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Regulation of glutamate homeostasis in the nucleus accumbens by astrocytic CB1 receptors and its role in cocaine-motivated behaviors

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Abstract

Cannabinoid type 1 receptors (CB1Rs) orchestrate brain reward circuitry and are prevalent neurobiological targets for endocannabinoids and cannabis in the mammalian brain. Decades of histological and electrophysiological studies have established CB1R as presynaptic G-protein coupled receptors (GPCRs) that inhibit neurotransmitter release through retrograde signaling mechanisms. Recent seminal work demonstrates CB1R expression on astrocytes and the pivotal function of glial cells in endocannabinoid-mediated modulation of neuron-astrocyte signaling. Here, we review key facets of CB1R-mediated astroglia regulation of synaptic glutamate transmission in the nucleus accumbens with a specific emphasis on cocaine-directed behaviors.

Keywords

Astrocyte; Cannabinoid type I receptors; Glutamate homeostasis; Anandamide; Reinstatement; Cocaine

1. Introduction

Marijuana, derived from the plant *Cannabis sativa*, has surged in popularity due to its recent legalization for medical and recreational use [1–3], spurring significant investigative efforts to explore its potentially harmful effects. Clinical studies show that cannabis exposure, especially during critical developmental periods such as adolescence, induces behavioral and cognitive deficits [1,3] and the development of neurological and behavioral sequelae, including elevated hyperactivity, impulsivity, sensitivity to drugs of abuse, and risk for

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Declaration of Competing Interest

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psychosis. ⁹ - tetrahydrocannabinol (THC) is the principal psychoactive compound in cannabis that engages signaling at CB1R. The endocannabinoid (eCB) system is comprised of endogenous lipid ligands [4,5] and receptors such as the cannabinoid type 1 (CB1R) [6], cannabinoid type 2 (CB2R) [7], vanilloid type 1 receptor (TRPV1) [8,9], acylethanolamides-GPR55 [10], and GPR119 [11]. While eCBs' functions as retrograde messengers have been well-characterized [12–17], mounting work suggests that the eCB system exerts complex effects dependent on inter- or intra-cellular CB1R localization across different brain regions [9,18–22].

The CB1R is the most abundant G-protein coupled receptor (GPCR) in the central nervous system and is present in a wide variety of brain regions including the hippocampus, nucleus accumbens (NAc), prefrontal cortex (PFC), amygdala, and ventral tegmental area (VTA) [4,23]. The density of CB1R distribution exhibits cell-type selectivity. In the VTA, for example, CB1R are richly concentrated on the axon terminals of GABAergic interneurons, sparsely expressed on glutamatergic axons, and surprisingly, absent on dopamine neurons [24–26]. It is critical to note that diminished expression of CB1R does not necessarily indicate less impact on their ability to modulate cellular activity. Slice electro-physiological experiments revealed that CB1R agonists robustly inhibited pre-synaptic glutamate release in the NAc [9], although CB1R are predominately expressed in the fast-spiking interneurons [27]. In the hippocampus, more than half of CB1R are located in GABAergic neurons [28]. Surprisingly, genetic deletion of CB1R on glutamatergic neurons caused a more robust decrease in HU-210 (a highly potent synthetic cannabinoid agonist) induced CB1R-G-protein binding compared to mice with GABAergic CB1R deletion in the hippocampus [28]. This study provides one possible explanation for the mismatch of expression level and functionality: every CB1R could bind with a larger quantity of G-proteins on glutamatergic neurons to compensate for its lower expression level [28].

Neuronal CB1R are mostly identified on axonal terminals and inhibit neurotransmitter release via intracellular $G_{i/o}$ -signaling cascades [29]. Conversely, extensive studies show that astroglia CB1R promote gliotransmission through G_q -, G_s -protein-induced intracellular calcium mobilization [9,18,19,22,30,31] or with mitochondrial activation [32]. CB1R-mediated glutamatergic gliotransmission controls the induction of long-term synaptic plasticity in the hippocampus through N-methyl-D-aspartate (NMDAR) [18] and presynaptic type I metabotropic glutamate receptors [19,20]. Accumulating evidence indicates that astroglia CB1R are necessary for long-term memory formation [22], and when activated by tetrahydrocannabinol (THC), are implicated in short-term spatial memory impairment [33].

Astroglia-mediated homeostatic regulation of extracellular glutamate is often disrupted in animal models of drug addiction [34–36]. Endogenous activation of CB1R promotes astrocytic exocytosis of glutamate in the NAc and curtails cocaine-seeking behavior in rats [9], suggesting that astroglia CB1R regulate reward-seeking behavior by fine-tuning glutamatergic signaling in the NAc. In this review, we discuss the recent advances in our understanding of astrocytic CB1R-mediated regulation of extracellular glutamate signaling and their change in function relevant to drug-seeking behaviors.

2. G-protein binding properties of CB1R

CB1R activation elicits paradoxical effects on neurons and astroglia. While CB1R inhibit intracellular calcium activity in neurons and concomitant synaptic transmitter release [16], in astroglia, CB1R signaling increases the probability of vesicular gliotransmission in a calcium-dependent manner [18]. In this section, we discuss two possible explanations for these cell-type-specific effects by CB1R agonism.

Astroglia possess intracellular signaling pathways that are distinct from those of neurons. In neurons, GPCR or Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based activation of G_q - and $G_{i/o}$ pathways induce depolarization and hyperpolarization, respectively, and elevate intracellular calcium following G_q , but not $G_{i/o}$, activation [37]. In astroglia, however, G_q - and $G_{i/o}$ -binding DREADD or GPCR activation increases the probability of intracellular calcium transients through non-overlapping intracellular signaling mechanisms in the hippocampus [37] and the striatum [38]. These findings suggest that CB1R-mediated activation of the $G_{i/o}$ -pathway diametrically increases intracellular calcium mobilization in astrocytes while decreasing that of neurons. In contrast, $G_{i/o}$ -DREADD-induced astroglial calcium transients are smaller in amplitude than those elicited by G_q -DREADD activation in the hippocampus [38]. Specifically, astrocytic $G_{i/o}$ -DREADD activation increases c-Fos expression and astroglia-mediated synaptic plasticity with little to no effect on intracellular calcium concentrations [38–40], suggesting that $G_{i/o}$ -pathways may be transduced in a calcium-independent manner in astroglia. Studies to date consistently report that G_q -DREADD activation elicits sustained intracellular calcium elevation.

Another possible explanation for the differential effects of CB1R-activation on neurons and astrocytes may be due to the promiscuous nature of CB1R themselves. In cultured HEK293 cells, the mixed CB1/2R agonist, WIN55,212-2, stimulates cyclic AMP (cAMP) synthesis via the G_s -protein pathway and simultaneously increases extracellular signal-regulated protein kinase 1/2 (ERK1/2) phosphorylation by $G_{i/o}$ -proteins [41]. Additional evidence further supports the notion that CB1R promiscuously interact with $G_{i/o}$ -, G_s - and G_q -proteins [18,31,41–43], whereas most GPCRs preferably bind with only one family of G-proteins, either $G_{i/o}$ -, G_s -, or G_q -proteins. To understand the mechanism of CB1R-G-protein binding properties, we must appreciate recent studies conducted on the biochemical composition of the CB1R. Most GPCRs have seven transmembrane domains and three intracellular loops (Fig. 1A). The second intracellular loop (ICL2) of CB1R is a hydrophobic residue accountable for the preference of G_s - or $G_{i/o}$ -protein binding (Fig. 1A,B) [41,44]. If the leucine at site 222 (Leu-222) of ICL2 is mutated to proline (this CB1R mutation is called Leu-222P), CB1R inhibits cAMP production via $G_{i/o}$ -protein activation [41]. Conversely, several CB1R mutations such as His-219A, Arg-220A, and Arg-226A enhance cAMP accumulation through activation of G_s -proteins [41]. In contrast, μ -opioid receptors (μ OR) almost exclusively couple with G_i -proteins, thus making them suitable to study the promiscuity of CB1R [45]. Recent cryogenic electron microscopy (cryo-EM) studies compared the structural properties of CB1R and μ OR and their G-protein binding domains. The Val-173 of ICL2 in μ OR is enveloped by the $\beta 2$ - $\beta 3$ loop, which is a hydrophobic pocket of the G_i -protein that couples with GPCRs [46]. In contrast, although CB1R agonists

induced CB1R-G_i-protein interaction, the bond between Leu-222 at ICL2 and the G_i-protein β 2- β 3 loop is relatively weaker than those involved in μ OR-G_i-protein interaction [46,47], which enables CB1R to interact with more than one G-protein. The differences in the amino acid sequence at ICL2 could be one mechanism underlying the differential signal transduction properties of neuronal and astroglia CB1R in regulating intracellular activity [41,46,48].

Dimerization refers to the reaction of two monomers combining to form a larger dimer molecule [49]. CB1R-CB1R homodimer molecules were first detected by Western blotting in the early 2000s [50]. Other GPCRs including adenosine A2A receptors (A2AR) [51], dopamine D2 receptors (D2R) [52], μ -Opioid receptors (μ R) [53], orexin OX1 receptors [54], serotonin 5-HT_{2A} receptors [55] and CB2 receptors [56], can also form heterodimers with CB1R. Dimerization can potentially change the attributes of CB1R activation. Different types of GPCRs have a distinct impact on CB1R function during the dimerization processes (Table 1). Here we discuss two examples of how dimerization could influence CB1R-G-protein binding. The A2AR-CB1R heterodimer induces ERK and protein kinase B (Akt) phosphorylation when the two monomers are activated separately in striatopallidal projection neurons. However, when agonists of A2AR and CB1R are co-administered, A2AR-CB1R-induced ERK and Akt phosphorylation is inhibited via cross-antagonism between A2AR and CB1R [51]. One explanation for the cross-antagonizing effect between A2AR and CB1R lies in the particular G-protein binding properties of a heterodimer receptor. As discrete monomers, A2AR and CB1R are exclusively G_s- and G_i-protein coupled, respectively, while the A2AR-CB1R dimer selectively interacts with G_q-proteins [51]. Dopamine D2R is a G_i-protein coupled receptor primarily located on presynaptic terminals and astrocytes [57,58]. Found in close proximity to CB1R, it is not surprising that D2R-CB1R heterodimers have been detected in the striatum. Bioluminescence resonance energy transfer (BRET) experiments show that antagonizing D2R inhibits CB1R-G_i mediated ERK phosphorylation and enhances CB1R-G_s binding in striatal medium spiny neurons (MSNs) [59]. However, slice electrophysiological studies show that D2R activation reverses CB1R-G_i induced synaptic depression in the globus pallidus [60], suggesting the functional diversity of D2R-CB1R heterodimer receptors across substructures of the basal ganglia. However, due to technical limitations, the functional impact of CB1R dimers has not been directly tested *in vivo*.

It is worth noting that a higher concentration of WIN55,212-2 is required to activate the G_{i/o}-ERK1/2 pathway (EC₅₀ = 500nM) compared to the G_s-cAMP-PKA (EC₅₀ = 50nM) pathway *in vitro* [41]. Activation by lower concentrations of WIN55,212-2 (higher affinity) suggests that the CB1R-G_s-cAMP-PKA pathway is more sensitive to eCB stimulation. Conversely, burst action potential firing induced eCB release could steer CB1R toward G_{i/o}-ERK1/2 signaling cascades to potentially inhibit cellular activity.

In summary, amino acid sequence variations at the CB1R-ICL2 hydrophobic pocket, distinctive dimerization subunits, and even ambient eCB concentrations can differentially regulate G-protein binding with CB1R in the brain. Further studies are required to elucidate whether the expression level of CB1R-ICL2 subtypes and CB1R dimerization dynamics occur during different activity states, which would provide functional insight of the

promiscuity of CB1R binding with G_q -, $G_{i/o}$ - and G_s -protein pathways as a form of switchboard in eCB signaling.

3. Dysregulated glutamate homeostasis at cortical-accumbens afferents underlies reinstatement of drug-seeking

Reinstatement of drug-seeking behavior is one of the diagnostic criteria for substance use disorders in the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), often appearing when exposed to the contextual environment, substance priming and stress. Vulnerability to relapse is attributed to drug-induced impairments in the cognitive control of motivated behavior [61], particularly when drug-seeking behaviors lead to aversive consequences [62,63]. Drug-related exteroceptive information is carried by glutamatergic afferents from the prefrontal cortex (PFC) to the NAc core [16,64]. Microdialysis measurements show that cocaine priming causes behavioral reinstatement and activates PFC to NAc core glutamatergic projections in rats that had prior cocaine self-administration experience [65]. Moreover, this transient glutamate input from the PFC is amplified in the local circuitry within the NAc core by a reduction in presynaptic autoreceptors and an increase in postsynaptic expression of α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors [9,66].

Group-II metabotropic glutamate receptors (mGluR2/3) are GPCRs on axonal terminals that inhibit presynaptic glutamate release through $G_{i/o}$ -protein-mediated inhibitory pathways [9]. In the NAc core, presynaptic mGluR2/3 function is dramatically reduced after chronic cocaine administration [67] or when cocaine-related contexts are extinguished [9,68]. Diminished presynaptic inhibition amplifies cocaine-evoked excitatory signals transduced by PFC-NAc glutamatergic afferents [65], and consequentially increases neuronal activity in the NAc core [68]. Spontaneous excitatory postsynaptic currents (sEPSCs) are changes in the membrane potential of postsynaptic neurons that represent the probability of presynaptic glutamate release. LY341495, a selective antagonist of mGluR2/3, increases sEPSCs frequencies on MSNs in the NAc core but failed to do so after cocaine self-administration [9,68]. The finding that LY341495 gives rise to synaptic glutamate release suggests that mGluR2/3 assist in maintaining homeostatic synaptic glutamate release in the NAc core, which is lost after cocaine exposure. The mGluR2/3 function is thought to be occluded by a lack of stimulation from ambient glutamate which can be re-recruited by selective agonists or by replenishing extracellular glutamate [9,68]. Taken together, enhanced fidelity of PFC to NAc core glutamatergic signaling, reinforced by depleted extracellular glutamate concentrations, are pathogenic markers of vulnerability to relapse-related behavior [34].

Basal concentrations of extracellular glutamate generally range from 1 to 5 $\mu\text{mol/L}$ in the rodent brain [69]. The critical balance between the release and clearance of extracellular glutamate hinges upon neuron-astrocyte interactions and is referred to as the 'glutamate homeostasis' [34]. Prolonged exposure to drugs of abuse, such as cocaine, is correlated with depleted extracellular glutamate and dysfunctional glutamate release mechanisms in the NAc [68,70]. Astrocytic processes exhibit morphological plasticity that induce changes to the perisynaptic extracellular environment that give rise to drug-induced glutamate

depletion. In the NAc, cystine-glutamate antiporters (xCT) on astrocytes are the primary source of the extracellular glutamate [71,72]. Impairment of cystine-glutamate exchange by the antiporter xCT due to chronic exposure to cocaine and withdrawal leads to the upregulation of cocaine-seeking behavior during reinstatement [68]. Restoring xCT-mediated astrocytic glutamate release with N-Acetylcysteine provides enduring protection against relapse [68]. Brain slice electrophysiological data show that N-Acetylcysteine stimulates glutamate release by driving xCT [70]. Increased extracellular glutamate potentiates EPSCs in MSNs [70], activates mGluR2/3 to reverse cocaine-induced synaptic potentiation [68], and restores glutamate transporter-1 (GLT-1), an astroglial transporter responsible for glutamate elimination [73].

Elimination of extracellular glutamate relies on excitatory amino acid transporters (EAAT) in astrocytes, such as GLT-1 [69,74–76]. Similar to xCT, GLT-1 expression levels in the NAc decrease following sustained administration and prolonged withdrawal from cocaine or heroin [77,78]. Additionally, repeated N-Acetylcysteine exposure increases GLT-1 expression in the NAc and inhibits reinstatement [73,77,78]. Similarly, pre-treating rats with systematic administration of ceftriaxone prevented cocaine or heroin relapse by elevating the GLT-1-mediated glutamate reuptake [77,79]. Although cocaine or heroin-induced loss of GLT-1 and xCT are rescued by ceftriaxone [77,79], polymerase chain reaction (PCR) and immunoprecipitation data indicate that their coregulation is not solely mediated by physical interaction between the two proteins [79]. Hence, GLT-1 expression is hypothesized to be rescued by xCT-mediated elevation of extracellular glutamate concentration. For detailed information about these maladaptive reductions to GLT-1 and xCT function implicated in drug relapse (Fig. 2C), we direct readers to this review [34]. Finally, we note that additional mechanisms contribute to astrocytic regulation of extracellular glutamate. For example, recent work has shown that stimulation of μ OR in hippocampal astrocytes induces glutamate release and drives conditioned place preference [151].

GLT-1 and xCT are enriched in astroglia processes, and their functional changes during withdrawal from drugs of abuse are generally coupled with morphometric changes in astrocytes. In the NAc core of rats experiencing heroin self-administration and extinction training, astrocytic processes are disassociated from Synapsin I, a biological marker for axonal terminals [80]. In tissue harvested during cue-induced reinstatement to heroin, colocalization of astrocytic processes and axons was recovered by rapid and transient volumetric expansion of these processes [80]. Similarly, extinction of cocaine and methamphetamine self-administration reduces astrocytic volume and lowers neuron-astrocyte colocalization [81–83]. Additional work shows that this atrophy of astrocytic processes following cocaine self-administration occurs in the NAc, but not in the PFC or basolateral amygdala [82]. The atrophy was not observed during cocaine self-administration but was pronounced after extinction [82]. As previously mentioned, extracellular glutamate concentration in the NAc decreases during extinction of drug self-administration. This is in accordance with the understanding that astrocytic presence near accumbal MSNs is reduced due to morphometric reduction that in turn impairs homeostatic regulation of glutamate concentration in the NAc.

Changes in astrocytic morphology and their effect on glutamate regulation impacts neuronal activity through functional interactions between neurons and astrocytes. In the central nervous system, astrocytic processes enwrap chemical synapses, forming a semi-isolated micro-environment termed the ‘tripartite synapse’ [84–86]. The sheathing formed by astrocytic processes physically insulates adjacent synapses to bolster the signal-to-noise ratio of synaptic transmission from other synapses [87–89]. A typical tripartite synapse consists of three principal components: presynaptic axon terminals, postsynaptic dendrites, and astroglia processes. The connecting space between the presynaptic terminal and the dendritic spine is the synaptic cleft (Fig. 2B). Action potential-induced synaptic activity raises the glutamate concentration from 20 nmol/L to 1 mmol/L in the synaptic cleft [90]. Almost instantaneously, glutamate in the synaptic cleft diffuses into the extrasynaptic space, which is referred to as glutamate spillover [69,74–76]. Spillover of glutamate is tightly regulated by EAAT expressed on astrocytes [69,91–95]. GLT-1 is the most prevalent type of EAAT and is responsible for ninety percent of the extracellular glutamate clearance [96]. Quantitative electron microscopy demonstrates that astrocytic processes interface with neurons asymmetrically, with three- to four-fold higher density in the postsynaptic than presynaptic regions [97]. As GLT-1 expression is closer in proximity to postsynaptic elements, quantitative models estimate the elimination rate of glutamate by GLT-1 to be two to four times faster around postsynaptic dendrites [97]. Spillover is thus more likely to stimulate glutamate receptors on presynaptic neurons (Fig. 2 A, B). Spillover glutamate binding with mGluR2/3 on axon terminals [97–99] prevents overaccumulation of glutamate in synaptic clefts.

Yet, considering the data presented above, several questions remain to be addressed in future studies. First, MSN projections from the dorsal striatum constitute the direct and indirect pathways of the reward circuits which promote and inhibit motivated behavior, respectively [100]. In comparison, the MSN projections from the NAc core are less distinctive [100–102]. However, whether the presynaptic internalization of mGluR2/3 differentially influences the targeted brain regions remains unknown. Secondly, MSNs are not the only PFC targets in the NAc core. Pyramidal neurons in the PFC directly project to and modulate the activity of fast-spiking interneurons [103] and dopamine neuron axons in the NAc core [16]. Less is known about the drug-induced changes of mGluR2/3 function in those projections. Finally, other brain regions like the basolateral amygdala [104] and hippocampus [105,106] send glutamatergic afferents onto MSNs in the NAc core, and whether mGluR2/3 potency is dynamically altered on those axons during drug-seeking behaviors remains to be investigated. As presynaptic mGluR2/3 expression and extracellular glutamate depletion in the NAc core promotes vulnerability to reinstatement of drug-seeking behavior, the cellular mechanisms involved in the regulation of glutamate homeostasis in this region serve as critical targets for treating substance use disorders (Fig. 2C) [9,34,35,68,77,107].

4. Astrocytic glutamate exocytosis is suppressed following prolonged drug exposure

Astrocytic glutamate exocytosis is another prominent contributor to ambient glutamate concentrations. Vesicular glutamate release from astrocytes occurs in the NAc [108], cortex [109], thalamus [110], and the hippocampus [111]. Astrocytes propagate cell-to-cell signaling by the release of glutamate from vesicular organelles approximately thirty nanometers in diameter that resemble synaptic vesicles of glutamatergic terminals [112,113]. Vesicular glutamate transporters (VGLUT), glutamate-proton exchanger proteins that package intracellular vesicles with glutamate [112–115], and the SNARE protein complex, which facilitates vesicular docking and membrane fusion [116–118], constitute the exocytotic machinery recruited in astroglia glutamate release.

Once triggered by increases in intracellular calcium signaling, astrocytic vesicular glutamate is exocytotically released into the extrasynaptic space. Considering the glutamate concentration in the synaptic cleft is similar or higher than that of the extrasynaptic space, astrocytic vesicular glutamate preferentially stimulates extrasynaptic glutamate receptors instead of AMPARs, which are primarily located within synapses (Fig. 2B) [108,119,120]. Neuronal activity to astrocytic glutamate exocytosis can be measured by whole-cell patch-clamp recording in hippocampal slices. Specifically, astrocytic activation by UV light-induced intracellular calcium uncaging enhances the potency of synaptic inputs to nearby neurons (within 200 μm) in a SNARE-dependent manner [121,122]. These findings demonstrate that astrocytic glutamate exocytosis potentiates presynaptic transmission. Concomitantly, astrocytic glutamate exocytosis elicits postsynaptic potentiation by stimulating GluN2B-containing NMDAR receptors on neuronal cell bodies [120,123]. Due to the slow activation kinetics of NMDAR, the rise time of astrocytic glutamate-induced postsynaptic depolarization currents is approximately 10 to 200 milliseconds, which is significantly longer than AMPAR-mediated EPSCs [9,108]. Due to this kinetic feature, neuronal depolarization currents induced by astrocytic glutamate exocytosis are conventionally referred to as slow inward currents (SICs; inward refers to positive charge that flows into the neuronal membrane) [111,124–126].

SIC frequency represents the probability of astrocytic glutamate exocytosis. In acute cultured brain slices, SICs are evoked mainly by GPCR-mediated intracellular calcium signals. (RS)-3,5-dihydroxyphenylglycine (DHPG), an agonist of mGluR1/5, significantly elevates the frequency of SIC events [108,120]. This effect of DHPG was absent in the NAc core after cocaine self-administration [9], which exacerbates glutamate depletion (Fig. 2C).

Potentiating astrocytic glutamate exocytosis alone is sufficient to reverse behavioral phenotypes caused by glutamate depletion. G_q -DREADD activation of NAc astrocytes reduces cue-induced reinstatement of ethanol- [127], cocaine- [128], and methamphetamine-seeking [83]. At the cellular level, augmenting astrocytic glutamate exocytosis rescues the functionality of presynaptic mGluR2/3, a hallmark for vulnerability to reinstatement [9,128].

Like G_q -DREADD activation, CB1R agonism potentiates astrocytic glutamate exocytosis [9]. In neurons, presynaptic CB1R inhibit neurotransmitter release through $G_{i/o}$ -pathways

when bound to eCB ligands released from postsynaptic terminals in a retrograde fashion [9,16]. Yet, in astrocytes, CB1R activation increases the probability of glutamate exocytosis [9,18]. In the NAc core, endogenous activation of synaptic and astrocytic CB1R are both dampened after cocaine exposure [9,27]. Thus, restoring CB1R-mediated synaptic and astrocytic glutamate release may be a promising strategy for mitigating reinstatement of drug-seeking behavior.

In this review, we emphasized calcium-dependent astrocytic vesicular release mechanisms. However, it is worth mentioning that non-vesicular, calcium-mediated release of glutamate is also crucial for regulating neuronal function and motivated behaviors [129].

5. Anandamide is a chief regulator of glutamate homeostasis in the NAc core

As stated previously, CB1R has unique hydrophobic pockets that can bind with either $G_{i/o}$ -, G_q -, and G_s -proteins depending on the amino acid sequence at ILC2. CB1R agonists inhibit synaptic neurotransmitter release by activating $G_{i/o}$ -proteins in presynaptic terminals [130]. On the contrary, CB1R on astrocytes potentiate G_q -proteins and intracellular calcium concentrations which promotes glutamatergic gliotransmission in multiple brain regions [9,18,31,33,43,131]. Pertussis toxin is a bacterial toxin that selectively catalyzes ADP-ribosylation of $G_{i/o}$ -proteins and is used as an inhibitor of intracellular $G_{i/o}$ -protein cascades. Elevation of extracellular eCB concentrations increases astrocytic calcium activity that is insensitive to pertussis toxin [18,19,31]. These findings suggest that CB1R do not activate $G_{i/o}$ -proteins in astrocytes. Thus, CB1R-mediated astrocytic calcium activity relies on intracellular G_q - or G_s -, and not, $G_{i/o}$ -protein signaling (Fig. 2B).

Data from whole-cell patch-clamp recordings show that synthetic cannabinoids enhance SICs event frequency in a CB1R-dependent manner in the NAc core after cocaine self-administration [9], implying that astrocytic CB1R-mediated glutamate exocytosis is resistant to cocaine exposure. Hypothetically, G_q - or G_s -proteins recruited by CB1R activation might compensate for reduced astrocytic mGluR1/5 function to potentially rescue diminished astrocytic glutamate exocytosis in the NAc core after cocaine exposure. Hence, administration of CB1R agonists or positive allosteric modulators could possibly utilize this pathway to stimulate astrocytic glutamate exocytosis and replenish extracellular glutamate concentrations [9].

N-arachidonylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG) are the two most studied eCBs. Anandamide is synthesized from N-arachidonoyl phosphatidyl ethanol (NAPE) and eliminated by the hydrolytic enzyme, fatty acid amido-hydrolase (FAAH). 2-AG is generated from 2-arachidonoyl-containing phospholipid diacylglycerol lipase alpha (DAGL) and hydrolyzed by monoacylglycerol lipase (MAGL; Fig. 2B) [130,132]. In general, eCB-mediated synaptic plasticity is elevated by administration of drugs of abuse and diminished following repeated exposure. We direct readers to these two outstanding reviews on the effects drug-induced adaptations on eCB signaling [4,133]. In the NAc core, URB597, a selective inhibitor for FAAH [16], elevates SICs frequency in saline-yoked rats but does not induce any changes in SICs in rats that experienced

12 days of extinction after cocaine self-administration [9]. The specific reduction of anandamide signaling in the NAc core emerges even after a single exposure to cocaine [134], as indicated by anandamide-mediated eCB-dependent long-term depression in the glutamatergic synapses (eCB-LTDe) in the NAc core [135]. Indeed, cocaine-induced downregulation of anandamide-mediated astrocytic glutamate exocytosis [9] and synaptic eCB-LTDe [135] interferes with the regulation of homeostatic glutamate in the NAc core.

Therefore, augmented anandamide signaling participates in restoring glutamate homeostasis in the NAc core to prevent reinstatement to drug-seeking. Consistent with this assumption, several research groups have reported that enhancing anandamide tone can prevent drug-seeking. Agonizing CB1R, one of the main targets for anandamide, with WIN55,212-2 elicits significant suppression of cocaine self-administration behavior [136]. Intra-NAc core infusion of methanandamide, a metabolically stable analog of anandamide [137], similarly diminishes cocaine reinstatement and rescues mGluR1/5-mediated astrocytic glutamate exocytosis and mGluR2/3-mediated synaptic inhibition [9]. Moreover, FAAH inhibitors prevent reinstatement to nicotine seeking and nicotine-induced dopamine release in the NAc shell [138,139].

Taken together, the above data suggest that drug-induced reductions in anandamide signaling are pathological markers associated with the reduction of astrocytic CB1R-mediated glutamate exocytosis in the NAc core. Therefore, anandamide analogs and indirect agonists may prevent reinstatement of drug-seeking by the augmentation of astrocytic glutamate exocytosis to rectify maladaptive encoding of environmental contingencies in cortical-accumbal afferents.

6. Outlook

Astrocytic CB1R are critical for maintaining extracellular glutamate homeostasis in the NAc core and serve as important therapeutic targets for preventing relapse to drug-seeking behavior. Here, we propose three major areas of exploration that require further investigation.

CB1R bind with G_q -, G_s -, and $G_{i/o}$ -proteins. Presynaptic CB1R binds to $G_{i/o}$ -proteins to inhibit synaptic neurotransmitter release, while astroglia CB1R preferentially target the G_q -protein pathways to increase gliotransmitter release. Whether CB1R G-protein binding selectivity can dynamically change in vivo and if so, its underpinning molecular mechanisms remain to be elucidated.

Measuring the relative changes in eCB concentration has been problematic in the past [140], since these molecules last on the order of seconds and are present over distances on the order of tens of micrometers. This gap in knowledge has been dramatically improved by very recent advances in genetically encoded tools for sensing neurotransmitters such as the GPCR activation-based eCB sensor (GRAB_{eCB2.0}) [141]. In brief, this sensor is a genetically encoded protein (derived from the human CB1R) that selectively binds and emits fluorescence response to 2-AG and anandamide, allowing for in vivo measurement of evoked and spontaneous changes in eCB dynamics within seconds [140,141]. Recent

studies leveraging this approach have demonstrated that 2-AG is dynamically coupled to physiological and pathological neural activity (i.e. seizure) in the hippocampus [142], confirming that neuronal 2-AG synthesis and release are driven by neuronal depolarization-induced increases in intracellular calcium levels. It is important to note that currently available eCB sensors cannot differentiate between anandamide and 2-AG. An AAV vector under the control of astrocyte-specific promoter (GfaABC1D) is available on the Addgene Repository, however data have yet to be reported demonstrating the efficacy of the GRAB_{eCB2.0} in tracking eCB signaling on astrocytes. The efficiency of GRAB_{eCB2.0} transduction in astrocytes also needs to be addressed in future studies.

A single astrocyte can interface with numerous synapses, modulate synaptic plasticity, and synchronize firing activity on multiple neurons [111,125,143–146]. For example, cortical astrocytic activity shifts neural circuits to a slow-oscillatory state, which is critical for memory consolidation [147]. Astrocytes may also be required to generate synchronized activity in neurons with similar genetic signatures. In the dorsal striatum, D1R-MSN activation-induced astrocytic glutamate signals may specifically potentiate other D1R-MSNs via eCB signaling but not influence D2R-MSN activity, and vice versa [43]. D1R-MSNs and D2R-MSNs in the dorsal striatum are segregated to direct pathway MSNs (dMSNs) and indirect pathway MSNs (iMSNs) according to their innervation target and impact on motivated behaviors [148–150]. This distinction has been called into question for MSNs in the NAc, since both D1R-MSNs and D2R-MSNs contribute to the indirect pathway [101]. Thus, the astrocyte-mediated synchronized activity of either D1R-MSNs or D2R-MSNs is anticipated to impact the activity states of both direct and indirect pathways of the basal ganglia circuitry. Yet, how eCB signaling modulates astrocyte-neuron ensembles operating the direct and indirect pathways with systemic insult from drugs of abuse remains to be studied.

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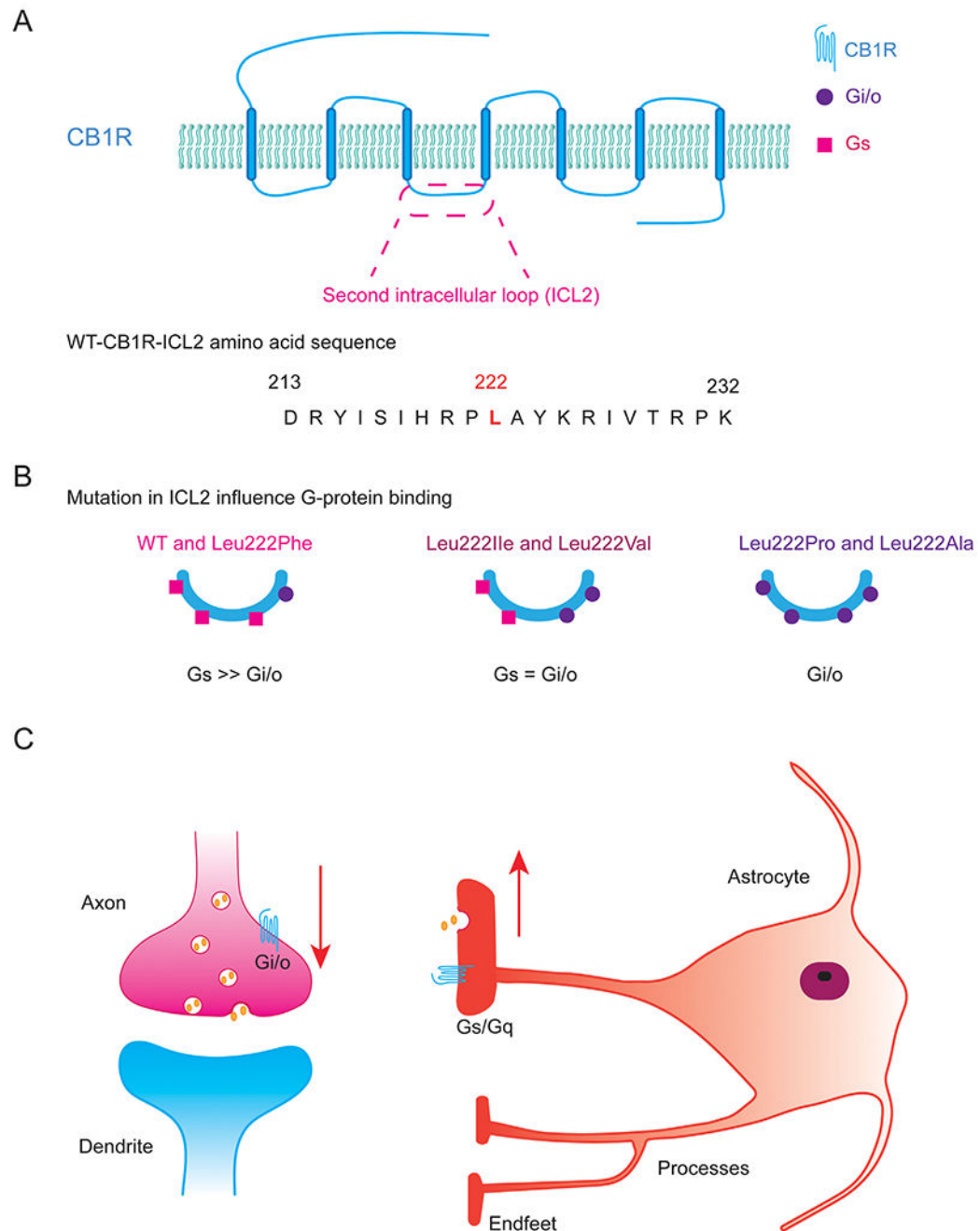


Fig. 1. G-protein binding properties of CB1R in the brain. (A), Schematic diagram of the transmembrane structure of the CB1R. The second intracellular loop (ICL2) is critical for G-protein recognition. (B), The amino acid sequence of the CB1R-ICL2 determines its binding preference to G_s - or $G_{i/o}$ -proteins. Mutations of the ICL2 sequence steers the CB1R toward $G_{i/o}$ -protein coupling. (C), The CB1R selectively couples to $G_{i/o}$ -proteins in presynaptic axon terminals to inhibit neurotransmitter release. In astrocytes, the CB1R activates G_s - or G_q -proteins to increase astrocytic activity.

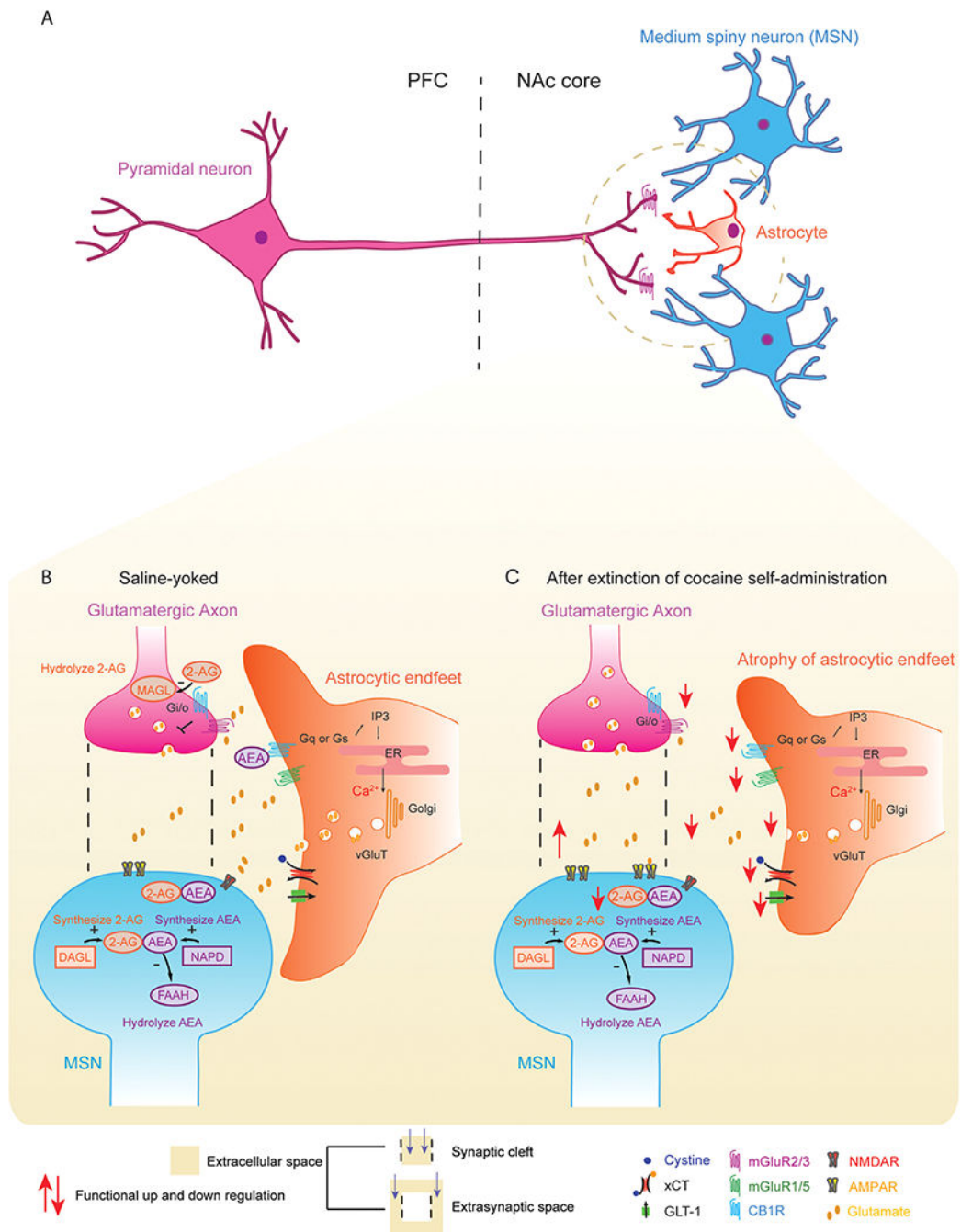


Fig. 2. Dysregulation of glutamate homeostasis and eCB signaling in the NAc of cocaine-addicted animals. (A), The prefrontal cortex innervates the NAc with glutamatergic axons that express mGluR2/3 autoreceptors. (B), Schematic of the glutamatergic tripartite synapse in the NAc. (C), Summary of cocaine-induced changes in extracellular glutamate and eCBs' actions in the NAc.

Table 1

CB1R heterodimer coupling to G-protein subtypes and signaling.

CB1R heterodimers	Receptor ligands	Prominent G-protein subtype / signaling	Substrate	Citation
CB1R [*] -	ACEA	G _{1/0} -dependent ERK phosphorylation	Striatum	[59]
D2R	ACEA + D2R antagonist: Haloperidol	G _s -dependent cAMP accumulation		
	ACEA + D2R agonist: Quinpirole	G _s -dependent cAMP accumulation	Globus pallidus	[60]
A2AR-CB1R [†]	WIN55,212-2 + A2AR agonist: CGS21680	G _q -mediated signaling	Dorsal striatum	[51]
CB1R [*] -CB2R	ACEA + CB2R agonist: JWH 133	G _{1/0} -Akt/PKB phosphorylation	Globus pallidus	[56]
5HT _{2A} -CB1R [†]	WIN55,212-2 + 5HT _{2A} R agonist: DOI	Cross antagonism: G _{1/0} -ERK 1/2 and Akt phosphorylation	HEK293T cells	[55]
μOR-CB1R [§]	CP55,940 + μOR agonist: DAMGO	G _q -phospholipase C signaling	<i>Xenopus</i> oocytes	[53]

Note. CB1R agonists:

^{*} ACEA (arachidonyl-2'-chloroethylamide, a stable synthetic analog of anandamide).

[†] WIN55,212-2.

[§] CP55,940.