Functional Interactions between Endogenous Cannabinoid and Opioid Systems: Focus on Alcohol, Genetics and Drug-Addicted Behaviors

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Abstract: Although the first studies regarding the endogenous opioid system and addiction were published during the 1940s, addiction and cannabinoids were not addressed until the 1970s. Currently, the number of opioid addiction studies indexed in PubMed-Medline is 16 times greater than the number of cannabinoid addiction reports. More recently, functional interactions have been demonstrated between the endogenous cannabinoid and opioid systems. For example, the cannabinoid brain receptor type 1 (CB1) and mu opioid receptor type 1 (MOR1) co-localize in the same presynaptic nerve terminals and signal through a common receptor-mediated G-protein pathway. Here, we review a great variety of behavioral models of drug addiction and alcohol-related behaviors. We also include data providing clear evidence that activation of the cannabinoid and opioid endogenous systems *via* WIN 55,512-2 (0.4-10 mg/kg) and morphine (1.0-10 mg/kg), respectively, produces similar levels of relapse to alcohol in operant alcohol self-administration tasks. Finally, we discuss genetic studies that reveal significant associations between polymorphisms in MOR1 and CB1 receptors and drug addiction. For example, the SNP A118G, which changes the amino acid aspartate to asparagine in the MOR1 gene, is highly associated with altered opioid system function. The presence of a microsatellite polymorphism of an (AAT)n triplet near the CB1 gene is associated with drug addiction phenotypes. But, studies exploring haplotypes with regard to both systems, however, are lacking.

Keywords: Drug addiction, CNR1, OPRM1, alcohol, prefrontal cortex, human, rat, mouse.

BRIEF HISTORICAL INTRODUCTION

The first article indexed in PubMed-MEDLINE that links addiction and cannabis was published in 1972 in Spain (and in Spanish) [1]. Throughout that decade, a limited number of studies focused on methodological problems, such as how to dissolve cannabinoids in water without loss of its biological activity [2], the development of specific radioimmunoassays to measure delta-9-tetrahydrocannabinol (D9-THC) in plasma and urine [3], and the arrangement of the first experiments of intravenous self-administration of D9-THC in rats [4]. The 1980s saw the expansion of clinical experiments. For example, the relationship between the use of marijuana and subjective effects, psychomotor performance, hormone release, as well as the validity of self-reported cannabis use was evaluated [5-7]. In addition, the release of the first NIDA (National Institute of Drug of Abuse) research monographs including cannabinoids [8] appeared in the 1980s. During the 1990s a series of relevant findings was published that linked cannabis use and increased risk of psychosis [9]. Moreover, one of the first studies demonstrating the association between a microsatellite polymorphism in the cannabinoid receptor gene (CNR1) and cannabis dependence, the generation of first knock-out (KO) mice lacking the CNR1 gene [10], and the first studies demonstrating functional interactions between the endocannabinoid and opioid systems [11] were published. However, it was not until 1997 that the cannabinoid brain receptor 1 (CB1) was directly linked to drug addiction by Comings and colleagues [12]. Currently, there is a novel cannabinoid receptor, the orphan receptor GPR55 [13], that has not been examined with regard to drug addiction. This will presumably soon change.

The endogenous opioid system has been more extensively linked to addiction and drug abuse. The first studies were published in 1947 [14], and the number of opioid and addiction works published and indexed in PubMed-MEDLINE is currently 16 times greater than that of cannabinoid and addiction studies (using the tag ALL - All Fields). About fifty years ago, little was known about the endogenous opioid system. Pharmacological studies using various opioid agonists and antagonists concluded that opioids exert pharmacological actions through various opioid receptor subtypes given the diversity of syndromes elicited by these substances. For a historical, in-depth review of all early pharmacological research see Martin [15]. Early in the 1970s, putative surface receptors were discovered in the brain [16-18], and they were named mu, kappa, and delta [19, 20]. In the 1980´s, receptor ligands were discovered [21], and in the 1990s, the endomorphins were characterized [22]. In addition, various attempts to elucidate the structure of opioid receptors were made during the 1980s, but the most successful results were obtained in the following decade using gene cloning methods [23]. The delta opioid receptor was the first to be cloned [24, 25]. This achievement facilitated the subsequent cloning of the mu opioid receptor (MOR) and kappa opioid receptor [26-31]. Mu, delta and kappa receptors are currently considered as "classical" opioid receptors. One of the first papers indexed in PubMed-

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MEDLINE describing the role of MOR and kappa receptors in addiction was a review from 1986 [32], in which the sigma receptor was included as an opioid receptor. Currently, this receptor is no longer considered to be an opioid receptor. Later, a novel opioid receptor with structural homology to other receptors, but with no ligand affinity, was discovered. This orphan receptor was termed the opioid-like receptor (ORL-1) [33, 34] and its corresponding endogenous ligand was called ligand orphanin FQ or nociceptin (OFQ/N) [35, 36]. In some studies, these orphan receptors were linked to drug addiction (e.g., Ciccocioppo *et al.* [37]). Throughout the 1980s-1990s, pharmacological research continued to identify numerous receptor subtypes within the main opioid receptor family [38-40], which likely reflected splice variants of the same gene. Finally, pharmacological research was complemented with opioid receptor and ligand knockout models [41-49], which in turn allowed the elucidation of the physiological role of each receptor and its putative ligands in pain, reward and addiction.

CO-LOCALIZATION AND FUNCTIONAL INTERACTION BETWEEN CB1 AND MU OPIOID RECEPTORS

The functional interaction between MOR and CB1 opioid receptors has only recently been studied. During the early 2000s, co-localization of MOR and CB1 receptors was demonstrated in the rat nucleus accumbens (shell and core), caudate putamen nucleus, and dorsal horn [50-52]. Co-localization of MOR and CB1 receptors in the first two brain structures (striatum) may be associated with drug-related behaviors, whereas co-localization in the dorsal horn may be related to the analgesic properties of both endogenous receptor systems.

Studies regarding the relationship between MOR and CB1 receptors at a morphological and functional level have shown that these receptors form a G-protein-coupled heterodimeric receptor that transmits signals via a common G protein [53, 54]. This is in contrast with the previous idea that G-protein-coupled receptors are monomeric structures. The physical association between MOR and CB1 receptors (an allosteric interaction) may explain how cell signaling and neural excitability could be mediated by the activation or blockade of cannabinoid and opioid receptors by cannabinergic and opioidergic molecules. For example, GABA and glutamate release is inhibited by the activation of MOR and CB1 receptors, and this is blocked by the administration of either a CB1 receptor antagonist or an MOR antagonist. Interestingly, previous administration of a cannabinoid receptor antagonist reverses the effects of MOR antagonists and *vice versa* [53]. Similarly, simultaneous activation of MOR and CB1 receptors causes a decreased response (measured by receptor-mediating signaling, i.e., GTPγS binding and mitogen-activated protein kinase (MAPK) phosphorylation) than the activation of each receptor individually [55]. Other studies have provided evidence that allosteric co-expression of MOR and CB1 receptors in the rat striatum (ventral and dorsal) has synergistic effects on cAMP/PKA signaling. It has been demonstrated that both MOR and CB1 receptors activate the same $G\alpha_{i3}$/AGS3/βγ pathway and that subthreshold concentrations of MOR and CB1 receptor agonists activate CRE-mediated gene expression [56]. Furthermore, the same authors showed that this signaling pathway requires purinergic signaling through adenosine and A2a receptors (methylxanthines are antagonists of these receptors). Blockade of A2a receptors prevents MOR-CB1 synergism and the same effect is seen with the removal of adenosine from striatal neural cells in culture [56].

On the other hand, studies by Canals and Milligan (2008) [57] suggested that the constitutive activity of CB1 receptors modulates the function of co-localized MOR (e.g., phosphorylation of extracellular signal-regulated kinases (ERK) 1/2 and MAP kinases). The authors used specific cells expressing human MOR and CB1 receptors to show that the presence of cannabinoid receptors leads to an increase in basal GTPγS binding. A D163N amino acid substitution was introduced into the primary sequence of the human CB1 receptor, causing a loss of its constitutive activity. As a result, the use of cells expressing MOR and mutated CB1 receptors showed that agonist opioid-mediated ERK1/2 MAP kinase phosphorylation is not affected by the presence of CB1 receptors, suggesting that constitutive activity (receptor signaling that is present in absence of an agonist) of CB1 receptors could explain, in part, functional interactions between both receptors. Furthermore, it has been reported that the expression of human CB1 receptors in cells (as well as application of the CB1 inverse agonist SR141716A) causes the sequestration of $G_{(i/o)}$ proteins, making them less available for other receptors [58, 59].

In rats, it has been reported that the cross-talk between both receptor systems can be altered by drug-related behaviors. For instance, operant self-administration of heroin in rats increases CB1 receptor function, while operant self-administration of the cannabinoid receptor agonist WIN 55,212-2 augments MOR binding and receptor efficacy. These changes have been evaluated in rat brain regions robustly associated with drug addiction such as the ventral tegmental area (VTA), nucleus accumbens (NAc), amygdala, and hippocampus [60]. Similar effects have been observed with nicotine treatment over a 10 day period. This subchronic treatment can increase and decrease the levels of the CB1 and MOR levels in the hippocampus, respectively [61].

CANNABINOID/OPIOID WITHDRAWAL, TOLERANCE, AND THE MESOLIMBIC DOPAMINE SYSTEM

In humans, there is some debate about cannabis addiction. One of the main points of the debate is the lack of a cannabinoid withdrawal syndrome, which is in contrast with regular opioid use. It is well known that individuals with severe opioid withdrawal symptoms may experience shaking, muscle and bone pain, nausea, depression, anxiety, and drug craving (NIDA, web page), whereas cannabinoid withdrawal syndrome is subtle. Basic research has contributed enormously to this misperception because most studies using animal models have utilized cannabinoid receptor antagonist injections in animals treated chronically with cannabinoid receptor agonists to trigger a clear and robust cannabinoid withdrawal syndrome (for example, [62, 63]). Obviously, these types of data are less applicable to humans when compared to clinical experiments and

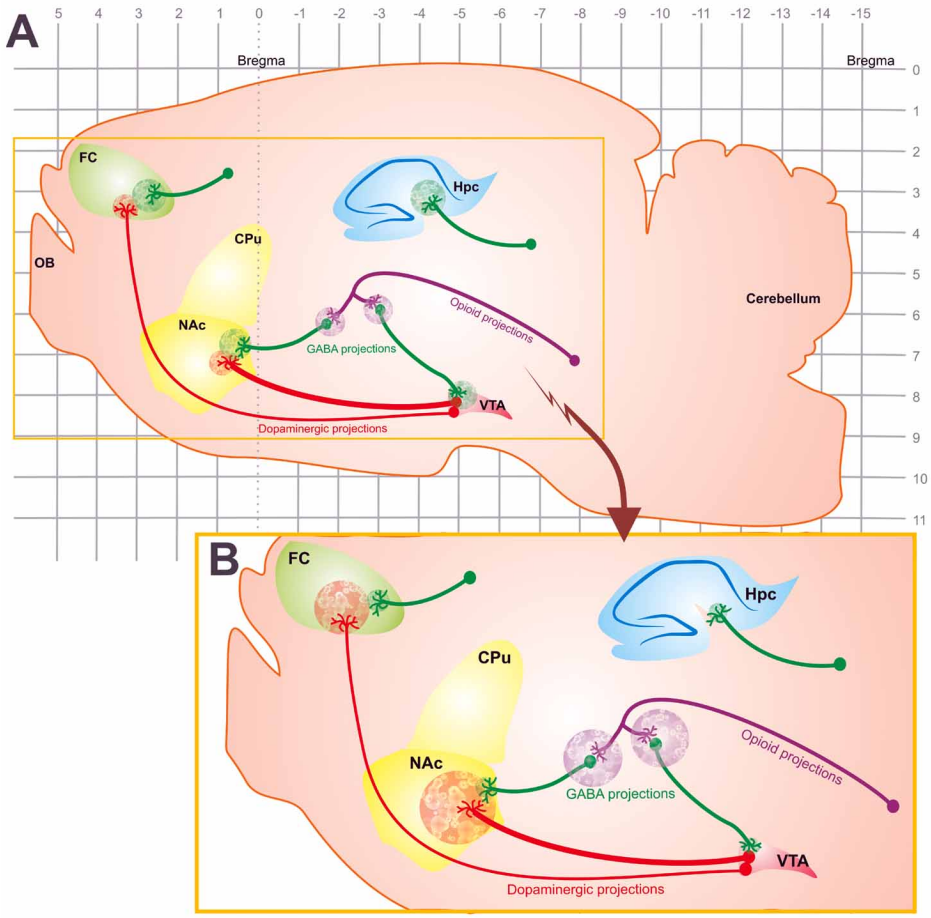

Fig. (1). A sagittal diagram of a rat brain and four structures involved in drug addiction: **a**) prefrontal cortex (PFC), **b**) striatum: ventral, nucleus accumbens (NAc) and dorsal, caudate putamen (CPu), **c**) ventral tegmental area (VTA), and **d**) hippocampus. CB1 receptors are highly expressed in these brain regions as well as in the olfactory bulb (OB, omitted here for the most part) and cerebellum. Some of the functional interactions between endogenous endocannabinoid and opioid systems in drug addiction are summarized. Primary PFC, NAc, Hpc, and VTA innervation and projections are shown. Approximately 40% of the projections from the VTA to the PFC are dopaminergic, while 80% of the projections from the VTA to the NAc are dopaminergic. Normally, GABAergic interneurons control the release of neurotransmitters and GABA release into the synaptic cleft, leading to decreased excitability of the cell. This controls dopamine release in the NAc and PFC **(A)**. Opioid receptor agonists, however, inhibit GABAergic neurons and GABA release, increasing the release of dopamine in the NAc and PFC **(B)**. Mu opioid receptors are highly expressed in GABAergic and dopaminergic neurons in the VTA. The localization of functional CB1 receptors is essentially presynaptic, modulating the extracellular release of multiple neurotransmitters such as dopamine, glutamate and GABA. Both endogenous systems share a common G protein-coupled signaling system.

evaluation of human cognition and other complex symptoms reported from a cannabinoid-addicted individual. However, accumulating evidence suggests that a marijuana/cannabis withdrawal syndrome exists. For example, irritability, restlessness, anxiety, thoughts of and cravings for marijuana/cannabis, decreased quality and quantity of sleep, and decreased food intake characterize marijuana/cannabis withdrawal. Moreover, these symptoms are more severe during the first week after withdrawal [64, 65]. Several groups are currently trying to elucidate whether marijuana/cannabis withdrawal symptoms should be included in future revisions of the Statistical Manual of Mental Disorders (DSM) and/or in the International Classification of Diseases (ICD) [66, 64]. Meanwhile, new findings have demonstrated that marijuana/cannabis withdrawal is related to alterations in brain activity (i.e., lower activity in the right and dorsolateral prefrontal regions and right occipital cortex) and spatial working memory [67]. Cerebral blood volume is increased in the right frontal region, bilateral temporal regions and cerebellum in chronic cannabis smokers after seven days of cessation [68]. In addition, as with treatment of opioid withdrawal syndrome, which uses opioid substitution-based therapy (i.e., methadone), there are clinical studies that demonstrate that oral administration of D9-THC suppresses cannabis withdrawal symptoms in a dose-dependent manner [69]. More interes-tingly, the combination of D9-THC and the alpha(2)-adrenergic receptor agonist Loxefidine generates the most significant improvements of several symptoms after marijuana with-drawal [65]. Currently, Loxefidine is being tested in clinical trials to treat opioid addiction [70].

The study of differences in response to the consumption of either opioid or cannabinoid compounds is very complex. Such responses differ from higher-toxicity profile of opioids to lower-toxicity profile of cannabinoids (opioid overdoses can frequently lead to respiratory depression and death, but this extremely unlikely to happen with cannabinoid over-dose), to differences in psychobiological processes of dependence and tolerance. There are compelling studies

showing how receptor desensitization may explain tolerance after MOR and CB1 acute/repeated activation. A reduction in the fraction of surface CB1 receptors together with a dramatic decrease of their mobility in synaptic terminals is one proposed mechanism that could lead to the desensitization of these receptors [71]. Interestingly, such effects can be observed after two hours of cannabinoid receptor agonist administration. Other studies reveal that MOR and CB1 receptors are desensitized due to endocytic events involving either recycling or for degradation [72, 73]. Thus, it seems to a certain extent that tolerance is due mainly to receptor internalization and uncoupling of receptor signal transduction, and not to the capacity of cannabinoid and opioid ligands to interact directly with MOR and CB1 receptors. Activation of ERK1/2 MAP kinase signaling has been linked to desensitization of MOR and CB1 receptors [74, 75], whereas natural and endogenous cannabinoid receptor agonists are not able to activate MOR, and vice versa with the MOR agonist DAMGO for CB1 receptors [76].

As summarized in Fig. (**1**), fiber projections from the VTA to NAc form the mesolimbic dopaminergic system. It is widely accepted that this dopaminergic pathway modulates the rewarding effects of drugs of abuse (for review, see [77]) as well as those of natural rewards such as sex and food [78, 79]. MOR and CB1 receptors are extensively present in the VTA and NAc nuclei, especially in the presynaptic compartments of GABAergic interneurons [80]. These facts reveal in part how both types of functional presynaptic receptors control neurotransmitter release and excitatory and inhibitory transmission in the NAc (for review, see [81]). Opioid receptors are implicated largely in drug addiction, and there is accumulating evidence that the endocannabinoid system participates in the rewarding effects of multiple drugs of abuse. For example, in rats, the systematic administration of cannabinoid receptor agonists leads to a reduction in alcohol-induced extracellular dopamine release in the NAc [82], as well as to a decrease in dopamine electrophysiological activity [83]. These physiological alterations are accompanied by structural alterations, among which there is an increase in the number of dendrite branches in the NAc and medial prefrontal cortex [84]. Moreover, the increase in cannabinoid endogenous tone by the inhibition of enzymes that degrade endocannabinoids results in mesolimbic dopaminergic alterations. For instance, FAAH inhibition increases extracellular dopamine levels in the shell of the NAc in rats [85], suppresses nicotine-induced electrophysiological activation of dopamine neurons in the VTA [86], and reduces nicotine-induced release of extracellular dopamine in the NAc [87].

PARALLELISM AND FUNCTIONAL INTERACTIONS BETWEEN THE CANNABINOID AND OPIOID SYSTEMS IN VARIOUS BEHAVIORAL MODELS OF DRUG ADDICTION

Cannabinoids and opioids are drugs of abuse with highly addictive properties but also with great therapeutic potential. Thus, research regarding the molecular mechanisms underlying cannabinoid and opioid system interactions could lead to new pharmacological approaches in the treatment of addiction. Behavioral data have served to establish some important parallels between both systems. Table **1** briefly shows some of the main findings from behavioral models of addiction related to cannabinoid-opioid interactions based on classic behavioral paradigms: conditioned place preference/aversion (CPP/CPA), self-administration paradigms (SA), intracranial self-stimulation (ICSS), withdrawal (WD), reinstatement/relapse, tolerance and sensitization.

Note that the first column indicates the drug that induces phenomena such as self-administration or withdrawal, and the second column shows the main experimental manipulations, which in order are cannabinoid/opioid agonists, antagonists, and also gene knock-outs.

The first experimental data showing behavioral evidence of the functional interaction between opioids and cannabinoids focused on withdrawal, tolerance and sensitization phenomena related to opioid dependence. For example, in 1975 Hine *et al.* [88] published the first study showing that natural cannabinoid agonists attenuate morphine withdrawal symptoms in rats. Other authors demonstrated cross-sensitization of locomotor activity in mice treated with opioids and cannabinoids [89].

Along with the development of refined models of addiction features, such as reward or reinstatement models, the characterization of the endocannabinoid system and the introduction of new potent cannabinoid agonists and antagonists have further supported behavioral evidence that both systems interact during reward and addiction. Research into the functional interaction between cannabinoids and opioids in reward is summarized in Table 1 with respect to three paradigms: CPP/CPA, SA and ICSS. The column next to the method shows the dosage of every drug used in the experiment in mg/kg as well as the administration route: intravenous (i.v.), intramuscular (i.m.), intraperitoneal (i.p.), subcutaneous (s.c.), intrathecal, oral or intracerebroventricular (i.c.v.). The fifth column shows the behavioral response or the main effect of the experimental manipulation for each paradigm. We listed the possible responses according to the original reports by the authors. Based on classical conditioning, conditioned place preference/aversion is arguably the most popular model due to its high reliability and low cost. Studies with this method have demonstrated the capability of both cannabinoid and opioid antagonists to attenuate or completely block the development of CPP for cannabinoids or opioids [90-93]. Furthermore, recent data have demonstrated the ability of naloxone to induce place aversion in rats [94, 95], which is blocked in MOR and proenkephalin KO mice [96, 97]. In contrast, the cannabinoid receptor antagonist SR141716A lacks intrinsic aversive effects and can block place aversion induced by high doses of WIN 55,212-2 [90]. The same opioid and cannabinoid antagonists can reduce self-administration behavior for opioids and cannabinoid drugs. Although there is clear evidence that CB1 KO animals fail to intravenously self-administer morphine or WIN-55,212-2 [10, 98], there is no consensus as to whether or not CB1 KO mice develop morphine CPP [99, 100].

Experimental data about opioid receptor KO mice in cannabinoid addiction models are also shown in Table **1** immediately after the pharmacological manipulations. There is clear evidence supporting the role of MOR in the rewarding properties of cannabinoid drugs, and there are also data supporting the role of Kappa opioid receptor/Dynorphin

in the aversive potential of cannabinoid agonists; this may account for the early lack of consensus regarding the ability of cannabinoids to produce CPP or stable self-administration. [101-103].

The effect of cannabinoid agonists in operant models of opioid self-administration is known to depend on the schedule program. Thus, there is evidence that under fixed schedules, pretreatment with D9-THC increases the rate of self-administration, but under progressive schedules, it increases the break-point, which is known to represent the motivational properties of the drug that is being self-administered [103].

There is also great interest concerning the potential teratogenical effects of cannabinoids and opioids during development, which could lead to increased vulnerability of individuals to addiction to these substances. For example, Rubio [104] showed that the administration of D9-THC during gestation facilitates the development of morphine CPP in adult rats, and there is also evidence that cannabinoid agonists can potentiate the self-administration of opioid drugs [105, 106]. In contrast, Riley and Vathy [107] found no evidence that morphine administered perinatally could enhance CPP or self-administration of morphine in adult rats.

Table **1** also shows the effects of cannabinoids and opioids in the ICSS model. To our knowledge there is less evidence regarding their interplay in this model, which might be related to the elevated cost of this model when compared to other methods.

Regarding dependence and withdrawal, we summarize cannabinoid-opioid functional interactions in three categories: withdrawal (WD), reinstatement and tolerance/sensitization phenomena. We have omitted cross-tolerance and cross-sensitization for pharmacological effects other than locomotor activity and reward.

Cannabinoid and opioid agonists share an ability to attenuate or block withdrawal signs and symptoms in cannabinoid- and opioid-dependent animals [108-110]. In contrast, the ability of non-selective opioid antagonists and cannabinoid antagonists to induce withdrawal signs in cannabinoid-dependent animals and to prevent reinstatement of drug seeking in both cannabinoid and opioid-dependent animals is well established, although there are no additive effects for Naloxone and SR141716A [11, 111, 112]. Research with opioid receptor KO mice has further demonstrated a role for the MOR in the manifestation of withdrawal signs [112, 63, 113] although there is also evidence of the opposite [101]. Moreover, CB1 KO mice with a history of morphine consumption show milder withdrawal signs when challenged with naloxone [10, 63], and do not develop behavioral sensitization to the locomotor effects induced by chronic morphine [100].

Table 1. Parallelism and Functional Interaction berween Cannabinoid and Opiod Systems in Various Behavioural Models of Addiction

CPP/CPA						
Drug-induced	**Effect of:**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
	CB1 Agonist					
-	D9-THC	CPP	1-4 i.p. 0.075-0.75 i.p.	Development	Rats	[114] [92]
-	WIN 55,212-2	CPA	0.3-1 s.c.	CPA	Rats	[90]
Morphine s.c.	D9-THC (chronic pretreatment)	CPP	10 i.p.	No effect	Mice	[109]
Morphine s.c.	WIN 55,212-2 (D. hippocampus)	CPP	µg/rat i.c.v.	No effect	Rats	[95]
	AM251 (D.hippocampus)		25-50-100 ng/rat i.c.v.			
Naloxone s.c.	WIN 55,212-2 (D.Hippocampus)	CPA	1 µg/rat i.c.v.	Place aversion	Rats	[95]
D9-THC i.p.	Cannabidiol	CPA	1-10 i.p.	Blocked aversion	Mice	[115]
Morphine s.c.	AM251 (intra accumbal)	CPP	5-125 ng/µl i.c.v.	Blocked	Rats	[116]
Morphine i.p.	D9-THC (during gestation)	CPP	1-5 i.p.	Facilitation in adults	Rats	[105]
	CB1 Antagonist					
Morphine s.c.	SR 141716A	CPP	0.03-3 i.p.	Blocked	Rats	[90]
WIN 55,212-2 s.c.	SR141716A	CPA	0.3-1 i.p.	Blocked	Rats	[90]
Morphine s.c.	SR141716A	CPP	0.1 i.p. 3 i.p.	Attenuation Blocked	Rats	[93]
CP 55.940 i.p.	SR141716A	CPP	0.5 i.p.	Blocked	Rats	[91]
Heroin i.p.	SR141716A	CPP	0.5 i.p.	Blocked	Rats	[91]
D9-THC i.p.	SR141716A	CPP	0.25-1 i.p.	Blocked	Rats	[92]

(Table 1) Contd…..

CPP/CPA						
Drug-induced	**Effect of:**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
	CB1 Agonist					
Morphine s.c.	SR141716A	CPP	3-5-10 i.p.	Blocked	Mice	[111] [117]
Salvinorin A i.m.	SR141716A	CPP	1 i.m.	Attenuation	Zebrafish	[118]
	Genetic Manipulation					
U50,488H	CB1 KO	CPA	-	Blocked CPA induced by K-agonist	Mice	[10]
Morphine s.c.	CB1 KO	CPP	-	Blocked acquisition	Mice	[100]
Morphine i.p.	CB1 KO	CPP	-	Development	Mice	[99]
	Opioid Agonist					
-	Endomorphine-2	CPP	30 µg/5µl i.c.v.	Development	Rats	[119]
Morphine i.p.	Nalbuphine	CPP	1 i.p.	Blocked acquisition	Rats	[120]
Morphine s.c.	Nociceptin	CPP	0-1.5 nmol i.c.v.	Blocked	Mice	[121]
Naloxone s.c.	Nociceptin	CPA	0-1.5 nmol i.c.v.	No effect	Mice	[121]
Morphine s.c.	Ro64-6198	CPP	1 i.p.	Blocked (acquisition)	Mice	[122]
			0.3-1 i.p.	No effect (expression)		
			1 i.p.	CPP reinstatement		
Morphine s.c.	Morphine (perinatally)	CPP/SA	10 s.c.	No effects	Rats	[107]
-	Salvinorin A	CPP	0.2-0.5 µg/kg i.m.	Place preference	Zebrafish	[118]
-	Salvinorin A	CPA	0.08 i.m.	Place aversion	Zebrafish	[118]
	Opioid Antagonist					
-	Naloxone (V. Pallidal)	CPP	0.0001-0.01 i.c.v.	Dose dependent aversion	Rats	[94]
-	Naloxone	CPA	1 s.c.	Place aversion	Rats	[95]
CP 55.940 i.p.	Naloxone	CPP	2 i.p.	Blocked	Rats	[91]
D9-THC i.p.	Naloxone	CPP	0.5-2 i.p.	Blocked	Rats	[92]
Morphine s.c.	B-funaltrexamine (M.Thalamus)	CPP	5 µg/side i.c.v.	Blocked acquisition but not expression	Rats	[123]
D9-THC i.p.	Nor-BNI (pretreatment)	CPA	5-10 s.c.	Blocked CPA	Mice	[49]
Salvinorin A i.m.	Nor-BNI/	CPP	10 i.m.	Attenuation	Zebrafish	[118]
	Genetic Manipulation					
Morphine i.p.	MOR KO	CPP	-	Blocked	Mice	[41]
D9-THC i.p.	MOR KO	CPP/CPA	-	Blocked CPP Reduced CPA	Mice	[101]
	DOR KO			No effect		
	KOR KO			Blocked CPA		
Morphine s.c.	PRODYN KO	CPP	-	Development (No effect)	Mice	[49]
D9-THC i.p.	PRODYN KO	CPA	-	Blocked	Mice	[49]
D9-THC i.p.	M/DOR KO	CPP	-	Reduced	Mice	[112]
Naloxone i.c. (ventral pallidum)	MOR KO PROENK KO	CPA	-	No place aversion	Mice	[96, 97]

(Table 1) Contd…..

CPP/CPA						
Drug-induced	**Effect of:** **Genetic Manipulation**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
Naloxone i.c. (ventral pallidum)	B-END KO	CPA	-	Robust CPA	Mice	[97]
Buprenorphin s.c.	MOR KO	CPP	-	Blocked	Mice	[124]
Morphine i.p.	KOR KO	CPP	-	No effect	Mice	[45]
SELF-ADMINISTRATION						
Drug	**Effect of** **CB1 Agonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
-	WIN 55,212-2	SA	0.01-0.1 i.v.	Self-administered	Mice	[125]
	WIN 55,212-2		12.5-50 μg/kg i.v.		Rats	[126]
	CP55,940		0.1-1.6 μg/2 μl i.c.v.		Rats	[127]
-	D9-THC	SA	0.01-0.02 μg/2 μl i.c.v.	SA	Rats	[92]
Heroin i.v.	D9-THC (pretreatment)	SA (FR1 schedule) (PR schedule)	2-4-8 i.p.	Increased only in FR1 schedule	Rats	[128]
Heroin i.v.	D9-THC	SA (PR schedule)	0.3-3 i.p.	Increased Break-point	Rats	[103]
	WIN 55,212-2		0.3-3 i.p.			
	AM 404		3-10 i.p.	Decreased Break-point at 10 mg/kg AM404 or in combination		
	URB 597		0.01-0.3 i.p.			
-	URB597	SA	0.3 i.v.	No SA	Squirrel Monkeys	[129]
D9-THC i.v.	URB597	SA	0.3 i.v.	No effect	Squirrel Monkeys	[129]
Anandamide i.v.				Decreased SA		
Anandamide i.v.				Increased SA		
Heroin i.v.	D9-THC (developement)	SA	1.5 i.p.	Increased	Rats	[105]
Heroin i.v.	D9-THC (prenatally)	SA	0.15 i.v.	Increased sensitivity to heroin SA	Rats	[106]
Morphine i.v.	D9-THC (perinatally)	SA (FR1 schedule)	5 p.o.	Increased SA (females only)	Rats	[130]
		SA (PR schedule)		No effect		[131]
	CB1 Antagonist					
WIN 55,212-2 i.v.	SR141716A	SA	0.25 i.p.	Prevented SA	Mice	[125]
CP55,940 i.c.v.		SA	0.5 i.p.	Reduced	Rats	[127]
Heroin i.c.v.		SA		Reduced		
D9-THC i.c.v.	SR141716A	SA	0.5 i.p.	Reduced	Rats	[92]
Heroin i.v.	SR141716A	SA	3 i.p.	Reduced	Rats	[113]
Heroin i.v.	SR141716A	SA	1-3 i.p.	Reduced	Rats	[132, 133]
Morphine i.v.	SR141716A	SA	0.25 i.p.	Reduced	Mice	[113]
	Genetic Manipulation					
WIN 55,212-2 i.v.	CB1 KO	SA	-	No SA	Mice	[10] [98]
Morphine i.v.						
Natural rewards	CB1 KO	SA	-	Reduction	Mice	[134]

(Table 1) Contd…..

SELF-ADMINISTRATION						
Drug	**Effect of Opioid Agonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
-	Heroin	SA	0.125-2 µg/2µl i.c.v.	SA	Rats	[127]
	Opioid Antagonist					
CP 55,940 i.c.	Naloxone	SA	2 i.p.	Reduced	Rats	[127]
Heroin i.c.v.				Reduced		
D9-THC i.c.v.	Naloxone	SA	2 i.p.	Reduced	Rats	[92]
D9-THC i.v.	Naltrexone	SA	0.03-0.3 i.m.	Reduced	Squirrel Monkeys	[135]
WIN 55,212-2 i.p.	NOR-BNI	SA	-	Enhancement	Mice	[102]
	Genetic Manipulation					
WIN 55,212-2 i.p.	PRODYN KO	SA	-	Enhancement	Mice	[102]
ICSS						
Drug	**Effect of CB1 Agonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
-	D9-THC	ICSS	1.5 i.p.	Lower Threshold	Rats	[136]
-	D9-THC	ICSS	1 i.p.	Lower Threshold	Fischer Rats	[137]
				Lower Threshold	Sprague Dawley	
				No effect	Lewis	
-	WIN 55,212-2	ICSS	0.1-1 i.p.	No change of threshold	Rats	[138]
-	WIN55,212-2	ICSS	3 i.p.	Increased threshold (reverted by SR141716A)	Rats	[139]
	CP55,940	ICSS	100 µg/kg i.p.	Increased threshold (reverted by SR141716A)	Rats	[139]
	HU-210	ICSS	30-100 µg/kg i.p.	Increased threshold (not reverted by SR141716A)	Rats	[139]
-	PMSF	ICSS	15, 30, 60 i.p.	Increased threshold (not reverted by SR 141716A)	Rats	[140]
	OMDM-2	ICSS	3, 10, 30 i.p.	Increased threshold (reverted by SR141716A)	Rats	[140]
	URB597	ICSS	0.3, 1, 3 i.p.	Increased threshold (reverted by SR141716A)	Rats	[140]
-	D9-THC	ICSS	1-2 i.p.	Increased Threshold (reverted by SR141716A)	Rats	[141]
	CB1 Antagonist					
-	SR141716A	ICSS	1, 3, 10 i.p.	Increased Threshold	Rats	[142]
HU-210 i.p.	AM251	ICSS	1-3 i.p.	Blocked Increased threshold by CB1 agonist.	Rats	[139]

(Table 1) Contd…..

ICSS						
Drug	**Effect of** / **Opioid Agonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
-	Morphine	ICSS (Prefrontal cortex)	7.5-10 i.p.	No effect	Rats	[143]
-	Morphine	ICSS	5.0 i.p.	Lower Threshold	Rats	[144]
-	Morphine spontaneus withdrawal (WD)	ICSS	-	Reduction in response rates	Rats	[145]
-	Fentanyl	ICSS	0.6 s.c.	Lower Threshold	Rats	[146]
Fentanyl s.c. spontaneus Withdrawal (WD)	Buprenorphine	ICSS	1-20 µg s.c.	Prevented increase of threshold	Rats	[146]
-	U-69,593	ICSS	0.25-0.5 i.p.	Increase thresholds	Rats	[147]
			0.5 i.p.			[148]
-	Salvinorin A	ICSS	0.5-2 i.p.	Increase Thresholds	Rats	[149]
	Opioid Antagonist					
-	Naloxone/Naltrexone	ICSS	2-20 i.p.	Increase of threshold	Rats	[150]
	Naloxone (chronic)	ICSS (VTA)	3 s.c.	Decreased response rate	Rats	[151]
Morphine s.c.	Naloxone	ICSS (Lateral Hypothalamus)	0.003-3 s.c.	Decreased response rate	Rats	[152]
	Naltrexone					
	Diprenorphine					
ANTI i.p.	U-69593	ICSS	2-4 i.p.	Blocked threshold increase	Rats	[147]
Fentanyl s.c. (Chronic)	Naloxone	ICSS	0.03 s.c.	Increased Thresholds	Rats	[146]
	Buprenorphine (pretreatment)/ Naloxone (Challenge)		1-20 µg s.c.	No increase of threshold		
WITHDRAWAL						
Drug	**Manipulation** / **CB1 Agonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
Morphine i.p. WD induced by Naloxone s.c.	D9-THC Pretreatment	Withdrawal	10 i.p.	Attenuation	Mice	[109]
Morphine s.c. WD precipitated by Naloxone i.p.	D9-THC Cannabidiol+THC	Withdrawal	2 i.p. 10 i.p.	Attenuation Increased attenuation	Rats	[88]
Morphine s.c. WD precipitated by Naloxone s.c.	D9-THC	Withdrawal	0.1-10 s.c.	Attenuation	Mice	[110]
Morphine s.c. WD precipitated by Naloxone i.p.	Anandamide	Withdrawal	5 i.v.	Attenuation	Rats	[108]
	CB1 Antagonist					
Morphine	SR141716A	Withdrawal	0.1-3 i.p.	Precipitation	Rats	[11]
	SR141716A/Naloxone		3 i.p. / 1 i.p.	No additive effect		
D9-THC s.c.	SR141716A	Withdrawal	5 i.p.	Precipitation	Rats	[153] [154]
			10 i.p.		Mice	[155] [156] [110]

(Table 1) Contd…..

WITHDRAWAL						
Drug	**Manipulation** / **CB1 Antagonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
D9-THC i.p. WD precipitated by SR141716A i.p.	Chronic SR141716A (cotreatment with THC)	Withdrawal	10 i.p.	No withdrawal	Mice	[117]
Morphine s.c. WD precipitated by Naloxone s.c.	SR141716A	Withdrawal	10 i.p.	No effect	Mice	[117]
Morphine s.c. WD precipitated by Naloxone i.p.	Chronic SR141716A (cotreatment with Morphine)	Withdrawal	5 i.p.	Attenuation	Rats	[157]
			10 i.p.		Mice	[117]
WIN 55,212-2 i.p.	SR141716A	Withdrawal	10 mg/kg s.c.	Precipitation	Mice	[158]
			1.5-3 µg i.c.v. (third ventricle)	Partial WD		
			0.75-3 µg i.c.v. (cerebellum)	Partial WD		
			1.5-3 µg i.c.v. (striatum)	No effect		
			1.5-3 µg i.c.v. (hippocampus)	Partial		
			1.5-3 µg i.c.v. (amygdala)	Partial		
Morphine i.p. WD precipitated by Naloxone i.p.	AM-251 (acute)	Withdrawal	1.6-3.2 µg intrathecal	No effect	Rats	[159]
	AM-251 (pretreatment w/ morphine)			Attenuation		
	Genetic Manipulation					
D9-THC WD precipitated by SR141716A i.p.	KO CB1	Withdrawal	-	No Withdrawal	Mice	[10] [110]
Morphine WD precipitated by Naloxone s.c.	KO CB1	Withdrawal	-	Attenuation	Mice	[10] [110]
Morphine i.p. WD precipitated by Naloxone s.c.	Double CB1/A2a KO	Withdrawal	-	Normal WD	Mice	[160]
	A2a KO			Increased WD		
	Opioid Agonist					
D9-THC precipitated WD by SR 141716A i.p.	Morphine	Withdrawal	0.01-0.3 s.c.	Attenuation of cannabinoid WD signs	Mice	[110]
Fentanyl s.c. WD precipitated by Naloxone s.c.	Buprenorphine (pretreatment)	Withdrawal	80 µg s.c.	Attenuation of somatic signs	Rats	[146]
	Opioid Antagonist					
D9-THC s.c.	Naloxone	Withdrawal	1-4 i.p.	Precipitation	Rats	[161, 162]
D9-THC p.o. (perinatally)	Naloxone	Withdrawal	5 i.p.	Precipitation	Rats	[108]
Morphine s.c.	Methylnaloxonium	Withdrawal	31-1000 ng i.c.v.	Higher partial WD (lateral ventricle)	Rats	[163]
				Higher WD (locus coeruleus)		
				Lower partial WD (NAc & Thalamus)		
HU-210 s.c.	Naloxone	Withdrawal	1 i.p.	Precipitation	Rats	[111]
	Naloxone / SR141716A		1 i.p. / 3 i.p.	No additive effect		

(Table 1) Contd…..

WITHDRAWAL						
Drug	**Manipulation** / **Opioid Antagonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
Morphine s.c.	Naloxone	Withdrawal	1 s.c.	Precipitation Opiate WD	Mice	[110]
D9-THC s.c.	Naloxone	Withdrawal	1-5 s.c.	Partial WD signs	Mice	[110]
Morphine s.c.	B-Funaltrexamine	Withdrawal	10 i.v.	Precipitation of WD signs	Rats	[164]
	Naltrindole		4 i.v.	Partial WD signs		
	Nor-Binaltorphimine		5 i.v.	Partial WD signs		
	Genetic Manipulation					
Morphine i.p. WD precipitated by Naloxone s.c.	KOR KO	Withdrawal	-	Attenuation	Mice	[45]
D9-THC s.c. WD precipitated by SR141716A i.p.	MOR KO	Withdrawal	-	Attenuation	Mice	[110]
Morphine s.c. WD precipitated by Naloxone s.c.	MOR KO	Withdrawal	-	Failure	Mice	[110]
D9-THC i.p. WD precipitated by SR141716A i.p.	MOR KO	Withdrawal	-	Unaffected	Mice	[101]
	DOR KO					
	KOR KO					
D9-THC i.p. WD preciptitated by SR141716A i.p.	PROENK KO	Withdrawal	-	Attenuation	Mice	[113]
Morphine i.p. WD precipitated by Naloxone s.c.	PRODYN KO	Withdrawal	-	Unchanged	Mice	[49]
D9-THC i.p.	Double MOR/DOR KO	Withdrawal	-	Attenuation	Mice	[112]
REINSTATEMENT						
Drug	**Manipulation** / **CB1 Agonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
Heroin i.v.	WIN55,212-2	Reinstatement	0.15-0.3 i.p.	Reinstatement	Rats	[165, 166]
	CP55,940		0.05-0.1 i.p.	Reinstatement		
	D9-THC		0.1-1 i.p.	No effect		
Heroin i.v.	HU-210	Reinstatement	0.02 s.c.	Reinstatement	Rats	[133]
Anandamide i.v.	D9-THC	Reinstatement	40 μg/kg i.v.	Reinstatement	Squirrel Monkeys	[129]
D9-THC i.v.	URB597		0.3 i.v.	No reinstatement		
	CB1 Antagonist					
WIN55,212-2 i.p. (cannabinoids or heroin priming)	SR141716A	Reinstatement	0.3 i.p.	Blocked	Rats	[167]
Heroin i.v. (cannabinoids priming)	SR141716A	Reinstatement	0.3 i.p.	Reduced	Rats	[166]
Heroin i.v. (heroin priming)				Prevented		
Heroin s.c. (heroin priming)	SR141716A	Reinstatement	1-3 s.c.	Reduced	Rats	[133]

(Table 1) Contd…..

REINSTATEMENT						
Drug	**Manipulation** / **Opioid Agonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
WIN55,212-2 i.p.	Heroin	Reinstatement	0.5 i.p.	Reinstatement	Rats	[167]
	Opioid Antagonist					
WIN 55,212-2 i.p. (cannabinoid priming)	Naloxone	Reinstatement	1 i.p.	Blocked	Rats	[167]
Heroin i.v. (heroin priming)	Naloxone	Reinstatement	1 i.p.	Blocked	Rats	[166]
SENSITIZATION						
Drug	**Manipulation** / **CB1 Agonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
-	D9-THC (chronic-WD-challenge)	Sensitization (motor)	5 i.p. (challenge)	Sensitization	Rats	[168]
-	D9-THC (intermittent schedule)	Sensitization (motor)	10 i.p.	No sensitization	Mice	[169]
Morphine s.c.	CP55,940 (pretreatment)	Sensitization (motor & reward)	0.1-0.2 i.p.	Enhancement	Rats	[170]
Morphine s.c.	D9-THC or WIN 55,212-2 (challenge)	Sensitization (motor)	75-150 μg/kg i.v.	Cross-Sens	Rats	[171]
	CB1 Antagonist					
Cannabinoids i.p. cross-sensitization by morphine s.c.	SR141716A (pretreatment)	Sensitization (motor)	1 i.p.	Blocked	Rats	[171]
WIN55,212-2 i.p. (heroin s.c. pre-treatment)	SR141716A	Sensitization (motor)	1 i.p.	Blocked	Rats	[172]
-	SR141716A	Sensitization (motor)	0.1-3 i.p.	No effect	Rats	[93]
Morphine s.c. induced sensitization	SR141716A	Sensitization (motor)	0.1-3 i.p.	No effect	Rats	[93]
	Genetic Manipulation					
Morphine s.c. (chronic)	CB1 KO	Sensitization (motor)	-	Failure	Mice	[100]
	Opioid Agonist					
D9-THC i.p.	Morphine (challenge)	Sensitization (motor)	0.5 i.v.	Cross-Sensitization	Rats	[171]
D9-THC i.p.	Heroin (challenge)	Sensitization (motor)	1 i.p.	Cross-sensitization	Rats	[173]
Morphine i.p.	Nalbuphine	Sensitization (motor)	1 i.p.	No effect	Rats	[120]
	Opioid Antagonist					
WIN 55,212-2 i.p. (heroin s.c. pre-treatment) / Heroin s.c. (WINi.p. pre-treatment)	Naloxone	Sensitization (motor)	0.1 s.c.	Blocked Cross-sensitization	Rats	[172, 174]
	Genetic Manipulation					
Buprenorphin s.c.	MOR KO	Motor Sensitization	-	Prevented	Mice	[124]
D9-THC i.p.	MOR/DOR KO	Motor sensitization	-	Unaffected	Mice	[112]

(Table 1) Contd…..

TOLERANCE						
Drug	**Manipulation** / **Cannabinoid Agonist**	**Paradigm**	**Dose(mg/kg)**	**Response**	**Animal**	**References**
Morphine s.c. (tolerant animals)	D9-THC	Tolerance	-	Antinociception Tolerance	Mice	[175]
	Opioid Agonist					
D9-THC s.c. (tolerant animals)	Morphine	Tolerance	-	Hypothermia Tolerance	Mice	[175]
D9-THC i.p.	PREPROENK KO	Tolerance (analgesia)	-	Slower Development	Mice	[113]
D9-THC i.p.	MOR KO	Tolerance (hypolocomotion)	-	Unaffected	Mice	[101]
	DOR KO			Unaffected		
	KOR KO			Reduced		
D9-THC i.p.	MOR KO	Tolerance (analgesia & hypothermia)	-	Unaffected	Mice	[101]
	DOR KO					
	KOR KO					

THE ENDOGENOUS CANNABINOID AND OPIOID SYSTEMS IN ALCOHOL ADDICTION

There is substantial evidence showing that cannabinoid and opioid systems may underlie the preference for, and consumption of, alcohol, in addition to other parameters associated with alcohol addiction. Most of these principles have been established with experimental animal models, mostly rats and mice, using genetic, pharmacological, and neuroanatomical-chemical methodologies. In the following paragraphs, we describe the main results of these studies with regard to alcohol self-administration (a more appropriate model for human alcohol consumption) and their relationship with cannabinoid and opioid endogenous systems.

a) Rats Selectively Bred for Alcohol Self-Administration (i.e., Alcohol-Preferring Rats) and Cannabinoid and Opioid Receptor KO Mice

There are very few lines of alcohol-preferring rats available that were developed by selective breeding using classic genetics. Two such lines have been used repeatedly to study the influence of the endocannabinoid and opioid systems on the preference for or excessive intake of alcohol. Alcohol-preferring rats present changes in both endogenous systems that are significantly associated with and could explain alcohol preference. For example, with regard to the endocannabinoid system, alcohol-preferring rats (Alko, Alcohol (AA); from the National Public Health Institute of Helsinki, Finland) have lower levels of monoacylglycerol lipase (MAGL) and especially of fatty acid amide hydroxylase (FAAH) in the prefrontal cortex [176]. In addition, they have decreased membrane-bound FAAH activity, together with reduced density of CB1 receptors and GTPγS binding, in the prefrontal cortex but not in the cerebellum or striatum, which are regions with higher contents of CB1 receptors. However, the pattern changes for the proteins. AA rats show lower expression levels of CB1 than non alcohol-preferring rats in the striatum but not in the prefrontal cortex. This suggests that AA rats have overactive endocannabinoid transmission into the prefrontal cortex and compensatory downregulation of CB1 receptor signaling.

In alcohol-naïve Sardinian alcohol-preferring rats (sP; from the University of Gagliari, Italy) the spontaneous activity of VTA dopaminergic neurons is increased, probably due to reduced endocannabinoid transmission in this region [177]. The authors used *in vivo* and *in vitro* electrophysiological recordings to demonstrate that endocannabinoid modulation of inhibitory transmission is compromised in sP rats as compared to non-preferring sNP rats. sP rats show a reduced probability of GABA release and a reduction in frequency but not amplitude of GABA-A inhibitory postsynaptic currents. Finally, Marchigian Sardinian alcohol-preferring rats (msP), a line derived from the rats from Gagliari but bred at the University of Camerino (Italy), exhibit an increase in CB1 receptor mRNA expression in several regions of the hippocampus (CA1, CA4), prefrontal cortex (PFC) and basal ganglia (caudate-putamen) [178].

With regard to the opioid system, upregulation of RNA expression and impairments in the orphanin FQ and nociceptin (OFQ/N) systems in the central amygdala have been linked to alcohol intake and preference in Marchigian Sardinian alcohol-preferring rats. For instance, msP rats possess upregulated OFQ/N messenger RNA expression in the central amygdala (CeA), bed nucleus of stria terminalis and primary motor cortex, whereas receptor density is increased in the CeA, primary motor cortex and basolateral amygdaloid nucleus as compared to Wistar rats. However, these rats also show a reduction of GTPγS binding in the CeA [179]. Alko rats have been found to have increased levels of mu and kappa opioid receptors. MOR is increased in the basolateral amygdala, medial preoptic area and substantia nigra pars reticulate as compared to levels in alcohol-avoiding rats [180]. Sommer *et al.* [181] concluded from Alko alcohol-preferring rats that the combination of higher opioidergic tone in the ventral striatum together with a decrease in endocannabinoid signaling in the PFC is one of

the main neurobiological substrates of alcohol preference in these rats. All of these studies with alcohol-preferring rats implicate specific alterations in endogenous endocannabinoid and opioid systems in the five key brain areas in drug addiction (e.g., NAc, VTA, PFC, amygdala, and hippocampus) and may explain the complex phenotype of alcohol consumption.

Up to now, another powerful tool to study the influence of genetics on endogenous cannabinoid and opioid systems during alcohol self-administration has been the use of genetically engineered KO mice. Several laboratories have developed viable and fertile CB1 KO mice that do not present critical phenotypic disturbances. Experimental work from these groups has shown that CB1 KO mice have lower alcohol preference and consumption, lack acute alcohol-induced dopamine release in the NAc, present hypolocomotor effects after 1.5, 2, and 2.5 g/kg alcohol injection, show no response to the cannabinoid receptor antagonist SR141716, show reduced alcohol-induced conditioned place preference, and have greater sensitivity to the acute intoxicating effects of alcohol as well as more severe alcohol withdrawal [182, 183, 184]. This is also accompanied by an increase in the level of D2 dopaminergic receptors in the striatum and alterations in GABA and NMDA transcripts [185, 186].

Similarly to CB1 KO mice, mice lacking opioid receptors show significant consequences in voluntary alcohol intake. For example, mice lacking MOR show reduced alcohol self-administration [187, 188, 189], and the same occurs in kappa opioid receptor KO mice [190]. On the other hand, delta-opioid receptor KO mice and other mice with reduced expression of the beta-endorphin complex show an increase in alcohol self-administration [191, 192]. Nevertheless, certain mutations resulting in the loss of beta-endorphin and enkephalin opioid peptides suggest that the rewarding effects of alcohol are not mediated by the receptors for these molecules [193, 194], or that these molecules could cause a reduction in alcohol consumption, particularly in female mice [195].

b) Pharmacological Manipulations of the Endogenous Cannabinoid and Opioid Systems Influence Alcohol-Related Behaviors

There are a number of important experimental studies showing that activation or blockade of endogenous cannabinoid and opioid systems affects alcohol intake and other alcohol-related behaviors. We will briefly describe some of the key findings, starting with the pharmacology of the endocannabinoid system. It has been shown that activation of CB1 receptors leads to an increase in alcohol relapse [196, 197], the reinstatement of alcohol-seeking behavior and motivation for beer [198, 199] and potentiation of the alcohol deprivation effect (for review, see [200]). There is an even larger number of studies demonstrating that the blockade of CB1 receptors affects alcohol-related behaviors. Antagonist compounds such as SR141716A and SR147778 reduce alcohol self-administration in operant and two-bottle choice paradigms when using alcohol-preferring and non-preferring rats [201-204]. In addition, these antagonists suppress the conditioned reinstatement of alcohol seeking [205] and significantly reduce the preference for and motivational properties of alcohol [206, 207]. All these effects have been found when antagonists are administrated either systematically or centrally, e.g., by microinjections into the NAc and VTA [208]. Due to the recent increase in the literature regarding CB1 receptors as a pharmacological target for the treatment of alcoholism, there have been several extensive reviews published [209-211]. Based on these studies and recent findings from our own group [212], it is plausible to conclude that the endocannabinoid system mediates not only alcohol intake but the motivational and emotional properties of alcohol intake, seeking and craving.

Currently, the number of studies focused on the role of the opioid system in alcohol self-administration is greater than the number of cannabinoid studies by a proportion of 5:1. This likely reflects the more recent discovery of the endogenous cannabinoid (approximately 20 years ago). However, these studies have demonstrated that use of the nonspecific opioid antagonist naltrexone decreases context-induced alcohol seeking and reinstatement of alcohol responses in rats [213, 214]. Similar results have been found with inhibition of the opioid-like orphan receptor NOP [7], MOR [215], and naloxone [216]. Using the "drinking in the dark" protocol with mice, i.e., exposure to higher concentrations of alcohol in the first hours of the dark cycle, naltrexone dose-dependently reduces alcohol intake [217]. Other studies, however, have shown that the effects of naltrexone are nonspecific for alcohol, as naltrexone also decreases spontaneous intake of saccharine and sucrose [218]. Other issues arise from the specific activation or blockade of the kappa opioid receptor. Whereas some studies have shown that blockade of this receptor does not alter alcohol response [219], or that it increases alcohol self-administration, other studies have revealed that activation of kappa opioid receptor causes a decrease in alcohol self-administration [220, 221]. An interesting study, however, revealed that blockade of kappa opioid receptors reduces alcohol operant self-administration if the animals are previously made alcohol-dependent [222].

Along with the endogenous cannabinoid system, the opioid system mediates alcohol intake and motivational aspects of alcohol addiction. The motivation to drink using animal models and the neuropharmacology of opioid receptors has been reviewed by Koob and colleagues [223]. Here, we have integrated the endogenous cannabinoid system and the endogenous opioid system (see Fig. **2**), and we have drawn parallels between both systems. In our laboratory, we have repeatedly demonstrated that activation of cannabinoid receptors by WIN 55,212-2 during periods of alcohol deprivation dose-dependently increases relapse by operant self-administration procedures in rats [196, 197, 224]. For the purposes of this review, we have included new original data. In this work, the treatment during the alcohol deprivation period was the opioid receptor agonist morphine; a similar dose-response increase in alcohol responding was obtained. In Fig. (**2**), panels a-d depict an adapted version of the work originally published in 2004. Panels e-h show the original data after the activation of opioid receptors by morphine. These results show that alcohol relapse is increased after activation of the G-protein coupled receptors, either MOR receptors or CB1 receptors, suggesting that both systems participate in alcohol addiction in a similar way. Although the mechanism is unknown, we assume that there

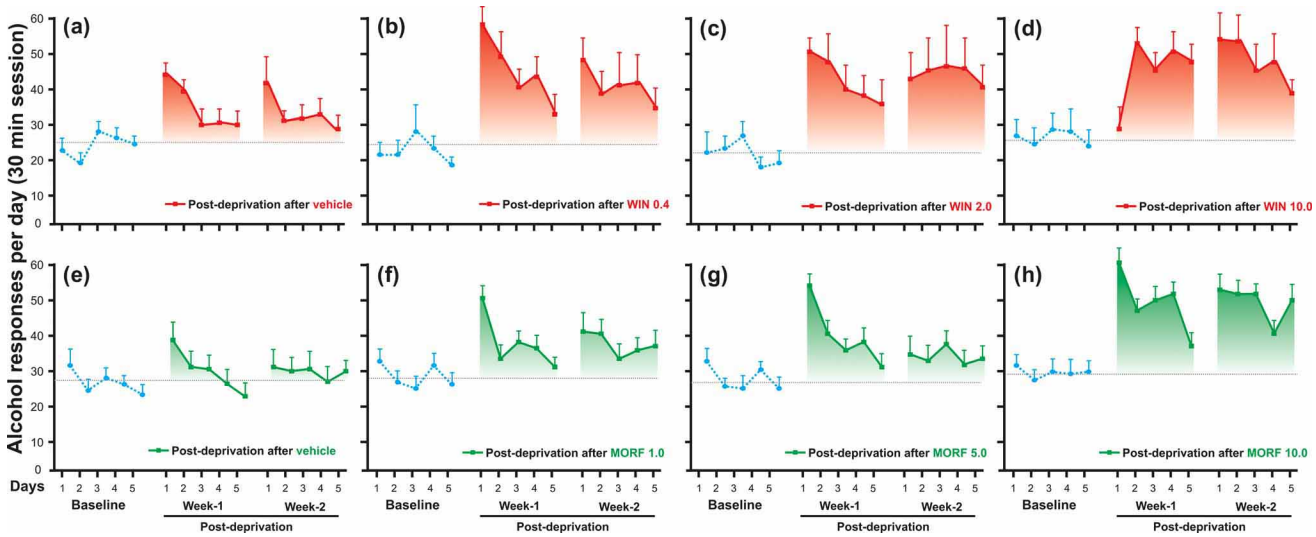

Fig. (2). Cannabinoid and opioid receptor agonists increase alcohol relapse. Wistar rats with an extended background of alcohol operant self-administration (at least 7 weeks of continuous 10% alcohol solution access, 30 min sessions) underwent an alcohol deprivation period in which the animals (n=9-10 by group) could not access alcohol operant chambers. During the deprivation period, rats were treated with either the synthetic cannabinoid receptor agonist WIN-55,212-2 (0.4-10 mg/kg, s.c.) (**a-d**) or the prototypical opioid (essentially mu-opioid) receptor agonist morphine (1.0-10 mg/kg, s.c.) (**e-h**). Subsequently, the animals were allowed to re-enter the alcohol operant chambers, and the number of alcohol responses was recorded (post-deprivation period). Data represent mean ± SEM of alcohol responses on the active lever. Blue dashed lines represent the baseline of alcohol responses before the alcohol deprivation period. Red and green lines correspond to the next two weeks (Monday to Friday) after cannabinoid or opioid treatment. Despite some differences between response patterns, both treatments induced a long-lasting response for obtaining alcohol in a dose-dependent manner. Grey dashed lines represent the mean from baseline. Red and green areas highlight the response after cannabinoid or opioid treatment compared with baseline and vehicle.

are other types of G-protein coupled receptors (discussed here previously) that could have this effect on alcohol relapse after their activation. Indeed, there are more than 490 genes in the mouse and more than 390 genes in humans that code for these proteins [225]. Therefore, further biochemical studies are needed.

THE GENETICS OF THE OPIOID AND ENDOGENOUS CANNABINOID SYSTEMS

Previously, we highlighted the effects of CB1, mu, kappa, and delta receptor deletions in mice, in addition to certain genetic clues from the endogenous cannabinoid and opioid systems regarding the phenotype of alcohol intake and preference in rats. In the following section, we will describe the main findings that address the relationships between specific polymorphisms in both the endogenous systems and drug addiction. First, when we grouped all of the results, we noticed that most studies could be clustered as follows: some studies compared different populations such as African-Americans, Caucasians, Hispanics, Chinese, and Indians, [226-228], as well as gender differences [229]. Others explored how certain haplotypes correlate with other mutations [227, 230] or studied associations between addiction phenotypes (e.g., to opiates or to alcohol) [231, 232] or associations with psychiatric traits such psychosis induced by a substance [233]. Finally, some studies compared different responses to pharmacological treatments such as therapies for drug addiction or pharmacodynamic responses (e.g., naloxone/naltrexone) [234, 235]. Currently, the number of opioid studies focusing on genetic polymorphisms and addiction is greater than that for cannabinoid studies at a proportion of nearly 4:1.

The mu opioid receptor gene (OPRM1) is the most commonly studied opioid receptor with regard to addiction. The OPRM1 gene contains several single nucleotide polymorphisms (SNPs) in exon I. Among these, the precoding variation A118G (SNP=rs1799971) is most often associated with an altered phenotype. This polymorphism results in a substitution of the amino acid asparagine by aspartate at position 40 (Asn40Asp, or in one-letter code: N40D). In consequence, it is possible to have the following genotypes (A/A, A/G and G/G). Such polymorphisms can lead to a reduction in the normal activity of this allele. It has been reported that the A118G genotype results in reduced expression of mRNA and lower protein levels [235]. As expected, this SNP is a common minor allele and has been calculated to have a frequency between 10-32% [236]. It would be very difficult to find an association between a widely distributed polymorphism and a specific disorder or disease that occurs in low frequency in the population. For example, subjects of Indian origin show a frequency of 12 % for the 118G allele. Interestingly, this frequency is multiplied by 2.5 fold in opioid-dependent subjects throughout this population [224]. Accordingly, this polymorphism has been associated with opioid dependence in the Indian population, and also in Chinese populations with positive responses for the first use of heroin [226, 227, 237]. It seems that specific phenotypes of this polymorphism can be modulated by gender. For instance, low-activity G alleles, either 118A/G or 118G/G genotypes, are associated with a reduction of the reinforcing value of nicotine, but only in women [238]. The 118G allele also has been related to alcohol sensitivity. In

one study, the craving for alcohol in heavy drinkers was evaluated with several questionnaires. Heterozygotic subjects carrying a copy of the 118G allele reported less alcohol craving and a higher alcohol-induced "high" after an intravenous alcohol challenge [239]. More recently, the polymorphism A118G has been mimicked in mice by the A112G SNP, producing similar biochemical responses as in humans, i.e., reduced mRNA expression [240].

However, some of the results described above have not been replicated using, for example, a German population [237]. In this study, the authors employed two large samples of heroin-dependent and alcohol-dependent German subjects. A statistically significant association was not found between endophenotypes and their genotypes (A118G polymorphism). Other concerns arise when linking the A118G polymorphism to the effects of naltrexone in alcoholic patients. Some studies have found that this polymorphism is responsible for thus opioidergic effect [231, 234] while others have not [230]. Although there are multiple opioid polymorphisms associated with drug abuse (for review, see Mayer and Höllt, [241]), one of the more promising is found in the kappa opioid receptor gene (OPRK1). This mutation is another SNP (36G>T) linked to opioid addiction [242, 243, 230]. Five SNPs in intron two of the OPRK1 gene together with nine SNPs are distributed throughout the PDYN gene, which encodes the ligand prodynorphin (dynorphins derives from this ligand), and has been significantly associated with alcohol dependence [244]. These studies also revealed overtransmitted haplotypes for alcohol-dependent and nonalcohol-dependent individuals, further supporting the association of the PDYN gene.

With regard to the genetics of the endocannabinoid system, one of the first reports identifying significant associations between CNR1 haplotypes and substance abuse phenotypes in three different samples (European-American, African-American and Japanese populations) came from Zhang and colleagues, 2004 [245]. This haplotype was composed of three SNPs (TAG haplotype) and displayed a clear association with polysubstance abuse in European-Americans and African-Americans, and with alcoholism in Japanese samples. In addition, these authors found a reduction in the level of mRNA transcribed from the TAG allele haplotype but not from other CNR1 haplotypes; additionally, the (AAT)n polymorphism of both American samples was associated with polysubstance abuse. Two studies identified that such a microsatellite polymorphism, an (AAT)n triplet repeat near the CNR1 gene, is also associated with cocaine dependence and addiction. In one study, a significant association was found when the number of triplet repeats reached (AAT)12 in an African-Caribbean population [243]. In another study, the significant number of triplet repeats in an American non-Hispanic Caucasian population was greater than or equal to five [246]. In addition, the latter study also found a significant association

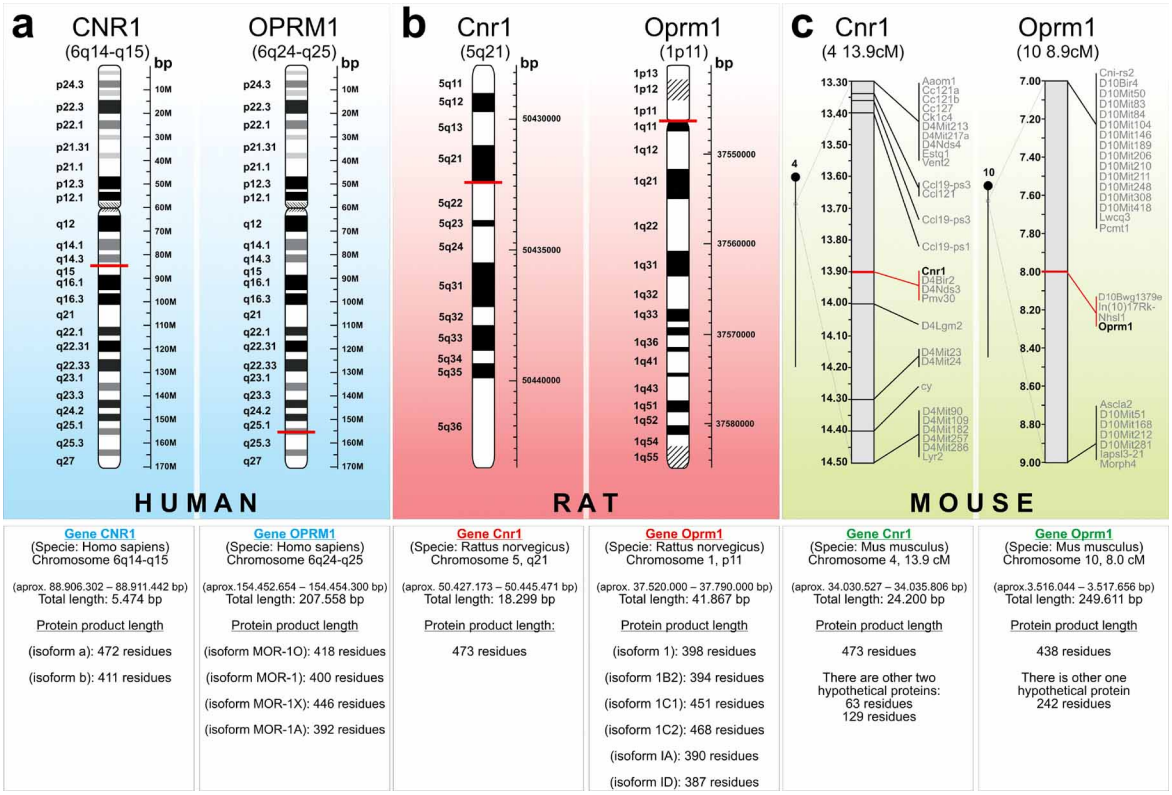

Fig. (3). Schematic and comparative representation of the location of CNR1 (cannabinoid brain receptor type 1 - CB1) and OPRM1 (mu-type opioid receptor type 1 - MOR1) genes in human, rat and mouse. In humans, **3a**, the CNR1 and OPRM1 genes are located on the long arm of chromosome 6 (6q14-q15 and 6q24-q25 respectively), but in rats and mice, they are located at different chromosomal positions. **3b** shows that in rats, the Cnr1 gene is located on chromosome 5 (5q21), and the Oprm1 gene is located on chromosome 1 (1p11). In mice, the Cnr1 gene is located on chromosome 4 (13.9 cM), and Oprm1 gene, on chromosome 10 (8.9 cM) (**3c**). The centromere is placed at the top (black circle) and the telomere at the bottom. The tables below summarize the localization of each gene in every species, the total length of the protein (base-pairs - bp), and the primary different isoforms of these proteins with their corresponding number of amino acids.

between (AAT)≥5 triplet repeats and amphetamine and cannabis dependence. In an unrelated opioid-addicted German population, however, the microsatellite polymorphism with (AAT)≥5 triplet repeats was not associated with intravenous opioid self-administration or opioid addiction [247]. Cravatt *et al.* [248, 249] found a SNP in the human FAAH gene that converts a proline residue to a threonine at position 129 (Pro129Thr, or in one-letter code: C129A) and is associated with drug and alcohol addiction when homozygous. More recently, the role of genetic polymorphisms related to cannabis abstinence, craving and dependence has been analyzed. Marijuana withdrawal has been associated with a specific SNP in the CNR1 gene, whereas marijuana craving is linked to another SNP in the FAAH gene [250]. There are also several more controversial studies regarding genetic polymorphisms within the endocannabinoid system and cannabis dependence. For instance, one study demonstrated that two SNPs in a large sample of individuals (1,923) were associated with cannabis dependence, with SNP rs806380 providing the largest effect [251]. However, another study failed to replicate these results [252].

Thus, taking into account studies of both endogenous systems, we can conclude that there is a lack of evidence showing that a combined haplotype of cannabinoid-opioid polymorphisms could have a significant influence on the drug addiction-phenotype or vulnerability to addiction. Moreover, it remains unclear how such genetic cannabinoid-opioid polymorphisms could confer different sensitivities to psychopharmacological agents. A schematic and comparative representation of the location of CNR1 and OPRM1 genes in humans, rats and mice, as well as their main protein isoforms, is shown in Fig. (**3**).

CONCLUSIONS AND FUTURE DIRECTIONS

CB1 receptors are widely expressed G-protein coupled receptors, and all opioid receptors are similarly coupled to a G-protein complex. This suggests a possible impact of receptor-mediated G-protein signaling. There is plentiful evidence showing that cannabinoid and opioid receptor subtypes co-localize in the presynaptic nerve terminal in brain regions that are highly implicated in drug addiction. As a result, the modulation of behavioral interactions, such as opioid antagonists precipitating withdrawal in cannabinoid-dependent animals (and vice versa) and alcohol-related behaviors mediated by cannabinoid/opioid receptor agonists/antagonists, may arise through functional interactions and co-localization. These interactions have the potential to become a powerful pharmacotherapeutic dual target; however, further studies are needed. A major issue to consider is how genetic interactions and distinct haplotypes in the endogenous cannabinoid and opioid systems can explain the human drug addiction phenotype. This knowledge will allow us to better understand some of the more intriguing aspects of drug addiction such as how different individuals respond in very different ways to the same drug, independent of social context.

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REFERENCES

[1] Repetto M, Menéndez M. Drug addiction to cannabis. Rev Sanid Hig Publica 1972; 46: 155-67.
[2] Zitko BA, Howes JF, Razdan RK, *et al.* Water-soluble derivatives of 1 –tetrahydrocannabinol. Science 1972; 17747: 442-4.
[3] Teale JD, Forman EJ, King LJ, *et al.* Radioimmunoassay of cannabinoids in blood urine. Lancet 1974; 7880: 553-5.
[4] van Ree JM, Slangen JL, de Wied DJ. Intravenous self-administration of drugs in rats. Pharmacol Exp Ther 1978; 2043: 547-57.
[5] Dax EM, Pilotte NS, Adler WH, *et al.* The effects of 9-ene-tetrahydrocannabinol on hormone release immune function. J Steroid Biochem 1989; 341(6): 263-70.
[6] Martin GW, Wilkinson DA, Kapur BM. Validation of self-reported cannabis use by urine analysis. Addict Behav 1988; 132: 147-50.
[7] Cone EJ, Johnson RE, Moore JD, *et al.* Acute effects of smoking marijuana on hormones, subjective effects performance in male human subjects. Pharmacol Biochem Behav 1986; 246: 1749-54.
[8] Cone EJ, Roache JD, Johnson RE. Effects of passive exposure to marijuana smoke. NIDA Res Monogr 1987; 76: 150-6.
[9] Williams JH, Wellman NA, Rawlins JN. Cannabis use correlates with schizotypy in healthy people. Addiction 1996; 6: 869-77.
[10] Ledent C, Valverde O, Cossu G, *et al.* Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 1999; 283: 401-4.
[11] Navarro M, Chowen J, Rocío A, *et al.* CB1 cannabinoid receptor antagonist-induced opiate withdrawal in morphine-dependent rats. Neuroreport 1998; 915: 3397-402.
[12] Comings DE, Muhleman D, Gade R, *et al.* Cannabinoid receptor gene (CNR1): association with i.v. drug use. Mol Psychiatry 1997; 22: 161-8.
[13] Ryberg E, Larsson N, Sjögren S, *et al.* The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol 2007; 152: 1092-101.
[14] Isbell H, Wikler A. Tolerance and addiction liability of 6-dimethylamino-4-4-diphenylheptanone-3 (methadon). J Am Med Assoc 1947; 135: 888-94.
[15] Martin WR. A steric theory of opioid agonists, antagonists, agonist-antagonists, and partial agonists. NIDA Res Monogr 1984; 2002; 49: 16-23.
[16] Pert CB, Snyder SH. Opiate receptor: demonstration in nervous tissue. Science 1973; 17977: 1011-4.
[17] Simon EJ, Hiller JM, Edelman I. Stereospecific binding of the potent narcotic analgesic (3H) Etorphine to rat-brain homogenate. Proc Natl Acad Sci USA 1973; 707: 1947-9.
[18] Terenius L. Characteristics of the "receptor" for narcotic analgesics in synaptic plasma membrane fraction from rat brain. Acta Pharmacol Toxicol 1973; 33: 377-84.
[19] Martin WR, Eades CG, Thompson JA, *et al.* The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. J Pharmacol Exp Ther 1976; 197: 517-32.
[20] Lord JA, Waterfield AA, Hughes J, *et al.* Endogenous opioid peptides: multiple agonists and receptors. Nature 1977; 267: 495-99.
[21] Akil H, Watson SJ, Young E, *et al.* Endogenous opioids: biology and function. Annu Rev Neurosci 1984; 7: 223-55.
[22] Zadina JE, Hackler L, Ge LJ, *et al.* A potent and selective endogenous agonist for the mu-opiate receptor. Nature 1997; 3866624: 499-502.
[23] Kieffer BL. Recent advances in molecular recognition and signal transduction of active peptides: receptors for opioid peptides. Cell Mol Neurobiol 1995; 156: 615-35.
[24] Evans CJ, Keith DE Jr, Morrison H, *et al.* Cloning of a delta opioid receptor by functional expression. Science 1992; 258: 1952-55.
[25] Kieffer BL, Befort K, Gaveriaux RC, *et al.* The delta-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. Proc Natl Acad Sci 1992; 89: 12048-52.

[26] Thompson RC, Mansour A, Akil H, *et al.* Cloning and pharmacological characterization of a rat mu opioid receptor. Neuron 1993; 11: 903-13.

[27] Chen Y, Mestek A, Liu J, *et al.* Molecular cloning and functional expression of a mu-opioid receptor from rat brain. Mol Pharmacol 1993; 44: 8-12.

[28] Meng F, Xie GX, Thompson RC, *et al.* Cloning and pharmacological characterization of a rat kappa opioid receptor. Proc Natl Acad Sci USA 1993; 9021: 9954-8.

[29] Minami M, Onogi T, Toya T, *et al.* Molecular cloning and in situ hybridization histochemistry for rat mu-opioid receptor. Neurosci Res 1994; 18: 315-22.

[30] Minami M, Toya T, Katao Y, *et al.* Cloning and expression of a cDNA for the rat kappa-opioid receptor. FEBS Lett 1993; 329: 291-5.

[31] Yasuda K, Raynor K, Kong H, *et al.* Cloning and functional comparison of kappa and delta opioid receptors from mouse brain. Proc Natl Acad Sci 1993; 90: 6736-40.

[32] Gillman MA. Nitrous oxide, an opioid addictive agent. Review of the evidence. Am J Med 1986; 81: 97-102.

[33] Bunzow JR, Saez C, Mortrud M, *et al.* Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a mu, delta or kappa opioid receptor type. FEBS Lett 1994; 347: 284-8.

[34] Mollereau C, Parmentier M, Mailleux P, *et al*. ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. FEBS Lett 1994; 341: 33-8.

[35] Meunier JC, Mollereau C, Toll L, *et al.* Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. Nature 1995; 377: 532-5.

[36] Reinscheid RK, Nothacker HP, Bourson A, *et al.* Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. Science 1995; 270: 792-4.

[37] Ciccocioppo R, Economidou D, Fedeli A, *et al.* Attenuation of ethanol self-administration and of conditioned reinstatement of alcohol-seeking behaviour by the antiopioid peptide nociceptin/ orphanin FQ in alcohol-preferring rats. Psychopharmacology (Berl) 2004; 172: 170-8.

[38] Paul D, Bodnar RJ, Gistrak MA, *et al.* Different mu receptor subtypes mediate spinal and supraspinal analgesia in mice. Eur J Pharmacol 1989; 168: 307-14.

[39] Zaki PA, Bilsky EJ, Vanderah TW, *et al.* Opioid receptor types and subtypes: the delta receptor as a model. Annu Rev Pharmacol Toxicol 1996; 36: 379-401.

[40] Zukin RZ, Eghali M, Olive D, *et al.* Characterization and visualization of rat and guinea pig brain kappa opioid receptors: evidence for kappa 1 and kappa 2 opioid receptors. Proc Natl Acad Sci USA 1998; 85: 4061-5.

[41] Matthes HW, Maldonado R, Simonin F, *et al.* Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature 1996; 383: 819-23.

[42] Konig M, Zimmer AM, Steiner H, *et al.* Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature 1996; 383: 535-8.

[43] Rubinstein M, Mogil JS, Japòn M, *et al.* Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. Proc Natl Acad Sci USA 1996; 93: 3995-4000.

[44] Sora I, Takahashi N, Funada M, *et al.* Opiate receptor knockout mice define mu receptor roles in endogenous nociceptive responses and morphine-induced analgesia. Proc Natl Acad Sci USA 1997; 94: 1544-9.

[45] Simonin F, Valverde O, Smadja S, *et al.* Disruption of the kappa-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective kappa-agonist U-50,488H and attenuates morphine withdrawal. EMBO J 1998; 17: 886-97.

[46] Zhu Y, King MA, Schuller AGP, *et al.* Retention of supraspinal delta-like analgesia and loss of morphine tolerance in delta opioid receptor knockout mice. Neuron 1999; 24: 243-52.

[47] Filliol D, GhozlS, Chluba J, *et al.* Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses. Nat Genet 2000; 25: 195-200.

[48] Sharifi N, Diehl N, Yaswen L, *et al.* Generation of dynorphin knockout mice. Mol Br Res 2001; 86: 70-5.

[49] Zimmer A, Valjent E, Köning M, *et al*. Absence of delta -9-tetrahydrocannabinol dysphoric effects in dynorphin-deficient mice. J Neurosci 2001; 21: 9499-505.

[50] Rodriguez JJ, Mackie K, Pickel VM. Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat Caudate putamen nucleus. J Neurosci 2001; 21: 823-33.

[51] Salio C, Fischer J, Franzoni MF, *et al.* CB1-cannabinoid and mu-opioid receptor co-localization on postsynaptic target in the rat dorsal horn. Neuroreport 2001; 12: 3689-92.

[52] Pickel VM, Chan J, Kash TL, *et al.* Compartment-specific localization of cannabinoid 1 (CB1) and mu-opioid receptors in rat nucleus accumbens. Neuroscience 2004; 127: 101-12.

[53] Schoffelmeer AN, Hogenboom F, Wardeh G, *et al*. Interactions between CB1 cannabinoid and mu opioid receptors mediating inhibition of neurotransmitter release in rat nucleus accumbens core. Neuropharmacology 2006; 51: 773-81.

[54] Hojo M, Sudo Y, Ando Y, *et al.* mu-Opioid receptor forms a functional heterodimer with cannabinoid CB1 receptor: electrophysiological and FRET assay analysis. J Pharmacol Sci 2008; 108: 308-19.

[55] Rios C, Gomes I, Devi LA. mu opioid and CB1 cannabinoid receptor interactions: reciprocal inhibition of receptor signaling and neuritogenesis. Br J Pharmacol 2006; 148: 385-6.

[56] Yao L, McFarland K, Fan P, *et al.* Adenosine A2a blockade prevents synergy between mu-opiate and cannabinoid CB1 receptors and eliminates heroin-seeking behavior in addicted rats. Proc Natl Acad Sci 2006; 103: 7877-82.

[57] Canals M, Milligan G. Constitutive activity of the cannabinoid CB1 receptor regulates the function of co-expressed Mu opioid receptors. J Biol Chem 2008; 283: 11424-34.

[58] Vasquez C, Lewis DL. The CB1 cannabinoid receptor can sequester G-proteins, making them unavailable to couple to other receptors. J Neurosci 1999; 19: 9271-80.

[59] Ross RA. Allosterism and cannabinoid CB(1) receptors: the shape of things to come. Trends Pharmacol Sci 2007; 28: 567-72.

[60] Fattore L, Viganò D, Fadda P, *et al.* Bidirectional regulation of mu-opioid and CB1-cannabinoid receptor in rats self-administering heroin or WIN 55,212-2. Eur J Neurosci 2007; 25: 2191-200.

[61] Marco EM, Granstrem O, Moreno E, *et al.* Subchronic nicotine exposure in adolescence induces long-term effects on hippocampal and striatal cannabinoid-CB1 and mu-opioid receptors in rats. Eur J Pharmacol 2007; 557: 37-43.

[62] Huang P, Liu-Chen LY, Unterwald EM, *et al.* Hyperlocomotion and paw tremors are two highly quantifiable signs of SR141716-precipitated withdrawal from delta9-tetrahydrocannabinol in C57BL/6 mice. Neurosci Lett 2009; 6: 4651: 66-70.

[63] Lichtman AH, Martin BR. Marijuana withdrawal syndrome in the animal model. J Clin Pharmacol 2002; 4211 Suppl: 20S-7S.

[64] Milin R, Manion I, Dare G, *et al.* Prospective assessment of cannabis withdrawal in adolescents with cannabis dependence: a pilot study. J Am Acad Child Adolesc Psychiatry 2008; 472: 174-8.

[65] Haney M, Hart CL, Vosburg SK, *et al.* Effects of THC and lofexidine in a human laboratory model of marijuana withdrawal and relapse. Psychopharmacol (Berl) 2008; 1971: 157-68.

[66] Chung T, Martin CS, Cornelius JR, *et al.* Cannabis withdrawal predicts severity of cannabis involvement at 1-year follow-up among treated adolescents. Addiction 2008; 1035: 787-99.

[67] Schweinsburg AD, Nagel BJ, Schweinsburg BC, *et al.* Abstinent adolescent marijuana users show altered fMRI response during spatial working memory. Psychiatry Res 2008; 1631: 40-51.

[68] Sneider JT, Pope HG Jr, Silveri MM, *et al.* Differences in regional blood volume during a 28-day period of abstinence in chronic cannabis smokers. Eur Neuropsychopharmacol 2008; 188: 612-9.

[69] Budney AJ, Vandrey RG, Hughes JR, *et al.* Oral delta-9-tetrahydrocannabinol suppresses cannabis withdrawal symptoms. Drug Alcohol Depend 2007; 861: 22-9.

[70] Montoya ID, Vocci F. Novel medications to treat addictive disorders. Curr Psychiatry Rep 2008; 105: 392-8.

[71] Mikasova L, Groc L, Choquet D, *et al.* Altered surface trafficking of presynaptic cannabinoid type 1 receptor in and out synaptic terminals parallels receptor desensitization. Proc Natl Acad Sci USA 2008; 10547: 18596-601.

[72] Wu DF, Yang LQ, Goschke A, *et al.* Role of receptor internalization in the agonist-induced desensitization of cannabinoid type 1 receptors. J Neurochem 2008; 1044: 1132-43.

[73] Martini L, Whistler JL. The role of mu opioid receptor desensitization and endocytosis in morphine tolerance and dependence. Curr Opin NeuroBiol 2007; 175: 556-64.

[74] Daigle TL, Kearn CS, Mackie K. Rapid CB1 cannabinoid receptor desensitization defines the time course of ERK1/2 MAP kinase signaling. Neuropharmacology 2008; 541: 36-44.

[75] Dang VC, Napier IA, Christie MJ. Two distinct mechanisms mediate acute mu-opioid receptor desensitization in native neurons. J Neurosci 2009; 2910: 3322-7.

[76] Kracke GR, Stoneking SP, Ball JM, *et al.* The cannabinoid receptor agonists, anandamide and WIN 55,212-2, do not directly affect mu opioid receptors expressed in Xenopus oocytes. Naunyn Schmiedebergs Arch Pharmacol 2007; 376: 285-93.

[77] Pierce RC, Kumaresan V. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 2006; 302: 215-38.

[78] Melis T, Succu S, Sanna F, *et al.* The cannabinoid antagonist SR 141716A (Rimonabant) reduces the increase of extra-cellular dopamine release in the rat nucleus accumbens induced by a novel high palatable food. Neurosci Lett 2007; 4193: 231-5.

[79] Pfaus JG, Wilkins MF, Dipietro N, *et al.* Inhibitory and disinhibitory effects of psychomotor stimulants and depressants on the sexual behavior of male and female rats. Horm Behav 2009; [Epub ahead of print] doi:10.1016/j.yhbeh.2009.10.004

[80] Hoffman AF, Lupica CR. Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. J Neurophysiol 2001; 85: 72-83.

[81] López-Moreno JA, González-Cuevas G, Moreno G, *et al.* The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction. Addict Biol 2008; 132: 160-87.

[82] López-Moreno JA, Scherma M, Rodríguez de Fonseca F, *et al.* Changed accumbal responsiveness to alcohol in rats pre-treated with nicotine or the cannabinoid receptor agonist WIN 55,212-2. Neurosci Lett 2008; 4331: 1-5.

[83] Diana M, Melis M, Muntoni AL, *et al.* Mesolimbic dopaminergic decline after cannabinoid withdrawal. Proc Natl Acad Sci 1998; 95: 10269-73.

[84] Kolb B, Gorny G, Limebeer CL, *et al.* Chronic treatment with Delta-9-tetrahydrocannabinol alters the structure of neurons in the nucleus accumbens shell and medial prefrontal cortex of rats. Synapse 2006; 606: 429-36.

[85] Solinas M, Justinova Z, Goldberg SR, *et al.* Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. J Neurochem 2006; 982: 408-19.

[86] Melis M, Pillolla G, Luchicchi A, *et al.* Endogenous fatty acid ethanolamides suppress nicotine-induced activation of mesolimbic dopamine neurons through nuclear receptors. J Neurosci 2008a; 2851: 13985-94.

[87] Scherma M, Panlilio LV, Fadda P, *et al.* Inhibition of anandamide hydrolysis by cyclohexyl carbamic acid 3'-carbamoyl-3-yl ester (URB597) reverses abuse-related behavioral and neurochemical effects of nicotine in rats. J Pharmacol Exp Ther 2008; 3272: 482-90.

[88] Hine B, Torrelio M, Gershon S. Interactions between cannabidiol and delta9-THC during abstinence in morphine-dependent rats. Life Sci 1975; 17: 851-7.

[89] Ayhan IH, Kaymakçalan S, Tulunay FC. Interaction between delta 9-tetrahydrocannabinol and morphine on the motor activity of mice. Psychopharmacol (Berl) 1979; 632: 169-72.

[90] Chaperon F, Soubrie P, Puech AJ, *et al.* Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. Psychopharmacology (Berl) 1998; 135: 324-32.

[91] Braida D, Pozzi M, Cavallini R, *et al.* Conditioned place preference induced by the cannabinoid agonist CP 55,940: interaction with the opioid system. Neuroscience 2001a; 104: 923-6.

[92] Braida D, Iosuè S, Pegorini S, *et al.* Delta9-tetrahydrocannabinol-induced conditioned place preference and intracerebroventricular self-administration in rats. Eur J Pharmacol 2004; 506: 63-69.

[93] Singh ME, Verty AN, McGregor IS, *et al.* A cannabinoid receptor antagonist attenuates conditioned place preference but not behavioural sensitization to morphine. Brain Res 2004; 1026: 244-53.

[94] Skoubis PD, Maidment NT. Blockade of ventral pallidal opioid receptors induces a conditioned place aversion and attenuates acquisition of cocaine place preference in the rat. Neuroscience 2003; 119: 241-9.

[95] Zarrindast MR, Nouri M, Ahmadi S. Cannabinoid CB1 receptors of the dorsal hippocampus are important for induction of conditioned place preference (CPP) but do not change morphine CPP. Brain Res 2007; 1163: 130-7.

[96] Skoubis PD, Mathes HW, Walwyn WM, *et al.* Naloxone fails to produce conditioned place aversion in mu-opioid receptor knock-out mice. Neuroscience 2001; 106: 757-63.

[97] Skoubis PD, Lam HA, Shoblock J, *et al.* Endogenous enkephalins, not endorphins, modulate basal hedonic state in mice. Eur J Neurosci 2005; 21: 1379-84.

[98] Cossu G, Ledent C, Fattore L, *et al.* Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. Behav Brain Res 2001; 118: 61-5.

[99] Rice OV, Gordon N. Gifford AN. Conditioned place preference to morphine in cannabinoid CB1 receptor knockout mice. Brain Res 2002; 945: 135-8.

[100] Martin M, Ledent C, Parmentier M, *et al.* Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice. Eur J Neurosci 2000; 12: 4038-46.

[101] Ghozland S, Matthes HW, Simonin F, *et al.* Motivational effects of cannabinoids are mediated by mu-opioid and kappa-opioid receptors. Neuroscience 2002; 22: 1146-54.

[102] Mendizábal V, Zimmer A, Maldonado R. Involvement of kappa/dynorphin system in WIN 55,212-2 self-administration in mice. Neuropsychopharmacology 2006; 31: 1957-66.

[103] Solinas M, Panlilio LV, Tanda G, *et al.* Cannabinoid agonists but not inhibitors of endogenous cannabinoid transport or metabolism enhance the reinforcing efficacy of heroin in rats. Neuropsychopharmacology 2005; 30: 2046-57.

[104] Rubio P, Rodriguez de Fonseca F, Martín-Calderon JL, *et al.* Maternal exposure to low doses of delta9-tetrahydrocannabinol facilitates morphine-induced place conditioning in adult male offspring. Pharmacol Biochem Behav 1998; 61: 229-38.

[105] Ellgren M, Spano SM, Hurd YL. Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. Neuropsychopharmacology 2007; 32: 607-15.

[106] Spano MS, Ellgren M, Wang X, *et al.* Prenatal cannabis exposure increases heroin seeking with allostatic changes in limbic enkephalin systems in adulthood. Biol Psychiatry 2007; 61: 554-63.

[107] Riley MA, Vathy I. Mid- to late gestational morphine exposure does not alter the rewarding properties of morphine in adult male rats. Neuropharmacology 2006; 51: 295-304.

[108] Vela G, Ruiz-Gayo M, Fuentes JA. Anandamide decreases naloxone-precipitated withdrawal signs in mice chronically treated with morphine. Neuropharmacology 1995; 34: 665-8.

[109] Valverde O, Noble F, Beslot F, *et al.* Delta9-tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. Eur J Neurosci 2001; 13: 1816-24.

[110] Lichtman AH, Sheikh SM, Loh HH, *et al.* Opioid and cannabinoid modulation of precipitated withdrawal in delta(9)-tetrahydrocannabinol and morphine-dependent mice. J Pharmacol Exp Ther 2001; 298: 1007-114.

[111] Navarro M, Carrera MR, Fratta W, *et al.* Functional interaction between opioid and cannabinoid receptors in drug self-administration. J Neurosci 2001; 21: 5344-50.

[112] Castañé A, Robledo P, Matifas A, *et al.* Cannabinoid withdrawal syndrome is reduced in double mu and delta opioid receptor knockout mice. Eur J Neurosci 2003; 17: 155: 159.

[113] Valverde O, Maldonado R, Valjent E, *et al.* Reduction of stress-induced analgesia but not of exogenous opioid effects in mice lacking CB1 receptors. J Neurosci 2000; 20: 9284-9.

[114] Lepore M, Vorel SR, Lowinson J, *et al.* Conditioned place preference induced by delta 9-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. Life Sci 1995; 56: 2073-80.

[115] Vann RE, Gamage TF, Warner JA, *et al.* Divergent effects of cannabidiol on the discriminative stimulus and place conditioning effects of Delta(9)-tetrahydrocannabinol. Drug Alcohol Depend 2008; 94: 191-8.

[116] Azizi P, Haghparast A, Hassanpour-Ezatti M. Effects of CB1 receptor antagonist within the nucleus accumbens on the acquisition and expression of morphine-induced conditioned place preference in morphine-sensitized rats. Behav Brain Res 2009; 197: 119-24.

[117] Mas-Nieto M, Pommier B, Tzavara ET, *et al.* Reduction of opioid dependence by the CB(1) antagonist SR141716A in mice: evaluation of the interest in pharmacotherapy of opioid addiction. Br J Pharmacol 2001; 132: 1809-16.

[118] Braida D, Limonta V, Pegorini S, *et al.* Hallucinatory and rewarding effect of salvinorin A in zebrafish: kappa-opioid and CB1-cannabinoid receptor involvement. Psychopharmacology (Berl) 2007; 190: 441-8.

[119] Huang EY, Chen CM, Tao PL. Supraspinal anti-allodynic and rewarding effects of endomorphins in rats. Peptides 2004; 25: 577-83.

[120] Tao PL, Liang KW, Sung WY, *et al.* Nalbuphine is effective in decreasing the rewarding effect induced by morphine in rats. Drug Alcohol Depend 2006; 84: 175-81.

[121] Sakoori K, Murphy NP. Expression of morphine-conditioned place preference is more vulnerable than naloxone-conditioned place aversion to disruption by nociceptin in mice. Neurosci Lett 2008; 443: 108-12.

[122] Shoblock JR, Wichmann J, Maidment NT. The effect of a systemically active ORL-1 agonist, Ro 64-6198, on the acquisition, expression, extinction, and reinstatement of morphine conditioned place preference. Neuropharmacology 2005; 49: 439-46.

[123] Guo N, Garcia MM, Taylor BK, *et al.* Blockade of micro-opioid receptors in the medial thalamus inhibits acquisition, but not expression, of morphine-induced conditioned place preference. Neuroscience 2008; 151: 948-54.

[124] Marquez P, Baliram R, Kieffer BL, *et al.* The mu opioid receptor is involved in buprenorphine-induced locomotor stimulation and conditioned place preference. Neuropharmacology 2007; 52: 1336-41.

[125] Martellotta MC, Cossu G, Fattore L, *et al.* Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naive mice. Neuroscience 1998; 85: 327-30.

[126] Fattore L, Cossu G, Martellotta CM, *et al.* Intravenous self-administration of the cannabinoid CB1 receptor agonist WIN 55,212-2 in rats. Psychopharmacology (Berl) 2001; 156: 410-6.

[127] Braida D, Pozzi M, Parolaro D, *et al.* Intracerebral self-administration of the cannabinoid receptor agonist CP 55,940 in the rat: interaction with the opioid system. Eur J Pharmacol 2001b; 413: 227-34.

[128] Solinas M, Panlilio LV, Goldberg SR. Exposure to delta-9-tetrahydrocannabinol (THC) increases subsequent heroin taking but not heroin's reinforcing efficacy: a self-administration study in rats. Neuropsychopharmacology 2004; 29: 1301-11.

[129] Justinova Z, Mangieri RA, Bortolato M, *et al.* Fatty acid amide hydrolase inhibition heightens anandamide signaling without producing reinforcing effects in primates. Biol Psychiatry 2008; 64: 930-7.

[130] Vela G, Martín S, García-Gil L, *et al.* 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 1998; 807: 101-9.

[131] González B, de Miguel R, Martin S, *et al.* Effects of perinatal exposure to delta 9-tetrahydrocannabinol on operant morphine-reinforced behavior. Pharmacol Biochem Behav 2003; 75: 577-84.

[132] Solinas M, Panlilio LV, Antoniou K, *et al.* The cannabinoid CB1 antagonist N-piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl) -4-methylpyrazole-3-carboxamide (SR-141716A) differentially alters the reinforcing effects of heroin under continuous reinforcement, fixed ratio, and progressive ratio schedules of drug self-administration in rats. J Pharmacol Exp Ther 2003; 306: 93-102.

[133] De Vries TJ, Homberg JR, Binnekade R, *et al.* Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. Psychopharmacology (Berl) 2003; 168: 164-9.

[134] Sanchis-Segura C, Cline BH, Marsicano G, *et al.* Reduced sensitivity to reward in CB1 knockout mice. Psychopharmacology (Berl) 2004; 176: 223-32.

[135] Justinova Z, Tanda G, Munzar P, *et al.* The opioid antagonist naltrexone reduces the reinforcing effects of Delta 9 tetrahydrocannabinol (THC) in squirrel monkeys. Psychopharmacol (Berl) 2004; 1731-2: 186-94.

[136] Gardner EL, Paredes W, Smith D, *et al.* Facilitation of brain stimulation reward by delta 9-tetrahydrocannabinol. Psychopharmacology (Berl) 1988; 96: 142-4.

[137] Lepore M, Liu X, Savage V, *et al.* 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 1996; 58: 365-72.

[138] Vlachou S, Nomikos GG, Panagis G. WIN 55,212-2 decreases the reinforcing actions of cocaine through CB1 cannabinoid receptor stimulation. Behav Brain Res 2003; 141: 215-22.

[139] Vlachou S, Nomikos GG, Panagis G. CB1 cannabinoid receptor agonists increase intracranial self-stimulation thresholds in the rat. Psychopharmacology (Berl) 2005; 179: 498-508.

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

[141] Vlachou S, Nomikos GG, Stephens DN, *et al.* Lack of evidence for appetitive effects of Delta 9-tetrahydrocannabinol in the intracranial self-stimulation and conditioned place preference procedures in rodents. Behav Pharmacol 2007; 18: 311-9.

[142] Deroche-Gamonet V, Le Moal M, Piazza PV, *et al*. SR141716, a CB1 receptor antagonist, decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. Psychopharmacology (Berl) 2001; 157: 254-9.

[143] Corbett D. Chronic morphine fails to enhance the reward value of prefrontal cortex self-stimulation. Pharmacol Biochem Behav 1992; 42: 451-5.

[144] Hayes RJ, Gardner EL. The basolateral complex of the amygdala mediates the modulation of intracranial self-stimulation threshold by drug-associated cues. Eur J Neurosci 2004; 20: 273-80.

[145] Schaefer GJ, Michael RP. Morphine withdrawal produces differential effects on the rate of lever-pressing for brain self-stimulation in the hypothalamus and midbrain in rats. Pharmacol Biochem Behav 1983; 18: 571-657.

[146] Bruijnzeel AW, Marcinkiewcz C, Isaac S, *et al.* The effects of buprenorphine on fentanyl withdrawal in rats. Psychopharmacology (Berl) 2007; 191: 931-41.

[147] Todtenkopf MS, Marcus JF, Portoghese PS, *et al.* Effects of kappa-opioid receptor ligands on intracranial self-stimulation in rats. Psychopharmacol (Berl) 2004; 172: 463-70.

[148] Tomasiewicz HC, Todtenkopf MS, Chartoff EH, *et al.* The kappa-opioid agonist U69,593 blocks cocaine-induced enhancement of brain stimulation reward. Biol Psychiatry 2008; 64: 982-8.

[149] Carlezon WA, Beguin C, DiNieri JA, *et al.* Depressive-like effects of the kappa-opioid receptor agonist salvinorin A on behavior and neurochemistry in rats. J Pharmacol Exp Ther 2006; 316: 440-7.

[150] Trujillo KA, Belluzzi JD, Stein L. Opiate antagonists and self-stimulation: extinction-like response patterns suggest selective reward deficit. Brain Res 1989; 492: 15-28.

[151] Hawkins M, Stein EA. Effects of continuous naloxone administration on ventral tegmental self-stimulation. Brain Res 1991; 560: 315-20.

[152] Easterling KW, Holtzman SG. In rats, acute morphine dependence results in antagonist-induced response suppression of intracranial self-stimulation. Psychopharmacology (Berl) 2004; 175: 287-95.

[153] Tsou K, Patrick SL, Walker JM. Physical withdrawal in rats tolerant to delta 9-tetrahydrocannabinol precipitated by a cannabinoid receptor antagonist. Eur J Pharmacol 1995; 280: 13 -5.

[154] Diana M, Melis M, Muntoni AL, *et al.* Mesolimbic dopaminergic decline after cannabinoid withdrawal. Proc Natl Acad Sci 1998; 95: 10269-73.

[155] Hutcheson DM, Tzavara ET, Smadja C, *et al.* Behavioural and biochemical evidence for signs of abstinence in mice chronically treated with delta-9-tetrahydrocannabinol. Br J Pharmacol 1998; 125: 1567-77.

[156] González S, Fernández-Ruiz J, Di Marzo V, *et al.* Behavioral and molecular changes elicited by acute administration of SR141716 to Delta9-tetrahydrocannabinol-tolerant rats: an experimental model of cannabinoid abstinence. Drug Alcohol Depend 2004; 74: 159-70.

[157] Rubino T, Massi P, Vigano D, *et al.* Long-term treatment with SR141716A, the CB1 receptor antagonist, influences morphine withdrawal syndrome. Life Sci 2000; 66: 2213-9.

[158] Castañé A, Maldonado R, Valverde O. Role of different brain structures in the behavioural expression of WIN 55,212-2 withdrawal in mice. Br J Pharmacol 2004; 8: 1309-17.

[159] Trang T, Ma W, Chabot JG, *et al.* Spinal modulation of calcitonin gene-related peptide by endocannabinoids in the development of opioid physical dependence. Pain 2006; 126: 256-71.

[160] Berrendero F, Castañe A, Ledent C, *et al.* Increase of morphine withdrawal in mice lacking A2a receptors and no changes in CB1/A2a double knockout mice. Eur J Neurosci 2003; 17: 315-24.

[161] Kaymakcalan S, Ayhan IH, Tulunay FC. Naloxone-induced or postwithdrawal abstinence signs in delta9-tetrahydrocannabinol-tolerant rats. Psychopharmacology (Berl) 1977; 55: 243-9.

[162] Vela G, Fuentes JA, Bonnin A, *et al.* Perinatal exposure to delta 9-tetrahydrocannabinol (delta 9-THC) leads to changes in opioid-related behavioral patterns in rats. Brain Res 1995b; 680: 142-7.

[163] Maldonado R, Stinus L, Gold LH, *et al.* Role of different brain structures in the expression of the physical morphine withdrawal syndrome. J Pharmacol Exp Ther 1992; 261: 669-77.

[164] Le Guen S, Gestreau C, Besson JM. Morphine withdrawal precipitated by specific mu, delta or kappa opioid receptor antagonists: a c-Fos protein study in the rat central nervous system. Eur J Neurosci 2003; 17: 2425-37.

[165] Fattore L, Spano MS, Cossu G, *et al.* Cannabinoid mechanism in reinstatement of heroin-seeking after a long period of abstinence in rats. Eur J Neurosci 2003; 17: 1723-6.

[166] Fattore L, Spano S, Cossu G, *et al.* Cannabinoid CB(1) antagonist SR 141716A attenuates reinstatement of heroin self-administration in heroin-abstinent rats. Neuropharmacology 2005; 48: 1097-104.

[167] Spano MS, Fattore L, Cossu G, *et al.* CB1 receptor agonist and heroin, but not cocaine, reinstate cannabinoid-seeking behaviour in the rat. Br J Pharmacol 2004; 143: 343-50.

[168] Rubino T, Vigano D, Massi P, *et al.* The psychoactive ingredient of marijuana induces behavioural sensitization. Eur J Neurosci 2001; 14: 884-6.

[169] Varvel SA, Martin BR, Lichtman AH. Lack of behavioral sensitization after repeated exposure to THC in mice and comparison to methamphetamine. Psychopharmacology (Berl) 2007; 193: 511-9.

[170] Norwood CS, Cornish JL, Mallet PE, *et al.* Pre-exposure to the cannabinoid receptor agonist CP 55940 enhances morphine behavioral sensitization and alters morphine self-administration in Lewis rats. Eur J Pharmacol 2003; 465: 105-14.

[171] Cadoni C, Pisanu A, Solinas M, *et al.* Behavioural sensitization after repeated exposure to Delta 9-tetrahydrocannabinol and cross-sensitization with morphine. Psychopharmacology (Berl) 2001; 158: 259-66.

[172] Pontieri FE, Monnazzi P, Scontrini A, *et al.* Behavioral sensitization to WIN55212.2 in rats pretreated with heroin. Brain Res 2001a; 898: 178-80.

[173] Lamarque S, Taghzouti K, Simon H. Chronic treatment with Delta(9)-tetrahydrocannabinol enhances the locomotor response to amphetamine and heroin. Implications for vulnerability to drug addiction. Neuropharmacology 2001; 41: 118-29.

[174] Pontieri FE, Monnazzi P, Scontrini A, *et al.* Behavioral sensitization to heroin by cannabinoid pretreatment in the rat. Eur J Pharmacol 2001b; 421: 1-3.

[175] Bloom AS, Dewey WL. A comparison of some pharmacological actions of morphine and delta9-tetrahydrocannabinol in the mouse. Psychopharmacology (Berl) 1978; 57: 243-8.

[176] Hansson AC, Bermudez-Silva FJ, Malinen H, *et al.* Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. Neuropsychopharmacology 2007; 32: 117-26.

[177] Melis M, Pillolla G, Perra S, *et al.* Electrophysiological properties of dopamine neurons in the ventral tegmental area of Sardinian alcohol-preferring rats. Psychopharmacology (Berl) 2008b; 201: 471-81.

[178] Cippitelli A, Bilbao A, Hansson AC, *et al*. Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. Eur J Neurosci 2005; 21: 2243-51.

[179] Economidou D, Hansson AC, Weiss F, *et al.* Dysregulation of nociceptin/orphanin FQ activity in the amygdala is linked to excessive alcohol drinking in the rat. Biol Psychiatry 2008; 64: 211-8.

[180] Soini SL, Honkanen A, Hyytiä P, *et al.* [3H]ethylketocyclazocine binding to brain opioid receptor subtypes in alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol 1999; 18: 27-34.

[181] Sommer W, Hyytiä P, Kiianmaa K. The alcohol-preferring AA and alcohol-avoiding ANA rats: neurobiology of the regulation of alcohol drinking. Addict Biol 2006; 11: 289-309.

[182] Hungund BL, Szakall I, Adam A, *et al.* Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. J Neurochem 2003; 84: 698-704.

[183] Naassila M, Pierrefiche O, Ledent C, *et al.* Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. Neuropharmacology 2004; 46: 243-53.

[184] Thanos PK, Dimitrakakis ES, Rice O, *et al.* Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors. Behav Brain Res 2005; 164: 206-13.

[185] Houchi H, Babovic D, Pierrefiche O, *et al.* CB1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. Neuropsychopharmacology 2005; 302: 339-49.

[186] Warnault V, Houchi H, Barbier E, *et al.* The lack of CB1 receptors prevents neuroadapatations of both NMDA and GABA(A) receptors after chronic ethanol exposure. J Neurochem 2007; 1023: 741-52.

[187] Roberts AJ, McDonald JS, Heyser CJ, *et al.* mu-Opioid receptor knockout mice do not self-administer alcohol. J Pharmacol Exp Ther 2000; 293: 1002-8.

[188] Becker A, Grecksch G, Kraus J, *et al.* Rewarding effects of ethanol and cocaine in mu opioid receptor-deficient mice. Schmiedebergs Arch Pharmacol 2002; 365: 296-302.

[189] Ghozland S, Chu K, Kieffer BL, *et al.* Lack of stimulant and anxiolytic-like effects of ethanol and accelerated development of ethanol dependence in mu-opioid receptor knockout mice. Neuropharmacol 2005; 49: 493-501.

[190] Kovacs KM, Szakall I, O'Brien D, *et al.* Decreased oral self-administration of alcohol in kappa-opioid receptor knock-out mice. Alcohol Clin Exp Res 2005; 29: 730-8.

[191] Grisel JE, Mogil JS, Grahame NJ, *et al.* Ethanol oral self-administration is increased in mutant mice with decreased beta-endorphin expression. Brain Res 1999; 835: 62-7.

[192] Roberts AJ, Gold LH, Polis I, *et al.* Increased ethanol self-administration in delta-opioid receptor knockout mice. Alcohol Clin Exp Res 2001; 25: 1249-56.

[193] Grahame NJ, Low MJ, Cunningham CL. Intravenous self-administration of ethanol in beta-endorphin-deficient mice. Alcohol Clin Exp Res 1998; 22: 1093-8.

[194] Hayward MD, Hansen ST, Pintar JE, *et al.* Operant self-administration of ethanol in C57BL/6 mice lacking beta-endorphin and enkephalin. Pharmacol Biochem Behav 2004; 79: 171-81.

[195] Racz I, Schürmann B, Karpushova A, *et al.* The opioid peptides enkephalin and beta-endorphin in alcohol dependence. Biol Psychiatry 2008; 1;6411: 989-97.

[196] López-Moreno JA, González-Cuevas G, Rodríguez de Fonseca F, *et al.* Long-lasting increase of alcohol relapse by the cannabinoid receptor agonist WIN 55,212-2 during alcohol deprivation. J Neurosci 2004; 24:8245-52.

[197] Alén F, Santos A, Moreno-Sanz G, *et al.* Cannabinoid-induced increase in relapse-like drinking is prevented by the blockade of the glycine-binding site of N-methyl-D-aspartate receptors. Neuroscience 2009; 158: 465-73.

[198] Gallate JE, Saharov T, Mallet PE, *et al.* Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. Eur J Pharmacol 1999; 370: 233-40.

[199] McGregor IS, Dam KD, Mallet PE, *et al.* Delta9-THC reinstates beer- and sucrose-seeking behaviour in abstinent rats: comparison with midazolam, food deprivation and predator odour. Alcohol Alcohol 2005; 40: 35-45.

[200] Colombo G, Serra S, Vacca G, *et al.* Endocannabinoid system and alcohol addiction: pharmacological studies. Pharmacol Biochem Behav 2005; 81: 369-80.

[201] Rodríguez de Fonseca F, Roberts AJ, Bilbao A, *et al.* Cannabinoid receptor antagonist SR141716A decreases operant ethanol self administration in rats exposed to ethanol-vapor chambers. Zhongguo Yao Li Xue Bao 1999; 20: 1109-14.

[202] Colombo G, Vacca G, Serra S, *et al.* Suppressing effect of the cannabinoid CB1 receptor antagonist, SR 141716, on alcohol's

motivational properties in alcohol-preferring rats. Eur J Pharmacol 2004; 498: 119-23.

[203] Gessa GL, Serra S, Vacca G, *et al.* Suppressing effect of the cannabinoid CB1 receptor antagonist, SR147778, on alcohol intake and motivational properties of alcohol in alcohol-preferring sP rats. Alcohol Alcohol 2005; 40: 46-53.

[204] Economidou D, Mattioli L, Cifani C, *et al.* Effect of the cannabinoid CB1 receptor antagonist SR-141716A on ethanol self-administration and ethanol-seeking behaviour in rats. Psychopharmacol (Berl) 2006; 183: 394-403.

[205] Cippitelli A, Bilbao A, Gorriti MA, *et al.* The anandamide transport inhibitor AM404 reduces ethanol self-administration. Eur J Neurosci 2007; 26: 476-86.

[206] Lallemand F, Soubrié P, De Witte P. Effects of CB1 cannabinoid receptor blockade on ethanol preference after chronic alcohol administration combined with repeated re-exposures and withdrawals. Alcohol Alcohol 2004; 39: 486-92.

[207] Lallemand F, Soubrié PH, De Witte PH. Effects of CB1 cannabinoid receptor blockade on ethanol preference after chronic ethanol administration. Alcohol Clin Exp Res 2001; 25: 1317-23.

[208] Malinen H, Hyytiá P. Ethanol self-administration is regulated by CB1 receptors in the nucleus accumbens and ventral tegmental area in alcohol-preferring AA rats. Alcohol Clin Exp Res 2008; 32: 1976-83.

[209] Le Foll B, Goldberg SR. Cannabinoid CB1 receptor antagonists as promising new medications for drug dependence. J Pharmacol Exp Ther 2005; 312: 875-83.

[210] Basavarajappa BS. The endocannabinoid signaling system: a potential target for next-generation therapeutics for alcoholism. Mini Rev Med Chem 2007; 7: 769-79.

[211] Colombo G, Orrù A, Lai P, *et al*. The cannabinoid CB1 receptor antagonist, rimonabant, as a promising pharmacotherapy for alcohol dependence: preclinical evidence. Mol Neurobiol 2007; 36:102-12.

[212] Alen F, Moreno-Sanz G, Isabel de Tena A, *et al.* Pharmacological activation of CB1 and D2 receptors in rats: predominant role of CB1 in the increase of alcohol relapse. Eur J Neurosci 2008; 27: 3292-8.

[213] Marinelli PW, Funk D, Juzytsch W, *et al.* The CRF1 receptor antagonist antalarmin attenuates yohimbine-induced increases in operant alcohol self-administration and reinstatement of alcohol seeking in rats. Eur J Neurosci 2007; 26: 2815-23.

[214] Burattini C, Gill TM, Aicardi G, *et al.* The ethanol self-administration context as a reinstatement cue: acute effects of naltrexone. Neuroscience 2006; 139: 877-87.

[215] Ciccocioppo R, Martin-Fardon R, Weiss F. Effect of selective blockade of mu(1) or delta opioid receptors on reinstatement of alcohol-seeking behavior by drug-associated stimuli in rats. Neuropsychopharmacology 2002; 27: 391-9.

[216] Sharpe AL, Samson HH. Effect of naloxone on appetitive and consummatory phases of ethanol self-administration. Alcohol Clin Exp Res 2001; 25: 1006-11.

[217] Kamdar NK, Miller SA, Syed YM, *et al.* Acute effects of naltrexone and GBR 12909 on ethanol drinking-in-the-dark in C57BL/6J mice. Psychopharmacology (Berl) 2007; 192: 207-17.

[218] Cichelli MJ, Lewis MJ. Naloxone nonselective suppression of drinking of ethanol, sucrose, saccharin, and water by rats. Pharmacol Biochem Behav 2002; 72: 699-706.

[219] Doyon WM, Howard EC, Shippenberg TS, *et al.* Kappa-opioid receptor modulation of accumbal dopamine concentration during operant ethanol self-administration. Neuropharmacology 2006; 51: 487-96.

[220] Mitchell JM, Liang MT, Fields HL. A single injection of the kappa opioid antagonist norbinaltorphimine increases ethanol consumption in rats. Psychopharmacology (Berl) 2005; 182: 384-92.

[221] Hölter SM, Henniger MS, Lipkowski AW, *et al.* Kappa-opioid receptors and relapse-like drinking in long-term ethanol-experienced rats. Psychopharmacology (Berl) 2000; 153: 93-102.

[222] Walker BM, Koob GF. Pharmacological evidence for a motivational role of kappa-opioid systems in ethanol dependence. Neuropsychopharmacology 2008; 33: 643-52.

[223] Koob GF, Roberts AJ, Kieffer BL, *et al.* Animal models of motivation for drinking in rodents with a focus on opioid receptor neuropharmacology. Recent Dev Alcohol 2003; 16: 263-81.

[224] Kapur S, Sharad S, Singh RA, *et al*. A118g polymorphism in mu opioid receptor gene (oprm1): association with opiate addiction in subjects of Indian origin. J Integr Neurosci 2007; 6: 511-22.

[225] Bjarnadóttir TK, Gloriam DE, HellstrSH, *et al.* Comprehensive repertoire and phylogenetic analysis of the G protein-coupled receptors in human and mouse. Genomics 2006; 883: 263-73.

[226] Zhang D, Shao C, Shao M, *et al.* Effect of mu-opioid receptor gene polymorphisms on heroin-induced subjective responses in a Chinese population. Biol Psychiatry 2007; 61:1244-51.

[227] Yuferov V, Fussell D, LaForge KS, *et al*. Redefinition of the human kappa opioid receptor gene (OPRK1) structure and association of haplotypes with opiate addiction. Pharmacogenetics 2004; 14: 793-804.

[228] Oosterhuis BE, LaForge KS, Proudnikov D, *et al*. Catechol-O-methyltransferase (COMT) gene variants: possible association of the Val158Met variant with opiate addiction in Hispanic women. Am J Med Genet B Neuropsychiatr Genet 2008; 147B: 793-8.

[229] Chen AC, LaForge KS, Ho A, *et al.* Potentially functional polymorphism in the promoter region of prodynorphin gene may be associated with protection against cocaine dependence or abuse. Am J Med Genet 2002; 114: 429-35.

[230] Tidey JW, Monti PM, Rohsenow DJ, *et al.* Moderators of naltrexone's effects on drinking, urge, and alcohol effects in non-treatment-seeking heavy drinkers in the natural environment. Alcohol Clin Exp Res 2008; 32: 58-66.

[231] Oslin DW, Berrettini WH, O'Brien CP. Targeting treatments for alcohol dependence: the pharmacogenetics of naltrexone. Addict Biol 2006; 11: 397-403.

[232] Kobayashi H, Hata H, Ujike H, *et al.* Association analysis of delta-opioid receptor gene polymorphisms in methamphetamine dependence/psychosis. Am J Med Genet B Neuropsychiatr Genet 2006; 141B: 482-6.

[233] Haile CN, Kosten TA, Kosten TR. Pharmacogenetic treatments for drug addiction: alcohol and opiates. Am J Drug Alcohol Abuse 2008; 34: 355-81.

[234] McGeary JE, Monti PM, Rohsenow DJ, *et al.* Genetic moderators of naltrexone's effects on alcohol cue reactivity. Alcohol Clin Exp Res 2006; 30:1288-96.

[235] Zhang Y, Wang D, Johnson AD, *et al.* Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. J Biol Chem 2005; 280: 32618-24.

[236] Shi J, Hui L, Xu Y, *et al*. Sequence variations in the mu-opioid receptor gene (OPRM1) associated with human addiction to heroin. Hum Mutat 2002; 19: 459-60.

[237] Franke P, Wang T, Nöthen MM, *et al.* Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. Am J Med Genet 2001; 105: 114-9.

[238] Ray R, Jepson C, Patterson F, *et al.* Association of OPRM1 A118G variant with the relative reinforcing value of nicotine. Psychopharmacology (Berl) 2006; 1883: 355-63.

[239] Ray LA, Hutchison KE. Effects of naltrexone on alcohol sensitivity and genetic moderators of medication response: a double-blind placebo-controlled study. Arch Gen Psychiatry 2007; 649: 1069-77.

[240] Mague SD, Isiegas C, Huang P, *et al.* Mouse model of OPRM1 (A118G) polymorphism has sex-specific effects on drug-mediated behavior. Proc Natl Acad Sci USA 2009; 10626: 10847-52.

[241] Mayer P, Höllt V. Pharmacogenetics of opioid receptors and addiction. Pharmacogenet Genom 2006; 16: 1-7.

[242] Gerra G, Leonardi C, Cortese E, *et al.* Human kappa opioid receptor gene (OPRK1) polymorphism is associated with opiate addiction. Am J Med Genet B Neuropsychiatr Genet 2007; 144B: 771-5.

[243] Ballon N, Leroy S, Roy C, *et al*. (AAT)n repeat in the cannabinoid receptor gene (CNR1): association with cocaine addiction in an African-Caribbean population. Pharmacogenomics 2006; J 6: 126-30.

[244] Xuei X, Dick D, Flury-Wetherill L, *et al.* Association of the kappa-opioid system with alcohol dependence. Mol Psychiatry 2006; 1111: 1016-24.

[245] Zhang PW, Ishiguro H, Ohtsuki T, *et al.* Human cannabinoid receptor 1: 5' exons, candidate regulatory regions, polymorphisms, haplotypes and association with polysubstance abuse. Mol Psychiatry 2004; 910: 916-31.

[246] Comings DE, Blum K. Reward deficiency syndrome: genetic aspects of behavioral disorders. Prog Brain Res 2000; 126: 325-41.

[247] Heller D, Schneider U, Seifert J, *et al.* The cannabinoid receptor gene (CNR1) is not affected in German i.v. drug users. Addict Biol 2001; 6: 183-7.

[248] Chiang KP, Gerber AL, Sipe JC, *et al.* Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. Hum Mol Genet 2004; 13: 2113-9.

[249] Sipe JC, Chiang K, Gerber AL, *et al.* A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci USA 2002; 99: 8394-9.

[250] Haughey HM, Marshall E, Schacht JP, *et al.* Marijuana withdrawal and craving: influence of the cannabinoid receptor 1 (CNR1) and fatty acid amide hydrolase (FAAH) genes. Addiction 2008; 10310: 1678-86.

[251] Agrawal A, Wetherill L, Dick DM, *et al.* Evidence for association between polymorphisms in the cannabinoid receptor 1 (CNR1) gene and cannabis dependence. Am J Med Genet B Neuropsychiatr Genet 2009; 150B5: 736-40.

[252] Hartman CA, Hopfer CJ, Haberstick B, *et al.* The association between cannabinoid receptor 1 gene (CNR1) and cannabis dependence symptoms in adolescents and young adults. Drug Alcohol Depend 2009; 1041-2: 11-6.

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