The vital role of constitutive GPCR activity in the mesolimbic dopamine system
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The midbrain dopamine system comprises a neural network critical in processing rewards and their cues. The importance of its functional integrity is strikingly underlined by some of the serious pathologies associated with its malfunction, such as drug abuse, obesity and depression. Several G protein-coupled receptors (GPCRs) have a pivotal role in the modulation of the activity of the midbrain dopamine system and its targets. Recent findings show that the in vivo activation mechanisms of these receptors go beyond agonist-dependent signaling. Indeed, agonist-independent constitutive GPCR activity (Figure 1) appears to have a vital role in native brain tissue, and interfering with its function can have deleterious effects. These findings have large repercussions for our understanding of GPCR control of neural networks and likely also for efficacious and safe drug design. The aim of the current review is to describe the role that the constitutive activity of several key GPCRs has in regulating the highly therapeutically relevant midbrain dopamine system.

**GPCR MODULATION OF THE MESOLIMBIC DOPAMINE SYSTEM**

Dopamine signaling patterns are largely dictated by the activity and firing mode of dopamine neurons in the ventral tegmental area (VTA), which project to several structures, including the nucleus accumbens (NAc), ventral pallidum (VP) and the prefrontal cortex (PFC). Especially the projection to the NAc has an important role in motivated appetitive behavior. Within the NAc, GABAergic medium spiny projection neurons (MSNs) are divided into those expressing the dopamine 1 receptor (D1R), which directly project back to the VTA (direct pathway), and those (expressing the dopamine 2 receptor (D2R)), which project back to the VP. Excitation of striatal D1R-MSNs is associated with reinforcing behavior, whereas activation of striatal D2R-MSNs has opposite effects. On top of the important role of these dopamine receptors, the activity of the VTA and its projection targets are modulated by several other GPCRs, some of which have been outlined in Figure 2. In this review we particularly focus on the role of three GPCRs in this neural circuit: the serotonin 2C receptor (HTR2C), the mu-opioid receptor (MOR) and the cannabinoid 1 receptor (CB1R). These are the GPCRs for which there is currently compelling evidence that their constitutive signaling contributes to their regulation of the VTA and its projection targets. In the following sections we elaborate on the functional role of the (constitutive) activation of these receptors.

Constitutively active serotonin 2C receptor: role in dopamine signaling and mood regulation

The Gq-coupled HTR2C serves an important function in mood regulation. HTR2C activation is anxiogenic (inducing anxiety), whereas interference with HTR2C signaling is anxiolytic (relieving anxiety). Part of the effects of HTR2C activity on emotional regulation may originate from its role in the mesocorticolimbic networks, which greatly contribute to emotional processing and depression-like behavior. Agonist-induced HTR2C activation counteracts dopamine signaling in the NAc. Instead, blockade of this receptor is associated with reduced effects of cocaine on locomotor activity and reduced motivation for cocaine seeking and drug relapse potential. Are any of these effects also affected by HTR2C constitutive activity?

In *in vitro* assays HTR2Cs exhibit constitutive activity for two downstream signaling cascades: the Gq-mediated phospholipase C (PLC) and the (G protein-independent) β-arrestin-dependent ERK1/2 pathways. Rather than this being restricted to such *in vitro* conditions, evidence has emerged that HTR2C constitutive activity regulates dopaminergic signaling in the NAC. Both HTR2C inverse agonists and neutral antagonists increase VTA dopamine neuron firing and dopamine release.
antagonist SB 242084 affects dopamine release to a lesser extent than the inverse agonist SB 206553. Importantly, the neutral antagonist was able to fully block the inverse agonistic effect of SB 206553 on striatal dopamine release. This pattern of effects suggests that both endogenous serotonin acting on HTR2Cs, as well as constitutive HTR2C activity, suppress striatal dopamine release.

There are indications that alterations in HTR2C constitutive activity are associated with psychiatric diseases like depression, an affliction often linked to a hypofunctional dopamine system. The evidence partly draws on the fact that the HTR2C is a seemingly unique GPCR for which mRNA editing occurs to produce different isoforms. These isoforms exhibit different functional properties, including (but not exclusively) different levels of constitutive activity. Mice expressing the unedited INI form of HTR2C (with high constitutive activity) exhibit depressive-like behavior in both the forced swim and tail-suspension tests. Contrarily, mice expressing the edited VGV form (with low constitutive activity) show the opposite phenotype in such tasks. Interestingly, alterations in HTR2C isoforms also occur in brain tissue of patients with depression and antidepressant treatment alters HTR2C mRNA editing. Moreover, antidepressants also directly affect HTR2C constitutive activity, with some acting as HTR2C neutral antagonists and others as inverse agonists. The tetracyclic antidepressants mianserin and mirtazapine are HTR2C inverse agonists for both the Gq-PLC-inositol phosphate and the ERK1/2 pathway. Instead, the selective serotonin reuptake inhibitor (SSRI) fluoxetine and the serotonin antagonist and reuptake inhibitor (SARI) trazodone are neutral antagonists for these HTR2C pathways. Mianserin and mirtazapine lead to large increases in dopamine signaling in the PFC (rather than the NAc), which has been postulated to underlie their antidepressant potential. Unfortunately, such antidepressant drugs have a broad array of effects on multiple receptors.
Mu-opioid receptors and addictive behavior

Opioid signaling in the VTA and NAc is triggered by intake of various drugs of abuse.\textsuperscript{27–29} In the VTA, MOR activation increases firing and burst frequency of dopamine neurons.\textsuperscript{30,31} This effect is mediated by suppression of GABAergic inhibition,\textsuperscript{32,33} but also requires the presence of a glutamatergic tone.\textsuperscript{33} Concomitant with an increase in dopamine levels in the NAc is the direct release of endogenous opioids in this area, which has a critical role in the motivational and hedonic properties of stimuli.\textsuperscript{1}

Antagonists for the MOR, such as naltrexone and naloxone, have been propagated to both help treat drug addiction and counteract opiate overdose. Interestingly, these compounds act as inverse agonists at the MOR, when the system has been pre-exposed to opiates.\textsuperscript{2} In such situations these compounds induce strong withdrawal symptoms,\textsuperscript{34} likely at least in part by causing hypoactivity of the midbrain dopamine system.\textsuperscript{35,36} There is now evidence to suggest that the drug withdrawal effects precipitated by MOR inverse agonists like naloxone, are at least partly due to suppression of constitutive MOR activity in the VTA and the (ventral) striatum (see below).

The relevance of constitutive MOR activity in drug withdrawal symptoms

Evidence for inverse agonistic effects at the MOR in physiological settings is compelling. Inverse MOR agonists reduce constitutive MOR recruitment of G proteins in brain homogenates of mice, but not in MOR knockout mice.\textsuperscript{36} Moreover, constitutively active MORs on GABAergic afferents to VTA dopamine neurons suppress GABAergic transmission in mouse brain slices. The latter was uncovered by the MOR inverse agonist KC-2-009, which itself strongly increased such GABAergic neurotransmission. That effect was not due to interference with endogenous opioid MOR-signaling, as the neutral MOR antagonist CTOP did not affect GABA transmission. CTOP importantly fully blocked the effect of KC-2-009, indicating that the inverse agonistic effect of the latter was MOR-dependent.\textsuperscript{32}

Beyond its presumed function in basal regulation regions like the VTA, constitutive MOR activity is modifiable. For instance, it increases after withdrawal from opiate (for example, morphine) treatment. This conclusion is derived from the observation that pretreatment with MOR agonists enhances the effect of MOR inverse agonists, but not of a neutral antagonist, on G protein signaling.\textsuperscript{38,39} Strikingly, pretreatment with morphine can fully reveal inverse agonistic function of certain MOR ligands such as naloxone and naltrexone. This is also true in regions like the midbrain and the striatum. There, morphine pretreatment reveals an inverse agonistic effect of naloxone, but not of neutral antagonist 6 β-naltrexol (a naltrexone metabolite), on G protein signaling and adenylyl cyclase activity.\textsuperscript{38,40} Withdrawal from chronic morphine treatment also enhances the effect of MOR inverse agonist KC-2-009, but not neutral antagonist CTOP, on GABAergic inputs to VTA dopamine neurons.\textsuperscript{32}

Such findings point towards an adaptive increase in MOR constitutive activity during opiate withdrawal. Potentially this is to partly compensate for the sudden lack of opiate-induced MOR stimulation that the system has grown accustomed to. Interference with constitutive MOR activity, in mesolimbic neural circuitry, may therefore especially have strong effects during states of opiate withdrawal. Indeed, withdrawal symptoms from morphine treatment, such as tremors, jumping and defecation, are precipitated more strongly by MOR inverse agonists than with an equipotent dose of MOR neutral antagonist 6 β-naltrexol. This is most likely due to constitutive MOR activity rather than any off-target effect, since 6 β-naltrexol attenuated the effects of an MOR inverse agonist on withdrawal symptoms.\textsuperscript{40} Chronic morphine intake, which would enhance constitutive MOR activity, also increases the conditioned place aversion induced by naloxone, but not that induced by MOR neutral antagonists.\textsuperscript{6} In view of these findings in animal models, MOR neutral antagonists may have the potential to provide fewer or less severe withdrawal symptoms than naloxone/naltrexone in humans. Relevant applications in this regard are treatment of opioid overdose and forms of drug addiction. However, it remains to be determined if neutral antagonists are indeed as efficacious as their inverse agonistic counterparts. There is tentative evidence that suggests that neutral antagonists are indeed capable of suppressing drug consumption,\textsuperscript{41} but additional studies are required.

Cannabinoid 1 receptors: role in food intake, obesity and negative emotions

The endocannabinoid system in the brain facilitates food intake, and its overactivity is implicated in the etiology of obesity.\textsuperscript{42,43} Key neural circuitry underlying these effects includes the hypothalamus and the limbic forebrain, where endocannabinoid levels rise during periods of hunger.\textsuperscript{44} In order to interfere with such endocannabinoid hunger signals, the CB1R inverse agonist rimonabant was marketed to treat obesity in humans. However, it (and similar compounds with it) was ultimately discontinued.

**Figure 2.** Schematic representation of the main connections of the midbrain dopamine neurons and their control by several key GPCRs. VTA dopamine neurons receive GABAergic inhibition from local GABA neurons, as well as GABA neurons from the rostromedial tegmental nucleus (RMTg). Medium spiny neurons in the NAc receive dopaminergic input from the VTA and project back either monosynaptically (direct pathway) or disynaptically through the ventral pallidum (VP; indirect pathway). The prefrontal cortex (PFC) provides an important glutamatergic (Glu) input to both the medium spiny neurons in the NAc and to neurons in the VTA, while also receiving dopaminergic input itself. Cannabinoid 1 (CB1), dopamine 1 (D1) and 2 (D2), serotonin 2C (HTR2C) and mu-opioid receptors (MORS) impinge on this network at various levels.
due to its association with feelings of anxiety and depression in a subgroup of subjects. A potential site of action for some of the therapeutic, and also the detrimental effects of CB1R inverse agonists, is the mesolimbic circuitry. Activity of dopamine neurons contributes to (food) reward-related behavior, and the hypo-functionality of these neurons has a crucial role in aversive behaviors. The constellation of effects observed with the CB1R inverse agonist rimonabant could potentially be parsed in effects that are solely due to interference with an endocannabinoid tone, and effects that are due to the additional suppression of constitutive CB1R activity. A prerequisite for this is that the CB1R constitutive activity that is described in heterologous expression systems, also occurs in vivo in relevant brain regions. It was recently shown that CB1R constitutive activity occurs in the VTA. In mouse brain slices, the CB1R inverse agonists rimonabant and AM251 increased GABAergic transmission onto VTA dopamine neurons: an effect not observed with two neutral CB1R antagonists O-2050 and NESS0327. In line with the inverse agonistic effect occurring due to suppression of CB1R constitutive activity, pretreatment with NESS0327 fully blocked any subsequent inverse agonistic effect of rimonabant. Similar evidence for CB1R constitutive activity was observed in the basolateral amygdala. The presence of CB1R constitutive activity in native tissue suggests that drugs like rimonabant can indeed have partially different effects than neutral CB1R antagonists. We now further discuss findings concerning this topic.

An important question is whether interference with endocannabinoid signaling at CB1Rs in the brain is sufficient to reduce food intake. If so, then CB1R neutral antagonists and inverse agonists should both be able to exert this therapeutic effect. In animal models, both neutral CB1R antagonists and inverse CB1R agonists lead to comparable reductions in food intake and body weight gain. Therefore, the suppression of constitutive CB1R activity (on top of the interference with endocannabinoid-CB1R signaling) does not appear to lead to an additional therapeutic effect (although there might be a component for CB1R constitutive activity in the periphery rather than in the brain).

With regard to side effects however, several reports of differences between CB1R inverse agonists and neutral antagonists exist in the domains of malaise, and anxiety- and depression-like behaviors: symptoms that were all reported by a subset of human subjects taking rimonabant. At higher doses, inverse CB1R agonists can cause a degree of illness, whereas neutral CB1 receptor antagonists appear to lack these side effects. Moreover, CB1R inverse agonists exert CB1R-dependent anxiogenic effects in mice, which may involve elevations of activity in amygdalar circuitry and hypofunctionality of the dopamine system. Interestingly, the neutral CB1R antagonists AM4113 and NESS0327 do not have these same effects on dopamine neurons and amygdalar circuitry, nor do they appear to raise anxiety. Finally, it is becoming clear that removal of CB1

**Figure 3.** In vivo relevance of CB1R constitutive activity. (a) Ventral tegmental area dopamine (DA) neurons are regulated by cannabinoid 1 receptor (CB1R)-expressing GABAergic neurons. Some of these CB1Rs (green; CB1R+) are constitutively active, whereas others are not (red, CB1R). (b) In mouse brain VTA dopamine neurons, CB1R agonist WIN55,212-2 reduces GABAergic input (miniature inhibitory postsynaptic currents; mIPSCs), whereas the inverse agonists SR141716A (rimonabant) and AM251 increase such GABAergic inhibition. This inverse agonistic effect is presumably by suppressing CB1R constitutive activity, because neutral CB1R antagonists O-2050 and NESS0327 do not affect GABAergic input themselves, whereas the latter does block the effect of both CB1R agonist WIN55,212-2 and SR141716A. Indeed, the effect of the inverse agonists in this slice preparation is not due to the interference with endocannabinoid-CB1R signaling, because indirect agonists URB597 and URB602, which act by preventing the degradation of the endocannabinoids anandamide and 2-Ag respectively, were ineffective in slice preparation. (c) Top) SR141716A reduces motivation for sucrose reward on a progressive ratio schedule. NESS0327 does not, but does block the effect of SR141716A. (Bottom) Similarly, SR141716A is anxiogenic in the elevated plus maze. NESS0327 is not, although it does block the effect of SR141716A. (d) SR141716A and NESS0327 reduce body weight gain to a similar extent. *P < 0.05; **P < 0.001.
Box 1. Outstanding questions

- What are the behavioral consequences of suppression of HTR2C constitutive activity versus interference with serotonergic signaling by neutral antagonists at this receptor?
- Do other prominent regulatory GPCRs in the mesolimbic dopamine system for which in vivo constitutive activity has been described (for example, dopamine, adenosine, ghrelin and melanocortin 4 receptors) exhibit in vivo constitutive activity?
- Does variation in in vivo constitutive activity of, for instance, CB1Rs and MORs contribute to dopamine-related pathologies?
- Are hemopressin and AgRP actual in vivo modulators of CB1R/MC4R constitutive activity beyond being antagonists, and do other such modulators exist for other GPCRs?
- To what extent is constitutive GPCR activity tissue- and/or cell-specific?
- Do constitutively active receptors necessarily engage in canonical signaling in vivo, or can they also engage in alternative signaling?
- Is it a general tendency in animal models that neutral antagonists for GPCRs acting in the midbrain dopamine system have fewer unwanted effects, and is this also true in humans?

signaling is associated with depression-like behavior in animal models. CB1R knockout animals, which obviously lack both agonist-dependent and constitutive CB1R signaling, are more susceptible to anhedonic effects of chronic mild stress.54 That effect may be particularly mediated by the lack of CB1Rs in dopamine receptive neurons.55 Furthermore, there is preclinical evidence that chronic use of the CB1R inverse agonist rimonabant induces a depression-like phenotype on a number of parameters, including more immobility in the forced swim test, reduction in prefrontal serotonin signaling and elevations in cytokine levels.56 Other hallmarks of depression are reductions in pleasure (anhedonia) and reductions in motivation and drive (avolition/ anergia).57 There are indications that CB1Rs ligands act on animal behaviors relevant to this. CB1R inverse agonists acutely decrease motivation for rewards and (after chronic use) reduce sucrose preference.48,56,58,59 It was recently found that the neutral CB1R antagonist NESS0327 does not reduce motivation for sucrose reward and could block such an effect of rimonabant.48 Interestingly, other studies showed that neutral CB1R antagonists do reduce operant responding for palatable food on schedules where the required effort to obtain a reward is relatively low.60,61 One study also suggested that, as effort costs increase, the potency of neutral antagonists to reduce motivated behavior may decrease.62 In an environment where (palatable) food is abundant and easily obtainable at low effort, this may be an appealing motivation for rewards with potential effects on depressive-like behavior.

Overall, these findings suggest that CB1R inverse agonists and neutral antagonists similarly reduce food intake and regulate body weight, while they differ in their propensity to affect anxiety and potentially depression-like behaviors. This preclinical evidence suggests that CB1R neutral antagonists have the potential to be efficacious and safer drugs (for instance for obesity treatment) than the therapeutically discarded CB1R inverse agonists (Figure 3).

Table 1. Constitutive GPCR activity in and beyond the reward system

| Receptor | Heterologous systems | Relevance in reward system/other systems |
|----------|----------------------|-----------------------------------------|
| Cannabinoid 1 (CB1R) | Gαi/0 binding62 | GABA on VTA dopamine neurons40 |
| | [35S]GTPγS66 | Motivation for rewards48 |
| | cAMP kinase activity63 | Lc-Fos in dorsal and ventral striatum7 |
| | GIRK flux64 | Constutitive activity in other systems |
| | VGCC flux65 | Anxiety7,48 |
| Mu-opioid (MOR) | [35S]GTPγS38,68 | GABA on VTA dopamine neurons48 |
| | cAMP68 | Lc-Fos in central amygdala neurons7 |
| | | Potentially [35S]GTPγS in several brain structures, although required inverse agonist concentrations were high.66 |
| | | Notably, hemopressin can act as an endogenous inverse agonist for the CB1R.67 |
| | | Constitutive activity in reward system |
| | | | [35S]GTPγS in striatum38 |
| | | | [35S]GTPγS in midbrain (after morphine treatment)46 |
| | | | GABA on VTA dopamine neurons48 |
| | | | Morphine withdrawal symptoms68,69 |
| | | Constitutive activity in other systems |
| | | | [35S]GTPγS in hippocampus and cortex (after morphine treatment)38 |
| | | | Nociception (in j2 arrestin−/− mice)70 |
| | | | Nociception after limb injury71 |
| | | | Spinal CAMP levels after limb injury71 |
| | | | Spinal intracellular Ca2+ levels after limb injury71 |
| | | Constitutive activity in reward system |
| | | Unknown |
| | | Constitutive activity in other systems |
| | | Unknown |
| Delta-opioid (DOR) | [35S]GTPγS72 | Constitutive activity in reward system |
| | | Unknown |
| | | Constitutive activity in other systems |
| | | | [35S]GTPγS in VTA dopamine neurons38 |
| | | | Nociception (in j2 arrestin−/− mice)70 |
| | | | Nociception after limb injury71 |
| | | | Spinal CAMP levels after limb injury71 |
| | | | Spinal intracellular Ca2+ levels after limb injury71 |
| Serotonin 2a (HTR2A) | PLC74 | Constitutive activity in reward system |
| | | Unknown |
| | | Constitutive activity in other systems |
| | | | [35S]GTPγS in VTA dopamine neurons38 |
| Serotonin 2c (HTR2C) | PLC (much more than for HTR2A)74 | Constitutive activity in reward system |
| | | [35S]GTPγS in VTA dopamine neurons38 |
| | | [35S]GTPγS in midbrain (after morphine treatment)38 |
| | | Nociception (in j2 arrestin−/− mice)70 |
| | | Nociception after limb injury71 |
| | | Spinal CAMP levels after limb injury71 |
| | | Spinal intracellular Ca2+ levels after limb injury71 |
| | | Constitutive activity in reward system |
| | | Unknown |
| | | Constitutive activity in other systems |
| | | | [35S]GTPγS in VTA dopamine neurons38 |
| | | | [35S]GTPγS in midbrain (after morphine treatment)38 |
| | | | Nociception (in j2 arrestin−/− mice)70 |
| | | | Nociception after limb injury71 |
| | | | Spinal CAMP levels after limb injury71 |
| | | | Spinal intracellular Ca2+ levels after limb injury71 |
| | | | Constitutive activity in reward system |
| | | Unknown |
| | | | Constitutive activity in other systems |
| | | | [35S]GTPγS in VTA dopamine neurons38 |
| | | | [35S]GTPγS in midbrain (after morphine treatment)38 |
| | | | Nociception (in j2 arrestin−/− mice)70 |
| | | | Nociception after limb injury71 |
| | | | Spinal CAMP levels after limb injury71 |
| | | | Spinal intracellular Ca2+ levels after limb injury71 |
| | | | Constitutive activity in reward system |
| | | Unknown |
| | | | Constitutive activity in other systems |
| | | | [35S]GTPγS in VTA dopamine neurons38 |
| | | | [35S]GTPγS in midbrain (after morphine treatment)38 |
| | | | Nociception (in j2 arrestin−/− mice)70 |
| | | | Nociception after limb injury71 |
| | | | Spinal CAMP levels after limb injury71 |
| | | | Spinal intracellular Ca2+ levels after limb injury71 |
the form of in vitro assays (for example, the dopamine receptors themselves), but sometimes there are also indications from other tissues or systems that GPCR may exhibit constitutive signaling (for example, the ghrelin receptor) (Table 1).

Current insights hint at the dynamism of constitutive GPCR activity. Instead of providing a fixed amount of background receptor activation, constitutive GPCR activity levels can be tuned by external factors, examples of which include cAMP-mediated MOR phosphorylation7 and mRNA editing of HTR2Cs.7 Moreover, constitutive (as well as agonist-dependent activation) can be modulated by endogenous inverse agonists, like the hypothalamic agouti-related peptide (AgRP) for the melanocortin 4 receptor, and the hemoglobin-derived hemopressin that acts as an inverse CB1R agonist in very similar ways as the synthetic inverse agonist rimonabant.67 Together these findings suggest that the extent and role of constitutive activity for GPCRs can be subject to modulation and can therefore be tissue-, region- and condition-specific.

The repercussions of the existence of constitutive GPCR activity are also evident on a behavioral level; in particular in relation to therapeutics for psychiatric disorders. If a GPCR population exhibits constitutive activity, it seems a necessary consequence that inverse agonists for such a GPCR will produce stronger (or more) effects than neutral antagonists. The question is whether this will prove to be beneficial (enhanced drug efficacy), detrimental (enhanced risk for side effects) or a combination of both. It is becoming clear that an imbalance in mesocorticlimbic dopamine signaling can have negative consequences, as it is associated with a variety of psychiatric disorders and aversive symptoms.4,11,34,35,37 Evidently, any pharmacotherapy aimed at remedying a dopamine-related disorder needs to take great care not to ‘overshoot’ its rebalancing objective. Preclinical evidence suggests that neutral antagonists for the CB1R and MOR produce fewer side effects than inverse agonists, while it appears that this does not necessarily happen at the cost of reduced efficacy in animal models. It stands to reason that as long as the primary aim of a treatment is to reduce signaling of an endogenous ligand at its GPCR in the midbrain dopamine system, neutral antagonists will be less prone to induce side effects compared to inverse agonists, and may be the primary drug of choice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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