Pharmacological Reports (2021) 73:971–983
https://doi.org/10.1007/s43440-021-00249-9

SPECIAL ISSUE: REVIEW

Integrative opioid-GABAergic neuronal mechanisms regulating dopamine efflux in the nucleus accumbens of freely moving animals

Tadashi Saigusa[1] · **Yuri Aono**[1] · **John L. Waddington**[2]

Received: 13 January 2021 / Revised: 26 February 2021 / Accepted: 11 March 2021 / Published online: 20 March 2021

Abstract

The nucleus accumbens (NAc) is a terminal region of mesocorticolimbic dopamine (DA) neuronal projections from the ventral tegmental area. Accumbal DA release is integrated by afferents from other brain regions and by interneurons, which involve a diversity of neurotransmitters and neuropeptides. These integrative processes, implicated in the pathobiology of neuropsychiatric disorders, are mediated via receptor subtypes whose relative roles in the regulation of accumbal DA release are poorly understood. Such complex interactions are exemplified by how selective activation of opioid receptor subtypes enhances accumbal DA efflux in a manner that is modulated by changes in neural activity through GABA receptor subtypes. This review delineates the roles of $GABA_A$ and $GABA_B$ receptors in GABAergic neural mechanisms in NAc that participate in delta- and mu-opioid receptor-mediated increases in accumbal DA efflux in freely moving rats, focusing on studies using in vivo brain microdialysis. First, we consider how endogenous GABA exerts inhibition of accumbal DA efflux through GABA receptor subtypes. We also consider possible intra-neuronal source of the endogenous GABA that inhibits accumbal DA efflux. As NAc contains GABAergic neurons that express delta- or mu-opioid receptors, inhibition of accumbal GABAergic neurons is a candidate for mediating delta- or mu-opioid receptor-mediated increases in accumbal DA efflux. Therefore, we provide a detailed analysis of the effects of GABA receptor subtype ligands on delta- and mu-opioid receptor-mediated accumbal DA efflux. Finally, we present an integrative model to explain the mechanisms of interaction among delta- and mu-opioid receptors, GABAergic neurons and DAergic neurons in NAc.

Keywords Dopamine release · GABA receptor subtypes · Opioid receptor subtypes · Nucleus accumbens · Neuronal interactions

Abbreviations

Allylglycine	L-allylglycine
DA	Dopamine
GAD	Glutamic acid decarboxylase
NAc	Nucleus accumbens
VTA	Ventral tegmental area

Introduction

The ventral striatum/nucleus accumbens (NAc) has been implicated in the regulation of multiple aspects of behaviour and in the pathobiology of neuropsychiatric disorders, and a critical element in these functions is its role as a terminal region for mesocorticolimbic dopamine (DA) neuronal projections from the ventral tegmental area (VTA: [1–3]). In the NAc, release of DA is integrated by afferents from distal and proximal brain regions and by interneurons, which involve a diversity of other neurotransmitters and neuropeptides [3, 4]. Furthermore, the integrative actions of essentially all of these neurotransmitters and neuropeptides are mediated via receptor subtypes whose differential roles in the regulation of DA release in NAc remain poorly understood.

These complex interactions in regulating accumbal DAergic neural activity are exemplified by (1) enhancement of accumbal DA efflux onto DA receptor subtypes following selective activation of opioid receptor subtypes [5–9], two

✉ Tadashi Saigusa
saigusa.tadashi@nihon-u.ac.jp

[1] Department of Pharmacology, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho-Nishi, Matsudo, Chiba 271-8587, Japan

[2] School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2, Ireland

families of G protein-coupled receptors, and (2) modulation of this effect by changes in neural activity through γ-aminobutyric acid (GABA) receptor subtypes [10–13]. This integration of neuronal effects prompts a number of challenging questions as follows: (a) To what extent do these effects of opioid drugs involve delta- and/or mu-opioid receptors? (b) To what extent do such effects involve higher-level subtypes such as delta1-, delta2- and mu1-opioid receptors? (c) To what extent do the modulating actions of endogenous GABA on these opioid receptor-mediated effects on accumbal DA efflux involve $GABA_A$ vs. $GABA_B$ receptors? (d) To what extent can we illuminate these questions and capture answers in a parsimonious neuronal network model?

For more than a decade, we have conducted microdialysis studies on accumbal DA efflux involving meticulous use of selective agonists (both exogenous and endogenous) and antagonists at opioid and GABA receptor subtypes in an effort to illuminate such complexities. Here, we review these findings and integrate them with the findings of other investigators to derive a parsimonious neuronal network model in a manner that can also be used as a template for other neuronal systems. This review describes the GABAergic neural mechanisms in NAc that are involved in delta- and mu-opioid receptor-mediated increases in accumbal DA efflux. In particular, we focus on findings from neuropharmacological studies that use in vivo microdialysis techniques. These methods allow local application of GABAergic and opioid drugs into NAc through a semipermeable microdialysis membrane by reverse dialysis and simultaneously analyse changes in accumbal DA levels. Accordingly, we are able to investigate the involvement of GABAergic and opioid neural activity in the regulation of accumbal DA efflux in freely moving rats.

First, we will briefly summarise the characteristics of DAergic and GABAergic neurons in NAc and then consider how endogenous GABA exerts inhibitory roles on accumbal DA efflux through GABA receptor subtypes. Next, we will discuss possible intra-neuronal sources of the endogenous GABA that inhibits such accumbal DA efflux. Furthermore, NAc is known to contain GABAergic neurons that incorporate delta- and mu-opioid receptors [14, 15]. Agonists at delta- and mu-opioid receptors have been shown to enhance accumbal DA efflux through two mechanisms: one involving opioid receptors that are sensitive to the non-selective opioid receptor antagonist naloxone; the other involving opioid receptors that are insensitive to naloxone (for review, see [9]). Thus, inhibition of GABAergic neurons in NAc could be involved in delta- or mu-opioid receptor-mediated increases in accumbal DA efflux. Therefore, we will overview the effects of GABA receptor subtype-selective ligands on delta- and mu-opioid receptor-mediated accumbal DA efflux to enhance understanding of the mechanisms of functional interactions among delta- or mu-opioid receptors and GABAergic and DAergic neurons in NAc.

Does endogenous GABA exert inhibition of accumbal DA efflux through $GABA_A$ and/or $GABA_B$ receptors?

The NAc and GABAergic systems

The NAc is a terminal area of mesolimbic DAergic neurons originating from the VTA that also receives non-DAergic, non-GABAergic excitatory projections from limbic nuclei such as the amygdala [16], hippocampus [17] and prefrontal cortex [18]. In addition, NAc sends GABAergic efferents to the ventral pallidum as well as to the VTA [19–21]. There are two types of GABA-containing striatal cells, called spiny and aspiny GABAergic nerve cells, on which GABA receptors are located [22]. It is suggested that the former cells are accumbal output neurons [23] and that the latter cells are interneurons [24, 25].

GABA receptors consist of two major subtypes, namely $GABA_A$ and $GABA_B$: the $GABA_A$ receptor is an inhibitory ionotropic receptor that consists of five subunits arranged around a ligand-gated Cl^- channel, which is opened to mediate inhibition via activation of the GABA binding sites; the $GABA_B$ receptor belongs to the metabotropic G-protein coupled receptor superfamily that consists of seven transmembrane domains and mediates slow and prolonged inhibitory action via activation of $G_{i/o}$-type proteins, inhibiting adenylate cyclase, activating inwardly rectifying postsynaptic K^+ channels and inactivating presynaptic voltage-gated Ca^{2+} channels.

$GABA_A$ and $GABA_B$ receptors are both distributed in NAc [22]. While both spiny and aspiny nerve cells in NAc contain $GABA_A$ receptors, immunoreactivity for its subunits is heterogeneously distributed [22]. $GABA_B$ receptors are reported to play a role as both autoreceptors and hetero-presynaptic receptors in NAc [26] or as hetero-presynaptic receptors in other brain regions [27–29], and $GABA_B$ receptors are not localised on axonal terminals that contain glutamic acid decarboxylase (GAD), the key enzyme for generating GABA from glutamate [30].

Both $GABA_A$ and $GABA_B$ receptors exert inhibitory roles in the regulation of basal accumbal DA efflux

$GABA_A$ receptors and basal accumbal DA efflux

The GABAergic system in VTA is known to regulate accumbal DAergic activity [31]. For example, neurochemical studies have shown that $GABA_A$ receptors within VTA exert an inhibitory role on accumbal DA release.

Intra-tegmental infusion of a $GABA_A$ receptor agonist decreased [32] and of a $GABA_A$ receptor antagonist increased [33] extracellular levels of DA in NAc of freely moving rats, respectively. Behavioural studies have also indicated that accumbal $GABA_A$ receptors can exert an inhibitory role on accumbal DAergic activity. For example, the non-competitive $GABA_A$ receptor antagonist picrotoxin enhanced [34, 35] and $GABA_A$ receptor agonists reduced [34, 36] hyperlocomotion induced by DA receptor agonists, respectively.

In vivo microdialysis studies have also revealed that $GABA_A$ receptors within NAc exert inhibitory control on accumbal DA efflux. The $GABA_A$ receptor antagonist bicuculline has been found to increase accumbal DA efflux in freely moving rats when infused into VTA [33] or directly infused into NAc ([10, 37, 38]; Table 1). Intra-accumbal infusion of the $GABA_A$ receptor agonist muscimol decreased accumbal DA efflux in halothane-anaesthetised rats [39]. However, it is notable that muscimol either failed to alter [38] or increased [40] accumbal DA efflux in freely moving rats when directly infused into NAc through the microdialysis probe. In fact, in agreement with these reports [38, 40], and in contrast to neurochemical evidence that local infusion of a $GABA_A$ receptor agonist into VTA reduced accumbal DA efflux [32], we have found that basal accumbal DA efflux was increased by intra-accumbal infusion of a high dose, but not a low dose, of muscimol ([10]; Table 1). The stimulatory effects of muscimol on DA efflux that we observed were indeed mediated by accumbal $GABA_A$ receptors, because intra-accumbal infusion of a low dose of bicuculline, which failed to alter basal DA efflux, significantly inhibited the increase of accumbal DA efflux induced by muscimol [10].

There is both behavioural and electrophysiological evidence that GABAergic interneurons exert inhibitory control on mesolimbic DAergic neurons via $GABA_A$ receptors in NAc. That bicuculline increased accumbal DA efflux is in accordance with this notion; thus, inhibition of $GABA_A$ receptors on the terminals of mesolimbic DAergic neurons would remove inhibitory control of these neurons and, accordingly, increase release of DA in their terminal areas, i.e. NAc. Specifically, we proposed that intra-accumbally applied bicuculline acted directly at $GABA_A$ receptors on the terminals of mesolimbic DAergic neurons and, accordingly, removed inhibitory GABAergic control of these neurons [10]. However, because muscimol also increased accumbal DA efflux, it is evident that this drug did not stimulate $GABA_A$ receptors on the terminals of mesolimbic DAergic neurons. A likely candidate may be $GABA_A$ receptors located on the cells and/or terminals of GABAergic interneurons that impinge upon the terminals of mesolimbic DAergic neurons. Thus, we proposed that intra-accumbally applied muscimol acted at $GABA_A$ receptors on GABAergic interneurons to inhibit their inhibitory control of DAergic neurons and, accordingly, disinhibited these neurons [10].

$GABA_B$ receptors and basal accumbal DA efflux

Activation of accumbal $GABA_B$ receptors has been found to induce suppression of locomotor activity [35] and blockade of accumbal $GABA_B$ receptors has been found to increase DA levels in NAc [37]. Because enhancement of locomotor activity can be elicited by activation of accumbal DA

Table 1 Summary of effects of $GABA_A$ and $GABA_B$ receptor ligands on endogenous and opioidergic facets of accumbal dopamine efflux

Accumbal DA efflux	$GABA_A$-R		$GABA_B$-R	
	Agonist	Antagonist	Agonist	Antagonist
	Muscimol	Bicuculline	Baclofen	Saclofen
(a) Baseline	Enhanced [10]	Enhanced	Failed to affect [12]	Enhanced
(b) Allylglycine-induced increase	Failed to affect [54]	Failed to affect	Inhibited [54]	Failed to affect
(c) δ_1-R-mediated increase	Failed to affect [11]	Failed to affect	Inhibited [13]	Failed to affect
(d) δ_2-R-mediated increase	Inhibited [11]	Failed to affect	Inhibited [13]	Failed to affect
(e) μ_1-R-mediated increase	Enhanced [10]	Failed to affect	Failed to affect [12]	Enhanced

Effects of $GABA_A$ and $GABA_B$ receptor (R) ligands on each of (a) baseline, (b) allylglycine-induced increase, (c) δ_1-R-mediated increase, (d) δ_2-R-mediated increase and (e) μ_1-R-mediated increase in accumbal dopamine (DA) efflux of freely moving rats. Low doses of GABA-R ligands that failed to alter baseline accumbal dopamine levels were chosen for co-administration experiments with allylglycine, δ_1-, δ_2-, and μ_1-R agonists (b–e). Numbers indicate references

receptors [41], these findings suggest that accumbal $GABA_B$ receptors exert inhibitory control of accumbal DAergic activity. Indeed, studies on the role of $GABA_B$ receptors in modulating cocaine-induced increase in accumbal DA have provided additional evidence for such an effect [42].

In line with these reports, we have found that higher doses of the $GABA_B$ receptor antagonist 2-hydroxysaclofen increase basal DA levels in accumbal dialysates in a dose-dependent manner ([12]; Table 1). Nevertheless, systemic administration of a $GABA_B$ receptor agonist did not alter basal accumbal DA levels [43] and, more specifically, intra-accumbal infusion of the $GABA_B$ receptor agonist baclofen failed to affect basal accumbal DA efflux [12]. Given the effectiveness of the antagonist and given the presence of functionally active $GABA_B$ receptors in NAc (see "The NAc and GABAergic systems"), this lack of any effect of the agonist may be due to high tonic GABAergic activity (ceiling effect). Nevertheless, these effects of $GABA_B$ receptor antagonists are in accordance with earlier findings suggesting that $GABA_B$ receptors are located presynaptically on DAergic nerve terminals in NAc and exert inhibitory control of accumbal DA efflux. As mentioned above (see "The NAc and GABAergic systems"), because $GABA_B$ receptors are reported to act as autoreceptors as well as hetero-presynaptic receptors in NAc [26] and other brain areas [27–29], $GABA_B$ receptors should not be distributed on GAD-positive GABAergic neurons in the rat brain [30]. On this basis, $GABA_B$ receptors could be localised on accumbal DAergic nerve endings, but not on GABAergic interneurons.

We have provided neurochemical evidence that accumbal GABA plays an inhibitory role in the regulation of accumbal DA efflux through $GABA_B$ receptors as well as $GABA_A$ receptors located on DAergic nerve endings in NAc. Specifically, we found that intra-accumbal infusion of either the $GABA_B$ receptor antagonist saclofen or the $GABA_A$ receptor antagonist bicuculline increases accumbal DA efflux of freely moving rats in a receptor-specific manner ([10, 12]; Table 1).

High tonic GABAergic activity through both GABA receptor subtypes located on DAergic nerve endings in NAc

That intra-accumbal infusions of $GABA_A$ or $GABA_B$ receptor antagonists each enhance accumbal baseline DA efflux, while intra-accumbal infusions of either $GABA_A$ or $GABA_B$ receptor agonists do not decrease accumbal baseline DA efflux of freely moving rats (Table 1), requires explanation. On the basis of these and related data, the most parsimonious explanation is that GABAergic tonus is high at the level of both $GABA_A$ receptors and $GABA_B$ receptors located on DAergic nerve endings in NAc. Thus, antagonists are able to reduce these high levels of tonic activity, giving release from inhibition; in contrast, agonists are unable to further increase receptor activation beyond these already high tonic levels (ceiling effect) and are hence unable to exert further inhibition (floor effect). Therefore, below we consider possible intracellular sources of endogenous GABA in NAc that subserves GABAergic tonus at the level of these GABA receptor subtypes located on accumbal DAergic nerve endings.

What are the sources of endogenous GABA that inhibit DA efflux in NAc?

GAD and its two isoforms: differences in location and function in accumbal cells

GAD is the main enzyme that synthesises GABA from glutamate and is the key enzyme for generating GABA for signal transduction between neurons. There are at least two distinct molecular isoforms of GAD, namely GAD65 and GAD67, where 65 and 67 refer to their molecular weights in kDa, respectively [44]. Evidence indicates that GABA generated by GAD65, but not by GAD67, is utilised for neurotransmission (for reviews see [45–47]) and GABA synthesised via GAD65 is known to exert tonic inhibition [48]. In NAc, GAD is present in (a) interneurons [49–51], (b) glia cells [52], (c) tyrosine hydroxylase-positive neurons that arise in the substantia nigra pars compacta/VTA and terminate in NAc [53] and (d) output neurons to the ventral pallidum and substantia nigra pars reticulata/VTA [19, 23].

Possible sources of extracellular GABA in NAc: insights from microdialysis experiments

While rat accumbal extracellular fluid collected by in vivo microdialysis contains appreciable amounts of GABA, these include moieties of GABA that could be released independent of neural firing. Accordingly, accumbal extracellular GABA levels are not fully sensitive to intra-accumbal infusion of the voltage-dependent Na^+ channel blocker tetrodotoxin, which inhibits neuronal release of transmitters [54]. The following sources may contribute additionally to extracellular GABA: (a) non-vesicular, cytosolic and action potential-independent leakage mechanisms [55–57]; (b) reversal of GABA transporters [58]; and (c) release from astrocytes [59, 60]. Further studies are necessary to clarify the extent to which GABA acting at $GABA_A$ receptors and GABA acting at $GABA_B$ receptors have a similar or dissimilar origin.

Allylglycine-induced accumbal DA efflux and GABA receptor subtypes

GAD in GABAergic nerve endings regulates presynaptic GABAergic tonus at GABA receptor subtypes located on DAergic nerve endings

Though GABA in the cytosol of GABAergic nerve endings has been implicated in non-neuronal processes (for reviews see [46, 47]), cytosolic GABA is mainly used in neural transmission, i.e. extracellular GABA that is released by a vesicular-dependent mechanism in GABAergic nerve terminals is synthesised by GAD in the cytosol of GABAergic neurons. The vesicular GABA transporter located on synaptic vesicles of GABA in neurons of the rat brain [61] transports cytosolic GABA into synaptic vesicles that can release GABA from GABAergic nerve terminals [45, 62, 63]. Since variation in amount of newly synthesised GABA in the cytosol can affect vesicular GABA levels [64] and, accordingly, the amount of GABA that can be released from the neuron, GAD in GABAergic nerve endings at least partly contributes to the strength of presynaptic GABAergic tonus mediated by GABA receptor subtypes on accumbal DAergic nerve endings. These assumptions were examined by in vivo microdialysis studies using the GAD inhibitor L-allylglycine (allylglycine) in which the effects of intra-accumbal infusion of allylglycine on DA levels in accumbal dialysates of freely moving rats were analysed.

The intra-accumbal infusion of allylglycine dose-dependently increased DA levels in dialysates from NAc ([54]; Table 1). Since these effects of allylglycine were almost fully suppressed by intra-accumbal infusion of tetrodotoxin, it is concluded that these effects are substantially dependent on neuronal activity [54].

Activation of accumbal $GABA_B$ receptors, but not $GABA_A$ receptors, inhibits allylglycine-induced increase in accumbal DA efflux

Intra-accumbal infusion of the $GABA_B$ receptor agonist baclofen, at a dose that did not alter basal accumbal DA efflux when administered alone, inhibited the allylglycine-induced increase in accumbal DA efflux ([54]; Table 1). This effect of baclofen appeared to be mediated by $GABA_B$ receptors because co-administration of the $GABA_B$ receptor antagonist saclofen, at a dose that did not alter either basal or allylglycine-induced accumbal DA efflux when given alone, was able to counteract the effect of baclofen to inhibit allylglycine-induced accumbal DA efflux ([54]; Table 1). The NAc contains GABA interneurons and GABAergic terminals of afferent neurons expressing an intense immunoreactivity to GAD [49–51]. Furthermore, NAc [26], like other brain regions [27–29, 65, 66], contains $GABA_B$ autoreceptors and $GABA_B$ hetero-presynaptic receptors, and $GABA_B$ receptors are not localised on GAD67-positive axonal terminals in the rat brain [30]. Therefore, it appears that the $GABA_B$ receptors under discussion are hetero-presynaptic receptors on DAergic terminals innervated by accumbal aspiny, GAD65-positive GABAergic interneurons. That inhibition of GAD dose-dependently increased accumbal DA efflux via $GABA_B$ receptors suggests that GABAergic tonus in the synapses involved is particularly high, in accordance with our previous report [12].

Behavioural [34–36], neurochemical [37–39] and immunohistochemical [22] studies have revealed that NAc contains functional $GABA_A$ receptors. However, $GABA_A$ receptors that control accumbal DA efflux are not activated by GABA that is synthesised in NAc by GAD. This is because neither the $GABA_A$ receptor agonist muscimol nor the $GABA_A$ receptor antagonist bicuculline altered the effects of either a low dose or a high dose of allylglycine on accumbal DA efflux ([54]; Table 1); a study on the effects of a low dose of muscimol was incorporated because of evidence that the high dose used disinhibits accumbal GABAergic interneurons that can increase accumbal DA efflux [11]. These data suggest that there is an allylglycine-insensitive GABA pool that could release GABA to exert inhibitory regulation on accumbal DAergic activity at the level of $GABA_A$ receptors.

Where does this allylglycine-insensitive pool of GABA originate? One possible candidate source is intra-vesicular GABA that has already been synthesised outside of NAc. Given the four different origins of accumbal GAD (see "GAD and its two isoforms: differences in location and function in accumbal cells"), the most likely candidate in this respect is tyrosine hydroxylase-positive neurons that arise in the substantia nigra and/or VTA and terminate in NAc. The GAD expressed in tyrosine hydroxylase-positive neurons may synthesise GABA for co-transmission with DA. Extracellular release of GABA from these neuronal terminals could reduce DA release and subsequently prevent over-activation of DAergic neural activity in NAc [53]. Other possibilities are action potential-independent leakage mechanisms [55–57] and/or reversal of GABA transporters [58]. Further studies are required to clarify the actual source of this allylglycine-insensitive pool of GABA. The notion that allylglycine treatment induces a biphasic increase in accumbal DA efflux [54] is in accordance with the possible existence of two GABA pools [45, 62, 63].

Microdialysis studies with the GABA synthesis inhibitor allylglycine provide in vivo neurochemical evidence that in NAc newly synthesised GABA exerts an inhibitory tonus on accumbal DAergic activity at the level of $GABA_B$ receptors but not of $GABA_A$ receptors. These studies also indicate that there is an allylglycine-insensitive GABA pool that can release GABA to exert inhibitory control of accumbal DAergic neural activity at the level of $GABA_A$ receptors.

These results indicate that inhibition of GAD has direct consequences for the effects of stimulation or inhibition of $GABA_B$ receptors, but not $GABA_A$ receptors, on accumbal DA efflux.

Accumbal GABAergic neurons are known to express opioid receptors, namely delta- and mu-opioid receptor subtypes. Therefore, stimulation of these receptors could reduce GABAergic inhibitory regulation of accumbal DAergic nerve endings and thus subsequently enhance release of DA from DAergic nerve terminals. In the next section, we discuss the roles of GABA receptor subtypes in delta- and mu-opioid receptor-mediated increases in accumbal DA efflux.

Table 2 Representative ligands to analyse the roles of delta- and mu1-opioid receptors in regulation of accumbal dopamine efflux

δ_1-R		δ_2-R		μ_1-R	
Agonist DPDPE	Antagonist BNTX	Agonist Deltorphin-II	Antagonist Naltriben	Agonist Endomorphin-1	Antagonist Naloxonazin
[9, 11, 13, 71]		[9, 11, 13, 71]		[8–10, 12]	

Numbers indicate references

What are the roles of GABA receptor subtypes in delta- and mu-opioid receptor-mediated DA efflux in NAc?

GABAergic neurons and delta- and mu-opioid receptor subtypes in VTA and NAc

Highly potent analgesics in clinical use are opioid receptor agonists and kappa-, delta- and mu-opioid receptor subtypes mediate these analgesic effects (for review see [67]). These opioid receptor subtypes belong to the G-protein coupled receptor superfamily. Kappa-, delta- and mu-opioid receptors each consist of seven transmembrane domains and are coupled with $G_{i/o}$ proteins to inhibit adenylate cyclase, open inwardly rectifying K^+ channels, and inhibit voltage-gated Ca^{2+} channels. In general, these receptor subtypes are considered to mediate reduction in excitability and firing of neurons.

In particular, delta- and mu-opioid receptor agonists are known to enhance DAergic neural activity in NAc through delta- and mu-opioid receptor activation in VTA (for reviews see [68, 69]). Disinhibition of mesolimbic DAergic neurons due to inhibition of inhibitory inputs to DAergic neurons from local GABAergic neurons, which express delta- and mu-opioid receptors, appears to be involved in this increase in accumbal DAergic neural activity (for review see [69]). The NAc also contains delta- and mu-opioid receptors. In fact, when infused directly into NAc, delta- and mu-opioid receptor agonists increase accumbal extracellular DA levels in freely moving rats [70–72]. Therefore, the roles of delta- and mu-opioid receptors in regulating accumbal DAergic neural activity have been analysed using delta- and mu-opioid receptor ligands (Table 2).

Delta1- and delta2-opioid receptor-mediated accumbal DA efflux and GABA receptor subtypes

The NAc contains delta-opioid receptors [14, 73, 74] that are encoded by a single gene [75] and exist as at least two types of pharmacologically distinct subtypes, namely delta1- and delta2-opioid receptors [76]. We have shown that intra-accumbal infusions of a delta1- and a delta2-opioid receptor agonist each enhance accumbal DA efflux [11, 13, 71]. Based on the expression of delta-opioid receptors on accumbal inhibitory neurons, many of which contain GABA, it has been suggested that delta-opioid receptors reduce accumbal inhibitory neurotransmission [14]. Therefore, decrease in activation of accumbal $GABA_A$ and $GABA_B$ receptors that inhibit DA efflux in NAc may mediate delta1- and delta2-opioid receptor agonist-induced increases in accumbal DA efflux. In order to obtain neuropharmacological evidence to support these propositions, our group first analysed the effects of the $GABA_B$ receptor agonist baclofen, which inhibited allylglycine-induced DA efflux in NAc, on delta1- and delta2-opioid receptor-mediated increases in accumbal DA efflux.

Delta1- and delta2-opioid receptor-mediated accumbal DA efflux are inhibited by activation of accumbal $GABA_B$ receptors

As shown in "Activation of accumbal $GABA_B$ receptors, but not $GABA_A$ receptors, inhibits allylglycine-induced increase in accumbal DA efflux", intra-accumbal infusion of baclofen supressed the increase in accumbal DA efflux induced by allylglycine (Table 1). Similar infusion of baclofen also supressed both accumbal delta1- and delta2-opioid receptor-mediated increases in accumbal DA efflux ([13]; Table 1). Since these effects of baclofen were counteracted by co-administration of the $GABA_B$ receptor antagonist saclofen, it was evident that stimulation of accumbal $GABA_B$ receptors could inhibit both delta1- and delta2-opioid receptor-mediated increases in accumbal DA efflux. As a corollary, reduction in accumbal $GABA_B$ receptor-mediated inhibition of accumbal DAergic activity would be necessary to induce delta1-opioid receptor- and delta2-opioid receptor-mediated increases in accumbal DA efflux [13].

Delta2-, but not delta1-, opioid receptor-mediated accumbal DA efflux is inhibited by activation of accumbal $GABA_A$ receptors

As discussed in "Activation of accumbal $GABA_B$ receptors, but not $GABA_A$ receptors, inhibits allylglycine-induced increase in accumbal DA efflux", intra-accumbal infusion of the $GABA_A$ receptor agonist muscimol did not affect the increase in accumbal DA efflux that followed inhibition of accumbal endogenous GABA synthesis (Table 1). Notably, however, intra-accumbal infusion of muscimol did supress delta2-, but not delta1-, opioid receptor-mediated increases in accumbal DA efflux. As this effect of muscimol was counteracted by co-administration of the $GABA_A$ receptor antagonist bicuculline, stimulation of accumbal $GABA_A$ receptors inhibits delta2-opioid receptor-mediated increases in accumbal DA efflux. As a corollary, reduction in accumbal $GABA_A$ receptor-mediated inhibition of accumbal DAergic activity would be necessary to induce delta2-, but not delta1-, opioid receptor-mediated increases in accumbal DA efflux [11].

How do GABAergic and DAergic neurons interact in mu- and delta-opioid receptor subtype-mediated processes in NAc?

As discussed in "GABAergic neurons and delta- and mu-opioid receptor subtypes in VTA and NAc", NAc expresses mu-opioid receptors [15] and selective stimulation of accumbal mu-opioid receptors has been shown to enhance accumbal DA efflux [70–72]. It is known that mu-opioid receptors are further subdivided into mu1- and mu2-opioid receptors based on their differential sensitivity to the selective mu1-opioid receptor antagonist naloxanazine [76, 77]. Evidence indicates that mu1-, but not mu2-, opioid receptors in NAc mediate such increases in accumbal DA efflux, because naloxanazine readily inhibited the increase in accumbal DA efflux induced by local administration of the endogenous opioid endomorphin-1 [8]. As mentioned above, the $GABA_B$ receptor agonist baclofen, which inhibited allylglycine-induced increases in accumbal DA efflux, was able to suppress both delta1- and delta2-opioid receptor-mediated increases in DA efflux in NAc of freely moving rats (Table 1). These inhibitory effects of baclofen on accumbal DA efflux were counteracted by co-administration of the $GABA_B$ receptor antagonist saclofen. As was assumed from allylglycine-induced increases in accumbal DA efflux, these findings clearly suggest that not only delta1- but also delta2-opioid receptor stimulation increases accumbal DA efflux by decreasing the availability of endogenous GABA for stimulating accumbal $GABA_B$ receptors to inhibit DA release. If accumbal mu1-opioid receptor stimulation increases DA efflux through the same mechanisms as delta1- and delta2-opioid receptor-mediated accumbal DA efflux, then endomorphin-1-induced accumbal DA efflux should be readily inhibited by co-administration of baclofen.

In contrast to these expectations, endomorphin-1-induced accumbal DA efflux was not inhibited by the $GABA_B$ receptor agonist baclofen; rather, it was enhanced by the $GABA_B$ receptor antagonist saclofen and this effect of saclofen was counteracted by baclofen (Table 1). Accordingly, it is suggested that blockade of accumbal $GABA_B$ receptors enhances mu1-opioid receptor-mediated accumbal DA efflux. As differences were observed in the effects of GABA receptor ligands on increases in accumbal DA efflux induced by allylglycine, the delta-opioid receptor agonists DPDPE and deltorphin-2, and endomorphin-1 [10–13, 54], it is possible that GAD inhibition, delta1-, delta2- and mu1-opioid receptor stimulation in NAc enhance DA efflux through different GABAergic neural mechanisms (Table 1).

As described previously ("Activation of accumbal $GABA_B$ receptors, but not $GABA_A$ receptors, inhibits allylglycine-induced increase in accumbal DA efflux" and "Delta2-, but not delta1-, opioid receptor-mediated accumbal DA efflux is inhibited by activation of accumbal $GABA_A$ receptors"), the $GABA_A$ receptor agonist muscimol did not alter allylglycine-induced and delta1-opioid receptor-mediated accumbal DA efflux, but did suppress delta2-opioid receptor-mediated accumbal DA efflux (Table 1). As these effects of muscimol were counteracted by the $GABA_A$ receptor antagonist bicuculline, these results clearly suggest that decreases in the availability of endogenous GABA for stimulating $GABA_A$ receptors could be at least partly responsible for the production of delta2-opoid receptor-mediated increases in accumbal DA efflux. If mu1-opioid receptor-mediated accumbal DA efflux was induced through similar accumbal GABAergic mechanisms, muscimol should supress the increase in accumbal DA efflux induced by endmorphin-1. However, muscimol enhanced the increase in accumbal DA efflux induced by endmorphin-1 ([10]; Table 1). As these effects of muscimol were counteracted by bicuculline, stimulation of accumbal $GABA_A$ receptors appears to enhance mu1-opioid receptor-mediated accumbal DA efflux. That the $GABA_A$ receptor agonist did not influence GAD inhibitor- and delta1-opioid receptor agonist-induced accumbal DA efflux, but inhibited and enhanced delta2- and mu1-opioid receptor agonist-induced DA efflux, respectively, clearly indicates heterogeneity in the mechanisms of GABA-opioid interaction that regulate accumbal DA efflux (Table 1).

In the next section, we consider the differential involvement of $GABA_A$ and $GABA_B$ receptor subtypes in how delta1-, delta2- and mu1-opioid receptors mediate increases in DA efflux in NAc.

Heterogeneous GABAergic mechanisms in delta-opioid receptor subtype- and mu1-opioid receptor-mediated accumbal DA efflux

In NAc, GABAergic neurons express delta-opioid receptor subtypes and mu1-opioid receptors that are known to inhibit neural firing. Accordingly, synaptic interactions between GABA and DA neurons via $GABA_A$ and/or $GABA_B$ receptors should explain at least in part the mechanisms by which delta- and mu-opioid receptor subtypes influence accumbal DA efflux.

Selective stimulation of delta- or mu-opioid receptor subtypes each enhance accumbal DA efflux and these increases in DA efflux are similarly or differently influenced by co-administration of $GABA_B$ or $GABA_A$ receptor ligands (Table 1). Within NAc these differences in the effects of GABA receptor ligands on DA efflux indicate heterogeneity in (1) the expression of delta- or mu-opioid receptor subtypes on GABA neurons that interact with accumbal DA neurons and/or (2) the expression of GABA receptor subtypes on GABA neurons that exert inhibition of DA neurons.

For two reasons, delta- and mu-opioid receptors do not appear to be co-localised on GABAergic neurons that exert inhibitory control on accumbal DAergic nerve terminals. Firstly, in contrast to $GABA_B$ receptor agonist-mediated inhibition of both delta1- and delta2-opioid receptor-mediated accumbal DA efflux, $GABA_B$ receptor stimulation did not alter mu1-opioid receptor-mediated accumbal DA efflux (Table 1); furthermore, antagonism of $GABA_B$ receptors, which failed to alter either delta1- or delta2-opioid receptor-mediated accumbal DA efflux, enhanced mu1-opioid receptor-mediated accumbal DA efflux (Table 1). Second, stimulation of $GABA_A$ receptors, which inhibited delta2- but not delta1-opioid receptor-mediated accumbal DA efflux, enhanced mu1-opioid receptor-mediated accumbal DA efflux (Table 1).

Synthesis

The challenge now is to identify a neuronal network and associated processes that can integrate and explain the breadth and depth of findings reviewed in "Does endogenous GABA exert inhibition of accumbal DA efflux through $GABA_A$ and/or $GABA_B$ receptors?" to "Heterogeneous GABAergic mechanisms in delta-opioid receptor subtype- and mu1-opioid receptor-mediated accumbal DA efflux" on interactions between $GABA_A$, $GABA_B$, delta1-, delta2- and mu1-opioid receptors in regulating accumbal DA efflux (Table 1).

Consider the neuronal networks of Figs. 1 and 2. Both $GABA_A$ and $GABA_B$ receptors are located postsynaptically on the terminals of accumbal DAergic neurons where they inhibit DA efflux; in addition, $GABA_A$ receptors, but not $GABA_B$ receptors, are also located presynaptically on the terminals of innervating GABAergic neurons where they inhibit GABA release onto postsynaptic $GABA_A$ receptors that inhibit DA efflux.

Each of delta1-, delta2- and mu1-opioid receptors are located presynaptically on the terminals of innervating

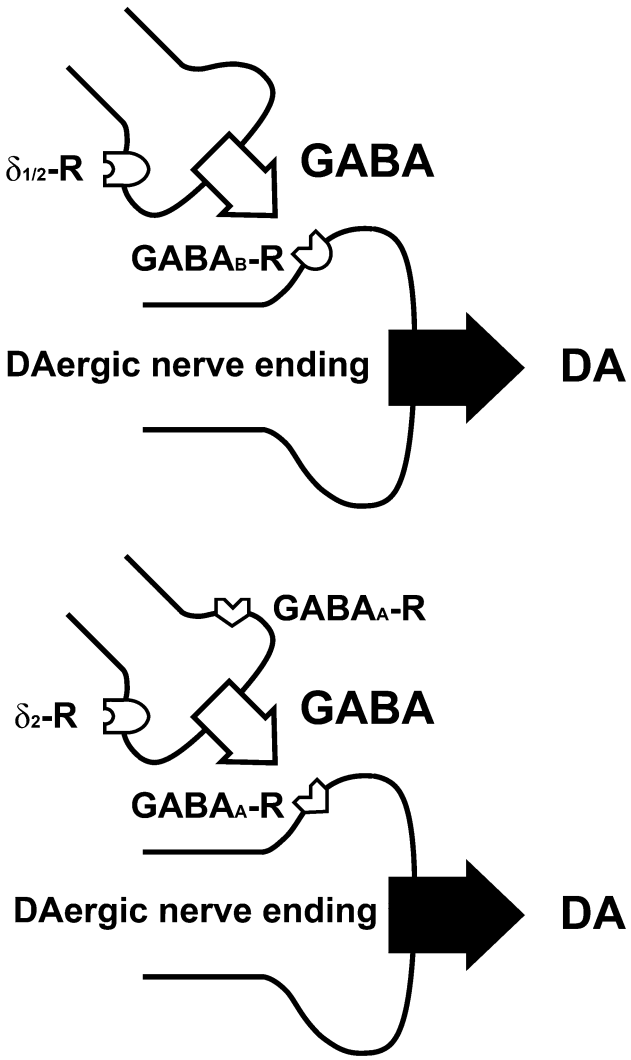

Fig. 1 Upper panel: a model indicating how GABAergic interneurons and dopaminergic neurons (DAergic nerve endings) interact in relation to $GABA_B$ as well as delta1- and/or delta2-opioid receptors in the nucleus accumbens. Lower panel: a model indicating how GABAergic interneurons and dopaminergic neurons (DAergic nerve endings) interact in relation to $GABA_A$ as well as delta1-opioid receptors in the nucleus accumbens. Glial cells that may contribute to regulation of extracellular neurotransmitter levels are not included in both panels. The arrows indicate GABA and dopamine (DA) release from the respective nerve endings

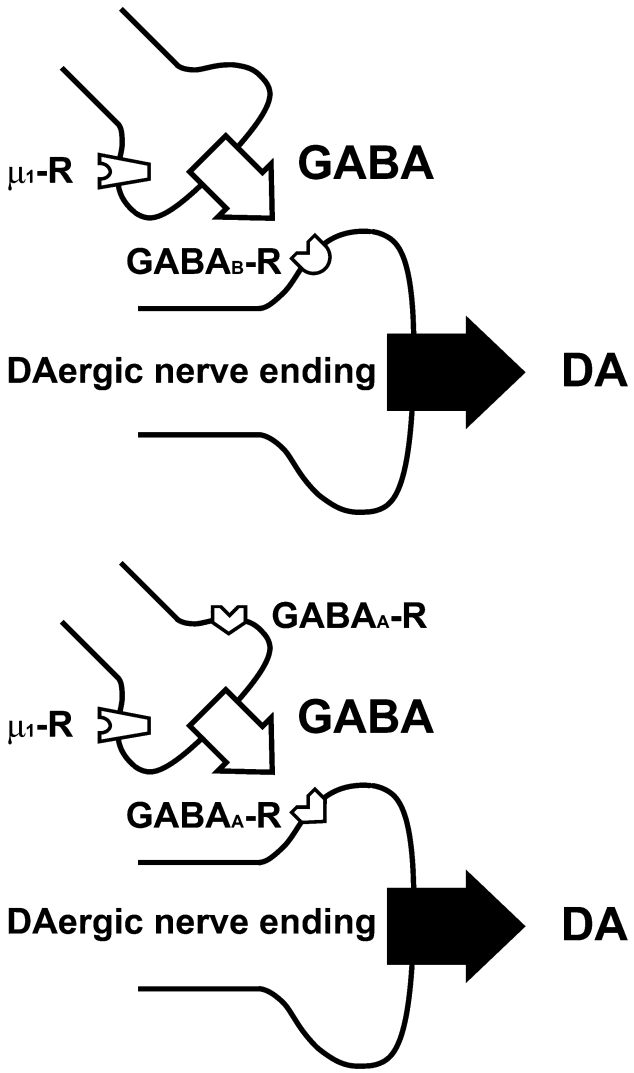

Fig. 2 Upper panel: a model indicating how GABAergic interneurons and dopaminergic neurons (DAergic nerve endings) interact in relation to $GABA_B$ as well as mu1-opioid receptors in the nucleus accumbens. Lower panel: a model indicating how GABAergic interneurons and dopaminergic neurons (DAergic nerve endings) interact in relation to $GABA_A$ as well as mu1-opioid receptors in the nucleus accumbens. Glial cells that may contribute to regulation of extracellular neurotransmitter levels are not included in both panels. The arrows indicate GABA and dopamine (DA) release from the respective nerve endings

GABAergic neurons, where they also inhibit GABA release onto postsynaptic $GABA_B$ receptors that inhibit DA efflux. Delta2- and mu1-opioid receptors, but not delta1-opioid receptors, are also located presynaptically on the terminals of innervating GABAergic neurons, proximal to presynaptic $GABA_A$ receptors, where they inhibit GABA release onto postsynaptic $GABA_A$ receptors that inhibit DA efflux. However, delta1-/delta2-opioid receptors and mu1-opioid receptors are not co-located; rather, they are located on distinct populations of GABAergic terminals.

Assume that tonic stimulation of accumbal $GABA_A$ and $GABA_B$ receptors by endogenous GABA is high. Then, (1) a $GABA_B$ receptor agonist would be without additional effect on DA efflux (ceiling effect), and (2) a $GABA_B$ receptor antagonist would reduce tonic $GABA_B$ receptor-mediated inhibition and increase DA efflux (Figs. 1 and 2, upper panels). If presynaptic $GABA_A$ receptors are more sensitive to $GABA_A$ receptor agonists than postsynaptic $GABA_A$ receptors (see "$GABA_A$ receptors and basal accumbal DA efflux"), then (3) a $GABA_A$ receptor agonist would act preferentially at presynaptic receptors to reduce tonic $GABA_A$ receptor-mediated inhibition and increase DA efflux, and (4) a $GABA_A$ receptor antagonist would act at postsynaptic receptors to reduce $GABA_A$ receptor-mediated inhibition and increase DA efflux (Figs. 1 and 2, lower panels).

Assume that delta1- and delta2-opioid receptor-mediated inhibition of tonic GABA release is similar between DA terminals containing $GABA_A$ and $GABA_B$ receptors. Then, (5) stimulation of presynaptic delta1- or delta2-opioid receptors would reduce tonic $GABA_B$ receptor-mediated inhibition and increase DA efflux (Fig. 1, upper panel); following this reduced tonic activity through $GABA_B$ receptors, (6) a $GABA_B$ receptor agonist would act at postsynaptic $GABA_B$ receptors to inhibit enhancement of DA efflux due to stimulation of delta1- or delta2-opioid receptors, and (7) a $GABA_B$ receptor antagonist would be without additional effect (floor effect) on enhancement of DA efflux due to stimulation of delta1- or delta2-opioid receptors. Also, (8) stimulation of presynaptic delta2-opioid receptors would reduce tonic $GABA_A$ receptor-mediated inhibition and increase DA efflux (Fig. 1, lower panel); following this reduced tonic activity through $GABA_A$ receptors, (9) a $GABA_A$ receptor agonist would act at postsynaptic $GABA_A$ receptors to inhibit enhancement of DA efflux due to stimulation of delta2-opioid receptors and (10) a $GABA_A$ receptor antagonist would be without additional effect (floor effect) on enhancement of DA efflux due to stimulation of delta2-opioid receptors. As delta1-opioid receptors are not co-located with presynaptic $GABA_A$ receptors, neither $GABA_A$ receptor agonists nor antagonists influence their effects on DA efflux.

Assume that mu1-opioid receptor-mediated inhibition of tonic GABA release on to GABA receptors is greater for DA terminals containing $GABA_A$ receptors than for those containing $GABA_B$ receptors. Then, (11) stimulation of presynaptic mu1-opioid receptors would exert only limited reduction in tonic $GABA_B$ receptor-mediated inhibition to only marginally increase DA efflux; following this limited reduction in tonic activity through $GABA_B$ receptors, (12) a $GABA_B$ receptor agonist would exert minimal additional effect and (13) a $GABA_B$ receptor antagonist would reduce tonic $GABA_B$ receptor-mediated inhibition and enhance DA efflux (Fig. 2, upper panel). However, (14) stimulation of presynaptic mu1-opioid receptors would induce marked

reduction of tonic postsynaptic $GABA_A$ receptor-mediated inhibition and increase DA efflux; then, (15) a $GABA_A$ receptor agonist would act preferentially at presynaptic $GABA_A$ receptors to further reduce tonic activity through $GABA_A$ receptors and enhance DA efflux, while (16) a $GABA_A$ receptor antagonist would exert only limited additional effect due to the marked reduction of tonic stimulation of postsynaptic $GABA_A$ receptors (floor effect; Fig. 2, lower panel).

Each of these effects (1)–(16) is in accordance with the experimental evidence described above (Table 1), indicating that the mechanisms outlined in Figs. 1 and 2 are plausible candidates for integrative GABAergic-opioid regulation of DA efflux in NAc. The present models (lower panels in Figs. 1 and 2) imply that the $GABA_A$ receptor agonist muscimol may act at postsynaptic $GABA_A$ receptors to reduce delta1-opioid receptor-mediated DA efflux, but it could act at presynaptic $GABA_A$ receptors to enhance mu1-opioid receptor-mediated DA efflux. Differences in changes in the available amount of endogenous GABA to act on $GABA_A$ receptors on accumbal DAergic nerve terminals after stimulation of delta2- or mu1-opioid receptors may explain the above-mentioned effects of muscimol. Thus, the available levels of endogenous GABA would be high before delta2- (lower panel of Fig. 1) or mu1- (lower panel of Fig. 2) opioid receptor activation, but this amount would decrease after activation of these opioid receptor subtypes. On this basis, via reduced competition from endogenous GABA, the exogenous $GABA_A$ receptor agonist would interact preferentially at $GABA_A$ receptors located on DAergic nerve terminals following stimulation of delta2-opioid receptors. The level of endogenous GABA available to postsynaptic $GABA_A$ receptors would also be high before mu1-opioid receptor activation, but this amount would markedly decrease after activation of mu1-opioid receptors. Due to this strong reduction of endogenous GABA acting at postsynaptic $GABA_A$ receptors, which could not compete with the exogenous $GABA_A$ receptor agonist (floor effect), muscimol would interact preferentially at presynaptic, but not postsynaptic, $GABA_A$ receptors following stimulation of mu1-opioid receptors.

Further studies are necessary to confirm and elaborate these integrative mechanisms and resolve proposed differences in the amounts of endogenous GABA available for acting at either $GABA_B$ or $GABA_A$ receptors to inhibit accumbal DA efflux in relation to selective activation of delta1-, delta2- and mu1-opioid receptors in NAc.

In the final section, we consider limitations in experimental conditions and explanatory scope. Then, we offer a perspective of such accumbal GABAergic neural mechanisms involved in delta- and mu-opioid receptor-mediated accumbal DA efflux in freely moving rats.

GABAergic mechanisms in basal, GAD inhibitor-induced, delta-opioid receptor subtype-mediated and mu-opioid receptor-mediated accumbal DA efflux

Limitations

In addition to findings on the locations of delta- and mu-opioid receptors and GABA receptor subtypes in NAc, the parsimonious neural network model presented in this review (Figs. 1, 2) is based on results from brain microdialysis experiments measuring accumbal extracellular DA levels. Accumbal DA in microdialysis samples should be released extracellularly consequent to neural firing, as intra-accumbal administration of the voltage-dependent Na^+ channel inhibitor tetrodotoxin strongly reduced DA levels [8, 54, 78]. These microdialysis studies, carried out in freely moving rats, used probes with a 2-mm dialysis membrane that could not distinguish between the core and shell regions of NAc. Therefore, the neuronal models presented in this review should be applied with caution to results obtained under other experimental conditions, such as analysis of DA levels in dialysates from anaesthetised experimental animals, species other than rats, and targeting specific sub-regions of NAc. Nevertheless, these models may be helpful for analysing results from experiments that use brain sections and isolated neurons.

As the models are simplified on the basis of synaptic relationships, other processes may be involved. For example, while glial cells could participate in the regulation of extracellular neurotransmitter levels and may express GAD, the key enzyme for generating GABA from glutamate, such processes are not reflected in these models. Furthermore, accumbal GAD has been found not only in interneurons but also in projections from the other locations in the brain (see "GAD and its two isoforms: differences in location and function in accumbal cells"). As mentioned in "Activation of accumbal $GABA_B$ receptors, but not $GABA_A$ receptors, inhibits allylglycine-induced increase in accumbal DA efflux", intra-vesicular GABA that has already been synthesised outside of NAc, such as in terminals of neurons that arise in the substantia nigra and/or VTA, is an important candidate for a source of allylglycine-insensitive GABA.

Delta1-, delta2- and/or mu-1-opioid receptors could be found not only on the terminals but also on the cell bodies of accumbal GABAergic interneurons [14, 15]. There may also be accumbal GABAergic output neurons with axon collaterals that input onto DAergic nerve endings to inhibit accumbal DAergic neural activity.

Conclusions

Though the above limitations in terms of experimental conditions and levels of explanation of results should be noted, the present review offers integrative models to explain opioidergic and GABAergic regulation of accumbal DA efflux in the following terms. First, endogenous GABA strongly stimulates $GABA_A$ and/or $GABA_B$ receptors on DAergic nerve endings in NAc. Furthermore, in NAc (1) GAD inhibitor-sensitive, newly synthesised GABA exerts inhibitory tonus on DAergic neural activity at the level of $GABA_B$ receptors, but not $GABA_A$ receptors, and (2) there is a GAD inhibitor-insensitive GABA pool that releases GABA and exerts inhibitory control of DAergic neural activity at the level of $GABA_A$ receptors. Second, NAc contains (1) GABAergic neurons that express delta1- and delta2-opioid receptors and inhibit DAergic neural activity through $GABA_B$ receptors on DAergic nerve endings, and (2) GABAergic neurons that express delta2-opioid receptors, but not delta1-opioid receptors and inhibit DAergic neural activity through $GABA_A$ receptors on DAergic nerve endings. Third, NAc also contains GABAergic neurons that express mu1-opioid receptors, but not delta-opioid receptors, and inhibit DAergic activity through $GABA_A$ and $GABA_B$ receptors on DAergic nerve endings. Finally, the magnitude of decrease in availability of endogenous GABA that interacts with $GABA_A$ and $GABA_B$ receptors to inhibit DAergic neural activity following stimulation of mu1-opioid receptors differs from that following stimulation of delta-opioid receptor subtypes.

These integrative models illuminate, at the level of individual receptor subtypes within an accumbal synaptic network, how selective activation of opioid receptors enhances accumbal DA efflux onto DA receptors and the critical role of GABAergic mechanisms in regulating interactions between these two families of G protein-coupled receptors. As such enhancement appears to be a critical factor in opioid dependence and associated morbidity and mortality [5, 6], improved understanding of these synaptic mechanisms may also provide clues to remediation.

Author contributions TS and JLW reviewed the recent literature and drafted the review. TS, YA and JLW were involved in many of the microdialysis studies that are included in the review. All authors contributed to and approved the final manuscript for submission.

Funding This study was supported by Grant-in-Aid for Scientific Research (C) (#17K11858 to TS; #18K09777 to YA) from the Ministry of Education, Culture, Sports, Science and Technology, Japan; The Private University Research Branding Project from MEXT, Japan; Nihon University President's Grant for Specified Interdisciplinary Research: Grant from The Nakatomi Foundation, Japan (TS, JLW); Grants from Suzuki Fund, Nihon University School of Dentistry at Matsudo; Grants from Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo (TS, YA).

Declarations

Conflict of interest The authors report no conflict of interest.

References

1. Di Chiara G. Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res. 2002;137:75–114.
2. Nicola SM. The nucleus accumbens as part of a basal ganglia action selection circuit. Psychopharmacology. 2007;191:521–50.
3. Floresco SB. The nucleus accumbens: an interface between cognition, emotion, and action. Annu Rev Psychol. 2015;66:25–52.
4. Castro DC, Bruchas MR. A motivational and neuropeptidergic hub: anatomical and functional diversity within the nucleus accumbens shell. Neuron. 2019;102:529–52.
5. Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, et al. Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology. 2004;47(Suppl 1):227–41.
6. Koob GF, Volkow ND. Neurobiology of addiction: a neurocircuitry analysis. Lancet Psychiatry. 2016;3:760–73.
7. Koob GF. Neurobiology of opioid addiction: opponent process, hyperkatifeia, and negative reinforcement. Biol Psychiatry. 2020;87:44–53.
8. Okutsu H, Watanabe S, Takahashi I, Aono Y, Saigusa T, Koshikawa N, et al. Endomorphin-2 and endomorphin-1 promote the extracellular amount of accumbal dopamine via nonopioid and mu-opioid receptors, respectively. Neuropsychopharmacology. 2006;31:375–83.
9. Saigusa T, Aono Y, Waddington JL. Mechanisms underlying δ- and μ-opioid receptor agonist-induced increases in extracellular dopamine level in the nucleus accumbens of freely moving rats. J Oral Sci. 2017;59:195–200.
10. Aono Y, Saigusa T, Mizoguchi N, Iwakami T, Takada K, Gionhaku N, et al. Role of $GABA_A$ receptors in the endomorphin-1-, but not endomorphin-2-, induced dopamine efflux in the nucleus accumbens of freely moving rats. Eur J Pharmacol. 2008;580:87–94.
11. Aono Y, Kiguchi Y, Watanabe Y, Waddington JL, Saigusa T. Stimulation of accumbal $GABA_A$ receptors inhibits delta2-, but not delta1-, opioid receptor-mediated dopamine efflux in the nucleus accumbens of freely moving rats. Eur J Pharmacol. 2017;815:18–25.
12. Saigusa T, Aono Y, Mizoguchi N, Iwakami T, Takada K, Oi Y, et al. Role of $GABA_B$ receptors in the endomorphin-1-, but not endomorphin-2-, induced dopamine efflux in the nucleus accumbens of freely moving rats. Eur J Pharmacol. 2008;581:276–82.
13. Watanabe Y, Aono Y, Komiya M, Waddington JL, Saigusa T. Stimulation of accumbal GABA_B receptors inhibits delta1- and delta2-opioid receptor-mediated dopamine efflux in the nucleus accumbens of freely moving rats. Eur J Pharmacol. 2018;837:88–95.
14. Svingos AL, Clarke CL, Pickel VM. Cellular sites for activation of δ-opioid receptors in the rat nucleus accumbens shell: relationship with Met^5-enkephalin. J Neurosci. 1998;18:1923–33.
15. Svingos AL, Moriwaki A, Wang JB, Uhl GR, Pickel VM. Mu-opioid receptors are localized to extrasynaptic plasma membranes of GABAergic neurons and their targets in the rat nucleus accumbens. J Neurosci. 1997;17:2585–2294.
16. Robinson TG, Beart PM. Excitant amino acid projections from rat amygdala and thalamus to nucleus accumbens. Brain Res Bull. 1988;20:467–71.

17. Blaha CD, Yang CR, Floresco SB, Barr AM, Phillips AG. Stimulation of the ventral subiculum of the hippocampus evokes glutamate receptor-mediated changes in dopamine efflux in the rat nucleus accumbens. Eur J Neurosci. 1997;9:902–11.
18. Christie MJ, James LB, Beart PM. An excitant amino acid projection from the medial prefrontal cortex to the anterior part of nucleus accumbens in the rat. J Neurochem. 1985;45:477–82.
19. Waddington JL, Cross AJ. Neurochemical changes following kainic acid lesions of the nucleus accumbens: implications for a GABAergic accumbal-ventral tegmental pathway. Life Sci. 1978;22:1011–4.
20. Berendse HW, Groenewegen HJ, Lohman AH. Compartmental distribution of ventral striatal neurons projecting to the mesencephalon in the rat. J Neurosci. 1992;12:2079–103.
21. Zahm DS, Heimer L. Specificity in the efferent projections of the nucleus accumbens in the rat: comparison of the rostral pole projection patterns with those of the core and shell. J Comp Neurol. 1993;8:220–32.
22. Schwarzer C, Berresheim U, Pirker S, Wieselthaler A, Fuchs K, Sieghart W, et al. Distribution of the major gamma-aminobutyric acid(A) receptor subunits in the basal ganglia and associated limbic brain areas of the adult rat. J Comp Neurol. 2001;433:526–49.
23. Chang HT, Kitai ST. Projection neurons of the nucleus accumbens: an intracellular labeling study. Brain Res. 1985;347:112–6.
24. Bolam JP, Clarke DJ, Smith AD, Somogyi P. A type of aspiny neuron in the rat neostriatum accumulates [^{3}H]gamma-aminobutyric acid: combination of Golgi-staining, autoradiography, and electron microscopy. J Comp Neurol. 1983;213:121–34.
25. Kita H, Kitai ST. Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations. Brain Res. 1988;447:346–52.
26. Xi ZX, Ramamoorthy S, Shen H, Lake R, Samuvel DJ, Kalivas PW. GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine. J Neurosci. 2003;23:3498–505.
27. Bettler B, Kaupmann K, Mosbacher J, Gassmann M. Molecular structure and physiological functions of $GABA_B$ receptors. Physiol Rev. 2004;84:835–67.
28. Davis LL, Trivedi M, Choate A, Kramer GL, Petty F. Growth hormone response to the $GABA_B$ agonist baclofen in major depressive disorder. Psychoneuroendocrinology. 1997;22:129–40.
29. Mouginot D, Kombian SB, Pittman QJ. Activation of presynaptic $GABA_B$ receptors inhibits evoked IPSCs in rat magnocellular neurons in vitro. J Neurophysiol. 1998;79:1508–17.
30. Margeta-Mitrovic M, Mitrovic I, Riley RC, Jan LY, Basbaum AI. Immunocytochemical localization of $GABA_B$ receptors in the rat central nervous system. J Comp Neurol. 1999;405:299–321.
31. Doherty M, Gratton A. Differential involvement of ventral tegmental GABA(A) and GABA(B) receptors in the regulation of the nucleus accumbens dopamine response to stress. Brain Res. 2007;1150:62–8.
32. Westerink BH, Kwint HF, deVries JB. The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. J Neurosci. 1996;16:2605–11.
33. Ikemoto S, Kohl RR, McBride WJ. GABA(A) receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. J Neurochem. 1997;69:137–43.
34. Pycock CJ, Horton RW. Dopamine-dependent hyperactivity in the rat following manipulation of GABA mechanisms in the region of the nucleus accumbens. J Neural Trans. 1979;45:17–33.
35. Wong LS, Eshel G, Dreher J, Ong J, Jackson DM. Role of dopamine and GABA in the control of motor activity elicited from the rat nucleus accumbens. Pharm Biochem Behav. 1991;38:829–35.
36. Scheel-Krüger J, Arnt J, Magelund G, Olianas M, Przewlocka B, Christensen AV. Behavioural functions of GABA in basal ganglia and limbic system. Brain Res Bull. 1980;5(Suppl 2):261–7.
37. Rahman S, McBride WJ. Involvement of GABA and cholinergic receptors in the nucleus accumbens on feedback control of somatodendritic dopamine release in the ventral tegmental area. J Neurochem. 2002;80:646–54.
38. Yan Q. Focal bicuculline increases extracellular dopamine concentration in the nucleus accumbens of freely moving rats as measured by in vivo microdialysis. Eur J Pharmacol. 1999;385:7–13.
39. Ferraro L, Tanganelli S, O'Connor WT, Antonelli T, Rambert F, Fuxe K. The vigilance promoting drug modafinil increases dopamine release in the rat nucleus accumbens via the involvement of a local GABAergic mechanism. Eur J Pharmacol. 1996;306:33–9.
40. Yoshida M, Yokoo H, Nakahara K, Tomita M, Hamada N, Ishikawa M, et al. Local muscimol disinhibits mesolimbic dopaminergic activity as examined by brain microdialysis and Fos immunohistochemistry. Brain Res. 1997;767:356–60.
41. Pijnenburg AJ, van Rossum JM. Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. J Pharm Pharmacol. 1973;25:1003–5.
42. Ashby CR Jr, Rohatgi R, Ngosuwan J, Borda T, Gerasimov MR, Morgan AE, et al. Implication of the $GABA_B$ receptor in gamma vinyl-GABA's inhibition of cocaine-induced increases in nucleus accumbens dopamine. Synapse. 1999;31:151–3.
43. Fadda P, Scherma M, Fresu A, Collu M, Fratta W. Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. Synapse. 2003;50:1–6.
44. Erlander MG, Tobin AJ. The structural and functional heterogeneity of glutamic acid decarboxylase: a review. Neurochem Res. 1991;16:215–26.
45. Buddhala C, Hsu CC, Wu JY. A novel mechanism for GABA synthesis and packaging into synaptic vesicles. Neurochem Int. 2009;55:9–12.
46. Martin DL, Rimvall K. Regulation of gamma-aminobutyric acid synthesis in the brain. J Neurochem. 1993;60:395–407.
47. Waagepetersen HS, Sonnewald U, Schousboe A. The GABA paradox: multiple roles as metabolite, neurotransmitter, and neurodifferentiative agent. J Neurochem. 1999;73:1335–42.
48. Walls AB, Nilsen LH, Eyjolfsson EM, Vestergaard HT, Hansen SL, Schousboe A, et al. GAD65 is essential for synthesis of GABA destined for tonic inhibition regulating epileptiform activity. J Neurochem. 2010;115:1398–408.
49. Bolam JP, Powell JF, Wu JY, Smith AD. Glutamate decarboylase-immunoreactive structures in the rat striatum: a correlated light and electron microscopic study including a combination of Golgi impregnation with immunocytochemistry. J Comp Neurol. 1985;237:1–20.
50. Cowan RL, Wilson CJ, Emson PC, Heizmann CW. Parvalbumin-containing GABAergic interneurons in the rat neostriatum. J Comp Neurol. 1990;302:197–205.
51. Oertel WH, Mugnaini E. Immunocytochemical studies of GABAergic neurons in rat basal ganglia and their relations to other neuronal systems. Neurosci Lett. 1984;47:233–8.
52. Lee M, Schwab C, McGeer PL. Astrocytes are GABAergic cells that modulate microglial activity. Glia. 2011;59:152–65.
53. González-Hernández T, Barroso-Chinea P, Acevedo A, Salido E, Rodríguez M. Colocalization of tyrosine hydroxylase and GAD65 mRNA in mesostriatal neurons. Eur J Neurosci. 2001;13:57–67.
54. Saigusa T, Aono Y, Sekino R, Uchida T, Takada K, Oi Y, Koshikawa N, Cools AR. In vivo neurochemical evidence that newly synthesised GABA activates GABA(B), but not GABA(A), receptors on dopaminergic nerve endings in the nucleus accumbens of freely moving rats. Neuropharmacology. 2012;62:907–13.
55. Attwell D, Barbour B, Szatkowski M. Nonvesicular release of neurotransmitter. Neuron. 1993;11:401–7.

56. Rossi DJ, Hamann M, Attwell D. Multiple modes of GABAergic inhibition of rat cerebellar granule cells. J Physiol. 2003;548:97–110.
57. Wall MJ, Usowicz MM. Development of action potential-dependent and independent spontaneous $GABA_A$ receptor-mediated currents in granule cells of postnatal rat cerebellum. Eur J Neurosci. 1997;9:533–48.
58. Richerson GB, Wu YM. Dynamic equilibrium of neurotransmitter transportrers; not just for reuptake anymore. J Neurophysiol. 2003;90:1363–74.
59. Kozlov AS, Angulo MC, Audinat E, Charpak S. Target cell-specific modulation of neuronal activity by atrocytes. Proc Natl Acad Sci USA. 2006;103:10058–63.
60. Liu QY, Schaffner AE, Chang YH, Maric D, Barker JL. Persistent activation of $GABA_A$ receptor/Cl^- channels by astrocyte-derived GABA in cultured embryonic rat hippocampal neurons. J Neurophysiol. 2000;84:1392–403.
61. Chaudhry FA, Reimer RJ, Bellocchio EE, Danbolt NC, Osen KK, Edwards RH, et al. The vesicular GABA transporter, VGAT, localizes to synaptic vesicles in sets of glycinergic as well as GABAergic neurons. J Neurosci. 1998;18:9733–50.
62. Jin H, Wu H, Osterhaus G, Wei J, Davis K, Sha D, et al. Demonstration of functional coupling between gamma-aminobutyric acid (GABA) synthesis and vesicular GABA transport into synaptic vesicles. Proc Natl Acad Sci USA. 2003;100:4293–8.
63. Walls AB, Eyjolfsson EM, Smeland OB, Nilsen LH, Schousboe I, Schousboe A, et al. Knockout of GAD65 has major impact on synaptic GABA synthesized from astrocyte-derived glutamine. J Cereb Blood Flow Metab. 2011;31:494–503.
64. Gasnier B. The loading of neurotransmitters into synaptic vesicles. Biochimie. 2000;82:327–37.
65. Galvan A, Hu X, Smith Y, Wichmann T. Localization and pharmacological modulation of GABA-B receptors in the globus pallidus of parkinsonian monkeys. Exp Neurol. 2011;229:429–39.
66. Tepper JM, Lee CR. GABAergic control of substantia nigra dopaminergic neurons. Prog Brain Res. 2007;160:189–208.
67. Azzam AAH, McDonald J, Lambert DG. Hot topics in opioid pharmacology: mixed and biased opioids. Br J Anaesth. 2019;122:e136–45.
68. Narita M, Funada M, Suzuki T. Regulations of opioid dependence by opioid receptor types. Pharmacol Ther. 2001;89:1–15.
69. Fields HL, Margolis EB. Understanding opioid reward. Trends Neurosci. 2015;38:217–25.
70. Yoshida Y, Koide S, Hirose N, Takada K, Tomiyama K, Koshikawa N, et al. Fentanyl increases dopamine release in rat nucleus accumbens: involvement of mesolimbic mu- and delta-2-opioid receptors. Neuroscience. 1999;92:1357–65.
71. Fusa K, Takahashi I, Watanabe S, Aono Y, Ikeda H, Saigusa T, et al. The non-peptidic delta opioid receptor agonist TAN-67 enhances dopamine efflux in the nucleus accumbens of freely moving rats via a mechanism that involves both glutamate and free radicals. Neuroscience. 2005;130:745–55.
72. Hirose N, Murakawa K, Takada K, Oi Y, Suzuki T, Nagase H, et al. Interactions among mu- and delta-opioid receptors, especially putative $delta_1$- and $delta_2$-opioid receptors, promote dopamine release in the nucleus accumbens. Neuroscience. 2005;135:213–25.
73. Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ. Autoradiographic differentiation of mu, delta and kappa opioid receptors in the rat forebrain and midbrain. J Neurosci. 1987;7:2445–64.
74. Gouarderes C, Tellez S, Tafani JAM, Zajac J-M. Quantitative autoradiographic mapping of delta-opioid receptors in the rat central nervous system using [^{125}I][D-Ala^2]deltorphin-I. Synapse. 1993;13:231–40.
75. Evans CJ, Keith DE, Morrison H, Magendzo K, Edwards RH. Cloning of a delta opioid receptor by functional expression. Science. 1992;258:1952–5.
76. Dietis N, Rowbotham DJ, Lambert DG. Opioid receptor subtypes: fact or artifact? Br J Anaesth. 2011;107:8–18.
77. Pasternak GW, Wood PJ. Multiple mu opiate receptors. Life Sci. 1986;38:1889–98.
78. Saigusa T, Fusa K, Okutsu H, Koshikawa N. Monitoring of extracellular dopamine levels in the dorsal striatum and the nucleus accumbens with 5-minute on-line microdialysis in freely moving rats. J Oral Sci. 2001;43:129–34.