

**Endocannabinoid system and drug addiction: new insights from mutant mice
approaches**

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Abstract

The involvement of the endocannabinoid system in drug addiction was initially studied by the use of compounds with different affinities for each cannabinoid receptor or for the proteins involved in endocannabinoids inactivation. The generation of genetically modified mice with selective mutations in these endocannabinoid system components has now provided important advances in establishing their specific contribution to drug addiction. These genetic tools have identified the particular interest of CB₁ cannabinoid receptor and endogenous anandamide as potential targets for drug addiction treatment. Novel genetic tools will allow determining if the modulation of CB₂ cannabinoid receptor activity and 2-arachidonoylglycerol tone can also have an important therapeutic relevance for drug addiction.

Introduction

Drug addiction is a chronic brain disease induced by repeated drug consumption leading to compulsive drug seeking, loss of control over drug use despite negative consequences, and repeated relapse. All drugs of abuse produce similar changes in specific brain pathways, including the reward circuits, which constitute the common neurobiological substrate for this brain disease. The endocannabinoid system has recently emerged as a crucial component of this common circuitry underlying drug addiction [1].

The endocannabinoid system consists of cannabinoid receptors, their endogenous ligands, and the enzymes involved in the synthesis and degradation of these endocannabinoids [2]. Two subtypes of cannabinoid receptors, CB₁ (CB₁R) and CB₂ (CB₂R), have been characterized and cloned, although compelling evidence supports the existence of other receptors that bind cannabinoid ligands, such as GPR55. Both CB₁R and CB₂R are G-protein-coupled receptors with quite different distributions in the central nervous system (CNS) and peripheral tissues [2]. CB₁R is highly expressed in the CNS, while CB₂R is mainly localized in immune cells, although it is also expressed in brain neurons [3].

The most relevant endogenous ligands for cannabinoid receptors are N-arachidonylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG). Additional endogenous molecules that bind to the cannabinoid receptors have been identified, although some of them may be artifacts [2]. These endocannabinoids are synthesized on demand, mainly postsynaptically and act as retrograde messengers regulating the presynaptic release of neurotransmitters [4]. Whether both endocannabinoids, or only 2-AG, act as retrograde synaptic messengers remains to be clarified. Anandamide and 2-AG are produced from cell membrane lipids via different biosynthetic pathways.

Anandamide acts as a partial agonist at both CB₁R and CB₂R, and also binds to the transient receptor potential vanilloid type 1 channel. 2-AG is the most abundant endocannabinoid in the CNS and activates both CB₁R and CB₂R [5]. Cannabinoid receptor activation by endocannabinoids is rapidly terminated through carrier-mediated uptake into cells followed by intracellular enzymatic degradation. Anandamide is degraded by the fatty acid amide hydrolase (FAAH), whereas 2-AG is primarily metabolized by monoacylglycerol lipase (MAGL) [4]. The molecular entities that transport anandamide and 2-AG into cells have not been yet identified, although this transport has been characterized pharmacologically [6]. The molecular characterization of proteins involved in these re-uptake processes will allow the future generation of genetically modified animals, which may clarify the relevance of these endocannabinoid inactivation mechanisms. In contrast, the genetically modified mice now available with constitutive or conditional mutations of the cannabinoid receptors (CB₁R and CB₂R) and endocannabinoid degrading enzymes (FAAH and MAGL) have provided important advances for understanding the physiological role of these endocannabinoid components in multiple functions, including drug addiction (Table 1).

Studies on CB₁R genetically modified mice

Genetic approaches have provided clear evidence regarding the involvement of CB₁R in drug addiction. CB₁R is the primary site of action for the rewarding and pharmacological responses of cannabinoids, although this receptor plays an overall modulatory effect on the addictive properties of all prototypical drugs of abuse [7]. Thus, CB₁R is involved in nicotine rewarding properties, as revealed by the abolishment of nicotine place preference

in CB₁R knockout mice (CB₁KO) [8,9], and the reduction of nicotine self-administration by CB₁R antagonists [10]. In contrast, the acquisition of nicotine self-administration in an acute reinforcement paradigm in mice with restrained mobility was not modified in CB₁ KO [11]. However, this acute paradigm fails to evaluate the maintenance of a stable operant self-administration responding, and the effects could be influenced by the stress induced by this restraint procedure. The influence of CB₁R in nicotine physical dependence is less clear. Thus, although the somatic expression of nicotine withdrawal was not modified in CB₁KO [8,9], the CB₁R antagonist rimonabant ameliorated somatic withdrawal in wild-type mice [9].

CB₁R also regulates ethanol rewarding properties. Thus, CB₁KO show a reduction of ethanol-induced place preference [12,13] and a decrease in voluntary ethanol intake [14,15], in agreement with pharmacological results using CB₁R antagonists [16]. Stress could participate in the regulation that CB₁R exerts on alcohol consumption since stress-induced increase in ethanol preference is blocked in CB₁KO [17]. CB₁R involvement in alcohol reward seems mediated through the modulation of its effects on the activation of mesolimbic dopamine transmission [14].

CB₁R also participates in opiate reward by modulating dopamine transmission. Thus, CB₁KO do not exhibit morphine place preference [18], although this effect was not observed in a later study [19], nor morphine self-administration [20,11]. In addition, morphine-enhanced extracellular dopamine in the nucleus accumbens (NAc) was attenuated in CB₁KO [21], although this effect was not replicated in the case of heroin when using rimonabant [22]. The severity of morphine withdrawal was also attenuated in CB₁KO [20].

In contrast to other drugs of abuse, psychostimulants enhance NAc dopamine levels by acting directly on dopaminergic terminals and do not require the modulatory role of mesolimbic CB₁R activity. Indeed, cocaine-enhanced NAc dopamine was unaltered in CB₁KO [23*], although another study reported a reduction of this cocaine effect [24]. Cocaine [18,12] and MDMA [25] place preference were preserved in CB₁KO. These knockout mice also learn to self-administer cocaine and amphetamine when using an acute paradigm in restrained animals [11]. In spite of these unaltered cocaine reward responses, chronic cocaine self-administration was attenuated in freely moving CB₁KO, and the motivation to obtain a cocaine infusion was dramatically decreased in these mutants using a progressive ratio schedule [23*].

Therefore, CB₁R is involved in the primary rewarding effects and the motivation to seek different drugs of abuse. Conditional knockout mice deficient in CB₁R at GABAergic interneurons or glutamatergic principal neurons are now available [26**]. These mice are promising tools to clarify the circuits mediating the effects of CB₁R on drug addiction.

Studies on CB₂R genetically modified mice

The presence of CB₂R in neurons is still a controversial issue mainly due to the difficulties to obtain reliable antibodies. However, previous pharmacological studies have shown an involvement of CB₂R in the modulation of some central effects of alcohol [27] and opioids [3]. A pivotal role of CB₂R in cocaine rewarding effects has been recently reported. Thus, systemic administration of the CB₂R agonist JWH133 decreased cocaine self-administration in wild-type and CB₁KO, but not in CB₂R knockout mice (CB₂KO) [28**]. Similar responses were revealed after intra-NAc infusion of JWH133 in wild-

type, but not in CB₂KO [28**]. Cocaine enhanced locomotion and NAc dopamine levels was also inhibited by JWH133 in wild-type mice, and in CB₁KO, but not in CB₂KO [28**]. In agreement, transgenic mice overexpressing CB₂R in the CNS showed a reduction of cocaine-induced place preference, self-administration and locomotor sensitization [29*]. All these new data reveal the interest of CB₂R pharmacological manipulation for cocaine addiction treatment. Future research will be necessary to investigate the possible mechanisms by which CB₂R modulates cocaine reward, and if this effect could be generalizable to other drugs of abuse. In this respect, nicotine self-administration and reinstatement of nicotine-seeking were not modified by the selective CB₂R agonist AM630 in rats [30].

Studies on FAAH genetically modified mice

The behavioural effects of anandamide can be effectively increased by pharmacological blockade of FAAH or by genetic FAAH deletion [5]. The effects of FAAH disruption on drug addictive properties have been evaluated using genetic approaches. Studies investigating the role of FAAH inhibition on THC dependence report that THC withdrawal was not modified in FAAH knockout mice (FAAHKO), although acute administration of the FAAH inhibitor, URB597 attenuated this withdrawal response [31]. These different results could be attributed to possible compensatory changes after the gene deletion, and the authors concluded that endocannabinoid modulation may be an effective treatment for cannabis withdrawal [31].

The enhancement of the anandamide tone increased sensitivity to nicotine reward. Indeed, FAAHKO exhibited place preference at sub-threshold doses of nicotine, and this

nicotine response was also augmented by repeated URB597 administration [9]. In addition, nicotine withdrawal was enhanced in FAAHKO, and after acute URB597 administration at high doses [9]. Therefore, anandamide may modulate the positive and negative effects of nicotine in opposite ways, i.e. increasing reward and worsening aversion. Pharmacological studies in rats have yielded opposite results to those obtained in mice. Thus, URB597 inhibited nicotine reward, decreased reinstatement of nicotine seeking behaviour [32], and reversed nicotine withdrawal-induced anxiety in rats, without modifying the somatic manifestations of nicotine withdrawal [33]. These discrepant results observed in mice and rats hinder a definite conclusion as to the role of anandamide on the behavioural effects of nicotine, and warrants further investigation.

Ethanol rewarding effects were also modified in FAAHKO. These mutants exhibit more preference for ethanol intake [34,15], although another study found this enhancement only in female FAAHKO [35]. These inconsistencies may be due to differences in the genetic background. Pharmacological studies showing that URB597 increases ethanol intake in mice [34,15] confirm that enhancing anandamide levels facilitates ethanol reward. In terms of ethanol withdrawal, FAAHKO display less intense handling-induced convulsions than control mice following withdrawal from chronic ethanol [15], although no differences were observed in an acute withdrawal paradigm [34].

The effects of increasing anandamide levels on opioid and cocaine addictive properties have only been investigated in pharmacological studies. Thus, pharmacological enhancement of anandamide levels reduces morphine withdrawal [36,37*] and reinstatement of cocaine seeking behavior [39], suggesting that this pharmacological intervention could prevent cocaine craving. Interestingly, URB597, in contrast to

anandamide [40] is not self-administered by monkeys, and does not reinstate drug craving in this animal species [41].

Studies on MAGL genetically modified mice

MAGL genetic disruption produces profound changes on the functional activity of the endocannabinoid system, which represents a potential limitation for the use of these genetic tools for pharmacological studies. Indeed, both constitutive MAGL disruption and chronic administration of the MAGL inhibitor JZL184, produce a sustained enhancement of 2-AG levels that leads to cross-tolerance to cannabinoid agonists, desensitization of brain CB₁R, and impaired endocannabinoid-dependent synaptic plasticity in mice [31]. This result is in contrast with the absence of adaptive changes on CB₁R function after FAAH disruption [5], and reveals a different role of anandamide and 2-AG in the functional activity of the endocannabinoid system. The use of pharmacological and genetic approaches has confirmed this differential role of anandamide and 2-AG in several behavioural responses [42].

Considering these adaptive changes in the MAGL knockouts, the study of the role of 2-AG in drug addiction has only been possible by using pharmacological tools [43]. The administration of JZL184 attenuated THC [31] and morphine abstinence [37*], revealing the potential interest of enhancing 2-AG tone to attenuate these withdrawal manifestations. Although 2-AG is self-administered by monkeys [44] in a similar way to anandamide [40], the possible reinforcing effects of MAGL inhibitors have not been reported yet.

A new genetic approach consisting in the overexpression of MAGL in forebrain neurons has been reported [45**]. These mutants show a significant decrease in forebrain 2-AG levels without compensatory changes in other endocannabinoid components, and have been used to evaluate the role of 2-AG in metabolic control. These transgenic mice now represent excellent tools to investigate the role of 2-AG in drug addiction.

Conclusions

The generation of genetically modified mice with selective mutations in specific components of the endocannabinoid system has provided important advances to identify the contribution of this system in drug addiction. However, several relevant issues have not been yet clarified mainly due to the limitations of the available experimental tools (Figure 1). The crucial role of CB₁R in drug addiction has been identified by using constitutive knockout mice. The recent generation of conditional knockouts lacking CB₁R in GABAergic interneurons or glutamatergic principal neurons [26**] will now allow to clarify the precise CB₁R circuits involved in drug addiction. A crucial limitation for the study of CB₂R localization and function is the lack of reliable antibodies. Recent studies have revealed that CB₂R in the CNS participate in drug reward. However, numerous questions remain open with regards to the possible mechanisms involved in these responses, particularly if they are mediated by CB₂R expressed in neurons or glial cells. The generation of novel conditional mutants with selective CB₂R deletion in these particular cells will be essential to further advance in this topic.

The finding that anandamide tone modulates the behavioural responses to drugs of abuse has provided an interesting target with potential therapeutic relevance. The attenuation of

drug withdrawal manifestations by enhancing 2-AG tone also suggests its potential therapeutic interest. Recent studies have clearly shown a different physiological role of anandamide and 2-AG. Considering the localization of the enzymes involved in endocannabinoid synthesis and degradation [46], and the differential responses of enhancing anandamide and 2-AG tone on memory and anxiety [42], we can speculate that anandamide will have a predominant role in processing cannabinoid receptor activity mostly at GABAergic terminals. Instead, 2-AG processing could be mainly involved in cannabinoid signalling in glutamatergic neurons. Each of these conditions could lead to differential responses on the modulation of drug rewarding properties. Constitutive MAGL disruption produces profound changes on the endocannabinoid system, limiting the use of these mutants. The recent generation of transgenic mice overexpressing MAGL in forebrain neurons without producing these adaptive changes [45**], has opened for the first time the possibility to use genetic tools to evaluate the role of 2-AG in drug addiction.

The new genetic tools recently generated will allow elucidating most of the relevant questions that are still opened about the role of the endocannabinoid system in drug addiction, which can open novel therapeutic approaches for this brain disease.

Acknowledgements

This work was supported by the Spanish “Ministerio de Ciencia e Innovación” (#SAF2007-64062 and SAF2011-29864), “Instituto de Salud Carlos III” (RETICS- Red de Trastornos Adictivos-Redes Temáticas de Investigación Cooperativa en Salud: #RD06/0001/0001, #RD06/0001/1004), grants #10/00316 and #10/01708, Plan Nacional sobre Drogas (PNSD #2009/026), the Catalan Government (SGR2009-00131) and the ICREA Foundation (ICREA Academia-2008). Important contributions from several authors could not be included due to space limitations. We thank Neus Morgui and Cristina Fernández for expert technical assistance.

Conflict of interest

All authors report no biomedical financial interests or potential conflicts of interest.

References

1. Serrano A, Parsons LH: **Endocannabinoid influence in drug reinforcement, dependence and addiction-related behaviors.** *Pharmacol Ther* 2011, **132**:215-241.
2. Mechoulam R, Parker L: **The endocannabinoid system and brain.** *Annu Rev Psychol* 2012, **64**:6.1-6.27.
3. Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA: **Identification and functional characterization of brainstem cannabinoid CB2 receptors.** *Science* 2005, **310**:329-332.
4. Castillo PE, Younts TJ, Chávez AE, Hashimoto Y: **Endocannabinoid signaling and synaptic function.** *Neuron* 2012, **76**:70-81.
5. Lucchicchi A, Pistis M: **Anandamide and 2-arachidonoylglycerol: pharmacological properties, functional features, and emerging specificities of the two major endocannabinoids.** *Mol Neurobiol* 2012, **46**:374-392.
6. Clapper JR, Mangieri RA, Piomelli D: **The endocannabinoid system as a target for the treatment of cannabis dependence.** *Neuropharmacology* 2009, **56** (Suppl 1):235-243.
7. Maldonado R, Valverde O, Berrendero F: **Involvement of the endocannabinoid system in drug addiction.** *Trends Neurosci* 2006, **29**:225-232.

8. Castañé A, Valjent E, Ledent C, Parmentier M, Maldonado R, Valverde O: **Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence.** *Neuropharmacology* 2002, **43**:857-867.
9. Merritt LL, Martin BR, Walters C, Lichtman AH, Damaj MI: **The endogenous cannabinoid system modulates nicotine reward and dependence.** *J Pharmacol Exp Ther* 2008, **326**:483-492.
10. Shoaib M: **The cannabinoid antagonist AM251 attenuates nicotine self-administration and nicotine-seeking behaviour in rats.** *Neuropharmacology* 2008, **54**:438-444.
11. Cossu G, Ledent C, Fattore L, Imperato A, Böhme GA, Parmentier M, Fratta W: **Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse.** *Behav Brain Res* 2001, **118**:61-65.
12. Houchi H, Babovic D, Pierrefiche O, Ledent C, Daoust M, Naassila M: **CB1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors.** *Neuropsychopharmacology* 2005, **30**:339-349.
13. Thanos PK, Dimitrakakis ES, Rice O, Gifford, Volkow ND: **Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors.** *Behav Brain Res* 2005, **164**: 206-213.
14. Hungund BL, Szakall I, Adam A, Basavarajappa BS, Vadasz C: **Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens.** *J Neurochem* 2003, **84**:698-704.

15. Vinod KY, Sanguino E, Yalamanchili R, Manzanares J, Hungund BL: **Manipulation of fatty acid amide hydrolase functional activity alters sensitivity and dependence to ethanol.** *J Neurochem* 2008, **104**:233-243.
16. Pava MJ, Woodward JJ: **A review of the interactions between alcohol and the endocannabinoid system: implications for alcohol dependence and future directions for research.** *Alcohol* 2012, **46**:185-204.
17. Racz I, Bilkei-Gorzo A, Toth ZE, Michel K, Palkovits M, Zimmer A: **A critical role for the cannabinoid CB1 receptors in alcohol dependence and stress-stimulated ethanol drinking.** *J Neurosci* 2003, **23**:2453-2458.
18. Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O: **Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice.** *Eur J Neurosci* 2000, **12**:4038-4046.
19. Rice OV, Gordon N, Gifford AN: **Conditioned place preference to morphine in cannabinoid CB1 receptor knockout mice.** *Brain Res* 2002, **945**:135-138.
20. Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, Böhme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, Parmentier M: **Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice.** *Science* 1999, **283**:401-404.
21. Mascia MS, Obinu MC, Ledent C, Parmentier M, Böhme GA, Imperato A, Fratta W: **Lack of morphine-induced dopamine release in the nucleus accumbens of cannabinoid CB(1) receptor knockout mice.** *Eur J Pharmacol* 1999, **383**:R1-2.

22. Caillé S, Parsons LH: **SR141716A reduces the reinforcing properties of heroin but not heroin-induced increases in nucleus accumbens dopamine in rats.** *Eur J Neurosci* 2003, **18**:3145-3149.
23. Soria G, Mendizábal V, Touriño C, Robledo P, Ledent C, Parmentier M, Maldonado R, Valverde O: **Lack of CB1 cannabinoid receptor impairs cocaine self-administration.** *Neuropsychopharmacology* 2005, **30**:1670-1680.
- * This study shows that suppression of CB₁R impairs cocaine self-administration mainly when the effort required to obtain a cocaine infusion is increased, but does not modify the effects of cocaine on extracellular levels of dopamine in the NAc. The authors suggest that CB₁R play an important role in the consolidation of cocaine reinforcement, although they are not required for its acute effects on mesolimbic dopaminergic transmission.
24. Li X, Hoffman AF, Peng XQ, Lupica CR, Gardner EL, Xi ZX: **Attenuation of basal and cocaine-enhanced locomotion and nucleus accumbens dopamine in cannabinoid CB1-receptor-knockout mice.** *Psychopharmacology (Berl)* 2009, **204**:1-11.
25. Touriño C, Ledent C, Maldonado R, Valverde O: **CB1 cannabinoid receptor modulates 3,4-methylenedioxymethamphetamine acute responses and reinforcement.** *Biol Psychiatry* 2008, **63**:1030-1038.
26. Bellocchio L, Lafenêtre P, Cannich A, Cota D, Puente N, Grandes P, Chaouloff F, Piazza PV, Marsicano G: **Bimodal control of stimulated food intake by the endocannabinoid system.** *Nat Neurosci* 2010, **13**:281-283.

** By using conditional mutant mice, the authors demonstrate an opposite role of CB₁R located in glutamatergic or GABAergic neurons in the control of food intake. The use of these two different conditional CB₁R knockout mouse lines opens new possibilities to dissect the functions of CB₁R on excitatory or inhibitory transmission in the modulation of the addictive properties of drugs of abuse.

27. Ishiguro H, Iwasaki S, Teasent L, Higuchi S, Horiuchi Y, Saito T, Arinami T, Onaivi ES: **Involvement of cannabinoid CB₂ receptor in alcohol preference in mice and alcoholism in humans.** *Pharmacogenomics J* 2007, **7**:380-385.
28. Xi ZX, Peng XQ, Li X, Song R, Zhang HY, Liu QR, Yang HJ, Bi GH, Li J, Gardner EL: **Brain cannabinoid CB₂ receptors modulate cocaine's actions in mice.** *Nat Neurosci* 2011, **14**:1160-1166.

** This study shows for the first time that activation of central CB₂R inhibits the rewarding properties of cocaine probably by a dopamine-dependent mechanism. The authors point to the NAc as an important brain region in the modulation of this effect. These results suggest that brain CB₂R may be a target for the pharmacotherapy of addiction.

29. Aracil-Fernández A, Trigo JM, García-Gutiérrez MS, Ortega-Álvaro A, Ternianov A, Navarro D, Robledo P, Berbel P, Maldonado R, Manzanares J: **Decreased cocaine motor sensitization and self-administration in mice overexpressing cannabinoid CB₂ receptors.** *Neuropsychopharmacology* 2012, **37**:1749-1763.

- * By using transgenic mice overexpressing CB₂R in the CNS, the authors provide evidence for a relevant role of CB₂R in the modulation of cocaine addictive properties. A decrease of cocaine-induced place preference and locomotor sensitization, and reduced cocaine intravenous self-administration was found in these transgenic animals.
30. Gamaledin I, Zvonok A, Makriyannis A, Goldberg SR, Le Foll B: **Effects of a selective cannabinoid CB₂ agonist and antagonist on intravenous nicotine self administration and reinstatement of nicotine seeking.** *PLoS One* 2012, **7**:e29900.
31. Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, Kinsey SG, Nguyen P, Ramesh D, Booker L, Burston JJ, Thomas EA, Selley DE, Sim-Selley LJ, Liu Q, Lichtman AH, Cravatt BF: **Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system.** *Nat Neurosci* 2010, **13**:1113-1119.
32. Forget B, Coen KM, Le Foll B: **Inhibition of fatty acid amide hydrolase reduces reinstatement of nicotine seeking but not break point for nicotine self-administration--comparison with CB(1) receptor blockade.** *Psychopharmacology (Berl)* 2009, **205**:613-624.
33. Cippitelli A, Astarita G, Duranti A, Caprioli G, Ubaldi M, Stopponi S, Kallupi M, Sagratini G, Rodríguez de Fonseca F, Piomelli D, Ciccocioppo R: **Endocannabinoid regulation of acute and protracted nicotine withdrawal: effect of FAAH inhibition.** *PLoS One* 2011, **6**:128-142.

34. Blednov YA, Cravatt BF, Boehm SL 2nd, Walker D, Harris RA: **Role of endocannabinoids in alcohol consumption and intoxication: studies of mice lacking fatty acid amide hydrolase.** *Neuropsychopharmacology* 2007, 32:1570-1582.
35. Basavarajappa BS, Yalamanchili R, Cravatt BF, Cooper TB, Hungund BL: **Increased ethanol consumption and preference and decreased ethanol sensitivity in female FAAH knockout mice.** *Neuropharmacology* 2006, 50:834-844.
36. Shahidi S, Hasanein P: **Behavioral effects of fatty acid amide hydrolase inhibition on morphine withdrawal symptoms.** *Brain Res Bull* 2011, 86:118-122.
37. Ramesh D, Ross GR, Schlosburg JE, Owens RA, Abdullah RA, Kinsey SG, Long JZ, Nomura DK, Sim-Selley LJ, Cravatt BF, Akbarali HI, Lichtman AH: **Blockade of endocannabinoid hydrolytic enzymes attenuates precipitated opioid withdrawal symptoms in mice.** *J Pharmacol Exp Ther* 2011, 339:173-185.
- * Inhibition of FAAH and MAGL attenuated naloxone-precipitated morphine withdrawal symptoms in mice through the activation of CB₁R, suggesting that increasing anandamide levels ameliorates the expression of opioid withdrawal.
38. Solinas M, Panlilio LV, Tanda G, Makriyannis A, Matthews SA, Goldberg SR: **Cannabinoid agonists but not inhibitors of endogenous cannabinoid transport or metabolism enhance the reinforcing efficacy of heroin in rats.** *Neuropsychopharmacology* 2005, 30:2046-2057.

39. Adamczyk P, McCreary AC, Przegalinski E, Mierzejewski P, Bienkowski P, Filip M: **The effects of fatty acid amide hydrolase inhibitors on maintenance of cocaine and food self-administration and on reinstatement of cocaine-seeking and food-taking behavior in rats.** *J Physiol Pharmacol* 2009, **60**:119-125.
40. Justinová Z, Solinas M, Tanda G, Redhi GH, Goldberg SR: **The endogenous cannabinoid anandamide and its synthetic analog R(+)-methanandamide are intravenously self-administered by squirrel monkeys.** *J Neurosci* 2005, **25**:5645-5650.
41. Justinová Z, Mangieri RA, Bortolato M, Chefer SI, Mukhin AG, Clapper JR, King AR, Redhi GH, Yasar S, Piomelli D, Goldberg SR: **Fatty acid amide hydrolase inhibition heightens anandamide signaling without producing reinforcing effects in primates.** *Biol Psychiatry* 2008, **64**:930-937.
42. Busquets-Garcia A, Puighermanal E, Pastor A, de la Torre R, Maldonado R, Ozaita A: **Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses.** *Biol Psychiatry* 2011, **70**:479-486.
43. Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, Pavón FJ, Serrano AM, Selley DE, Parsons LH, Lichtman AH, Cravatt BF: **Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects.** *Nat Chem Biol* 2009, **5**: 37-44.
44. Justinová Z, Yasar S, Redhi GH, Goldberg SR: **The endogenous cannabinoid 2-arachidonoylglycerol is intravenously self-administered by squirrel monkeys.** *J Neurosci* 2011, **31**:7043-7048.

45. Jung K, Jason C, Fu J, D'Agostino G, Guijarro A, Thongkham D, Avanesian A, Astarita G, DiPatrizio NV, Frontini A, Cinti S, Diano S, Piomelli D: **2-Arachidonoylglycerol signaling in forebrain regulates systemic energy metabolism.** *Cell Metabolism* 2012, **15**:299-310.

** This study describes the generation of a novel line of transgenic mice that overexpress MAGL in forebrain neurons without producing adaptive changes in other endocannabinoid components. These transgenic mice have revealed the role of 2-AG signalling in forebrain neural circuits in the control of energy dissipation and represent excellent genetic tools to investigate the role of 2-AG in drug addiction.

46. Katona I, Urbán GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF: **Molecular composition of the endocannabinoid system at glutamatergic synapses.** *J Neurosci* 2006, **26**:5628-5637.

47. Kenny PJ: **'Macrophage' cannabinoid receptor goes up in smoke.** *Nat Neurosci* 2011, **9**:1100-1102.

Figure legend

Mechanisms involved in the modulation of drug rewarding effects by the endocannabinoid system and remaining open questions based on recent findings using genetically modified mice. The enhancement of anandamide tone facilitates nicotine and alcohol reward as shown in FAAHKO. Nicotine and alcohol could increase dopaminergic neuron firing rates and induce anandamide release in the ventral tegmental area (VTA). Anandamide acts as retrograde messenger on presynaptic CB₁R and inhibits both GABAergic and glutamatergic inputs to VTA dopaminergic neurons. The possible effects of the genetic FAAH deletion in psychostimulant and opioid rewarding properties remains to be elucidated. The role of 2-AG in drug reward by using MAGL genetically modified mice is still unknown. On the other hand, the activation of CB₂R in the NAc decreases cocaine reward in wild-type, but not in CB₂KO. Cocaine enhances dopamine levels in the NAc directly blocking the dopamine transporter (DAT) located on dopaminergic axon terminals. CB₂R localized on astrocytes or microglia could decrease dopamine levels by regulating the release of inflammatory cytokines from these cells. Alternatively, the activation of CB₂R possibly located on dopaminergic terminals could also inhibit dopamine release in the NAc [47].