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Unraveling the dynamics of dopamine release and its actions on target cells

Tanya Sippy[1,2], **Nicolas X. Tritsch**[1,3]

[1]Neuroscience Institute, New York University Grossman School of Medicine, United States

[2]Department of Psychiatry, New York University Grossman School of Medicine, United States

[3]Fresco Institute for Parkinson's and Movement Disorders, New York University Langone Health, United States

Abstract

The neuromodulator dopamine (DA) is essential for regulating learning, motivation, and movement. Despite its importance, however, the mechanisms by which DA influences the activity of target cells to alter behavior remain poorly understood. In this review, we describe recent methodological advances that are helping overcome challenges that have historically hindered the field. We discuss how the employment of these methods is shedding light on the complex dynamics of extracellular DA in the brain as well as how DA signaling alters the electrical, biochemical and population activity of target neurons *in vivo*. These developments are generating novel hypotheses about the mechanisms through which DA release modifies behavior.

Keywords

neuromodulation; movement; reinforcement learning; reward; striatum; excitability; synaptic plasticity

Bridging the actions of DA from cells to behavior

The neuromodulator DA is firmly established as a key regulator of learning, motivation, and movement under both physiological and pathological conditions [1,2]. Over the past several decades, significant efforts have been devoted to revealing how DA contributes to behavior in mammals by characterizing the activity of DA-releasing neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) *in vivo*, and by dissecting the effects of DA on target cells *in vitro*. These studies provide the foundations upon which current models of DA function are built. *In vivo* studies established, for example, that DA neurons

*Correspondence: Tanya.Sippy@nyulangone.org (T. Sippy) and Nicolas.Tritsch@nyulangone.org (N.X. Tritsch).

Declarations of Interests

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show a variety of discharge patterns that have broadly been assigned to one of two categories called tonic and phasic [3,4]. Tonic refers to the spontaneous – possibly cell-intrinsic [5,6] – firing of 2 to 8 action potentials per second that provide target neurons with a basal level of DA and has been proposed to serve a permissive role in movement [2]. Phasic refers to brief (a few hundred milliseconds-long) increases or decreases in firing of DA neurons (i.e. bursts and pauses, respectively) that have been strongly linked to encoding of reward prediction errors (RPEs) and promoting reinforcement learning [2].

Upon release, DA can act on any one of 5 subtypes of G protein-coupled receptors (GPCRs) D1 through D5, which can localize to various cellular compartments (e.g., soma, dendrites or axons) and target cells (e.g., excitatory and inhibitory neurons, as well as glial cells). These receptors segregate into two families, depending on the class of G protein they activate: D1-like receptors (D1 and D5) couple to $G\alpha_{s/olf}$ to stimulate cyclic adenosine monophosphate (cAMP) production by adenylate cyclases, whereas D2-like receptors (D2, D3 and D4) couple to $G\alpha_{i/o}$ to inhibit adenylate cyclases and cAMP production [7]. cAMP activates several effector molecules, the main one being protein kinase A (PKA), which acts as a key regulator of cellular function through protein phosphorylation. However, the specific physiological mechanisms and time course through which intracellular effectors recruited by DA modulate the activity of target neurons to affect behavior remain poorly understood. In this review, we first discuss new approaches for studying the dynamics of DA release *in vivo*. We then consider some of the challenges associated with studying the functional effects of DA signaling on target cells and discuss how novel approaches to study of DA signaling *in vivo* are challenging traditional models of DA function. This review mainly focuses on dopaminergic neurons of the SNc and VTA and their densest target – the striatum – in the nervous system of rodents.

Understanding DA through its release dynamics

A key element to understanding how DA functions is to define when and where DA is released during behavior. Microdialysis and voltammetry both provide quantitative measures of DA concentration, but these methods have notable limitations: microdialysis lacks the ability to resolve fast DA signals, and voltammetry is relatively difficult to implement in behaving animals [8]. Extracellular spike recordings from SNc and VTA neurons have been used extensively *in vivo* as a proxy for DA release in the striatum. However, the validity of this approach has been called into question; one study, for instance, reported that the slow changes in DA in the ventral striatum that correlate with a rat's motivation to engage in an operant task are not reflected in the firing rate of VTA neurons [9]. Others, building upon prior work [10–14], showed that acetylcholine (ACh) release in striatal slices from mice and primates is sufficient to evoke action potentials in DA axons via direct activation of nicotinic ACh receptors on DA terminals [15,16]. Although the extent to which these mechanisms contribute to DA release *in vivo* remains unknown, it is clear at this point that the release dynamics of DA on both fast and slow timescales cannot be presumed to be a simple outcome of DA neuron firing, but needs to be directly and specifically monitored in the brain region of interest during behavior.

Two imaging techniques have been adopted to address this need. The first relies on the expression of bright and sensitive genetically-encoded calcium indicators (GECIs) like GCaMP6 [17] and its more recent variants [18,19] to infer DA release based on the activity of specific populations of DA axons. The other enables direct monitoring of extracellular DA levels by expressing a genetically-encoded fluorescent biosensor for DA belonging to the dLight [20,21] and GRAB-DA [22,23] families. Both methods are amenable to either one- or two-photon microscopy in behaving animals. One concern with these families of sensors is that they are typically overexpressed and might function to buffer signaling molecules (e.g. calcium or released neuromodulator) away from their intended molecular targets, thereby depriving sensor-expressing cells of important intracellular signaling cascades. Despite these caveats, studies employing these approaches have already yielded notable insights. For example, mesoscopic imaging revealed the existence of wave-like spatiotemporal patterns of DA axon activity across the medio-lateral axis of the dorsal striatum of mice during periods of immobility and in the absence of sensory stimuli [24]. Intriguingly, the directionality of these waves depended on the conditioning paradigm (Pavlovian vs. operant). Moreover, calcium imaging from DA axons in the striatum and cell bodies in the SNc and VTA demonstrated that DA neurons respond phasically not only to reward and to reward-predicting cues, but also to sensory, motor and cognitive variables [25–27]. This observation was independently confirmed using electrophysiological recordings from optogenetically-identified DA neurons [28–32]. In addition, optogenetic manipulations of DA neurons affect the probability of initiating movement [27] and contribute to resilience-associated behavior [33], further expanding the known influences of phasic DA beyond reward signaling.

Phasic increases or decreases in DA release may not be the only modes by which DA encodes reward-based signals. A recent study utilizing GCaMP6 photometry identified a slow increase in the activity of VTA axons ~10 minutes after thirsty mice consumed water [34]. This protracted ‘post-ingestive’ response was required for learning about the hydration properties of fluids, indicating that prolonged changes in DA levels may also contribute to behavioral reinforcement. This is consistent with other recent studies showing that RPE signals can manifest as slow, gradual ramps in the discharge of DA neurons and in the accumulation of extracellular DA in the striatum during operant tasks [35–37]. Together, these findings blur the simplistic distinction between tonic and phasic signaling and their respective roles in movement and learning, and suggest that DA signaling is far more complex than previously thought. Indeed, there is a growing appreciation that phenomena previously described as steady ‘tonic’ levels of DA, based on the seemingly-random spontaneous discharge of individual DA neurons, might actually reflect population-level DA ramps and periodic fluctuations that take place on time scales ranging from seconds to minutes or even hours [38,39].

The challenges of deciphering the actions of DA on target cells

Despite considerable progress in characterizing the activity of DA-releasing neurons and the dynamics of extracellular DA levels (see above), the specific physiological mechanisms and time course through which DA modulates the activity of neurons in target brain regions to affect behavior remain poorly understood (Figure 1A). An important challenge associated

with deciphering the effects of DA on downstream circuits is that DA release may occur via mechanisms beyond those established at canonical synapses: DA axons form varicosities [40], many of which are not competent to release DA through activity-dependent vesicular exocytosis [41,42] and undergo pronounced depression upon repeated stimulation [38,43]. Once released, DA is believed to act via volume transmission, binding to receptors that lie some distance away from release sites and that are not localized within well-defined postsynaptic specializations [44]. In addition, DA neurons also release DA through vesicular exocytosis from their soma and dendrites, where it acts on D2 auto-receptors to negatively modulate somatic firing [45,46].

Another major challenge is that unlike neurotransmitters such as glutamate and GABA that act on ligand-gated ion channels, DA acts exclusively through GPCRs. Thus, DA receptors do not directly evoke membrane currents that depolarize or hyperpolarize target neurons [7,47]. Instead, the actions of DA on any given cell vary with the type of receptor(s), second messengers and effector proteins expressed, and with the electrical and biochemical state of that cell, making the effects of DA remarkably diverse and highly cell- and context-dependent. For example, in brain slices, DA has been shown to increase or decrease the release probability of presynaptic GABAergic and glutamatergic synapses, strengthen or weaken postsynaptic GABAergic and glutamatergic signaling, and elevate or depress the intrinsic excitability of neurons [7,48]. DA also alters synaptic transmission by evoking calcium signals and transmitter release from astrocytes [49,50], and promotes or hinders the formation of new dendritic spines and their structural plasticity [51–54]. Recent studies have also drawn attention to the fact that optogenetic manipulations need to be properly calibrated, as non-physiological stimulation can produce behavioral effects that endogenous DA release cannot [55].

Lastly, it is technically and conceptually difficult to appreciate the time course of modulation by DA; DA levels fluctuate constantly on both short and long timescales *in vivo* [2,38] and GPCRs impose their own temporal constraints, affecting cellular physiology over a multitude of time scales ranging from hundreds of milliseconds to minutes or even hours [56] (Figure 1B). Thus, the effects of DA on target neurons are complex and time-varying, and any simplification of DA signaling depicted in circuit diagrams as a generic ‘plus’ or ‘minus’ sign should be approached with caution.

Classical view of DA modulation in the striatum

The striatum is a subcortical brain region whose neuronal population is largely composed of GABAergic spiny projection neurons (SPNs) that express either D1 or D2 receptors. The mutually exclusive expression of D1 or D2 receptors by SPNs is well established in the mouse, particularly so in the dorsal striatum [57]. This distinction may not be as clear in the ventral striatum or in other species, and other neurons in the striatum and elsewhere have been shown to co-express D1 and D2-type DA receptors. While the ultimate goal is to understand how DA acts in these more complex scenarios, a logical first step is to clarify the effects of D1 and D2 receptor activation in a system where they can be clearly separated at the cellular level. Within this framework, pioneering work in the rodent dorsal striatum converged on a model in which DA elevates the activity of D1 receptor-expressing striato-

nigral SPNs and depresses that of D2 receptor-expressing striato-pallidal SPNs [58,59] (Figure 2). This view is largely supported by studies in dissociated preparations and brain slices utilizing prolonged pharmacological stimulation of DA receptors. For example, DA has been shown to promote the long-term potentiation (LTP) of glutamatergic synapses from neocortical projections that impinge on D1-SPNs and the long-term depression (LTD) of excitatory inputs onto D2-SPNs through a combination of pre- and post-synaptic mechanisms [60,61]. DA has also been suggested to bias striatal output in favor of D1-SPNs through short-term plasticity of postsynaptic glutamate receptors, and through presynaptic modulation of GABA release from SPN collaterals [7,57,62].

Another important way in which DA is believed to alter striatal output is by dynamically modifying the somatodendritic excitability of SPNs. Stimulating DA receptors does not evoke any apparent changes in the resting membrane potential of SPNs, as these cells do not express G protein-gated inwardly rectifying potassium (GIRK) or hyperpolarization-activated cyclic nucleotide–gated (HCN) channels [7,57]. However, DA affects the gating properties and conductances of several voltage-gated sodium, potassium and calcium channels that control how many action potentials SPNs fire in response to a constant excitatory input [7,47]. A recent study demonstrated that a single bout of optogenetic stimulation of DA axons evokes a robust increase in the intrinsic excitability of D1-SPNs [63], consistent with previous work using pharmacological activation of DA receptors [64,65]. The synaptic effects of DA arose within half a second, indicating that DA can rapidly adjust the gain of striatal output. Surprisingly, the effects of DA can be extremely long-lasting, persisting for over 10 minutes, suggesting that the excitability of D1-SPNs exposed to constant DA neuron firing *in vivo* may be persistently elevated (Figure 1B). Evidence for negative modulation of excitability in D2-SPNs has been harder to yield, with several studies showing inconsistent effects of D2 receptor agonists on voltage-gated potassium and sodium channels, and little-to-no effects on depolarization-evoked somatic spiking [66–69].

Despite these mechanistic insights, important questions remain about the specific modulatory effects of DA, their magnitude and time course, particularly *in vivo* and in behaving animals. Indeed, the striatal environment *in vivo* is markedly different from that *ex vivo*. First, SPNs in brain slices rest at a membrane potential approximately 20 mV more negative than *in vivo*, in part because SPNs are exposed to a constant barrage of excitatory synaptic inputs in the awake brain [70–72]. Second, extracellular DA levels are exceedingly low in striatal slices as DA afferents are severed, unlike the situation *in vivo*, where SPNs are constantly exposed to fluctuating DA levels [9,26,73]. It is therefore conceivable that some of the prolonged modulatory effects described *in vitro* are occluded *in vivo*. Lastly, striatal output is shaped by the dynamic interplay of many cellular and synaptic elements, including striatal interneurons and afferent activity patterns [74–76]. For instance, phasic activation of DA neurons can rapidly pause the spontaneous discharge of cholinergic interneurons via the activation of D2 receptors [77–80] and trigger a delayed burst of activity in these neurons through glutamate corelease [81,82]. In turn, acetylcholine release has been shown to modulate the activity of SPNs and striatal interneurons, as well presynaptic release from glutamatergic and dopaminergic afferents [10–16,83–91]. The modulatory effects of DA axons on striatal output can therefore only be adequately evaluated by factoring in

population dynamics during behavior. Indeed, recent work has begun to incorporate neural activity dynamics across recurrently-connected networks to better account for how DA shapes learning and behavior [92,93].

Novel insights into DA signaling in target neurons

Many of the methods discussed above that are used to record from DA neurons are also being utilized to identify and record from specific populations of DA-recipient neurons, including D1- and D2-SPNs. For example, the activity of these neurons can be monitored as a population using bulk fluorescence from fiber photometry [94–96], or at single-cell resolution using one-photon head-mounted miniature microscopes in freely behaving animals or two-photon imaging in head-restrained animals [97–101]. In addition, electrophysiology methods have been employed in combination with optogenetics to “phototag” recorded cells, thereby enabling spiking measurements from genetically-identified DA-recipient neurons [102,103]. These recordings offer high temporal resolution but are typically lower-yield than imaging methods and do not easily allow for longitudinal recordings over days.

A burgeoning and promising avenue of research entails imaging of downstream molecular targets of DA receptors in specific cell types. In recent years, significant progress has been made in developing genetically-encoded optical sensors for intracellular cAMP levels and PKA activity [104–106]. Most of these sensors consist of protein sequences that undergo conformational changes upon physically binding to cAMP or being phosphorylated by PKA, and rely on Förster resonance energy transfer (FRET) or circularly permutated fluorescent proteins to generate photons that can be imaged by conventional fluorescence or lifetime microscopy and photometry. Their sensitivity and kinetics depend on the specifics of the molecular sensor being used, in much the same way that various calcium dynamics can be revealed using GCaMP variants that show different on/off binding kinetics and that localize to different subcellular compartments [17,18]. Importantly, these sensors reflect the net balance between the rate of cAMP production/PKA activation and the activity of enzymes catalyzing the degradation of cAMP (e.g., phosphodiesterases) and the dephosphorylation of PKA substrates (e.g., phosphatases). Thus, the fluorescence of PKA sensors may increase as a result of an elevation in cAMP or a decrease in the activity of intracellular phosphatases. An additional consideration is that these sensors should not be construed as reflecting DA receptor signaling exclusively or in its entirety, as DA receptors can signal through other pathways than adenylate cyclases, cAMP can activate other second messengers in addition to PKA, and other GPCRs and intracellular signaling cascades can converge onto the same effectors, including intracellular $G\alpha_q$-coupled receptors, intracellular calcium levels and other protein kinases and phosphatases [107–110]. Still, these approaches have already led to a flurry of observations highlighting how incompletely-understood *in vivo* DA signaling is, as well as calling for significant revisions to the classic view that DA bi-directionally controls the discharge of D1- and D2-SPNs in equal yet opposite ways.

For instance, a recent study using fluorescent lifetime photometry of the PKA phosphorylation sensor FLIM-AKAR uncovered that net PKA activity in D1- and D2-SPNs in the nucleus accumbens is differentially sensitive to phasic increases and decreases in

DA [111]. During a behavior that elicited positive and negative RPEs, dopamine transients resulted in changes in PKA activity within seconds that lasted for tens of seconds. In D1-SPNs, net PKA activity increased when extracellular DA levels rose, but it did not decrease when DA levels fell below baseline. By contrast, net PKA activity in D2-SPNs increased in response to phasic dips in extracellular DA, but remained unchanged upon phasic elevations in DA (Figure 2C, middle). This asymmetric modulation of PKA activity has been suggested to reflect differences in the basal occupancy of D1 and D2 receptors resulting from the higher affinity of D2 receptors for DA relative to D1 receptors [112]. However, previous work *in vitro* leveraging the ability of D2 receptors to recruit GIRK channels demonstrated that they are capable of detecting phasic increases in DA [113,114]. In addition, D1 and D2 receptor-based fluorescent DA sensors are both capable of reporting increases and decreases in extracellular DA, suggesting that the observed asymmetric modulation of PKA may arise downstream of DA receptors. Other GPCRs expressed by SPNs converge on the same intracellular signaling cascades and compete with DA for PKA activation, the best-known being $G\alpha_i$-coupled muscarinic M4 receptors in D1-SPNs and $G\alpha_s$-coupled adenosine A2A receptors in D2-SPNs. It is therefore increasingly evident that the actions of DA on SPNs cannot be understood without also factoring in modulators like acetylcholine and adenosine, whose levels in the brain, like DA, vary dynamically ([90,115,116], as well as preliminary data from our group [117]). In fact, net PKA activity was surprisingly found to increase in D2-SPNs during locomotion because locomotion is also associated with an increase in adenosine, which counters the inhibitory effects of DA on adenylate cyclase via the activation of A2A receptors [115]. Similarly, a recent *in vivo* study reported that synaptic potentiation in SPNs necessitates synaptic depolarization to coincide with both an increase in extracellular DA and a decrease in ACh, consistent with the idea that $G\alpha_i$-coupled M4 receptors oppose the actions of DA on PKA signaling in D1-SPNs [118].

As mentioned, these recent findings indicate that D1- and D2-SPNs specialize in the respective detection of phasic increases and decreases in DA. This notion is in line with previous theoretical models suggesting that both populations of SPNs serve independent, temporally-dissociated functions during learning [119,120], with D1-SPNs specializing in learning from unexpected rewards and positive outcomes (i.e. positive RPEs), and D2-SPNs specializing in signaling when expected rewards are omitted (i.e. negative RPEs). Indeed, experiments in brain slices showed that PKA-dependent structural plasticity is only engaged in D1-SPNs when DA levels increase [51] and in D2-SPNs when DA levels decrease [54]. These plasticity mechanisms have been proposed to underlie stimulus generalization during Pavlovian conditioning to sensory cues via D1 detection of DA phasic increases, whereas stimulus discrimination required D2 signaling in response to phasic DA decreases. [54]. DA dip-evoked structural long-term potentiation in D2-SPNs relied on the activation of the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) downstream from PKA [54].

Of note, the time course of fluorescence changes of current sensors in response to brief elevations in DA are remarkably prolonged (10s of seconds to minutes) [111,115]. This raises fundamental questions about the ability of SPNs to resolve individual DA transients that occur on sub-second time scales, such as those evoked by RPEs that occur in close succession during Pavlovian and operant conditioning paradigms (see Outstanding questions).

What about the effects of DA on the spiking output of target neurons? Several studies have leveraged the ability to express genetically-encoded calcium indicators in either D1- or D2-SPNs to monitor the activity of both populations either simultaneously or in separate cohorts of mice. These studies have repeatedly found that calcium signals in D1- and D2-SPNs are comparable in time course and magnitude, indicating that striatal DA does not persistently depress D2-SPNs relative to D1-SPNs [94,98,100]. Instead, the spatiotemporal patterns of both D1 and D2 SPN activation are strongly correlated with ongoing behavior, with separate groups of neurons showing preferential activation during specific motor actions [95,97,101].

Importantly, SPN calcium activity is strongly modulated by acute pharmacological manipulations of DA signaling [98,100,101]: blocking DA receptors elevated the activity of D2-SPNs and depressed that of D1-SPNs, suggesting that decreasing DA levels unbalances striatal output towards D2-SPNs. By contrast, elevating DA receptor signaling using either pharmacological agonists or blockers of presynaptic DA transporters such as cocaine depressed the activity of both D1- and D2-SPNs within minutes while still biasing striatal output in favor of D1-SPNs (Figure 2C**, right**). This latter observation is inconsistent with classic models of DA modulation that predict a net potentiation of D1-SPN activity relative to baseline with elevated DA (Figure 2C**, left**). Interestingly, these modifications were most evident in the total number of D1- and D2-SPNs recruited during behavior, rather than in the frequency or amplitude of calcium transients displayed by individual SPNs, revealing an additional dimension of DA signaling at the level of neuronal populations [98].

It is important to note that the frequency of calcium transients in SPNs is significantly lower than the rate of action potential firing in SPNs, indicating that calcium imaging may not be appropriate for estimating the discharge of SPNs. Intracellular calcium signals in SPNs instead reflect bursts of action potentials [121,122] and may therefore best highlight somato-dendritic calcium events likely to promote synaptic plasticity [123,124]. By modulating the likelihood that SPNs respond to glutamatergic inputs with a burst of action potentials and a rise in somato-dendritic calcium, DA may control the number of neurons positioned to participate in calcium-dependent synaptic plasticity.

Concluding Remarks and Future Perspectives

In this review, we discussed how recent methodological advances are prompting a more nuanced and dynamical view of DA release and DA signaling in target neurons. These advances also blur the clear-cut distinction between phasic and tonic DA, and indicate that the differential effects of DA on reward-based learning vs. movement cannot simply be attributed to the patterns of DA neuron spiking or DA release. In addition, it is becoming increasingly clear that phasic DA does not exclusively signal RPEs, but also errors in sensory predictions (including from internal states) and in motor performance [125–127]. Classical notions of how DA acts *in vivo* on target neurons are also being revisited, as it is increasingly recognized that the effects of DA at the circuit-level are space-, time- and context-dependent, and as such cannot easily be inferred from single-cell studies *in vitro*. Much work therefore remains to understand how the rich patterns of DA release modify brain circuits to affect behavior over different time scales and in different contexts.

To this end, first, chronic lesion and systemic pharmacological manipulations that lack specificity and allow for cellular/network adaptations need to be complemented by spatially- and temporally-precise (and carefully-calibrated) manipulations like opto- and chemogenetics to manipulate endogenous DA activity. Second, methods of single-cell resolution need to be employed more frequently, to properly document the diversity of responses that DA produces and to allow more nuanced understanding of their physiological roles. Third, *in vivo* mechanistic studies need to be expanded, using electrophysiological approaches capable of assessing subthreshold membrane dynamics. For instance, whole-cell recordings in awake, behaving mice [70,71] combined with opto-tagging [128] should be levied to investigate the effects of DA on intrinsic excitability and synaptic strength in a cell type-specific manner. Alternatively, voltage imaging methods could be used; these methods are improving continuously and are showing great promise in revealing subthreshold dynamics across many cells simultaneously [129–132].

While this review focused primarily on the role of DA in the striatum of rodents, DA is released throughout the brain, and its physiological actions are likely to display both similarities and differences across brain regions and species. As mentioned above, even in the context of cells expressing D1- or D2-type DA receptors, the effects of DA are likely to vary depending on the patterns of DA release, the concentration and time course of DA in the extracellular space, the constellation of GPCRs expressed by target cells, the release patterns of other neuromodulators, the number of cellular elements sensitive to modulation by GPCRs, and the timing of DA release relative to neuronal activity. Thus, studies of DA function in other brain areas including the cortex [133] hippocampus [134], amygdala [135–137] and hypothalamus [138] of mammals as well as in the nervous system of songbird [127] , flies [139] and worms [140] will be key for garnering a holistic view of the range of modulatory mechanisms elicited by DA. Importantly, the approaches we reviewed here can be leveraged to study other neuromodulatory systems that work in concert with DA, including serotonin, norepinephrine and acetylcholine across several species. Such studies will provide a deeper appreciation of how neuromodulators exert their powerful effects on behavior in both health and disease.

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References

1. Klaus A et al. (2019) What, If, and When to Move: Basal Ganglia Circuits and Self-Paced Action Initiation. Annu Rev Neurosci 42, 459–483 [PubMed: 31018098]
2. Schultz W (2007) Multiple Dopamine Functions at Different Time Courses. Annual Review of Neuroscience 30, 259–288
3. Grace AA and Bunney BS (1984) The control of firing pattern in nigral dopamine neurons: single spike firing. J. Neurosci 4, 2866–2876 [PubMed: 6150070]
4. Grace AA and Bunney BS (1984) The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4, 2877–2890 [PubMed: 6150071]

5. Kita T et al. (1986) Electrical membrane properties of rat substantia nigra compacta neurons in an in vitro slice preparation. Brain Res 372, 21–30 [PubMed: 3708356]
6. Fujimura K and Matsuda Y (1989) Autogenous oscillatory potentials in neurons of the guinea pig substantia nigra pars compacta in vitro. Neurosci Lett 104, 53–57 [PubMed: 2812536]
7. Tritsch NX and Sabatini BL (2012) Dopaminergic Modulation of Synaptic Transmission in Cortex and Striatum. Neuron 76, 33–50 [PubMed: 23040805]
8. Wu Z et al. (2022) Pushing the frontiers: tools for monitoring neurotransmitters and neuromodulators. Nat Rev Neurosci 23, 257–274 [PubMed: 35361961]
9. Mohebi A et al. (2019) Dissociable dopamine dynamics for learning and motivation. Nature 570, 65–70 [PubMed: 31118513]
10. Rice ME and Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. Nat Neurosci 7, 583–584 [PubMed: 15146188]
11. Cachope R et al. (2012) Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: Setting the tone for reward processing. Cell Reports 2, 33–41 [PubMed: 22840394]
12. Zhou FM et al. (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci 4, 1224–1229 [PubMed: 11713470]
13. Zhang H and Sulzer D (2004) Frequency-dependent modulation of dopamine release by nicotine. Nat Neurosci 7, 581–582 [PubMed: 15146187]
14. Threlfell S et al. (2012) Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. Neuron 75, 58–64 [PubMed: 22794260]
15. Kramer PF et al. (2022) Synaptic-like axo-axonal transmission from striatal cholinergic interneurons onto dopaminergic fibers. Neuron DOI: 10.1016/j.neuron.2022.07.011
16. Liu C et al. (2022) An action potential initiation mechanism in distal axons for the control of dopamine release. Science 375, 1378–1385 [PubMed: 35324301]
17. Chen T-W et al. (2013) Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300 [PubMed: 23868258]
18. Dana H et al. (2019) High-performance calcium sensors for imaging activity in neuronal populations and microcompartments. Nat Methods 16, 649–657 [PubMed: 31209382]
19. Zhang Y et al. (2021) Fast and sensitive GCaMP calcium indicators for imaging neural populations. bioRxiv, 2021.11.08.467793
20. Patriarchi T et al. (2018) Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. Science DOI: 10.1126/science.aat4422
21. Patriarchi T et al. (2020) An expanded palette of dopamine sensors for multiplex imaging in vivo. Nat Methods 17, 1147–1155 [PubMed: 32895537]
22. Sun F et al. (2018) A Genetically Encoded Fluorescent Sensor Enables Rapid and Specific Detection of Dopamine in Flies, Fish, and Mice. Cell 174, 481–496.e19 [PubMed: 30007419]
23. Sun F et al. (2020) Next-generation GRAB sensors for monitoring dopaminergic activity in vivo. Nat Methods 17, 1156–1166 [PubMed: 33087905]
24. Hamid AA et al. (2021) Wave-like dopamine dynamics as a mechanism for spatiotemporal credit assignment. Cell 184, 2733–2749.e16 [PubMed: 33861952]
25. Engelhard B et al. (2019) Specialized coding of sensory, motor and cognitive variables in VTA dopamine neurons. Nature 570, 509–513 [PubMed: 31142844]
26. Howe MW and Dombeck DA (2016) Rapid signalling in distinct dopaminergic axons during locomotion and reward. Nature 535, 505–10 [PubMed: 27398617]
27. da Silva JA et al. (2018) Dopamine neuron activity before action initiation gates and invigorates future movements. Nature DOI: 10.1038/nature25457 https://www.nature.com/articles/nature25457#supplementary-information
28. da Silva JA et al. (2018) Dopamine neuron activity before action initiation gates and invigorates future movements. Nature DOI: 10.1038/nature25457 https://www.nature.com/articles/nature25457#supplementary-information
29. Panigrahi B et al. (2015) Dopamine Is Required for the Neural Representation and Control of Movement Vigor. Cell 162, 1418–1430 [PubMed: 26359992]

30. Dodson PD et al. (2016) Representation of spontaneous movement by dopaminergic neurons is cell-type selective and disrupted in parkinsonism. Proceedings of the National Academy of Sciences 113, E2180–E2188
31. Coddington LT and Dudman JT (2018) The timing of action determines reward prediction signals in identified midbrain dopamine neurons. Nat Neurosci 21, 1563–1573 [PubMed: 30323275]
32. Kremer Y et al. (2020) Context-Dependent Multiplexing by Individual VTA Dopamine Neurons. J Neurosci 40, 7489–7509 [PubMed: 32859713]
33. Willmore L et al. (2022) Behavioural and dopaminergic signatures of resilience. Nature 611, 124–132 [PubMed: 36261520]
34. Grove JCR et al. (2022) Dopamine subsystems that track internal states. Nature 608, 374–380 [PubMed: 35831501]
35. Kim HR et al. (2020) A Unified Framework for Dopamine Signals across Timescales. Cell 183, 1600–1616.e25 [PubMed: 33248024]
36. Hamilos AE et al. (2021) Slowly evolving dopaminergic activity modulates the moment-to-moment probability of reward-related self-timed movements. eLife 10, e62583 [PubMed: 34939925]
37. Howe MW et al. (2013) Prolonged dopamine signalling in striatum signals proximity and value of distant rewards. Nature 500, 575–579 [PubMed: 23913271]
38. Liu C et al. (2021) Spatial and temporal scales of dopamine transmission. Nat Rev Neurosci 22, 345–358 [PubMed: 33837376]
39. Collins AL and Saunders BT (2020) Heterogeneity in striatal dopamine circuits: Form and function in dynamic reward seeking. Journal of Neuroscience Research 98, 1046–1069 [PubMed: 32056298]
40. Descarries L et al. (1996) Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: a quantitative autoradiographic and immunocytochemical analysis. J Comp Neurol 375, 167–186 [PubMed: 8915824]
41. Liu C et al. (2018) Dopamine Secretion Is Mediated by Sparse Active Zone-like Release Sites. Cell 172, 706–718.e15 [PubMed: 29398114]
42. Bamford NS et al. (2004) Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. Neuron 42, 653–663 [PubMed: 15157425]
43. Condon MD et al. (2019) Plasticity in striatal dopamine release is governed by release-independent depression and the dopamine transporter. Nat Commun 10, 4263 [PubMed: 31537790]
44. Sulzer D et al. (2016) Striatal dopamine neurotransmission: Regulation of release and uptake. Basal Ganglia 6, 123–148 [PubMed: 27141430]
45. Rice ME and Patel JC (2015) Somatodendritic dopamine release: recent mechanistic insights. Philos Trans R Soc Lond B Biol Sci 370, 20140185 [PubMed: 26009764]
46. Gantz SC et al. (2018) The Evolving Understanding of Dopamine Neurons in the Substantia Nigra and Ventral Tegmental Area. Annual Review of Physiology 80, 219–241
47. Gerfen CR and Surmeier DJ (2011) Modulation of striatal projection systems by dopamine. Annu Rev Neurosci 34, 441–466 [PubMed: 21469956]
48. Gerfen CR and Surmeier DJ (2011) Modulation of Striatal Projection Systems by Dopamine. 10.1146/annurev-neuro-061010-113641. [Online]. Available: https://www.annualreviews.org/doi/abs/10.1146/annurev-neuro-061010-113641. [Accessed: 12-May-2021]
49. Corkrum M et al. (2020) Dopamine-Evoked Synaptic Regulation in the Nucleus Accumbens Requires Astrocyte Activity. Neuron 105, 1036–1047.e5 [PubMed: 31954621]
50. Pittolo S et al. (2022) Dopamine activates astrocytes in prefrontal cortex via α1-adrenergic receptors. Cell Reports 40, 111426 [PubMed: 36170823]
51. Yagishita S et al. (2014) A critical time window for dopamine actions on the structural plasticity of dendritic spines. Science 345, 1616–1620 [PubMed: 25258080]
52. Kozorovitskiy Y et al. (2015) Neuromodulation of excitatory synaptogenesis in striatal development. Elife 4, e10111 [PubMed: 26551563]

53. Wu M et al. (2021) Ketamine Rapidly Enhances Glutamate-Evoked Dendritic Spinogenesis in Medial Prefrontal Cortex Through Dopaminergic Mechanisms. Biol Psychiatry 89, 1096–1105 [PubMed: 33637303]
54. Iino Y et al. (2020) Dopamine D2 receptors in discrimination learning and spine enlargement. Nature 579, 555–560 [PubMed: 32214250]
55. Coddington LT and Dudman JT (2021) In Vivo Optogenetics with Stimulus Calibration. Methods Mol Biol 2188, 273–283 [PubMed: 33119857]
56. Grundmann M and Kostenis E (2017) Temporal Bias: Time-Encoded Dynamic GPCR Signaling. Trends Pharmacol Sci 38, 1110–1124 [PubMed: 29074251]
57. Gerfen CR and Surmeier DJ (2011) Modulation of Striatal Projection Systems by Dopamine. Annual Review of Neuroscience 34, 441–466
58. Nelson AB and Kreitzer AC (2014) Reassessing Models of Basal Ganglia Function and Dysfunction. Annual Review of Neuroscience 37, 117–135
59. Calabresi P et al. (2014) Direct and indirect pathways of basal ganglia: a critical reappraisal. Nat Neurosci 17, 1022–1030 [PubMed: 25065439]
60. Shen W et al. (2008) Dichotomous Dopaminergic Control of Striatal Synaptic Plasticity. Science 321, 848–851 [PubMed: 18687967]
61. Calabresi P et al. (2007) Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci 30, 211–219 [PubMed: 17367873]
62. Dobbs LK et al. (2016) Dopamine Regulation of Lateral Inhibition between Striatal Neurons Gates the Stimulant Actions of Cocaine. Neuron 90, 1100–1113 [PubMed: 27181061]
63. Lahiri AK and Bevan MD (2020) Dopaminergic Transmission Rapidly and Persistently Enhances Excitability of D1 Receptor-Expressing Striatal Projection Neurons. Neuron 106, 277–290.e6 [PubMed: 32075716]
64. Ericsson J et al. (2013) Dopamine Differentially Modulates the Excitability of Striatal Neurons of the Direct and Indirect Pathways in Lamprey. J. Neurosci 33, 8045–8054 [PubMed: 23637194]
65. Planert H et al. (2013) Membrane properties of striatal direct and indirect pathway neurons in mouse and rat slices and their modulation by dopamine. PLoS One 8, e57054 [PubMed: 23469183]
66. Hu X-T et al. (2005) Repeated Cocaine Administration Decreases Calcineurin (PP2B) but Enhances DARPP-32 Modulation of Sodium Currents in Rat Nucleus Accumbens Neurons. Neuropsychopharmacol 30, 916–926
67. Surmeier DJ et al. (1992) Dopamine receptor subtypes colocalize in rat striatonigral neurons. Proc Natl Acad Sci USA 89, 10178 [PubMed: 1332033]
68. Zhang X-F et al. (1998) Whole-Cell Plasticity in Cocaine Withdrawal: Reduced Sodium Currents in Nucleus Accumbens Neurons. J. Neurosci 18, 488 [PubMed: 9412525]
69. Lemos JC et al. (2016) Enhanced GABA Transmission Drives Bradykinesia Following Loss of Dopamine D2 Receptor Signaling. Neuron 90, 824–838 [PubMed: 27196975]
70. Sippy T et al. (2015) Cell-Type-Specific Sensorimotor Processing in Striatal Projection Neurons during Goal-Directed Behavior. Neuron 88, 298–305 [PubMed: 26439527]
71. Sippy T et al. (2021) Cell type-specific membrane potential changes in dorsolateral striatum accompanying reward-based sensorimotor learning. Function DOI: 10.1093/function/zqab049
72. Gertler TS et al. (2008) Dichotomous Anatomical Properties of Adult Striatal Medium Spiny Neurons. J. Neurosci 28, 10814–10824 [PubMed: 18945889]
73. Hamid AA et al. (2021) Wave-like dopamine dynamics as a mechanism for spatiotemporal credit assignment. Cell 184, 2733–2749.e16 [PubMed: 33861952]
74. Tepper JM et al. (2018) Heterogeneity and Diversity of Striatal GABAergic Interneurons: Update 2018. Front Neuroanat 12, 91 [PubMed: 30467465]
75. Lee K et al. (2019) Gain Modulation by Corticostriatal and Thalamostriatal Input Signals during Reward-Conditioned Behavior. Cell Rep 29, 2438–2449.e4 [PubMed: 31747611]
76. Peters AJ et al. (2021) Striatal activity topographically reflects cortical activity. Nature 591, 420–425 [PubMed: 33473213]

77. Gallo EF et al. (2022) Dopamine D2 receptors modulate the cholinergic pause and inhibitory learning. Mol Psychiatry 27, 1502–1514 [PubMed: 34789847]
78. Chuhma N et al. (2014) Dopamine neurons control striatal cholinergic neurons via regionally heterogeneous dopamine and glutamate signaling. Neuron 81, 901–912 [PubMed: 24559678]
79. Straub C et al. (2014) Multiphasic Modulation of Cholinergic Interneurons by Nigrostriatal Afferents. J. Neurosci 34, 8557–8569 [PubMed: 24948810]
80. Wieland S et al. (2014) Phasic dopaminergic activity exerts fast control of cholinergic interneuron firing via sequential NMDA, D2, and D1 receptor activation. J Neurosci 34, 11549–11559 [PubMed: 25164653]
81. Cai Y and Ford CP (2018) Dopamine Cells Differentially Regulate Striatal Cholinergic Transmission across Regions through Corelease of Dopamine and Glutamate. Cell Rep 25, 3148–3157.e3 [PubMed: 30540946]
82. Chuhma N et al. (2018) Dopamine neuron glutamate cotransmission evokes a delayed excitation in lateral dorsal striatal cholinergic interneurons. Elife 7, e39786 [PubMed: 30295607]
83. Morgenstern NA et al. (2022) Pyramidal tract neurons drive amplification of excitatory inputs to striatum through cholinergic interneurons. Sci Adv 8, eabh4315 [PubMed: 35138902]
84. Shen W et al. (2005) Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. Journal of Neuroscience 25, 7449–7458 [PubMed: 16093396]
85. Shin JH et al. (2017) Distinctive Modulation of Dopamine Release in the Nucleus Accumbens Shell Mediated by Dopamine and Acetylcholine Receptors. J Neurosci 37, 11166–11180 [PubMed: 29030431]
86. Tanimura A et al. (2019) Cholinergic Interneurons Amplify Thalamostriatal Excitation of Striatal Indirect Pathway Neurons in Parkinson's Disease Models. Neuron 101, 444–458.e6 [PubMed: 30658860]
87. Dorst MC et al. (2020) Polysynaptic inhibition between striatal cholinergic interneurons shapes their network activity patterns in a dopamine-dependent manner. Nat Commun 11, 5113 [PubMed: 33037215]
88. English DF et al. (2011) GABAergic circuits mediate the reinforcement-related signals of striatal cholinergic interneurons. Nat Neurosci 15, 123–130 [PubMed: 22158514]
89. Witten IB et al. (2010) Cholinergic interneurons control local circuit activity and cocaine conditioning. Science 330, 1677–1681 [PubMed: 21164015]
90. Fleming W et al. (2022) Cholinergic interneurons mediate cocaine extinction in male mice through plasticity across medium spiny neuron subtypes. Cell Rep 39, 110874 [PubMed: 35649378]
91. Nelson AB et al. (2014) Striatal Cholinergic Interneurons Drive GABA Release from Dopamine Terminals. Neuron 82, 63–70 [PubMed: 24613418]
92. Wang JX et al. (2018) Prefrontal cortex as a meta-reinforcement learning system. Nat Neurosci 21, 860–868 [PubMed: 29760527]
93. Parker NF et al. (2022) Choice-selective sequences dominate in cortical relative to thalamic inputs to NAc to support reinforcement learning. Cell Reports 39, 110756 [PubMed: 35584665]
94. Cui G et al. (2013) Concurrent activation of striatal direct and indirect pathways during action initiation. Nature 494, 238–42 [PubMed: 23354054]
95. Markowitz JE et al. (2018) The Striatum Organizes 3D Behavior via Moment-to-Moment Action Selection. Cell 174, 44–58.e17 [PubMed: 29779950]
96. Meng C et al. (2018) Spectrally Resolved Fiber Photometry for Multi-component Analysis of Brain Circuits. Neuron 98, 707–717.e4 [PubMed: 29731250]
97. Klaus A et al. (2017) The Spatiotemporal Organization of the Striatum Encodes Action Space. Neuron 95, 1171–1180.e7 [PubMed: 28858619]
98. Maltese M et al. (2021) Dopamine differentially modulates the size of projection neuron ensembles in the intact and dopamine-depleted striatum. eLife 10, e68041 [PubMed: 33983121]
99. Sheng M et al. (2019) Emergence of stable striatal D1R and D2R neuronal ensembles with distinct firing sequence during motor learning. PNAS 116, 11038–11047 [PubMed: 31072930]
100. Parker JG et al. (2018) Diametric neural ensemble dynamics in parkinsonian and dyskinetic states. Nature 557, 177–182 [PubMed: 29720658]

101. Barbera G et al. (2016) Spatially Compact Neural Clusters in the Dorsal Striatum Encode Locomotion Relevant Information. Neuron 92, 202–213 [PubMed: 27667003]
102. Isomura Y et al. (2013) Reward-modulated motor information in identified striatum neurons. J. Neurosci 33, 10209–10220 [PubMed: 23785137]
103. Kravitz AV et al. (2013) Optogenetic identification of striatal projection neuron subtypes during in vivo recordings. Brain research 1511, 21–32 [PubMed: 23178332]
104. Massengill CI et al. (2021) Genetically encoded sensors towards imaging cAMP and PKA activity in vivo. Journal of Neuroscience Methods 362, 109298 [PubMed: 34339753]
105. Lee SJ et al. (2019) Monitoring Behaviorally Induced Biochemical Changes Using Fluorescence Lifetime Photometry. Frontiers in Neuroscience 13
106. Massengill CI et al. (2022) Sensitive genetically encoded sensors for population and subcellular imaging of cAMP in vivo. Nat Methods 19, 1461–1471 [PubMed: 36303019]
107. Chen Y et al. (2017) Endogenous Gαq-Coupled Neuromodulator Receptors Activate Protein Kinase A. Neuron 96, 1070–1083.e5 [PubMed: 29154125]
108. Hanoune J and Defer N (2001) Regulation and role of adenylyl cyclase isoforms. Annu Rev Pharmacol Toxicol 41, 145–174 [PubMed: 11264454]
109. Massengill CI et al. (2021) Genetically encoded sensors towards imaging cAMP and PKA activity in vivo. J Neurosci Methods 362, 109298 [PubMed: 34339753]
110. Sassone-Corsi P (2012) The cyclic AMP pathway. Cold Spring Harb Perspect Biol 4, a011148 [PubMed: 23209152]
111. Lee SJ et al. (2021) Cell-type-specific asynchronous modulation of PKA by dopamine in learning. Nature 590, 451–456 [PubMed: 33361810]
112. Beaulieu J-M and Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 63, 182–217 [PubMed: 21303898]
113. Marcott PF et al. (2014) Phasic dopamine release drives rapid activation of striatal D2-receptors. Neuron 84, 164–176 [PubMed: 25242218]
114. Beckstead MJ et al. (2004) Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. Neuron 42, 939–946 [PubMed: 15207238]
115. Ma L et al. (2022) Locomotion activates PKA through dopamine and adenosine in striatal neurons. Nature DOI: 10.1038/s41586-022-05407-4
116. Peng W et al. (2020) Regulation of sleep homeostasis mediator adenosine by basal forebrain glutamatergic neurons. Science 369, eabb0556 [PubMed: 32883833]
117. Krok AC et al. (2022) Intrinsic reward-like dopamine and acetylcholine dynamics in striatum. bioRxiv, 2022.09.09.507300
118. Reynolds JNJ et al. (2022) Coincidence of cholinergic pauses, dopaminergic activation and depolarisation of spiny projection neurons drives synaptic plasticity in the striatum. Nat Commun 13, 1296 [PubMed: 35277506]
119. Collins AGE and Frank MJ (2014) Opponent actor learning (OpAL): modeling interactive effects of striatal dopamine on reinforcement learning and choice incentive. Psychol Rev 121, 337–366 [PubMed: 25090423]
120. Gurney KN et al. (2015) A new framework for cortico-striatal plasticity: behavioural theory meets in vitro data at the reinforcement-action interface. PLoS Biol 13, e1002034 [PubMed: 25562526]
121. Kerr JND and Plenz D (2002) Dendritic Calcium Encodes Striatal Neuron Output during Up-States. J. Neurosci 22, 1499–1512 [PubMed: 11880480]
122. Owen SF et al. (2018) Fast-Spiking Interneurons Supply Feedforward Control of Bursting, Calcium, and Plasticity for Efficient Learning. Cell 172, 683–695.e15 [PubMed: 29425490]
123. Carter AG and Sabatini BL (2004) State-Dependent Calcium Signaling in Dendritic Spines of Striatal Medium Spiny Neurons. Neuron 44, 483–493 [PubMed: 15504328]
124. Jędrzejewska-Szmek J et al. (2017) Calcium dynamics predict direction of synaptic plasticity in striatal spiny projection neurons. Eur J Neurosci 45, 1044–1056 [PubMed: 27233469]
125. Stalnaker TA et al. (2019) Dopamine neuron ensembles signal the content of sensory prediction errors. eLife 8, e49315 [PubMed: 31674910]

126. Gardner MPH et al. (2018) Rethinking dopamine as generalized prediction error. Proceedings of the Royal Society B: Biological Sciences 285, 20181645

127. Gadagkar V et al. (2016) Dopamine neurons encode performance error in singing birds. Science 354, 1278–1282 [PubMed: 27940871]

128. Ketzef M et al. (2017) Dopamine Depletion Impairs Bilateral Sensory Processing in the Striatum in a Pathway-Dependent Manner. Neuron 94, 855–865.e5 [PubMed: 28521136]

129. Knöpfel T and Song C (2019) Optical voltage imaging in neurons: moving from technology development to practical tool. Nature Reviews Neuroscience 20, 719–727 [PubMed: 31705060]

130. Marshall JD et al. (2016) Cell-Type-Specific Optical Recording of Membrane Voltage Dynamics in Freely Moving Mice. Cell 167, 1650–1662.e15 [PubMed: 27912066]

131. Liu Z et al. (2022) Sustained deep-tissue voltage recording using a fast indicator evolved for two-photon microscopy. Cell 185, 3408–3425.e29 [PubMed: 35985322]

132. Villette V et al. (2019) Ultrafast Two-Photon Imaging of a High-Gain Voltage Indicator in Awake Behaving Mice. Cell 179, 1590–1608.e23 [PubMed: 31835034]

133. Lee JY et al. (2021) Dopamine facilitates associative memory encoding in the entorhinal cortex. Nature 598, 321–326 [PubMed: 34552245]

134. Takeuchi T et al. (2016) Locus coeruleus and dopaminergic consolidation of everyday memory. Nature 537, 357–362 [PubMed: 27602521]

135. Lutas A et al. (2022) History-dependent dopamine release increases cAMP levels in most basal amygdala glutamatergic neurons to control learning. Cell Rep 38, 110297 [PubMed: 35081349]

136. Lutas A et al. (2019) State-specific gating of salient cues by midbrain dopaminergic input to basal amygdala. Nat Neurosci 22, 1820–1833 [PubMed: 31611706]

137. Hasegawa E et al. (2022) Rapid eye movement sleep is initiated by basolateral amygdala dopamine signaling in mice. Science 375, 994–1000 [PubMed: 35239361]

138. Zhang SX et al. (2021) Hypothalamic dopamine neurons motivate mating through persistent cAMP signalling. Nature 597, 245–249 [PubMed: 34433964]

139. Handler A et al. (2019) Distinct Dopamine Receptor Pathways Underlie the Temporal Sensitivity of Associative Learning. Cell 178, 60–75.e19 [PubMed: 31230716]

140. Cermak N et al. (2020) Whole-organism behavioral profiling reveals a role for dopamine in state-dependent motor program coupling in C. elegans. eLife 9, e57093 [PubMed: 32510332]

Outstanding questions

- How rapidly can DA alter the physiology of target cells *in vivo* to affect behavior, and how long do these effects persist?
- Are the effects of DA on neurons lacking HCN and GIRK channels necessarily downstream of PKA, and thus limited to the kinetics of PKA activation and deactivation?
- Do the kinetics of PKA signaling in subcellular compartments (e.g. dendritic spines) match those imaged *in vivo* across entire cells at the level of populations of neurons?
- If DA modulation only occurs on slow timescales, then what is the role of phasic signaling? And how can DA assign credit to specific actions and sensory stimuli that predict reward when its downstream signaling pathways remain elevated for tens of seconds?
- Are there yet undiscovered ways in which DA affects physiology on faster timescales than currently recognized? Could it be, for example, that PKA signaling in spines is faster than the signal reported by photometry, which is presumably driven mostly by much slower nuclear signaling?
- Will sensors other than PKA, such as cAMP sensors, reveal dynamics on similar timescales?
- Are there DA signaling cascades that are independent from PKA and that enable more immediate forms of modulation?
- How does DA affect the intrinsic excitability of D1- and D2-SPNs, and the strength of synapses that impinge on them on both short- and long timescales?
- How is DA receptor signaling affected by other striatal modulators like acetylcholine or adenosine, which act on GPCRs and converge onto the same downstream effectors as DA receptors (i.e. cAMP and PKA)?

Highlights

- Dopamine is an intensely studied neuromodulator that has been linked to reward signaling, motivation and movement production, and has been implicated in a multitude of neuropsychiatric diseases.
- Despite the established role of dopamine in health and disease, the molecular, cellular and circuit mechanisms of dopamine action *in vivo* remain poorly understood.
- New tools for monitoring the activity of dopamine neurons and extracellular dopamine in target brain regions have revealed that the patterns of dopamine release are considerably more complex and diverse than previously thought.
- Advances in cell type specific recording techniques, including intracellular effectors like calcium and PKA are challenging current understanding of the modulatory effects of dopamine on target cells.

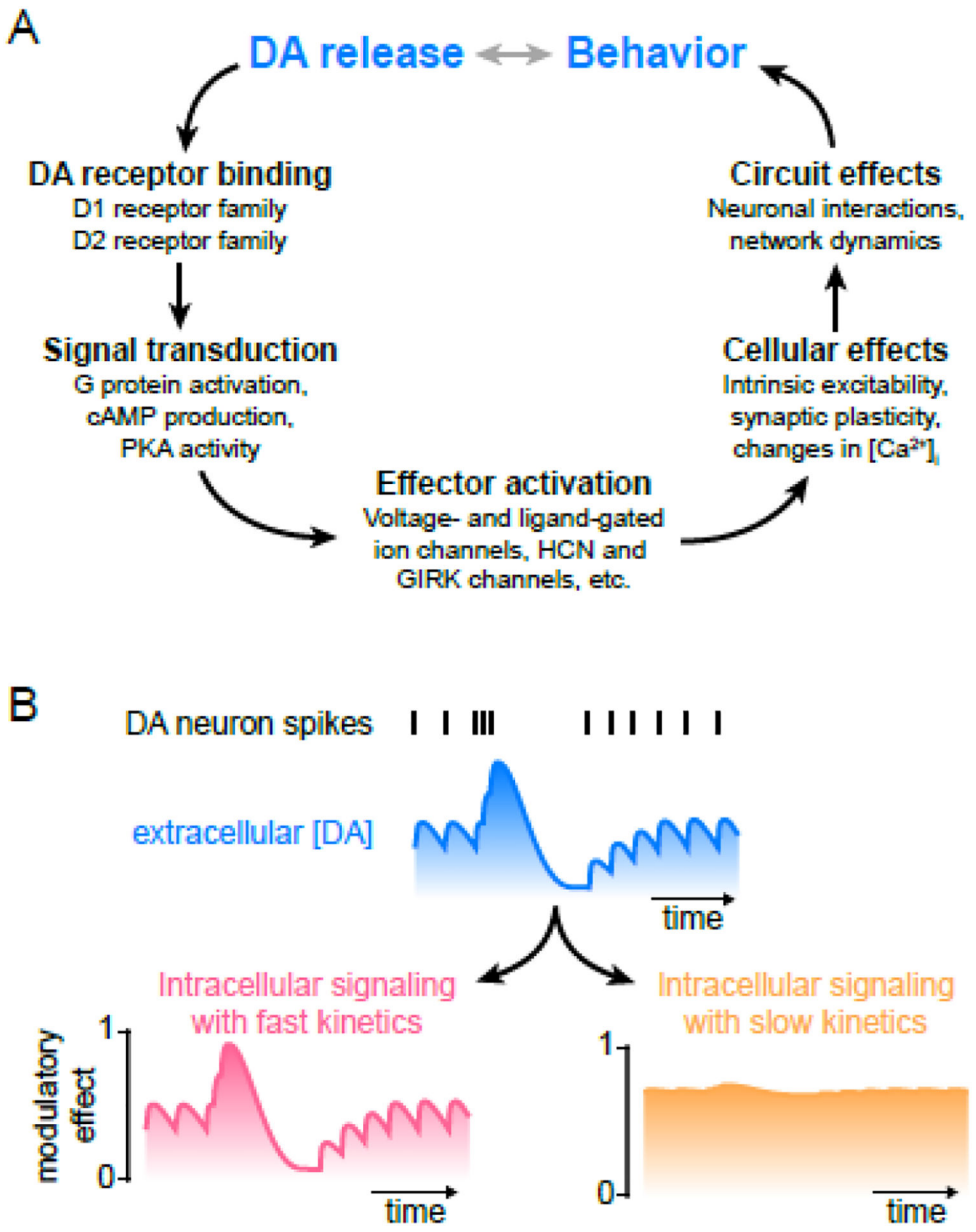

Figure 1. Unraveling the dynamics of dopamine's molecular, cellular and circuit effects.

(A) Extensive research has addressed the ability of DA to alter behavior, and conversely, how behavioral state modulates DA release and function (blue). However, the specific mechanisms through which DA modifies the activity of target neurons and circuits *in vivo* to affect behavior are not fully understood. **(B)** Among the most poorly understood aspects is the exact time course of DA modulation *in vivo*, combining the activity patterns of populations of DA neurons and the kinetics of intracellular signaling cascades activated by DA receptors. The schematic shows the discharge of a single hypothetical DA neuron (*top*; each bar represents an action potential) and the resulting changes in extracellular DA concentration in target brain regions (*blue*). *Bottom panels*: the time course of any given modulatory effect (e.g. PKA activation, dendritic excitability, neuronal ensemble size,

etc.) varies with the kinetics of the signal transduction pathway recruited by DA. Slow kinetics limit the ability of target cells to detect and differentially respond to frequent phasic increases and decreases in extracellular DA, reporting instead slow changes in the mean discharge of populations of DA neurons. Note that distinct modulatory effects within a given cell can exhibit different kinetics, depending on the signal transduction pathway and/or effector being recruited.

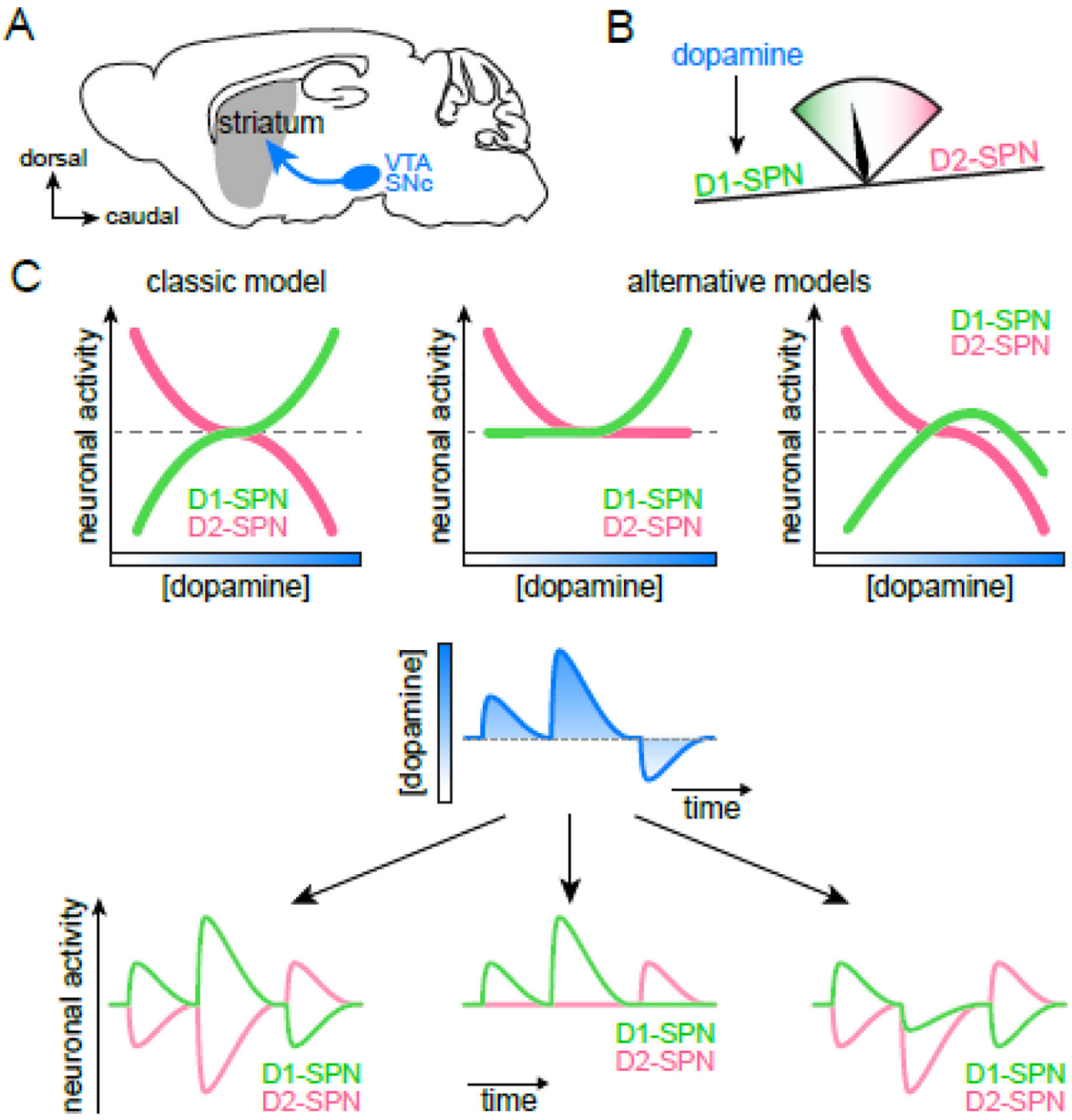

Figure 2. DA modulation of striatal output.

(A) Schematic of a mouse brain in the sagittal plane showing the striatum (gray) innervated by SNc and VTA DA neurons in the ventral midbrain (blue). **(B)** DA is widely believed to bias striatal output in favor of the direct pathway, which originates in striatal projection neurons (SPNs) expressing D1-type DA receptors. **(C)** Several models have been proposed to account for the effects of DA on the activity of D1- and D2-SPNs. Three models are shown, each depicting the net effect of DA on cellular processes positively correlated with 'neuronal activity' (e.g. the probability that a SPN fires an action potential to a given synaptic input, or the likelihood that an action potential triggers the release of a synaptic vesicle) as a function of extracellular DA concentrations (*top panels*) or time (*bottom panels*). The classic model (*left*) posits that DA exerts roughly equal and opposite effects on

D1-SPNs and D2-SPNs. Two recent studies investigating PKA signaling [111] and synaptic plasticity [54] suggest that D1- and D2-SPNs specialize in the detection of phasic increases and decreases in DA, respectively (*middle*). Others (*right*), using cellular-resolution Ca^{2+} imaging from populations of SPNs, have reported that strong stimulation of D1 receptors paradoxically depresses D1-SPN activity while maintaining striatal output biased in favor of the direct pathway [98,100,101].