Addiction Biology

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The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction

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ABSTRACT

Addiction is a chronic, recurring and complex disorder. It is characterized by anomalous behaviors that are linked to permanent or long-lasting neurobiological alterations. Furthermore, the endocannabinoid system has a crucial role in mediating neurotransmitter release as one of the main neuromodulators of the mammalian central nervous system. The purpose of the present review is to instruct readers about the functional and structural interactions between the endocannabinoid system and the main neurotransmitter systems of the central nervous system in the context of drug addiction. With this aim, we have systematically reviewed the main findings of most of the existing literature that explores cross-talk in the five brain areas that are most traditionally implicated in addiction: amygdala, prefrontal cortex, nucleus accumbens, hippocampus and ventral tegmental area (VTA). The neurotransmission systems influenced by the pharmacology of the endocannabinoid system in these brain areas, which are reviewed here, are gamma-aminobutyric acid (GABA), glutamate, the main biogenic amines (dopamine, noradrenaline and serotonin), acetylcholine and opioids. We show that all of these neurotransmitter systems can be modulated differentially in each brain area by the activation or deactivation of cannabinoid CB1 brain receptors. Specifically, most of the studies relate to the hippocampus and nucleus accumbens. Moreover, the neurotransmitter with the fewest number of related studies is acetylcholine (excepting in the hippocampus), whereas there is a large number that evaluates GABA, glutamate and dopamine. Finally, we propose a possible interpretation of the role of the endocannabinoid system in the phenomenon of addiction.

Keywords Amygdala, cannabinoid, hippocampus, nucleus accumbens, prefrontal cortex, VTA.

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INTRODUCTION

Addiction is a chronic disorder. Consequently, it is not possible to understand the phenomenon of addiction without assuming that persistent changes in the central nervous systems have occurred. Prototypical examples of these changes include tolerance, dependence and/or sensitization after repeated drug exposure with corresponding neurochemical changes in the brain (for reviews, see Chao & Nestler 2004; Nestler 2004; Ron & Jurd 2005). Currently, growing evidence supports the notion that the endocannabinoid system is implicated strongly in such neuroadaptations induced by the repeated exposure to drugs of abuse (see, for example, Fattore *et al.* 2005; Gonzalez, Cebeira & Fernandez-Ruiz 2005).

However, in contrast to the idea that addiction is a chronic disorder, we found two principal constraints in most of the relevant scientific studies that have been published. Firstly, these studies were performed in an acute manner. Secondly, the administration of drugs was non-voluntary. Despite these limitations, herein, we review consistent and reliable evidence that indicates a role for the endocannabinoid system in the phenomenon of drug abuse and addiction. There are a few published reviews on this topic (e.g. De Vries & Schoffelmeer 2005; Parolaro, Vigano & Rubino 2005; Rodríguez de Fonseca *et al.* 2005;

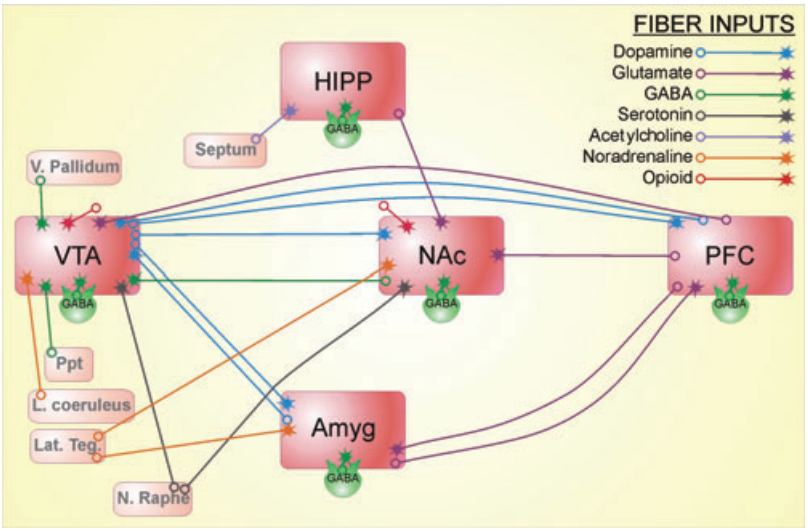


Figure 1 The main neural projections between the five brain areas more studied in addiction. Highly summarized and schematic representation of the main interconnections between the five brain areas (in red) that are significantly involved and investigated in drug addiction. Other brain areas are also illustrated because of project-relevant efferences to those five key brain areas. As shown throughout the text, there are a large number of studies that demonstrate the role of the endocannabinoid system in the neuromodulation of all of the neurotransmitter systems depicted here. Logically, the effects of the cannabinoids would depend on the brain area that is specifically affected, as well as the inputs and outputs under the control of cannabinoid-mediated neurotransmitter release. Also, it is interesting to note that the principal excitatory and neuroinhibitory neurotransmission is under the control of endocannabinoids. For instance, most gamma-aminobutyric acid (GABA)ergic inhibitory interneurons express CB1 presynaptic receptors in abundance, modulating the release of GABA at the synapses (Hájos & Freund 2002; Berghuis *et al*. 2007). The soma of the fiber projection is illustrated as a circle and its corresponding axonic terminal as a star. Amyg, amygdala; HIPP, hippocampus; L. coeruleus, locus coeruleus; Lat. Teg., lateral tegmental noradrenergic cell groups; N. Raphe, raphé nuclei of the brain stem; NAc, nucleus accumbens; PFC, prefrontal cortex; Ppt, pedunculopontine nucleus; V. Pallidum, ventral pallidum; VTA, ventral tegmental area

Maldonado, Valverde & Berrendero 2006), but the present review provides a new structure and methodology Firstly, we decided to extend a table that summarizes the main natural, endogenous and synthetic cannabinoids. Secondly, we have classified the functional and structural interactions between the endocannabinoid and the major neurotransmitter systems into five subheadings. These neurotransmitters are gamma-aminobutyric acid (GABA)/glutamate, some principal biogenic amines (dopamine, noradrenaline and serotonin), opioids and acetylcholine. Each subheading corresponds to a specific brain structure or area: amygdala, prefrontal cortex, nucleus accumbens, hippocampus and the ventral tegmental area (VTA). We have chosen these structures and areas because they are the main areas that have been classically related to addiction (Lupica, Riegel & Hoffman 2004; Kalivas & Volkow 2005; Gould 2006; Hyman, Malenka & Nestler 2006; Koob 2006). Please note that the order of these brain areas in the text is arbitrary. Thirdly, we have depicted and highly simplified the main interconnections between those five brain areas in Fig. 1 in order to clarify some of the interactions described throughout the present review. Finally, in Fig. 2, we have conceptually integrated the possible role of the endocannabinoid system in the phenomenon of addiction. This was done in an attempt to provide new insights into the persistent changes observed after drug exposure. This would eventually lead to the aberrant behavior of an individual addicted to drugs.

The methodology of the bibliographic search relied on use of the MEDLINE database through the service of the U.S. National Library of Medicine, PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez/query.fcgi). We have coupled the word 'cannabinoid' with the rest of the neurotransmitters and brain areas described above. Thus, due to the character of this work and the specific subdivisions that have been generated, results from unique published works may be found throughout the text.

The aim of the present review is to provide some clues about the functional and structural interactions between the endocannabinoid system and the main neurotransmitter systems of the central nervous system. With this purpose, we have reviewed the main findings of most of the existing studies that have explored such interactions in the five brain areas in the field of addiction and drug abuse.

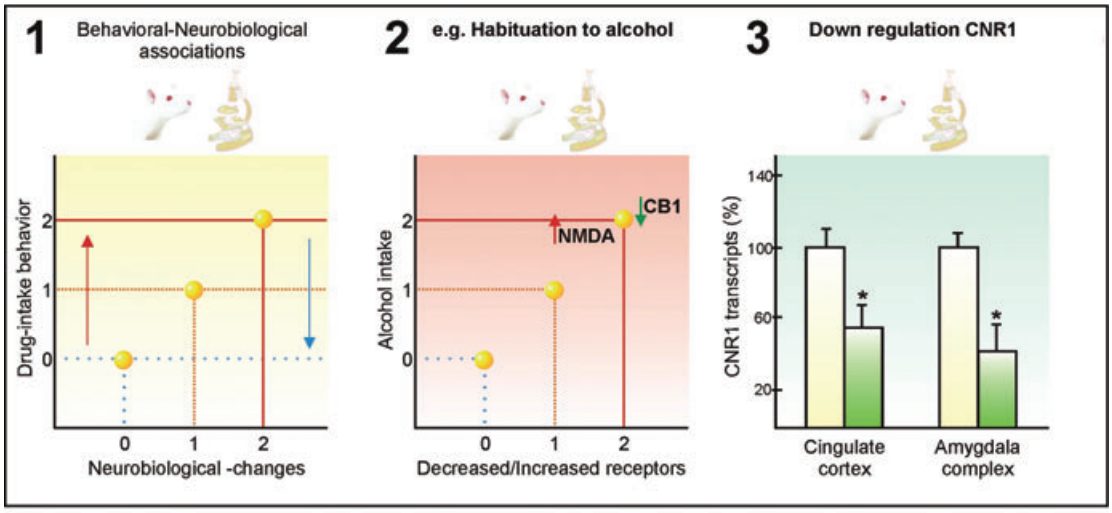
PRINCIPAL NATURAL, ENDOGENOUS AND SYNTHETIC CANNABINOIDS

Due to the complexity of the cannabinoid system, our description of cannabinoids is not intended to be exhaustive (see Table 1), and consequently, the reader is referred to further reviews to gain more detailed knowledge specific to this topic. The categories we used are the following: (1) natural (phytocannabinoids); (2) endogenous (endocannabinoids and endocannabinoid-related

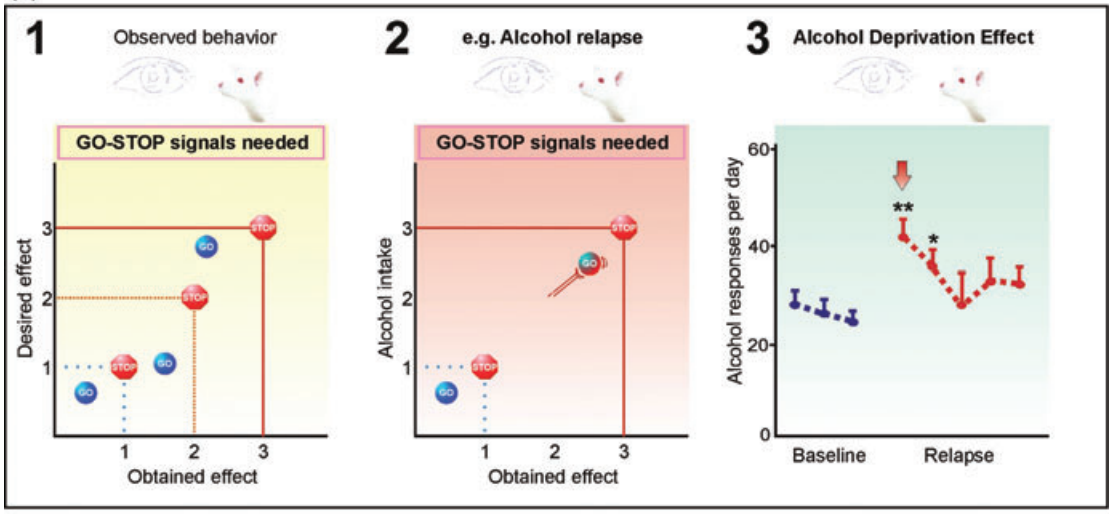
Table 1 Summary of cannabinoids classified as natural (phytocannabinoids), endogenous (endocannabinoids and endocannabinoid-related substances) and synthetic (cannabinoid receptor agonists, cannabinoid receptor antagonists, uptake blockers and inhibitors of fatty acid amide hydrolase) compounds.

- Natural
  - Phytocannabinoids
    - Delta(9)-tetrahydrocannabinol (THC)
    - Delta(8)-THC
    - Cannabidiol
    - Cannabigerol
    - Cannabichromene
    - Cannabicyclol
    - Cannabielsoin
    - Cannabitriol
    - Miscellaneous
- Endogenous
  - Endocannabinoids
    - N-arachidonoylethanolamide (anandamide; CB1-CB2 partial agonist)
    - 2-arachidonoylglycerol (CB1 complete agonist, CB2 agonist)
    - 2-arachidonoylglyceryl ether (noladin ether; CB1 complete agonist)
    - O-arachinoyl-ethanolamine (virodhamine; CB2 partial agonist, CB1 antagonist, inverse agonist)
    - N-arachidonyl-dopamine (CB1 agonist)
    - Docosatetraenoylethanolamide?
    - Oleamide?
    - N-Oleoyl dopamine?
    - Dihomo-linolenoylethanolamide?
  - Endocannabinoid-related compounds
    - Fatty acid derivatives
      - Oleamide
      - Oleoylethanolamide
      - 2-oleoylglycerol
      - Stearoylethanolamide
      - Palmitoylethanolamide
      - 2-palmitoylglycerol
      - Linoleoylethanolamide
      - 2-linoleoylglycerol
    - Archidonoyl-aminoacid
- Synthetic
  - Cannabinoid receptor agonists
    - Classical cannabinoids
      - Delta (8)-THC (CB1-CB2 agonist)
      - HU-210 (CB1-CB2 agonist)
      - AM411 (CB1 agonist)
      - O-1184 (CB1 agonist, CB2 inverse agonist)
      - O-1057 (complete CB1-CB2 agonist)
    - Non-classical cannabinoids
      - CP-55 940 (complete CB1-CB2 agonist)
      - JWH-015 (CB2 agonist)
      - L-768242 (CB2 agonist)
    - Specific CB-2 receptor agonists
      - AM-1241
      - HU-308
      - L-759633
      - L-759656
      - JWH-015
      - JWH-133
      - GW405833
    - Eicosanoids
      - R-(+)-WIN-55, 212-2 (complete CB1-CB2 agonist)
    - Aminoalkylindoles
      - R-(+)-methanandamide
      - Arachidonoyl-2¢-chloroethylamide
      - Arachidonylcyclopropylamide
      - O-1812
    - 2-arylimino-5,6 dihydro-4H-1, 3-thiazines
    - Arylsulfonamides (CB1 agonists)
  - Cannabinoid receptor antagonists
    - Diarylpyrazoles
      - SR141716A (rimonabant; CB1 antagonist, inverse agonist)
      - AM251 (CB1 antagonist, inverse agonist)
      - SR147778 (CB1 antagonist, inverse agonist)
      - AM281 (CB1 antagonist, inverse agonist)
      - SR144528 (CB2 antagonist, inverse agonist)
    - Substituted benzofuranes
      - LY 320135 (CB1 antagonist)
    - Aminoalkylidoles
      - AM 630 (CB2 antagonist, partial CB1 agonist)
    - Triazole derivatives
      - LH-21 (CB1 antagonist)
  - Uptake blockers
    - AM 404
    - UCM 707
    - AM1172
    - VDM11
    - VDM13
    - OMDM1
    - OMDM2
    - LY 2183240
    - LY 2318912
    - O-2093
  - Inhibitors of fatty acid amide hydrolase (FAAH)
    - Carbamate FAAH inhibitors
      - OL-135
      - URB 597
      - URB 532
  - Bisarylimidazole derivative

(a)



(b)



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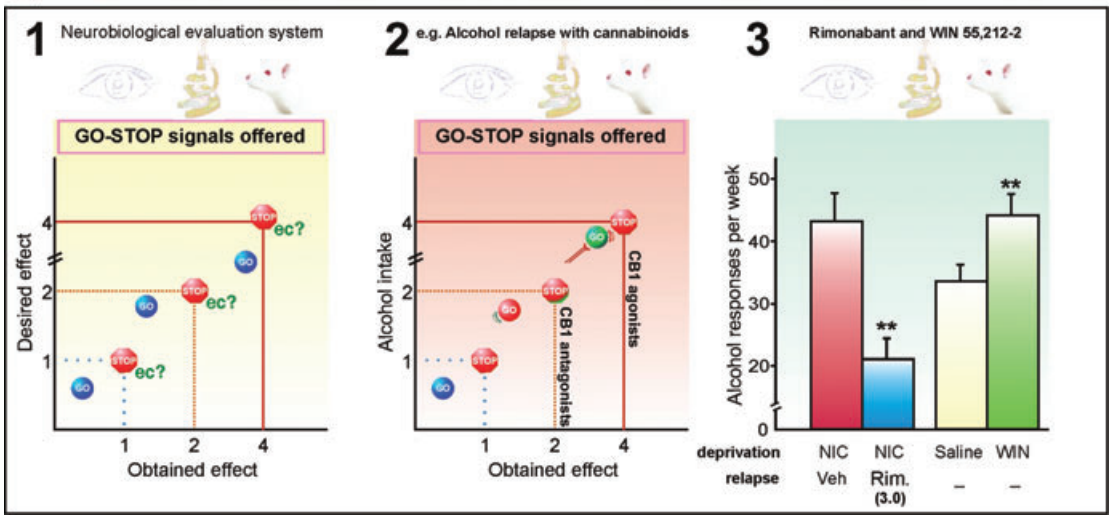

Figure 2 A possible interpretation of the role of the endocannabinoid system in the phenomenon of addiction. It is well known that repeated drug exposure is associated with neurochemical and neurobiological long-lasting changes. For example, changes in strength synaptic transmission are induced by long-term potentiation after repeated drug treatment. Furthermore, such neurobiological changes are associated with behaviors that can be easily evaluated: i.e. recording the animal's work for obtaining a drug. This idea is summarized in (a). In (a1), it is illustrated, in a general way, that the increase/decrease of drug intake is associated with neurobiological changes, and that this association keeps, in some way, a linear relation: higher/lower drug intake is correlated with higher/lower specific neurobiological changes. Also, it is necessary to keep in mind that there exists a reciprocal interaction between drug intake increase/decrease and neurobiological changes. They are a cause and/or consequence of each other. In (a2), a schematic example of these concepts based on demonstrated evidences is shown. It shows how different levels of alcohol intake can produce an increase in the number of glutamatergic NMDA (Fadda & Rossetti 1998), but on the contrary, produce a decrease in the number of cannabinoid CB1 receptors (Basavarajappa, Cooper & Hungund 1998). Note that in all panels, the *xy* coordinate axes are in absolute values (in consequence, a decrease would also be represented by a displacement to the right of the coordinate planes). In (a3), the relative abundance of CB1 transcripts in the amygdala complex and cingulate cortex (from Wistar rats with an extended history of operant alcohol self-administration and that were treated with the cannabinoid receptor agonist WIN 55,212-2) during alcohol deprivation (green bars) is shown. Their corresponding vehicle groups are represented by yellow bars. These are preliminary results from our laboratory. This correlation between drug intake and neurobiological changes would be accompanied by other physiological and psychological components of difficult evaluation. Only we can infer such changes by observing the new behaviors of the animals during drug intake. For example, we can observe that the animals progressively consume more/less drug. And similarly, we can infer that the animals will stop their drug intake once they reach the desired effect. It is not possible to think that when a behavior starts, it does not finish. In consequence, it is plausible to consider that there exist specific internal indicators that motivate and drive the animals to begin/maintain drug consumption (called GO signals), and that there are other concrete internal indicators that stop drug intake (called STOP signals); and we can conclude that the animals will stop their drug intake behavior when GO signals disappear and only STOP signals remain, that is, when an adjustment between the desired effect and the obtained effect happens; (b1) summarizes these ideas. Observing animal behavior, we hypothesize that some signals that would increase the probability of beginning/maintaining a drug-related behavior (GO signals) would exist, and other signals that would increase the probability of stopping a drug-related behavior (STOP signals) would also exist. For example, during a drug intake session, the GO signals will become progressively reduced and only STOP signals will remain; an adjustment between the desired effect of the organism and the obtained effect would have occurred. These adjustments can occur at many different degrees. In (b2), a simplified scheme of such a hypothesis is shown. It can be reliably observed that animals with a long-extended history of alcohol self-administration, following a period of alcohol deprivation, exhibit a significant temporal increase in alcohol consumption called alcohol-deprivation effect (Lopez-Moreno *et al*. 2004; Lopez-Moreno, Gonzalez-Cuevas & Navarro 2007). According to our hypothesis, it is obvious that the STOP signals in alcohol consumption have been displaced to another point during alcohol relapse (i.e. values 3) that is different from the coordinates of origin: the alcohol intake baseline (values 1). In (b3), the characteristic increase in the number of responses for obtaining alcohol in Wistar rats after a period of alcohol deprivation (alcohol-deprivation effect, marked with red arrow) is illustrated. There were significant differences when compared with baseline. These robust and significant differences are limited to 1 or 2 days. (Graphics adapted from Lopez-Moreno *et al*. (2004).) Following our reasoning, the adjustment process between the desired drug effect and the obtained drug effect would have to be linked to specific neurobiological changes. Such non-directly observable changes would explain the different behavioral patterns of drug intake observed. This represents the existence of neurobiological substrates for the corresponding GO and STOP signals called *neurobiological system of evaluation* of GO and STOP signals; (c1) graphically represents this hypothesis. Due to enormous evidence that one of the main roles of the endocannabinoid system is the neuromodulation of many neurotransmitter systems, we include this system as a putative functional component of the *neurobiological system of evaluation*. This would indicate that alterations in the endocannabinoid system would be related, for example, to an increase in the STOP-signal threshold. This bigger distance between the drug's obtained effect and the drug's desired effect would be translated into different observable drug intake patterns. Contrary to this, the *neurobiological system of evaluation* is not directly observable, but it can be studied by different techniques (electrophysiology, microdialysis, immunohistochemistry, microscopic immunocytochemistry, etc.). In (c2), a schematic example of the effects of the CB1 cannabinoid receptor agonists in alcohol relapse is shown. Cannabinoid agonists significantly increase the relapse to alcohol and make the alcohol-deprivation effect long-lasting; that is, the higher alcohol intake rates are maintained through several consecutive days (Lopez-Moreno *et al*. 2004). Complementary to this, cannabinoid receptor antagonists prevent the relapse to alcohol even when there is a nicotine–alcohol interaction (Lopez-Moreno *et al*. 2007). On one hand, (c3) depicts that the dose of 3.0 mg/kg of the cannabinoid receptor antagonist rimonabant fully prevented the relapse to alcohol in Wistar rats that were exposed to nicotine during the alcohol-deprivation period (blue bar). Animals that were not pre-treated with rimonabant 30 minutes before the alcohol test showed a significant number of alcohol responses (red bar) when compared with rimonabant and vehicle groups (this last group not shown). On the other hand, this panel shows that the cannabinoid receptor agonist WIN 55,212-2 increases the relapse to alcohol in rats (green bar) when compared with the vehicle group (yellow bar). All these data correspond to the weekly number of alcohol responses (Graphics adapted from Lopez-Moreno *et al*. (2004, 2007).)

compounds; and (3) synthetic [cannabinoid receptor agonists, cannabinoid receptor antagonists, uptake blockers and inhibitors of fatty acid amide hydrolase (FAAH)]. At least 65 cannabinoids have been identified in the cannabis plant, of which delta(9)-tetrahydrocannabinol is responsible for many of its psychoactive effects (Mechoulam & Hanus 2000). Furthermore, five different types of endogenous ligands of cannabinoid receptors have been discovered so far. These are N-arachidonoylethanolamide (anandamide), 2-arachidonoylglycerol (2-AG), 2-arachidonoylglyceryl ether (noladin ether), O-arachidonoylethanolamine (virodhamine) and N-arachidonoyl-

dopamine (NADA) (Hashimotodani, Ohno-Shosaku & Kano 2007). However, other endocannabinoids have also been proposed (Pertwee 2005). Apart from the endocannabinoids, there are other related endogenous compounds that are structurally similar that are called endocannabinoid-related compounds (Kogan & Mechoulam 2006). According to the International Union of Pharmacology (Howlett *et al.* 2002), whereas cannabinoid agonists can be divided into classical cannabinoids, non-classical cannabinoids, aminoalkylindoles and eicosanoids, cannabinoid antagonists can be split into diarylpyrazoles, substituted benzofuranes, aminoalkylindoles and triazole derivatives. In addition, we have added some new novel cannabinoids (Muccioli & Lambert 2005). Finally, the uptake blockers of anandamide and inhibitors of FAAH, as synthetic compounds, are also taken into account in order to provide a comprehensive view of the cannabinoid system.

THE AMYGDALA COMPLEX AND THE CANNABINOID SYSTEM

Emotions play a key role in human and animal behavior, and most behaviors are, in major or minor degree, regulated by emotions. Drug-addictive behaviors are essentially caused, affected or aggravated by emotional components: e.g. the rewarding effects of drug intake, craving and/or aversive withdrawal effects. One of the main brain structures involved in emotion process is the amygdala. There are a meaningful number of scientific reports demonstrating the role of the amygdala complex in drug-addicted behaviors (for reviews, see Kilts 2001; Koob 2003; See *et al.* 2003).

GABA and glutamate

There are several studies that have shown a functional interaction between GABAergic/glutamatergic neurotransmission and the endocannabinoid system. Most of these authors explain such interactions in the basolateral as well as in the lateral subregions of the amygdala.

Firstly, in the basolateral complex, it has been demonstrated that treatment of rats with WIN 55,212-2 and CP 55,940 suppresses the amplitude of $GABA_A$ receptor-mediated evoked and spontaneous inhibitory postsynaptic currents from spiny principal cells, neurons that connect fundamentally to each other with GABAergic synapses (Katona *et al.* 2001). The inhibition of spontaneous GABAergic currents has been demonstrated with other experimental set-ups, such as mechanically isolated neurons from the basolateral amygdala or neuron/bouton preparations. Furthermore, the selective treatment of the rat basolateral amygdala with several cannabinoid agonists (e.g. WIN 55,212-2, HU-210 and delta(9)-tetrahydrocannabinol) causes a reduction of the excitatory neurotransmission to the shell of the nucleus accumbens, which is fully prevented by the CB1 receptor antagonist rimonabant (Pistis *et al.* 2002). The inhibitory effect exerted by WIN 55,212-2 is thought to be dependent on calcium (Zhu & Lovinger 2005) and $GABA_A$ receptors that contain the α1-subunit (Marowsky, Fritschy & Vogt 2004). Although the activation of CB1 receptors on presynaptic axon terminals by WIN 55,212-2 causes a reduction of glutamate-excitatory transmission within the basolateral amygdala, CB1 knockout mice do not show such neuromodulation in neural cells, except for GABAergic neurons (Domenici *et al.* 2006). In an auditory fear-conditioning task, it has been demonstrated that the release of endocannabinoids in the basolateral complex is critical for the extinction of aversive memories. The activation of CB1 receptors by endogenous cannabinoids (anandamide and 2-AG) is a determinant in the long-term depression (LTD) of inhibitory GABAergic currents (Marsicano *et al.* 2002).

Secondly, as with the basolateral complex, the pharmacological activation of presynaptic CB1 receptors with WIN 55,212-2 in the lateral amygdala leads to a decrease of glutamatergic and GABAergic neurotransmission. This presynaptic mechanism is mediated through activation of Gi/o proteins and the modulation of K^+ conductance (Azad *et al.* 2003). It seems that this control of neurotransmission in the lateral amygdala is similar to that observed in the nucleus accumbens, but it is different than that observed in the hippocampus (Azad *et al.* 2003). *In vitro* studies show that low-level stimulation of the lateral amygdala induces the release of endocannabinoids postsynaptically from neurons of the basolateral amygdala, causing LTD of GABAergic transmission. More specifically, the endogenous cannabinoid anandamide could decrease the inhibitory activity of interneurons in the amygdala (Azad *et al.* 2004).

Interestingly, a functional link between the GABAergic and cannabinoid systems is phylogenetically highly conserved. Functional innervations have been observed between both systems. In addition, an abundance of CB1 receptors have been found in the amygdala of amphibian brains (Cesa *et al.* 2001).

Biogenic amines (dopamine, noradrenaline, serotonin)

Regarding the studies on serotonin and the endocannabinoid system in the amygdala, it has been shown that the CB1 receptor is co-expressed in the rat amygdala with the ligand-gated ion channel receptor for serotonin: the 5-hydroxytryptamine $(5\text{-HT})_{3A}$ receptor. Both receptors co-exist in GABAergic interneurons in the amygdala

and the hippocampal formation, among other regions (Morales *et al.* 2004). In addition, these interneurons co-express transcripts of CB1 mRNA and the 5-HT_{3A} subunit. This suggests a possible interaction between cannabinoid and serotonergic systems in GABAergic neurotransmission. Moreover, it has been reported that CB1 receptors and 5-HT transporter proteins are widely co-distributed in the amygdala of the rat (Ashton, Darlington & Smith 2006). Very recent findings indicate the existence of CB1 proteins on serotonergic fibers from the raphe nuclei and synapses in the amygdala. Furthermore, CB1 receptors are detected in synapses that express the serotonin reuptake transporter (5-HT transporter) (Häring *et al.* 2007). Therefore, putative cross-talk between serotonergic and cannabinoid systems in this brain structure is suggested.

Regarding the studies on the dopaminergic and the endocannabinoid systems in the amygdala, it has been reported that administration of delta(9)-tetrahydrocannabinol leads to a reduction in dopamine levels. This reduction is also observed in the nucleus accumbens, although a lack of effect is found in the striatum (Hernandez-Tristan *et al.* 2000). In humans, the CB1 receptor is present in the amygdala and neocortex, but cannabinoid-modulated release of dopamine can only be observed in the neocortex (Steffens *et al.* 2004). Moreover, prenatal studies in humans show that CB1 receptor mRNA expression is predominantly and intensely localized in the amygdala and the hippocampus. There is a significant correlation between the expression of amygdalar D2 mRNA and prenatal cannabis exposure such that increased marijuana use by the mother is related to decreased D2 mRNA expression levels in the amygdala of the human fetus (Wang *et al.* 2004).

Other studies, evaluating the noradrenergic–cannabinoid interaction, show that lesions on the basolateral amygdala of rats during certain neonatal periods (postnatal-day 7 and postnatal-day 21) result in a reduction of dopamine D2-like receptor density in mesolimbic, but not in striatal regions. Curiously, CB1 receptors show an inverse pattern, wherein there is an increment in the striatum but not in the mesolimbic regions, although noradrenergic transmission is reduced in both regions (Bouwmeester *et al.* 2006). Moreover, administration of delta(9)-tetrahydrocannabinol does not modify the noradrenaline level in several brain regions, including the amygdala. Consequently, this discredits a possible link between the delta(9)-tetrahydrocannabinol-induced increase in mouse killing behavior and noradrenergic neurotransmission (Yoshimura & Ueki 1981).

Finally, it is well-known that amphetamines result in the release of biogenic amines, principally dopamine and serotonin. Amphetamines have also been found to induce LTD in the amygdala. However, it has been demonstrated that dopamine, serotonin and noradrenaline α2-receptor antagonists are not able to reverse this phenomenon in the rat amygdala. The ability of the CB1 receptor antagonist AM251 to reverse amphetamine-induced LTD (Huang *et al.* 2003) suggests that CB1 receptors may be critical for the establishment of amphetamine-induced LTD in the amygdala.

Opioids

Rats with a chronic background of intravenous heroin self-administration (39 days) exhibit a significant decrease of μ-opioid-stimulated [^{35}S]GTPγS binding in the amygdala, whereas no relevant changes occur with CB1 receptors (Sim-Selley *et al.* 2000). Furthermore, non-voluntary chronic exposure to morphine results in a reduction of the binding of CB1 receptors in the basolateral amygdala and an increase in the nucleus accumbens (González *et al.* 2002). More recently, Fattore and colleagues have demonstrated that intravenous self-administration of heroin in rats increases not only the density of CB1 receptors but also their functionality. In addition, intravenous self-administration of WIN 55,212-2 causes a modest increase of CB1 and μ-opioid receptor levels in the amygdala, but only μ-opioid receptors increase their functionality after self-administration of the cannabinoid receptor agonist (Fattore *et al.* 2007).

There are sexual dimorphisms in the expression of μ-opioid receptors after chronic treatment with delta(9)-tetrahydrocannabinol. For example, male rats born from mothers treated with this cannabinoid during the gestation and lactation periods have lower densities of μ-opioid receptors in the amygdala when compared with male controls. In contrast, female rats exhibit higher densities of μ-opioid receptors in the amygdala (posteromedial cortical nucleus) when compared with female controls (Vela *et al.* 1998). Furthermore, adult male and female rats perinatally exposed to delta(9)-tetrahydrocannabinol experience differential regulation of proenkephalin mRNA levels in the caudate-putamen, but no significant differences are found by exploring the central amygdala or other brain regions (Corchero *et al.* 1998). In humans, marijuana use during pregnancy leads to an increase in μ-opioid receptor expression in the amygdala. Similarly, prenatal exposure to alcohol, but not tobacco, reduces mRNA expression levels of another opioid receptor type in the amygdala, in this case, the kappa receptor (Wang *et al.* 2006).

The marker for neural activation, Fos-immunoreactivity (Fos-IR), which was used to elucidate the functional interaction between cannabinoid and opioid systems, is increased after treatment with morphine (10 mg/kg) in the central, basolateral and medial nuclei of the amygdala. A similar increase of Fos-IR is found after rimonabant treatment alone (3 mg/kg);

however, the combination of both compounds attenuates morphine-induced Fos-IR in the basolateral amygdala (Singh *et al.* 2004). Although Fos-IR is increased in the central nucleus of the amygdala of rats after acute treatment with heroin, pre-exposure to delta(9)-tetrahydrocannabinol reduces it (Singh, McGregor & Mallet 2005). Similarly, if rats are perinatally exposed to delta(9)-tetrahydrocannabinol (5 mg/kg, from PD 4 to 14), an important reduction of the heroin-induced Fos-IR, along with an enhancement of the rewarding properties of heroin as evaluated in the conditioned place preference paradigm, is observed (Singh, McGregor & Mallet 2006). Along this line, experiments from Allen and colleagues have evaluated an immediate early gene, the c-Fos transcription factor. They showed that naloxone as well as delta(9)-tetrahydrocannabinol increased the number of Fos-IR in the basolateral and central nuclei of the amygdala. The combination of both molecules caused an additive effect in the central nucleus of the amygdala, increasing Fos-IR (Allen *et al.* 2003). Together with these intracellular data, acute administration of delta(9)-tetrahydrocannabinol (1 mg/kg) or morphine (5 mg/kg) in mice (CD-1) results in an equal increase of extracellular signal-regulated kinase (ERK) phosphorylation in the central nuclei of the amygdala, but no significant differences are found in the basolateral and lateral complexes of the amygdala (Valjent *et al.* 2004).

In another set of experiments that included some behavioral variables, administration of WIN 55,212-2 into the central nucleus of the rat amygdala caused a dose-dependent antinociceptive effect on the tail-flick latency test. Together with other results (e.g. Manning *et al.* 2001), this indicates that antinociception is induced by opioids and cannabinoids. However, whereas Manning, Martin & Meng (2003) suggest that this effect is almost absent in the basolateral complex of the amygdala, other authors have revealed elevated tail-flick latencies when WIN 55,212-2 is microinjected into the basolateral nuclei (Martin *et al.* 1999). Likewise, treatment with the amide hydrolase inhibitor URB597 or the monoacylglycerol lipase inhibitor URB602 does not modify tail-flick latencies in the central nucleus of the amygdala (Connell *et al.* 2006). This is likely due to the existence of a greater number of CB1 receptors in the basolateral amygdala as compared with the central nucleus of the amygdala. However, in the same study, Connell and co-workers showed that microinjection of rimonabant into the basolateral amygdala of the rat suppressed analgesia that was induced by stress. Finally, evaluation of non-human primates with a bilateral lesion of the amygdala complex by magnetic resonance has shown that these animals have altered antinociceptive responses to morphine and WIN 55,212-2 when compared with unoperated control monkeys (Manning *et al.* 2001).

Acetylcholine

As for noradrenaline, the role of acetylcholine in the delta(9)-tetrahydrocannabinol-induced mouse-killing behavior in rats has been studied. Delta(9)-tetrahydrocannabinol (6 mg/kg) increased the amount of acetylcholine in the amygdala in both rats (killer versus non-killer) when compared with untreated rats. This suggests that acetylcholine does not mediate the delta(9)-tetrahydrocannabinol-induced mouse-killing behavior in rats (Yoshimura, Fujiwara & Ueki 1974).

THE NUCLEUS ACCUMBENS AND THE CANNABINOID SYSTEM

The nucleus accumbens, together with the VTA, is a key structure of the brain reward system. The nucleus accumbens receives important dopaminergic projections from the VTA, and practically, the exposure (voluntary or not voluntary) to every drug of abuse leads to an increase in the dopamine release in this region. However, there seem to be scientific discrepancies regarding the functional mean of this dopamine increase after repeated drug exposure (e.g. Ungless 2004). Also, the nucleus accumbens can be divided mainly into two regions: core and shell. Most evidences reveal that the nucleus accumbens shell region is more sensitive to an increase in dopamine release after exposure to any drug of abuse (Di Chiara 2002; Di Chiara *et al.* 2004).

GABA and glutamate

The most common type of neurons in the nucleus accumbens are the medium spiny GABAergic neurons, which are highly innervarted by GABAergic interneurons located in the same nucleus accumbens and by glutamatergic afferences out of the amygdala, hippocampus and prefrontal cortex.

By using the *in vitro* whole-cell patch clamp technique, the effects of WIN 55,212-2 and CP 55,940 on GABA-induced postsynaptic currents were investigated in mice nucleus accumbens. Both cannabinoids inhibited the stimulus-evoked GABA-mediated inhibitory postsynaptic currents (Manzoni & Bockaert 2001). Other electrophysiological results in the same study point to a presynaptic localization of cannabinoid CB1 receptors in mice nucleus accumbens. Similar results have been obtained in the shell of nucleus accumbens of rats, where GABA-mediated inhibitory postsynaptic currents are reduced by WIN 55,212-2 and reversed by rimonabant. Similarly, WIN 55,212-2 is able to inhibit glutamatergic excitatory postsynaptic currents at a postsynaptic level (Hoffman & Lupica 2000).

There are very relevant studies exploring the role of the endocannabinoid system in the long-lasting decrease

of synaptic effectiveness; that is, in the phenomenon of LTD. It has been demonstrated that the activation of CB1 receptors with WIN 55,212-2 or with an endocannabinoid transporter blocker, AM-404, mediates long-term synaptic depression in mice nucleus accumbens. The induction of presynaptic endocannabinoid-mediated LTD requires the activation of the metabotropic glutamate 5 (mGlu5) receptor and the increase of postsynaptic Ca^{++} from intracellular stores. This leads to a postsynaptic release of endocannabinoids that would activate the presynaptic CB1 receptors and cause LTD (Robbe *et al.* 2002). The extension of this study from Robbe and colleagues, which explored the connections between the prefrontal cortex and the nucleus accumbens, showed that WIN 55,212-2 inhibits spontaneous and evoked glutamate-mediated transmission through a presynaptic mechanism. Such mechanism would include the activation of presynaptic K^+ channels and GABA neurotransmission (Robbe, Alonso & Manzoni 2003). Along this line, another study shows that a single *in vivo* exposure to delta(9)-tetrahydrocannabinol causes suppression of endocannabinoid-mediated LTD in mouse cortico-accumbens synapses. In addition, subchronic treatment with delta(9)-tetrahydrocannabinol (1 week) causes a reduction in the coupling efficiency at CB1 receptors to Gi/o transduction proteins and in the CB1-induced inhibition of cortico-accumbens excitatory synapses. However, the endocannabinoid-mediated LTD remained because the presynaptic mGlu receptor 2/3 (mGluR2/3) replaced the impaired endocannabinoid system (Mato *et al.* 2005). In rats, a single exposure to cocaine blocks endocannabinoid-mediated retrograde signalling at prefrontal cortex–nucleus accumbens synapses. It seems that CB1 and mGlu5 receptors in the nucleus accumbens are the principal mediators of endocannabinoid-mediated retrograde LTD (Fourgeaud *et al.* 2004).

Other studies focused on the functional interaction between GABAergic and endocannabinoid systems in the nucleus accumbens. To that end, it has been shown that acute administration of morphine (3 mg/kg) and WIN 55,212-2 (5 mg/kg), as well as heroin self-administration leads to a reduction of GABA efflux into the nucleus accumbens of rats. These effects are reversed by the cannabinoid CB1 receptor antagonist rimonabant (Caillé & Parsons 2006). However, perinatal treatment of rats with delta(9)-tetrahydrocannabinol does not change the activity of glutamic acid decarboxylase or the level of GABA in the nucleus accumbens of adult rats (Garcia-Gil *et al.* 1999). In addition, local perfusion of the selective CB1 receptor antagonist AM251 into the nucleus accumbens shows that whereas GABAergic neurotransmission is not altered, a dose-dependent inhibition of the cocaine-induced increase of glutamate is observed. This might explain the prevention of the cocaine-primed reinstatement of drug-seeking behavior in rats by systemic treatment with AM251 (Xi *et al.* 2006).

There are also several results concerning glutamate–cannabinoid interactions in the nucleus accumbens that can be summarized as follows: the administration of the glutamate receptor antagonist MK801 reverses the delta(9)-tetrahydrocannabinol-induced progressive and transient activation of mitogen protein kinase/ERK in the shell and core of the rat nucleus accumbens. Furthermore, exposure of the rat nucleus accumbens to WIN 55,212-2 results in a dose-dependent reduction in the levels of glutamate, which is antagonized by rimonabant. This cannabinoid-induced inhibition of glutamate release does not seem to be related to the cyclic adenosine monophosphate–protein kinase A (cAMP–PKA) cascade or to inhibition of a different kind of Ca^{2+} channel. Instead, this effect seems to be modulated by activation of K^+ channels (Robbe *et al.* 2001; Valjent *et al.* 2001). Finally, acute administration of alcohol diminishes the levels of the endogenous cannabinoid anandamide in the rat nucleus accumbens, along with inhibition of the levels of glutamate; however, it does not change the levels of enzymatic activity of the FAAH or anandamide precursors (Ferrer *et al.* 2007).

Biogenic amines (dopamine, noradrenaline, serotonin)

The dynamic formation of CB1/D2heterodimers was recently discovered (Kearn *et al.* 2005). It was suggested that their formation is based on the concurrent activation of both receptors, which are located mainly in the nucleus accumbens (Matyas *et al.* 2006). Likewise, two recent studies reported that chronic treatment with delta(9)-tetrahydrocannabinol leads to an increase in the length of dendrites as well as in the number of dendritic branches in the rat shell nucleus accumbens (Kolb *et al.* 2006). Also, these studies found that D2 and CB1 receptors have very similar subcellular distributions in the dendrites and axons of the rat shell and core of the nucleus accumbens, as evaluated by electron microscopic immunocytochemistry. These results not only indicate D2 and CB1 heterodimerization, but they also suggest cross-talk between the release of endocannabinoids and the control of dopamine neurotransmission (Pickel *et al.* 2006).

There are a significant number of studies that have explored the modulation of dopamine transmission by cannabinoids. For example, intracerebroventricular administration of cannabidiol, one of the major constituents of *Cannabis sativa*, leads to an increase in the extracellular levels of dopamine in the nucleus accumbens of rats, as evaluated by *in vivo* microdialysis (Murillo-Rodríguez *et al.* 2006). Also, intravenous self-administration of WIN 55,212-2 in two different strains

of rats (Lister Hooded and Long Evans) was found to be linearly associated with the release of dopamine into the shell of the nucleus accumbens. As measured by microdialysis during operant cannabinoid self-administration sessions (Fadda *et al*. 2006), the release of dopamine was greater in the shell than in the core of the nucleus accumbens (Lecca *et al*. 2006). Intravenous self-administration of anandamide, as well as of methanandamide, also enhanced the extracellular levels of dopamine in the rat shell nucleus accumbens. Although administration of the analogue of anandamide, AM-404, an anandamide re-uptake inhibitor, does not potentiate anandamide-induced elevation of dopamine in this region, inhibition of FAAH facilitates the anandamide-induced increase of dopamine (Solinas *et al*. 2006; Solinas *et al*. 2007b). In addition, the extracellullar levels of dopamine in the rat shell nucleus accumbens are augmented after intake of a novel high palatable food. Whereas this increase in dopamine is reversed by rimonabant, either WIN 55,212-2 or HU-210 can block the effect that is induced by rimonabant (Melis *et al*. 2007). By using CB1 receptor knockout mice, it has been shown that there is a complete lack of alcohol-induced dopamine release in the nucleus accumbens (1.5 mg/kg, intraperitoneal alcohol injection). These genetically modified mice also present a significantly lower consumption of alcohol than their corresponding wild-type controls (Hungund *et al*. 2003).

These cannabinoid-induced dopamine alterations have been monitored with more sophisticated techniques (i.e. using *in vivo* voltammetry) that provide higher temporal resolution than classical microdialysis techniques. For example, it has been demonstrated that the release of dopamine induced by nicotine, alcohol and cocaine in the rat shell nucleus accumbens is inhibited by the cannabinoid antagonist rimonabant (Cheer *et al*. 2007). Furthermore, using fast-scan cyclic voltammetry, it has been shown that intravenous treatment with WIN 55,212-2 increases the frequency of dopamine concentration transients in the rat nucleus accumbens, but it reduces the amplitude of electrically evoked dopamine release. These effects are prevented by rimonabant (Cheer *et al*. 2004).

Logically, due to low basal levels of serotonin in the nucleus accumbens, there are only a few studies that have explored this neurotransmitter in this brain region. It has been found that rimonabant causes a rise in extracellullar levels of serotonin and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the rat nucleus accumbens, as evaluated by *in vivo* microdialysis, without altering the levels of norepinephrine dopamine or their corresponding metabolites, in contrast to what occurs in the prefrontal cortex (Tzavara *et al*. 2003). Interestingly, attenuation of the cannabinoid withdrawal syndrome by 3,4-methylenedioxy-N-methylamphetamine (MDMA) in mice may be related to alterations in serotonergic transmission in the prefrontal cortex, but not in the nucleus accumbens (Touriño, Maldonado & Valverde 2007).

Other findings, including behavioral data, reveal that local perfusion of the cannabinoid agonist AM251 into the rat nucleus accumbens prevents cocaine-primed relapse. This effect does not seem to be associated with inhibition of the release of dopamine, as it is opposed to occurring in glutamatergic neurotransmission (Xi *et al*. 2006). However, no significant changes were found by evaluating the cannabinoid-induced antinociceptive effect on the tail-flick latency test after microinjection of WIN 55,212-2 in the rat nucleus accumbens (Martin *et al*. 1999).

Finally, we describe the main results of two different studies. The first one finds that cocaine, delta(9)-tetrahydrocannabinol, nicotine and morphine cause ERK phosphorylation in mouse nucleus accumbens. It is likely that this effect is mediated through a dopamine receptor D1 mechanism (Valjent *et al*. 2004). The second one shows that while the antagonist rimonabant enhances the electrically evoked activity of the dopaminergic medial forebrain bundle, which projects to the nucleus accumbens, the agonist WIN 55,212-2 depresses this response (Pillolla *et al*. 2007).

Opioids

Several studies have explored the functional interaction between the opioid and cannabinoid systems in the nucleus accumbens in relation to the release of dopamine. By using *in vivo* brain microdialysis, it has been demonstrated that delta(9)-tetrahydrocannabinol, WIN 55,212-2 and heroin elevate the extracellular levels of dopamine in the shell of rat nucleus accumbens. Previous administration of the antagonist rimonabant reverses delta(9)-tetrahydrocannabinol- but not heroin-induced increases of dopamine transmission. However, naloxone and the μ-opioid receptor antagonist naloxonazine are able to reverse the enhancement of this heroin-related dopamine efflux (Tanda, Pontieri & Di Chiara 1997). In addition, it is also known that the acute morphine injection-induced elevation of dopamine in the shell of rat nucleus accumbens is not reversed by rimonabant. However, during heroin self-administration, local administration of rimonabant into the nucleus accumbens reduces the heroin-induced increase of dopamine (Caillé & Parsons 2006). In another study, it was found that rimonabant treatment did not modify the release of extracellular dopamine levels in the shell of rat nucleus accumbens (Alonso *et al*. 1999). Moreover, the activation of CB1 cannabinoid receptors by the cannabinoid agonists WIN 55,212-2 and HU-210, as well as that of the μ-opioid receptor agonists by morphine and [D-Ala^2,N-Me-Phe^4,Gly-ol]-enkephalin, inhibited the release of

glutamate and GABA in the core of rat nucleus accumbens. These effects are selectively and allosterically antagonized by the cannabinoid receptor antagonists, rimonabant and AM251, and by the μ-opioid receptor antagonists, naloxone and D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (Schoffelmeer *et al.* 2006).

In the shell nucleus accumbens, Fos-IR was increased after treatment with morphine (10 mg/kg), as was previously shown in the amygdala. Although a similar increase of Fos-IR was found after rimonabant treatment alone (3 mg/kg), the combination of both compounds attenuated this Fos-IR increment (Singh *et al.* 2004). Chronic exposure to delta(9)-tetrahydrocannabinol results in a significant increase of heroin-induced Fos-IR in the core of rat nucleus accumbens, while acute administration of heroin elevates Fos-IR in the shell and core rat nucleus accumbens (Singh *et al.* 2005). However, if rats are perinatally exposed to delta(9)-tetrahydrocannabinol (5 mg/kg, from PD 4 to 14), an important reduction of acute heroin-induced Fos-IR is observed in the shell region of the nucleus accumbens (Singh *et al.* 2006).

Several authors have studied the roles of serotonin, adenosine A2A receptors and inhibitory postsynaptic currents in order to explain functional interactions between the cannabinoid and opioid systems in the nucleus accumbens. In one study, repeated treatment with the selective serotonin reuptake inhibitor fluoxetine was found to diminish proenkephalin gene expression in the shell, not in the core, of the rat nucleus accumbens. A similar decrement of prodynorphin gene expression was observed in the core and shell of this region, whereas CB1 receptor was decreased in the caudate-putamen, but not in the accumbens (Oliva *et al.* 2005). In another study, synergism between CB1 and δ-opioid receptors was found to be mediated by adenosine A2 receptors. This synergistic mechanism implies the activation of cAMP–PKA intracellular signaling after activation of CB1 and δ-opioid receptors by subthreshold doses of cannabinoid/opioid agonists (Yao *et al.* 2003). Likewise, local and systemic administration of the adenosine A2a receptor antagonist 3,7-dimethyl-1-propargylxanthine suppresses heroin-primed reinstatement of drug-seeking behavior in rats (Yao *et al.* 2006). Furthermore, treatment of rat brain slices of the shell of nucleus accumbens with WIN 55,212-2 did not alter the resting membrane potential or the whole cell conductance, which seems to dismiss the idea of a WIN 55,212-2-induced postsynaptic effect. However, while cannabinoid-evoked GABAergic inhibitory postsynaptic currents are inhibited by a non-specific selective-opioid receptor antagonist (μ/δ opioid), no effect was found using a μ-opioid receptor antagonist (DAMGO) (Hoffman & Lupica 2000).

Another set of experiments was performed in order to explore functional interactions between cannabinoid and opioid systems in the nucleus accumbens; however, other variables were evaluated in this case, including heroin self-administration, cross-tolerance to an opioid antagonist, food intake, behavioral sensitization, antinociception and antidepressant-like properties. The main findings of these experiments can be summarized as follows: heroin self-administration in rats, morphine self-administration and morphine-induced place preference in mice are blocked by rimonabant. In addition, whereas morphine-dependent mice exhibit an opiate-like withdrawal syndrome after rimonabant treatment, naloxone precipitates a mild cannabinoid-like withdrawal syndrome. Acute treatment with morphine leads to a reduction of CB1 receptor mRNA expression in the rat nucleus accumbens, but this effect disappears following chronic morphine treatment (Navarro *et al.* 2001). Also, intravenous self-administration of heroin in rats increases the density of the binding of μ-opioid receptors in the nucleus accumbens, but it does not affect their functionality. On the contrary, CB1 receptors drastically increase their activity in the nucleus accumbens. Additionally, intravenous self-administration of WIN 55,212-2 causes an increase of μ-opioid receptors in this brain region (Fattore *et al.* 2007). Chronic exposure to delta(9)-tetrahydrocannabinol or WIN 55,212-2 in rats produces cross-tolerance to the inhibitory effects of an opioid agonist, as well as a decrease in the plasticity and the sensitivity of glutamatergic and GABAergic synapses to cannabinoids and opioids (Hoffman *et al.* 2003). Systemic and intracerebral (into the rat nucleus accumbens or paraventricular nucleus of the hypothalamus) administration of morphine increases food intake. Rimonabant reverts this effect when it is administered either systemically or locally into the hypothalamus, but not when it is injected locally into the nucleus accumbens (Verty *et al.* 2003). Some of the signs of morphine-induced behavioral sensitization are prevented by the antagonist rimonabant. However, whereas the anandamide level in the nucleus accumbens is increased after an acute morphine injection, the level of 2-AG in the same structure is decreased (Vigano *et al.* 2004). Acute co-administration of subthreshold doses of the cannabinoid agonist CP-55,940 and morphine causes a significant analgesic effect using the tail-flick test. When this subthreshold cannabinoid treatment is chronic, CP-55,940 produces a significant antinociceptive effect in morphine-tolerant rats. However, if CP-55,940-tolerant rats receive a morphine challenge, this analgesic response is not present. A reduction in μ-opioid receptor binding in the nucleus accumbens of CP-55,940-tolerant rats has also been observed (Vigano *et al.* 2005). Acute treatment with delta(9)-tetrahydrocannabinol in mice facilitates enkephalins-induced antinociceptive- and antidepressant-like properties, and it increases the extracellular levels of met-enkephalin in the mouse

nucleus accumbens. Chronic treatment with delta(9)-tetrahydrocannabinol (3 weeks) reverses the naloxone-induced withdrawal syndrome in mice that are addicted to morphine (Valverde *et al.* 2001).

Acetylcholine

Consistent with the lower expression of CB1 receptors in the rat nucleus accumbens in comparison with other brain regions, the WIN 55,212-2-induced reduction of acetylcholine is lower in this region than in the hippocampus. However, potentiation of the release of acetylcholine after treatment with rimonabant is observed in the hippocampal region, but not in the nucleus accumbens (Gifford & Ashby 1996). Using *in vivo* microdialysis, it has been observed that different doses of rimonabant (3.0 and 10.0 mg/kg) do not change the efflux of acetylcholine in the rat nucleus accumbens, but they do increase acetylcholine neurotransmission in the medial prefrontal cortex (Tzavara *et al.* 2003). Recently, it has been demonstrated that delta(9)-tetrahydrocannabinol-induced dopamine release in the rat shell of nucleus accumbens can be reversed by the selective α-7 nicotinic acetylcholine receptor antagonist methyllycaconitine (Solinas *et al.* 2007a). However, the inactivation of μ-opioid and CB1 cannabinoid receptors do not result in significant changes in the inhibition of acetylcholine release in the rat nucleus accumbens core (Schoffelmeer *et al.* 2006).

THE PREFRONTAL CORTEX AND THE CANNABINOID SYSTEM

The prefrontal cortex is characterized, among other functions, by the guidance, control, planning, perseverance and inhibition of behaviors. Part of these functions is carried out by the glutamatergic projections to the nucleus accumbens and amygadala, as well as the glutamatergic and dopaminergic efferences to the VTA (see Fig. 1 and Kalivas, Volkow & Seamans 2005). In addicted individuals and in animal models of drug addiction, this region seems to be affected and can cause significant disregulations of normal brain functioning after repeated drug exposure, such as alcohol or classical psychostimulants (Porrino & Lyons 2000; Volkow *et al.* 2007).

GABA and Glutamate

As previously noted in the section describing the nucleus accumbens, Robbe and colleagues explored the connections between the prefrontal cortex and the nucleus accumbens, and showed that WIN 55,212-2 is able to inhibit spontaneous and evoked glutamate-mediated transmission through a presynaptic mechanism (Robbe *et al.* 2003). There is also evidence that a single exposure to delta(9)-tetrahydrocannabinol *in vivo* causes the suppression of endocannabinoid-mediated LTD in the mouse cortico-accumbens synapse. In addition, subchronic treatment with delta(9)-tetrahydrocannabinol reduces both the coupling efficiency at CB1 receptors to Gi/o transduction proteins and the inhibition of cortico-accumbens excitatory synapses induced by the activation of CB1 receptors (Mato *et al.* 2005).

More specifically, with regard to cannabinoid and GABA–glutamate interactions in the prefrontal cortex, there have been very interesting findings focused on the layers of the prefrontal cortex. Here, we briefly review three of these studies. The electrophysiological study of WIN 55,212-2 and CP-55,940 in slices of rat prefrontal cortex (layer V pyramidal afferents) has shown that both molecules suppress glutamatergic excitatory postsynaptic currents. In addition to suppressing these effects, rimonabant, by itself, was also able to increase excitatory glutamatergic neurotransmission. WIN 55,212-2 and rimonabant act differentially in 'non-plastic' cells [neuronal cells that are resistant to tetanic stimulation-induced LTD or long-term potentiation (LTP)] and 'plastic' cells. They exhibited a lack of effect in the former neurons. Indeed, in the presence of tetanic stimulation in plastic neurons, WIN 55,212-2 potentiates LTD rather than LTP, while rimonabant causes the inverse effect, that is, it potentiates LTP rather than LTD (Auclair *et al.* 2000). Moreover, administration of WIN 55,212-2 is able to suppress excitatory postsynaptic currents in pyramidal neurons from layer V of the cortex, although not in layers II or III, even though these three layers express high levels of the CB1 receptor. It is known that the activation of pyramidal neurons causes endocannabinoid-mediated depolarization-induced suppression of excitation while their inhibition is not sensitive to endocannabinoid release. Another set of experiments permit the conclusion that the superficial cortex layers II and III are less sensitive to cannabinoid actions in glutamatergic neurotransmission than the profound cortex layer V (Fortin & Levine 2007). Additionally, the existence of a functional interaction between group II metabotropic glutamate receptors and CB1 receptors has been demonstrated. Accordingly, the activation of group II metabotropic glutamate receptors caused a similar long-lasting depression of excitatory neurotransmission relative to that exerted by cannabinoids at presynaptic locations in layer V of pyramidal neurons of the rat prefrontal cortex. At the postsynaptic level, the interaction between CB1 and group II metabotropic glutamate receptors occurs through activation of ERK (Barbara *et al.* 2003).

Another two groups of researchers have studied the extracellular release of GABA and glutamate in the prefrontal cortex by *in vivo* microdialysis. It has been shown that acute treatment with delta(9)-tetrahydrocannabinol

causes an increase in the extracellular levels of dopamine and glutamate while it reduces the level of GABA (Pistis *et al.* 2002). Acute administration of WIN 55,212-2 into the rat frontal cortex causes a dose-dependent inhibition of the extracellular levels of GABA. This inhibition is prevented by SR141716, which is ineffective by itself (Ferraro *et al.* 2001). Regarding glutamatergic transmission in the prefrontal cortex, a dose of 1 mg/kg of WIN 55,212-2 is able to increase the extracellular levels of glutamate, whereas doses of 0.01 and 2 mg/kg do not have such an effect; however, different results are obtained *in vitro*. Thus, in primary cultures of rat prefrontal cortex neurons, the administration of WIN 55,212-2 causes a clear dose-dependent release of glutamate (Ferraro *et al.* 2001). The fact that rimonabant is able to prevent all of the effects exerted by these cannabinoid agonists reported in the above studies suggests altered GABAergic and glutamatergic transmission under CB1 receptor control.

Biogenic amines (dopamine, noradrenaline, serotonin)

Similar to the basolateral amygdala, stimulation of the rat prefrontal cortex causes excitation of the nucleus accumbens neurons. This activity can be prevented by WIN 55,212-2, HU-210 or delta(9)-tetrahydrocannabinol treatment. Rimonabant reverses this cannabinoid-induced inhibitory effect (Pistis *et al.* 2002). Furthermore, it is recognized that electrical stimulation of the VTA leads to a phasic inhibition of rat prefrontal cortex pyramidal neurons. This inhibition is reversed by delta(9)-tetrahydrocannabinol and WIN 55,212-2 treatment. As the cannabinoid-induced reduction of inhibition is reversed by rimonabant, it has been suggested that the activation of CB1 receptors controls the excitability of prefrontal cortex pyramidal neurons (Pistis *et al.* 2001). Furthermore, *in vitro* and *in vivo* studies have shown that stimulation of the rat prefrontal cortex induces a transient suppression of excitatory efferences onto dopamine mesolimbic projections mediated by the release of 2-arachidonyl-glycerol. This activates metabotropic glutamate receptors and intracellular calcium. These effects are mimicked by the agonist WIN 55,212-2 and reversed by the antagonist rimonabant (Melis *et al.* 2004).

The extracellular release of the main biogenic amines and their metabolites in this brain region after cannabinoid treatment has also been studied. For example, using *in vivo* microdialysis, different doses of rimonabant cause an increase in the extracellullar levels of the following monoamines in the rat medial prefrontal cortex: serotonin (and its metabolite 5-HIAA), norepinephrine and dopamine [and their metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] (Tzavara *et al.* 2003). While acute treatment with delta(9)-tetrahydrocannabinol increases dopamine turnover (DOPAC/dopamine) in the rat prefrontal cortex, but not in the nucleus accumbens or striatum (Jentsch *et al.* 1997), subchronic and chronic treatments with delta(9)-tetrahydrocannabinol and WIN 55,212-2 cause a selective and persistent (up to 14 days) reduction of dopamine turnover in the rat medial prefrontal cortex. No significant changes were found in the nucleus accumbens or striatum (Verrico, Jentsch & Roth 2003). On the contrary, oral treatment with cannabinoid receptor antagonists (rimonabant or SLV319) results in an increase in the efflux of dopamine and norepinephrine in the rat prefrontal cortex (Need *et al.* 2006). Interestingly, subchronic treatment with WIN 55,212-2 leads to an enhancement of the norepinephrine level in the rat prefrontal cortex after acute challenge with the same cannabinoid receptor agonist. Also, an increase in the expression of the catecholamine-synthesizing enzyme tyrosine hydroxylase in the rat locus coeruleus was observed (Page *et al.* 2007). Finally, acute and chronic administration of MDMA attenuates the rimonabant-induced withdrawal syndrome in a dose-dependent manner in animals that are chronically treated with delta(9)-tetrahydrocannabinol. These effects are related to an increase in the extracellular levels of serotonin in the mouse prefrontal cortex, but the responses in the nucleus accumbens were not altered (Touriño *et al.* 2007).

Alternatively, the cocaine-induced increase of dopamine in rat prefrontal cortex synapses was evaluated in relation to the endocannabinoid system. Blockade of CB1 receptors by rimonabant as well as acute administration of cocaine result in a dose-dependent increase of Fos-like immunoreactivity in the rat prefrontal cortex, with a higher number of Fos-positive cells across the infralimbic and prelimbic cortices. However, antagonism of D1 and D2-like receptors is not able to prevent the rimonabant-induced increase of Fos-like immunoreactivity (Alonso *et al.* 1999). In addition, in rats, a single exposure to cocaine blocks endocannabinoid-induced LTD. This effect was not present in mice lacking the dopaminergic D1 receptor or those suppressed by inactivation of D1 receptors by a selective antagonist (Fourgeaud *et al.* 2004).

Other types of studies have explored the co-localization of CB1 receptors, alterations of neuromorphology after cannabinoid treatment and activation of G-proteins. Analysis of the rat frontal cortex by confocal immunofluorescence and immunoelectron microscopy has shown that one-third of axon terminals co-express CB1 receptors and the catecholamine-synthesizing enzyme dopamine-β-hydroxylase. Indeed, CB1 receptors are located at noradrenergic presynaptic terminals (Oropeza, Mackie & Van Bockstaele 2007). There is an increase in the length of dendrites as well as

in the number of dendritic branches into the rat medial prefrontal cortex after chronic treatment with delta(9)-tetrahydrocannabinol. Also, a similar alteration occurs in the shell of the nucleus accumbens (Kolb *et al.* 2006). In prefrontal slices of post-mortem human brain, the localization of receptor-activated G-proteins was analyzed by autoradiography, with the highest binding being found after activation of CB1 receptors following WIN 55,212-2 treatment. By contrast, others agonists, including μ-opioid, serotonin-1A, serotonin-1B/D and α-adrenoreceptors, slightly increased the binding of receptor-activated G-proteins (Rodríguez-Puertas *et al.* 2000).

An interesting study used spontaneously hypertensive rats, which are considered a validated animal model of attention-deficit hyperactivity disorder, to elucidate the reduction of CB1 receptor expression in the spontaneously hypertensive rat prefrontal cortex in impulsive rats when compared with their respective control Wistar–Kyoto rats. Additionally, when impulsive rats were treated with WIN 55,212-2, an increase in self-control in their impulsive-like behavior was observed, whereas there was no effect in Wistar–Kyoto control rats (Adriani *et al.* 2003).

Opioids

Most of the studies regarding the interactions between opioid and cannabinoid systems in the prefrontal cortex are based on voluntary or non-voluntary treatment with opioid receptor agonists. For example, an acute injection of morphine led to an increase in anandamide levels in several rat brain regions, including the nucleus accumbens and caudate-putamen, but not in the prefrontal cortex. Similar results were obtained after chronic treatment with morphine. However, following a 15-day withdrawal period, anandamine levels had increased significantly in the prefrontal cortex, suggesting an endocannabinoid adaptation after chronic morphine treatment in this brain region (Vigano *et al.* 2004). While intravenous self-administration of heroin in rats increased functionality in the prefrontal cortex, intravenous self-administration of WIN 55,212-2 caused an increase of μ-opioid receptors in this same region (Fattore *et al.* 2007). Moreover, in mice, acute administration of delta(9)-tetrahydrocannabinol and morphine resulted in an increase in ERK phosphorylation in the deep layers of the prefrontal cortex. A dopaminergic receptor antagonist D1 is able to reverse these effects, which suggests involvement of an underlying dopamine D1 receptor-dependent mechanism (Valjent *et al.* 2004). Also, in the rat prefrontal cortex, there is a sexual dimorphic expression of μ-opioid receptors after chronic treatment with delta(9)-tetrahydrocannabinol during the gestation and lactation periods. Female rats born from these rats, but not males, had a higher density of μ-opioid receptors in the prefrontal cortex compared with their controls (Vela *et al.* 1998). In another type of study, it was determined that the opioid antagonist naloxone was able to reverse the cannabinoid-induced inhibition of action potentials of dopaminergic neurons from the rat nucleus accumbens shell in response to electrical stimulation of the prefrontal cortex (Pistis *et al.* 2002).

Acetylcholine

Endocannabinoid/acetylcholine-related studies in the prefrontal cortex are almost exclusively focused on the release of acetylcholine. For instance, using *in vivo* microdialysis, intravenous administration of the cannabinoid receptor agonists WIN 55,212-2, HU-210 and delta(9)-tetrahydrocannabinol resulted in a dose-dependent increase in the efflux of acetylcholine levels in the rat prefrontal cortex. These effects were reversed by the antagonist rimonabant. The dose of rimonabant used was unable to change the extracellular levels of acetylcholine in this brain area (Acquas *et al.* 2000, 2001). However, in an earlier study, it was demonstrated that intraperitoneal administration of WIN 55,212-2 and delta(9)-tetrahydrocannabinol caused long-lasting decrease in the amount of acetylcholine in the rat medial prefrontal cortex. Cannabinoid-induced inhibition of acetylcholine release was suppressed by rimonabant. Furthermore, when rimonabant was administrated alone at a modestly high dose (3.0 mg/kg), the extracellular acetylcholine levels were increased; however, a low dose of rimonabant alone (0.1 mg/kg) was ineffective in the medial prefrontal cortex (Gessa *et al.* 1998). More recently, it has been reported that delta(9)-tetrahydrocannabinol stimulates the release of acetylcholine in the rat prefrontal cortex, which can be prevented by several opioid and dopaminergic antagonists in different experimental set-ups. In this way, systemic injection of naloxone, naltrexone or a D1 receptor antagonist inhibited cannabinoid-induced acetylcholine release. Likewise, bilateral infusion of μ-opioid receptor agonists into the rat VTA had the same effects (Pisanu *et al.* 2006). Another study showed that systemic administration of WIN 55,212-2 and delta(9)-tetrahydrocannabinol dose- and time-dependently enhanced the acetylcholine level in the prefrontal cortex region. Although rimonabant reverses these effects, it appears that this stimulation of acetylcholine [induced by delta(9)-tetrahydrocannabinol] is not caused by the activation of cannabinoid receptors in the prefrontal cortex, because the administration of delta(9)-tetrahydrocannabinol by reverse microdialysis does not modify the acetylcholine efflux in this region (Verrico

et al. 2003). Finally, Tzavara and colleagues showed that different doses of rimonabant (3.0 and 10.0 mg/kg) resulted in an increase in the extracellullar level of acetylcholine in the rat medial prefrontal cortex, but not in the nucleus accumbens (Tzavara *et al.* 2003).

THE HIPPOCAMPUS AND THE CANNABINOID SYSTEM

It is well known that the hippocampus region is involved in memory, and mainly, in the acquisition and consolidation of episodic memories. Furthermore, this region controls different types of learning and process of habit formation. One key feature in becoming addicted is the association of particular environments with drug effects, as well as the association of drug intake with internal emotional states. In consequence, such learning and long-lasting associations perpetuate the search for drug intake. In the origin of this memory, we can find synaptic plasticity processes, essentially LTP and LTD (Kauer & Malenka 2007).

Currently, four endocannabinoid mechanisms are known to exist within CA1-interneuron cell populations. First, it has been postulated that inhibition of GABA release (also known as depolarization-induced suppression of inhibition) can be induced by endocannabinoids. Moreover, the activation of G-protein coupled receptors (e.g. metabotropic acetylcholine receptors and group I mGluRs), in addition to an increase in the depolarization-induced suppression of inhibition induced by cannabinoids, can lead to persistent suppression of evoked inhibitory postsynaptic currents. Finally, the activation of group I mGluRs induces (but does not maintain) cannabinoid-related LTD of GABA release (i.e. inhibitory LTD) (Edwards, Kim & Alger 2006).

GABA and glutamate

Numerous studies have been devoted to functional interactions between these amino acids and the endocannabinoid system in the hippocampus. One part of these studies has generally explored the cannabinoid-induced neuromodulation throughout the hippocampus. The main findings of these studies can be described as follows: administration of WIN 55,212-2 to presynaptic axon terminals in mouse slices of the hippocampus reduced glutamate-excitatory transmission. However, mice that were deficient for the CB1 receptor in all neurons, except for GABAergic neurons, did not show such cannabinoid-induced glutamate-excitatory neuromodulation. In contrast, when the lack of CB1 receptors was selective and exclusive to GABAergic neurons, cannabinoid-induced glutamate-excitatory neuromodulation was observed (Domenici *et al.* 2006). Another study using similar conditional mutant mice lacking CB1 receptors on the GABAergic interneurons showed that excitotoxic lesions induced by kaininic acid were reduced in hippocampal pyramidal neurons. This effect was presumably mediated by an increase in anandamide levels in this brain region (Marsicano *et al.* 2003). Similarly, by using synaptosomes isolated from the rat hippocampus, it has been shown that both GABA and aspartate release were inhibited by synthetic cannabinoids (WIN 55,212-2, CP-55,940 and ACEA), as well as by endogenous cannabinoids (anandamide and 2-arachidonoylglycerol) and capsaicin. However, the antagonists AM-251 and rimonabant were not able to reverse these cannabinoid effects (D'amico *et al.* 2004). Therefore, the authors suggest that cannabinoid-induced inhibition of aspartate and GABA release was not mediated by presynaptic CB1 receptors. Further studies using glutamatergic nerve endings isolated from rat hippocampus have found that the effects of endogenous and synthetic cannabinoids were not always identical (Cannizzaro *et al.* 2006). These authors show that anandamide caused a concentration-dependent inhibition of aspartate release, whereas the synthetic agonist WIN 55,212-2 inhibited its release. In both cases, the release was evoked by KCl. Along this line of results, we review three electrophysiological studies. In the first study, it was shown that hippocampal LTD, which was generated by high-frequency stimulation, was blocked when animals were treated repeatedly with delta(9)-tetrahydrocannabinol. This effect can persist for 3 days after cannabinoid withdrawal. In contrast, treatment with the antagonist AM-251 alone increased LTD and prevented the cannabinoid-induced inhibition of LTD. Interestingly, while WIN 55,212-2 did not show tolerance to glutamate release, delta(9)-tetrahydrocannabinol produced tolerance to the inhibition of synaptic GABA release (Hoffman *et al.* 2007). In the second study, it was demonstrated that CP-55,940 inhibited the vesicular GABA release evoked by low-level stimulation in the rat hippocampus. This effect was reversed by the antagonist AM-251. However, when the stimulus frequency was increased, the cannabinoid-induced inhibition of GABA exocytosis was absent (Brager *et al.* 2003). In the third study, the authors reported that both cannabinoid agonists, WIN 55,212-2 and CP-55,940, modulated depolarization-induced suppression of inhibition and prevented inhibitory postsynaptic currents in the rat dentate gyrus, whereas the antagonist rimonabant prevented these effects (Isokawa & Alger 2005).

Most of the following studies are related to the CA1 and CA3 pyramidal cells of the hippocampus. There are a number of relevant findings indicating a close relationship between cholecystokinin interneurons and CB1 receptors in the CA1 area of the hippocampus. The principal cell populations of the adult mice hippocampus

(CA1 and CA3 pyramidal neurons) express high levels of the enzyme that generates the endocannabinoid 2-AG: diacylglycerol lipase α. Also, this enzyme is highly expressed in granule cells of the dentate gyrus. However, diacylglycerol lipase α is only present at a low level, if at all, in GABAergic interneurons and glial cells. It seems that diacylglycerol lipase α is expressed in postsynaptic terminals on glutamatergic neurons of the mouse hippocampus, whereas CB1 receptors are expressed in presynaptic regions. In addition, CB1 receptors are abundant in GABAergic axon terminals (Katona *et al.* 2006). It is known that Schaffer collaterals project to the CA1 area of the hippocampus and that repetitive stimulation of these fibers activates group I (mGlu1 and mGlu5) receptors at CA1 pyramidal cells, leading to presynaptic cannabinoid-induced reduction of GABA release. It seems that CB1 receptors are necessary for the induction of LTD at inhibitory synapses, as they are reverted by the antagonist AM-251, but are not needed for its maintenance (Chevaleyre & Castillo 2003). WIN 55,212-2 reduces the excitatory postsynaptic currents in rat CA1 pyramidal neurons. The cannabinoid-induced suppression of inhibitory postsynaptic currents is mediated presynaptically and can be prevented by rimonabant. The authors suggest that cannabinoid receptors are located exclusively in the inhibitory synapses of the hippocampal neurons (excluding excitatory synapses) and that CA1 pyramidal neurons, but not interneurons, are able to produce endocannabinoids after prolonged states of depolarization (Hoffman *et al.* 2003). Recently, it has been found that these effects are mediated by the activation of ryanodine receptors (the major cellular mediator of calcium-induced calcium release) in rat CA1 pyramidal cells, thereby suggesting their critical role in endocannabinoid release (Isokawa & Alger 2006).

However, in the CA1 region, there is some discrepancy between the results of various authors. The WIN 55,212-2-induced inhibition of glutamatergic excitatory postsynaptic currents is present in the hippocampus of CD-1 strain mice and Sprague Dawley strain rats, but not in C57BL/6J strain mice. Both wild-type mice strains were used as the background for the generation of two independent lines of CB1 receptor-deficient mice (Hoffman *et al.* 2005). However, further studies did not find such differences, and they claim that in wild-type C57BL/6 mice, the cannabinoid-induced inhibition of glutamatergic excitatory postsynaptic currents is present at Schaffer collateral/commissural fiber-CA1 pyramidal cells of the hippocampus (Takahashi & Castillo 2006).

With regard to cholecystokinin-expressing interneurons in the CA1 pyramidal cells of the hippocampus, it is known that they express CB1 receptors on their presynaptic terminals. Moreover, when they are activated, GABA release is suppressed (Klausberger *et al.* 2005). The major source of perisomatic GABAergic input to CA1 pyramidal cells is the cholecystokinin-positive basket cells. Analyses of these interneurons have demonstrated that the proportion of action that fails to evoke GABA release is decreased after administration of the antagonist AM-251, suggesting persistent suppression of synaptic neurotransmission by CB1 receptors. Likely, the activation of metabotropic glutamate receptors, as well as muscarinic receptors, causes the inhibition of neurotransmission that is mediated by the release of endocannabinoids (Neu, Foldy & Soltesz 2007). Moreover, endocannabinoid exposure facilitates the induction of LTP in the rat CA1 pyramidal cells of the hippocampus. This effect would be mediated by participation of endocannabinoids in depolarization-induced suppression of inhibition (Carlson, Wang & Alger 2002). It seems that cannabinoid-mediated depolarization-induced suppression of inhibition may differ depending on the firing rates of presynaptic interneurons. With low-frequency action potentials in rat cholecystokinin-positive CA1 basket cells, the administration of WIN 55,212-2 suppresses the inhibitory postsynaptic currents in postsynaptic pyramidal neurons. However, with high-frequency action potentials, the cannabinoid-induced inhibition of GABA release can be abolished (Földy *et al.* 2006). GABA-evoked currents in rat hippocampus mossy fibers-CA3 are also sensitive to stimulation of CB1 receptors by WIN 55,212-2. This cannabinoid reduces spill-over peak amplitudes, whereas the cannabinoid CB1 receptor antagonist AM-251 reverses this effect (Alle & Geiger 2007). From a neurodevelopmental perspective, prenatal treatment with delta(9)-tetrahydrocannabinol increases the density of cholecystokinin-expressing interneurons in the rat hippocampus. In addition, anandamide induces migration and morphogenesis of CB1 receptor-expressing interneurons in this brain area. It is thought that the trans-activation of tyrosine kinase receptor B-dependent signaling would mediate such effects (Berghuis *et al.* 2005).

Another group of studies provided findings that were relevant to neuroprotection and exposure to alcohol. The first study demonstrated that CB1 receptors co-localize with the vesicular glutamate transporter 1 at glutamatergic terminals of the mouse hippocampus, and by using mice with a CB1-receptor-gene deletion in the hippocampus, that the CB1 receptor is necessary and sufficient to protect against kainic acid-induced seizures caused by aberrant glutamatergic neurotransmission (Monory *et al.* 2006). In addition, pre-treatment with WIN 55,212-2 and delta(9)-tetrahydrocannabinol reduced glutamatergic activity-induced neural death in hippocampal neurons in culture. After prolonged exposure to either of the cannabinoids, a desensitization of CB1 receptors occurs and cannabinoid-induced neuroprotection is

reduced (Gilbert *et al*. 2007). The second study shows that rats exposed to chronic intermittent alcohol exposure present not only significant reductions in CB1 receptor mRNA and protein levels on GABAergic synapses of the rat hippocampus after two days of withdrawal, but also a decrease in the frequency of spontaneous inhibitory currents. However, after an alcohol-withdrawal period of 40 days, CB1 receptor mRNA and protein levels are increased, with no significant changes in spontaneous inhibitory currents (Mitrirattanakul *et al*. 2007). Furthermore, acute administration of alcohol decreases the levels of the endogenous cannabinoid anandamide in the rat hippocampus without altering the enzymatic activity levels of the FAAH or anandamide precursors (Ferrer *et al*. 2007).

Using a more integrated approach to analyze the functional interactions between GABA/glutamate and cannabinoid neurotransmission, some studies suggest, on the one hand, that the activation of CB1 receptors by WIN 55,212-2 on GABAergic interneurons could modulate the flow and encoding of information in the hippocampus, as well as the selection of the corresponding behavioral strategy (Hampson & Deadwyler 1999). On the other hand, the studies also indicate that the endocannabinoid system and the cannabinoid-induced reduction of neurotransmitter release would be critical parts of the cellular and molecular mechanisms of learning and memory in the hippocampus (for review, see Sullivan 2000).

Biogenic amines (dopamine, noradrenaline, serotonin)

In this brain region, regarding the biogenic amines and the endocannabinoid system, a large portion of the reviewed studies have explored either the extracellular release of these neurotransmitters or their implication in working memory. The first type of study shows that delta(9)-tetrahydrocannabinol and WIN 55,212-2 have different effects in aminergic neurotransmission within the rat hippocampus. Both cannabinoid agonists increase dopa/noradrenaline synthesis and reduce dopa/dopamine and 5-hydroxytryptophan (5-HTP)/5-HT synthesis in this brain region, whereas cannabinoid receptor antagonists (rimonabant or AM-281) reverse these effects (Moranta, Esteban & Garcia-Sevilla 2004). In addition, the synthesis of these monoamines can be modified if rats are treated subchronically with alcohol (7 days). While cannabinoid agonists increase dopa/noradrenaline and reduce 5-HTP/5-HT synthesis, both alterations are reduced in chronic alcohol-withdrawn rats (Moranta, Esteban & Garcia-Sevilla 2006). Using human and guinea pig hippocampal slices, it has been shown that CB1 receptors could be mediating the release of noradrenaline in this brain area (Schlicker *et al*. 1998). This was confirmed later using guinea pig hippocampal slices. Both agonists, WIN 55,212-2 and CPP-55,940, suppress NMDA- and kainate-stimulated noradrenaline release. However, the antagonist rimonabant has the opposite effect; it increases noradrenaline release (Kathmann *et al*. 1999). Unlike the inhibitory effects of CP-55,940 in the electrically evoked acetylcholine release observed in rat brain slices, norepinephrine is not modified after CP-55,940 or rimonabant treatment (Gifford *et al*. 1997). Although acute administration of delta(9)-tetrahydrocannabinol leads to an increase in serotonin levels in the rat dorsal hippocampus, this effect is not present following chronic prenatal or postnatal delta(9)-tetrahydrocannabinol treatment (Molina-Holgado *et al*. 1993). Another study, which linked food intake and monaminergic release, shows that chronically low doses of delta(9)-tetrahydrocannabinol (0.001 mg/kg) increase mouse food intake and activity. Whereas cannabinoid-induced food consumption is reversed by rimonabant, this is not the case for the effect on mouse activity. This dose of delta(9)-tetrahydrocannabinol decreases dopamine and serotonin levels in the hippocampus, although no significant changes are found with norepinephrine (Avraham *et al*. 2004).

In the next few lines we review the main findings of three studies related to working memory. Delta(9)-tetrahydrocannabinol-induced impaired working memory in rats is reversed by the antagonist rimonabant and by a D2 dopamine receptor antagonist. Also, the cannabinoid-induced memory impairment can be potentiated by the administration of a D2 dopamine receptor agonist (Nava *et al*. 2000). Another study examined the role of serotonin in delta(9)-tetrahydrocannabinol-induced impaired working memory and found that delta(9)-tetrahydrocannabinol increased the amount of serotonin in the ventral hippocampus, while it reduced the release of serotonin from the ventral hippocampus. In addition, the serotonin precursor 5HTP, a serotonin reuptake inhibitor, a serotonin receptor agonist and a 5-HT_2 serotonin receptor antagonist reversed the delta(9)-tetrahydrocannabinol-induced impaired working memory in rats (Egashira *et al*. 2002). More recently, these authors examined the selective serotonin reuptake inhibitor, citalopram, in delta(9)-tetrahydrocannabinol-induced impairment of spatial memory, showing that low doses of this compound are able to diminish the cannabinoid effect on spatial memory (Egashira *et al*. 2006).

A series of results from studying the serotonin and cannabinoid systems and their relations have shown significant co-expression of the functional 5-HT_{3A} subunit of the 5-HT_3 receptor and CB1 receptors in all neural populations of the hippocampus and the subgranular layer of the dentate gyrus. In addition, these receptors co-exist on

GABAergic neurons (Morales & Bäckman 2002). Furthermore, and similar to the amygdalar region, very recent findings demonstrate the existence of CB1 proteins on serotonergic fibers from the raphe nuclei and in the synapses of the hippocampus (Häring *et al.* 2007). Also, serotonin and endocannabinoid systems seem to be related in alcohol addiction. For example, WIN 55,212-2 has different effects on alcohol consumption depending on the mice strains in such a way that it increases alcohol intake in the alcohol-avoiding DBA/2J mice, whereas chronic treatment has no effect on the alcohol-preferent C57BL/6J mice strain. The cannabinoid-induced increase of alcohol consumption in DBA/2J mice is reversed by rimonabant or by a serotonergic receptor 5-HT_{1A} agonist. In addition, in both mice strains, chronic WIN 55,212-2 treatment reduced [^{35}S]guanosine triphosphate-γ-S binding in the hippocampus (Kelaï *et al.* 2006).

Finally, we summarize two studies related to the dopaminergic and endocannabinoid systems in this brain area. In humans, prenatal studies show that CB1 mRNA receptor expression is predominant and is intensely localized to the amygdala and hippocampus. An increase of marijuana use from the mother is related to a decrease of D2 mRNA expression levels in the amygdala of the human fetus, but not in the hippocampus. Also, no significant changes are found in the D1 and CB1 mRNA levels in the human hippocampus (Wang *et al.* 2004). Furthermore, the acute administration of delta(9)-tetrahydrocannabinol (1 mg/kg) increases the ERK phosphorylation in the CA1, CA2 and CA3 areas of the mouse hippocampus, but not in the dentate gyrus. The blockade of dopaminergic D1 receptors is not able to reverse this effect (Valjent *et al.* 2004).

Opioids

There is clear evidence of the functional interactions between endocannabinoid and opioid systems (Navarro *et al.* 2001). Most of these articles are based on the non-voluntary or voluntary administration of opioids, followed by an evaluation of neurochemical changes from the endocannabinoid system. Acute and chronic injections of morphine lead to an increase of anandamide in the rat hippocampus. However, if rats are treated chronically with morphine, a new challenge of morphine significantly decreases the high levels of morphine-induced increases of anandamide. By contrast, 2-AG levels are decreased when the animals receive chronic and acute exposures to morphine. Also, the new challenge of morphine in chronic-morphine treated rats leads to an increase in 2-AG in this brain area (Vigano *et al.* 2004). However, in a previous study, these authors did not find significant changes in anandamide levels in the rat hippocampus after chronic morphine treatment (Vigano *et al.* 2003). This discrepancy may be due to the different regimen doses of chronic morphine treatment. In another study using autoradiographic-binding in rats that received chronic morphine, a significant reduction of CB1 receptors in the hippocampus is shown, whereas chronic treatment with the agonist cannabinoid receptor CP-55,940 does not change μ-opioid receptor levels in this brain area (Vigano *et al.* 2005). Also, there are sexual dimorphisms in the hippocampus (CA3 area) in animals born from mothers treated with delta(9)-tetrahydrocannabinol during gestation and lactation periods. In this brain region, cannabinoid exposure results in a higher density of μ-opioid receptors in females, but not in males (Vela *et al.* 1998). Whereas intravenous self-administration of heroin in rats increases the density of the μ-opioid receptors in the hippocampus and the functionality of CB1 receptors in this region, intravenous self-administration of WIN 55,212-2 causes a modest decrease in the levels of CB1 receptors in the hippocampus, but increases μ-opioid receptor levels and dramatically enhances their functionality (Fattore *et al.* 2007).

Acetylcholine

There are a large number of studies exploring the interconnections between this neurotransmitter and the endocannabinoid system in the hippocampus. Most of them have studied the release of acetylcholine after exposure to cannabinoids, which were essentially measured by microdialysis. Another group investigated the role of muscarinic acetylcholine receptors as part of the mechanism implicated in the pharmacological effects of cannabinoids. In the following section, we describe, in chronological order, the main findings about the release of acetylcholine mediated by cannabinoids. One of the first studies is from Gessa and coworkers in 1997. This study and others have shown that acute systemic injection of delta(9)-tetrahydrocannabinol, WIN 55,212-2 and CP-55,940 dose-dependently reduces the extracellular release of acetylcholine in the rat hippocampus, and this effect is reversed by the antagonist rimonabant. Furthermore, chronic systemic injection of delta(9)-tetrahydrocannabinol does not produce tolerance to its inhibitory effects (Gessa *et al.* 1997; Carta, Nava & Gessa 1998). In another study, these results are extended to show that intraperitoneal administration of WIN 55,212-2 and delta(9)-tetrahydrocannabinol causes a long-lasting inhibition of the release of acetylcholine levels in the rat hippocampus. This effect is suppressed by rimonabant, but when rimonabant is administrated alone in a modestly high dose (3.0 mg/kg), the extracellular acetylcholine levels are increased. This is not achieved with a low dose (0.1 mg/kg) (Gessa *et al.* 1998).

These findings can be replicated in rat hippocampal slices; in these slices, electrically evoked acetylcholine release is dose-dependently inhibited by WIN 55,212-2 (Gifford *et al*. 1999). Also, in rat hippocampal synaptosomes, it has been shown that WIN 55,212-2 reverses the release of acetylcholine; this inhibition is less potent or completely absent in cortical and striatal synaptosomes, respectively. In contrast, the antagonist rimonabant produces an increase in the release of acetylcholine in hippocampal synaptosomes (Gifford *et al*. 2000). However, a study shows that intravenous administration of WIN 55,212-2 and HU-210 increases, in a dose-dependent manner, the efflux of acetylcholine levels in the rat hippocampus. These effects are reversed by the antagonist rimonabant, which by itself does not change the extracellular levels of acetylcholine in this brain area (Acquas *et al*. 2000). Similar results have been found in CB1 knockout mice. In these mice, acetylcholine release is increased significantly after cannabinoid agonist treatment. However, in wild-type mice, while WIN 55,212-2 induces suppression of acetylcholine release, rimonabant increases acetylcholine efflux (Kathmann, Weber & Schlicker 2001).

Other studies correlate changes in acetylcholine levels with alterations in working memory. Delta(9)-tetrahydrocannabinol impairs working memory and reduces the extracellular levels of acetylcholine in the rat hippocampus. Both effects are reversed by the antagonist rimonabant and by a D2 dopamine receptor antagonist. In addition, the cannabinoid-induced memory impairment is potentiated by the administration of a D2 dopamine receptor agonist (Nava *et al*. 2000). These same authors show that repeated treatment with delta(9)-tetrahydrocannabinol does not produce tolerance to the reduction of extracellular hippocampal acetylcholine release (Nava *et al*. 2001). Moreover, this reduction in acetylcholine efflux is not temporally linked to the reduction of correct alternation tasks in the working-memory test T-maze: the reduction of acetylcholine release occurs 60 minutes after the memory deficit in the T-maze.

Previous findings related to those mentioned before are also demonstrated in different mice strains: NMRI, CD-1 and C57BL/6J. In all of them, it has been found that presynaptic CB1 receptors can mediate the release of acetylcholine in the hippocampus. These effects have been shown with the agonists WIN 55,212-2 and CP-55,940. Both agonists inhibit electrically evoked tritium overflow in hippocampal slices (pre-incubated with [^{3}H]choline), whereas the antagonist rimonabant prevents this effect (Kathmann *et al*. 2001). Further studies by these authors show that cannabinoid-induced inhibition of acetylcholine release is preserved in C57BL/6J aged mice that are up to 28 months old (Redmer, Kathmann & Schlicker 2003). In a different study, it is demonstrated that cannabinoid-mediated acetylcholine release is dependent on the dose of cannabinoid that is used. Low doses of WIN 55,212-2 cause a transient inhibition of acetylcholine release, whereas higher doses result in a prolonged inhibition in the rat hippocampus. The high-dose cannabinoid-induced inhibition of acetylcholine efflux is blocked by rimonabant, but this is not the case for the increase of acetylcholine release after the low-dose cannabinoid treatment. Only D1 and D2 receptor antagonists suppress the biphasic effects of WIN 55,212-2 (Tzavara *et al*. 2003). Two more recent studies have shown that delta(9)-tetrahydrocannabinol stimulates the release of acetylcholine in the rat hippocampus, but that the systemic injection of naloxone, naltrexone or a D1 receptor antagonist causes the inhibition of cannabinoid-induced acetylcholine release in the hippocampus. Similarly, bilateral infusion of an antagonist of μ-opioid receptors into the rat VTA has the same effects (Pisanu *et al*. 2006). By using *in vivo* microdialysis, CB1 knockout mice and immunochemistry, it has been shown that hippocampal acetylcholine release is specifically controlled by CB1 receptors. In addition, local infusion of CB1 receptor antagonists, rimonabant and AM-251 dose-dependently increased the release of acetylcholine in the hippocampus. However, in knockout mice and by the antagonism of the D1 dopaminergic receptor, the stimulation of acetylcholine efflux after cannabinoid antagonist treatment was abolished. Also, significant co-expression of CB1 receptors with cholinergic and dopaminergic receptors was found in nerve terminals (Degroot *et al*. 2006).

The main results of three studies including the muscarinic acetylcholine receptors and the endocannabinoid system in this brain area are described below. Studies of interneurons in the rat hippocampus CA1 region slices have found that activation of muscarinic acetylcholine receptors increases the endocannabinoid-mediated depolarization-induced suppression of inhibition and induces persistent endocannabinoid release. It seems that the functional interactions between muscarinic and glutamatergic mechanisms of endocannabinoid release take place at an intracellular level, but not at a receptor level (Kim *et al*. 2002). Other results point out that cholinergic enhancement of depolarization-induced suppression of inhibition may involve M_1 and M_3 receptors, and that activation of postsynaptic muscarinic M_1 and M_3 receptors facilitates the depolarization-induced release of endocannabinoids from postsynaptic neurons. This effect is suppressed by metabotropic cholinergic receptor antagonists (Ohno-Shosaku *et al*. 2003). Along this line, it has been proposed that the inhibition of neurotransmission into the hippocampus obeys two distinct mechanisms: cannabinoid-dependent and cannabinoid-independent. Accordingly, one neuronal population of

synapses would respond to activation of muscarinic M_2 receptors, thereby directly suppressing the release of GABA, while in the other neuronal population, activation of muscarinic M_1 and M_3 receptors leads to an endocannabinoid release that would suppress GABAergic release after presynaptic activation of CB1 receptors (Fukudome *et al.* 2004).

Finally, another series of experiments revealed functional and structural interactions between endocannabinoid, cholinergic and GABAergics systems. In rats, the existence of two septohippocampal cholinergic neurons has been described. One of them would express $GABA_B$ and CB1 receptors and would have large neural somata. The other one would not express both kinds of receptors and would have a smaller neural somata (Nyíri *et al.* 2005). Similar specific studies in the rat hippocampus have shown that depolarization of a single hilar mossy cell of the dentate gyrus results in inhibition of local GABAergic afferents. Moreover, the activation of CB1 receptors on these GABAergic afferents, by endocannabinoids or WIN 55,212-2, considerably inhibits calcium-dependent exocytosis (Hoffman & Lupica 2006). However, regarding excitatory neurotransmission, it has been suggested that cholinergic inputs from the septum to the middle molecular layer of the hippocampus are modulated by endocannabinoid release and that this regulates the primary excitatory afference of the hippocampus (Colgin *et al.* 2003).

THE VTA AND THE CANNABINOID SYSTEM

This area is also classically related to drug addiction. This area projects important dopaminergic efferences to the nucleus accumbens, amygdala and prefrontal regions (see Fig. 1), and it receives relevant dopaminergic inputs from the amygdala and prefrontal cortex. Similar to the nucleus accumbens, multiple drugs of abuse (cocaine, morphine, amphetamines, nicotine, alcohol) increase the release of dopamine in this region and also exhibit relevant process of LTP (Kauer & Malenka 2007). Such events lead to changes in synaptic function as the strength of synaptic connections in dopaminergic cells, forming reward-related learning in addicted behaviors (Jones & Bonci 2005),

GABA and glutamate

Three of the few existing experiments in this brain area that address amino acidergic neurotransmission and the endocannabinoid system are fundamentally electrophysiological in their methodology. The first study verified that WIN 55,212-2 produces a reduction of $GABA_A$ receptor-mediated inhibitory postsynaptic currents in the rat VTA, which is prevented by rimonabant. These results indicate that, most likely, the reduction of GABAergic neurotransmission in the VTA would lead to an increase of the firing rate of dopaminergic VTA neurons (Szabo, Siemes & Wallmichrath 2002). *In vitro* electrophysiological experiments by Melis *et al.* (2004) show that WIN 55,212-2 and HU-210 reduce, in a dose-dependent manner, NMDA and AMPA excitatory postsynaptic potentials in VTA dopamine neurons, and these effects are reversed by the antagonists AM-281 and rimonabant (Melis *et al.* 2004). Along with further results by these same authors, it is suggested that depolarization-induced suppression of excitation is present in the VTA, and presumably, is mediated by a calcium-dependent mechanism. A more recent study exploring the implication of the glycine neurotransmitter describes how, through an allosteric mechanism, delta(9)-tetrahydrocannabinol and anandamide potentiate the function of glycine receptors in the rat VTA. Furthermore, it has been shown that delta(9)-tetrahydrocannabinol and anandamide produce dose-dependent potentiation of glycine-activated currents in isolated neuronal cells of the VTA. This cannabinoid-induced potentiation of glycine-activated currents is maximal with lower concentration of glycine, whereas a high concentration decreases this effect. These effects seem to be independent of CB1 receptors (Hejazi *et al.* 2006).

Biogenic amines (dopamine, noradrenaline, serotonin)

As in the studies on amino acidergic neurotransmission, the role of monoamines and the endocannabinoid system have been investigated mostly by electrophysiological studies. Here, we review some of them. Two of the earliest studies have shown that intravenous administration of delta(9)-tetrahydrocannabinol and WIN 55,212-2 causes a dose-dependent increase in dopamine neuron firing in the rat VTA while pre-treatment with rimonabant dose-dependently reverses this dopaminergic response. However, different doses of naloxone do not modify the cannabinoid-induced increase of the VTA dopamine firing rate. Furthermore, it seems that VTA dopaminergic neurons are more sensitive to the effects of delta(9)-tetrahydrocannabinol than neurons from the substantia nigra pars compacta (French 1997; French, Dillon & Wu 1997). Other authors have also demonstrated that the stimulation of dopaminergic neurons of the rat VTA results in a phasic inhibition of prefrontal cortex pyramidal neurons. Intravenous administration of delta(9)-tetrahydrocannabinol and WIN 55,212-2 increases the firing rate of the pyramidal prefrontal cortex to VTA, whereas rimonabant reverses these effects (Pistis *et al.* 2001). However, it seems that a heterogeneous neuronal response exists between the populations

of dopaminergic neurons in the rat VTA. Indeed, HU-210 predominantly causes an enhancement of the firing rate of dopaminergic neurons, whereas other neurons are unaffected or their activity is decreased after a cannabinoid challenge. Rimonabant has no effect when it is injected alone, but it reverses HU-210-mediated changes in the VTA (Cheer *et al.* 2003). Interestingly, in this brain region, the phenomenon known as depolarization-induced suppression of excitation has been described to be calcium-dependent. This process can be blocked by administration of cannabinoid receptor antagonists (AM-281, rimonabant) and a dopaminergic D2 antagonist (eticlopride). Moreover, it can be potentiated by a dopaminergic D2 agonist (quinpirole) (Melis *et al.* 2004). More recently, further studies have provided evidence that the stimulation of a medial forebrain bundle can elicit modulation of endocannabinoid-mediated dopaminergic neuron activity in the short-term in the rat VTA (Pillolla *et al.* 2007). Additionally, another study has shown that VTA dopamine neurons continue to exhibit an increase in the firing rate after a delta(9)-tetrahydrocannabinol challenge in rats chronically treated with delta(9)-tetrahydrocannabinol (Wu & French 2000).

Other types of studies have tried to elucidate how the endocannabinoid system modulates the release of several monoamines. An example of these experiments is the dose-dependent increase of somatodendritic levels of dopamine and their metabolites (DOPAC and HVA) in this area, but not in the nucleus accumbens after local injection of delta(9)-tetrahydrocannabinol into the rat VTA (Chen *et al.* 1993).

Opioids

Here, we review four recent findings providing strong evidence for neuro-interactions between opioid and cannabinoid systems in the VTA. The first one shows that delta(9)-tetrahydrocannabinol stimulates the release of acetylcholine in several brain regions and that this effect can be prevented by several opioid and dopaminergic antagonists (i.e. bilateral infusion of the pseudo-irreversible μ1-antagonist naloxonazine into the rat VTA prevented the release of acetylcholine). Consequently, it has been suggested that acetylcholine release is related to the production of endogenous opioids in the VTA (Pisanu *et al.* 2006). The second one shows that intravenous self-administration of heroin in rats increases the density of μ-opioid and CB1 receptors in the rat VTA. However, intravenous self-administration of WIN 55,212-2 causes a modest decrease in CB1 receptors in the VTA (Fattore *et al.* 2007). The other two studies, by Singh and colleagues, have verified that treatment with morphine (10 mg/kg) enhances Fos-IR in the rat VTA, and this effect is suppressed by the antagonist rimonbant. However, rimonabant alone (3 mg/kg) also increases Fos-IR in the VTA (Singh *et al.* 2004). Also, the administration of heroin increases the Fos-IR in the rat VTA. However, if animals are treated perinatally with delta(9)-tetrahydrocannabinol, there is a reduction of heroin-induced Fos-IR (Singh *et al.* 2006). Additionally, using the conditioned-place-preference paradigm test, these same authors observed that animals perinatally exposed to delta(9)-tetrahydrocannabinol show a potentiation of the rewarding properties of heroin.

Acetylcholine

With the criteria used in our research and which was explained in the Introduction section, we have found only one reference in the PubMed database concerning acetylcholine and cannabinoids in the VTA. This study states that delta(9)-tetrahydrocannabinol stimulates the release of acetylcholine in several brain regions. Among them are the prefrontal cortex and hippocampus. It seems that this acetylcholine release is related to the production of endogenous opioids in the VTA and dopaminergic cells with efferences to the shell of nucleus accumbens (Pisanu *et al.* 2006).

CONCLUSIONS

Essentially, we have reviewed the pharmacology of the endocannabinoid system based on the functional and structural interactions that this system establishes with the main neurotransmitter systems (e.g. GABA, glutamate, biogenic amines, opioids and acetylcholine). Such interactions have focused on five key brain areas traditionally related to addiction (i.e. amygdala, nucleus accumbens, prefrontal cortex, hippocampus and VTA).

From a general perspective, it is noteworthy to mention the current interest in the endocannabinoid system, as indicated by the great number of studies that were found (mostly from the year 2000). Although some of the earlier studies came from the hippocampus, the more recent findings investigated cannabinoid-induced neuromodulation in the GABAergic and glutamatergic systems, regardless of the brain area that was studied. This is an important fact, because it indicates that the main excitatory and inhibitory systems of the mammalian central nervous system are under the influence of the endocannabinoid system. In the addicted individual, the imbalance in glutamatergic neurotransmission is common. It is also known that a dysregulation of excitatory signaling could lead to the relapse of drug use and cravings (for reviews, see Dackis & O'Brien 2003; Tzschentke & Schmidt 2003; Addolorato *et al.* 2005), supporting the notion of addicted behavior as a chronic disorder. Therefore, it is easy to estimate the importance

of the endocannabinoid system in the phenomenon of addiction, especially when its neuromodulation is compromised, for example, by an altered performance of receptors and cellular signaling, fundamentally of cannabinoid CB1 receptors.

Also, we have summarized the main findings to demonstrate that the endocannabinoid system is highly implicated in relevant brain processes, such as neuroplasticity: LTD and LTD. Other results show, on the one hand, that endogenous and exogenous administration of several CB1 receptor agonists cause a decrease in the inhibitory activity of GABAergic interneurons, and on the other hand, that cannabinoids can change the reward sensitivity to other drugs of abuse, and as equally shown with other drugs of abuse, increase the dopamine extracellular levels in the nucleus accumbens. However, the efflux of acetylcholine after activation of CB1 receptors will depend on the brain area that is studied. Most evidence reveals that there is an increase in acetylcholine release in the prefrontal cortex after CB1 stimulation, whereas a large number of studies in the hippocampus prove its reduction. Other findings show that cannabinoid receptors are closely linked to dopaminergic D2 and opioid receptors. In both cases, there are important modifications in the number and functionality of these receptors following cannabinoid exposure, as well as activation of the immediate early gene c-fos and metabotropic cascades triggered by the activation of G-proteins.

Despite the great number of results showing that these neurotransmitter systems are under the influence of the endocannabinoid system, it is difficult, in many cases, to correlate subtle neurochemical changes with a discrete behavior. This difficulty is even greater if we consider that some neuroadaptations can occur with the objective of overcoming the deficits observed in cannabinoid signaling, or the presence of other functional neurotransmitter systems that can also regulate the control of neurotransmitter release, such as presynaptic mGluR2/3 (Mato *et al.* 2005). Thus, at present, we know from animal and human studies that the endocannabinoid system can directly modulate addiction-related behaviors and that its repercussion in brain areas related with reward (nucleus accumbens and VTA), with memory and learning (hippocampus), with basic emotions and emotional memories (amygdala), and with inhibition, self-control and planning of behavior (prefrontal cortex) are noticeable. We propose that the endocannabinoid system would function as a neuronal mediator between a craving (or need) and its satisfaction. The malfunctioning of this supposed underlying neuronal mechanism (i.e. the endocannabinoid system) could cause a greater discrepancy between both the desired and obtained psychological states. Finally, we can conclude that although the precise role of the endocannabinoid system in different brain process remains to be elucidated, its importance in behavioral addiction is evident.

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