

VIEWPOINT

Roles of dopamine and glutamate co-release in the nucleus accumbens in mediating the actions of drugs of abuse

Silas A. Buck^{1,2}, Mary M. Torregrossa² , Ryan W. Logan^{2,3}  and Zachary Freyberg^{2,4} 

1 Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA

2 Department of Psychiatry, Translational Neuroscience Program, University of Pittsburgh, Pittsburgh, PA, USA

3 Center for Systems Neurogenetics of Addiction, The Jackson Laboratory, Bar Harbor, ME, USA

4 Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA, USA

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Correspondence

Z. Freyberg, Department of Psychiatry, University of Pittsburgh, 3811 O'Hara Street, Biomedical Science Tower W1640, Pittsburgh, PA 15213, USA

Tel: +1 (412) 648 0033

E-mail: freyberg@pitt.edu

and

R. W. Logan, Department of Psychiatry, University of Pittsburgh, 450 Technology Drive, Pittsburgh, PA 15219, USA

Tel: +1 (412) 383-3084

E-mail: loganrw@upmc.edu

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Projections of ventral tegmental area dopamine (DA) neurons to the medial shell of the nucleus accumbens have been increasingly implicated as integral to the behavioral and physiological changes involved in the development of substance use disorders (SUDs). Recently, many of these nucleus accumbens-projecting DA neurons were found to also release the neurotransmitter glutamate. This glutamate co-release from DA neurons is critical in mediating the effect of drugs of abuse on addiction-related behaviors. Potential mechanisms underlying the role(s) of dopamine/glutamate co-release in contributing to SUDs are unclear. Nevertheless, an important clue may relate to glutamate's ability to potentiate loading of DA into synaptic vesicles within terminals in the nucleus accumbens in response to drug-induced elevations in neuronal activity, enabling a more robust release of DA after stimulation. Here, we summarize how drugs of abuse, particularly cocaine, opioids, and alcohol, alter DA release in the nucleus accumbens medial shell, examine the potential role of DA/glutamate co-release in mediating these effects, and discuss future directions for further investigating these mechanisms.

Introduction

Addictive substances including cocaine, opioids, and alcohol, and the ensuing substance use disorders (SUDs) produced by these drugs, place high economic and medical burdens on society [1–4], and drug overdose deaths continue to rise [5]. Despite this enormous public health impact, there is still no clear understanding of the mechanisms by which these drugs of abuse

work, particularly across cellular and behavioral levels. Additionally, most treatments are aimed at symptomatic relief rather than targeting the mechanisms responsible for the reinforcing aspects of drugs of abuse. This presents an urgent need to uncover the underlying neurological mechanisms behind SUDs so that more effective treatments can be created.

Abbreviations

DA, dopamine; DAT, dopamine transporter; ERK, extracellular signal-related kinase; IF, interfascicular nucleus; mNAcSh, medial nucleus accumbens shell; NAc, nucleus accumbens; PBP, parabrachial pigmented nucleus; RLl, rostral linear nucleus; SUD, substance use disorder; TH, tyrosine hydroxylase; VGLUT2, vesicular glutamate transporter 2; VMAT2, vesicular monoamine transporter 2; VTA, ventral tegmental area.

Drug-induced changes in dopamine (DA) neurotransmission have classically been the mainstay of our understanding of how drugs of abuse produce motivating and reinforcing properties [6–8]. Virtually, all addictive drugs enhance DA neurotransmission [6,9–11], either by: (a) enhancing presynaptic DA release, (b) reducing DA reuptake, (c) modifying presynaptic release probability, or via some combination of these presynaptic mechanisms [6,12–14]. Additionally, the regional specificity of this DAergic neurotransmission is equally critical for the addictive properties of drugs. DA neurons that project from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) play a major role in SUDs [15–22]. Indeed, blocking DA neurotransmission in the NAc greatly reduces the reinforcing properties of addictive drugs [23–25]. Furthermore, recent improvements in imaging and optogenetic techniques have identified the medial shell of the nucleus accumbens (mNAcSh) to be crucial for the addictive behaviors associated with drugs like cocaine, opioids, and alcohol [17,22,26–32]. These mNAcSh-projecting DA neurons are found in the medial portion of the VTA [27,33], suggesting that the medial VTA-mNAcSh projection is fundamentally critical in drug reinforcement, motivation, and reward-related behaviors.

Medial VTA DA neurons: unique properties

mNAcSh-projecting medial VTA DA neurons possess unique properties that distinguish these cells from other DAergic neuron subpopulations. Unlike mid-brain DA neurons projecting to the dorsal striatum which fire periodically at frequencies < 10 Hz, these mNAcSh-projecting medial VTA DA neurons fire at high frequencies (> 20 Hz) over extended periods of time [17,34–36]. These neuron population-specific differences in firing frequency are correlated with higher basal firing rates, burst activity, and heightened vulnerability to drugs like cocaine [37]. Additionally, modulatory afferent inputs and gene expression patterns in medial VTA DA neurons are different from other DAergic areas [17,34,38]. Perhaps most importantly, a subset of medial VTA DA neurons co-release the neurotransmitter glutamate in addition to DA [39,40]. A majority of these medial VTA DA/glutamate co-releasing neurons project their terminals to the mNAcSh [15,41–44]. This is based on a growing body of evidence from multiple approaches. First, stimulation of medial VTA DA neurons evokes excitatory postsynaptic potentials in the mNAcSh [17,45]. Second, the majority of medial VTA DA neurons projecting to the mNAcSh express vesicular glutamate transporter 2

(VGLUT2), which packages glutamate into vesicles for synaptic release [17,39,44,46,47]. In particular, studies have identified the rostral linear (RLi) and interfascicular (IF) nuclei of the VTA in rodent midbrain [43,46] and the parabrachial pigmented (PBP) nucleus in humans [48] as areas containing the majority of VGLUT2-expressing DA neurons (Fig. 1). Finally, retrograde tracing of the mNAcSh shows that labeled VTA neurons almost exclusively express tyrosine hydroxylase (TH), the rate-limiting enzyme of DA synthesis [17,27]. Nevertheless, apparent discrepancies remain in the precise numbers of medial VTA TH⁺/VGLUT2⁺ neurons. Immunocytochemistry studies by Chuhma *et al.* [17] demonstrated that 79% of all medial VTA neurons were TH⁺/VGLUT2⁺ in juvenile mice, with the number of double-positive neurons declining to 48% by adulthood. This result is consistent with earlier work in isolated postnatal rat mesencephalic DA neurons where ~80% of the neurons expressed VGLUT2 protein [39,47]. In contrast, only ~40% of TH⁺ VTA DA neurons in P25 juvenile mice express VGLUT2, at least at the mRNA level [41]. In contrast, other studies have reported significantly lower numbers of VGLUT2⁺ DA neurons in the VTA ranging from 0.1% to 25% [39,40,49]. Such variability in VTA TH⁺/VGLUT2⁺ neuron numbers may depend on the age/developmental stage sampled, the approaches used and potential differences between mRNA versus protein expression of VGLUT2 and TH markers. Despite this potential variability in the neuron number, there is substantial evidence that glutamate co-release from this unique subset of medial VTA DA neurons plays a critical role in mediating the cellular response to addictive drugs (Fig. 2).

While the role of DA release from medial VTA DA neurons in SUDs has been well studied, new research is beginning to investigate the role of glutamate co-release from these same neurons, presenting a potentially novel mechanism of addictive drug action. Here, we review recent findings on relationships between addictive drugs with an emphasis on cocaine, opioids, and alcohol and their actions on mNAcSh DA release, the potential role(s) of glutamate co-release in mediating this relationship, and future areas of research to further uncover the intertwined roles of DA and glutamate co-transmission from DA neurons in SUDs and their potential as novel therapeutic strategies for treating addiction. To date, there has been very little investigation of the postsynaptic effects of DA and glutamate co-transmission from VTA terminals and the possible impact of co-transmission on reward-related behaviors. There are hints that the integration of DA and glutamate may be necessary for reward-related learning. For

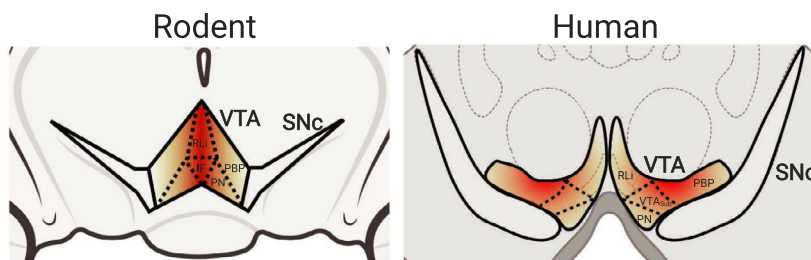


Fig. 1. Spatial distribution of VTA TH⁺/GLUT2⁺ neurons. In rodents (left panel), the highest concentration of VGLUT2-expressing DA neurons (TH⁺/GLUT2⁺) is in the medial regions of the VTA (darker shading), including the RLi and IF nuclei [43,46]. In humans (right panel), most TH⁺/GLUT2⁺ co-expressing neurons are found in the PBP [48]. PN, paranigral nucleus; VTA_{sub}, VTA subdivision.

example, cue-evoked food learning induces a greater extracellular signal-related kinase (ERK) and phosphoERK response in the NAc via the coordinated activation of D₁ and NMDA receptors [50]. In striatum, ERK acts to integrate separate DA and glutamate inputs that converge on medium spiny neurons involved in drug-induced synaptic and behavioral plasticity [51]. However, recent findings suggest DA and glutamate can be co-released from neuronal subpopulations in the midbrain, which may be used to further tune DA neurotransmission in response to drugs and other reward-related stimuli. This opens the possibility that integration of DA and glutamate neurotransmission may similarly potentiate the reinforcing actions of drugs of abuse (Fig. 3). Future studies will elucidate the precise signaling mechanisms for this integration, particularly in the context of different SUDs.

Medial VTA-mNAcSh DA release and SUDs

Cocaine

Cocaine is known to elevate DA neurotransmission in the striatum by inhibiting DA reuptake via dopamine transporter (DAT) blockade [6,7,13,52,53]. Recent evidence suggests that cocaine also acts independently of DAT to increase striatal DA neurotransmission by increasing VTA DA neuron firing, which is consistent with cocaine's continued reinforcing properties in self-administration and place paradigms in DAT knockout mice [11,54]. Cocaine can also alter the timing and magnitude of DA release by enhancing both tonic and phasic DA neuron activity, uncoupling the relative contributions of both firing modes to DA-modulated behaviors [6,11]. Within the striatum, the NAc shell is particularly important for cocaine-related behaviors, as the NAc shell exhibits greater increases in extracellular DA relative to other striatal regions after cocaine

administration [7,33,55–58]. Additionally, NAc shell-projecting VTA DA neurons are important in cocaine-related behaviors such as locomotor sensitization and conditioned place preference [17,26]. The mNAcSh, more specifically, shows larger transient increases in DA release in response to cocaine [6,59,60] and is crucial for cocaine reinforcement and related behaviors [17,26,27]. The medial VTA-mNAcSh projection has also been shown to encode aversive behaviors [61] and plays a role in incentive salience to environmental stimuli predictive of behaviorally relevant events [17,27,62]. Overall, these findings highlight that the medial VTA-mNAcSh projection is a critical location of cocaine action, where cocaine leads to further increases in DA neurotransmission via the inhibition of DA reuptake and the increased firing frequency of medial VTA DA neurons relative to other subpopulations of midbrain DA neurons.

Opioids

Opioids including morphine and heroin enhance DA neurotransmission by stimulating burst firing of VTA DA neurons that project to the NAc [6,22,56,63,64], which is especially critical for drug self-administration [11]. Opioids also block the actions of local inhibitory GABA neurons, leading to increased VTA DA neuron activity [65,66] and enhance presynaptic release probability in a stimulus frequency-dependent manner [67]. In the mNAcSh, heroin increases DA release by activating a subset of medial VTA DA neurons [22]. Thus, while opioids may not act mainly through DAT inhibition like cocaine, these drugs induce a similar increase in medial VTA-derived DA neurotransmission in the mNAcSh.

Alcohol

Like cocaine and opioids, DA release has been heavily implicated in the reinforcing effects of alcohol [68–70].

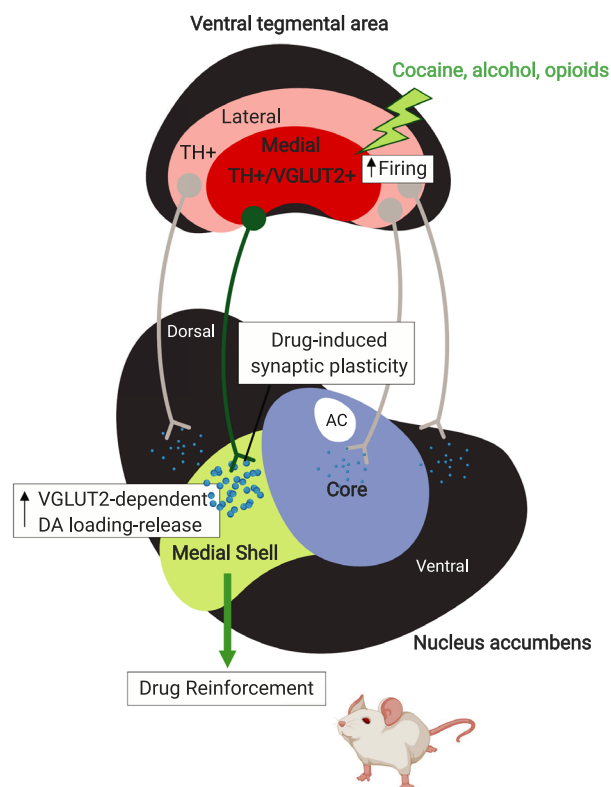


Fig. 2. A novel role for TH⁺/VGLUT2⁺ neurons in drug reinforcement. Drugs of abuse including cocaine, opioids, and alcohol preferentially increase vesicular DA loading and release in a distinct subpopulation of DA neurons that co-transmit both DA and glutamate and express both TH and VGLUT2 (TH⁺/VGLUT2⁺). The cell bodies of these DA/glutamate co-transmitting neurons predominantly localize to the medial VTA and project to the medial nucleus accumbens shell (mNAcSh). In response to drug-induced increases in cell firing, these medial VTA TH⁺/VGLUT2⁺ DA neurons that project to the mNAcSh have functional features unique from other midbrain DA neurons including the DA-only neurons that project to the nucleus accumbens (NAc) core, including distinct modulatory afferent inputs and high-frequency firing (> 20 Hz). Moreover, DA/glutamate co-transmission by this distinct TH⁺/VGLUT2⁺ subpopulation of DA neurons may be a critical mechanism for regulating reward-related behaviors and associated with vulnerability to SUDs.

Though the precise mechanisms remain poorly understood, there is evidence suggesting that alcohol increases DA neurotransmission by increasing the activity of VTA DA neurons [68,71–73]. Interestingly, while this increased activity of VTA DA neurons in response to alcohol leads to large DA release in the NAc, the NAc shell exhibits larger transient increases in DA compared to the core, consistent with other drugs of abuse [30,32,59,60]. In contrast, one study found that chronic ethanol exposure has no effect on

DA reuptake in the mNAcSh [74] and other recent work showed that optogenetically activating NAc-projecting VTA neurons reduced ethanol consumption in a subset of mice exhibiting high ethanol consumption [75]. Clearly, many unanswered questions remain. For example, to date, there are no known studies of the effect of ethanol on presynaptic DA release probability in the mNAcSh. Together, these findings suggest that alcohol increases DAergic neurotransmission, particularly in the mNAcSh, via an upregulation of VTA DA neuron firing, and that alcohol may produce additional effects on DA release via unexplored mechanisms.

Overall, comparing the actions of cocaine, opioids, and alcohol, all three types of drugs increase DAergic neurotransmission in the NAc, and even more specifically in the mNAcSh, which plays a number of important roles in the addictive, reinforcing properties of these drugs (Fig. 2). In all three cases, the neurons, which are built to fire rapidly and over prolonged periods, are especially well suited to potentiate drugs' behavioral effects. Since a large proportion of these medial VTA DA neurons release glutamate, this leads us to ask: What role does glutamate co-release from this special population of DA neurons play in potentiating the actions of drugs of abuse?

Glutamate co-release from medial VTA DA neurons projecting to mNAcSh mediates the effects of addictive drugs

An important clue concerning the role of glutamate in DA neurons and its potential relevance to DA/glutamate co-transmission from medial VTA DA neurons stems from findings showing that glutamate and its vesicular transporter VGLUT modulate activity-dependent DA loading in NAc terminals [76]. This concept of 'vesicular synergy', where one neurotransmitter synergistically enhances vesicular packaging and release of another neurotransmitter, was first demonstrated in striatal cholinergic interneurons and subsequently extended to serotonergic neurons [77,78]. Hnasko *et al.* [16] were first to report vesicular synergy between DA and glutamate, resulting in glutamate-induced acidification of DA synaptic vesicles. Additional work showed that DA/glutamate vesicular synergy was attributed to the expression of VGLUT2 in these subsets of DA/glutamate co-releasing neurons [16,79]. Consistent with these earlier findings, we recently demonstrated that VGLUT2 expression in NAc DA terminals is especially important for tuning vesicular DA loading and release in response to high neuronal activity [76]. Specifically, we found that VGLUT2 and

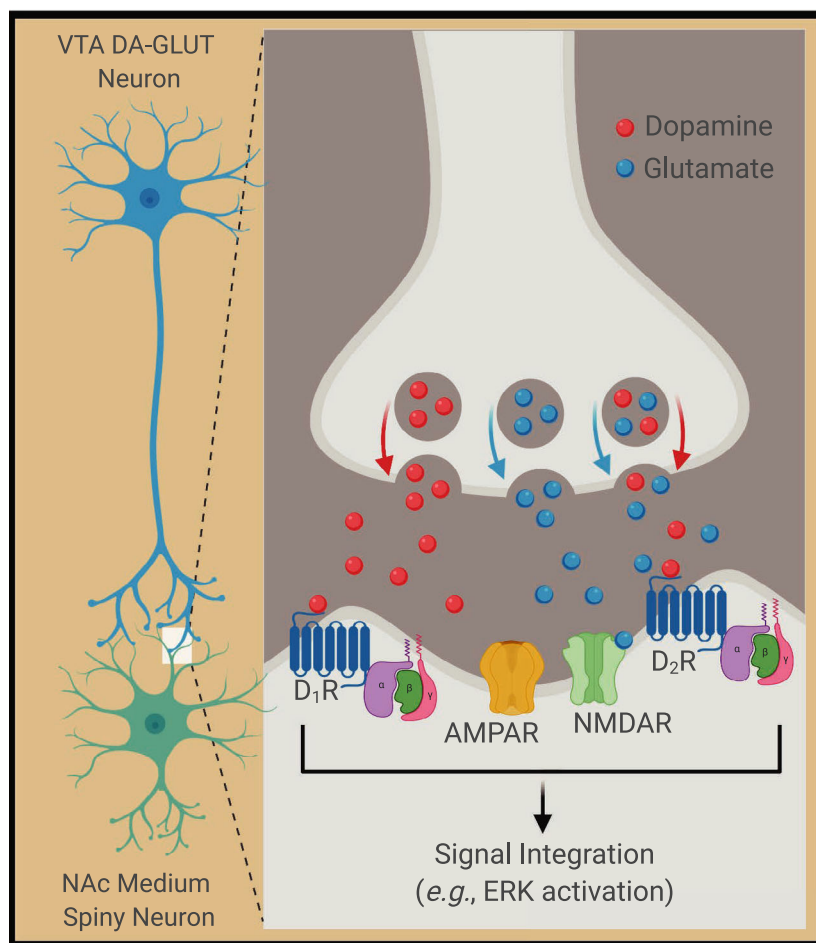


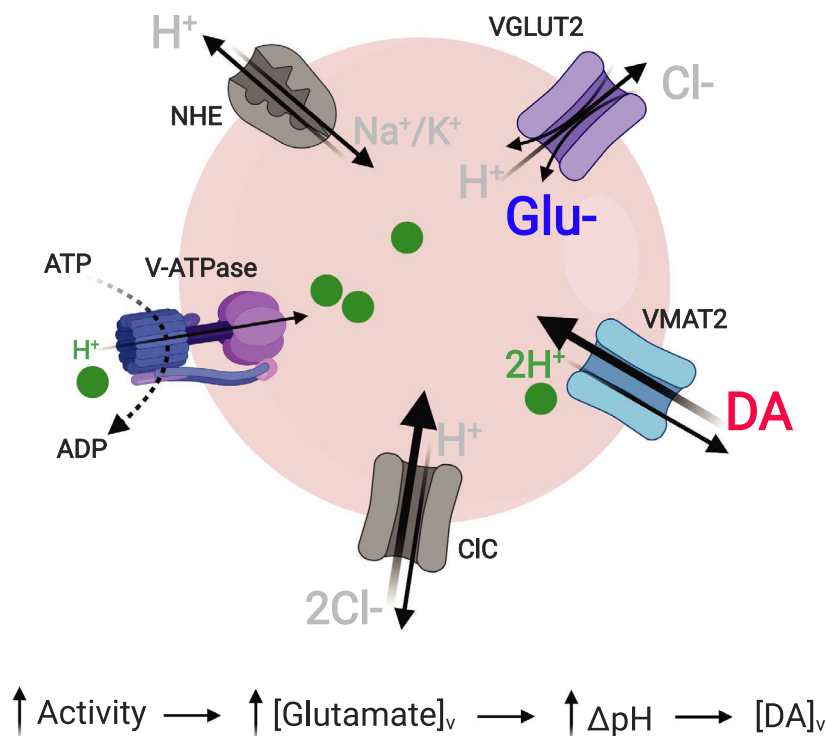
Fig. 3. Model for drug-induced synaptic plasticity via signal integration of DA and glutamate co-transmission from VTA to NAc. Drugs of abuse increase DA cell firing in the VTA and induce the co-release of DA and glutamate from presynaptic medial VTA DA neurons (left panel). DA and glutamate signaling is integrated by postsynaptic medium spiny neurons in the NAc via activation of DA receptors (D₁ and D₂), and glutamatergic AMPA receptors (AMPA) and NMDA receptors (NMDAR) (right panel); all four receptor subtypes (D₁, D₂, AMPAR, NMDAR) are shown on the same cell for simplicity. Concurrent activation of postsynaptic DA and glutamate receptors in the NAc integrates signaling from these different receptors that may be unique from signaling induced by stimulation of either DA or glutamate receptors alone. For drugs of abuse, this DA/glutamate receptor co-stimulation can lead to induction of ERK-dependent intracellular signaling pathways events involved in regulating synaptic function and plasticity critical for drug reward and reinforcement.

glutamate are necessary to increase acidification of synaptic vesicles during periods of heightened neuronal firing. Since the vesicular pH gradient (ΔpH) is the primary driving force for DA loading [80–83], activity-dependent vesicular hyperacidification enables tuning of vesicular DA loading and release in response to firing frequency [76,79]. These findings place presynaptic glutamate into a unique physiological context within DA neurons as facilitators of neurotransmitter loading through effects on vesicular pH regulation, rather than strictly as a neurotransmitter acting on postsynaptic receptors. Moreover, these data suggest that glutamate enables DA neurons to meet the demands of high-frequency burst firing and therefore to sustain adequate vesicular DA loading and release over time during exposure to drugs of abuse, enabling these drugs' reinforcing properties at the molecular level (Fig. 4). Lastly, we and others showed that VGLUT2 expression can be modified in response to cell stress, particularly in VTA DA neurons, emphasizing the importance of glutamate and VGLUT2 in the

plasticity of these DA neurons [84–87]. Furthermore, we find that DA neuron VGLUT expression is similarly altered in response to agents that change synaptic DA levels such as psychostimulants like amphetamine or reserpine (unpublished data, Silas A. Buck, Mary M. Torregrossa, Ryan W. Logan, Zachary Freyberg), further suggesting that DA neuron VGLUT2 expression may be a critical component of the machinery for neuronal plasticity by maintaining homeostatic control over synaptic DA levels.

Despite the above findings, DA/glutamate vesicular synergy remains controversial. There are currently conflicting reports on the extent to which (a) DA and glutamate are released from the same presynaptic site within a neuron [88,89], and (b) VGLUT2 and vesicular monoamine transporter 2 (VMAT2), the vesicular DA transporter, are localized to the same vesicle [16,76,90–92]. Support for vesicular co-localization of VMAT2 and VGLUT2 primarily comes from earlier biochemical and functional data. Hnasko *et al.* [16] showed that VGLUT2⁺ synaptic vesicles isolated from

Fig. 4. Model for VGLUT2-mediated synaptic vesicle hyperacidification and increased DA loading. We propose a model for VGLUT2- and glutamate-mediated increases in synaptic vesicle acidification and content in DA terminals in response to neuronal stimulation, as follows: (a) Increased neuron activity causes a rise in cytoplasmic Na^+ . (b) Influx of Na^+ into vesicles via Na^+/K^+ exchangers (NHE) increases the vesicle membrane potential ($\Delta\Psi$), the main driving force for glutamate entry. (c) The resulting buildup of intraluminal negative charge from the increased vesicular glutamate ($[\text{glutamate}]_v$) increases the vesicular proton-motive force, causing the V-ATPase to pump more H^+ into the vesicle, increasing the vesicular pH gradient (ΔpH). (d) The elevated ΔpH increases the driving force for vesicular DA loading via VMAT2, increasing overall DA vesicle content ($[\text{DA}]_v$).



rat ventral striatum are also VMAT2⁺ and *vice versa* via reciprocal immunoprecipitation. Additional electrophysiological data also demonstrated that VGLUT2 knockdown in DA neurons decreases evoked DA release according to *ex vivo* fast-scan cyclic voltammetry, and that glutamate stimulates vesicular DA uptake [16]. Similarly, VGLUT2 knockdown eliminates activity-dependent vesicular DA loading, further suggesting a direct role for VGLUT2 in DA vesicle function [76]. Nevertheless, to date, there is no ultrastructural evidence demonstrating co-localization of VMAT2 and VGLUT2 to the same synaptic vesicles. Instead, several studies have shown that VGLUT2 and VMAT2 segregate in discrete subpopulations of vesicles in the same axons of mesoaccumbens fibers, including at separate sites of release unique to DA versus glutamate vesicles [39,40,89,90,93,94]. Consistent with this, recent work showed that midbrain DA neurons release DA and glutamate with different properties (i.e., release probabilities, modes of release), reflecting storage in different DAergic and glutamatergic synaptic vesicle populations [90].

It may still be possible that a subset of DA neurons that co-transmit glutamate release both glutamate and DA from the same synaptic site and/or contain VGLUT2 and VMAT2 on the same vesicle. Because VGLUT2 is specifically required for activity-dependent changes in DA vesicle loading, the above differences in

findings may reflect activity-dependent changes in vesicular co-release and co-localization of VGLUT2 and VMAT2. We propose that changes in neuronal activity may alter the trafficking of VMAT2 or VGLUT2 to synaptic vesicles to facilitate vesicular synergy and co-release. Indeed, work suggests that VGLUT2 expression and trafficking in neurons is altered in response to changes in activity [95,96]. Further work is required to clarify these outstanding issues and their relevance to mechanisms of action for drugs of abuse. Together, these findings point to a role of DA neuron glutamate release in mediating the effects of drugs of abuse. Below, we review the current findings on DA neuron glutamate release in mediating cocaine-, opioid-, and alcohol-induced changes in DA neuron function and in the behaviors associated with these drugs.

Psychostimulants: cocaine and amphetamine

Recent studies have increasingly linked glutamate release from medial VTA DA neurons to the cellular and behavioral effects of cocaine. Conditional knockout of VGLUT2 in DA neurons, which eliminates the ability of DA neurons to release glutamate, blunts locomotor sensitization to cocaine [16,21,76] and disrupts reward consumption in response to cocaine [20]. At the postsynaptic level, conditional knockout of

VGLUT2 in presynaptic DA neurons, which reduces glutamate co-release from DAergic terminals in the NAc, leads to an upregulation of AMPA/NMDA receptor ratios on accumbal D₁ receptor-expressing medium spiny neurons [97]. VGLUT2 conditional knockout also occludes cocaine-induced upregulation of cell excitability mediated by AMPA/NMDA receptors [97]. In parallel, the coordinated release of DA alongside glutamate seems to participate in integrating cocaine-associated synaptic plasticity. These are precisely the types of synaptic changes that have been linked previously to cocaine reward-related behaviors [51,98,99].

DA/glutamate co-release is also important for the behavioral effects of other psychostimulants including amphetamines. Conditional VGLUT2 knockout in mice reduces amphetamine-induced locomotion [21,97]. Similar deficits in amphetamine sensitization have also been described after DA neuron-specific conditional heterozygous reduction of phosphate-activated glutaminase expression, the enzyme responsible for glutamate recycling [41]. Likewise, in *Drosophila*, RNAi-mediated VGLUT knockdown in DA neurons decreases amphetamine-stimulated hyperlocomotion [76]. Together, these findings indicate that glutamate release from medial VTA DA neurons in the mNAcSh is a critical regulator of the behavioral effects of psychostimulants.

Opioids

The precise contributions of glutamate co-released with DA to opioid-related behaviors remain poorly characterized. However, recent work by Luscher and colleagues showed that, in mice, reinforcement by optogenetic VTA GABA self-inhibition and reinforcement by heroin self-administration share underlying neural circuits and are compatible with a disinhibitory mechanism where heroin targets GABA neurons. This leads to an increase in activity of medial VTA DA neurons that project to the mNAcSh [22]. In fact, a single heroin injection can obstruct this optogenetically driven VTA GABA self-inhibition mechanism and increase DA activity [22]. To date, no studies have specifically manipulated DA neuron glutamate co-release in the context of opioid-related DA neuron function or behavior. However, recent studies, including those using intersectional genetic strategies, confirmed that the majority of medial VTA-mNAcSh-projecting DA neurons significantly express VGLUT2 and co-release glutamate [15,41–44]. Because virtually all DA neurons transiently express VGLUT2 during development [39,84,100], we cannot rule out that the joint expression of Cre and Flp recombinases in VGLUT2⁺

DA neurons (required for intersectional genetic strategies) may nonphysiologically modify DA neuron VGLUT2 expression; additional studies are required to validate these results. Nevertheless, the above data strongly suggest that the co-release of glutamate from these DA neurons specifically in the mNAcSh plays a critical role in mediating the effects of opioids. Future experiments are highly warranted to test this intriguing hypothesis.

Alcohol

Glutamate release plays a pivotal role in alcohol reinforcement and motivation. Ethanol administration elevates extracellular glutamate in the NAc in a dose-dependent manner [25], and these changes are critical for establishing and maintaining learning and motivation events associated with alcohol dependence and relapse behaviors [101–105]. Furthermore, genetic vulnerability to alcohol use disorders involves alterations in the glutamatergic system, including those involving VGLUT2 expression and function [25,106]. Indeed, VGLUT2 expression changes in ethanol-consuming rats that were previously exposed to early-life stress [107]. Moreover, chronic stress leads to altered expression of glutamatergic receptors in the NAcSh and increases both the locomotor activating effects and motivation for alcohol. These data suggest that glutamate-related plasticity and potentially glutamate release from VTA DA neurons underlie vulnerability to the effects of alcohol [108]. While the direct role of DA neuron glutamate release on alcohol-related neurological and behavioral changes has not been investigated, these findings provide indirect evidence of the importance of glutamate release in the NAc, from DA neurons and other inputs, on mediating the effects of alcohol.

Future directions and conclusions

There are many outstanding questions around the roles of DA/glutamate co-release that need to be resolved. First, some medial VTA neurons that project to the mNAcSh may be purely glutamatergic [42,44,109] and play a role either complementary to or separate from DA/glutamate co-releasing neurons. For example, the NAc shell receives glutamatergic projections from other brain regions including the ventromedial prefrontal cortex which has been implicated in the reinstatement of opioid-seeking behaviors [110–113]. Second, since VGLUT2 function and expression may be critical for tuning DA vesicle loading and release in the context of neuronal activity, there is the intriguing

possibility that VGLUT2 expression in presynaptic DA neurons may serve as a rheostat of DA neuron function. Thus, when synaptic DA levels diminish, DA/glutamate neurons may upregulate VGLUT2 expression and/or function to facilitate activity-dependent DA loading and release to maintain homeostasis of synaptic DA levels. Additionally, at the presynaptic level, besides the respective single and combined roles of DA and glutamate from the medial VTA-mNACSh-projecting neurons, the mechanisms regulating the synthesis and loading of both neurotransmitters remain poorly understood. Furthermore, the local circuitry responsible for direct and indirect control over release from these co-transmitting terminals, and how these regulatory circuits respond to drugs of abuse, are relatively unknown. For example, Rayport and colleagues have identified mNACSh cholinergic interneurons as a preferential target of glutamate release from DA neurons, as glutamate co-release from DA neurons drives burst firing of mNACSh cholinergic interneurons [15,114]. Cholinergic interneurons in the mNACSh have also been implicated in reward processing and aversive learning, and they are activated by cocaine self-administration [115–119]. Together, these findings suggest glutamate release from DA neurons may mediate some actions of drugs of abuse by activating cholinergic interneurons in the mNACSh. Future studies should investigate this possibility as well as potential downstream effects of cholinergic interneuron activation by DA/glutamate co-release.

At the postsynaptic level, the specific functional relevance of DA/glutamate co-transmission remains unclear. Just as glutamate may facilitate tuning of activity-dependent vesicular DA loading and release presynaptically, we propose that concurrent stimulation of DA and glutamate receptors may similarly tune postsynaptic signaling in the striatum, particularly in the mNACSh. Co-stimulation of different populations of DA and glutamate receptors would therefore enable an integration of different intracellular signaling pathways. Importantly, this raises the possibility that different receptor combinations (i.e., D₁/AMPA versus D₂/AMPA versus D₃/NMDA) give rise to unique postsynaptic responses (Fig. 3). Such a model would provide a broad palette of potential postsynaptic responses in keeping with the dynamic nature of the striatal circuitry. Just as importantly, such a model may enable further tuning of the postsynaptic signal based on the different frequencies of presynaptic firing—that is, phasic versus tonic DA release, particularly in the context of different drugs of abuse. For example, do different opioids differen-

tially modulate the relative amounts of DA and glutamate co-released in the mNACSh based on their distinct effects on firing and release from the nerve terminals? These drug-specific effects on frequency and duration of firing could have vast implications with regards to different combinations of receptors being stimulated in different sequences and at different temporal intervals. This may ultimately explain drug-specific differences in time-scale and magnitude of changes to the DA system, including among drugs of the same class.

In summary, the discovery of DA/glutamate co-releasing neurons within the last two decades has opened up a wide range of speculation of these cells' possible roles, from reward and SUDs [15,21,41,97,120] to DA neuron degeneration and Parkinson's disease [84–87]. Research has established that the majority of these co-transmitting neurons project to the mNACSh, where DA release is modulated by drugs of abuse. Indeed, glutamate release from DA neurons has been directly linked to cocaine consumption and locomotor sensitization, as well as indirectly linked to opioid- and alcohol-related changes in behavior. The role of DA neuron glutamate release on mediating the effects of drugs of abuse appears to be linked to its excitatory action on cholinergic interneurons in the mNACSh. Ultimately, we propose that the medial VTA neurons that co-release both DA and glutamate act as 'integrators' of these two signals. We also introduce the idea that there may be 'emergent' or synergistic properties that arise at the postsynaptic level in the targeted postsynaptic neurons that either transmitter alone could not accomplish. Might this foster increased synaptic plasticity that gives rise to the learned or reinforced behaviors associated with drugs of abuse? In all, current research points to DA neuron glutamate release as an essential aspect of how drugs of abuse exert their effects, opening up a new therapeutic avenue for treating SUDs.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

ZF, RWL, SAB, and MMT conceived the ideas and wrote the manuscript.

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