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# Targeting corticostriatal transmission for the treatment of cannabinoid use disorder

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| **Abstract:** | It is generally assumed that the rewarding effects of cannabinoids are mediated by CB1 receptors (CB1Rs) whose activation disinhibits dopaminergic neurons in the ventral tegmental area. However, this mechanism can hardly explain novel results indicating that dopaminergic neurons also mediate the aversive effects of cannabinoids in rodents, and previous results showing that preferentially presynaptic adenosine A2A receptor (A2AR) antagonists counteract self-administration of delta-9-tetrahydrocannabinol in non-human primates. Based on recent experiments in rodents and imaging studies in humans, we propose that the activation of frontal corticostriatal glutamatergic transmission constitutes an additional and necessary mechanism. We review the evidence supporting that cortical astrocytic CB1Rs are involved in the activation of corticostriatal neurons and that A2AR receptor heteromers localized in striatal glutamatergic terminals mediate the counteracting effects of the presynaptic A2AR antagonists, constituting potential targets for the treatment of cannabinoid use disorder. |

cover letter

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Jerry Madukwe, PhD
Editor-in-chief
Trends in Pharmacological Sciences

Dear Dr. Madukwe,

We thank you very much for the opportunity to resubmit our manuscript TIPS-D-23-00062, entitled "Targeting corticostriatal transmission for the treatment of cannabinoid use disorder" to be considered as an Opinion article in Trends in Pharmacological Sciences. This revised version of the manuscript takes into consideration all the suggestions of the reviewers and our editorial suggestions, which include a modification of Fig. 1. All modifications are marked in red in the new text file.

Looking forward to your hopefully favorable final decision,

Best wishes,

Sergi Ferré, MD, PhD

**Highlights**

- Recent studies in rodents and humans indicate that activation of frontal corticostriatal glutamatergic transmission is a main mechanism involved in the rewarding and striatal dopamine-releasing effects of THC and synthetic cannabinoids.
- This mechanism involves stimulatory cannabinoid $CB_1$ receptors ($CB_1Rs$) localized in cortical astrocytes, which activation promotes astrocytic glutamate release.
- Dopamine release in the nucleus accumbens (NAc) induced by an increased frontal corticostriatal transmission is mediated by a local striatal microcircuit that involves the corticostriatal glutamatergic terminal, the cholinergic interneuron, and the dopaminergic terminal.
- The selective pharmacological targeting of corticostriatal terminals, to counteract their stimulated glutamate release, provides a new therapeutic approach for cannabinoid use disorder.

# Targeting corticostriatal transmission for the treatment of cannabinoid use disorder

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**Abstract:** **It is generally assumed that the rewarding effects of cannabinoids are mediated by $CB_1$ receptors ($CB_1Rs$) whose activation disinhibits dopaminergic neurons in the ventral tegmental area. However, this mechanism can hardly explain novel results indicating that**

**dopaminergic neurons also mediate the aversive effects of cannabinoids in rodents, and previous results showing that preferentially presynaptic adenosine $A_{2A}$ receptor ($A_{2A}R$) antagonists counteract self-administration of delta-9-tetrahydrocannabinol in non-human primates. Based on recent experiments in rodents and imaging studies in humans, we propose that the activation of frontal corticostriatal glutamatergic transmission constitutes an additional and necessary mechanism. We review the evidence supporting that cortical astrocytic $CB_1$Rs are involved in the activation of corticostriatal neurons and that $A_{2A}R$ receptor heteromers localized in striatal glutamatergic terminals mediate the counteracting effects of the presynaptic $A_{2A}R$ antagonists, constituting potential targets for the treatment of cannabinoid use disorder.**

## The VTA hypothesis of the rewarding effects of cannabinoids

The mounting consumption of cannabis products with increased concentrations of the **cannabinoid** (see Glossary) delta-9-tetrahydrocannabinol (THC), and the incessant appearance of more potent **synthetic cannabinoids** (see Glossary) represent a continuous increase in the risk of adverse health outcomes for cannabis users [1,2]. However, currently there is no treatment for cannabinoid use disorder. Elucidation of the mechanisms that mediate the reinforcing effects of cannabinoids should provide clues for a possible pharmacological treatment for cannabinoid use disorder. The consensus is that the **rewarding effects** (see Glossary) of cannabinoids are ultimately related to their ability to increase the extracellular concentration of dopamine in the most ventral part of the **striatum** (see Glossary), the **nucleus accumbens (NAc)** (see Glossary), a property shared by all addictive drugs [3]. This was first demonstrated in 1997 by Gianluigi Tanda, in the laboratory of Gaetano Di Chiara [4], with systemic administration of THC in rats. Four years later, in the laboratory of Steven Goldberg, Gianluigi Tanda was also the first to demonstrate that THC could be self-administered by an experimental animal, the squirrel monkey [5]. It was later found that rats learn to intracranially self-administer THC in the NAc and in the **ventral tegmental area (VTA)** (see Glossary), site of origin of the dopaminergic cells that project to the NAc [6].

There are two subtypes of classical cannabinoid receptors, named $CB_1$ and $CB_2$ receptors ($CB_1R$ and $CB_2R$), which belong to the G protein-coupled receptor (GPCR) family and are usually coupled to inhibitory $G_{i/o}$ proteins. It is well established that the rewarding effects of cannabinoids depend on their ability to act as agonists at $CB_1Rs$, although their precise location

in the brain and at the cellular level has not yet been fully elucidated. Although $CB_1R$ ligands would be logically considered for the treatment of cannabinoid use disorder, significant adverse effects (depression and suicidality) of the $CB_1R$ **inverse agonist** (see Glossary) rimonabant, led to its withdrawal from clinical trials in 2008, and boosted the search for other strategies to either directly target $CB_1Rs$ (**partial agonists** or **neutral antagonists**; see Glossary) or to modulate the endocannabinoid system [7]. It is a tenet of this article that the elucidation of the main mechanisms involved in the rewarding effects of cannabinoids, should lead to new therapeutic approaches for the treatment of cannabinoid use disorder.

It is generally believed that a major mechanism involved in the dopamine-releasing effects of THC is the disinhibition of the activity of the VTA dopaminergic cells, mediated by $CB_1Rs$ located at the terminals of GABAergic neurons that target the dopaminergic cells [8]. These GABAergic inputs would mostly originate in the adjacent rostromedial tegmental nucleus, also known as the tail of the VTA [9] (**Fig. 1**), but they could also include the terminals of GABAergic efferent neurons from the NAc [10]. In fact, since these striatal neurons also express $CB_1Rs$ in their somatic-dendritic area [10], they could mediate the ability of THC to be self-administered in the NAc.

However, cannabinoids also produce aversive effects, which seem to dominate over the rewarding effects in mice and are predominantly mediated by $CB_2Rs$ that are located in VTA dopaminergic neurons [11]. Although originally thought to be only present in the periphery, $CB_2Rs$ were later found to be localized in the brain, including the VTA. Their functional and pharmacological properties, including their affinity for THC, are very similar to those of $CB_1Rs$, and their preferential $G_{i/o}$ coupling explains their inhibitory role on dopaminergic cell function

[7]. In addition, a recent study by the group of Zheng-Xiong Xi using genetic and optogenetic techniques showed that a subset of VTA dopaminergic cells also express $CB_1$Rs, whose activation promotes aversive effects [12]. Furthermore, as discussed here, the VTA mechanism falls short of explaining the previously demonstrated counteracting effects of specific adenosine receptor antagonists on THC self-administration in non-human primates.

The present article provides evidence for a necessary and yet neglected mechanism involved in the reinforcing and dopamine-releasing effects of THC and other $CB_1$R agonists: the activation of the frontal corticostriatal glutamatergic transmission. First, we review the evidence that shows significant prefrontal corticostriatal activation induced by THC both in the experimental animal and in humans. Second, we examine the VTA-independent mechanism that promotes striatal dopamine release upon glutamate release secondary to corticostriatal activation and present evidence indicating that this mechanism is involved in the dopamine-releasing effects of cannabinoids. This also implies reviewing the recently demonstrated key role of adenosine and **endocannabinoids** (see Glossary) in modulating striatal glutamate release, mediated by complexes (heteromers) of $CB_1$Rs and adenosine receptors located in corticostriatal glutamatergic terminals [13]. Third, we present evidence indicating that targeting the corticostriatal glutamatergic terminals, with ligands that decrease the ability of the terminal to release glutamate, is sufficient to counteract the rewarding effects of THC, providing a mechanistic basis for the treatment of cannabinoid use disorder**.**

**THC induces prefrontal corticostriatal activation in experimental animals and humans**

There is now significant experimental evidence indicating that THC promotes strong activation of the frontocortical pyramidal glutamatergic neuron that projects to the NAc, and this mechanism seems to involve astrocytic $CB_1$Rs. Importantly, correlative results have been obtained with imaging studies in humans. In the experimental animal, systemic administration of THC in rats was initially shown to increase the extracellular levels of dopamine and glutamate and decrease GABA levels in the prefrontal cortex [14]. The increase in dopamine could be explained by activation of VTA neurons that also project to the prefrontal cortex, and the decrease in GABA levels could be explained by activation of inhibitory $CB_1$Rs localized in the terminals of **cortical GABAergic interneurons** [15,16] (see Glossary). The increase in extracellular levels of glutamate could then be related to the decreased inhibitory GABAergic input to **cortical glutamatergic neurons** (see Glossary) and, possibly, to an increased stimulatory dopaminergic input. More recent evidence indicates the involvement of astrocytes, which express $CB_1$Rs that can couple to stimulatory $G_q$ proteins and to $G_{i/o}$ proteins, which differently to neurons, exert a stimulatory function in astrocytes (**Box 1**). The activation of astrocytic $CB_1$Rs promotes intracellular transient $Ca^{2+}$ elevations and a consequent astrocytic glutamate release, capable of influencing the function of adjacent neurons (**Fig. 1** and **Box 1**).

The notion that astrocytic $CB_1$R plays an imortant role in the cortical glutamate-releasing effects of THC was reinforced by the finding of a significant counteraction of THC-induced glutamate release in the rat frontal cortex and cortical astrocytic cultures by kynurenic acid (KYNA), an astrocytic-derived endogenous negative allosteric modulator of the $\alpha_7$-nicotinic acetylcholine receptor ($\alpha_7$nAChR) [17]. In the same study, a significant co-localization of $CB_1$Rs and $\alpha_7$nAChRs was demonstrated in rat frontocortical astrocytes [17]. The combined increase in

astrocytic glutamate release and dopamine release and the decrease in GABA should be expected to induce a strong activation of corticostriatal pyramidal neurons (**Fig. 1**). This was demonstrated in the same study, where rat frontocortical cells identified as output neurons projecting to the NAc showed a very significant increase in their firing rate after systemic administration of THC [17]. Importantly, this THC-induced increase in firing rate was more robust than the one also observed in VTA cells projecting to the NAc and was counteracted by increasing the brain extracellular levels of KYNA with the systemic administration of an inhibitor of kynurenine 3-monooxygenase (KMO), the enzyme that metabolizes kynurenine, the precursor of KYNA [17].

That THC produces a significant activation of the prefrontal cortex and is associated with its rewarding effects has been supported by recent and previous imaging studies in chronic marijuana users and non-users during the administration of THC. With **positron emission tomography (PET)** (see Glossary) using 2deoxy-2-[$^{18}$F]-fluoro-D-glucose, Volkow et al. [18] showed that during "intoxication" associated with systemic administration of THC, glucose metabolism selectively increased in the frontal and prefrontal cortex and in the right temporal cortex. Furthermore, those effects were stronger in chronic marijuana users than in non-users [18]. Concomitantly, Mathew et al. [19] in PET experiments with $^{15}$O-labelled water, showed that systemic THC administration increasd cerebral blood flow, specially in the frontal cortex, insula and cingulate gyrus of subjects with a history of exposure to marijuana, which was correlated with intoxication ratings and feeling "high". Similar results have recently been obtained in subjects regularly consuming marijuana after a variable oral dose of THC, using **near-infrared spectroscopy** (see Glossary) [20]. This study also showed that THC induces

frontocortical activation, which correlated with the level of intoxication but not with the dose [20].

Other recent imaging studies in humans have also provided evidence for an overlap between the psychotomimetic effects of THC and its effects on the frontal corticostriatal circuit. A recent study with **magnetic resonance spectroscopy (MRS)** (see Glossary) by Colizzi et al. [21] showed that systemic THC produced a striatal increase (in the head of the caudate nucleus) of the "**Glx peak"** (see Glossary), the compound MRS signal for glutamate plus glutamine, which was significantly higher in individuals who were more sensitive to its psychotomimetic effects. Another recent study also found an altered striatal volume/Glx relationship in patients with early psychosis with a history of cannabis use [22]. Furthermore, in correlation with the role of astrocytes in the THC-induced corticostriatal activating effects, interindividual differences in transient psychosis-like effects of THC have recently been associated with a differential impact on cortical astrocytic function, measuring **myo-inositol** levels (see Glossary) with MRS [23].

Altogether, these findings indicate that the activation of prefrontal corticostriatal neurons is a main pharmacological effect of the systemic administration of THC, which most probably underlies its psychotomimetic and possibly its rewarding effects. The significant contribution of cortical astrocytes would support the search for compounds able to selectively counteract $CB_1R$ signaling in astrocytes. This can be achieved either directly or indirectly, such as with $\alpha_7$nAChRs ligands or KMO inhibitors, which have been pursued for the treatment of other neuropsychiatric disorders [24,25]. However, less predictive would be the therapeutic effect of increasing brain extracellular levels of KYNA with KMO inhibitors. Thus, KYNA levels are increased in the brain of individuals with schizophrenia [26] and early exposure to THC in rats

causes enduring cognitive deficits, which have been attributed to chronic increases in brain KYNA levels [27].

**THC induces striatal dopamine release by a VTA-independent and glutamate-dependent mechanism**

To understand how increased corticostriatal neurotransmission is involved in the dopamine-releasing and therefore rewarding effects of cannabinoids, an examination of the VTA-independent and glutamate-dependent striatal mechanism that promotes dopamine release is needed. Also, how this mechanism can bypass the well-established presynaptic inhibitory role of $CB_1$Rs located in the corticostriatal glutamatergic terminals needs examination.

Dopamine is directly involved in reinforcement, in the learning ("stamping-in") of stimulus-**reward** (see Glossary) and reward-response associations that follows the receipt of reward [28]. Concomitantly, dopamine is involved in reward-oriented behavior, in the increased responsiveness to rewarding stimuli, with orienting and approaching responses to those stimuli [28]. These two complementary functions are dependent on two temporally different operating modes of dopamine release: first, a fast millisecond-scale phasic response, which codes for reward prediction errors [29], which therefore provides a rapid response to reward-related signals and can significantly contribute to the role of dopamine in reinforcement; and second, a prolonged, minute-scale tonic dopamine response [29], which provides signals of proximity and value of distant rewards [30] and therefore can significantly contribute to the role of dopamine in reward-oriented behaviors.

Although both types of dopamine release have been attributed to two corresponding phasic and tonic responses in dopamine cell firing [29], it was recently shown that a significant component of the tonic response is independent of somatic dopamine cell spiking and is, in fact, generated at the terminal level [31]. This component is glutamate-dependent and is mediated by a local striatal microcircuit that involves the corticostriatal glutamatergic terminal, the cholinergic interneuron, and the dopaminergic terminal [32]. Several studies using optogenetic techniques have provided significant evidence indicating that striatal glutamate release by corticostriatal nerve terminals contacting cholinergic interneurons leads to acetylcholine release and activation of α4-β2*nAChRs (the asterisk indicates the possible presence of additional subunits) located in the striatal dopaminergic terminals, leading to the local release of dopamine [33–35]. This mechanism is independent of somatic dopamine cell firing and depends on the initiation of action potentials at the dopamine nerve terminal [36,37]. If the rewarding effects of THC involve this striatal microcircuit (**Fig. 1**), it would imply that a strong frontocortical activation should be rewarding. In fact, this has been demonstrated in mice that optogenetically self-stimulate the corticostriatal terminals in the NAc [35,38].

However, several studies from different research groups, including ours, have also demonstrated the existence of functional $CB_1$Rs in the corticostriatal terminals, whose activation promotes the inhibition of glutamate release [35,39–41]. This raises the question of how the systemic administration of THC can promote corticostriatal glutamate release upon activation of cortical $CB_1$Rs, while simultaneously activating presynaptic striatal inhibitory $CB_1$Rs. The conundrum can be explained by invoking the concept of G protein-coupled receptor (GPCR) heteromers (**Box 2**) and by the finding that $CB_1$Rs form heteromers with adenosine $A_{2A}$

receptors ($A_{2A}$Rs) in corticostriatal terminals, mediating a unique fine-tune modulation of glutamate release by adenosine and endocannabinoids [13,32].

$A_{2A}$Rs also form heteromers with adenosine $A_1$ receptors ($A_1$Rs) at the corticostriatal nerve terminals, and both $A_1$R-$A_{2A}$R and $A_{2A}$R-$CB_1$R heteromers play a very important and elaborated role in the adenosine- and endocannabinoid-mediated control of corticostriatal glutamatergic transmission. This depends on the multiple allosteric interactions conveyed by the GPCR heteromers, which include ligand-dependent and independent allosteric interactions and interactions through a plasma membrane effector that complexes with the heteromer (labelled type I, II and III **allosteric interactions**, respectively; see Glossary) (**Box 2**).

As reviewed elsewhere [32], adenosine has more affinity for the $A_1$R than for the $A_{2A}$R and, under conditions of low corticostriatal transmission, endogenous adenosine predominantly originates from ATP released from astrocytes and targets the $A_1$R in the $A_1$R-$A_{2A}$R heteromer, which promotes inhibition of glutamate release. This is counterbalanced by the constitutive activity of the $A_{2A}$R in the $A_{2A}$R-$CB_1$R heteromer, and both mechanisms provide the basal sensitivity of the corticostriatal terminal to release glutamate (**Fig. 2A**). On the other hand, a type II allosteric interaction (**Box 2**) impedes the constitutive activity of the $A_{2A}$R in the $A_1$R-$A_{2A}$R heteromer [13] (**Fig. 2A**). It could be shown that the main mechanism by which $CB_1$R activation inhibits corticostriatal glutamate release is by inhibiting the constitutive activation of adenylyl cyclase (AC) by $A_{2A}$R in the $A_{2A}$R-$CB_1$R heteromer, a type III allosteric interaction [13] (**Box 2**).

Importantly, activation of $A_{2A}$R in the $A_1$R-$A_{2A}$R and $A_{2A}$R-$CB_1$R heteromers leads to a type I allosteric interaction (**Box 2**), which decreases the signaling of the corresponding

heteromeric partner, the $A_1R$ and $CB_1R$ [13,42] (**Fig. 2B**). This mechanism can explain the results of a series of electrophysiological experiments in corticostriatal slices and KCl-induced glutamate release experiments in striatal synaptosomes, where $A_{2A}R$ agonists significantly counteract the depressant effect of $A_1R$ and $CB_1R$ agonists on corticostriatal transmission and glutamate release [42–44]. Although we recently suggested that the activation of $A_{2A}Rs$ in the glutamatergic terminals would prmarily occur under pathological conditions associated with excessive production of extracellular adenosine [32], recent studies by Rodrigo Cunha's group indicate that elevated synaptic adenosine levels that are sufficient to activate presynaptic $A_{2A}Rs$ are generated under conditions of strong activation of the corticostriatal neurons [45] (**Fig. 2B**).

The origin of this synaptic adenosine is ATP which is co-released with glutamate at high stimulation intensities and quickly converted to adenosine by the ectonucleotidase CD73 [45]. In fact, presynaptic $A_{2A}Rs$, and not even neighboring ATP receptors, appear to be the preferential targets of synaptically released corticostriatal ATP release [45]. Activation of these presynaptic $A_{2A}Rs$ can then disinhibit glutamate release by counteracting the effect of adenosine and endogenous or exogenous cannabinoids on $A_1Rs$ and $CB_1Rs$ in the respective $A_{2A}R$ heteromers. Therefore, the $A_1R$-$A_{2A}R$ and $A_{2A}R$-$CB_1R$ heteromers provide an adenosine-mediated mechanism that counteracts the local inhibitory effect of THC on striatal glutamate release (**Fig. 2**).

In summary, the local striatal microcircuit that involves the corticostriatal glutamatergic terminal, the cholinergic interneuron, and the dopaminergic terminal seems to provide the bases for the dopamine-releasing and therefore reinforcing effects of cannabinoids. This being the case, it implies that specific pharmacological targeting of the different neuronal elements of

the circuit could provide new approaches for the treatment of cannabinoid use disorder. In the next section, we elaborate on the selective targeting of the corticostriatal glutamatergic terminal based on the expression of specific adenosine receptor heteromers. However, other putative targets localized in the cholinergic interneurons and the dopaminergic terminals could also be considered.

**Targeting corticostriatal glutamatergic terminals to counteract the rewarding effects of cannabinoids**

From the previous considerations, it follows that targeting $A_{2A}R$ in the $A_1R$-$A_{2A}R$ and $A_{2A}R$-$CB_1R$ heteromers of the corticostriatal terminals should counteract the dopamine-releasing and rewarding effects of THC. Antagonizing the constitutive activity and the effect of adenosine on these $A_{2A}R$ heteromers should provide a significant decrease in the sensitivity of corticostriatal terminals to release glutamate after cannabinoid-induced strong activation of the corticostriatal neuron. However, striatal $A_{2A}Rs$ are highly expressed postsynaptically, in the GABAergic striatopallidal neuron, where they form heteromers with dopamine $D_2Rs$, which mediate the psychostimulant effects of the non-selective adenosine receptor antagonist caffeine [46,47]. The blockade of striatal postsynaptic $A_{2A}Rs$ would then be expected to produce the opposite effect than the blockade of presynaptic $A_{2A}Rs$, an increase in the reinforcing effects of THC by enhancing the effect of dopamine release on the signaling of the $D_2R$ in the $A_{2A}R$-$D_2R$ heteromer.

In concert with this, we have previously found significant differences in the pharmacodynamic properties of different $A_{2A}R$ antagonists that depend on the heteromeric partner of $A_{2A}R$. In rodents, different profiles were obtained when comparing the ability of the different selective $A_{2A}R$ antagonists to act postsynaptically, in the striatal $A_{2A}R$-$D_2R$ heteromer, by analyzing their ability to stimulate locomotor activity, and presynaptically, in the $A_1R$-$A_{2A}R$ and $A_{2A}R$-$CB_1R$ heteromers, by analyzing their ability to counteract striatal glutamate release induced by electrical or optogenetically-triggered corticostriatal stimulation. MSX-3 produced both effects at similar systemic doses, although it was about three times more potent at counteracting corticostriatal transmission; KW-6002 (istradefylline) had a postsynaptic profile, with a strong locomotor activating effect and no effect at counteracting corticostriatal transmission; and SCH-442416 showed a presynaptic profile, with a strong blockade of corticostriatal transmission at doses without locomotor activation [46,48,49].

The mechanism for the presynaptic profile of SCH-442416 (or more precisely, for the lack of its postsynaptic effect) was related to a selective decrease in its affinity for the $A_{2A}R$ forming heteromers with $D_2R$ (a type II allosteric interaction, **Box 2**) [48], while the explanation for the lack of presynaptic effect of KW-6002 lies within its neutral antagonism. On the other hand, SCH-442416 and MSX-3 are inverse agonists, and therefore capable of counteracting the constitutive activity of the $A_{2A}R$ [32]. The three $A_{2A}R$ antagonists were tested for their ability to modify THC self-administration in squirrel monkeys. As predicted, SCH-442416 or a low dose of MSX-3 significantly counteracted THC self-administration, while the opposite qualitative response was observed with KW-6002 or a high dose of MSX-3 [44,45].

These results, which depend on the selective ability of SCH-442416 or a low dose of MSX-3 to target striatal presynaptic $A_{2A}$Rs, constitute an additional indirect demonstration of the significant role of corticostriatal transmission in the dopamine-releasing and rewarding effects of THC. On the other hand, the facilitation of THC self-administration induced by the selective postsynaptic $A_{2A}$R antagonist KW-6002 can be explained by a potentiation of the effect of the non-opposed THC-induced dopamine release on the $A_{2A}$R-$D_2$R heteromer. Caffeine is also a neutral $A_{2A}$R antagonist [32] and, in addition, it locally promotes striatal glutamate and dopamine release through its $A_1$R antagonistic properties [47]. Therefore, although, to our knowledge, the effect of caffeine has not been evaluated on the reinforcing effects of THC in animal models, we should expect a significant potentiating effect.

As expected from the involvement of cortical astrocytic $CB_1$Rs in the frontocortical activating properties of THC and their interactions with $\alpha_7$nAChRs, increasing the endogenous extracellular levels of KYNA with the systemic administration of a KMO inhibitor also counteracted THC-induced dopamine release in the rat NAc and THC self-administration in squirrel monkeys [52]. However, it could be demonstrated that local application of KYNA in the NAc also counteracts THC-induced dopamine release [52]. This can be explained by the also well-established existence of $\alpha_7$nAChRs in the striatal glutamatergic terminals [32], and indicates that, regardless of the effect of KYNA on cortical astrocytes or other putative brain areas, inhibition of $\alpha_7$nAChR function in the corticostriatal terminals is sufficient to explain the counteracting effect of systemic administration of KMO inhibition on THC self-administration.

In summary, different results converge on $A_{2A}$R heteromers and $\alpha_7$nAChR receptors localized in the frontal corticostriatal glutamatergic terminals as promising targets for the

treatment of cannabinoid use disorder. Yet, the putative therapeutic effects of selective $\alpha_7$nAChR receptor antagonists could also depend on their concomitant targeting at cortical astrocytes. Similarly, the pharmacological effect of $A_{2A}$R ligands might also include localizations other than the corticostriatal terminal. In fact, functional $A_{2A}$Rs have also been demonstrated in cortical GABAergic interneurons and astrocytes [53,54]. The demonstrated significant counteraction of the rewarding effects of THC by the selective presynaptic $A_{2A}$R inverse agonist, SCH-442416, encourages its clinical investigation, or of other compounds with similar pharmacodynamic properties. Unfortunately, the selective postsynaptic neutral $A_{2A}$R antagonist KW-6002 is the only approved $A_{2A}$R ligand for clinical use, in Parkinson's disease.

**Concluding Remarks and Future Perspectives**

The studies reviewed here indicate that activation of frontal corticostriatal transmission is a necessary mechanism involved in the rewarding and dopamine-releasing effects of THC and other cannabinoids. We need to resolve the real relevance of the generally accepted VTA mechanism (see outstanding questions). We have also reviewed the evidence supporting the involvement of cortical astrocytic $CB_1$Rs in the activation of frontal corticostriatal pyramidal neurons and their cholinergic modulation mediated by astrocytic $\alpha_7$nAChR receptors. More studies are needed to determine whether an increase in frontal corticostriatal transmission is also a main mechanism involved in the psychotomimetic effects of cannabinoids, and to determine the real contribution of cortical astrocytes (see outstanding questions).

We have also reviewed the elaborate control by adenosine of the glutamate released by coticostriatal terminals, mediated by presynaptic $A_{2A}R$ heteromers. We have then proposed the investigation of $\alpha_7$nAChR receptor and specific presynaptic $A_{2A}R$ ligands for the treatment of cannabinoid use disorder (see outstanding questions). Another strategy would be to evaluate compounds already available for other clinical indications that have been shown experimentally to inhibit glutamate release upon selective stimulation of corticostriatal terminals. Those include ligands of the α2δ calcium channel subunit, such as gabapentin, and the $D_2$-like receptor agonists pramipexole and ropinirole, which have therapeutic application in Restless Legs Syndrome (RLS). By local striatal application, these compounds were able to counteract the optogenetically-induced glutamate release of supersensitive corticostriatal terminals in a rodent model of RLS [55]. Significantly the same effect was obtained with the inhibitor of adenosine transport dipyridamole [56], which predicted its recently demonstrated beneficial effect in RLS patients [57]. The effect of dipyridamole was dependent on its ability to promote an increase in the striatal extracellular concentration of adenosine and its preferential binding and activation to presynaptic $A_1Rs$ [56]. Therefore, this strategy offers an alternative to systemically administered $A_1R$ agonists, which would not be clinically useful due to their pronounced depressant cardiovascular and respiratory effects. Nevertheless, a recent study by Frenguelli's group found an $A_1R$ agonist with functional selectivity for the activation of the G protein subtype $G_{oB}$ with potent analgesic effects, but without causing sedation, bradycardia, hypotension, or respiratory depression in rodents [58].

Finally, the demonstration of the key role of frontal corticostriatal transmission in the rewarding, dopamine-releasing and psychotomimetic effects of THC should also drive attention

to non-pharmacological treatments and non-invasive methods of cortical function modulation, such as repetitive **transcranial magnetic stimulation (TMS)**. Recent reviews point to early encouraging results, but recommend larger, randomized and more standardized clinical trials [59,60].

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**Glossary**

**Adenylyl cyclase (AC):** Plasma membrane effector of GPCRs, which activation promotes the synthesis of the signaling molecule cAMP from ATP. GPCRs coupled to $G_s$ and $G_i$ proteins stimulate and inhibit its activity, respectively.

**Allosteric:** From allosterism, the process by which the interaction of a chemical or protein at one location on a protein or macromolecular complex (the *allosteric* site) influences the binding or function of the same or another chemical or protein at a topographically distinct site.

**Cannabinoids:** Naturally occurring biologically active compounds from cannabis, including THC and cannabidiol.

**Constitutive activity:** Constitutive activity is the basal activation of a receptor in the absence of a ligand, which is counteracted by inverse agonists, but not by neutral antagonists. The name inverse agonist is then related to its ability to promote the opposite effect of an agonist.

**Cortical GABAergic interneurons:** Neurons of the cerebral cortex that release the inhibitory neurotransmitter GABA and act locally within cortical networks.

**Cortical glutamatergic neurons:** Neurons of the cerebral cortex that release the excitatory neurotransmitter glutamate and are classified largely as pyramidal and nonpyramidal according to their morphology. Pyramidal neurons are projecting neurons that communicate with other

cortical or subcortical regions of the brain, such as the corticostriatal neurons, which connect the cortex to the striatum.

**Endocannabinoids:** Endogenous neurotransmitters that bind and activate cannabinoid receptors, including anandamide and 2-arachidonoglycerol.

**Glx peak:** See magnetic resonance spectroscopy.

**Inverse agonist:** See constitutive activity.

**Magnetic resonance spectroscopy (MRS):** MRS allows the detection of some relatively small molecules within cells or in the extracellular space. The most prominent signals commonly observed in MR spectra include Glx, which is a composite of glutamate plus glutamine, and myo-inositol, which is a marker of glial activity.

**Myo-inositol:** See magnetic resonance spectroscopy.

**Neutral antagonist:** See constitutive activity.

**Near-infrared spectroscopy:** Optical imaging technique that provides an indirect measure of brain activity by quantifying oxygenated and deoxygenated hemoglobin.

**Nucleus accumbens:** See striatum.

**Partial agonist:** Ligand that binds to a receptor and promotes a weaker activation (lower efficacy) than a full agonist (usually the endogenous ligand).

**Positron emission tomography (PET):** Imaging technique that detects the differential accumulation of low amounts of different radioactive tracers in the brain, such as 2deoxy-2-[$^{18}$F]-fluoro-D-glucose and $^{15}$O water, as measures of increased metabolism and cerebral blood flow, as indirect measures of increased neuronal activity.

**Reward:** In the noun form (a reward), an object or event that elicits approach and is worked for; its analogue is 'a reinforcer'. In the verb form (to reward) the term is synonymous with 'to reinforce' [23]. See the text for the definition of reinforcement.

**Transcranial magnetic stimulation (TMS):** Non-invasive method that uses magnetic fields to promote repetitive activation of superficial (cortical) brain areas, currently under investigation for several neuropsychiatric disorders.

**Rewarding effects:** Ability to act as a reward. See reward.

**Striatum:** Subcortical brain structure which is the main target of the dopaminergic system and is classically subdivided in a dorsal compartment, which includes the caudate and putamen nuclei (caudate-putamen in rodents) and a ventral compartment, which includes the nucleus accumbens (NAc) and the olfactory tubercle.

**Synthetic cannabinoids:** Continuously emerging structurally different compounds with powerful cannabinoid receptor agonistic properties.

**Ventral tegmental area (VTA):** Brainstem nucleus which harbors the cell bodies of the dopaminergic cells that project to the NAc and prefrontal cortex.

**Box 1. Stimulatory role of astrocytic $CB_1$Rs.** Astrocytes are major players in brain function by exchanging signals with neurons. Unlike neurons, they do not show electrical excitability, but they show intracellular $Ca^{2+}$ transients that evoke the release of their own signaling molecules. These gliotransmitters include glutamate and ATP, which can influence neuronal excitability, synaptic transmission, and plasticity [61]. Araque's research group showed that hippocampal astrocytes express $CB_1$Rs, whose activation promotes the opposite than in neurons, a stimulatory effect, an astrocytic glutamate release that can influence the function of adjacent pyramidal neurons [62]. Later they showed that this influence includes synaptic plastic changes [63], as confirmed by other studies in the cerebral cortex [64], and the same functional role has been ascribed to astrocytic $CB_1$Rs in the NAc and VTA [65,66]. Although initial studies indicated that the stimulatory effect of astrocytic $CB_1$Rs was mediated by $G_q$ proteins [62], a recent study using a chemogenetic approach indicated that activation of both $G_q$ and $G_{i/o}$ ead to the same excitatory effect in astrocytes in hippocampal slice preparations and *in vivo* in the primary somatosensory cortex [67]. Therefore, unlike in neurons, not only $G_q$, but also $G_{i/o}$ protein activation promotes astrocytic activation, with the concomitant release of gliotransmitters [67].

The same as $CB_1$R, the GABAB receptor (GABABR) is a classical $G_{i/o}$-coupled inhibitory receptor in neurons that has stimulatory properties in astrocytes [68], and astrocytic GABABRs play an important role in prefrontal cortical function [69]. Significantly, a study on stimulus discrimination in humans showed that the GABABR agonist baclofen can substitute for THC and can also potentiate THC-induced discriminative-stimulus effects [70]. This could therefore be related to the common astrocytic mechanisms of $CB_1$Rs and GABABRs. In addition, the same as $CB_1$Rs, activation of GABABRs located in cortical GABAergic interneurons promotes the

disinhibition of cortical pyramidal neurons [71]. However, GABABRs are also located in corticostriatal glutamatergic terminals, where their activation inhibits glutamatergic transmission [72]. Lastly, GABABRs are also located in the VTA, both in dopaminergic cells and GABAergic interneurons, where they show a different sensitivity that depends on the different subunit composition of their associated potassium channels [73]. This determines that low concentrations of baclofen disinhibit while high concentrations inhibit dopaminergic cell activity [73]. The existence of similar differential functional properties among the multiple GABABRs involved could determine a similar predominant pharmacological effect of systemically administered baclofen than with THC, an increased corticostriatal transmission.

Astrocytes, with their ability to release glutamate, can therefore exert a stimulatory role on adjacent neurons. However, their ability to also release ATP constitutes an apparent enigma, since, in most studied cases, this implies a rapid conversion to adenosine and activation of presynaptic $A_1$Rs, which inhibits glutamatergic transmission (see main text). A seminal study by Araque's group has nevertheless shown that a single astrocyte can decode the activity of adjacent GABAergic interneurons and release the gliotransmitters glutamate or ATP depending on the neuronal activity, by sensing different patterns of activation of the astrocytic GABABR elicited by different patterns of GABA release [74].

**Box 2. G protein-coupled receptor heteromers.** During the A large amount of experimental evidence from many different studies has accumulated during the last two decades indicating that G protein-coupled receptors (GPCRs) form functional heteromers, which are defined as macromolecular complexes composed of at least two different GPCR units (called protomers)

with biochemical properties that are demonstrably different from those of its individual components [75]. A common GPCR functional unit is constituted by a homodimer, with two equal protomers, and one G protein, with its α, β and γ subunits. Heteromers then can be constituted by heterotetramers, with two homodimers each one coupled to its preferred G protein [76,77]. Moreover, a common heterotetramer seems to be constituted by one homodimer coupled to a stimulatory $G_{s/olf}$ ($G_s$ for short) protein and the other homodimer coupled to an inhibitory $G_{i/o}$ ($G_i$ for short) protein, which provides the framework for the canonical $G_s$-$G_i$ antagonistic interaction at the AC level, by which the activation of a $G_i$-coupled receptor inhibits the activation of AC by a $G_s$-coupled receptor [76]. This includes the heteromers of the $G_s$-coupled adenosine $A_{2A}R$ ($A_{2A}R$) with the $G_i$-coupled dopamine $D_2$ receptor ($D_2R$), the adenosine $A_1$ receptor ($A_1R$) or the $CB_1R$ [13]. Thus, different transmembrane domains (TMs) are involved in GPCR oligomerization, and the same GPCR can display different oligomeric interfaces when forming heteromers with different GPCRs, which determines different quaternary structures and properties [13].

GPCR heteromers provide the framework for different allosteric interactions, which have been recently classified into three types [78]. In Type I, a ligand of one protomer changes the properties (affinity or efficacy) of a ligand for the other protomer, or its **constitutive activity** (see Glossary). Type II corresponds to a ligand-independent interaction, where one protomer, without ligands, changes the properties of a ligand for the other protomer or its constitutive activity. Type III corresponds to an allosteric interaction through a plasma membrane effector that also oligomerizes with the GPCR heteromer. This includes **adenylyl cyclase (AC)** (see Glossary), in which TMs can establish molecular interactions with TMs of GPCRs [79]. The

canonical $G_s$-$G_i$ antagonistic interaction at the AC level corresponds to a type III allosteric interaction. The existence of these functional oligomeric complexes led to introduce the GPCR-effector macromolecular membrane assembly concept or GEMMA, defined as an assembly of directly interacting specific GPCRs, G proteins and effectors localized in the plasma membrane with emergent functional and pharmacological characteristics [78].

**Figure 1. Cellular elements of the VTA, prefrontal cortex and NAc that mediate the dopamine-releasing effects of THC.** The classical VTA mechanism involves $CB_1$Rs localized in terminals of GABAergic neurons from the rostromedial tegmental nucleus (RMTgN) and terminals of GABAergic efferent neurons from the NAc, promoting activation of dopamine (DA) neurons by disinhibition. The corticostriatal mechanism results from activation of the pyramidal cells of the prefrontal cortex (PFC) induced by, first, DA release from the disinhibited VTA DA neurons; second, activation of $CB_1$Rs localized in the terminals of GABAergic interneurons; and third, by $CB_1$Rs localized in astrocytes, which produces astrocytic glutamate (glu) release by a $G_q/G_{i/o}$-dependent increase in calcium transients. THC-induced increase in the activity of PFC-NAc neurons leads to glu release in the NAc, which promotes DA release by the intermediate activation of ACh interneurons. $CB_1$Rs are also localized in corticostriatal terminals, but their potential inhibition of glu release is counteracted by the concomitant increase in synaptic adenosine generated by ATP released upon the strong corticostriatal input (see text and Fig. 2).

**Figure 2. Schematic representation of corticostriatal glutamatergic terminals and their modulatory $A_1R$-$A_{2A}R$ and $A_{2A}R$-$CB_1R$ heteromers.** Glutamate (glu) release by the terminals of

corticostriatal pyramidal neurons depends on the firing rate of the pyramidal neuron and on the local levels of adenosine (Ado) and endocannabinoids, mainly 2-arachidonoylglycerol (2-AG). A fine-tune modulation is mediated by $A_1R$-$A_{2A}R$ and $A_{2A}R$-$CB_1R$ heteromers. With a low firing rate (**A**), local Ado originates mostly from astrocytic ATP and binds with more affinity to the $A_1R$ than to the $A_{2A}R$ in the $A_1R$-$A_{2A}R$ heteromer, where a type II allosteric mechanism blunts the constitutive activity of the $A_{2A}R$; on the other hand, the $A_{2A}R$ in the $A_{2A}R$-$CB_1R$ heteromer shows significant constitutive activity, which is negatively modulated by activation of the $CB_1R$ by a type III allosteric interaction. With a high firing rate (**B**), including that induced by THC, a higher synaptic concentration of Ado is produced upon ATP co-released with glu and metabolized by synaptic ectonucleotidase CD73; increased levels of Ado lead to activation of $A_{2A}Rs$, which counteract $A_1R$ and $CB_1R$ signaling in the $A_1R$-$A_{2A}R$ and $A_{2A}R$-$CB_1R$ heteromers by type I allosteric interactions.

**Outstanding questions**

- What is the real relevance of the VTA mechanism, the activation of dopaminergic cells in the VTA, in the rewarding effects of cannabinoids?
- Would clinically applicable $A_{2A}R$ or $\alpha_7$nAChR ligands be of therapeutic use in cannabinoid use disorder?
- Would compounds that are clinically available and have been shown experimentally to inhibit stimulated corticostriatal glutamate release be useful for the treatment of cannabinoid use disorder and cannabinoid-induced psychosis?
- Is also the increase in frontal corticostriatal transmission a main mechanism involved in the psychotomimetic effects of cannabinoids?
- What is the real contribution of cortical astrocytes in the rewarding and psychotomimetic effects of cannabinoids?

**Answers to comments by reviewer #1:**

**1-A major inference derived from their proposed mechanism underlying THC addiction is that the selective decrease of glutamate release should alleviate the rewarding effects of THC. One of the most effective presynaptic mechanisms decreasing glutamate release is the activation of adenosine A1 receptors. It may be of interest to consider potential effects of the direct activation of A1 receptors in the control of THC self-administration apart from the impact of dipyridamole. Another powerful system presynaptically controlling the release of glutamate is operated by GABA-B receptors. I remember struggling with an apparently paradoxical study reporting a potentiation by baclofen on the effects of THC (Lile et al., 2012, Drug Alcohol Depend 126:216-23), which might not fully align with the hypothesis proposed in this review. If it makes sense, a comment might be inserted in the text.**

The mechanisms of dipyridamole in the corticostriatal glutamatergic terminal have now been better explained in the Concluding Remarks and Future Perspectives section, and, as the reviewer indicates, explicitly described as related to the increase activation of presynaptic striatal A1R, and consequent inhibition of glutamate release. We have then included an additional reference about the experiment that demonstrated the A1R-mediated inhibition of optogenetically-induced corticostriatal glutamate release by dipyridamole both in naïve rats and in a rat model of restless legs syndrome (RLS; Ferre et al., 2019). We have also added a reference about the existence, in this animal model, of supersensitivity of cortico-striatal terminals to release glutamate (Yepes et al., 2017). The importance of adding this additional information is, first, because our results predicted that dipyridamole or other adenosine transport inhibitors would be effective for the treatment of RLS, which was recently demonstrated in a randomized, placebo, crossover study (Garcia-Borreguero et al., 2021; ref. also added in the text). Second, because results obtained with this animal model provide the rationale for our concluding remarks (already included in the previous version of the manuscript) about the possibility "…to evaluate compounds already available for other clinical indications that have been shown experimentally to inhibit glutamate release upon selective stimulation of corticostriatal terminals". We have therefore expanded the following sentence: "those compounds include ligands of the α2δ calcium channel subunit such as gabapentin and the $D_2$-like receptor agonists pramipexole and ropinirole, which have therapeutic application in Restless Legs Syndrome (RLS). By local striatal application, these compounds were able to counteract the optogenetically-induced glutamate release of supersensitive corticostriatal terminals in a rodent model of RLS (Yepes et al., 2017)". About the possibility of using A1R agonists, instead of indirect agonists like dipyridamole, we have added the following sentences and new reference in the last section: "…this strategy offers an alternative to systemically administered $A_1$R agonists, which would not be clinically useful due to their pronounced depressant cardiovascular and respiratory effects. Nevertheless, a recent study by Frenguelli's group found an $A_1$R agonist with functional selectivity for the activation of the G protein subtype GoB with potent analgesic effects, but without causing sedation, bradycardia, hypotension, or respiratory depression in rodents".

We are very grateful to the reviewer's comment about the GABABR agonist baclofen and its yet unexplained results in the discriminative stimulus study in human by Lyle et al, showing that it can substitute for THC and can also potentiate THC-induced discriminative-

stimulus effects. The fact is that this allowed us to bring more support to the role of astrocytes in the central effects of THC. As we have now explained in the manuscript (in Box 1), "the same as $CB_1R$, GABAB receptor (GABABR) is a classical Gi/o-coupled inhibitory receptor in neurons that has stimulatory properties in astrocytes, and astrocytic GABABRs play an important role in prefrontal cortical function." We have added references from Araque's group that support these statements. See also our answer to the point 4 raised by the reviewer. We have then suggested that their common pharmacological effects might depend on activation of the frontal corticostriatal pyramidal neurons, and not only from astrocytic glutamate release, but also from activation of CB1Rs and GABABRs localized in cortical GABAergic interneurons, promoting disinhibition of cortical pyramidal neurons (see new ref. 71). However, as discussed in the manuscript (Box 1), and as indicated by the reviewer, GABABRs are also localized in corticostriatal glutamatergic terminals, where their activation inhibits glutamatergic transmission. We are not aware of the existence of mechanisms such as those mediated by adenosine and adenosine receptor heteromers that allow surmounting the potential inhibitory effect mediated by CB1R in glutamatergic terminals. Nevertheless, we have suggested a heuristic explanation based on the already demonstrated different sensitivity of GABABRs that depends on the different subunit composition of their associated potassium channels (see ref. 73).

**2-Adenosine A2A receptors are also present in the PFC, regulating the activity of the output neurons of layer V. Would it make sense to entertain the hypothesis that the benefits afforded by some A2A receptor antagonist could also or mostly involve a control of PFC excitability (in a manner similar to the A2AR-CB1R interaction in the hippocampus to control reference memory)? Considering this hypothesis would mean that the behavioral benefits afforded by A2A receptor antagonists could involve the control of PFC circuits (see Kerkhofs et al., 2018, Front Pharmacol 9:133) rather than mostly the local control of glutamate release probability at corticostriatal terminals.**

We agree with the reviewer, and in page 16 (first paragraph), we added to the text the sentence: "…the pharmacological effect of $A_{2A}R$ ligands might also include localizations other than the corticostriatal terminal. In fact, functional $A_{2A}Rs$ have also been demonstrated in cortical GABAergic interneurons and astrocytes". To support this statement, we have added the ref. suggested by the reviewer by Kerkhofs et al. and a recent ref. from Lopes et al. (ref. 54).

**3-Given that caffeine is the most widely consumed psychoactive drug and has been a core interest of some of the authors, would it be relevant to introduce a small comment on the expected effects of coffee consumption on THC rewarding effects? Although this might be a detail outside the central scope of this review, I would be curious to know if there is any evidence supporting a putative selective action of caffeine on pre vs. postsynaptic A2AR in corticostriatal synapses, especially since it has been argued that caffeine might be more effective to antagonize heteromeric rather than homomeric adenosine receptors.**

We do agree with the reviewer that we should add some comments about caffeine. This comment is also related to point 7 raised by the reviewer and, from that comment, we believe we did not make clear enough the distinction of the functional role of striatal presynaptic versus postsynaptic $A_{2A}R$. This is crucial for supporting our claim about seeking selective striatal

presynaptic $A_{2A}R$ antagonists, and more specifically $A_{2A}R$ inverse agonists. Now, caffeine is totally comparable to KW-6602 in its pharmacodynamic properties at the $A_{2A}R$, as a neutral antagonist. But also, caffeine is non-selective and binds and blocks $A_1R$ signaling, which, as we previously demonstrated promotes striatal glutamate and dopamine release. We have therefore added the following sentences in the text: in page 13 (last paragraph), "however, striatal $A_{2A}Rs$ are highly expressed postsynaptically, in the GABAergic striatopallidal neuron, where they form heteromers with dopamine $D_2Rs$, which mediate the psychostimulant effects of the non-selective adenosine receptor antagonist caffeine [46,47]. The blockade of striatal postsynaptic $A_{2A}Rs$ would then be expected to produce the opposite effect than blockade of presynaptic $A_{2A}Rs$, an increase in the reinforcing effects of THC by enhancing the effect of dopamine release on the signaling of the $D_2R$ in the $A_{2A}R$-$D_2R$ heteromer"; and in page 15 (first paragraph), "caffeine is also a neutral $A_{2A}R$ antagonist [32] and, in addition, it locally promotes striatal glutamate and dopamine release through its $A_1R$ antagonistic properties [47]. Therefore, although, to our knowledge, the effect of caffeine has not been evaluated on the reinforcing effects of THC in animal models, we should expect a significant potentiating effect.

**4-The proposed role of astrocytic CB1 receptors to activate astrocytes and trigger an increased PFC excitability is certainly tempting, and most importantly supported by direct experimental evidence. However, the mechanistic proposal that this might occur through an increased release of gliotransmitters such as ATP and glutamate is not so easy to align with some published studies. In fact, the release of ATP from astrocytes, namely in the PFC, has been associated with antidepressant effects through P2X2 receptors (e.g. Cao et al., 2013, Nature Med 19:773-7) and with heterosynaptic depression through adenosine A1 receptor activation (e.g. Pascual et al., 2005, Science 310:113-6). The former evidence would argue for direct emotional responses encoded in prefrontocortical circuits and the former would trigger a decreased corticostriatal response. If an attempt to rebut these comments may led to a dilution of the focus of the text, an alternative may simply be to remove the idea that there are changes in the release of gliotransmitters, should the authors agree with this suggestion.** We agree with the reviewer about the mostly opposite effect of astrocytic ATP versus astrocytic glutamate on neuronal function, with its rapid conversion to adenosine and preferential activation of inhibitory A1Rs. In fact, depending on the circuit and glutamate receptors involved, glutamate has also been shown to be inhibitory. Again, the work of Araque's group and particularly that related to astrocytic GABABR (see answer to point 1) provided significant results that strongly suggest that the astrocyte can release both gliotransmitters, but differentially, depending on the neuronal activity, by sensing different patterns of activation of the astrocytic GABABR elicited by different patterns of GABA release (reference 74). This is now elaborated in an additional paragraph included at the end of Box 1.

**5-The proposed centrality of a deregulation of PFC circuits upon THC consumption makes it rather compelling to consider TMS as a candidate strategy to manipulate THC addition, in view of the particularly easy manipulation of the PFC with TMS. Although I not aware of such studies, would it make sense to mention such a possibility as a future prospect prompted by the groundbreaking hypothesis presented in this review?**

We agree with the reviewer in that, with our here proposed key role of corticostriatal neurotransmission in the central effects of cannabinoids, TMS should be considered as an alternative treatment for cannabinoid use disorder. This has been added at the end of the last section. In fact, several studies have already been performed, and as added to the text, recent reviews point to early encouraging results, but recommend larger, randomized and more standardized clinical trials (refs. 59 and 60).

**6-A question that remains untested is the stability of the heteromers upon THC regular consumption. The presented hypothesis seems to assume that they are 'immutable', but the beautiful work of Ciruela showed that A2A-D2 receptor heteromers were (rather profoundly) altered in PD. Could it be that, apart from altered levels of neurotransmitters/ neuromodulators with regular THC consumption, the modification of the relative amounts of the different heteromers in corticostriatal terminals may play a decisive role in altered signaling associated with the modification over time of THC rewarding perception?**
We agree with the reviewer, since we believe that GPCR heteromers and their stoichiometry can change significantly with different sustained treatments and pathologies. At this point, in relation to $CB_1R$ and adenosine receptor heteromers, we would prefer not confusing the reader with an added putative complexity, for which we still do not experimental results.

**7-My final comment is related to my surprise to see that a recent review on the role of A2A receptors, in particular that A2A receptors in corticostriatal synapses may have a central role in the homeostatic balance between emotional and cognitive behavior (Chen et al., 2023, Neuropharmacology 226: 109421), was apparently ignored. It is my impression that the core ideas of this previous review are fully aligned with the present proposal and the present proposal would certainly benefit from considering these previously published ideas. I would challenge the authors to consider aligning their proposal with these of Chen's review.**
Please see answer to point 3 raised by this reviewer. The review by Chen et al. makes only reference to the striatal postsynaptic $A_{2A}R$ and the $A_{2A}R$-$D_2R$ in the striatopallidal neuron.

**Answers to the minor comments by reviewer #2:**

**1-Page 3 , line 17: …could be self-administered by the experimental animal, the squirrel monkey..**
Corrected.

**2-Page 4, line 6-7: Regarding the role of the CB2 receptor, I recommend adding further information, the proposed mechanism, the ligand used, etc.**
We have added a few more sentences about the function and pharmacology of CB2R in page 4 (last paragraph).

**3-Page 5, line 14: Regarding the ability of the CB1 receptor stimulation to increase dopamine levels, the authors could also refer to the paper by Kędziora et al., 2023.**
We only detected a paper by Kędziora et al related to $CB_1Rs$: “Kędziora, M. et al. (2023) Inhibition of anandamide breakdown reduces pain and restores LTP and monoamine levels in

the rat hippocampus via the $CB_1$ receptor following osteoarthritis. *Neuropharmacology* 222, 109304". However, the study does not explore the activation of the dopaminergic system by THC or other cannabinoids. Unless we missed another paper by the same author, we honestly do not see the value of adding this reference.

**4-Page 7, line 11: Perhaps a brief reference could be made at this point to the role of glutamine on glutamate synthesis/turnover.**
We kindly disagree with the reviewer. We do not see the value of commenting on the glutamate/glutamine cycle when describing the "Glx peak". But also following the suggestions from the editor, we have made it more clearly in the text that the "Glx peak" (also in the Glossary) is the compound MRS signal for glutamate plus glutamine.

**5-Page 8, lines 10-16: The sentence could be split in two**
Corrected.

**6-Perhaps a brief introduction could be made to the three types of allosteric interaction although it is well explained in box 2**
We have added the following sentence in page 11 (second paragraph): This depends on the multiple allosteric interactions conveyed by the GPCR heteromers, which includes ligand-dependent an independent allosteric interactions and interactions through a plasma membrane effector that complexes with the heteromer (labelled type I, II and III allosteric interactions, respectively; see Glossary) (Box 2).

**Answers to comments by reviewer #3:**

**1-Although the data available to date are not yet sufficient to fully support the authors' hypotheses, these are well described and scientifically relevant. For this reason I think the manuscript deserves to be published as "opinion" in TIPS. However, the authors should discuss how the hypothesis of a therapeutic intervention aimed at increasing the levels of KYNA can be reconciled with the numerous scientific evidences demonstrating the association between KYNA and the risk of psychosis, a relevant aspect for those who abuse, or have abused of cannabinoid consumption.**
We agree with the reviewer in downgrading the possible therapeutic use of KMO inhibitors. We have now added the following sentences and references in the text (page 8, last paragraph): "However, less predictive would be the therapeutic effect of increasing the brain extracellular levels of KYNA with KMO inhibitors. Thus, KYNA levels are increased in the brain of individuals with schizophrenia (ref. 26] and early exposure to THC in rats causes enduring cognitive deficits, which have been attributed to chronic increases in brain KYNA levels (ref. 27).

**2-Furthermore, in several parts of the article the author refer to "endocannabinoid", discussing results obtained by exogenous cannabinoids. This should be made clear.**
These were typos in a couple of instances "cannabinoids" was meant instead of "endocannabinoids".

Figure 1



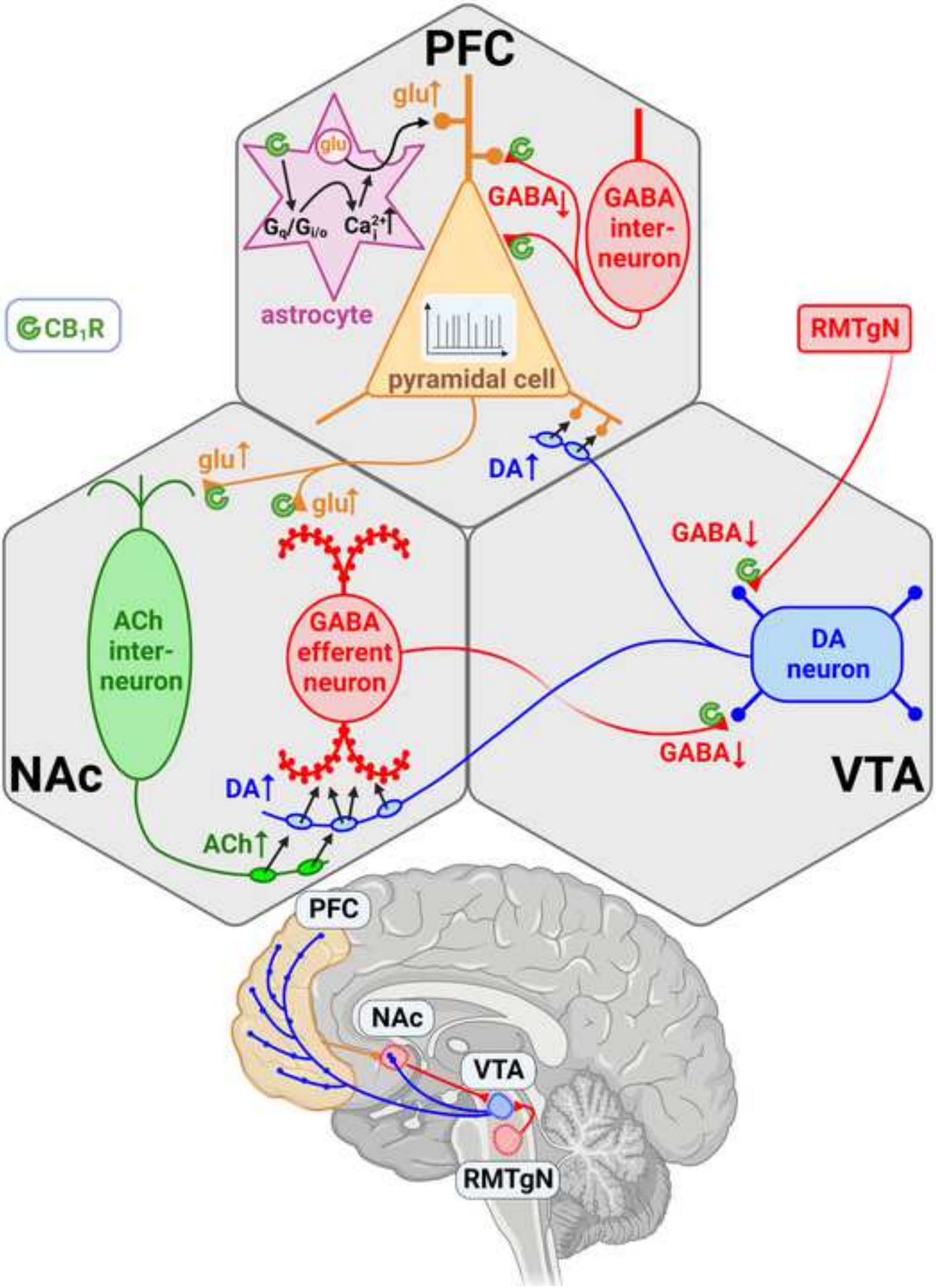

Figure 2