reward processes in rats: studies using the intracranial self-stimulation paradigm. *Eur. Neuropsychopharmacol.* 19, 205–214.

McSweeney, F. K., and Roll, J. M. (1993). Responding changes systematically within sessions during conditioning procedures. *J. Exp. Anal. Behav.* 60, 621–640.

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

Miliaressis, E., Rompre, P.-P., Laviolette, P., Philippe, L., and Coulombe, D. (1986). The curve-shift paradigm in self-stimulation. *Physiol. Behav.* 37, 85–91.

Nelson, A., and Killcross, S. (2006). Amphetamine exposure enhances habit formation. *J. Neurosci.* 26, 3805–3812.

Olds, J. (1962). Hypothalamic substrates of reward. *Physiol. Rev.* 42, 554–604.

Olds, J., and 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.

Paxinos, G., and Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates.* San Diego: Academic Press.

Robbe, D., Kopf, M., Remaury, A., Bockaert, J., and Manzoni, O. J. (2002). Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8384–8388.

Schoenbaum, G., and Setlow, B. (2005). Cocaine makes actions insensitive to outcomes but not extinction: implications for altered orbitofrontal-amygdalar function. *Cereb. Cortex* 15, 1162–1169.

Solinas, M., and Goldberg, S. R. (2005). Motivational effects of cannabinoids and opioids on food reinforcement depend on simultaneous activation of cannabinoid and opioid systems. *Neuropsychopharmacology* 30, 2035–2045.

Solinas, M., Goldberg, S. R., and Piomelli, D. (2008). The endocannabinoid system in brain reward processes. *Br. J. Pharmacol.* 154, 369–383.

Solinas, M., Panlilio, L. V., Tanda, G., Makriyannis, A., Matthews, S. A., and Goldberg, S. R. (2005). Cannabinoid agonists but not inhibitors of endogenous cannabinoid transport or metabolism enhance the reinforcing efficacy of heroin in rats. *Neuropsychopharmacology* 30, 2046–2057.

Tomasiewicz, H. C., Mague, S. D., Cohen, B. M., and Carlezon, W. A. Jr. (2006). Behavioral effects of short-term administration of lithium and valproic acid in rats. *Brain Res.* 1093, 83–94.

Trujillo-Pisanty, I., Hernandez, G., Moreau-Debord, I., Cossette, M.-P., Conover, K., Cheer, J. F., and Shizgal, P. (2011). Cannabinoid receptor blockade reduces the opportunity cost at which rats maintain operant performance for rewarding brain stimulation. *J. Neurosci.* 31, 5426–5435.

Valenstein, E. S., Cox, V. C., and Kakolewski, J. W. (1970). Reexamination of the role of the hypothalamus in motivation. *Psychol. Rev.* 77, 16–31.

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

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

Vlachou, S., Nomikos, G. G., and Panagis, G. (2005). CB1 cannabinoid receptor agonists increase intracranial self-stimulation thresholds in the rat. *Psychopharmacology (Berl.)* 179, 498–508.

Ward, S. J., and Dykstra, L. A. (2005). The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940). *Behav. Pharmacol.* 16, 381–388.

Xi, Z. X., Gilbert, J. G., Peng, X. Q., Pak, A. C., Li, X., and Gardner, E. L. (2006). Cannabinoid CB1 receptor antagonist AM251 inhibits cocaine-primed relapse in rats: role of glutamate in the nucleus accumbens. *J. Neurosci.* 26, 8531–8536.

Xi, Z. X., Spiller, K., Pak, A. C., Gilbert, J., Dillon, C., Li, X., Peng, X. Q., and Gardner, E. L. (2008). Cannabinoid CB1 receptor antagonists attenuate cocaine' rewarding effects: experiments with self-administration

and brain-stimulation reward in rats. *Neuropsychopharmacology* 33, 1735–1745.

Yin, H. H., and Knowlton, B. J. (2002). Reinforcer devaluation abolishes conditioned cue preference: evidence for stimulus-stimulus associations. *Behav. Neurosci.* 116, 174–177.

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Acute effects of delta-9-tetrahydrocannabinol on performance monitoring in healthy volunteers

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Rationale: The error-related negativity (ERN) is a negative event-related potential that occurs immediately after an erroneous response and is thought to reflect human performance monitoring. Delta-9-Tetrahydrocannabinol (THC) administration in healthy volunteers has been linked to impaired performance monitoring in behavioral studies, but to date no studies have examined the effects of cannabinoids on the ERN. **Methods:** EEG data from 10 healthy volunteers was recorded during execution of a speeded choice-reaction-time task (Flankers task) after administration of THC or placebo vapor in a double-blind randomized crossover design. **Results:** The findings of this study show that the ERN was significantly reduced after administration of THC. The behavioral outcomes on the Flankers task showed no indications of drug-induced impairments. **Discussion:** The diminished ERN reflects impairments in the process of performance monitoring. The task design was not optimized to find behavioral effects. The study shows that cannabinoids impair performance monitoring.

Keywords: performance monitoring, error-related negativity, ERN, cannabinoids, THC, human, cannabis

INTRODUCTION

Several studies have shown that acute cannabis administration is associated with impairments of several cognitive processes (Gonzalez, 2007). One important process is the identification and correction of differences between intended and executed actions, also known as performance monitoring. This performance-monitoring system enables us to detect failures in our actions and to adapt our behavior accordingly. Therefore, it is an essential process for safe and efficient functioning in everyday situations. The functionality of the performance-monitoring system may vary with conditions such as fatigue, psychiatric disease, and drug taking (Scheffers et al., 1999; de Bruijn et al., 2004; Lorist et al., 2005; Schrijvers et al., 2009; Schellekens et al., 2010). Many drugs of abuse are known to increase the risk of engaging in maladaptive behavior, suggesting that drugs of abuse may impair human performance monitoring. Cannabis is the most frequently used illegal drug in Europe, most often self-administered for its mood-altering or "relaxing" effects (Green et al., 2003; Vicente et al., 2008). The use of cannabis and other cannabinoids for medical purposes as an analgesic or antiemetic for example is on the rise (Machado Rocha et al., 2008; Elikkottil et al., 2009). Surprisingly, to date the effect of cannabinoids on human performance monitoring is not sufficiently understood.

Cannabis contains a number of chemicals that belong to the class of cannabinoids. Delta-9-tetrahydrocannabinol (THC) is the main and most potent psychoactive cannabinoid of cannabis and is probably of greatest importance in the recreational use of the drug (Ashton, 1999; Russo and Guy, 2005). In pharmacological challenge studies in humans THC in isolation as well as cannabis has been administered. Administration of THC activates the cannabinoid receptors (CB1 and CB2). CB1 receptors are widespread in the brain, which probably accounts for the great variety of associated effects (Glass et al., 1997). These effects can be classified into two categories: affective and cognitive. Studies addressing the affective effects have shown that THC administration may cause an increase in anxiety and sedation and a decrease in motivation (Fusar-Poli et al., 2009; Dumont et al., 2011). Studies addressing the cognitive effects of THC, have often demonstrated that THC is associated with impairments in, e.g., working memory and attention (Crean et al., 2011). Studies of both human and animal subjects have also demonstrated that cannabis administration impairs behavioral flexibility and inhibitory control (McDonald et al., 2003; Ramaekers et al., 2006; Pattij et al., 2008).

Performance monitoring is a process that allows humans to respond actively and safely to changing environmental demands. Neural correlates of this process can be assessed by means of electroencephalography (EEG). When humans make an error in speeded choice-reaction tasks, a sharp negative peak is seen in the EEG around 50–100 ms after the erroneous response. Because of these characteristics, this event-related potential (ERP) component was named the error-related negativity (ERN; Falkenstein et al., 1990; Gehring et al., 1993). The ERN is considered a valid and reliable index of performance monitoring (Segalowitz et al., 2010). ERP recordings present a major advantage over behavioral outcomes, because ERP measures enable us to objectively investigate mechanisms underlying changes in cognitive functioning, for example as a result of drug effects (Kenemans and Kähkönen, 2011). Three influential theories have been developed that have

thoroughly modeled the functional significance of the component: the Mismatch hypothesis (e.g., Falkenstein et al., 1991; Bernstein et al., 1995), the reinforcement-learning theory (RL; Holroyd and Coles, 2002), and the response-conflict theory (Yeung et al., 2004). The Mismatch hypothesis presumes that the ERN reflects a process that compares a representation of a correct response with the actual response. The RL-theory has been developed as an extension of the Mismatch Theory. According to the RL-theory the ERN reflects a learning process mediated by dopaminergic signaling in the mesencephalic dopaminergic nuclei when an outcome is worse than expected. The response-conflict theory, on the other hand, states that the ERN is generated when response-conflict occurs, i.e., in situations where a choice between several incompatible responses has to be made. Various imaging studies have implicated the anterior cingulate cortex (ACC) as the most likely candidate structure for generating the ERN (Herrmann et al., 2004; Stemmer et al., 2004; Debener et al., 2005). In line with this assumption, Debener et al. (2005) showed that larger ERN amplitudes are associated with a larger BOLD response in the ACC and that this is accompanied by stronger behavioral adaptations following errors.

To the authors' knowledge no previous studies have specifically addressed the effects of acute THC intoxication on the ERN. However, a number of other cognitive processes that are tightly coupled with performance monitoring have been investigated. First, Lane et al. (2005) found that cannabis decreases sensitivity to choice outcome during decision making tasks (Lane et al., 2005). The sensitivity to choice outcome can be interpreted as the behavioral consequence of performance monitoring. Second, working memory impairments following THC administration are probably one of the most consistently reported cognitive effects of THC (Ranganathan and D'Souza, 2006). Previously it was shown that working memory improvement was positively correlated with the ERN (Horowitz-Kraus and Breznitz, 2009). This coupling between working memory and performance monitoring also suggests that performance monitoring will be impaired after THC. Third, in a study on the long-term effects of cannabis use on error awareness was shown that regular cannabis users demonstrated less error awareness. Impaired error awareness is indicative of impaired performance monitoring. In the same report the authors also showed that this impairment was associated with hypoactivity in the ACC (Hester et al., 2009). Several imaging studies have shown that THC administration is associated with a reduction in cerebral blood flow in frontal brain regions (Borgwardt et al., 2008; Martín-Santos et al., 2010). Brain areas that are also of importance in performance monitoring. Together, these studies strongly suggest that THC administration is associated with compromised performance monitoring.

Jocham and Ullsperger (2009) mentioned in a recent review that investigating the effects of cannabinoids on the ERN is of particular relevance (Jocham and Ullsperger, 2009). They arrived at this conclusion because of the widespread distribution of cannabinoid receptors in the brain together with the growing use of THC. However, they also note in their review that to date these studies are lacking. Nonetheless, previous research can provide some hypotheses about the effects of cannabinoids on the ERN. Pharmacological studies, for example, have suggested that ERN characteristics depend on changes in dopaminergic neurotransmission. Specifically, in healthy volunteers the amplitude is increased after administration of the indirect dopaminergic agonist amphetamine (de Bruijn et al., 2004), and decreased by the dopamine 2 receptor antagonist haloperidol (Zirnheld et al., 2004; de Bruijn et al., 2006). Importantly, THC has also been shown to interact with the dopamine system, i.e., THC administration is followed by an increase in dopamine release in the striatum (Bossong et al., 2009). On this premise, it can be expected that ERN amplitudes are larger after THC administration.

The ERN may also be dependent on levels of motivation and sedation. Administration of alcohol or benzodiazepines (both substances known to induce sedation) has shown a reduction in the ERN amplitude (Johannes et al., 2001; Ridderinkhof et al., 2002; de Bruijn et al., 2004). Non-pharmacological studies have repeatedly demonstrated a positive correlation between ERN amplitude and motivation and arousal (de Bruijn et al., 2006; Ganushchak and Schiller, 2008). From this research it may be expected that THC may have a sedative and de-motivational effect which may reduce the ERN amplitudes post THC administration.

In summary, there may be two competing effects. Based on pharmacological studies we expect to observe an increased ERN following THC administration. Conversely, based on results from cognitive studies, we predict that THC will impair performance monitoring and that the ERN will therefore be reduced. At this point, we do not know which is the dominant effect. In order to investigate the effect of THC administration on the ERN, we subjected participants to the Flankers task after acute THC administration on two separate testing days in a placebo-controlled manner.

MATERIAL AND METHODS

SUBJECTS

Sixteen healthy volunteers (12 male, four female), regular users between the ages of 18 and 27 were recruited through advertisement on the internet and at local drug testing services. All subjects met inclusion criteria of on average at least two exposures of THC per week in the last year and at least eight ecstasy exposures in the last 2 years. Detailed demographic data can be found in other reports (see e.g., Dumont et al., 2011). Exclusion criteria included pregnancy, (history of) psychiatric illness (assessed using the Structured Clinical Interview for DSM-IV axis I disorders, non-patient version (First et al., 1994) Axis II disorders were excluded using the Temperament and Character Inventory (Svrakic et al., 1993), use of over-the-counter medication within 2 months prior to the commencement of the study, (history of) treatment for addiction problems as assessed by a structured interview, excessive smoking (>10 cigarets/day), and orthostatic dysregulation. Physical and mental health was determined by assessment of medical history, a physical, and electrocardiographic examination as well as standard hematological and chemical blood examinations. A total number of 10 subjects (eight male, two female, average age of 20.6 years) were included in the current analyses. Subjects smoked on average 4.6 exposures of THC per week for an average period of 5.9 years. Of the subjects excluded, one did not refrain from drug use, after which further study participation was denied. Two subjects experienced an adverse event that was judged to be likely related to study drug administration.

Furthermore, for three subjects no EEG data could be analyzed due to technical problems. These six subjects were not included in the final data-analysis. All subjects provided their written informed consent before participating in the study, and were paid for their participation.

The study was approved by the Medical Ethics Committee of the Radboud University Nijmegen Medical Centre. The study is registered at The Netherlands Trial Registry (No. NTR1317).

STUDY DRUGS

THC was purified according to good manufacturing practice (GMP)-compliant procedures (Farmalyse BV, Zaandam, The Netherlands) from the flowers of *Cannabis sativa* grown under Good Agricultural Practice (Bedrocan BV Medicinal Cannabis, Veendam, The Netherlands; Choi et al., 2004; Hazekamp et al., 2004) and was dissolved in 200 µl 100 vol% alcohol. THC was stored in a dark room at −20°C in 1-ml amber glass vials containing a Teflon screw cap secured with Parafilm to minimize evaporation. The 200-µl 100% alcohol solution without THC was used as placebo. On each study day, three subsequent dosages of THC (4, 6, and 6 mg) or placebo were administered at 90-min intervals. Placebo and THC were administered by means of using a Volcano® vaporizer (Storz-Bickel GmbH, Tüttlingen, Germany), a validated method of intrapulmonary THC administration (Hazekamp et al., 2006; Abrams et al., 2007). Five minutes before administration, THC was vaporized at a temperature of 225°C and the vapor was stored in a polythene bag equipped with a valved mouthpiece, preventing the loss of THC in between inhalations. The transparent bag was covered with a black plastic bag to prevent unblinding. Subjects were not allowed to speak, and were instructed to inhale deeply and hold their breath for 10 s after each inhalation. Subjects were instructed to empty the bag within 2–3 min. The inhalation procedure was practiced at screening using the mouthpiece of the vaporizer only.

DESIGN AND PROCEDURE

Placebo and THC were administered according to a balanced protocol in a randomized, double-blind, and crossover design. Every subject participated in both conditions with at least 7 days in between in which no other drug exposure was allowed. The current study was part of a larger study. Pharmacokinetic, cognitive, and neurophysiological data obtained from the study sample have been published previously (Dumont et al., 2009, 2011; Lansbergen et al., 2011).

To elicit ERNs, the participants performed a modified Flankers task (Eriksen and Eriksen, 1974; de Bruijn et al., 2004, 2006) in which they had to respond with either their left or right index finger to the central letter (H or S) of a congruent (HHHHH or SSSSS) or incongruent (HHSHH or SSHSS) letter string. First, a fixation point was presented (lasting 100 ms) followed 300 ms later by the stimulus (also lasting 100 ms). During the next 900 ms the screen remained blank, after which a visual feedback stimulus appeared for 1000 ms. The next trial was presented after an inter-trial interval of 100 ms. Visual feedback consisted of a yellow, a blue, or a red rectangle indicating whether the preceding response had been correct, incorrect or too late, respectively. Participants were instructed to respond as fast as possible to avoid feedback indicating that their response was too slow according to a preset reaction-time (RT) deadline. After written and verbal instructions, the participants familiarized themselves with the task in a practice block consisting of 60 trials, during which the initial RT deadline was set at a relatively liberal limit of 800 ms. At the end of this practice block, the average RT and SD of the correct responses were computed. Next, for each individual participant and test day the RT deadline was determined by adding 0.5 SD to the mean RT. For each subject and per each condition an individualized deadline was computed. Because previous studies on action monitoring have shown that ERN amplitude is affected by accuracy (see e.g., Gehring et al., 1993) including this RT deadline was essential to ensure that error rates did not vary across treatment conditions (de Bruijn et al., 2004, 2006). The experimental phase consisted of five blocks of 100 trials with a self-paced pause halfway through each block. After each block, participants were informed on the number of incorrect responses and responses whose latency exceeded the deadline. Verbal encouragement was given to keep performance accuracy around 80–90%.

PHARMACOKINETIC MEASUREMENTS

Blood samples (4.5 ml covered with aluminum foil) were taken at baseline 5, 20, 95, 110, 185, 200 min after the first THC administration. Plasma samples were immediately put on ice and were processed within 30 min after collection. Plasma samples were stored at a temperature of −80°C for less than 3 months before laboratory analysis.

EEG RECORDING

The electroencephalogram (EEG) was recorded from 27 tin electrodes mounted in an elastic electrode cap (Electrocap International). Electrodes were placed at seven midline and 20 lateral locations in accordance with the international 10–20 system. All electrodes were referenced to the left mastoid. The vertical electro-oculogram (EOG) was recorded bipolarly from electrodes placed above and below the right eye. The horizontal EOG was also recorded bipolarly from electrodes lateral to each eye. All electrode impedances were kept below 5 kΩ at the start of the recording session. The EEG and EOG signals were amplified using a time-constant of 8 s and a bandpass between 0.02 and 30 Hz. All signals were digitized with a sampling rate of 200 Hz using a 16-bit A/D converter.

STATISTICAL ANALYSES

Electro-oculogram artifact correction was carried out using the procedure proposed by Gratton et al. (1983). For the ERP analyses all responses with reaction times faster than 150 ms (placebo 1.5% and THC 1.0%) were removed from the data sets. Epochs associated with correct and incorrect responses were averaged separately and time-locked to response onset, starting 100 ms before and ending 500 ms after response onset relative to a 100-ms pre-response baseline. Correct responses were also averaged separately for congruent and incongruent stimuli time-locked to stimulus onset. The ERN was determined on correct and error trials in separate subject averages by subtracting the most negative peak in the 0- to 200-ms time-window after response onset from the most positive peak in the time-window starting 80 ms before and

ending 80 ms after response onset at electrode FCz/Cz, where ERN amplitude was largest (Holroyd et al., 2003; de Bruijn et al., 2004). The stimulus-locked ERPs were computed separately for correct congruent and incongruent trial types, in both treatment conditions. The amplitude of the N1 component was defined as the most negative deflection occurring in the 50- to 150-ms post stimulus time-window at electrodes FCz, Cz, and Pz. The N2 component was defined on incongruent trials as the most negative peak between 200–350 ms after stimulus onset at electrode FCz. The amplitude of the P300 was defined on incongruent stimuli as the largest positive deflection between 300 and 500 ms at electrodes FCz, Cz, and Pz.

Individual averages for error rates and RTs were entered in a general linear model (GLM) with repeated measures (SPSS version 16.0, Chicago, IL, USA). The possible factors of the different GLMs were condition (two levels: THC or placebo), congruency (two levels: congruent vs. incongruent), and correctness (two levels: correct vs. incorrect). Adaptive behavior following erroneous responses was investigated by examining reaction times on correct responses following either correct or incorrect trials. To avoid serial congruency effects, only incongruent trials were included in these analyses. This type of performance adjustment is also known as post-error slowing (Rabbitt, 1966). A GLM analysis was performed with the factor condition (two levels: THC or placebo), and post-correctness (two levels: post-correct vs. post-error). The response-locked ERN was entered in a GLM, again with condition, congruency, and correctness as within subject factors. The stimulus-locked ERPs were analyzed by a GLM including condition (two levels: THC and placebo), congruency (congruent vs. incongruent), and electrode sites (three levels only for P300 and N1 analyses).

RESULTS

THC PLASMA CONCENTRATIONS

THC concentrations have previously been published (Dumont et al., 2011) but are reported here for the current sample selection. Average THC peak and trough plasma concentrations are shown in **Table 1**. THC concentrations during the placebo condition were always zero.

BEHAVIORAL EFFECTS

Performance

The percentage of Trial responses for each of the five possible response types for each condition and trial type is given in **Table 2**. The average error rate and average percentage of "too late" trial responses did not differ between the two drug conditions (both $p > 0.1$). The ANOVA revealed that the error rate of incongruent trial types was higher than on congruent trial types [$F(1,9) = 125.60$, $p < 0.001$]. Similarly, there were more "too late" responses at incongruent trials than at congruent trials [$F(1,9) = 32.67$, $p < 0.001$]. The interaction between congruency and condition did not reach significance for "incorrect" and "too late" trial responses ($p > 0.05$). The percentages for "too early" and "omission" responses showed that they constitute less than 4% of the responses in each condition.

Table 1 | Mean (SEM) THC peak (5 min after drug administration) and trough (20 min after drug administration) plasma levels (in ng/ml).

	4 mg (1st)	6 mg (2nd)	6 mg (3rd)
Peak	59.8 (7.5)	71.9 (10.9)	89.2 (18.0)
Trough	9.5 (1.1)	13.4 (1.8)	17.8 (2.0)

Reaction times

A repeated measures ANOVA for correct and incorrect trials only ("too late" trials were excluded) showed that there were no differences between the placebo and THC condition on the reaction time (see **Figure 1**). There was a significant main effect of congruency [$F(1,9) = 43.46$, $p < 0.001$] and correctness [$F(1,9) = 66.39$, $p < 0.001$]. Subjects were faster in general on the incorrect trials (314 ms) in comparison to correct trials (347 ms) and performed faster on the congruent trials (322 ms) compared to incongruent trials (339 ms). No interaction effects were observed (all $p > 0.1$).

Performance adjustments

First, we compared reaction times of correct responses on trials that followed a correct response (post-correct) or an erroneous response (post-error). This post-error slowing analysis revealed neither a main effect for condition [$F(1,9) = 0.11$, $p = 0.743$], nor

Table 2 | Mean percentages of correct, incorrect, too late, too early, and omission responses to congruent and incongruent trials for the placebo and THC condition.

	Congruent		Incongruent	
	Placebo	THC	Placebo	THC
% Correct	77.6	76.1	52.8	53.7
% Incorrect	12.5	15.3	27.3	32.3
% Too late	7.4	7.2	16.6	12.1
% Too early	1.4	1.0	1.5	1.0
% Omission	1.1	0.4	1.8	0.9

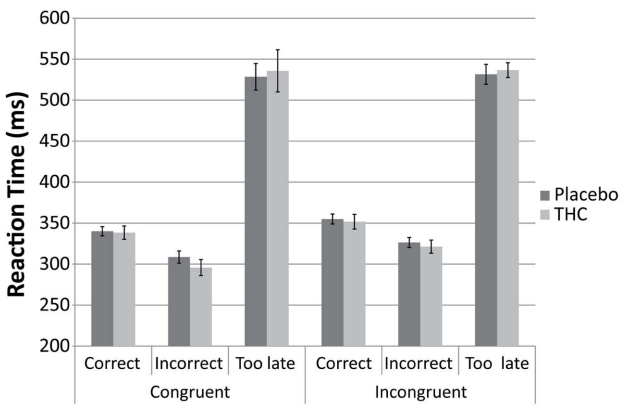

FIGURE 1 | Bar graphs showing average Reaction Time for "congruent" and "incongruent" trials for placebo (black), and THC (gray) condition. Results are displayed separately for "correct," "incorrect," and "too late" responses. Error bars represent SE of the mean.

for post-correctness [$F(1,9) = 2.48$, $p = 0.150$], nor an interaction between the two [$F(1,9) = 0.92$, $p = 0.362$].

Second, we compared reaction times of correct responses that preceded an error (pre-error) or that followed an error (post-error). This post-error slowing analyses did reveal a main effect of post-error slowing [$F(1,9) = 19.77$, $p = 0.002$]. There was neither a significant main effect for condition [$F(1,9) = 0.41$, $p = 0.538$], nor a significant interaction between the two [$F(1,9) = 0.65$, $p = 0.442$]. The main effect of post-error slowing showed that reaction times following an error (349 ms) were significantly slower than reaction times preceding the erroneous response (336 ms).

ERP ANALYSES

Response-locked ERPs

Figure 2 depicts the response-locked ERNs for the two treatment conditions. No overall significant effects of drugs were observed [$F(1,9) = 0.072$, $p = 0.795$], nor was there a significant main effect of correctness ($p > 0.1$). There was a significant interaction between condition and correctness [$F(1,9) = 7.00$, $p = 0.027$]. Planned contrasts showed that the difference in the "ERN" for correct and incorrect trial responses was significant in the placebo condition [$F(1,9) = 19.28$, $p = 0.002$, -0.9 vs. $-4.9\,\mu V$] but not in the THC condition [$F(1,9) = 2.90$, $p = 0.123$, $-2.4\,\mu V$ vs. $-3.9\,\mu V$].

STIMULUS-LOCKED ERPs

To investigate whether the effects of THC on response-locked ERPs were not caused by an overall reduction in general stimulus processing or attention, additional stimulus-locked ERPs were conducted. **Figure 3** depicts the grand average ERP wave for correct and incorrect trial responses separately for both conditions and for the three selected electrode sites. The waveform is in accordance with typical stimulus-locked waveforms.

N1 amplitude

For the N1 amplitude, the GLM only revealed a significant main effect of electrode [$F(1,9) = 4.516$, $p = 0.040$]. The *post hoc* tests showed that the effect was caused by larger N1 amplitudes at frontal and central sites (-2.5 and $-2.4\,\mu V$) in comparison to parietal sites ($-1.8\,\mu V$, $p < 0.05$). There was no effect of condition, nor a significant interaction effect between electrode and condition (all $p > 0.1$).

P300 amplitude

For the P300 amplitude, there was only a significant main effect of electrode [$F(1,9) = 6.829$, $p = 0.023$]. The *post hoc* tests showed that the P300 amplitudes over the central and posterior electrode sites (9.6 and $10.1\,\mu V$) were significantly higher than over the frontal electrode site ($6{,}7\,\mu V$, $p < 0.05$). Drug condition had no effect on the P300 amplitude ($p > 0.1$).

N2 amplitude

The analyses on the N2 amplitude showed a main effect of congruency [$F(1,9) = 18.575$, $p = 0.002$]. As expected, the N2 amplitude was larger for incongruent trials than for congruent trials (-2.7 vs. $-0.9\,\mu V$). There was no main effect of condition, nor was there a condition by congruency interaction effect ($p > 0.1$).

DISCUSSION

The current study investigated the effects of THC administration on performance monitoring. Results showed that THC leads to reduced performance monitoring, as reflected in decreased ERN amplitudes compared to the placebo condition. The two conditions did not differ however, with respect to either behavioral performance measures or stimulus-locked ERP components.

THC AND PERFORMANCE MONITORING

Compromised performance monitoring as reflected by a reduced ERN under acute THC administration is consistent with a number of previous behavioral reports. For example, impairments in associated cognitive processes after THC and cannabis administration were demonstrated for reversal learning, inhibitory control, risk taking, and working memory (Curran et al., 2002; Ramaekers et al., 2006; Pattij et al., 2008; Hunault et al., 2009). We did not find any effects of condition on the behavioral measures of error rate, RT, and post-error slowing. The employment of individually determined RT deadlines results in a limited time-window in which participants are able to give a correct response. This procedure leads to a considerable limitation in the possible variance in performance and reaction times, but with the aim of maintaining similar performance levels between the conditions. The absence of an effect in performance measures is therefore not surprising and is a direct consequence of the individualized deadline. The reason we employed this method was to ensure that effects on the ERN would be due to the pharmacological condition, and not caused by differences in performance levels. This procedure is rather common in ERN studies as differences in performance may have an effect on ERN amplitude (see e.g., Gehring et al., 1993) and was employed in a number of other studies including from our own lab (e.g., Luu et al., 2000; de Bruijn et al., 2004, 2006; Debener et al., 2005). Comparable to our findings, in a number of other studies not always an association between the ERN and performance measures could be demonstrated (see e.g., Ullsperger et al., 2002; de Bruijn et al., 2004; Ullsperger and von Cramon, 2006). The absence of behavioral effects may, among other factors, be depending on sample size and the employed task design. The task design is likely to be the main contributing factor in our study. It is not unthinkable that employment of the Flankers task with different task parameters *will* yield behavioral effects in future experiments.

As stated in the introduction performance monitoring is a process that allows humans to respond actively and safely to changing environmental demands. Existing theories agree that this process reflected by the ERN is the result of a warning signal in the brain –error or conflict– that triggers the need for behavioral adaptation. In order to modify and improve behavior, other functions are recruited such as motor responses, attention, or learning. Although the relation with behavioral performance is often not that evident in highly controlled paradigms designed to investigate the ERN, the relevance of performance monitoring in daily life is evident. Everyday actions like safely driving a car require continuous performance monitoring and are obviously much more complex than choice-reaction tasks like the one currently used. Thus it is highly plausible that reductions in performance monitoring may be even more obvious in such complex behaviors. When drivers accidentally reach the verge of a road, they need to

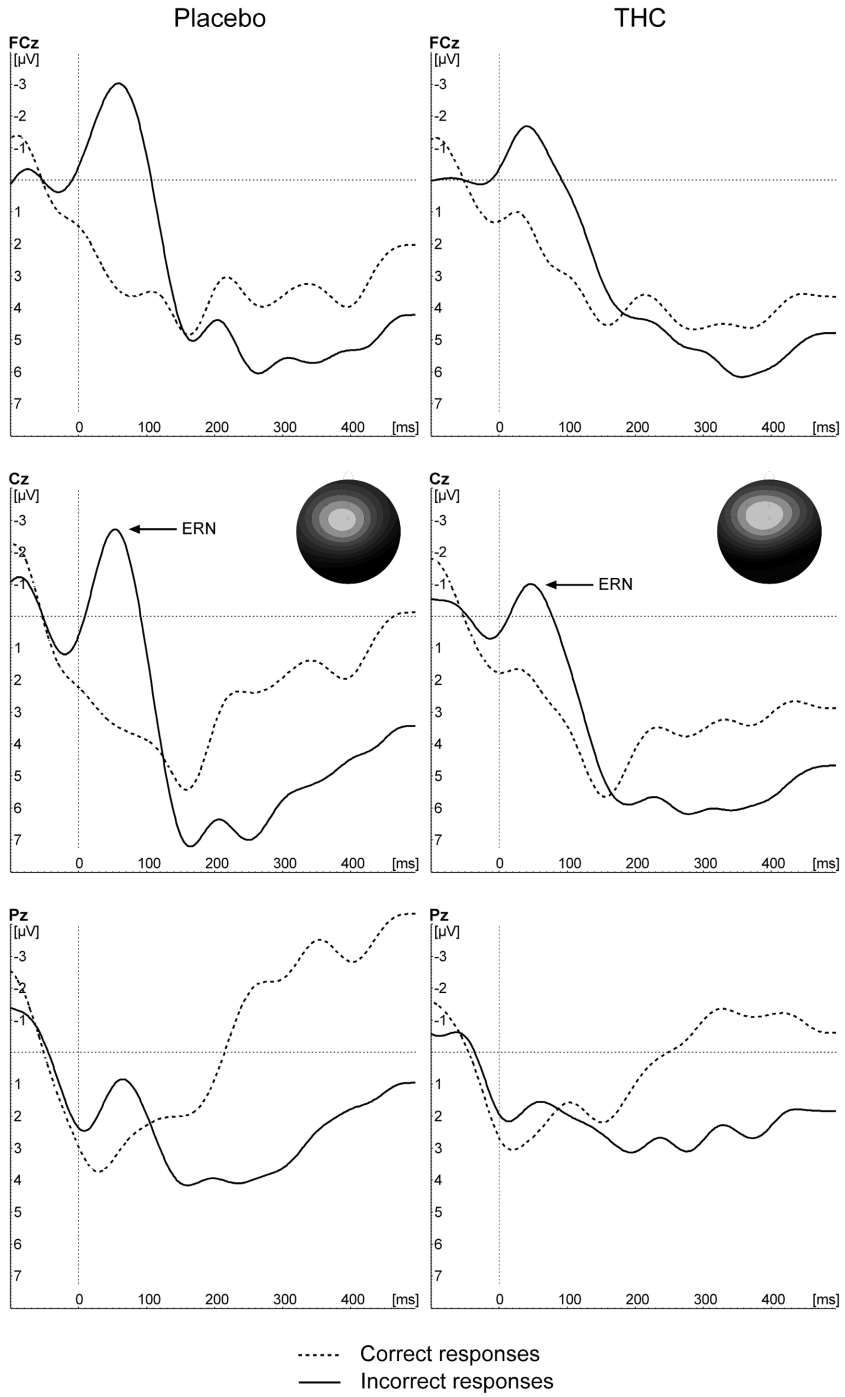

FIGURE 2 | Grand average waveforms of incorrect and correct responses to incongruent trial types for placebo and THC conditions.

recognize this and correct their steering in order to prevent the car from slipping off the road. Our results suggest that impairments in performance monitoring caused by THC administration may result in diminished warning signals and less efficient behavioral adaptations in a daily task like driving. In practice this could mean that the risk of slipping off the road is not timely notified and the required motor response to keep the car on the road is not operating correctly or fast enough. This suggestion is in line with recent findings from Calabria et al. (2010) and Penning et al. (2010) demonstrating that cannabis users show impaired driving abilities and have an increased risk to die in motor accidents.

In our study all subjects were regular users, i.e., at least 1–2 exposures per week in the last year. Also, the age range was small and all subjects had comparable durations of cannabis use. We

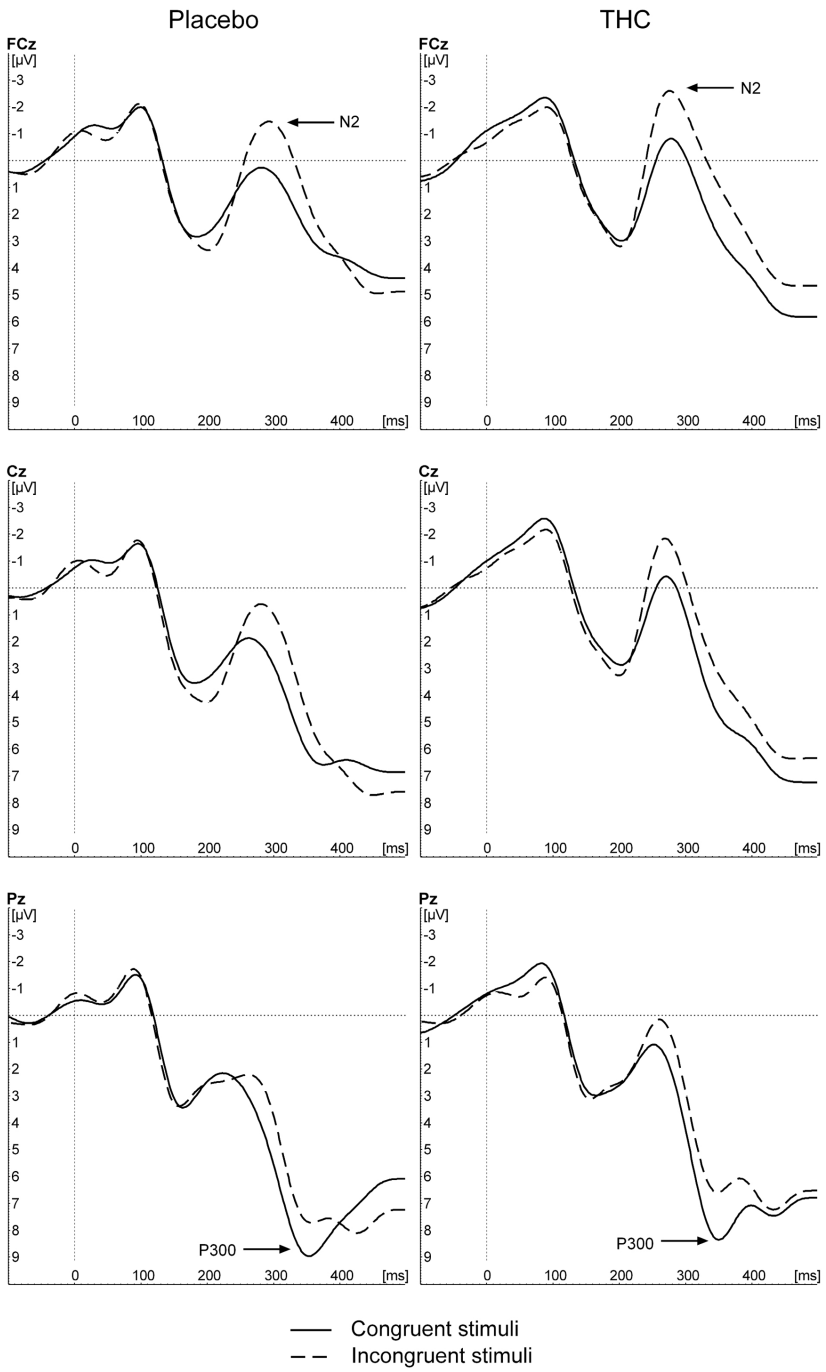

FIGURE 3 | Grand average stimulus-locked waveforms in response to congruent and incongruent trial types for placebo, and THC conditions.

observed an effect of THC on performance monitoring in regular users, however, it is of interest if the effect is also observed in occasional cannabis users. Studies in which the effects of THC on performance monitoring are directly compared between occasional and heavy users are warranted in order to directly investigate potential differences in affected cognitive processes. It is also imperative to compare acute drug effects with long-term drug effects in order to identify to what extent the cognitive profiles are different. For example, memory problems have repeatedly been found among heavy and long-term cannabis users, but may also occur under acute administration (see for a review Solowij and Battisti, 2008). It is also of importance to dissociate between acute drug effects in short-term occasional users vs. long-term/heavy users. For example, Ramaekers et al. (2009) compared the cognitive effects of THC administration between heavy and occasional users. They reported that THC significantly impaired performance

on critical tracking, divided attention, and the stop signal task in occasional users, while in the heavy user group only stop signal performance was affected. Therefore, it is of importance to assess user history and to select subjects with comparable histories as this may interact with the cognitive process under investigation.

Another important question to address is to what extent the effect of THC on performance monitoring differs from other substances. It has been shown that alcohol and benzodiazepines also produce reductions in the ERN (de Bruijn et al., 2004; Ridderinkhof et al., 2002). In contrast to our results, benzodiazepine administration was associated with greater cognitive impairments as indicated by a slowed reaction time and absence of the N2 congruency effect. Despite control measures that were taken to ensure similar performance levels, benzodiazepine administration overruled this. In order to systematically address potential differences between THC and other pharmacological compounds, future studies in which THC, benzodiazepines, and alcohol are directly compared are recommended.

PHARMACOLOGY

Our study showed that activation of the cannabinoid system results in a reduction of the amplitude of the ERN. Previous studies have demonstrated ERN modulations by *dopamine*, i.e., DA agonists increase the amplitude and DA antagonists result in amplitude reductions (de Bruijn et al., 2004, 2006). THC administration is thought to increase dopaminergic release through disinhibition of GABAergic neurons (Pistis et al., 2002; Lupica et al., 2004), which implies an effect equivalent to a DA agonist. In keeping with previous pharmacological literature an increase in ERN amplitude would be expected, while we have observed the opposite in the present study. The dopamine system is also of importance in one of the three influential theories that have modeled the ERN: the RL-theory (Holroyd and Coles, 2002). The theory states that whenever a response is worse than expected, i.e., during commitment of an error, a negative error signal is generated which is coded as a phasic dopaminergic dip in the tonic activity of the mesencephalic dopaminergic system (Holroyd and Yeung, 2003). Holroyd and Yeung (2003) have outlined how the finding of the supposed increase in tonic mesencephalic dopaminergic neurotransmission by alcohol, may lead to a decreased ERN according to the RL-theory. One of the mechanisms they proposed is that increased tonic activity of the mesencephalic dopamine system, could lead to an increased inhibition of the ACC that in turn yields a reduction of the ERN. Similar to alcohol, cannabis also increases tonic dopaminergic neurotransmission in the mesencephalic brain areas (Boileau et al., 2003; Bossong et al., 2009). We therefore speculate that a similar mechanism occurs following THC administration.

The predictions from other pharmacological work and the RL-theory are contradictory and imply that there is a discrepancy within current opinions about dopaminergic pharmacology and the ERN/performance monitoring. Contributing to this conundrum is that drugs may affect dopaminergic neurotransmission via different pathways. Cannabis, e.g., may increase dopamine release via inhibition of the GABAergic system after activation of the endocannabinoid system. Amphetamine for example, interacts with dopamine by the redistribution of dopamine from the synaptic vesicle into the cytosol and the induction of reverse transport of dopamine through pre-synaptic reuptake transporters of dopamine through pre-synaptic reuptake transporters (Sulzer et al., 2005). We also do not sufficiently know how drugs induced changes in tonic mesencephalic dopamine neurotransmission relate to phasic dopaminergic in- and decreases and how this exactly translates to reinforcement-learning. Caution should thus be exercised in interpretation of our results in terms of the RL-theory. Future research into the underlying mechanisms of the RL-theory as well as the pharmacology of THC administration is needed.

Drugs rarely only affect dopaminergic neurotransmission, and this certainly also applies for administration of THC. Cannabinoid administration has also been associated with altered noradrenergic (Muntoni et al., 2006), GABAergic, and glutamatergic changes (Pistis et al., 2002). These other systems may also directly have an effect on the ERN. For example, it has been proposed that noradrenergic activation results in enlarged ERN amplitude (de Bruijn et al., 2004; Riba et al., 2005). This is also nicely illustrated with the example of alcohol administration, which is known to increase the release of GABA and of dopamine in the midbrain. Like THC, alcohol is associated with a reduction of the ERN amplitude (Ridderinkhof et al., 2002; Bartholow et al., in press). This example shows that it is hard to show which neurotransmission system the observed findings should be attributed to.

The endocannabinoid system has relatively recently been discovered and new perspectives and insights are booming. One new perspective is, for example, that THC administration in rats with a history of regular THC exposure yields a decrease in dopamine rather than an increase (Jentsch et al., 1998; Verrico et al., 2004). Although this preclinical work might not be directly comparable to the situation in our study, it is important to consider in the interpretation and discussion of our results in light of other pharmacological studies and the RL-theory. All subjects included in the current study used at least 1–2 cannabis exposures per week in the last year and can thus be considered as regular users. In order to better address this issue, it is highly recommended for future research to investigate if and how cannabis administration affects dopaminergic signaling in short vs. long-term users.

MOTIVATION, ATTENTION, AND ALERTNESS

To further explore the decreased ERN post THC administration, we evaluated three potential factors that could have influenced the decreased ERN. First, based on data obtained from a partial overlapping study sample, we previously published that THC administration causes a decrease in motivation (Dumont et al., 2011). These findings are in accordance with other reports (Böcker et al., 2010). Also, the ERN is known to be dependent on motivation levels (Bush et al., 2000; Boksem et al., 2006) and therefore a decrease in motivation levels could have indirectly modulated the observed reduction of the ERN. In order to address this with more objective measures we analyzed stimulus-locked ERPs. The amplitude of the stimulus-locked P300 component is most relevant for motivation, as its amplitude has previously been positively correlated with motivation (Nijboer et al., 2010). Despite the fact that no P300 differences could be found in our data, an effect of motivation cannot be excluded, because the self-report scales obtained from

the same sample suggested that motivation decreased under THC affects. Similar to the effects of motivation, THC was shown to reduce attention and the ERN was previously shown to depend on subjects' attention levels (Pailing and Segalowitz, 2004; Böcker et al., 2010; Larson and Clayson, 2011). The N100 and P300 components are among the group of ERPs that are known to be reduced by decreased attention (Coull, 1998). Because we did not find an effect on these outcomes following THC administration, we could not provide support for the possibility that THC affects the ERN through reduction of attention.

Finally, we investigated the effect of sedation by analyzing the stimulus-locked N2 amplitude to congruent and incongruent trials. Previous work showed that this N2 congruency effect (i.e., increased conflict-induced N2 amplitudes on incongruent trials), is affected by strong sedative effects of drugs. Administration of benzodiazepines, for example, induces a reduction in this N2 effect (de Bruijn et al., 2004). We did not find an effect on the N2 after THC administration, which suggests our subjects were not heavily sedated. Alternatively, reduced N2 amplitude may be a specific biomarker of sedative effects of benzodiazepines and might not extrapolate to other sedative substances. Interestingly enough the administration of alcohol, which is also known to induce moderate sedative effects, also did not affect the amplitude of N2 (Ridderinkhof et al., 2002). Saccadic eye movement can also be used to measure sedation. Again, previously published data of a partly overlapping subject sample showed no effects of THC on saccadic eye-movements (Dumont et al., 2011). In contrast, the subjective alertness scale showed a significant decrease in the THC condition (Dumont et al., 2011). Taken together, the *subjective* measures suggest that the ERN could be mediated by sedation. However, this could not be supported by the objective measures and thus suggests a discrepancy between the two. Consequently, more research should be conducted in this area to better address the sedative drug effects and their relation with performance monitoring.

CONCLUSION

To conclude, our findings suggest that administration of THC has a diminishing effect on human performance monitoring as reflected by reduced ERN amplitudes. Given the small size of the study consisting of only 10 subjects, the results should be considered as preliminary and need to be confirmed with larger samples. Nevertheless, the results are relevant for several reasons. First, THC is the most important component of cannabis, which is a drug that is recreationally used by many people over the world. The study provides a better understanding of the risks of cannabis use during performance of complex functions like driving which require a high level of performance monitoring. Second, as THC is increasingly examined and applied for clinical applications, mapping the potential (cognitive) side-effects are crucial aspects of patient's safety and drug compliance. We for the first time demonstrated that activation of the endocannabinoid system influences the ERN. We believe that the results of this study have extended our understanding of the cognitive effects associated with cannabinoids. The effects of cannabinoids on performance monitoring and cognitive process in general, need further evaluation.

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REFERENCES

Abrams, D. I., Vizoso, H. P., Shade, S. B., Jay, C., Kelly, M. E., and Benowitz, N. L. (2007). Vaporization as a smokeless cannabis delivery system: a pilot study. *Clin. Pharmacol. Ther.* 82, 572–578.

Ashton, C. H. (1999). Adverse effects of cannabis and cannabinoids. *Br. J. Anaesth.* 83, 637–649.

Bartholow, B. D., Henry, E. A., Lust, S. A., Saults, J. S., and Wood, P. K. (in press). Alcohol effects on performance monitoring and adjustment: affect modulation and impairment of evaluative cognitive control. *J. Abnorm. Psychol.* doi: 10.1037/a0023664. [Epub ahead of print].

Bernstein, P. S., Scheffers, M. K., and Coles, M. G. H. (1995). "Where did I go wrong?" A psychophysiological analysis of error detection. *J. Exp. Psychol. Hum. Percept. Perform.* 21, 1312–1322.

Böcker, K. B., Gerritsen, J., Hunault, C. C., Kruidenier, M., Mensinga, T. T., and Kenemans, J. L. (2010). Cannabis with high δ9-THC contents affects perception and visual selective attention acutely: an event-related potential study. *Pharmacol. Biochem. Behav.* 96, 67–74.

Boileau, I., Assaad, J. M., Pihl, R. O., Benkelfat, C., Leyton, M., Diksic, M., Tremblay, R. E., and Dagher, A. (2003). Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse* 49, 226–231.

Boksem, M. A., Meijman, T. F., and Lorist, M. M. (2006). Mental fatigue, motivation and action monitoring. *Biol. Psychol.* 72, 123–132.

Borgwardt, S. J., Allen, P., Bhattacharyya, S., Fusar-Poli, P., Crippa, J. A., Seal, M. L., Fraccaro, V., Atakan, Z., Martin-Santos, R., O'Carroll, C., Rubia, K., and McGuire, PK. (2008). Neural basis of delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. *Biol. Psychiatry* 64, 966–973.

Bossong, M. G., van Berckel, B. N., Boellaard, R., Zuurman, L., Schuit, R. C., Windhorst, A. D., van Gerven, J. M., Ramsey, N. F., Lammertsma, A. A., and Kahn, R. S. (2009). Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology* 34, 759–766.

Bush, G., Luu, P., and Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn. Sci. (Regul. Ed.)* 4, 215–222.

Calabria, B., Degenhardt, L., Hall, W., and Lynskey, M. (2010). Does cannabis use increase the risk of death? Systematic review of epidemiological evidence on adverse effects of cannabis use. *Drug Alcohol Rev.* 29, 318–330.

Choi, Y. H., Hazekamp, A., Peltenburg-Looman, A. M., Frédérich, M., Erkelens, C., Lefeber, A. W., and Verpoorte, R. (2004). NMR assignments of the major cannabinoids and cannabiflavonoids isolated from flowers of *Cannabis sativa*. *Phytochem. Anal.* 15, 345–354.

Coull, J. T. (1998). Neural correlates of attention and arousal: insights from electrophysiology, functional neuroimaging and psychopharmacology. *Prog. Neurobiol.* 55, 343–361.

Crean, R. D., Crane, N. A., and Mason, B. J. (2011). An evidence based review of acute and long-term effects of *cannabis* use on executive cognitive functions. *J. Addict. Med.* 5, 1–8.

Curran, H. V., Brignell, C., Fletcher, S., Middleton, P., and Henry, J. (2002). Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology (Berl.)* 164, 61–70.

de Bruijn, E. R. A., Hulstijn, W., Verkes, R. J., Ruigt, G. S. F., and Sabbe, B. G. C. (2004). Drug-induced stimulation and suppression of action monitoring in healthy volunteers. *Psychopharmacology (Berl.)* 177, 151–160.

de Bruijn, E. R. A., Sabbe, B. G., Hulstijn, W., Ruigt, G. S. F., and Verkes, R. J. (2006). Effects of antipsychotic and antidepressant drugs on action monitoring in healthy volunteers. *Brain Res.* 1105, 122–129.

Debener, S., Ullsperger, M., Siegel, M., Fiehler, K., von Cramon, D. Y., and Engel, A. K. (2005). Trial-by-trial coupling of concurrent electroencephalogram and functional magnetic resonance imaging identifies the dynamics of performance monitoring. *J. Neurosci.* 25, 11730–11737.

Dumont, G. J. H., Kramers, C., Sweep, F. C., Touw, D. J., van Hasselt, J. G., de Kam, M., van Gerven, J. M., Buitelaar, J. K., and Verkes, R. J. (2009). *Cannabis* coadministration potentiates the effects of "ecstasy" on heart rate and temperature in humans. *Clin. Pharmacol. Ther.* 86, 160–166.

Dumont, G. J. H., van Hasselt, J. G., de Kam, M., van Gerven, J. M., Touw, D. J., Buitelaar, J. K., and Verkes, R. J. (2011). Acute psychomotor, memory and subjective effects of MDMA and THC co-administration over time in healthy volunteers. *J. Psychopharmacol. (Oxford)* 25, 478–489.

Elikkottil, J., Elikottil, J., Gupta, P., and Gupta, K. (2009). The analgesic potential of cannabinoids. *J. Opioid Manag.* 5, 341–357.

Eriksen, B. A., and Eriksen, C. W. (1974). Effects of noise letters upon the identification of a target letter in a nonsearch task. *Percept. Psychophys.* 16, 143–149.

Falkenstein, M., Hohnsbein, J., Hoormann, J., and Blanke, L. (1990). "Effects of errors in choice reaction tasks on the ERP under focused and divided attention," in *Psychophysiological Brain Research,* eds C. H. M. Brunia, A. W. K. Gaillard, and A. Kok (Tilburg: Tilburg University Press), 192–195).

Falkenstein, M., Hohnsbein, J., Hoormann, J., and Blanke, L. (1991). Effects of crossmodal divided attention on late ERP components. II. Error processing in choice reaction tasks. *Electroencephalogr. Clin. Neurophysiol.* 78, 447–455.

First, M. B., Frances, A. J., Pincus, H., Vettorello, N., and Davis, W. W. (1994). DSM-IV in progress. Changes in substance-related, schizophrenic, and other primarily adult disorders. *Hosp. Community Psychiatry* 45, 18–20.

Fusar-Poli, P., Crippa, J. A., Bhattacharyya, S., Borgwardt, S. J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S. A., O'Carrol, C., Atakan, Z., Zuardi, A. W., and McGuire, P. K. (2009). Distinct effects of δ9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Arch. Gen. Psychiatry* 66, 95–105.

Ganushchak, L. Y., and Schiller, N. O. (2008). Motivation and semantic context affect brain error-monitoring activity: an event-related brain potentials study. *Neuroimage* 39, 395–405.

Gehring, W. J., Goss, W., Coles, M. G. H., Meyer, D. E., and Donchin, E. (1993). A neural system for error detection and compensation. *Psychol. Sci.* 4, 385–390.

Glass, M., Dragunow, M., and Faull, R. L. (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 77, 299–318.

Gonzalez, R. (2007). Acute and non-acute effects of cannabis on brain functioning and neuropsychological performance. *Neuropsychol. Rev.* 17, 347–361.

Gratton, G., Coles, M. G., and Donchin, E. (1983). A new method for off-line removal of ocular artifact. *Electroencephalogr. Clin. Neurophysiol.* 55, 468–484.

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

Hazekamp, A., Choi, Y. H., and Verpoorte, R. (2004). Quantitative analysis of cannabinoids from *Cannabis sativa* using 1H-NMR. *Chem. Pharm. Bull.* 52, 718–721.

Hazekamp, A., Ruhaak, R., Zuurman, L., van Gerven, J., and Verpoorte, R. (2006). Evaluation of a vaporizing device (Volcano) for the pulmonary administration of tetrahydrocannabinol. *J. Pharm. Sci.* 95, 1308–1317.

Herrmann, M. J., Römmler, J., Ehlis, A. C., Heidrich, A., and Fallgatter, A. J. (2004). Source localization (LORETA) of the error-related-negativity (ERN/Ne) and positivity (Pe). *Brain Res. Cogn. Brain Res.* 20, 294–299.

Hester, R., Nestor, L., and Garavan, H. (2009). Impaired error awareness and anterior cingulate cortex hypoactivity in chronic cannabis users. *Neuropsychopharmacology* 34, 2450–2458.

Holroyd, C. B., and Coles, M. G. (2002). The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. *Psychol. Rev.* 109, 679–709.

Holroyd, C. B., Nieuwenhuis, S., Yeung, N., and Cohen, J. D. (2003). Errors in reward prediction are reflected in the event-related brain potential. *Neuroreport* 14, 2481–2484.

Holroyd, C. B., and Yeung, N. (2003). Alcohol and error processing. *Trends Neurosci.* 26, 402–404.

Horowitz-Kraus, T., and Breznitz, Z. (2009). Can the error detection mechanism benefit from training the working memory? A comparison between dyslexics and controls–an ERP study. *PLoS ONE* 4, e7141. doi: 10.1371/journal.pone.0007141

Hunault, C. C., Mensinga, T. T., Böcker, K. B., Schipper, C. M., Kruidenier, M., Leenders, M. E., de Vries, I., and Meulenbelt, J. (2009). Cognitive and psychomotor effects in males after smoking a combination of tobacco and cannabis containing up to 69 mg delta-9-tetrahydrocannabinol (THC). *Psychopharmacology (Berl.)* 204, 85–94.

Jentsch, J. D., Verrico, C. D., Le, D., and Roth, R. H. (1998). Repeated exposure to delta 9-tetrahydrocannabinol reduces prefrontal cortical dopamine metabolism in the rat. *Neurosci. Lett.* 246, 169–172.

Jocham, G., and Ullsperger, M. (2009). Neuropharmacology of performance monitoring. *Neurosci. Biobehav. Rev.* 33, 48–60.

Johannes, S., Wieringa, B. M., Nager, W., Dengler, R., and Münte, T. F. (2001). Oxazepam alters action monitoring. *Psychopharmacology (Berl.)* 155, 100–106.

Kenemans, J. L., and Kähkönen, S. (2011). How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. *Neuropsychopharmacology* 36, 26–51.

Lane, S. D., Cherek, D. R., Tcheremissine, O. V., Lieving, L. M., and Pietras, C. J. (2005). Acute marijuana effects on human risk taking. *Neuropsychopharmacology* 30, 800–809.

Lansbergen, M. M., Dumont, G. J. H., van Gerven, J. M., Buitelaar, J. K., and Verkes, R. J. (2011). Acute effects of MDMA (3,4-methylenedioxymethamphetamine) on EEG oscillations: alone and in combination with ethanol or THC (delta-9-tetrahydrocannabinol). *Psychopharmacology (Berl.)* 213, 745–756.

Larson, M. J., and Clayson, P. E. (2011). The relationship between cognitive performance and electrophysiological indices of performance monitoring. *Cogn. Affect. Behav. Neurosci.* 11, 159–171.

Lorist, M. M., Boksem, M. A., and Ridderinkhof, K. R. (2005). Impaired cognitive control and reduced cingulate activity during mental fatigue. *Brain Res. Cogn. Brain Res.* 24, 199–205.

Lupica, C. R., Riegel, A. C., and Hoffman, A. F. (2004). Marijuana and cannabinoid regulation of brain reward circuits. *Br. J. Pharmacol.* 143, 227–234.

Luu, P., Flaisch, T., and Tucker, D. M. (2000). Medial frontal cortex in action monitoring. *J. Neurosci.* 20, 464–469.

Machado Rocha, F. C., Stéfano, S. C., De Cássia Haiek, R., Rosa Oliveira, L. M., and Da Silveira, D. X. (2008). Therapeutic use of *Cannabis sativa* on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. *Eur. J. Cancer Care (Engl.)* 170.17, 431–443.

Martín-Santos, R., Fagundo, A. B., Crippa, J. A., Atakan, Z., Bhattacharyya, S., Allen, P., Fusar-Poli, P., Borgwardt, S., Seal, M., Busatto, G. F., and McGuire, P. (2010). Neuroimaging in cannabis use: a systematic review of the literature. *Psychol. Med.* 40, 383–398.

McDonald, J., Schleifer, L., Richards, J. B., and de Wit, H. (2003). Effects of THC on behavioral measures of impulsivity in humans. *Neuropsychopharmacology* 28, 1356–1365.

Muntoni, A. L., Pillolla, G., Melis, M., Perra, S., Gessa, G. L., and Pistis, M. (2006). Cannabinoids modulate spontaneous neuronal activity and evoked inhibition of locus coeruleus noradrenergic neurons. *Eur. J. Neurosci.* 23, 2385–2394.

Nijboer, F., Birbaumer, N., and Kübler, A. (2010). The influence of psychological state and motivation on brain-computer interface performance in patients with amyotrophic lateral sclerosis – a longitudinal study. *Front. Neurosci.* 4:5 doi: 10.3389/fnins.2010.00055

Pailing, P. E., and Segalowitz, S. J. (2004). The effects of uncertainty in error monitoring on associated ERPs. *Brain Cogn.* 56, 215–233.

Pattij, T., Wiskerke, J., and Schoffelmeer, A. N. (2008). Cannabinoid modulation of executive functions. *Eur. J. Pharmacol.* 585, 458–463.

Penning, R., Veldstra, J. L., Daamen, A. P., Olivier, B., and Verster, J. C. (2010). Drugs of abuse, driving and traffic safety. *Curr. Drug Abuse Rev.* 3, 23–32.

Pistis, M., Ferraro, L., Pira, L., Flore, G., Tanganelli, S., Gessa, G. L., and Devoto, P. (2002). Delta(9)-tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study. *Brain Res.* 948, 155–158.

Rabbitt, P. M. A. (1966). Errors and error correction in choice-response tasks. *J. Exp. Psychol.* 71, 264–272.

Ramaekers, J. G.,Kauert, G., vanRuitenbeek, P., Theunissen, E. L., Schneider, E., and Moeller, M. R. (2006). High-potency marijuana impairs executive function and inhibitory motor control. *Neuropsychopharmacology* 31, 2296–2303.

Ramaekers, J. G., Kauert, G., Theunissen, E. L., Toennes, S. W., and Moeller, M. R. (2009). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *J Psychopharmacol.* 23, 266–277.

Ranganathan, M., and D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl.)* 188, 425–444.

Riba, J., Rodríguez-Fornells, A., Münte, T. F., and Barbanoj, M. J. (2005). A neurophysiological study of the detrimental effects of alprazolam on human action monitoring. *Brain Res. Cogn. Brain Res.* 25, 554–565.

Ridderinkhof, K. R., de Vlugt, Y., Bramlage, A., Spaan, M., Elton, M., Snel, J., and Band, G. P. (2002). Alcohol consumption impairs detection of performance errors in mediofrontal cortex. *Science* 298, 2209–2211.

Russo, E., and Guy, G. W. (2005). A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Med. Hypotheses* 66, 234–246.

Scheffers, M. K., Humphrey, D. G., Stanny, R. R, Kramer, A. F., and Coles, M. G. H. (1999). Error-related processing during a period of extended wakefulness. *Psychophysiology* 36, 149–157.

Schellekens, A. F., De Bruijn, E. R. A., van Lankveld, C. A., Hulstijn, W., Buitelaar, J. K., de Jong, C. A., and Verkes, R. J. (2010). Alcohol dependence and anxiety increase error-related brain activity. *Addiction* 105, 1928–1934.

Schrijvers, D., De Bruijn, E. R. A., Maas, Y. J., Vancoillie, P., Hulstijn, W., and Sabbe, B. G. (2009). Action monitoring and depressive symptom reduction in major depressive disorder. *Int. J. Psychophysiol.* 71, 218–224.

Segalowitz, S. J., Santesso, D. L., Murphy, T. I., Homan, D., Chantziantoniou, D. K., and Khan, S. (2010). Retest reliability of medial frontal negativities during performance monitoring. *Psychophysiology* 47, 260–270.

Solowij, N., and Battisti, R. (2008). The chronic effects of cannabis on memory in humans: a review. *Curr. Drug Abuse Rev.* 1, 81–98.

Stemmer, B., Segalowitz, S. J., Witzke, W., and Schönle, P. W. (2004). Error detection in patients with lesions to the medial prefrontal cortex: an ERP study. *Neuropsychologia* 42, 118–130.

Sulzer, D., Sonders, M. S., Poulsen, N. W., and Galli, A. (2005). Mechanisms of neurotransmitter release by amphetamines: a review. *Prog. Neurobiol.* 75, 406–433.

Svrakic, D. M., Whitehead, C., Przybeck, T. R., and Cloninger, C. R. (1993). Differential diagnosis of personality disorders by the seven-factor model of temperament and character. *Arch. Gen. Psychiatry* 50, 991–999.

Ullsperger, M., and von Cramon, D. Y. (2006). The role of intact frontostriatal circuits in error processing. *J. Cogn. Neurosci.* 18, 651–664.

Ullsperger, M., von Cramon, D. Y., and Müller, N. G. (2002). Interactions of focal cortical lesions with error processing: evidence from event-related brain potentials. *Neuropsychology* 16, 548–561.

Verrico, C. D., Jentsch, J. D., Roth, R. H., and Taylor, J. R. (2004). Repeated, intermittent delta(9)-tetrahydrocannabinol administration to rats impairs acquisition and performance of a test of visuospatial divided attention. *Neuropsychopharmacology* 29, 522–529.

Vicente, J., Olszewski, D., and Matias, J. (2008). "Prevalence, patterns and trends of cannabis use among adults in Europe," in *A Cannabis Reader: Global Issues and Local Experiences.* eds S. Rödner Sznitman, B. Olsson, and R. Room (Lisbon: European Monitoring Center on Drug and Drug Addiction), 1–26.

Yeung, N., Botvinick, M. M., and Cohen, J. D. (2004). The neural basis of error detection: conflict monitoring and the error-related negativity. *Psychol. Rev.* 111, 931–959.

Zirnheld, P. J., Carroll, C. A., Kieffaber, P. D., O'Donnell, B. F., Shekhar, A., and Hetrick, W. P. (2004). Haloperidol impairs learning and error-related negativity in humans. *J. Cogn. Neurosci.* 16, 1098–1112.

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Beyond THC: the new generation of cannabinoid designer drugs

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Synthetic cannabinoids are functionally similar to delta9-tetrahydrocannabinol (THC), the psychoactive principle of cannabis, and bind to the same cannabinoid receptors in the brain and peripheral organs. From 2008, synthetic cannabinoids were detected in herbal smoking mixtures sold on websites and in "head shops" under the brand name of Spice Gold, Yucatan Fire, Aroma, and others. Although these products (also known as "Spice drugs" or "legal highs") do not contain tobacco or cannabis, when smoked they produce effects similar to THC. Intoxication, withdrawal, psychosis, and death have been recently reported after consumption, posing difficult social, political, and health challenges. More than 140 different Spice products have been identified to date. The ability to induce strong cannabis-like psychoactive effects, along with the fact that they are readily available on the Internet, still legal in many countries, marketed as natural safe substances, and undetectable by conventional drug screening tests, has rendered these drugs very popular and particularly appealing to young and drug-naïve individuals seeking new experiences. An escalating number of compounds with cannabinoid receptor activity are currently being found as ingredients of Spice, of which almost nothing is known in terms of pharmacology, toxicology, and safety. Since legislation started to control the synthetic cannabinoids identified in these herbal mixtures, many new analogs have appeared on the market. New cannabimimetic compounds are likely to be synthesized in the near future to replace banned synthetic cannabinoids, leading to a "dog chasing its tail" situation. Spice smokers are exposed to drugs that are extremely variable in composition and potency, and are at risk of serious, if not lethal, outcomes. Social and health professionals should maintain a high degree of alertness for Spice use and its possible psychiatric effects in vulnerable people.

Keywords: spice, designer drugs, synthetic cannabinoids, addiction, Internet, herbal blends, natural highs, cannabimimetics

INTRODUCTION

Cannabis is one of the oldest drugs of abuse and its consumption is still high worldwide. During the past two decades, knowledge of its pharmacology and the role of the endocannabinoid system in brain function and physiology has improved greatly (Thakur et al., 2009; Alger and Kim, 2011). Despite its long history of use and abuse for both medical and recreational purposes, a new generation of synthetic cannabinoids has recently emerged on the market, which are sold on the Internet as herbal mixtures under the brand names of "Spice," "Spice Gold," "Spice Diamond," "Arctic Spice," "Silver," "Aroma," "K2," "Genie," "Scene" or "Dream," and advertised as incense products, meditation potpourris, bath additives, or air fresheners. These products are often referred to as "herbal highs" or "legal highs" because of their legal status and purported natural herbal make-up. They are distributed in the form of dried leaves or resin, although more recently powdery products have also begun to emerge (Kikura-Hanajiri et al., 2011), and are sold without age restriction in metal-foil sachets, usually containing 3 g of vegetable matter, to which one or more of the synthetic cannabinoids have been added. Spice is typically smoked, using a pipe or by rolling in a cigaret paper, but can also be ingested as an infusion, or inhaled. These novel and increasingly popular recreational drugs first appeared on websites and in specialized shops ("head shops," which sell paraphernalia for cannabis users) around 2004, if not earlier (Dresen et al., 2010), and are sold as mild hallucinogens with prominent cannabis-like effects. They soon became popular in Central European countries, began to catch the attention of a broader public in 2007, and gained a high degree of popularity in 2008. Consequently, they have also attracted the attention of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) early warning system on new drugs (Early Warning System, 2009; EMCDDA, 2009). However, it was not before the end of 2008 that two synthetic cannabinoids were identified for the first-time as the main active (not declared) ingredients of an herbal blend called "Spice": the C8 homolog of the non-classical cannabinoid CP-47,497, CP-47,497-C8, and a cannabimimetic aminoalkylindole called JWH-018 (Auwärter et al., 2009; Uchiyama et al., 2009a). At the beginning of 2009, legislation in several European countries (Austria, Germany, France, Luxembourg, Poland,

Lithuania, Sweden, and Estonia) subjected all products containing these substances to the Narcotics Law, so that Spice and the other cannabinoid-containing "natural" mixtures were no longer accessible in head shops and online stores (**Figure 1**). In the same year, following advice from the Advisory Council on the Misuse of Drugs (ACMD, 2009) and the EMCDDA, the Misuse of Drugs Act 1971 was amended and classified synthetic cannabinoids as controlled substances in the UK (UK Statutory Instrument, 2009). Far from stopping their sale, prohibition rather had the effect of facilitating the generation of new follow-up designer cannabimimetic substances: the aminoalkylindole JWH-073 (Auwärter et al., 2009; Lindigkeit et al., 2009), the hexyl homolog JWH-019 (Dresen et al., 2011), and the two more recent aminoalkylindoles, JWH-250 (Westphal et al., 2010) and JWH-398 (Hudson et al., 2010). During 2009, the potent synthetic cannabinoid agonist HU-210 was identified in Spice products in the UK (EMCDDA, 2009). Starting from the middle of 2010, the EMCDDA warned about the presence of JWH-015 in an herbal mixture called "Topaz" that is sold in Austria, along with the discovery of a methyl derivative of JWH-073 (1-butyl-3-(1-(4-methyl)naphthoyl)indole), JWH-122 (1-pentyl-3-(4-methyl-1-naphthoyl)indole) being a methyl derivative of JWH-018, and AM-694 (1-(5-fluoropentyl)-3-(2-iodobenzoyl)indole) as the first halogenated aminoalkylindole found in an herbal mixture (Ernst et al., 2011). On March 2011, the Drug Enforcement Administration (DEA) issued the final order to temporarily ban five synthetic cannabinoids, namely JWH-018, JWH-073, JWH-200, CP-47,497, and CP-47,497-C8. Currently, there are no more doubts that these Spice-like products are no longer limited to European Countries but rather have spread worldwide, from Ukraine, to Taiwan, to the USA (Vardakou et al., 2010), and a growing number of countries are currently implementing their own law or policy of controlling at one or more of these synthetic cannabinoids (Drug Policy Alliance, 2011). In Europe, the emergence of Spice drugs has became a major alarm for the Europol's Organised Crime Threat Assessment (OCTA, 2011), while in the USA starting from February 2011, the Uniform Code of Military Justice (UCMJ) banned Spice and US Air Force is now screening urine tests for synthetic cannabinoids (Air Force Times, 2011; UCMJ, 2011).

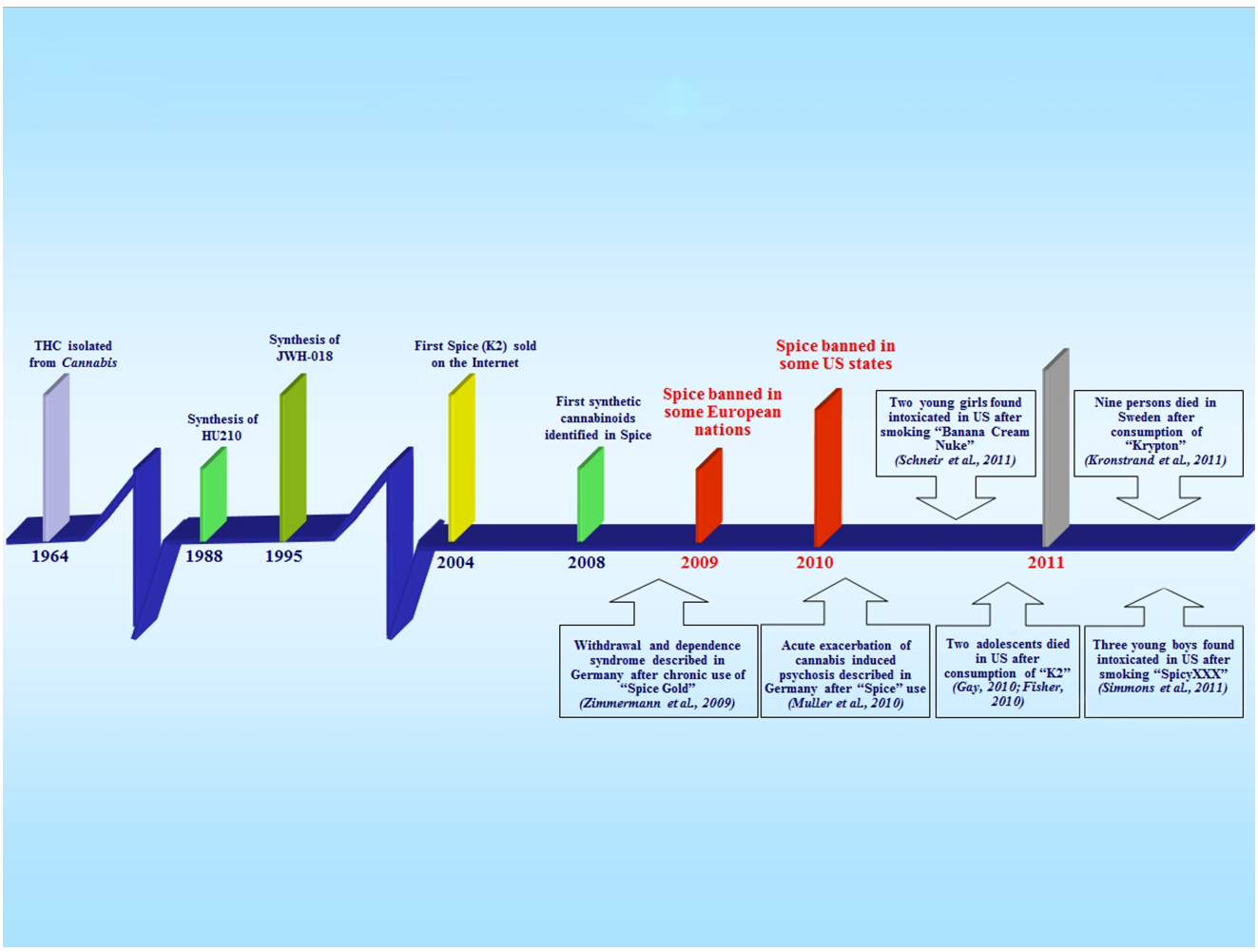

FIGURE 1 | Timeline of synthetic cannabinoids and Spice products.

DRUG QUEEN OF THE WEB

The presence of websites dedicated to the use of recreational controlled drugs as well as the use of cyberspace for the assessment of the drug abuse market are not recent phenomena (Rawaf and Schifano, 2000; Schifano et al., 2003, 2006). Yet, the role played by the Internet as one of the major markets for novel designer drugs is increasingly alarming (Corazza et al., 2011). To date, new Spice products that purportedly contain synthetic cannabinoids appear in the online market on a regular basis and their popularity has grown rapidly in the past few years (Fabrizio Schifano, personal communication). Tracking the world wide web, it is clear that the vast majority of legal highs are purchased online, which partly explains why Spice use is so widespread and causes social and medical concern (Burley, 2008; Vardakou et al., 2011). There are an increasing number of websites where users can order Spice blends or pure JWH compounds without age restriction or any type of control. Amazingly, with a few clicks of a mouse, many highly psychoactive substances can be obtained cheaply and legally (Schmidt et al., 2011). On a growing number of blogs, users report their own mixtures and testing procedures, describe subjective side effects, and express their preference for their favorite smoking blends. That is, in a smoking blend competition, "Spice Diamond" was elected as the best smoking product among 41 different mixtures (Vardakou et al., 2010). Looking at the different Internet fora, it appears that users are perfectly conscious of the cannabis-like effects of these herbal blends; they know that their psychoactive properties are mainly due to synthetic cannabinoids, and more surprisingly, in most cases, they are aware that these compounds have never been tested for human consumption.

A dramatic online snapshot of the Spice phenomenon as an emerging trend has been recently given by an important web mapping program, the Psychonaut Web Mapping Project, a European Commission-funded project involving researchers from seven European countries (Belgium, Finland, Germany, Italy, Norway, Spain, and UK), which aims to develop a web scanning system to identify newly marketed psychoactive compounds, and their combinations (e.g., ketamine and Spice, cannabis and Spice), on the basis of the information available on the Internet (Psychonaut Web Mapping Research Group, 2010). As a major result of the Project, a new and updated web-based database is now widely accessible to implement a regular monitoring of the web for novel and recreational drugs[1].

By monitoring fora, blogs, and chats, as well as e-newsgroups, chat rooms, mailing lists, e-newsletters, and bulletin boards in eight languages (Dutch, English, Finnish, German, Italian, Norwegian, Spanish, and Swedish), Professor Fabrizio Schifano (the Scientific Coordinator of the Psychonaut Web Mapping Project) and collaborators have found that online users mostly appreciate Spice products for their psychoactive effects, lack of detection in body fluids, ease of online access, and legal status, although some consumers have reported unpleasant side effects, such as paranoia, cramps, strong headache, mild hallucinations, and vomiting (Schifano et al., 2009). Although the provenance of these herbal mixture is often not clear, the project has identified potential wholesalers and manufacturers of Spice, including a UK company (Psyche Deli) and a Dutch company (Zonged.eu/MultiNETional), although some Spice products seem to be imported from China (Jack, 2009). Notably, not only Spice mixtures but also related merchandise, such as an informative CD explaining their psychoactive effects, can be found on sale on eBay (Aurazendoctor, 2009). It is clear that the Internet offers an overabundance of drug-related data that are constantly one step ahead of those available to clinicians and law enforcement authorities (Boyer et al., 2001). Likewise, the poor quality of product information provided to consumers is particularly worrying, because the majority of the websites have inconsistent information available to users (Hillebrand et al., 2010).

WHY CANNABINOID DESIGNER DRUGS ARE SO POPULAR

THEY INDUCE PSYCHOACTIVE EFFECTS

According to discussions on retailers' websites, Spice smokers find drug effects similar to those of marijuana, leading to the hypothesis that many users smoke it as a legal alternative to cannabis. Apparently labeled as incense, the herbal constituents listed on the packaging of Spice have been deliberately contaminated with synthetic cannabinoids (mainly JWH-018) to induce cannabimimetic effects (Steup, 2009). Thus, while the natural ingredients of these herbal mixtures seem not to possess psychoactive properties *per se*, synthetic cannabinoids are probably mixed into a solvent and then sprayed on a plant-derived base for delivery, leading to a final product with potent cannabis-like properties (Vardakou et al., 2010). Their agonistic activity on the CB1 receptor is responsible for elevating mood and inducing a feeling of well-being. Some Spice users have reported effects similar to or even stronger than those obtained by smoking cannabis, such as physical relaxation, changes in perception, and mild euphoria. The higher potency of action of these synthetic cannabinoids might be explained by *in vitro* experiments that have suggested that, while THC acts as a partial agonist on the CB1 receptor, JWH-018 acts as a full and potent agonist (Atwood et al., 2010). Moreover, compared with THC, JWH-018 possesses approximately a fourfold higher affinity to the cannabinoid CB1 receptor and 10-fold higher affinity to CB2 receptor (Aung et al., 2000; Huffman and Padgett, 2005). With respect to other products containing non-controlled plants and fungi and marketed as legal highs (e.g., *Salvia divinorum* or khat), Spice blends better satisfy users' expectations, in that their psychoactive effects are perceived to be even stronger than cannabis (Griffiths et al., 2010). Regrettably, safety information provided is sparse and of uncertain utility, with only a few products warnings about potential adverse effects or drug interactions.

THEY ARE LEGAL

Spices are often referred to as legal highs, in that they are neither controlled by the 1971 Misuse of Drugs Act, nor licensed for legal use such as alcohol and nicotine. Both the use and possession of these drugs have been long officially authorized, and their supply tolerated as long as they are sold for purposes other than human consumption. The relatively recent identification of the first synthetic cannabinoids as not declared ingredients of Spice

[1]http://www.psychonautproject.eu

marked a significant turning point in this situation. Indeed, although structurally distinct from THC, synthetic cannabinoids are part of the well-characterized aminoalkylindole class of ligands that also bind and activate CB1 receptors. Although their government regulation is still inconsistent or even lacking in many countries, Spice products are currently controlled in 14 European nations (Austria, Denmark, Estonia, France, Germany, Ireland, Italy, Latvia, Lithuania, Luxembourg, Poland, Romania, Sweden, and UK), where they are classified as pharmaceuticals or narcotics. Yet, they are still legal and uncontrolled in the remaining parts of Europe and many other countries, leading to heavy global marketing.

In the USA, some states (Alabama, Arkansas, Georgia, Kansas, Kentucky, and Missouri) have taken legislative action against the distribution and use of Spice, and until recently, only one synthetic cannabinoid, namely HU-210, was considered a Schedule I substance (unsafe, highly abused, no medical usage). On November 24, 2010, the United States Drug Enforcement Administration temporarily added to the list the following synthetic cannabinoids: JWH-018, JWH-073, JWH-200, CP-47,497, and cannabicyclohexanol (US Department of Justice Drug Enforcement Administration Drugs and Chemicals of Concern, 2010). JWH compounds (i.e., JWH-018, JWH-015, and JWH-073) are also currently unregulated in New Zealand and are easily obtainable in head shops and from many websites (Every-Palmer, 2011).

However, regulatory mechanisms are weak and difficult to enforce when controlled products are available on the Internet, because online retailers can evade easily national jurisdiction and supply Spice products to other countries but not their own. In addition, experience has shown that, as legal authorities adopt control measures, other synthetic cannabinoids are soon added to existing Spice-like products, suggesting that the producers expect prohibition and are ready to synthesize an assortment of substitutes (Lindigkeit et al., 2009). It looks like the producers are moving onto the next product, always one step ahead of the law (Dargan et al., 2011). This is not totally unexpected because there is a high demand for legal highs (United Nations Office on Drugs and Crime, 2011; Zawilska, 2011), implying that it will be satisfied by products containing chemical ingredients that are not yet prohibited. Lack of consistency in the measures adopted to control the market, ranging from medical legislation to formal drug control instruments, is another major point of concern in monitoring and responding to worldwide circulation of Spice (ACMD, 2009; McLachlan, 2009).

THEY ARE READY AVAILABLE AND HIGHLY ATTRACTIVE

Marketed under the generic brand name of Spice, an increasing number of herbal mixtures are sold mainly on the Internet, and in some countries, in dedicated shops that offer legal alternatives to prohibited drugs. The price is affordable also by young people, roughly €9–12 per gram: each sachet typically contains 3 g of smoking mixture, sufficient for around eight joints, and costs €27–36. As reported in a warning editorial by Griffiths et al. (2010), the Spice phenomenon represents a clear example of how globalization brings major challenges to the control of new drugs marketing, in that a user living in a country where Spice is controlled can buy it from a foreign retailer.

It is noteworthy that the multicolored packaging of these herbal blends is very attractive and highly sophisticated; many of them have a wide-open-eye imprint and circulate under exotic brand names, such as "Tropical Synergy" or "Yucatan Fire" (Sobolevsky et al., 2010). Although the product label often states that the product is "not for human consumption" or "for aromatherapy only," the composite blend of ingredients listed on the pack suggests the opposite, suggesting that the purpose of such a statement is to elude the interest of the medicines and healthcare products regulatory agencies. Because of their packaging resembling incense or tea and their scented smell, Spice products are far less noticeable as drugs, are not easily identified by parents or carers, and can comfortably be consumed at home. Thus, they are extremely tempting for young people that are willing to try cannabis but are afraid of the legal consequences and/or the reputation.

THEY ARE PERCEIVED AS SAFE DRUGS

Commercial advertisements describing Spice as "natural herbs" or "harmless incense blend" are very colorful and use intuitive (figurative) language, resulting in greater attractiveness for vulnerable individuals who might not otherwise smoke cannabis, in particular adolescents wishing to have a "safe" experience. Lack of safety information could lead to the incorrect supposition that herbal mixtures are safe, especially amongst first-time users. These herbal blends typically have a pleasurable smell and taste (i.e., honey or vanilla), are delivered in attractive packaging, often as pre-rolled cigarets, and are marketed as "incense cones." For some people, undesirable psychoactive effects and public perception of cannabinoid use could represent major limitations in the use of marijuana: Spice circumvents these limits by appearing as a safer alternative to cannabis. People that have been warned against using cannabis for legislative (i.e., after a period in prison or a forensic hospital) or medical (i.e., predisposition to psychotic illness) reasons might also find Spice a safe (and undetectable) drug to use. Moreover, Spice products represent a tempting alternative for those who have experienced adverse effects from smoking marijuana (ACMD, 2009; EMCDDA, 2009). However, all Spice products introduced into the market lack any published *in vivo* testing, even in animal models, and very little information is available in international medical databases.

THEY ARE NOT EASILY DETECTABLE IN URINE AND BLOOD SAMPLES

Most of the synthetic cannabinoids added as not-listed ingredients to Spice products are very difficult to detect by commonly used drug screening procedures. Apart from the analogs of THC such as HU-210, the structure of these new synthetic cannabinoids differs from that of THC, so that they probably will not trigger a positive test for cannabinoids in immunoassays of body fluids. This has important consequences, because it encourages not only cannabis users but also curious people with no previous experience of illicit drugs to use these products to attain cannabis-like effects, without having to fear prosecution. Furthermore, wherever drug screening is routinely performed to guarantee abstinence from drugs (i.e., hospital or institutions carrying out detoxification, forensic psychiatric centers), people can be motivated to substitute cannabis with Spice products. Maximal research effort is currently focusing

on the development of new analytical procedure for measuring urinary concentrations of synthetic cannabinoids and several potential metabolites of each (Beuck et al., 2011; Grigoryev et al., 2011; Hudson and Ramsey, 2011; Moran et al., 2011).

WHAT DO THEY CONTAIN?

It is not easy to determine exactly what is in Spice products, due to the lack of reference samples and the presence of masking agents of natural origin such as tocopherol (vitamin E), eugenol, or fatty acids (Dresen et al., 2010), which are commonly added to confuse identification. Spice is supposed to contain up to 15 different vegetal compounds, which gives rise to a wide variety of drug combinations (Zuba et al., 2011), among which are the psychoactive herbs known as "Wild Dagga" (*Leonotis leonurus*) and "Indian warrior" (*Pedicularis densiflora*). Potentially psychoactive alkaloids, such as betonicine, aporphine, leonurine nuciferine, or nicotine, are often declared as ingredients in these products; yet, only some herbal mixtures have the constituents stated, and most samples of Spice contain inert vegetable matter. The synthetic psychoactive compounds added to Spice were initially almost unknown substances, which forensic laboratories had difficulties in recognizing and finding reference samples. A decisive improvement in the identification of these chemicals is the recent development of a GC–MS screening procedure that combines high chromatographic resolution with the existence of well-established libraries, offering huge collections of spectra that can be adapted by adding spectra of emerging psychoactive compounds that have probably been added to herbal mixtures (Auwärter et al., 2009; Dresen et al., 2010, 2011).

The active compounds present in Spice products are placed principally at the surface of the herbal ingredients, and the extraction procedure is able to remove them without significant contamination by the vegetable components of the herb. Unfortunately, trafficking and detection of these drugs is hampered by the fact that the exact content of many Spice products still remains unpredictable, because it changes constantly over time as a reaction to prevention and legal actions, leading to an ever-expanding array of synthetic cannabinoids being available on the market. It is clear that comprehension of the clinical pharmacology of these compounds is essential for practitioners and scientists to discriminate the relative toxicity associated with the different synthetic cannabinoid mixtures and routes of administration. The major psychoactive ingredients of Spice products are illustrated in **Figure 2**.

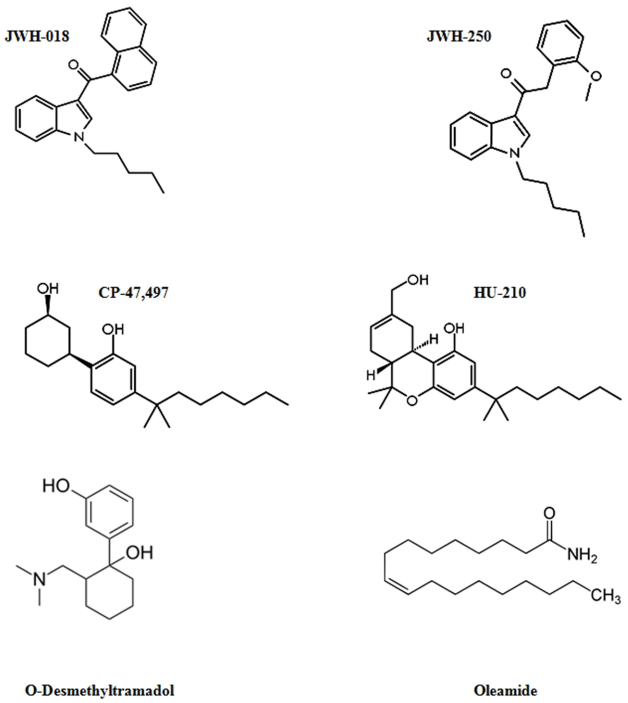

FIGURE 2 | Structures of most common psychoactive ingredients of Spice products: synthetic cannabinoids (JWH-018, JWH-250, CP-47,497, HU-210), μ-opioid agonists (*O*-Desmethyltramadol), and fatty acid derivatives with cannabinoid-like activity (Oleamide).

CANNABINOIDS

Unlike cannabis, Spice products do not contain the phytocannabinoids cannabidiol (rarely THC) but synthetic cannabinoid drugs, which originate from four chemically distinct groups: (i) the JWH compounds, synthesized by John W. Huffman (JWH) in the 1980s, of which JWH-018 is the most studied and best characterized to date; (ii) the CP-compounds, a cyclohexylphenol series synthesized by Pfizer in the 1970s, with the identified CP-47,497 and its modified version CP-47,497-C8 (obtained by extending the dimethylheptyl side chain to dimethyloctyl); (iii) the HU-compounds, synthesized in the 1960s at the Hebrew University; and (iv) the benzoylindoles, such as AM-694 and RCS-4 (Huffman et al., 2008; EMCDDA, 2009; Lindigkeit et al., 2009; Uchiyama et al., 2009b, 2010, 2011a,b; United States Drug Enforcement Administration, 2009; Hudson et al., 2010; Nakajima et al., 2011). The show binding affinities for the CB1 and/or the CB2 receptors (see EMCDDA, 2009 for a comprehensive review), are lipid soluble and non-polar, and consist of 22 to 26 carbon atoms, which explains why they volatilize readily when smoked. Contrary to nabilone, a synthetic analog of tetrahydrocannabinol approved by the US Food and Drug Administration (FDA) to treat chemotherapy-induced nausea and vomiting, no therapeutic effects have been documented so far for synthetic cannabinoids detected in these herbal mixtures.

The family of the JWH compounds is the most numerous and, although their chemical structures differ greatly from those of THC, they have a higher affinity to CB1 and/or CB2 receptors and are more potent than THC (Huffman et al., 2003; Huffman, 2009). Conversely, JWH-015 [(2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone] acts as a selective CB2 receptor agonist (Aung et al., 2000). At the end of 2008, the synthetic cannabinoid naphthoylindole, JWH-018, and the cyclohexylphenol CP-47,497, along with one of its active homologs, CP-47,497-C8, were detected using nuclear magnetic resonance spectroscopy (Auwärter et al., 2009). JWH-018 (naphthalen-1-yl-(1-pentylindol-3-yl)methanone) was first synthesized during an analysis aiming at developing new cannabimimetic indole compounds with potential therapeutic effects comparable with those

of THC (Chin et al., 1999). It belongs to the aminoalkylindole family and has been shown to have a binding affinity for the CB1 receptors in the low nanomolar range (∼9 nM; Aung et al., 2000; Atwood et al., 2010). In cannabinoid receptor expressing CHO cells, JWH-018 inhibits forskolin-stimulated cAMP production (Chin et al., 1999), whereas in HEK293 cells stably expressing this receptor, it was recently found to activate multiple cannabinoid receptor signaling pathways, including the phosphorylation of ERK1/2 mitogen activated protein kinase and the internalization of CB1 receptors (Atwood et al., 2010). Specifically, JWH-018 dose-dependently inhibits glutamate release in autaptic excitatory hippocampal neurons, probably acting on the CB1 receptor, an effect reversed by administration of the CB1 receptor antagonist rimonabant (Atwood et al., 2010). *In vivo* studies showing that JWH-018 induces analgesia, catalepsy, hypomotility, and hypothermia, namely the tetrad of behaviors classically caused by cannabinoids administration (Wiley et al., 1998), have confirmed that this compound acts as a potent and effective CB1 receptor agonist. Specifically, JWH-018 displayed fourfold affinity to the CB1 receptor and about 10-fold affinity to the CB2 receptor compared with THC (Aung et al., 2000; Huffman et al., 2005). It is worth nothing that unlike metabolites of most synthetic cannabinoids, JWH-018 hydroxylated metabolites retain *in vitro* and *in vivo* activity at CB1 receptors (Brents et al., 2011), a finding that in conjunction with the higher CB1 receptor affinity and activity relative to THC may contribute to the greater prevalence of adverse effects observed with JWH-018-containing products relative to marijuana. Other JWH compounds have been identified in herbal mixtures, such as JWH-250, which shows high affinity for the CB1 and CB2 receptors (Dresen et al., 2010, 2011), and the butyl homolog of JWH-018, JWH-073 (naphthalen-1-yl-(1-butylindol-3-yl)methanone), which seems to bind more specifically to the CB1 receptor (Wiley et al., 1998; Aung et al., 2000). The latter has been recently shown to act similarly to JWH-018, although it is less potent in inhibiting neurotransmission and slower in producing internalization of cannabinoid receptors (Atwood et al., 2011). Several studies have reported a recent decrease in the content of JWH-018 in Spice products, replaced by JWH-073 (Lindigkeit et al., 2009) or other synthetic psychoactive cannabinoids (Hudson et al., 2010; Uchiyama et al., 2010, 2011a), of which JWH-398 is the most recently identified in the UK and Germany (Vardakou et al., 2010). JWH-398 was found to be a very potent non-selective CB1/CB2 receptor agonist (Huffman, 2009), while in a recent study, the *N*-alkyl-3-(1-naphthoyl)indole JWH-122, a very potent CB1 receptor agonist with a structure closely related to JWH-018 and JWH-073, has been identified as a new ingredient of commercial samples of a Spice product called "Lava Red" (Ernst et al., 2011).

The CP-47,497 cannabinoid compound (2-(1R,3S)-3-hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol) lacks the classical cannabinoid chemical structure (tricyclic benzopyran system) and is 3–28 times more potent than THC (Weissman et al., 1982). Like its homolog cannabicyclohexanol (CP-47,497-C8), it shows higher affinity to the receptor CB1 compared to CB2 (Auwärter et al., 2009), and has THC-like activity in animals (Weissman et al., 1982; Compton et al., 1992). The concentration–response curve of CP-47,497-C8 in inhibiting neurotransmission in autaptic hippocampal neuron cultures is nearly identical to that described for JWH-018 (Atwood et al., 2010, 2011).

The classical cannabinoid HU-210 [(6aR,10aR)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c] chromen-1-ol)], whose agonistic activity on the CB1 receptor has been long recognized, is an ingredient of herbal mixtures in the UK and USA European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2009), where it has been placed under control since 2009 and 2010, respectively. This synthetic analog of THC was shown to be a full non-selective agonist at the CB1 and CB2 receptors, and to possess intrinsic affinities for cannabinoid receptors that exceed those of the high-efficacy agonists, CP 55,940 and WIN 55,212-2 (Howlett et al., 2002). Notably, the pharmacological effects of HU-210 *in vivo* are also exceptionally long lasting, and in animal models it has been shown to negatively affect learning (Ferrari et al., 1999) and memory (Robinson et al., 2007; Mackowiak et al., 2009) processes as well as sexual behavior (Ferrari et al., 2000).

Among the benzoylindoles, AM-694 [(1-(5-fluoropentyl)-3-(2-iodobenzoyl)indole)] binds strongly to CB1 and CB2 receptors, and now represents an example of the latest synthetic cannabinoid agonists added to Spice that is currently available on the UK market, but still not controlled by current UK legislation (Dargan et al., 2011). Another hazardous benzoylindole is RCS-4 [(4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone)], which is a synthetic JWH-018 cannabinoid analog with uncertain biological activity that, under the name of "Devil Smoke," is currently used in combination with JWH-073 (Drugs-Forum, 2011). On March 11, 2011, it was banned as euphoriant substance by the Danish Ministry of Health (2011).

It seems reasonable to hypothesize that additional compounds beside the above-mentioned might also contribute to the behavioral and subjective effects produced by smoking Spice, and that their different pharmacology might explain the different psychoactive effects experienced after smoking Spice. Although the marijuana-like effects of smoked Spice products are probably due to activation of CB1 receptor, the potential role of CB2 receptors in such effects is still to be investigated. Regrettably, cannabinoids identified so far in Spice products are believed to be only the tip of an iceberg; the first of a larger number of synthesized substances with cannabis-like effects mediated by their agonist activity at the CB1 (and/or CB2) receptor. Currently, more than 100 compounds with cannabimimetic activities are waiting for identification.

OPIOIDS

Besides cannabinoids, other psychoactive substances can be part of Spice products, such as the synthetic opioid *O*-desmethyltramadol (Dresen et al., 2010). This opioid is an active metabolite of the opioid tramadol, a centrally acting analgesic drug with suspected abuse liability (Raffa, 2008). Very recently, *O*-desmethyltramadol was found as an ingredient of a Spice-like blend called "Krypton," in combination with Kratom (*Mitragyna speciosa*), an Asiatic medicinal plant that has been used as an herbal drug for a long time (Arndt et al., 2011; Philipp et al., 2011). Mitragynine, an alkaloid present in Kratom, acts as a μ-opioid receptor agonist, and

when combined with *O*-desmethyltramadol, another potent μ-opioid agonist, can lead to fatal consequences. Indeed, in less than 1 year, consumption of Krypton had fatal results and caused the unintentional deaths of nine persons (Kronstrand et al., 2011).

OTHER SUBSTANCES

Oleamide (cis-9,10-octadecenoamide), a fatty acid derivative with cannabinoid-like activity (Leggett et al., 2004) and hypnotic properties (Fedorova et al., 2001) is one of the most frequent non-cannabinoid ingredients associated with Spice products (Dresen et al., 2010). In association with JWH-018, oleamide is present in an herbal mixture sold as "Aroma" (Every-Palmer, 2011). In particular, it was found that "Aroma" contained the highest concentration of oleamide and the second highest concentration of JWH-018 (Uchiyama et al., 2010). Harmine and harmaline, two reversible inhibitors of the monoamine oxidase enzyme with stimulating central effects (Fortunato et al., 2009, 2010), have also been found in one of these products in combination with myristicin and asarone (Dresen et al., 2010, 2011). Benzophenone (HM 40) is another undeclared substance found in an herbal mixture, although most likely it was not added purposely but rather should be considered a contamination from synthesis (Dresen et al., 2010, 2011).

Many other ingredients are listed on the Spice packets, with their combinations greatly varying in number and concentration, often depending on the country of distribution. For example, in a packet of Spice called "Banana Cream Nuke" bought in an USA smoke shop, the following ingredients were listed: alfalfa, blue violet, nettle leaf, comfrey leaf, *Gymnema sylvestre*, passion flower leaf, horehound, and neem leaf (Schneir et al., 2011). Notably, this product caused acute intoxication in two young girls, probably due to the co-presence of THC, JWH-018, and JWH-073 identified among 15 other synthetic cannabinoids, whereas none of the listed ingredients were detectable (Schneir et al., 2011), with the only exception of passion flower (*Passiflora* sp.) that is well known to possess anxiolytic properties (Dhawan et al., 2004). Conversely, some packets of Spice sold in the UK declare beach bean (*Canavalia maritima* or *Canavalia rosea*), blue lotus (*Nelumbo nucifera*), and dwarf skullcap (*Scutellaria nana*) as ingredients of the mixture, for which no safety data are available (Burley, 2008). Moreover, in Germany, in the past 2 years, head shops were selling different varieties of herbal mixtures by combining the above-mentioned plants with white or blue water lily (*Nymphaea alba* or *Nymphaea caerulea*), Indian Warrior (*Pedicularis densiflora*), Lion's Ear (also known as Lion's Tail or Wild Dagga; *L. leonurus*), Maconha Brava (*Zornia latifolia*), and Honeyweed or Siberian Motherwort (*Leonurus sibiricus*; Teske et al., 2010). Other plants commonly used in Spice products in combination with synthetic cannabinoids included Marshmallow (*Althaea officinalis*) and Dog Rose or Rosehip (*Rosa canina*; Seely et al., 2011). Not surprisingly, no natural cannabinoids were declared as constituents.

WHAT ARE THEIR MAIN EFFECTS?

In a growing number of Internet blogs, Spice is described by users as able to exert strong cannabis-like effects, but inter- and intra-batch variations, both in terms of substances present and their quantity, have also resulted in accidental overdosing that requires hospitalization (Auwärter et al., 2009). Worryingly, very little is known about its pharmacology and toxicology in humans, and virtually nothing has been investigated thus far about the health implications of its use, either in humans or animals, which hampers appropriate medical treatment of Spice-induced side effects. The carcinogenic potential caused by inhaling smoke containing these substances has also not been evaluated (EMCDDA, 2009). Only limited data on the pharmacological properties of CP-47,497 in animal models and on the metabolism of JWH-015 in rat liver microsomes are available (Compton et al., 1992; Zhang et al., 2006). It is noteworthy that CP-47,497 generalized with THC in drug discrimination studies in rats, that is, it produces subjective effects similar to those of THC, with an absolute threshold dose 3–14 times lower than that of THC (Weissman et al., 1982). Very recently, JWH-018 and CP-47,497 were found to significantly decrease the locomotor activity and increase the electroencephalogram power spectra in rats (Uchiyama et al., 2011b).

In general, the desired effects of Spice include a sense of empathy and well-being. However, there is an increasing number of clinical reports describing patients presenting for emergency medical care after smoking Spice products, the most common symptoms being nausea, anxiety, agitation/panic attacks, tachycardia, paranoid ideation, and hallucinations (Piggee, 2009; Banerji et al., 2010; Bebarta et al., 2010; Vearrier and Osterhoudt, 2010). On the Internet, it is possible to find a quantity of self-reports of users experiencing anxiety and psychotic symptoms after using synthetic cannabinoids[2]. Finally, in the literature, there is one published case report of tolerance and withdrawal phenomena (Zimmermann et al., 2009), another of drug-induced psychosis (Müller et al., 2010), and two clinical studies conducted on psychotic patients (Every-Palmer, 2010, 2011).

CENTRAL EFFECTS AND COGNITIVE DEFICITS

Spice blends are often described as energizing, euphoric, and disinhibiting (Schifano et al., 2009), which are likely among the most desirable effects pursued by users. However, halting speech and avoidant eye contact were observed in a young student who smoked Spice for 3 weeks (Benford and Caplan, 2011). Moreover, after chronic (8 months) daily use, Spice can induce serious cognitive impairment (Zimmermann et al., 2009). Loss of consciousness and confusion have also been described, as well as unresponsiveness, seizures, agitation, and irritation (Seely et al., 2011; Simmons et al., 2011b).

EMOTIONAL ALTERATIONS

Anxiety is one of the main side effects experienced during acute intoxication, which resolves within 1–2 h after consumption (Schneir et al., 2011). A sense of extreme anxiety and sudden depression has been reported during withdrawal from chronic Spice use (Zimmermann et al., 2009). Paranoia and hallucinations have been observed in some patients (Banerji et al., 2010; Bebarta et al., 2010; Simmons et al., 2011a). Alterations in mood and perception after Spice have also been described (Auwärter

[2]http://www.erowid.org/experiences/subs/expJWH018.shtml#Train Wrecks & Trip Disasters

et al., 2009), and two studies has associated the use of synthetic cannabinoids with exacerbation of cannabis-induced psychosis (Müller et al., 2010; Benford and Caplan, 2011). Interestingly, unlike cannabis, Spice blends do not contain cannabidiol, a phytocannabinoids known to possess anxiolytic properties, which is able to reduced anxiety in both animals (Guimarães et al., 1990; Moreira et al., 2006) and humans (Bergamaschi et al., 2011; Crippa et al., 2011). More importantly, cannabidiol displays high potency as an antagonist of CB1 and CB2 receptor agonists (Thomas et al., 2007; Pertwee, 2008), and is able to revert not only THC-induced social withdrawal in rats (Malone et al., 2009) but also THC-induced anxiety in normal volunteers (Zuardi et al., 1982), suggesting that lack of this cannabinoid in Spice drugs may exacerbate the detrimental effects of these herbal mixtures on emotion and sociability.

DEPENDENCE AND WITHDRAWAL

To the best of our knowledge, only one case report in Germany has described thus far a withdrawal syndrome after discontinuation from smoking Spice (Zimmermann et al., 2009). Specifically, withdrawal phenomena and a dependence syndrome have been described after chronic consumption of an herbal mixture called "Spice Gold" (typically containing CP-47,497-C8 and JWH-018) in a 20-year-old man, who had a history of smoking this product daily for 8 months as the only relief from his internal unrest and nervousness. He found Spice relaxing and sedative, with psychoactive effects very similar to those of cannabis. He entered hospital voluntarily, requesting medical treatment for detoxification of Spice after experiencing a similar syndrome a few weeks earlier during a phase of abstinence owing to a short supply. Internal unrest and profuse sweating were among the first symptoms observed by doctors, followed by drug craving, nocturnal nightmares, tremor, and headache. Other physical withdrawal symptoms included palpitation, nausea and vomiting, and were not dissimilar from those described during cannabis withdrawal (Budney and Hughes, 2006). Besides a clear withdrawal syndrome, a diagnosis of dependency was confirmed by the development of drug tolerance (the patient had to increase rapidly the dose from 1 to 3 g/day), persistence of drug craving (he felt a continuous strong desire for the drug), the continuous urge to consume it despite the adverse consequences (cognitive impairments and risk of losing his professional training position), and the scarce attention to other interests or duties (participation in practical work).

PSYCHOTIC EFFECTS

The link between cannabis use and the occurrence of psychotic episodes is widely recognized, although it has not been determined yet whether abuse of the drug in psychotic patients antedates the onset of the pathology or it is a consequence of the disorder (Arseneault et al., 2002). Indeed, regular cannabis use is thought to increase the risk of developing psychosis and to facilitate the manifestation of the disorder in vulnerable individuals. On the other hand, patients smoke cannabis to self-medicate the negative symptoms of schizophrenia or the side effects of antipsychotic medications. The great medical interest in examining the effects of these synthetic cannabinoids on the psychotic population is therefore not surprising. Few data are available on the psychological and other risks of synthetic cannabinoids; nevertheless, despite the limited number of clinical observations, in the Internet fora, a growing number of users have reported experiencing psychotic symptoms after smoking Spice.

A first case report described the effects of Spice on a 25-year-old man who had a history of cannabis-induced recurrent psychotic episodes (Müller et al., 2010). It was found that Spice triggered not only acute exacerbation of cannabis-induced recurrent psychotic episodes, but also the manifestation of new symptoms, such as recurrent paranoid hallucinations (Müller et al., 2010). To such an acute reactivation of symptoms after abuse of Spice could have contributed the absence of cannabidiol, which is presumed to have antipsychotic potency (Zuardi et al., 2006; Zullino et al., 2007), thus suggesting a higher potency for psychosis of these substances. In line with this, relapses following the use of a Spice product in psychotic patients have been reported by forensic services (Every-Palmer, 2010). More recently, psychotic relapse after smoking Spice was confirmed in 15 psychotic New Zealand patients, all familiar with a locally available JWH-018-containing product called "Aroma" (Every-Palmer, 2011). Intriguingly, no one of these patients reported withdrawal symptoms or physical distress after using the Spice product, three of them described developing some tolerance to the product, and 13 acknowledged having smoked "Aroma" as a cannabis substitute. A latest study described the case of a young student experiencing severe anxiety, paranoia, and both visual and auditory hallucinations after repeated (3 weeks) use of Spice (Benford and Caplan, 2011). Thus, evidence seems to indicate that Spice products can precipitate psychosis in vulnerable individuals, implying the necessity of advising people with risk factors for psychosis against using synthetic cannabinoids.

PERIPHERAL EFFECTS

Although gastrointestinal effects, such as nausea, vomiting, and retching, are the most common after consumption of Spice, cardiovascular effects, such as extremely elevated heart rate and blood pressure, chest pain, and cardiac ischemia are among the most dangerous consequences (Canning et al., 2010; Schneir et al., 2011; Seely et al., 2011). Occasional inappropriate laughter, injected conjunctiva, xerostomia, and nystagmus have been described as well (Auwärter et al., 2009; Schneir et al., 2011). Spice also induces metabolic effects, such as hypokalemia, hyperglycemia, and acidosis, and autonomic effects, such as fever and mydriasis (Seely et al., 2011; Simmons et al., 2011a).

LETHAL EFFECTS

Contrary to the partial action of THC at the CB1 receptor, synthetic cannabinoids identified so far in Spice products have been shown to act as full agonists with increased potency, thus leading to longer durations of action and an increased likelihood of adverse effects. Although limited at the moment, some life-threatening symptoms have been reported by subjects that use these products as marijuana substitutes, and coma and suicide attempts have been reported after smoking the dangerous spice drug K2 (Missouri Department of Health and Senior Services, 2010). Dramatically, two adolescents died in the USA after ingestion of a

Spice product called "K2," one due to a coronary ischemic event (Fisher, 2010), and the other committed suicide due to the unbearable sense of extreme anxiety (Gay, 2010). The non-cannabinoid ingredient of many Spice products, namely the opioid agonist *O*-desmethyltramadol, when used in combination with Kratom (as in the mixture known as Krypton), may have lethal consequences (Kronstrand et al., 2011).

CONCLUDING REMARKS

Spice drugs include a large range of products sold as "ethno drugs" or legal substitutes for cannabis since 2004. From the end of 2008, some potent synthetic cannabinoids, such as JWH-018 and CP-47,497, were identified as psychoactive ingredients of these herbal blends, and some countries placed them under control. Due to their powerful psychoactivity, ready availability on the Internet, legal status, and non-detection in drug testing, Spice products have acquired an unexpected popularity, especially among youngest and first-time consumers, including college students (Hu et al., 2011). Synthetic cannabinoids currently available on the market have been shown to induce severe peripheral and central effects, including drug dependence and psychosis. Yet, these products provide very limited safety information about their effects and possible health consequences, so that uninformed users risk serious adverse effects. This renders it very complicated, if not impossible, for health professionals and clinicians to carry out accurate assessments of possible drug-related medical and psychiatric consequences of their use. The emergence of the Internet as the major player in shaping the international Spice market has led to significant public health concerns. Continuous monitoring of herbal mixtures available online is essential for timely detection of new chemicals, which will continue to be developed as a reaction to the newly implemented control measures (Uchiyama et al., 2009b, 2010, 2011a; Dresen et al., 2010, 2011; Dowling and Regan, 2011; Westphal et al., 2011).

FUTURE PERSPECTIVES

Very limited information is available on the safety of the Spice ingredients in humans, and the occurrence of serious health damage in their abusers is highly probable. Rapid detection and identification of new synthetic cannabinoids in human urine and blood samples would greatly restrict their use and diffusion. Up to the beginning of 2010, the first methodologies for the quantification of synthetic cannabinoids in human serum have been developed and validated in accordance with conventional screening protocols based on enzymatic hydrolysis, liquid–liquid extraction, and liquid chromatography/electrospray tandem mass spectrometry analysis (Müller et al., 2010; Teske et al., 2010). Researchers should continue to develop rapid and reliable detection screening procedures, because they would be crucial for medical staff in assisting patients at the emergency units, to psychiatrists in recognizing and treating psychiatric symptoms, and to police authorities in assessing fitness to drive. Evidence that the synthetic cannabinoids are not detectable in human body fluids underlines the need to elucidate their metabolic pathways and identify their metabolites, which could shed light on their pharmacokinetics and toxicity (Wintermeyer et al., 2010). Cannabinoids have been classified as doping substances by the World Anti-Doping Agency (WADA, 2011), thus, screening for the synthetic cannabinoids and their major metabolites could also be applied to human urine doping controls (Müller et al., 2010).

In countries where synthetic cannabinoids are under control, incessant monitoring of the manufacture, distribution, and use of marketed Spice-like products is necessary. Given the worldwide spread of these herbal mixtures, an international cooperation system is mandatory for sharing analytical information and improving monitoring of the global drug market. In countries where synthetic cannabinoids are marketed legally, accurate labeling of products containing psychoactive compounds should be requested, so that users can be conscious of the potential risks associated. In both cases, however, it will be an ongoing challenge to detect synthetic cannabinoids in herbal mixtures and to include them in analytical methods. Future research should focus on the study of their pharmacological effects, both at the central (dependence, psychosis, anxiety, depression) and peripheral (tremor, nausea, tachycardia, headache) levels, as well as on the evaluation of the health consequences of smoking Spice repeatedly. This is also to avoid the risk of banning compounds with not health hazarding profile which would probably be replaced quickly by more dangerous substances (Hammersley, 2010). More importantly, understanding of the neurobiological bases of such compounds activity might encourage the development of synthetic cannabinoids that produce therapeutic effects with a minimum of psychoactive effects, such as the synthetic THC dronabinol (Marinol), the synthetic THC analog nabilone (Cesamet), and the standardized cannabis extract (Sativex).

REFERENCES

Advisory Council on the Misuse of Drugs (ACMD). (2009). *Consideration of the Major Cannabinoid Agonists*. London: Home Office.

Air Force Times. (2011). *AF Using Urine Tests to Detect "Spice" Use*. Available at: http://www.airforcetimes.com/news/2011/03/air-force-using-urine-tests-for-spice-031211w/ [accessed August 9, 2011].

Alger, B. E., and Kim, J. (2011). Supply and demand for endocannabinoids. *Trends Neurosci.* 3, 304–315.

Arndt, T., Claussen, U., Güssregen, B., Schröfel, S., Stürzer, B., Werle, A., and Wolf, G. (2011). Kratom alkaloids and O-desmethyltramadol in urine of a "Krypton" herbal mixture consumer. *Forensic Sci. Int.* 208, 47–52.

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A., and Moffitt, T. E. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *BMJ* 325, 1212–1213.

Atwood, B. K., Huffman, J., Straiker, A., and Mackie, K. (2010). JWH018, a common constituent of "spice" herbal blends, is a potent and efficacious cannabinoid CB1 receptor agonist. *Br. J. Pharmacol.* 160, 133–140.

Atwood, B. K., Lee, D., Straiker, A., Widlanski, T. S., and Mackie, K. (2011). CP47,497-C8 and JWH073, commonly found in "spice" herbal blends, are potent and efficacious CB(1) cannabinoid receptor agonists. *Eur. J. Pharmacol.* 659, 139–145.

Aung, M. M., Griffin, G., Huffman, J. W., Wu, M., Keel, C., Yang, B., Showalter, V. M., Abood, M. E., and Martin, B. R. (2000). Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB1 and CB2 receptor binding. *Drug Alcohol Depend.* 60, 133–140.

Aurazendoctor. (2009). *Make Your Own Herbal Spice Gold Smoke Blends eBook CD, eBay, ID Number: 270362517411*. Available at: http://cgi.ebay.com/Make-Your-Own-Herbal-Spice-Gold-Smoke-Blends-eBook-CD_W0QQitemZ270362517411 [accessed March 24, 2009].

Auwärter, V., Dresen, S., Weinmann, W., Müller, M., Pütz, M., and Ferreirós, N. (2009). "Spice" and other herbal blends: harmless incense or cannabinoid designer drugs? *J. Mass Spectrom.* 44, 832–837.

Banerji, S., Deutsch, C. M., and Bronstein, A. C. (2010). Spice ain't so nice. *Clin. Toxicol. (Phila.)* 48, 632.

Bebarta, V. S., Varney, S., and Sessions, D. (2010). Spice: a new "legal" herbal mixture abused by young active duty military personnel. *Clin. Toxicol. (Phila.)* 48, 632.

Benford, D. M., and Caplan, J. P. (2011). Psychiatric sequelae of spice, k2, and synthetic cannabinoid receptor agonists. *Psychosomatics* 52, 295.

Bergamaschi, M. M., Queiroz, R. H., Chagas, M. H., de Oliveira, D. C., De Martinis, B. S., Kapczinski, F., Quevedo, J., Roesler, R., Schröder, N., Nardi, A. E., Martín-Santos, R., Hallak, J. E., Zuardi, A. W., and Crippa, J. A. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. *Neuropsychopharmacology* 36, 1219–1226.

Beuck, S., Möller, I., Thomas, A., Klose, A., Schlörer, N., Schänzer, W., and Thevis, M. (2011). Structure characterisation of urinary metabolites of the cannabimimetic JWH-018 using chemically synthesised reference material for the support of LC-MS/MS-based drug testing. *Anal. Bioanal. Chem.* 401, 493–505.

Boyer, E. W., Shannon, M., and Hibberd, P. L. (2001). Web sites with misinformation about illicit drugs. *N. Engl. J. Med.* 345, 469–471.

Brents, L. K., Reichard, E. E., Zimmerman, S. M., Moran, J. H., Fantegrossi, W. E., and Prather, P. L. (2011). Phase I hydroxylated metabolites of the K2 synthetic cannabinoid JWH-018 retain in vitro and in vivo cannabinoid 1 receptor affinity and activity. *PLoS One* 6, e21917. doi: 10.1371/journal.pone.0021917

Budney, A. J., and Hughes, J. R. (2006). The cannabis withdrawal syndrome. *Curr. Opin. Psychiatry* 19, 233–238.

Burley, B. (2008). *Doctors Worries Over Legal Highs. BBC Newsbeat Oct 7, 2008.* Available at: http://news.bbc.co.uk/newsbeat/hi/health/newsid_7656000/7656172.stm [accessed May 3, 2011].

Canning, J. C., Ruha, A.-M., Pierce, R., Torrey, M., and Reinhart, S. J. (2010). Severe GI distress after smoking JWH-018. *Clin. Toxicol. (Phila.)* 48, 618.

Chin, C. N., Murphy, J. W., Huffman, J. W., and Kendall, D. A. (1999). The third transmembrane helix of the cannabinoid receptor plays a role in the selectivity of aminoalkylindoles for CB2, peripheral cannabinoid receptor. *J. Pharmacol. Exp. Ther.* 291, 837–844.

Compton, D. R., Johnson, M. R., Melvin, L. S., and Martin, B. R. (1992). Pharmacological profile of a series of bicyclic cannabinoid analogs: classification as cannabimimetic agents. *J. Pharmacol. Exp. Ther.* 260, 201–209.

Corazza, O., Schifano, F., Farre, M., Deluca, P., Davey, Z., Torrens, M., Demetrovics, Z., Di Furia, L., Flesland, L., Siemann, H., Skutle, A., Van Der Kreeft, P., and Scherbaum, N. (2011). Designer drugs on the internet: a phenomenon out-of-control? The emergence of hallucinogenic drug Bromo-Dragonfly. *Curr. Clin. Pharmacol.* 6, 125–129.

Crippa, J. A., Derenusson, G. N., Ferrari, T. B., Wichert-Ana, L., Duran, F. L., Martin-Santos, R., Simões, M. V., Bhattacharyya, S., Fusar-Poli, P., Atakan, Z., Santos Filho, A., Freitas-Ferrari, M. C., McGuire, P. K., Zuardi, A. W., Busatto, G. F., and Hallak, J. E. (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *J. Psychopharmacol. (Oxford)* 25, 121–130.

Danish Ministry of Health. (2011). *Amendment of executive order on euphoriant substances 11 March 2011.* Available at: http://laegemiddelstyrelsen.dk/en/service-menu/product-areas/euphoriant-substances/nyheder-om-euforiserende-stoffer/amendment-of-executive-order-on-euphoria-march 2011 [accessed August 8, 2011].

Dargan, P. I., Hudson, S., Ramsey, J., and Wood, D. M. (2011). The impact of changes in UK classification of the synthetic cannabinoid receptor agonists in "spice." *Int. J. Drug Policy* 22, 274–277.

Dhawan, K., Dhawan, S., and Sharma, A. (2004). *Passiflora*: a review update. *J. Ethnopharmacol.* 94, 1–23.

Dowling, G., and Regan, L. (2011). A method for CP 47, 497 a synthetic non-traditional cannabinoid in human urine using liquid chromatography tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 879, 253–259.

Dresen, S., Ferreirós, N., Pütz, M., Westphal, F., Zimmermann, R., and Auwärter, V. (2010). Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. *J. Mass Spectrom.* 45, 1095–1232.

Dresen, S., Kneisel, S., Weinmann, W., Zimmermann, R., and Auwärter, V. (2011). Development and validation of a liquid chromatography-tandem mass spectrometry method for the quantitation of synthetic cannabinoids of the aminoalkylindole type and methanandamide in serum and its application to forensic samples. *J. Mass Spectrom.* 46, 163–171.

Drug Enforcement Administration. (2011). *Schedules of Controlled Substances: Temporary Placement of Five Synthetic Cannabinoids Into Schedule I. Federal Register 2011, 76(40).* Available at: http://www.deadiversion.usdoj.gov/fed_regs/rules/2011/fr0301.htm [accessed August 8, 2011].

Drug Policy Alliance. (2011). *Legislation to Ban K2/Spice, "Bath Salts" and Other Synthetic Drugs Sailing Through Congress Today.* Available at: http://www.drugpolicy.org/news/2011/07/legislation-ban-k2spice-bath-salts-and-other-synthetic-drugs-sailing-through-congress-t [accessed August 9, 2011].

Drugs-Forum. (2011). Herbal incense recipe (JWH-073 + RCS-4). Available at: http://www.drugs-forum.com/forum/showthread.php?t=146867 [accessed June 3, 2011].

Early Warning System. (2009). Available at: http://www.emcdda.europa.eu/themes/new-drugs/early-warning [accessed October 23, 2009].

Ernst, L., Schiebel, H. M., Theuring, C., Lindigkeit, R., and Beuerle, T. (2011). Identification and characterization of JWH-122 used as new ingredient in "spice-like" herbal incenses. *Forensic Sci. Int.* 208, 31–35.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). (2009). *Understanding the "Spice" Phenomenon. Lisbon: EMCDDA; 2009.* Available at: http://www.emcdda.europa.eu/html.cfm/index90917EN.html [accessed April 23, 2011].

Every-Palmer, S. (2010). Warning: legal synthetic cannabinoid-receptor agonists such as JWH018 may precipitate psychosis in vulnerable individuals. *Addiction* 105, 1859–1860.

Every-Palmer, S. (2011). Synthetic cannabinoid JWH-018 and psychosis: an explorative study. *Drug Alcohol Depend.* 117, 152–157.

Fedorova, I., Hashimoto, A., Fecik, R. A., Hedrick, M. P., Hanus, L. O., Boger, D. L., Rice, K. C., and Basile, A. S. (2001). Behavioral evidence for the interaction of oleamide with multiple neurotransmitter systems. *J. Pharmacol. Exp. Ther.* 299, 332–342.

Ferrari, F., Ottani, A., and Giuliani, D. (2000).Inhibitory effects of the cannabinoid agonist HU 210 on rat sexual behaviour. *Physiol. Behav.* 69, 547–554.

Ferrari, F., Ottani, A., Vivoli, R., and Giuliani, D. (1999). Learning impairment produced in rats by the cannabinoid agonist HU 210 in a water-maze task. *Pharmacol. Biochem. Behav.* 64, 555–561.

Fisher, W.G. (2010). *Inhaled Incense "K2" May Cause Heart Damage.* Available at: http://drwes.blogspot.com/2010/08/inhaled-incense-k2-may-cause-heart.html? [accessed June 2, 2011].

Fortunato, J. J., Réus, G. Z., Kirsch, T. R., Stringari, R. B., Fries, G. R., Kapczinski, F., Hallak, J. E., Zuardi, A. W., Crippa, J. A., and Quevedo, J. (2010). Effects of beta-carboline harmine on behavioral and physiological parameters observed in the chronic mild stress model: further evidence of antidepressant properties. *Brain Res. Bull.* 81, 491–496.

Fortunato, J. J., Réus, G. Z., Kirsch, T. R., Stringari, R. B., Stertz, L., Kapczinski, F., Pinto, J. P., Hallak, J. E., Zuardi, A. W., Crippa, J. A., and Quevedo, J. (2009). Acute harmine administration induces antidepressive-like effects and increases BDNF levels in the rat hippocampus. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 1425–1430.

Gay, M. (2010). *Synthetic Marijuana Spurs State Bans. In The New York Times, July 10.* Available at: http://www.nytimes.com/2010/07/11/us/11k2.html [accessed April 3, 2011].

Griffiths, P., Sedefov, R., Gallegos, A., and Lopez, D. (2010). How globalization and market innovation challenge how we think and respond to drug use: "spice" a case study. *Addiction* 105, 951–953.

Grigoryev, A., Savchuk, S., Melnik, A., Moskaleva, N., Dzhurko, J., Ershov, M., Nosyrev, A., Vedenin, A., Izotov, B., Zabirova, I., and Rozhanets, V. (2011). Chromatography-mass spectrometry studies on the metabolism of synthetic cannabinoids JWH-018 and JWH-073, psychoactive components of smoking mixtures. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 879, 1126–1136.

Guimarães, F. S., Chiaretti, T. M., Graeff, F. G., and Zuardi, A. W. (1990). Antianxiety effect of cannabidiol in the elevated plus-maze. *Psychopharmacology (Berl.)* 100, 558–559.

Hammersley, R. (2010). Dangers of banning spice and the synthetic cannabinoid agonists. *Addiction* 105, 373.

Hillebrand, J., Olszewski, D., and Sedefov, R. (2010). Legal highs on the Internet. *Subst. Use Misuse* 45, 330–340.

Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., Felder, C. C., Herkenham, M., Mackie, K., Martin, B. R., Mechoulam, R., and Pertwee, R. G. (2002). International union of pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.* 54, 161–202.

Hu, X., Primack, B. A., Barnett, T. E., and Cook, R. L. (2011). College students and use of K2: an emerging drug of abuse in young persons. *Subst. Abuse Treat. Prev. Policy* 6, 16–19.

Hudson, S., and Ramsey, J. (2011). The emergence and analysis of synthetic cannabinoids. *Drug Test Anal.* 3, 466–478.

Hudson, S., Ramsey, J., King, L., Timbers, S., Maynard, S., Dargan, P. I., and Wood, D. M. (2010). Use of high-resolution accurate mass spectrometry to detect reported and previously unreported cannabinomimetics in "herbal high" products. *J. Anal. Toxicol.* 34, 252–260.

Huffman, J. W. (2009). "Cannabimimetic indoles, pyrroles, and indenes: structure-activity relationships and receptor interactions," in *The Cannabinoid Receptors*, ed. P. H. Reggio (New York, Humana Press), 49–94.

Huffman, J. W., Mabon, R., Wu, M. J., Lu, J., Hart, R., Hurst, D. P., Reggio, P. H., Wiley, J. L., and Martin, B. R. (2003). 3-Indolyl-1-naphthylmethanes: new cannabimimetic indoles provide evidence for aromatic stacking interactions with the CB1 cannabinoid receptor. *Bioorg. Med. Chem.* 11, 539–549.

Huffman, J. W., and Padgett, L. W. (2005). Recent developments in the medicinal chemistry of cannabinomimetic indoles, pyrroles and indenes. *Curr. Med. Chem.* 12, 1395–1411.

Huffman, J. W., Thompson, A. L., Wiley, J. L., and Martin, B. R. (2008). Synthesis and pharmacology of 1-deoxy analogs of CP-47,497 and CP-55,940. *Bioorg. Med. Chem.* 16, 322–335.

Huffman, J. W., Zengin, G., Wu, M. J., Lu, J., Hynd, G., Bushell, K., Thompson, A. L., Bushell, S., Tartal, C., Hurst, D. P., Reggio, P. H., Selley, D. E., Cassidy, M. P., Wiley, J. L., and Martin, B. R. (2005). Structure–activity relationships for 1-alkyl-3-(1-naphthoyl)indoles at the cannabinoid CB(1) and CB(2) receptors: steric and electronic effects of naphthoyl substituents. New highly selective CB(2) receptor agonists. *Bioorg. Med. Chem.* 13, 89–93.

Jack, A. (2009). *The Story of Spice. The Financial Times Online, February 13.* Available at: http://www.ft.com/cms/s/2/1721e2da-f8a0-11dd-aae8-000077b07658.html [accessed May 5, 2011].

Kikura-Hanajiri, R., Uchiyama, N., and Goda, Y. (2011). Survey of current trends in the abuse of psychotropic substances and plants in Japan. *Leg. Med. (Tokyo)* 13, 109–115.

Kronstrand, R., Roman, M., Thelander, G., and Eriksson, A. (2011). Unintentional fatal intoxications with mitragynine and o-desmethyltramadol from the herbal blend krypton. *J. Anal. Toxicol.* 35, 242–247.

Leggett, J. D., Aspley, S., Beckett, S. R., D'Antona, A. M., Kendall, D. A., and Kendall, D. A. (2004). Oleamide is a selective endogenous agonist of rat and human CB1 cannabinoid receptors. *Br. J. Pharmacol.* 141, 253–262.

Lindigkeit, R., Boehme, A., Eiserloh, I., Luebbecke, M., Wiggermann, M., Ernst, L., and Beuerle, T. (2009). Spice: a never ending story? *Forensic Sci. Int.* 191, 58–63.

Malone, D. T., Jongejan, D., and Taylor, D. A. (2009). Cannabidiol reverses the reduction in social interaction produced by low dose delta(9)-tetrahydrocannabinol in rats. *Pharmacol. Biochem. Behav.* 93, 91–96.

Mackowiak, M., Chocyk, A., Dudys, D., and Wedzony, K. (2009). Activation of CB1 cannabinoid receptors impairs memory consolidation and hippocampal polysialylated neural cell adhesion molecule expression in contextual fear conditioning. *Neuroscience* 158, 1708–1716.

McLachlan, G. (2009). Taking the spice out of legal smoking mixtures. *Lancet* 374, 600.

Missouri Department of Health and Senior Services. (2010). *Health Advisory (3.05.10): K2 Synthetic Marijuana Use among Teenagers and Young Adults in Missouri Missouri.* Available at: http://health.mo.gov/emergencies/ert/alertsadvisories/index.php [accessed August 8, 2011].

Moran, C. L., Le, V. H., Chimalakonda, K. C., Smedley, A. L., Lackey, F. D., Owen, S. N., Kennedy, P. D., Endres, G. W., Ciske, F. L., Kramer, J. B., Kornilov, A. M., Bratton, L. D., Dobrowolski, P. J., Wessinger, W. D., Fantegrossi, W. E., Prather, P. L., James, L. P., Radominska-Pandya, A., and Moran, J. H. (2011). Quantitative measurement of JWH-018 and JWH-073 metabolites excreted in human urine. *Anal. Chem.* 83, 4228–4236.

Moreira, F. A., Aguiar, D. C., and Guimarães, F. S. (2006). Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 30, 1466–1471.

Müller, H., Sperling, W., Köhrmann, M., Huttner, H. B., Kornhuber, J., and Maler, J. M. (2010). The synthetic cannabinoid Spice as a trigger for an acute exacerbation of cannabis induced recurrent psychotic episodes. *Schizophr. Res.* 118, 309–310.

Nakajima, J., Takahashi, M., Seto, T., and Suzuki, J. (2011). Identification and quantitation of cannabimimetic compound JWH-250 as an adulterant in products obtained via the internet. *Forensic Toxicol.* 29, 51–55.

Organised Crime Threat Assessment (OCTA). (2011). *Europol Public Information.* Available at: http://www.europol.europa.eu/content/publication/octa-2011-eu-organised-crime-threat-assessment-655 [accessed August 9, 2011].

Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br. J. Pharmacol.* 153, 199–215.

Philipp, A. A., Meyer, M. R., Wissenbach, D. K., Weber, A. A., Zoerntlein, S. W., Zweipfenning, P. G., and Maurer, H. H. (2011). Monitoring of kratom or Krypton intake in urine using GC-MS in clinical and forensic toxicology. *Anal. Bioanal. Chem.* 400, 127–135.

Piggee, C. (2009). Investigating a not-so-natural high. *Anal. Chem.* 81, 3205–3207.

Psychonaut Web Mapping Research Group. (2010). *Psychonaut Web Mapping Project: Final Report.* London: Institute of Psychiatry, King's College London.

Raffa, R. B. (2008). Basic pharmacology relevant to drug abuse assessment: tramadol as example. *J. Clin. Pharmacol. Ther.* 33, 101–108.

Rawaf, S., and Schifano, F. (2000). The internet, children, young people and substance misuse: co-ordinated approaches are needed to minimise the use of Ecommerce to obtain drugs and prevent substance misuse. *Public Health Med.* 2, 96.

Robinson, L., Goonawardena, A. V., Pertwee, R. G., Hampson, R. E., and Riedel, G. (2007). The synthetic cannabinoid HU210 induces spatial memory deficits and suppresses hippocampal firing rate in rats. *Br. J. Pharmacol.* 151, 688–700.

Schifano, F., Corazza, O., Deluca, P., Davey, Z., Di Furia, L., Flesland, L., Mannonen, M., Pagani, S., Peltoniemi, T., Pezzolesi, C., Scherbaum, N., Siemann, H., Skutle, A., Torrens, M., and Van Der Kreeft, P. (2009). Psychoactive drug or mystical incense? Overview of the online available information on spice products. *Int. J. Cult. Ment. Health* 2, 137–144.

Schifano, F., Deluca, P., Baldacchino, A., Peltoniemi, T., Scherbaum, N., Torrens, M., Farre, M., Flores, I., Rossi, M., Eastwood, D., Guionnet, C., Rawaf, S., Agosti, L., Di Furia, L., Brigada, R., Majava, A., Siemann, H., Leoni, M., Tomasin, A., Rovetto, F., and Ghodse, A. H. (2006). Drugs on the web; the psychonaut 2002 EU project. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 30, 640–646.

Schifano, F., Leoni, M., Martinotti, G., Rawaf, S., and Rovetto, F. (2003). Importance of cyberspace for the assessment of the drug abuse market: preliminary results from the psychonaut 2002 project. *Cyberpsychol. Behav.* 6, 405–410.

Schmidt, M. M., Sharma, A., Schifano, F., and Feinmann, C. (2011). "Legal highs" on the net-evaluation of UK-based Websites, products and product information. *Forensic Sci. Int.* 206, 92–97.

Schneir, A. B., Cullen, J., and Ly, B. T. (2011). "Spice" girls: synthetic cannabinoid intoxication. *J. Emerg. Med.* 40, 296–299.

Seely, K. A., Prather, P. L., James, L. P., and Moran, J. H. (2011). Marijuana-based drugs: innovative therapeutics or designer drugs of abuse? *Mol. Interv.* 11, 36–51.

Simmons, J., Cookman, L., Kang, C., and Skinner, C. (2011a). Three cases of "spice" exposure. *Clin. Toxicol. (Phila.)* 49, 431–433.

Simmons, J. R., Skinner, C. G., Williams, J., Kang, C. S., Schwartz, M. D., and Wills, B. K. (2011b). Intoxication from smoking "spice." *Ann. Emerg. Med.* 57, 187–188.

Sobolevsky, T., Prasolov, I., and Rodchenkov, G. (2010). Detection of JWH-018 metabolites in smoking mixture post-administration urine. *Forensic Sci. Int.* 200, 141–147.

Steup, C. (2009). *Drugs Shipped as Incense Seized as DHL. Wilmington News Journal.* Available at: http://usualredant.de/drogen/download/analyse-thc-pharm-Spicejwh-018.pdf [accessed March 4, 2009].

Teske, J., Weller, J.P., Fieguth, A., Rothämel, T., Schulz, Y., and Tröger, H.D. (2010). Sensitive and rapid quantification of the cannabinoid receptor agonist naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) in human serum by liquid chromatography–tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 878, 265–269.

Thakur, G. A., Tichkule, R., Bajaj, S., and Makriyannis, A. (2009). Latest advances in cannabinoid receptor agonists. *Expert Opin. Ther. Pat.* 19, 1647–1673.

Thomas, A., Baillie, G. L., Phillips, A. M., Razdan, R. K., Ross, R. A., and Pertwee, R. G. (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br. J. Pharmacol.* 150, 613–623.

Uchiyama, N., Kawamura, M., Kikura-Hanajiri, R., and Goda, Y. (2011a). Identification and quantitative analyses of two cannabimimetic phenylacetylindoles, JWH-251 and JWH-250, and four cannabimimetic naphthoylindoles, JWH-081, JWH-015, JWH-200 and JWH-073, as designer drugs in illegal products. *Forensic Toxicol.* 29, 25–37.

Uchiyama, N., Kikura-Hanajiri, R., Matsumoto, N., Huang, Z. L., Goda, Y., and Urade, Y. (2011b). Effects of synthetic cannabinoids on electroencephalogram power spectra in rats. *Forensic Sci. Int.* 1. doi: 10.1016/j.forsciint.2011.05.005. [Epub ahead of print].

Uchiyama, N., Kikura-Hanajiri, R., Kawahara, N., and Goda, Y. (2009a). Identification of a cannabimimetic indole as a designer drug in a herbal product. *Forensic Toxicol.* 27, 61–66.

Uchiyama, N., Kikura-Hanajiri, R., Kawahara, N., Haishima, Y., and Goda, Y. (2009b). Identification of a cannabinoid analog as a new type of designer drug in a herbal product. *Chem. Pharm. Bull.* 57, 439–441.

Uchiyama, N., Kikura-Hanajiri, R., Ogata, J., and Goda, Y. (2010). Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic Sci. Int.* 198, 31–38.

UK Statutory Instrument. (2009). *No. 3209. Dangerous Drugs. The Misuse of Drugs Act 1971 (Amendment) Order 2009.* Available at: http://www.opsi.gov.uk/si/si2009/pdf/uksi_20093209_en.pdf [accessed August 13, 2010].

Uniform Code of Military Justice (UCMJ). (2011). *UCMJ Bans Spice.* Available at: http://court-martial.com/spice/ [accessed August 9, 2011].

United Nations Office on Drugs and Crime (UNODC). (2011). *World Drug Report, 2010.* Available at: http://www.idpc.net/publications/unodc-world-drug-report-2011 [accessed August 9, 2011].

United States Drug Enforcement Administration, Office of Forensic Sciences (2009). *"Spice"-Plant Material(S) Laced With Synthetic Cannabinoids or Cannabinoid Mimicking Compounds. Microgram Bulletin 42(3).* Available at: http://www.justice.gov/dea/programs/forensicsci/microgram/mg0309/mg0309.html [accessed June 1, 2010].

US Department of Justice Drug Enforcement Administration Drugs and Chemicals of Concern. (2010). Spice – cannabinoids. Available at: http://www.deadiversion.usdoj.gov/drugs_concern/spice/index.html [accessed April 23, 2011].

Vardakou, I., Pistos, C., and Spiliopoulou, C. H. (2010). Spice drugs as a new trend: mode of action, identification and legislation. *Toxicol. Lett.* 197, 157–162.

Vardakou, I., Pistos, C., and Spiliopoulou, C. H. (2011). Drugs for youth via Internet and the example of mephedrone. *Toxicol. Lett.* 201, 191–195.

Vearrier, D., and Osterhoudt, K. C. (2010). A teenager with agitation: higher than she should have climbed. *Pediatr. Emerg. Care* 26, 462–465.

Weissman, A., Milne, M., and Melvin, M. S. Jr. (1982). Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. *J. Pharmacol. Exp. Ther.* 223, 516–523.

Westphal, F., Junge, T., Sonnichsen, F., Rosner, P., and Schaper, J. (2010). Ein neuer Wirkstoff in SPICE-artigen Kräutermischungen: Charakterisierung von JWH-250, seinen Methyl-und Trimethylsilylderivaten (A new compound in herbal mixtures: characterisation of JWH-250, its methyl- and trimethylsilyl-derivatives). *Toxichem. Krimtech.* 77, 46–58.

Westphal, F., Sönnichsen, F. D., and Thiemt, S. (2011). Identification of 1-butyl-3-(1-(4-methyl)naphthoyl)indole in a herbal mixture. *Forensic Sci. Int.* doi: 10.1016/j.forsciint.2011.03.031. [Epub ahead of print].

Wiley, J. L., Compton, D. R., Dai, D., Lainton, J. A., Phillips, M., Huffman, J. W., and Martin, B. R. (1998). Structure-activity relationships of indole- and pyrrole-derived cannabinoids. *J. Pharmacol. Exp. Ther.* 285, 995–1004.

Wintermeyer, A., Möller, I., Thevis, M., Jübner, M., Beike, J., Rothschild, M. A., and Bender, K. (2010). In vitro phase I metabolism of the synthetic cannabimimetic JWH-018. *Anal. Bioanal. Chem.* 398, 2141–2153.

World Anti-Doping Agency (WADA). (2011). Available at: http://www.wada-ama.org/rtecontent/document/2010_Prohibited_List_FINAL_EN_Web.pdf [accessed April 5, 2011].

Zawilska, J. B. (2011). "Legal highs" – new players in the old drama. *Curr. Drug Abuse Rev.* 4, 122–130.

Zhang, Q., Ma, P., Cole, R. B., and Wang, G. (2006). Identification of in vitro metabolites of JWH-015, an aminoalkylindole agonist for the peripheral cannabinoid receptor (CB2) by HPLC-MS/MS. *Anal. Bioanal. Chem.* 386, 1345–1355.

Zimmermann, U. S., Winkelmann, P. R., Pilhatsch, M., Nees, J. A., Spanagel, R., and Schulz, K. (2009). Withdrawal phenomena and dependence syndrome after the consumption of "spice gold." *Dtsch. Artzebl. Int.* 106, 464–467.

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Moreira, F. A., and Guimarães, F. S. (2006). Cannabidiol, a cannabis sativa constituent, as an antipsychotic drug. *Braz. J. Med. Biol. Res.* 39, 421–429.

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

Zuba, D., Byrska, B., and Maciow, M. (2011). Comparison of "herbal highs" composition. *Anal. Bioanal. Chem.* 400, 119–126.

Zullino, D. F., Rathelot, T., and Khazaal, Y. (2007). Cannabis and psychosis. *Lancet* 370, 15–40.

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Neuroimaging evidence for cannabinoid modulation of cognition and affect in man

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Cannabinoid receptors (CB1 and CB2) are ubiquitous within the brain (Wilson and Nicoll, 2002; Eggan and Lewis, 2007). Their distribution and role in the modulation of different neurotransmitter systems (Pertwee and Ross, 2002; Pertwee, 2008a) clearly indicate that cannabinoids are involved in the modulation of different cognitive and emotional processes (Solowij and Michie, 2007). The role of the endocannabinoid system in this has attracted the attention of basic scientists for decades (Zanettini et al., 2011). The modulation of cognitive and emotional processes in man by the extracts of *Cannabis sativa* (*C sativa*), the most commonly used illicit drug consumed by an estimated 4% of the adult population worldwide (Copeland and Swift, 2009), has also been known for a long time and extensively investigated in experimental and observational studies (Solowij, 1998; Ranganathan and D'Souza, 2006; Solowij and Michie, 2007; Crippa et al., 2009; D'Souza et al., 2009). However, only over the last 20 years has it been possible to precisely investigate the neural basis of the acute effects of cannabinoids on cognition by employing sophisticated neuroimaging techniques (Bhattacharyya et al., 2009a, 2012a; Martin-Santos et al., 2010). A renewed interest in the link between regular cannabis use and development of psychotic disorders has provided further impetus, coupled with interest in the therapeutic potential of certain cannabinoids.

Pharmacological challenge studies involving the administration of cannabinoids present in the extract of *C sativa* or their synthetic counterparts in combination with neuroimaging have served to complement current understanding regarding the role of the endocannabinoid system in regulating human cognitive and emotional processes (Zanettini et al., 2011), to model aspects of various psychiatric illnesses in man and understand their neural underpinnings (Bhattacharyya et al., 2009a). Among the more than 60 different cannabinoids (Mechoulam and Gaoni, 1967) present in the extract of *C sativa*, delta-9-tetrahydrocannabinol (THC) is thought to be responsible for most of the psychotropic effects of cannabis (Mechoulam et al., 1970) and modulation of cognitive domains such as learning and memory (Hall and Solowij, 1998; Curran et al., 2002; Ranganathan and D'Souza, 2006), psychomotor control (Hart et al., 2001; McDonald et al., 2003; Ramaekers et al., 2006, 2009), and attention (Hall and Solowij, 1998; Ilan et al., 2004), as evident from systematic acute experimental studies. The purpose of this article is to provide a brief critical overview of current neuroimaging evidence of the acute effects of THC in man as evident from neuroimaging studies. The studies are organized into groups based on neuroimaging domains examined.

MEMORY AND VERBAL LEARNING

To date, three functional magnetic resonance imaging (fMRI) studies have examined the neural correlates of the effects of cannabinoids on memory processing, the only cognitive domain robustly affected in chronic cannabis users and following acute administration (Grant et al., 2003; Ranganathan and D'Souza, 2006; Solowij and Michie, 2007; D'Souza et al., 2008). Bhattacharyya et al. (2009b) examined the effects of THC on neural activation while healthy occasional cannabis users performed a learning task that involved the repeated presentation of verbal stimuli. Consistent with previous reports (Zeineh et al., 2003), most of the learning under the placebo condition occurred during the first presentation of the encoding block and there was a linear decrement in the engagement of the parahippocampal gyrus, which is involved in the encoding of contextual information about stimuli that may be reactivated later to aid in recollection (Eichenbaum et al., 2007). Administration of THC disrupted the normal linear decrement present with placebo in the engagement of the parahippocampal cortex, which is involved in the encoding of contextual information about stimuli (Eichenbaum et al., 2007). Furthermore, the relationship between the change in parahippocampal activation and memory performance present with placebo was disrupted by THC, consistent with evidence that THC impairs medial temporal function in animals (Robbe et al., 2006; Puighermanal et al., 2009; Wise et al., 2009) and memory performance in animals and man (Curran et al., 2002; D'Souza et al., 2004; Robbe et al., 2006; Puighermanal et al., 2009; Wise et al., 2009). These results may reflect increased demands on encoding under the influence of THC as a result of an impairment in the efficient encoding of contextual information in the medial temporal cortex, which has a central role in relational memory binding (Hannula and Ranganath, 2008). Its activation has been shown previously to correlate with the quantity of novel and successful mnemonic processing (Stern et al., 1996; Brewer et al., 1998; Wagner et al., 1998; Eldridge et al., 2000; Zeineh et al., 2000, 2003). During the recall condition of the task, THC augmented activation in the left medial prefrontal and dorsal anterior cingulate cortex (ACC), areas that have been related to retrieval monitoring and verification (Simons et al., 2005; Fleck et al., 2006). THC also attenuated left rostral ACC and bilateral striatal activation, and its effect in the ventral striatum was directly correlated with the severity of psychotic symptoms induced by it concurrently, demonstrating that the acute induction of psychotic symptoms by THC is related to its effects on striatal function. This study also provided the first human evidence that impairments in learning and memory induced by cannabis are mediated through its effects on medial temporal and prefrontal function.

Subsequently, Bossong et al. (2011) reported an attenuation of activity under the influence of THC in the insula and inferior frontal gyrus on the right side and in the middle occipital gyrus on the left side during the encoding condition of an associative memory task involving pictorial stimuli. During the recall condition, THC enhanced the engagement of the cuneus and

precuneus. As the authors did not observe any significant effect of THC on task performance, the neural effects may be interpreted as being related to the pharmacological effects of the drug rather than being confounded by differential task performance.

More recently, Bhattacharyya et al. (2012b) employed their previously established design (Bhattacharyya et al., 2009b) and examined the genetic moderation of the neural effects of orally administered THC during memory processing. Variations in genes modulating central dopaminergic neurotransmission, such as *AKT1* (rs1130233) and dopamine transporter (*DAT1*) (40 base-pair variable number of tandem repeats in the 3′ untranslated region) were found to modulate the effects of THC on medial temporal, striatal, and midbrain function during encoding and recall conditions. Furthermore, the effects of THC on striatal and midbrain activation during the encoding and recall conditions, respectively, of the verbal memory task were greater in those individuals carrying the risk variants of both the genes compared to the rest.

ATTENTION AND RESPONSE INHIBITION

O'Leary et al. (2002) examined the neural correlates of the attentional deficits reported following both acute administration and chronic use of cannabis (Hall and Solowij, 1998; Solowij and Michie, 2007). During a dichotic listening task performed by a group of regular abstinent cannabis users they observed an increase in regional cerebral blood flow (rCBF) in the temporal poles bilaterally, cerebellum, insula, and putamen on the right side and the left ventral frontal cortex and a decrease in rCBF in the left superior temporal gyrus, right occipital lobe and bilateral frontal cortical regions areas that form an integral component of the attentional network (Berger and Posner, 2000). In a subsequent study (O'Leary et al., 2007), the authors employed an improved design that allowed them to minimize the carryover effects of THC and reported a significant increase in rCBF bilaterally in the anterior insula, anterior cingulate, orbital frontal lobe, temporal poles, and cerebellum and decrease in rCBF in the mesial occipital lobes and precuneus under the influence of THC.

Borgwardt et al. (2008) examined the neural substrates for the impairments in psychomotor control reported in cannabis users (Ramaekers et al., 2009; Crean et al., 2011) and reported that administration of THC resulted in a decrease in the normal activation associated with response inhibition in the right inferior frontal gyrus as well as the ACC – key regions implicated in inhibitory control (Garavan et al., 1999; Rubia et al., 2001).

EMOTIONAL AND SENSORY PROCESSING

Several studies have employed neuroimaging to study the effects of THC on emotional and sensory processing. Phan et al. (2008) investigated the effect of a small dose of THC during the processing of social signals of threat by using angry and fearful faces and reported an attenuation of amygdalar activation. Although this was not associated with any changes in anxiety ratings, the authors interpreted their results as indicative of a potential anxiolytic role of THC. It is likely that lack of a significant anxiogenic effect in this study was related to the lower dose of THC employed by Phan and colleagues as in a subsequent study, Fusar-Poli et al. (2009) reported a significant increase in anxiety ratings under the influence of a higher dose of THC. However, these effects were not associated with modulation of amygdala activity under the influence of THC. Instead, THC produced an increase in engagement of the right inferior parietal lobule and attenuation of engagement of the left medial frontal gyrus while viewing mildly fearful faces. While viewing intensely fearful faces, there was an increase in engagement of the left precuneus and primary sensorimotor corex bilaterally and decrease in engagement of the middle frontal gyrus bilaterally and in the posterior cingulate gyrus. In a subsequent three-way comparison between the effects of THC and cannabdiol, a non-psychoactive ingredient in cannabis, relative to the placebo condition, the same group reported a modulatory effect of THC on amygdalar processing (Bhattacharyya et al., 2010), which was directly correlated with the increase in anxiety induced by it suggesting that the lack of effect on amygdala activation in the former study (Fusar-Poli et al., 2009) may have been related to a modestly powered sample.

Winton-Brown et al. (2011) examined the modulation of activation during auditory and visual processing in healthy subjects as the acute abnormalities in sensory processing (Tart, 1970) under the influence of cannabis are similar to those experienced during psychotic episodes (Koethe et al., 2006). During an auditory processing condition, THC attenuated activation bilaterally in the anterior and posterior superior temporal gyrus and middle temporal gyrus, the insulae and in the supramarginal gyri and in the right inferior frontal gyrus and left cerebellum relative to the placebo condition. During a visual processing condition, THC attenuated activation in the extrastriate visual cortex and enhanced activation in lingual and middle occipital gyri (corresponding to the primary visual cortex) on the right side and parts of the lingual and fusiform gyri extending anteriorly on the left side.

REWARD AND SALIENCE PROCESSING

Bhattacharyya et al. (2012c) examined the effect of THC on attentional salience processing and its relationship with psychotic symptoms induced under its influence. Employing a visual oddball detection task, they observed that relative to placebo THC attenuated activation in the right caudate but augmented it in the right prefrontal cortex, including the inferior frontal gyrus, during the processing of "salient" oddball stimuli relative to "non-salient" standard stimuli. This was associated with a reduction in response latency to standard relative to oddball stimuli under THC, suggesting that the non-salient standard stimuli may have appeared relatively more salient under the influence of THC. This is consistent with evidence that insignificant sensory stimuli or commonplace conversations acquire new meanings and significance under the influence of cannabis (Tart, 1970). The effect of THC in the right caudate was negatively correlated with the severity of the psychotic symptoms it induced and its effect on response latency. These results provide experimental support for the salience model of psychosis (Kapur, 2003), are consistent with evidence of abnormal salience attribution in patients with schizophrenia (Jensen et al., 2008; Murray et al., 2008) as well as linking aberrant salience attribution and the presence of psychotic symptoms (Roiser et al., 2009). Furthermore, they provide the first evidence that the effects of cannabis on psychosis may be mediated by influencing the neural substrate of attentional salience processing.

van Hell et al. (2012) employed a monetary reward task involving reward anticipation and feedback conditions to

explore the role of the endocannabinoid system during human reward processing (Gardner, 2005; Solinas et al., 2007). Under the influence of THC there was reduced feedback-related activity in the left inferior parietal cortex and the inferior temporal gyrus bilaterally during the rewarding trials, but no effect during the non-rewarding trials. These neural effects were associated with a trend-level slowing down of the speed of task performance for both the rewarding and neutral trials under THC influence, although this effect was more prominent for the reward trials. This was in contrast to the faster responding to the rewarding relative to the neutral trials observed across all conditions. THC did not have any significant effect on neural activation during the anticipation of reward. This may suggest that under THC influence, salient and rewarding trials may appear less striking (van Hell et al., 2012). This is indicated by a greater slowing down during the rewarding trials as well as attenuation of activation under the influence of THC in the inferior parietal cortex, which functions as a "behavioral integrator" providing a "salience representation" of the external world and signals attentional priority for behaviorally salient signals (Gottlieb, 2007).

CONCLUSION

Neuroimaging studies reviewed here suggest that, consistent with the polymorphic and heterogeneous nature of the cognitive and symptomatic effects of cannabis, THC has modulatory effects over widely distributed neural networks in man. While the earliest neuroimaging studies involving cannabinoids [reviewed in Bhattacharyya et al. (2009a) and Martin-Santos et al. (2010)] mainly investigated the effects of chronic use or acute administration of cannabis on rCBF, more recent studies have employed neuroimaging technologies with better spatial resolution to investigate the modulation of the neural correlates of cognitive and emotional processes by cannabinoids (Bhattacharyya et al., 2012). These studies demonstrate the neural basis of the effects of THC across a number of cognitive (learning, attention, response inhibition, salience, and reward) and emotional (anxiety) processes (Borgwardt et al., 2008; Bhattacharyya et al., 2009b, 2012a,b). These effects are consistent with the ubiquitous distribution of the main cannabinoid receptor (CB1; Wilson and Nicoll, 2002; Eggan and Lewis, 2007) and are likely to be mediated through the modulation of different neurotransmitter systems (Pertwee, 2008a,b). Delineation of the precise neural mechanisms underlying the distinct and often opposite acute cognitive and symptomatic effects of different cannabinoids in man complements existing evidence from basic science regarding the role of endocannabinoids in cognitive and emotional processing. This may not only help in modeling different aspects of the psychopathology of mental disorders such as schizophrenia and offer insights into their underlying mechanisms, but may suggest potentially new therapeutic targets for drug discovery.

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REFERENCES

Berger, A., and Posner, M. I. (2000). Pathologies of brain attentional networks. *Neurosci. Biobehav. Rev.* 24, 3–5.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J. A., Fusar-Poli, P., and McGuire, P. (2012a). Neural mechanisms for the cannabinoid modulation of cognition and affect in man: a critical review of neuroimaging studies. *Curr. Pharm. Des.* (in press).

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J. A., Kambeitz, J., Prata, D., Willams, S., Brammer, M., Collier, D. A., and McGuire, P. K. (2012b). Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: Akt1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and striatal function. *Mol. Psychiatry* doi: 10.1038/mp.2011.187. [Epub ahead of print].

Bhattacharyya, S., Crippa, J. A., Allen, P., Martin-Santos, R., Borgwardt, S., Fusar-Poli, P., Rubia, K., Kambeitz, J., O'Carroll, C., Seal, M., Giampietro, V., Brammer, M., Zuardi, A. W., Atakan, Z., and McGuire, P. K. (2012c). Induction of psychosis by delta-9-tetrahydrocannbinol reflects modulation of prefrontal and striatal function during attentional salience processing. *Arch. Gen. Psychiatry* 69, 27–36.

Bhattacharyya, S., Crippa, J. A., Martin-Santos, R., Winton-Brown, T., and Fusar-Poli, P. (2009a). Imaging the neural effects of cannabinoids: current status and future opportunities for psychopharmacology. *Curr. Pharm. Des.* 15, 2603–2614.

Bhattacharyya, S., Fusar-Poli, P., Borgwardt, S., Martin-Santos, R., Nosarti, C., O'Carroll, C., Allen, P., Seal, M. L., Fletcher, P. C., Crippa, J. A., Giampietro, V., Mechelli, A., Atakan, Z., and McGuire, P. (2009b). Modulation of mediotemporal and ventrostriatal function in humans by delta-9-tetrahydrocannabinol: a neural basis for the effects of cannabis sativa on learning and psychosis. *Arch. Gen. Psychiatry* 66, 442–451.

Bhattacharyya, S., Morrison, P. D., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., Nosarti, C., O'Carroll, C., Seal, M., Allen, P., Mehta, M. A., Stone, J. M., Tunstall, N., Giampietro, V., Kapur, S., Murray, R. M., Zuardi, A. W., Crippa, J. A., Atakan, Z., and McGuire, P. K. (2010). Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology* 35, 764–774.

Borgwardt, S. J., Allen, P., Bhattacharyya, S., Fusar-Poli, P., Crippa, J. A., Seal, M. L., Fraccaro, V., Atakan, Z., Martin-Santos, R., O'Carroll, C., Rubia, K., and McGuire, P. K. (2008). Neural basis of delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. *Biol. Psychiatry* 64, 966–973.

Bossong, M. G., Jager, G., van Hell, H. H., Zuurman, L., Jansma, J. M., Mehta, M. A., van Gerven, J. M. A., Kahn, R. S., and Ramsey, N. F. (2011). Effects of δ9-tetrahydrocannabinol administration on human encoding and recall memory function: a pharmacological fmri study. *J. Cogn. Neurosci.* 24, 588–599.

Brewer, J. B., Zhao, Z., Desmond, J. E., Glover, G. H., and Gabrieli, J. D. (1998). Making memories: brain activity that predicts how well visual experience will be remembered. *Science* 281, 1185–1187.

Copeland, J., and Swift, W. (2009). Cannabis use disorder: epidemiology and management. *Int. Rev. Psychiatry* 21, 96–103.

Crean, R. D., Crane, N. A., and Mason, B. J. (2011). An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *J. Addict. Med.* 5, 1–8.

Crippa, J. A., Zuardi, A. W., Martin-Santos, R., Bhattacharyya, S., Atakan, Z., McGuire, P., and Fusar-Poli, P. (2009). Cannabis and anxiety: a critical review of the evidence. *Hum. Psychopharmacol.* 24, 515–523.

Curran, H. V., Brignell, C., Fletcher, S., Middleton, P., and Henry, J. (2002). Cognitive and subjective dose-response effects of acute oral delta 9-tetrahydrocannabinol (thc) in infrequent cannabis users. *Psychopharmacology (Berl.)* 164, 61–70.

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

D'Souza, D. C., Ranganathan, M., Braley, G., Gueorguieva, R., Zimolo, Z., Cooper, T., Perry, E., and Krystal, J. (2008). Blunted psychotomimetic and amnestic effects of delta-9-tetrahydrocannabinol in frequent users of cannabis. *Neuropsychopharmacology* 33, 2505–2516.

D'Souza, D. C., Sewell, R. A., and Ranganathan, M. (2009). Cannabis and psychosis/schizophrenia: human studies. *Eur. Arch. Psychiatry Clin. Neurosci.* 259, 413–431.

Eggan, S. M., and Lewis, D. A. (2007). Immunocytochemical distribution of the cannabinoid cb1 receptor in the primate neocortex: a regional and laminar analysis. *Cereb. Cortex* 17, 175–191.

Eichenbaum, H., Yonelinas, A. P., and Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annu. Rev. Neurosci.* 30, 123–152.

Eldridge, L. L., Knowlton, B. J., Furmanski, C. S., Bookheimer, S. Y., and Engel, S. A. (2000). Remembering

episodes: a selective role for the hippocampus during retrieval. *Nat. Neurosci.* 3, 1149–1152.

Fleck, M. S., Daselaar, S. M., Dobbins, I. G., and Cabeza, R. (2006). Role of prefrontal and anterior cingulate regions in decision-making processes shared by memory and nonmemory tasks. *Cereb. Cortex* 16, 1623–1630.

Fusar-Poli, P., Crippa, J. A., Bhattacharyya, S., Borgwardt, S. J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S. A., O'Carrol, C., Atakan, Z., Zuardi, A. W., and McGuire, P. K. (2009). Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Arch. Gen. Psychiatry* 66, 95–105.

Garavan, H., Ross, T. J., and Stein, E. A. (1999). Right hemispheric dominance of inhibitory control: an event-related functional mri study. *Proc. Natl. Acad. Sci. U.S.A.* 96, 8301–8306.

Gardner, E. L. (2005). Endocannabinoid signaling system and brain reward: emphasis on dopamine. *Pharmacol. Biochem. Behav.* 81, 263–284.

Gottlieb, J. (2007). From thought to action: the parietal cortex as a bridge between perception, action, and cognition. *Neuron* 53, 9–16.

Grant, I., Gonzalez, R., Carey, C. L., Natarajan, L., and Wolfson, T. (2003). Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. *J. Int. Neuropsychol. Soc.* 9, 679–689.

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

Hannula, D. E., and Ranganath, C. (2008). Medial temporal lobe activity predicts successful relational memory binding. *J. Neurosci.* 28, 116–124.

Hart, C. L., van Gorp, W., Haney, M., Foltin, R. W., and Fischman, M. W. (2001). Effects of acute smoked marijuana on complex cognitive performance. *Neuropsychopharmacology* 25, 757–765.

Ilan, A. B., Smith, M. E., and Gevins, A. (2004). Effects of marijuana on neurophysiological signals of working and episodic memory. *Psychopharmacology (Berl.)* 176, 214–222.

Jensen, J., Willeit, M., Zipursky, R. B., Savina, I., Smith, A. J., Menon, M., Crawley, A. P., and Kapur, S. (2008). The formation of abnormal associations in schizophrenia: neural and behavioral evidence. *Neuropsychopharmacology* 33, 473–479.

Kapur, S. (2003). Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. *Am. J. Psychiatry* 160, 13–23.

Koethe, D., Gerth, C. W., Neatby, M. A., Haensel, A., Thies, M., Schneider, U., Emrich, H. M., Klosterkotter, J., Schultze-Lutter, F., and Leweke, F. M. (2006). Disturbances of visual information processing in early states of psychosis and experimental delta-9-tetrahydrocannabinol altered states of consciousness. *Schizophr. Res.* 88, 142–150.

Martin-Santos, R., Fagundo, A. B., Crippa, J. A., Atakan, Z., Bhattacharyya, S., Allen, P., Fusar-Poli, P., Borgwardt, S., Seal, M., Busatto, G. F., and McGuire, P. (2010). Neuroimaging in cannabis use: a systematic review of the literature. *Psychol. Med.* 40, 383–398.

McDonald, J., Schleifer, L., Richards, J. B., and de Wit, H. (2003). Effects of thc on behavioral measures of impulsivity in humans. *Neuropsychopharmacology* 28, 1356–1365.

Mechoulam, R., and Gaoni, Y. (1967). Recent advances in the chemistry of hashish. *Fortschr. Chem. Org. Naturst.* 25, 175–213.

Mechoulam, R., Shani, A., Edery, H., and Grunfeld, Y. (1970). Chemical basis of hashish activity. *Science* 169, 611–612.

Murray, G. K., Corlett, P. R., Clark, L., Pessiglione, M., Blackwell, A. D., Honey, G., Jones, P. B., Bullmore, E. T., Robbins, T. W., and Fletcher, P. C. (2008). Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Mol. Psychiatry* 239, 267–276.

O'Leary, D. S., Block, R. I., Koeppel, J. A., Flaum, M., Schultz, S. K., Andreasen, N. C., Ponto, L. B., Watkins, G. L., Hurtig, R. R., and Hichwa, R. D. (2002). Effects of smoking marijuana on brain perfusion and cognition. *Neuropsychopharmacology* 26, 802–816.

O'Leary, D. S., Block, R. I., Koeppel, J. A., Schultz, S. K., Magnotta, V. A., Ponto, L. B., Watkins, G. L., and Hichwa, R. D. (2007). Effects of smoking marijuana on focal attention and brain blood flow. *Hum. Psychopharmacol.* 22, 135–148.

Pertwee, R. G. (2008a). The diverse cb1 and cb2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br. J. Pharmacol.* 153, 199–215.

Pertwee, R. G. (2008b). Ligands that target cannabinoid receptors in the brain: from thc to anandamide and beyond. *Addict. Biol.* 13, 147–159.

Pertwee, R. G., and Ross, R. A. (2002). Cannabinoid receptors and their ligands. *Prostaglandins Leukot. Essent. Fatty Acids* 66, 101–121.

Phan, K. L., Angstadt, M., Golden, J., Onyewuenyi, I., Popovska, A., and de Wit, H. (2008). Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. *J. Neurosci.* 28, 2313–2319.

Puighermanal, E., Marsicano, G., Busquets-Garcia, A., Lutz, B., Maldonado, R., and Ozaita, A. (2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mtor signaling. *Nat. Neurosci.* 12, 1152–1158.

Ramaekers, J. G., Kauert, G., Theunissen, E. L., Toennes, S. W., and Moeller, M. R. (2009). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *J. Psychopharmacol. (Oxford)* 23, 266–277.

Ramaekers, J. G., Moeller, M. R., van Ruitenbeek, P., Theunissen, E. L., Schneider, E., and Kauert, G. (2006). Cognition and motor control as a function of delta9-thc concentration in serum and oral fluid: limits of impairment. *Drug Alcohol Depend.* 85, 114–122.

Ranganathan, M., and D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl.)* 188, 425–444.

Robbe, D., Montgomery, S. M., Thome, A., Rueda-Orozco, P. E., McNaughton, B. L., and Buzsaki, G. (2006). Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nat. Neurosci.* 9, 1526–1533.

Roiser, J. P., Stephan, K. E., den Ouden, H. E., Barnes, T. R., Friston, K. J., and Joyce, E. M. (2009). Do patients with schizophrenia exhibit aberrant salience? *Psychol. Med.* 39, 199–209.

Rubia, K., Russell, T., Overmeyer, S., Brammer, M. J., Bullmore, E. T., Sharma, T., Simmons, A., Williams, S. C., Giampietro, V., Andrew, C. M., and Taylor, E. (2001). Mapping motor inhibition: conjunctive brain activations across different versions of go/no-go and stop tasks. *Neuroimage* 13, 250–261.

Simons, J. S., Gilbert, S. J., Owen, A. M., Fletcher, P. C., and Burgess, P. W. (2005). Distinct roles for lateral and medial anterior prefrontal cortex in contextual recollection. *J. Neurophysiol.* 94, 813–820.

Solinas, M., Yasar, S., and Goldberg, S. R. (2007). Endocannabinoid system involvement in brain reward processes related to drug abuse. *Pharmacol. Res.* 56, 393–405.

Solowij, N. (1998). *Cannabis and Cognitive Functioning*. Cambridge: Cambridge University Press.

Solowij, N., and Michie, P. T. (2007). Cannabis and cognitive dysfunction: parallels with endophenotypes of schizophrenia? *J. Psychiatry Neurosci.* 32, 30–52.

Stern, C. E., Corkin, S., Gonzalez, R. G., Guimaraes, A. R., Baker, J. R., Jennings, P. J., Carr, C. A., Sugiura, R. M., Vedantham, V., and Rosen, B. R. (1996). The hippocampal formation participates in novel picture encoding: evidence from functional magnetic resonance imaging. *Proc. Natl. Acad. Sci. U.S.A.* 93, 8660–8665.

Tart, C. T. (1970). Marijuana intoxication common experiences. *Nature* 226, 701–704.

van Hell, H. H., Jager, G., Bossong, M. G., Brouwer, A., Jansma, J. M., Zuurman, L., van Gerven, J., Kahn, R. S., and Ramsey, N. F. (2012). Involvement of the endocannabinoid system in reward processing in the human brain. *Psychopharmacology (Berl.)* 219, 981–990.

Wagner, A. D., Schacter, D. L., Rotte, M., Koutstaal, W., Maril, A., Dale, A. M., Rosen, B. R., and Buckner, R. L. (1998). Building memories: remembering and forgetting of verbal experiences as predicted by brain activity. *Science* 281, 1188–1191.

Wilson, R. I., and Nicoll, R. A. (2002). Endocannabinoid signaling in the brain. *Science* 296, 678–682.

Winton-Brown, T. T., Allen, P., Bhattacharyya, S., Borgwardt, S. J., Fusar-Poli, P., Crippa, J. A., Seal, M. L., Martin-Santos, R., Ffytche, D., Zuardi, A. W., Atakan, Z., and McGuire, P. K. (2011). Modulation of auditory and visual processing by delta-9-tetrahydrocannabinol and cannabidiol: an fmri study. *Neuropsychopharmacology* 36, 1340–1348.

Wise, L. E., Thorpe, A. J., and Lichtman, A. H. (2009). Hippocampal cb(1) receptors mediate the memory impairing effects of delta(9)-tetrahydrocannabinol. *Neuropsychopharmacology* 34, 2072–2080.

Zanettini, C., Panlilio, L. V., Alicki, M., Goldberg, S. R., Haller, J., and Yasar, S. (2011). Effects of endocannabinoid system modulation on cognitive and emotional behavior. *Front. Behav. Neurosci.* 5:57. doi: 10.3389/fnbeh.2011.00057

Zeineh, M. M., Engel, S. A., and Bookheimer, S. Y. (2000). Application of cortical unfolding techniques to functional mri of the human hippocampal region. *Neuroimage* 11, 668–683.

Zeineh, M. M., Engel, S. A., Thompson, P. M., and Bookheimer, S. Y. (2003). Dynamics of the hippocampus during encoding and retrieval of face-name pairs. *Science* 299, 577–580.

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