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Choosing the right drug: Status and future of endocannabinoid research for the prevention of drug-seeking reinstatement

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Author Comments:	

Cover Letter

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Current Opinion in Pharmacology
Special Issue - Targeting drug addiction mechanisms and processes: beyond reward

Dear Christian and Marco,

Please, find attached our manuscript entitled *" Choosing the right drug: Status and future of endocannabinoid research for the prevention of drug-seeking reinstatement"* for your consideration for publication as a short review in the Special Issue "Targeting drug addiction mechanisms and processes: beyond reward".

This short manuscript reviews the current and future status of art of how the endocannabinoid system can be exploited for the treatment of substance use disorders. As the evidence for its role keeps growing, it was difficult to keep the manuscript within the 2700 word limit. For this reason, the topics like the possible use of biased ligands or other promising phytocannabinoids, other than CBD, for the treatment of relapse have not been covered.

Within the pharmacology community, and the broader scientific and lay communities there is considerable interest in the roles of endocannabinoids, and an enduring interest in drug addiction. Hence, we believe that, in addition to its importance for the field, this manuscript will be of interest to your broad readership.

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Therefore, we would appreciate if you chose other reviewers for our manuscript.

Finally, we confirm that the paper is submitted with the full consent of all co-authors and the paper has not been published and is not under consideration in other journals.

The authors also declare no competing financial interests.

We look forward to the comments of the editors and referees. Thank you very much in advance for consideration.

All the best (on behalf of all co-authors)

Miriam Melis

Choosing the right drug: Status and future of endocannabinoid research for the prevention of drug-seeking reinstatement

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Abstract

Prolonged exposure to drugs of abuse leads to severe alterations in mesocorticolimbic dopamine circuitry deeply implicated in substance use disorders. Despite considerable efforts, few medications to reduce relapse rates are currently available. To solve this issue, researchers are uncovering therapeutic opportunities offered by the endocannabinoid system. The cannabinoid receptor type 1 (CB_1R), and its endogenous ligands, participate in orchestration of cue- and stress-triggered responses leading to obtain natural and drug rewards. Here, we review the evidence supporting the use of CB_1R neutral antagonists, allosteric modulators, indirect agonists, as well as multi-target compounds, as improved alternatives compared to classical CB_1R antagonists. The promising therapeutic value of other substrates participating in endocannabinoid signaling, like peroxisome proliferator-activated receptors, is also covered. Overall, a wide body of pre-clinical evidence avails novel pharmacological strategies interacting with the endocannabinoid system as clinically amenable candidates able to counteract drug-induced dopamine maladaptations contributing to increased risk of relapse.

Introduction

With approximately 35 million regular users of abuse drugs in the world [1], drug addiction, also referred to as 'substance use disorders (SUD)', remains a serious public health issue. SUD is a chronic, relapsing brain disease characterized by the compulsive pursue and use of the drug despite its harmful consequences [2]. Despite considerable research efforts aimed at deciphering the neurobiological substrates underpinning SUD, few if any medications have been introduced clinically.

The mesocorticolimbic dopaminergic (DA) system undergoes profound, multi-faceted alterations after prolonged exposure to drugs of abuse (see [3], for a recent review). The current framework of drug addiction includes three phases: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (craving) [4]. Because of the particular etiology of each of these stages, new pharmacotherapies can be designed to specifically cope with each maladaptation [5], giving rise to identifiable points of intervention [6]. Here, we capitalize on the potential of the endocannabinoid (eCB) system to modulate motivation and anxiety in normal and physiological conditions. Specifically, we will summarize the most relevant evidence pointing towards the therapeutic properties of eCB-targeting compounds with improved features when compared to classical rimonabant-like medications. The focus of the present review will be on the disruption of elevated anxiety states contributing to significant dysphoria and increased risk of relapse [7,8] during the first abstinence attempts [9], as well as the prevention of conditioned responses leading to craving that characterize the pernicious abstinent/relapse stage of the drug addiction cycle [10].

Brief overview of the eCB system

Cannabinoid receptors type1 (CB_1R) are predominantly expressed in the central nervous system (CNS) [11], comprising the most abundant G protein-coupled receptor (GPCR) brain system [12], whereas CB_2R are preferentially found in the periphery [13]. A consensus on whether CB_2R are expressed at a significant functional level in CNS neurons has not yet been achieved [14]. Both receptors are generally coupled to $G_{i/o}$ proteins [16] and are activated by anandamide (AEA) and 2-arachidonoylglycerol (2-AG) [15]. 2-AG derives from the hydrolysis of 1,2-diacylglycerol (DAG) via the DAG lipase (DAGL), while its breakdown is mediated by the monoacylglycerol lipase (MAGL). AEA is produced from *N*-arachidonoyl phosphatidyl ethanol (NAPE) by the NAPE phospholipase D (NAPE-PLD) and metabolized by the fatty acid amide hydrolase (FAAH). AEA also binds to the transient receptor potential channel 1 (TRPV1) [16], as well as the nuclear peroxisome proliferator-activated receptors alpha and gamma (PPARα/γ) [17]; these are often considered part of the "extended eCB system" (for a thorough review, see [15]).

Unlike classical neurotransmitters, eCBs are not stored in vesicles but are synthesized and released *de novo* in response to sustained neuronal activation [18], predominantly acting in a retrograde fashion. Upon activation, CB_1R inhibit neurotransmitter release from the presynaptic compartment by inactivating N- and P/Q-type voltage-gated Ca^{2+} channels and opening inward-rectifying K^+ channels [19,20]. This characteristic mode of action provides neurons with a negative feedback mechanism able to filter and modulate neurotransmitter inputs -including DA- with synaptic resolution [21].

eCB mechanisms with potential for therapeutic intervention

CB_1R in the mesolimbic circuitry

CB_1R and its endogenous ligands are present throughout the mesocorticolimbic circuitry [22], fine-tuning DA-signaled processes governing motivated behavior and decision-making [23–25]. In the ventral tegmental area (VTA), activation of CB_1R localized in GABAergic terminals leads to the disinhibition of VTA DA cell bodies, thereby increasing DA outflow in terminal regions, such as the striatum and prefrontal cortex (PFC), an adaptation further exacerbated following drug repeated exposure [26–28] (Figure 1b). It is hypothesized that the functional relevance of CB_1R in animal models of drug abuse [29–34] derives from the regulation of DA signaling during cue-elicited behavior [28,35,36]. In this way, DA transients progressively shift from signaling the reward by itself to signaling the cues that predict its availability, a change sculpted by 2-AG/CB_1R neurotransmission [37,38]. In contrast, AEA, which binds CB_1R with sub-maximal potency [39], hinders cue-evoked DA events [37,40]. In humans, places and stimuli associated with drug use trigger craving and, therefore, pose a major risk factor contributing to relapse [41]. Hence, control of cue-elicited DA events in the presence of discrete associated stimuli by CB_1R and 2-AG, but also AEA, is a primary target for eCB-based pharmacotherapies of drug addiction [42].

CB_1R-mediated stress processing in medial prefrontal cortices

Recent advances have extended the neuronal mechanisms by which CB_1R-mediated signaling underlies other pathological components of SUD. Stressful events can serve as cues that enhance anxiety states and are associated with higher risk of relapse [43] to contribute to the dysphoric state experienced by addicted individuals [9,44]. Crucially, the eCB system plays a major role regulating these stress-induced changes in sensitivity to natural and drug rewards [45,46]. In cocaine-abstinent rats, stress-induced changes in corticosterone PFC levels drive cocaine-seeking reinstatement by mobilizing 2-AG in the prelimbic part of the medial PFC (PL mPFC), which in turn activates CB_1R located in the pre-synaptic terminals of GABAergic cortical (probably cholecystokinin, CCK) interneurons [47–49] (Figure 1a). This disinhibition allows for amplified excitatory projections to the nucleus accumbens (NAc), eliciting motivated behavior towards drugs of abuse [50–52] (Figure 1c). Hence, targeting CB_1R to counteract stress-promoted drug seeking during abstinence is a promising strategy, although unanswered questions remain; e.g., the mechanism by which glucocorticoids mobilize 2-AG

[53]. 2-AG also mitigates anxiety and stress-induced maladaptations through alternative mechanisms by activating CB_1R in other brain areas [54]. These include the reduction in prostaglandin production [55], the normalization of stress-induced decreases in the mammalian target of rapamycin (mTOR) pathway [56] or the facilitation of adult HPC neurogenesis [57]. Although the functional relevance of such additional mechanisms has not yet been directly assessed in animal models of SUD, its implications warrant further examination for the management of hypersensitized stress responses in this disorder.

Beyond CB_1R: AEA, N-acylethanolamines and PPARα for the treatment of nicotine addiction

Unlike 2-AG, a set of mechanistic rules for a putative role of AEA in the neurobiology of drug-motivated behavior is still lacking [37]. Evidence that repeated drug exposure alters AEA brain levels in the striatum, limbic forebrain and HPC [58–60] suggests a yet to be determined mechanism of involvement. Interestingly, changes in AEA tone often oppose those of 2-AG [61]. This divergence suggests that the implications of both eCB moieties are segregated, thus corroborating the hypothesis that AEA and 2-AG subserve distinct physiological roles [62]. For instance, while the 2-AG-degrading enzyme MAGL is expressed pre-synaptically, the main AEA-catabolic enzyme is preferentially located in post-synaptic compartments [63]. Additionally, AEA targets a broader spectrum of receptors: it acts as a partial agonist of TRPV1 channels [59,64], whose activity favors cocaine- [65] and ethanol- [66] seeking in mice. AEA also activates PPARα, which regulate gene transcription and control homeostatic cell functions such as inflammation [67,68]. Of note, PPARα activation attenuates nicotine reinforcement and nicotine-induced DA outflow in the NAc [69–71]. The mechanism hypothesized involves the interaction between cholinergic signaling and PPARα shaping DA cell activity via phosphorylation of $\beta_2$containing-nicotinic acetylcholine receptors [72], which would prevent nicotine actions on DA neurons [73]. These pre-clinical studies, along with many others [74], support that pharmacological manipulations either elevating the tone of N-acylethanolamines including AEA or directly activating PPARα are potential targets for the treatment of nicotine use disorders.

Drugs and phytopharmaceuticals with potential for SUD treatment

Since the synthesis and approval for clinical use of the CB_1R antagonist rimonabant (SR141716A), clinical studies proved that it could promote smoking cessation and reduce nicotine relapse rates [42]. Pre-clinical studies suggested that it could also prevent psychostimulant, opioid, and alcohol reinstatement [75–79]. However, its poor tolerability due to psychiatric side effects (e.g., anxiety, depression, suicidality) prompted its withdrawal from the market [80–82]. Since its side effects might relate to its inverse agonist profile [77], a great effort has been devoted to developing eCB-based medications with favorable risk-benefit ratios [83].

Novel CB_1R-targeting compounds

CB_1R neutral antagonists and negative allosteric modulators (NAMs) are listed among the NIDA's medication development priorities in response to the opioid crisis [84]. CB_1R neutral antagonists should exert similar protective effects of rimonabant and its congeners but have less severe adverse effects. This is because, unlike with inverse agonists, neutral antagonists do not modify CB_1R ligand-independent control of basal cAMP accumulation, which is thought to play a crucial role in maintaining cellular homeostasis [85,86]. The neutral antagonist AM4113 [87] does not induce forskolin-stimulated cAMP formation *in vitro* [88], malaise or anxiety-like effects [89]. AM4113 also attenuates cue-induced reinstatement of nicotine, Δ^9-tetrahydrocannabinol (THC) and cocaine operant seeking [90,91]. AM4113 treatment also suppresses methamphetamine, heroin and alcohol voluntary intake [92,93]. Consistently, AM4113 also blocks nicotine-induced increases of VTA DA neuronal activity [90] and alcohol-induced accumbal DA release [92]. As promising as these results may appear, there is an additional intrinsic caveat for CB_1R antagonists: the ubiquity of its substrate. CB_1R are expressed across most brain structures, neuron populations, and cell lines of the CNS, and can be coupled to various G proteins other than canonical $G_{i/o}$ [94]. Therefore, systemic blockade of CB_1Rs interferes with a considerable number of off-target circuits. For instance, AM4113 increases stereotype-like behaviors, alters satiation and precipitates cannabinoid withdrawal signs [83], effects that can be ascribed to an indiscriminate blockade of CB_1R function. To circumvent this issue, alternative approaches have been exploited to target CB_1R function, including the development of allosteric modulators [95]. Allosteric modulation only influences downstream effects of CB_1R upon orthosteric ligand binding without affecting CB_1R activity. Little pre-clinical information is currently available on their therapeutic effects. Org27569, an atypical CB_1R NAM (see [83]), reduces cue- and drug-induced reinstatement of cocaine and methamphetamine seeking to a similar extent than rimonabant [96], and is devoid of CB_1R-related adverse effects [97]. Its selectivity has been challenged as it reduces food intake in CB_1R-knockout mice [97], thus suggesting that further studies are mandatory. At this stage, the most promising CB_1R NAM appears to be the neurosteroid pregnenolone [98–100]. Vallée et al., [101] elegantly probed its effects in reducing voluntary intake of the cannabimimetic drug WIN55,212-2 and preventing THC-induced increase in both NAc DA levels and firing activity of VTA neurons in a CB_1R-dependent manner. Pregnenolone is also well tolerated [102], and a FDA-approved drug in diverse clinical trials including two for the treatment of cannabis use disorder (NCT02439814, NCT02811939).

Inhibition of eCB-degrading enzymes

Nowadays, alternative pharmacological approaches, such as the use of indirect CB_1R agonists through inhibition of either FAAH and MAGL, appear as the preferred drug pipelines for targeting eCB system. This intervention is theorized to only interfere at synapses where eCBs are released "on demand". URB597, the 'gold standard' among classical FAAH inhibitors, heightens AEA brain levels [103] with relatively good selectivity [104], and attenuates cue-

induced reinstatement of nicotine seeking [69,105]. URB597 also reduces both cue- and stress-induced cocaine reinstatement of operant seeking responses [106,107] in a CB_1R-dependent manner [105]. Paradoxically, FAAH inhibition opposes to rimonabant effects, and yet similar anti-abuse effects are achieved. This is probably due to sub-maximal agonist potency of AEA acting as a partial agonist of CB_1R and competing with 2-AG [103]. Alternatively, the therapeutic potential of elevated AEA tone may reside in its ability to bind to other targets, such as PPARα/γ [108], TRPV1 channels [59] and CB_2R [109], or in the modification of other FAAH substrates. Available studies already demonstrate that the N-acylethanolamines palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) [74,110], endogenous PPARα ligands [72,110], reduce nicotine-induced excitation of VTA DA neurons, DA concentrations in the NAc and prevent nicotine-seeking reinstatement [111]. Alternatively, PEA-OEA/PPARα signaling can also be facilitated through the inhibition of the PEA- and OEA-degrading enzyme N-acylethanolamine acid amidase (NAAA), a strategy that also prevents nicotine-induced DA activation and reward in rats [112]. Noteworthy, FAAH inhibitors reduce anxiety-related outcomes, a treatment priority in SUDs. In fact, both FAAH and MAGL inhibitors display effective anxiolytic effects in pre-clinical studies [54,113–116]. However, the use of JZL184 for SUDs appears negligible since it facilitates cue-induced reinstatement of nicotine seeking [117], and increases the motivation to respond for alcohol [118].

Finally, it is worth noting that molecules interacting with eCB-synthesizing enzymes have not been sufficiently regarded, unlike FAAH or MAGL blockers, mainly due to major interferents in their functional interaction with upstream components involved in lipid-synthesis pathways. Anabolic enzymes for AEA and 2-AG synthesis (NAPE-PLD and DAGL, respectively) serve broader functions that greatly impact the lipidome throughout the brain and other organs [21,55,119]. In contrast, such interactions should occur to a lesser extent with FAAH and MAGL inhibitors, as these enzymes are placed downstream in bran lipid synthesis pathways, but compound-specific unexpected interactions may arise [120]. For instance, substantial alterations in lipid networks of cortical neurons due to a metabolic dysregulation of the CNS were specifically ascribed to BIA 10-2474 but not to other FAAH inhibitors [121]. Consequently, one could speculate that manipulation of AEA tone via FAAH inhibition would be of great therapeutic efficacy, specifically when reducing craving triggered by anxiogenic cues during abstinence, but this intervention requires particular consideration to avoid lipidome-related neurological complications [121].

Cannabidiol: One Ring to rule them all?

The eCB-CB_1R drug portfolio extends to phytocannabinoids, compounds that often exhibiting multi-target activity [122], such as those of cannabidiol (CBD) [123]. Unlike THC, CBD is devoid of psychotomimetic [124] and rewarding [125] effects, and neither directly activates CB_1R nor CB_2R [123]. Its pharmacodynamic profile includes its actions as a NAM of CB_1R [126–129] and of CB_2R [129,130] at physiologically-relevant concentrations. CBD also

inhibits FAAH activity [131–135] and activates both PPARγ [136–139] and TRPV1 receptors [131,132,140]. Of note, its pharmacodynamics combines all the appealing abovementioned alternative therapeutic strategies. Additionally, CBD has unrelated eCB effects, such as agonism at 5-HT_{1A} receptor and blockade of adenosine reuptake [141]. Nonetheless, its anti-relapse effects in pre-clinical studies are not definitive. Some authors report that it decreases cue- [142,143] and stress-induced [143] reinstatement of cocaine and alcohol seeking [143], attenuates alcohol- and methamphetamine-induced relapse [144–146], and cue-induced reinstatement of heroin responding [147]. However, other studies find that CBD does not modify priming-induced cocaine seeking [142,148,149], and even increased stress-induced cocaine-seeking reinstatement [142]. Notably, this latter study shows a bimodal effect of CBD on cocaine-seeking reinstatement depending on the triggering source, and that both its beneficial and undesirable effects were CB_1R-mediated [142]. Previous work also describes a CBD-induced reduction of cocaine self-administration to CB_2R, TRPV1 and 5-HT_{1A} receptor mechanisms [150], and to facilitation of adult hippocampal neurogenesis [151]. Alternatively, a 5-HT_{1A} mechanism might account for its anti-reward effects through modulation of VTA neuronal network dynamics [152]. Interestingly, CBD reduced attentional bias to cigarette cues in tobacco smokers [153], and lessened cue-induced craving and anxiety in heroin abstinent individuals [154]. All the remaining clinical studies (reviewed in [155]) were discouraging in terms of efficacy, though highlighted its safety profile [156–160].

Concluding remarks

Overall, a growing body of pre-clinical evidence highlights the therapeutic opportunities derived from the deeper knowledge of the eCB system. CB_1R, 2-AG, AEA, their metabolic enzymes, as well as other candidate components of the eCB signaling, such as PPAR, are key modulators of diverse neuronal processes involved in the orchestration of cue-directed behavior toward natural and drug rewards. eCBs are also efficient regulators of stress-induced responses at the circuit level, which might be exploited to manage deleterious behavioral consequences in drug addicts. Accordingly, the eCB system has been an important candidate in the search for new pharmacotherapies against SUDs. However, only a few compounds have reached clinical trial phases. Alternative eCB-targeting compounds are reinvigorating previously tempered expectations due to their ability to influence eCB function depending on the endogenous state of the targeted circuit. As presented herein, CB_1R NAMs, eCB degradation inhibitors, as well as the phytocannabinoid CBD are among the most appealing classes of drugs filling a gap already shown promising for counter-rewarding effects in animal models of drug addiction. Finally, it is worth mentioning that the observations reported by clinical trials have been mostly focused on measures of craving feelings and that reductions of relapse rates are still relatively modest, indicating that the journey ahead in the exploration of therapeutic properties of the diverse compounds covered here is still long.

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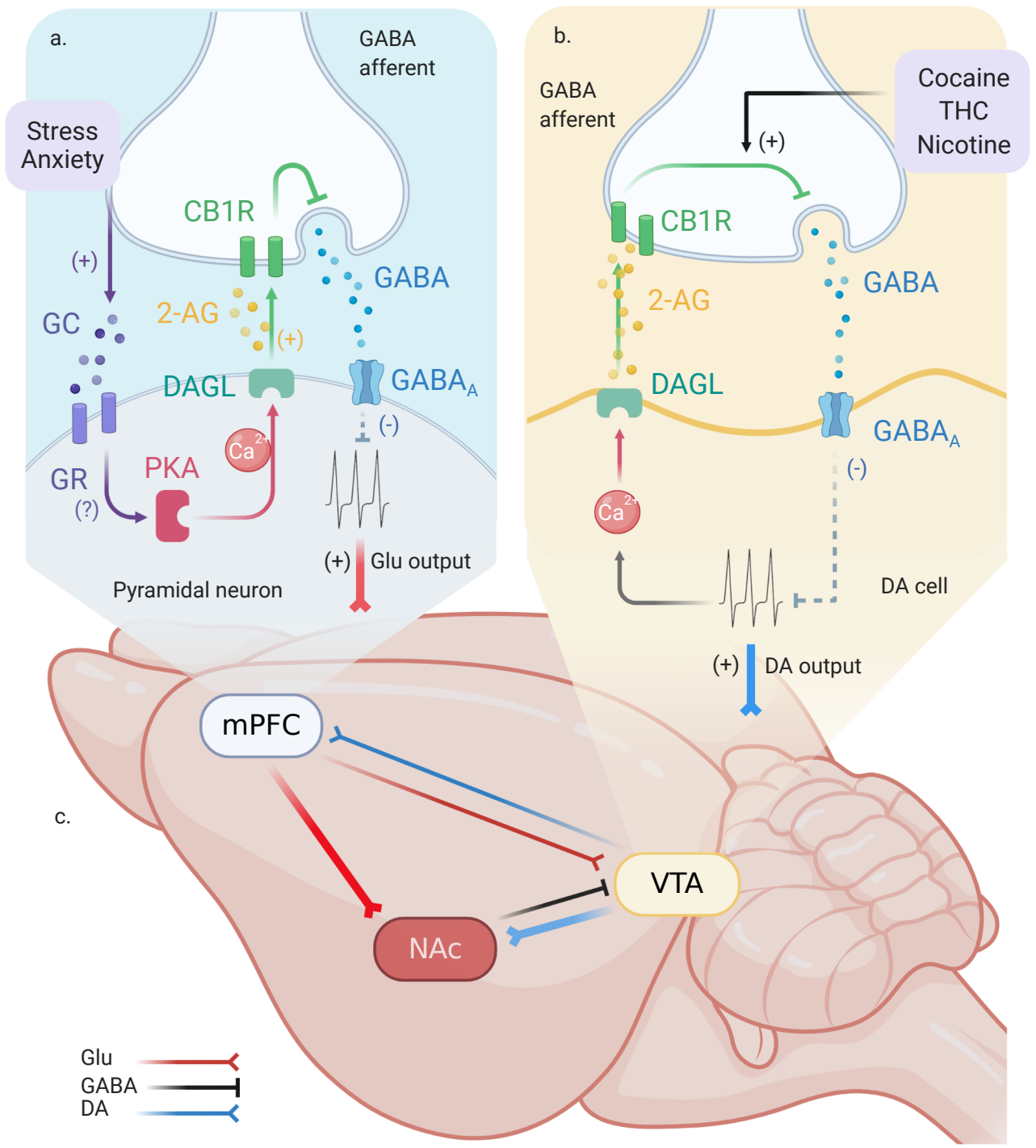
Figure legends

Figure 1. Schematic representation of two signaling pathways by which CB_1R participate in the regulation of cue and stress reactivity contributing to enhanced risk of drug use relapse. (**a**) In the mPFC, stressful events can trigger the release of glucocorticoids that start a series of intracellular downstream cascades –through an unknown receptor [53]– leading to 2-AG mobilization in pyramidal neurons and the inhibition of pre-synaptic GABA afferents. The subsequent disinhibition of NAc-projecting pyramidal neurons directs motivated behavior towards the procurement of the drug. (**b**) Following VTA DA cell activation, increased intracellular Ca^{2+} enhances DAGL activity, which may in turn produce the synthesis and release of 2-AG. Upon release, 2-AG binds presynaptic CB_1R, mostly found in GABA afferents. Then, activated CB_1R inhibit the release of GABA, thus disinhibiting DA neuron firing. 2-AG/CB_1R signalling within the VTA is increasingly activated following repeated drug exposure. This mechanism is thought to shape cue-evoked DA transients during the pursuit of drugs of abuse, opening a therapeutic window to tackle cue-induced relapse. (**c**) A simplified representation of the circuitry changes undergone between the mPFC, NAc and VTA that are subjected to the modulatory action of the eCB system. Abbreviations: GABA, γ-aminobutyric acid; GC: glucocorticoids; GR, glucocorticoid receptor; PKA, protein kinase A; DAGL, 1,2-diacylglycerol lipase; 2-AG, 2-arachidonoylglycerol; CB_1R: cannabinoid receptor 1. THC; Δ^9-tetrahydrocannabinol; DA, dopamine; Glu, glutamate; VTA, ventral tegmental area; NAc, nucleus accumbens; mPFC, medial prefrontal cortex. Created with Biorender.com.

Drs Lujàn, Cheer and Melis declare no conflict of interest.

Figure 1


a.
GABA afferent
Stress Anxiety
CB1R
(+)
GABA
GC
2-AG
(+)
DAGL
GABA$_A$
(-)
GR
(?)
PKA
Ca^{2+}
(+) Glu output
Pyramidal neuron
b.
GABA afferent
Cocaine THC Nicotine
(+)
CB1R
2-AG
GABA
DAGL
GABA$_A$
(-)
Ca^{2+}
DA cell
(+) DA output
c.
mPFC
VTA
NAc
Glu
GABA
DA