Spatial and temporal scales of dopamine transmission

Changliang Liu, Pragya Goel and Pascal S. Kaeser ✉

Abstract | Dopamine is a prototypical neuromodulator that controls circuit function through G protein-coupled receptor signalling. Neuromodulators are volume transmitters, with release followed by diffusion for widespread receptor activation on many target cells. Yet, we are only beginning to understand the specific organization of dopamine transmission in space and time. Although some roles of dopamine are mediated by slow and diffuse signalling, recent studies suggest that certain dopamine functions necessitate spatiotemporal precision. Here, we review the literature describing dopamine signalling in the striatum, including its release mechanisms and receptor organization. We then propose the domain-overlap model, in which release and receptors are arranged relative to one another in micrometre-scale structures. This architecture is different from both point-to-point synaptic transmission and the widespread organization that is often proposed for neuromodulation. It enables the activation of receptor subsets that are within micrometre-scale domains of release sites during baseline activity and broader receptor activation with domain overlap when firing is synchronized across dopamine neuron populations. This signalling structure, together with the properties of dopamine release, may explain how switches in firing modes support broad and dynamic roles for dopamine and may lead to distinct pathway modulation.

Dopamine has multidimensional importance in the control of brain function and is associated with a broad spectrum of brain disorders, including Parkinson disease and addiction. As an evolutionarily ancient neuromodulator, roles of dopamine are preserved from *Caenorhabditis elegans* to humans[1,2]. In the mammalian brain, most dopamine neurons reside in two nuclei in the ventral midbrain: the pars compacta of the substantia nigra and the ventral tegmental area. Substantia nigra dopamine neurons project to the dorsal striatum, giving rise to the nigrostriatal pathway, important for motor control and action selection. Ventral tegmental area dopamine neurons predominantly project to the ventral striatum and the prefrontal cortex, forming the mesocorticolimbic pathway, and are responsible for reward processing and reinforcement learning[3–6].

A central unresolved question is how dopamine signalling is organized to mediate these broad functions[6–8]. The classical view is that dopamine transmission lacks speed and accuracy; however, recent studies have found hallmarks of precise signalling.

Early work by Carlsson led to the discovery of dopamine as a neurotransmitter and suggested that its functions are slow and imprecise[9,10]. Rabbits treated with reserpine, a vesicular monoamine transporter (VMAT) blocker that depletes brain dopamine, were paralysed. Upon injection of L-DOPA (a dopamine precursor), movement was restored under the continued presence of VMAT blockade. Hence, the brain was able to metabolize L-DOPA and use it to drive locomotion despite the absence of vesicular dopamine loading and of precise exocytotic release. Morphological studies have supported the hypothesis that spatial precision is not built into dopamine signalling, as dopamine receptors and transporters are predominantly extrasynaptic[11–14]. Furthermore, the vertebrate dopamine receptors, which are all G protein-coupled receptors (GPCRs), operate at speeds that are orders of magnitude slower than ionotropic receptors[15]. Together, these observations have led to the model that dopamine signals through volume transmission and does not rely on the high spatiotemporal precision that defines synaptic transmission (FIG. 1).

However, striatal circuit function is highly dynamic and relies on precise dopamine modulation. Indeed, dopamine signals are local and short-lived[16–18] and dopamine-mediated behaviours and target-cell modulation can correlate with dopamine neuron firing on the order of tens to hundreds of milliseconds[19–27]. Further supporting spatiotemporal precision, resupplying L-DOPA improves some symptoms caused by dopamine decline, yet only partially alleviates learning deficits[28–31].

Department of Neurobiology, Harvard Medical School, Boston, MA, USA.

✉*e-mail: kaeser@hms.harvard.edu*

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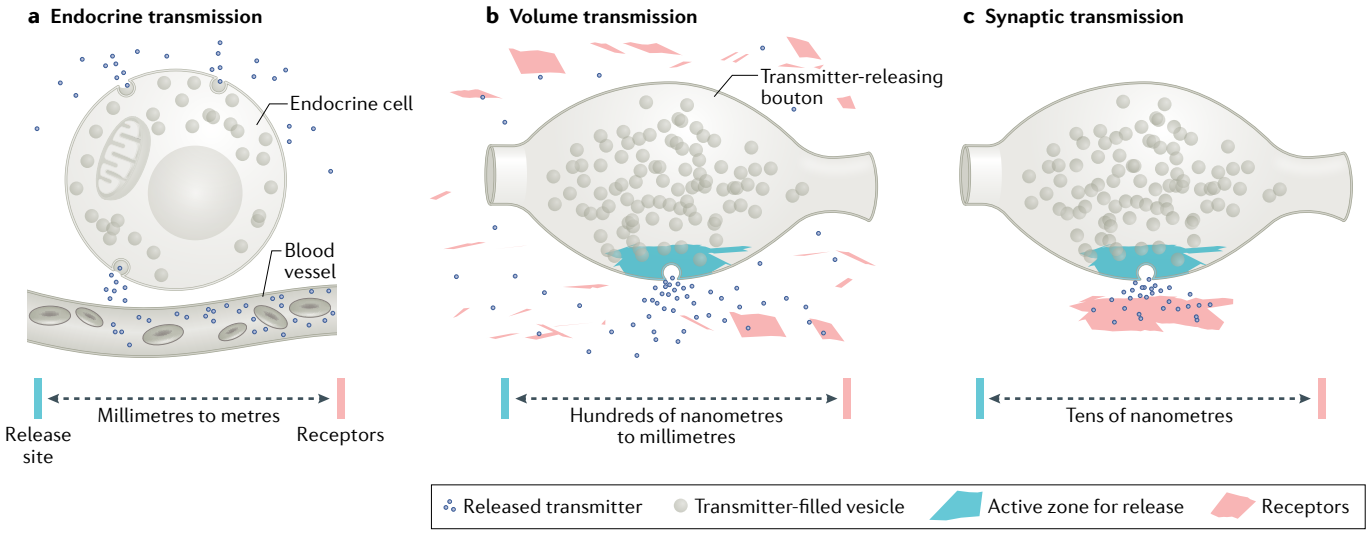


Fig. 1 | **Modes of chemical transmission.** Overview of the fundamental modes of chemical transmission differing with regard to release precision and the organization of receptors. **a** | Endocrine cells release their transmitters, generally hormones, from the cell surface. The transmitters often travel long distances through the extracellular space and the bloodstream to receptors residing far away from the release sites. Typically, no specialized release-site architecture is evident in these cells. **b** | Volume transmission relies on the diffusion of transmitters in the extracellular space, and the receptors are only loosely coupled with the release sites. Often, specialized active zone-like release sites mediate neuromodulator secretion. A steep transmitter concentration gradient is built upon release, and the degree of receptor activation depends on their distance to these release sites. **c** | Synaptic transmission relies on tight spatial coupling between the active zone and receptor clusters, which are often aligned with one another at the subsynaptic scale. Signal transmission is confined to the synaptic cleft to ensure accuracy and efficient receptor activation.

On the basis of these and other findings, the field has developed a compelling framework on the importance of dopamine dynamics in behaviour[4–6,32]. However, reconciling the physiology of dopamine transmission with the broad and sometimes rapid coding properties is challenging. Here, we review progress on the mechanisms of dopamine release and the functional organization of dopamine receptors to evaluate how the signalling architecture may support dopamine functions. We focus on the vertebrate striatum because of the well-established importance of dopamine in this circuit, and draw parallels to other dopamine systems when appropriate. We then develop a generalized framework for dopamine neurotransmission. We propose the domain-overlap model, which relies on rapid release followed by diffusion, with the organization of release and receptors on a micrometre scale. The presence of these signalling domains potentially explains how switches in firing modes can lead to distinct pathway modulation and broad coding dynamics.

Dopamine release

Striatal dopamine levels are highly dynamic and fluctuate on different timescales, with sub-second transients, ramps that may last for several seconds and oscillations on the timescale of hours[20,33–37]. It has long remained uncertain from where on the extensively branched striatal axons dopamine is released and how the secretory mechanisms involved can account for these dynamic signals[7,38]. Because most dopamine transmission is not naturally associated with postsynaptic currents, many studies rely on electrochemical measurements that sample dopamine from a large area and lack either temporal resolution or dopamine selectivity. This has started to change with the development of fluorescent reporters and dopamine-sensitive ion channels (FIG. 2). Accumulating evidence now indicates that dopamine signalling is not only temporally dynamic but also spatially organized[16–18,22,39–41].

Dopamine varicosities. The dopamine terminals are called varicosities, and many of them do not form classical synapses and are not associated with defined postsynaptic structures[42–44]. The findings that vesicular dopamine loading is essential for dopamine release[45–47] and that quantal release events can be detected[48–51] established that most dopamine is released through vesicle fusion[7,8]. In principle, each varicosity could embody a release site. However, a recent study suggested functional heterogeneity across varicosities, with only 20% of them secreting above the detection threshold[18]. Hence, it is possible that not all varicosities are release competent, or that there is strong heterogeneity in the release properties between varicosities.

Dopamine neurons co-release glutamate and GABA (reviewed in depth elsewhere[52,53]). In striatal dopamine axons, glutamate release is segregated from dopamine release[13,54–56]. Symmetric synapses, such as those detected in the dopamine system[43,57,58], are typically GABAergic, and the optogenetic activation of dopamine axons indeed robustly triggers the release of dopamine and GABA from the same vesicular compartment[59,60]. Therefore, the identification of sites for GABA transmission of dopamine neurons may reveal the source of dopamine release. However, morphological analyses suggest that most GABAergic markers are not detected in the subset of varicosities that form synaptic contacts with target cells[13]. Thus, whether these synaptic varicosities of dopamine axons are the source of GABA and dopamine

Varicosities
Enlarged compartments of dopamine axons filled with small, clear vesicles; varicosities are similar to presynaptic boutons but are often not associated with well-defined postsynaptic specializations.

Quantal release events
Events involving fusion of a single vesicle, leading to the release of neurotransmitters from that vesicle.

Symmetric synapses
Synapses in which the presynaptic and postsynaptic electron densities appear similar, contrasting asymmetric synapses in which the postsynaptic densities are more prominent.

release remains uncertain. Studies in cultured midbrain dopamine neurons confirm that only a small proportion of dopamine terminals form synapses, but that both synaptic and non-synaptic terminals can release dopamine irrespective of the target cell contact[61]. Together, these studies suggest that there are specific sites for exocytosis, but that the synapse-like morphology of a subset of the dopamine varicosities does not reliably allow the identification of these sites.

Active zone-like dopamine release sites. If the varicosities themselves do not define secretory hotspots, such sites may be accounted for by specific molecular machinery. Evoked exocytosis is triggered by Ca^{2+} entry through voltage-gated Ca^{2+} (Ca_V) channels and is mediated by SNARE (soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptor) complexes that drive vesicle fusion. At conventional synapses, evoked release is triggered by vesicular Ca^{2+} sensors

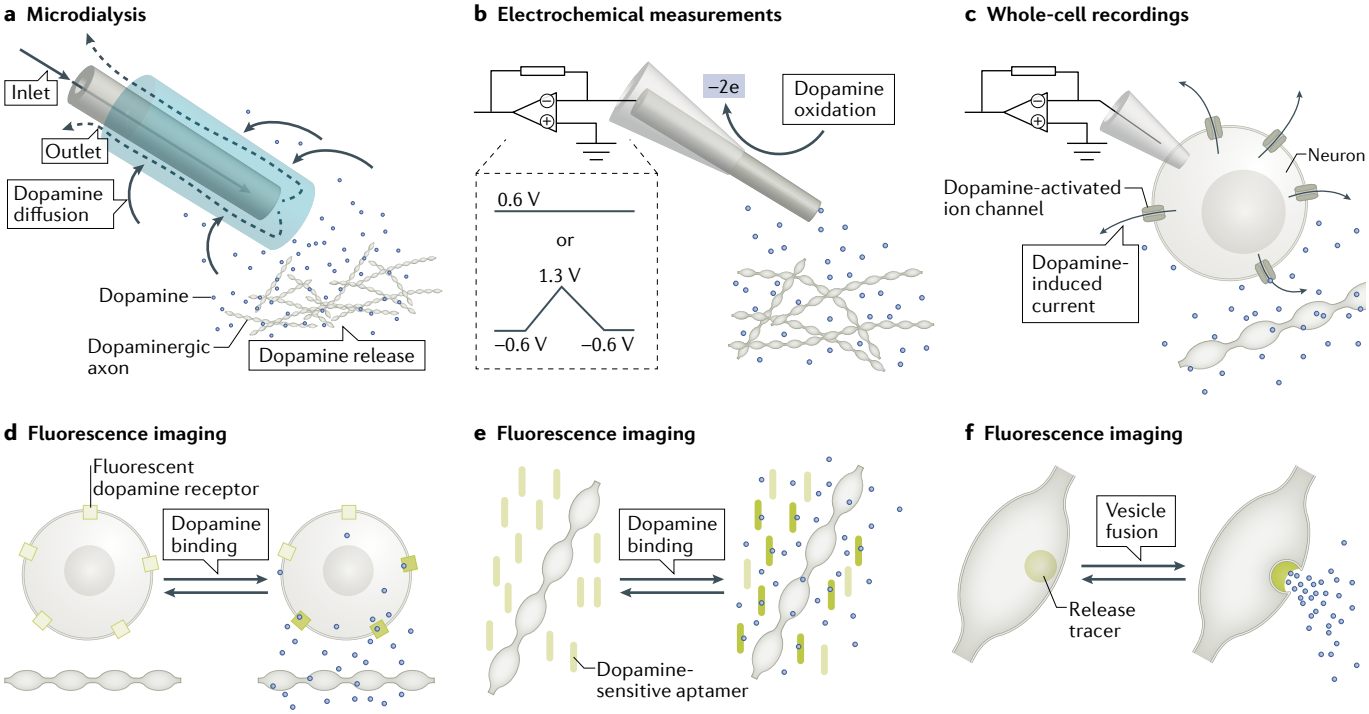


Fig. 2 | **Measurements of dopamine transmission.** Microdialysis enables sampling of the chemical environment in the brain[124] (panel **a**). A semipermeable probe inserted into the brain is perfused to exchange solutes with the surrounding tissue. The method can be used to quantitatively measure multiple neurotransmitters and molecules in vivo and is sensitive enough to detect as little as 50 fg of dopamine in the sample. Microdialysis provides the best measurement for baseline dopamine levels, but its temporal resolution (minutes) and spatial resolution (several hundred micrometres) are too low to report the subcellular organization of dopamine transmission or to detect fast dopamine transients. Electrochemical measurements rely on the oxidation of dopamine at the surface of a carbon fibre electrode[147] (panel **b**). Constant-potential amperometry, typically performed at 0.6 V for dopamine, provides the best temporal resolution of current techniques (sub-millisecond, limited by the sampling frequency), but suffers from a low chemical selectivity, as any molecule that can be oxidized at the applied voltage will contribute to the signal. Fast-scan cyclic voltammetry improves the chemical selectivity at the cost of temporal resolution (typically one data point approximately every 100 ms). Different molecules are oxidized at distinct voltages and can be distinguished by scanning across holding voltages (typically with a triangular wave ranging from –0.6 V to 1.3 V and a scan speed of 400–800 V per second). Because electrochemical measurements rely on the subtraction of a reference current, they are suited to measure changes, but not for assessing baseline dopamine concentrations. Whole-cell electrophysiology can be used to measure currents mediated by ion channels that are activated by dopamine (panel **c**). This method relies on the natural coupling of the G protein-activated inward rectifier K^+ channel 2 (GIRK2) to dopamine type 2 (D2) receptors for somatodendritic dopamine release in the midbrain[95], on the exogenous expression of GIRK2 channels to report striatal D2 receptor activation[16] or on introducing dopamine-sensitive ion channels called ligand-gated channel 53 (lgc-53) from *Caenorhabditis elegans*[49]. These whole-cell recordings have high temporal precision and report dopamine at the target cell. Various fluorescence imaging techniques can be used to assess dopamine release (panels **d**–**f**). The GPCR-activation-based dopamine ($GRAB_{DA}$) sensor and dLight are genetically engineered dopamine receptors (panel **d**) in which a circularly permuted green fluorescence protein (GFP) is inserted such that their fluorescence increases upon dopamine binding[79,83]. The sensors exhibit good spatiotemporal resolution and can be used in vivo and in vitro. Although the sensors are engineered dopamine receptors, their expression pattern may not mimic that of endogenous dopamine receptors, and hence the signal reports dopamine release but is not suited to infer the spatial organization of dopamine sensing on target cells. Synthetic optical probes (panel **e**) are made by conjugating oligonucleotides to single-wall carbon nanotubes to render the spectrum of the nanotubes selective and sensitive to dopamine. Their key advantages are their resistance to photobleaching and their ability to report dopamine release with very high spatiotemporal resolution[22,144]. Vesicular monoamine transporter 2 conjugated on its intraluminal side with a pH-sensitive fluorophore (VMAT-pHluorin) and fluorescent false neurotransmitters (FFNs) represent two tracing strategies (panel **f**) for assessing vesicle fusion and content release, respectively, different from other methods that measure extracellular dopamine levels. The fluorescence of VMAT-pHluorin increases when the acidic vesicular lumen is neutralized upon fusion with the plasma membrane[72]. FFNs are VMAT2 substrates and/or dopamine transporter (DAT) substrates and can be used to monitor dopamine vesicle fusion via dye release[18,148]. Both methods may permit the detection of quantal release events under certain conditions, but low signal-to-noise ratios often limit their detection, and the translation of measurements to absolute dopamine levels is difficult.

Chromaffin cells
Endocrine cells in the adrenal medulla that secrete catecholamines; chromaffin cells are a widely used model to study exocytosis.

called synaptotagmins and is restricted to presynaptic active zones on the target membrane[62,63] (FIG. 1). Active zones are present at every synapse, where they precisely target release towards postsynaptic receptors. Their regulatory mechanisms shape the relationship between firing and release, and they contain the scaffolding proteins RIM (Rab-3-interacting molecule; also known as regulating synaptic membrane exocytosis protein), RIM-BP (RIM-binding protein), Piccolo, Bassoon, MUNC13 (also known as protein unc-13 homologue), liprin-α and ELKS (also known as ERC, CAST and RAB6-interacting protein 2)[62,64]. In contrast to synapses, exocytosis from other secretory cells, for example chromaffin cells, occurs across broad membrane domains and may not need active zone-like specializations[63,65] (FIG. 1). Until recently, whether a central neuromodulator such as dopamine relies on active-zone machinery has remained unclear.

Evoked dopamine release is now established to require active zone-like sites (FIG. 3). First, dopamine release occurs on a millisecond timescale and has a high release probability, indicating that vesicles are rendered ready for release before the arrival of an action potential[17,40]. Second, the clustering of active-zone proteins, including Bassoon, RIM, ELKS and MUNC13, can be detected using confocal, super-resolution and immunoelectron microscopy[13,17,41,55,66,67]. Finally, evoked dopamine release is abolished upon dopamine

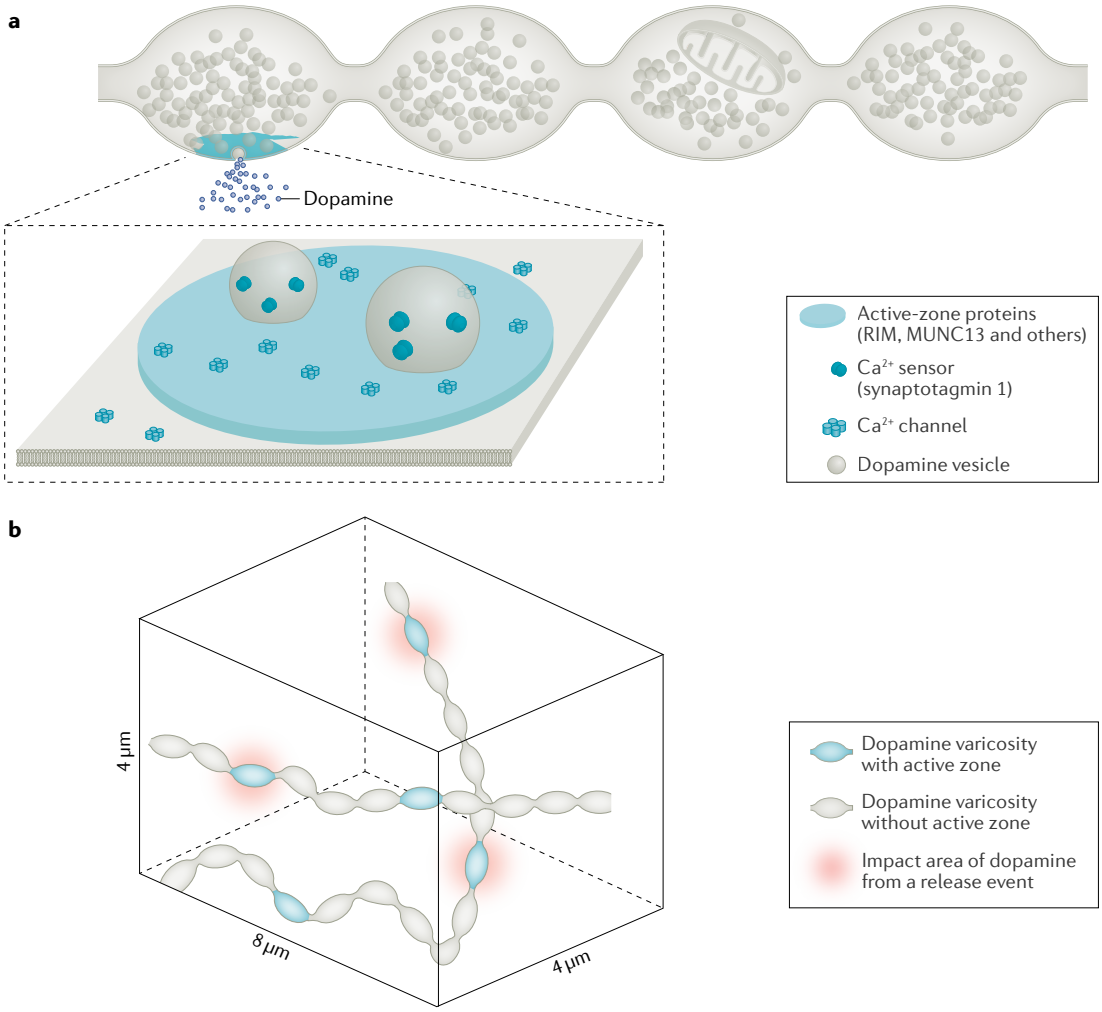


Fig. 3 | **Sparse dopamine release sites. a** | Cellular (top) and molecular (bottom) organization of dopamine release sites. Approximately 25% of dopamine varicosities contain functional release sites composed of active-zone proteins. RIM (Rab-3-interacting molecule) and MUNC13 are essential for action potential-triggered dopamine release and mediate the coupling of release-ready vesicles to Ca^{2+} entry for fast release triggered by the Ca^{2+} sensor synaptotagmin 1 (REFS[17,40,41,68]). The exact identities and distributions of Ca^{2+} channels in dopamine axons are not well understood, but they may be more broadly distributed than active-zone proteins, and multiple different channel subtypes contribute to release[18,78]. Similarly, additional Ca^{2+} sensors are probably present, but their identities and roles are not known[40]. **b** | Distribution of dopamine release sites and the impact area of individual dopamine release events in the striatum. The sparsity of active zone-containing varicosities, the long-lasting depression of individual sites after a release event and the rapid dilution of dopamine into the extracellular space suggest that, at any given time, only a small fraction of the space reaches high-enough dopamine levels for a sufficient period of time for efficient receptor activation, and large striatal areas are not within reach of these varicosities during baseline activity. If dopamine receptors reside in this distant space, they are unlikely to be activated by single vesicule-release events.

neuron-specific knockout of the genes encoding RIM or MUNC13, establishing essential roles[17,41,68].

Notably, the frequency of active-zone scaffolds in dopamine axons is much lower than that of varicosities[17,41]. This result matches with the observation that only approximately 20% of varicosities show detectable release of fluorescent false neurotransmitters, supporting the model that many varicosities are release-incompetent[18]. The examination of dopamine distribution in the striatum using a fluorescent sensor further revealed that evoked dopamine release is not widespread but is limited to sparse hotspots[22]. Together, these findings evidence a model in which action potential-induced dopamine release occurs at a limited number of sites that are defined by RIM and MUNC13 (FIG. 3). On average, there is one dopamine release site approximately every 4 µm of dopamine axon, which leads to an estimate of one site per 25 µm^3 of the striatum[17,41]. Hence, the vast majority of striatal space is a few micrometres away from a dopamine release site (FIG. 3b). This sparsity of dopamine sources, together with the release properties discussed below, has important implications for dopamine coding and forms the foundation of the domain-overlap model that we propose.

Properties of dopamine release. The active zone of a synapse docks and primes synaptic vesicles to generate a readily releasable pool (RRP) and it positions those vesicles at defined distances from presynaptic Ca^{2+} channels to control their vesicular release probability (*P*)[62,64,69–71]. RRP and *P* control the efficacy of release; the greater they are, the more dopamine is released when an action potential arrives. A high *P* also leads to rapid RRP depletion during repetitive firing[71].

RRP measurements in dopamine neuron cultures and synaptosomes revealed that only a few per cent of the vesicles are part of the RRP[72,73]. Therefore, mechanisms to generate releasable vesicles must be present. We propose that, similar to conventional synapses[70,74–76], this function is mediated by RIM and MUNC13, given the strong dependence of dopamine release on these proteins and their presence in dopamine axons[17,41,55,68]. One observation consistent across studies is that dopamine RRP replenishment is slow, with the depression of dopamine release lasting for tens of seconds after a single stimulus[16,17,40,77–80]. Thus, recovery after depletion of the RRP is 10–100-fold slower than that of glutamatergic or GABAergic synapses that undergo depression[71,80,81]. As the replenishment speed is critical for the frequency range at which a transmission system can operate, and as dopamine receptors are 'slow' GPCRs, the dopamine system is not well suited for high-frequency information transfer.

P is determined by the amount of Ca^{2+} entry, by its localization relative to release-ready vesicles and by the Ca^{2+} sensitivity of the release machinery. It is often estimated by assessing responses to paired stimuli (paired-pulse ratios)[71]. If *P* is high, the release triggered by the first stimulus depletes the RRP and reduces the second response when the interstimulus interval is short. Dopamine axons exhibit strong depression, with the first pulse depleting approximately 60% of the RRP[17,40,41,55,80,81], indicating that *P* is very high.

Estimating *P* via paired-pulse stimulation necessitates the detection of peak release, the specific activation of dopamine axons and the activation of the same axon population by both stimuli — requirements that are often not met. Peak release is best assessed by measuring peak dopamine levels, which are detected within less than 5 ms after stimulation if the sampling rate is fast[17,49,80]. Hence, estimating *P* via paired pulse ratios requires sampling at >400 Hz, which is typical for amperometry. Lower-frequency measurements, such as voltammetry (often performed at less than 10 Hz in vivo) or certain imaging approaches, report dopamine levels at a specific time point while extracellular dopamine levels decay or report average dopamine levels over the sampling time window. The amount of dopamine detected using these methods highly depends on the sampling time point and is dominated by dopamine accumulation and clearance but not by release. During stimulus trains or burst firing, low sampling frequencies may further magnify the apparent contributions of later responses owing to prolonged dopamine decay, sometimes leading to low estimates of *P*. However, when the sampling frequency is fast enough to capture the release peak, depression indicative of a high *P* is seen in vivo[79,82,83], similar to observations in brain slices[17,40,41,55,80,81]. Ultimately, approaches that are not fast enough to measure peak release are not well suited to determine *P*.

A further confound in early studies is that electrical stimulation co-activates striatal cholinergic interneurons, which locally drive the dopamine release that dominates the evoked response[17,84–87]. This mechanism leads to complete depression after the first stimulus and can be avoided through the use of pharmacology or by dopamine neuron-specific optogenetic activation. Optogenetic stimulation enables dopamine axon firing at frequencies of up to 10 Hz when a fast actuator is used[17]. Given the complexity of the dopamine axonal arbor and the distinct properties of the axon versus the soma, it is important to establish the reliability of activation in the stimulated cellular compartment in optogenetic experiments.

Dopamine release in the striatum is steeply dependent on extracellular Ca^{2+}, supporting the reliance on plasma membrane Ca^{2+} channels[40,78,88] (FIG. 3a). A recent study used optogenetics to stimulate dopamine axons and found that Ca_V1 (L-type) channels, Ca_V2 (P/Q-type, N-type and R-type) channels and Ca_V3 (T-type) channels all contribute to dopamine release[78], unlike classical synapses, which nearly exclusively rely on Ca_V2 channels[89,90]. In contrast to these mixed Ca^{2+} sources, synaptotagmin 1 is the main fast Ca^{2+} sensor for synchronous axonal dopamine secretion[40], bolstering the model of a fast and precise mode of release. Remarkably, although the removal of synaptotagmin 1 dramatically reduced dopamine release in response to single action potentials, KCl-induced depolarization still triggered release from synaptotagmin 1-deficient dopamine neurons, suggesting that additional release modes and Ca^{2+} sensors exist[40].

In addition to axonal release, midbrain dopamine neurons also release dopamine through vesicular exocytosis from their somata and dendrites[91–94]. The activation of somatic dopamine D2 autoreceptors in turn

Readily releasable pool (RRP). The subset of vesicles within a nerve terminal that can be quickly released by an action potential; readily releasable vesicles are often docked.

Vesicular release probability (*P*). The probability with which a vesicle from the readily releasable pool fuses with the presynaptic plasma membrane in response to an action potential.

Depression
A decrease in release during or following repetitive firing; depression is typically a result of deletion of the readily releasable pool owing to a high *P*.

Somatodendritic release
Release from neuronal somata and dendrites (as opposed to axons); somatodendritic dopamine release is mediated by exocytosis and is an important feature of dopamine neurons.

Pacemaker currents
Cell-autonomous, spontaneous currents that drive the tonic firing of dopamine neurons; they are mainly mediated by Ca^{2+} channels.

Refractory sites
Sites at which a vesicle has fused become refractory and are unavailable for immediate reuse because the readily releasable pool is depleted and because material from the preceding fusion event needs to be cleared.

triggers inhibitory postsynaptic currents (D2-IPSCs), which are mediated by the G protein-activated inward rectifier K^+ channel 2 (GIRK2) and can be measured by whole-cell recordings[95] (FIG. 2c). Although we are only beginning to understand the somatodendritic release machinery of dopamine neurons, a recent study established that it relies on the release-site organizer RIM[68], similar to axonal release[17]. D2-IPSCs can be measured in the striatum when GIRK2 channels are virally expressed, and their characterization strongly supports a high *P* of dopamine release and strong RRP depletion followed by depression[16,55]. As discussed further below, this line of work has revealed important insights into dopamine receptor activation.

Tonic and phasic release. Switching between firing modes is a hallmark feature of dopamine neurons[96,97] (BOX 1). Tonic firing at 0.2–10 Hz relies on cell-autonomous pacemaker currents[98,99], whereas burst (or phasic) firing at more than 10 Hz is driven by excitatory inputs[100,101] and results in synchronized dopamine neuron activity. It has been proposed that tonic firing generates steady-state dopamine concentrations, whereas burst firing leads to phasic release to produce short and fast dopamine transients[101,102]. Switching between firing modes may account for dynamic dopamine signalling[4]. The somatic firing rates of dopamine neurons, however, are not linearly translated into axonal dopamine release, as the latter is subject to strong short-term depression, as discussed above. Furthermore, a small amount of extracellular dopamine can be detected in the absence of somatic firing or when the protein machinery for action potential-triggered release is ablated[17,33,86]. For these reasons, tonic and phasic firing are not equal to tonic and phasic release[4] (BOX 1).

The notion that the dopamine release machinery responds robustly to initial activity but rapidly depresses for tens of seconds[16,17,40,77–80] makes important predictions (FIG. 4). First, tonic firing should lead to depletion and generate refractory sites, and dopamine release in response to each action potential should be largely determined by the recovery of these sites. As such, neurons with a lower spontaneous activity might contribute more during phasic release because their RRP is less depleted during tonic firing. Second, during burst firing, only the first few action potentials should lead to substantial dopamine release from a single axon. It is thus the synchrony of population firing, and not the firing pattern of individual neurons, that should dominate signalling during phasic release. In support of this view, burst firing is strongly impaired in mice that lack NMDA receptors, but phasic dopamine transients and the behaviours mediated by them persist[100,103].

The terms phasic release and burst (or phasic) firing are widely and often interchangeably used (BOX 1). We emphasize that, even though they are correlated[4], they represent very different aspects of dopamine coding. Phasic release depends on the simultaneous recruitment of a dopamine neuron population, which relies on synchrony across dopamine neurons and does not require burst firing[33,100,103,104]. By contrast, burst firing is an activity pattern of a single dopamine neuron and does not strongly enhance dopamine release from that neuron, owing to prominent use-dependent depression[16,17,40,77–80]. In most cases, burst firing is synchronized across dopamine neurons and the first spike efficiently elevates extracellular dopamine levels whereas subsequent activity triggers less dopamine release owing to reduced synchrony and the presence of refractory sites[79,83,105]. Whereas high-frequency sampling appropriately reports high initial release rates, low-frequency measurements may suggest an increase in release late in the burst, because they overestimate the dopamine levels caused by the prolonged decay and because dopamine reuptake is overwhelmed[17,79,82,83,106] (FIG. 4). The later spikes during bursts are probably not efficient at further increasing dopamine levels but help to maintain the elevated levels caused by the first spike.

Dopamine receptors

Medium spiny neurons (MSNs) are the main output neurons in the striatum and contain most of the dopamine receptors of this region. The striatum displays considerable regional heterogeneity and is subdivided into patches

Box 1 | Tonic versus phasic dopamine neuron activity

The terms 'tonic' and 'phasic' have been used to describe several features of dopamine neurons[4]. In the context of dopamine transmission, it is important to distinguish between firing, release and signalling.

Firing

'Tonic firing' refers to activity of a dopamine neuron sustained at 0.2–10 Hz and mediated by cell-autonomous pacemaker conductances[98,99]. It is estimated that 50–98% of dopamine neurons exhibit tonic firing in vivo[101,149].

'Burst firing' is characterized by short bursts of action potentials (3–10 spikes, at more than 10 Hz) of a dopamine neuron. It is typically caused by the activation of its NMDA receptors via excitatory inputs and embodies the response to environmental stimuli[100,101]. Burst firing is sometimes called 'phasic firing', a term that emphasizes the synchrony of activity across dopamine neurons, which arises from shared inputs.

Release and signalling

'Tonic release' generates short-lived dopamine transients of a few milliseconds at a small, variable subset of release sites. Dopamine is quickly diluted into the extracellular space, and the balance between tonic release and reuptake via the dopamine transporter (DAT) determines the measured baseline dopamine levels, which are approximately 2–20 nM (REFS[123,124]). These levels are below the activation threshold of most dopamine receptors[16,127–129] but are probably composed of many short-lived dopamine peaks that are averaged over time. 'Tonic signalling' is most probably caused by these short-lived dopamine signals close to release sites, rather than by steady-state dopamine levels per se. Remarkably, only about 70% of the baseline dopamine measured in microdialysis is caused by action-potential firing[17,41]. The remaining 30% or so is independent of action potentials and the active-zone proteins RIM (Rab-3-interacting molecule) and MUNC13, and may, for example, be accounted for by spontaneous vesicular fusion[17,40,41,48,68].

'Phasic release' is an important form of dopamine coding and occurs when a large number of dopamine release sites are simultaneously activated. Dopamine reuptake mechanisms are transiently overpowered, resulting in substantial crosstalk between dopamine signalling domains and prolonged dopamine dwell times (FIG. 6). For 'phasic signalling', the rapid dopamine elevation through domain-overlap across multi-micrometre-sized areas may lead to the activation of dopamine receptors distant from release sites.

The synchrony of release across populations of dopamine neurons is required for phasic release and phasic signalling. In experimental paradigms, low-frequency stimulation is often used to mimic tonic release. However, this does not replicate the stochastic nature of release-site activation that is typical for tonic release; it instead recruits many axons simultaneously and thus mimics the synchrony across neurons, which is an essential feature of phasic release.

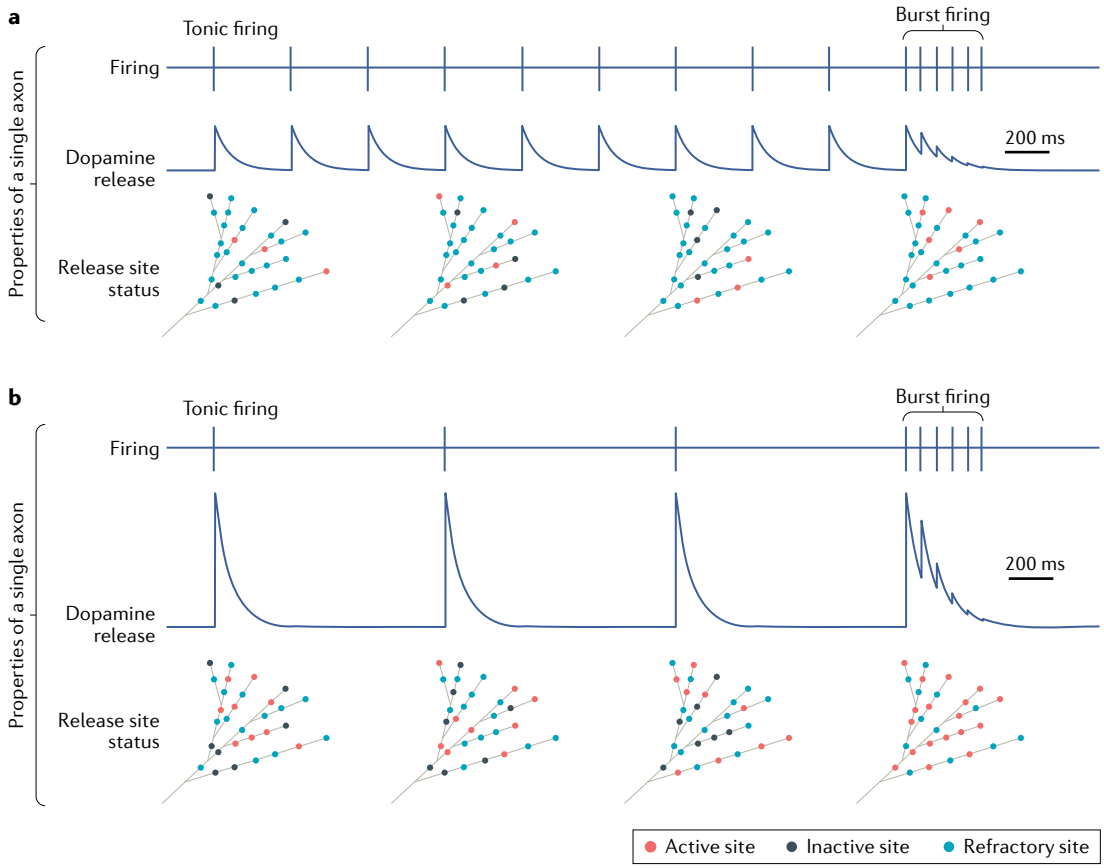


Fig. 4 | **Dopamine neuron firing and release.** A model of dopamine release of a single dopamine axon during tonic firing and burst firing. The amount of dopamine released from a single axon depends on how many sites release dopamine (red dots, active sites). Many sites do not release dopamine, either because the initial release probability of the available sites is below 1 (black dots, inactive sites) or because sites are in a depressed state (blue dots, refractory sites). Neurons with a higher tonic firing frequency (panel **a**) will have more refractory sites and release less dopamine in response to each action potential than neurons with a lower tonic firing frequency (panel **b**), which will have fewer refractory sites and release more dopamine in response to each action potential. Burst firing leads to the rapid depression of release from a single axon, with neurons with a low tonic firing frequency contributing more dopamine. Note that this speculative model presents the total amount of release from a single axon, which cannot currently be measured. Typical measurements using electrochemical methods (FIG. 2b) reflect the average dopamine in a large volume and from many neurons, not the sum of peak dopamine at release sites from a single axon. In addition, measurements taken in vivo using voltammetry may reveal a build-up of dopamine (instead of a decay) during rapid stimulation because dopamine reuptake is overwhelmed and because low-frequency measurements overestimate dopamine levels during the decay phase.

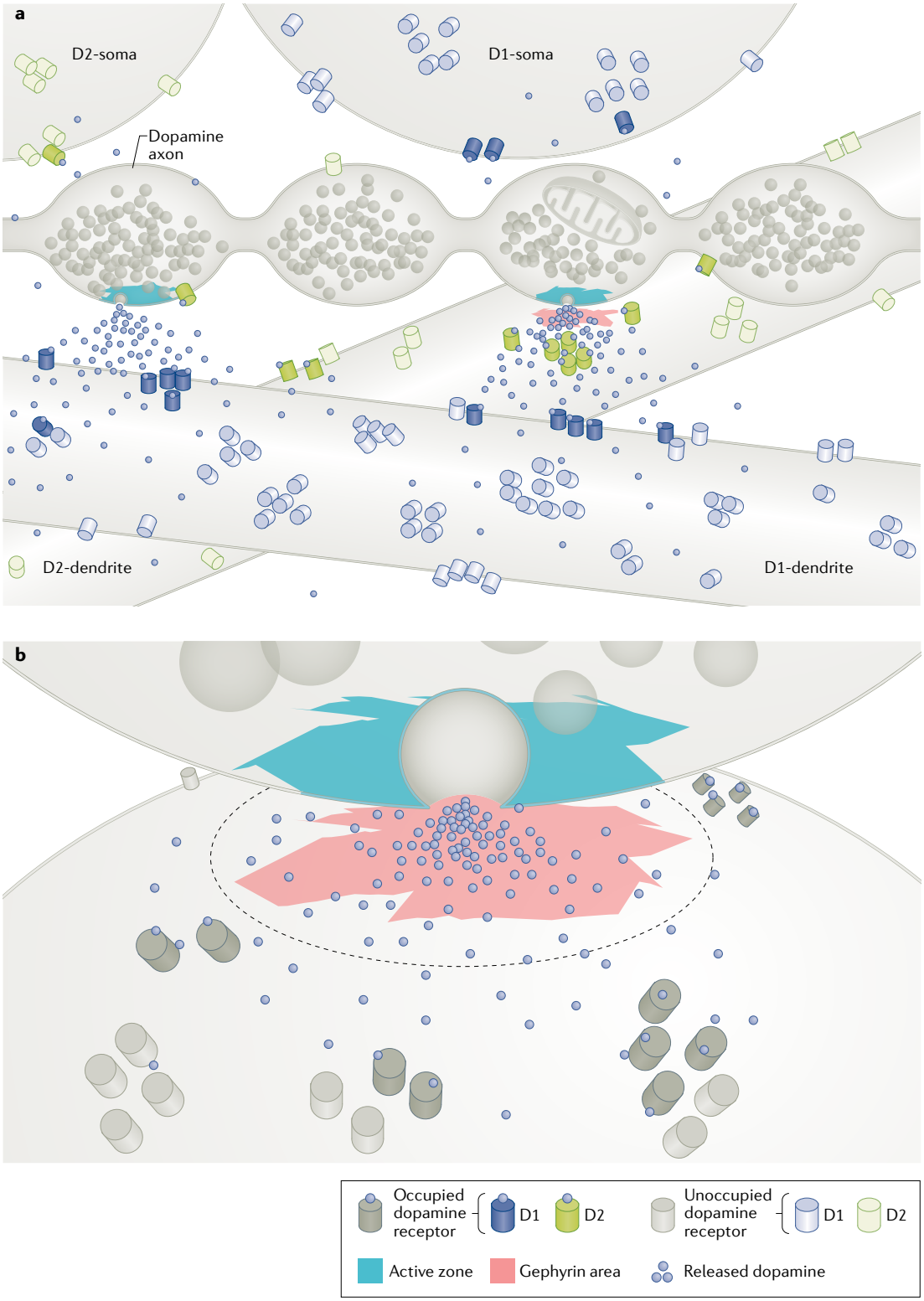
(or striosomes) and matrix[107]; MSNs are localized in both areas. Here, we focus on the nanometre-to-micrometre scale organization of dopamine receptors on MSNs and how their organization could contribute to the control of dopamine signalling.

Cell type-specific dopamine receptor distribution. Dopamine receptors are GPCRs. In vertebrates, five genes encode two major classes of dopamine receptors: the D1-like (D1, D5) and the D2-like (D2, D3 and D4) receptors[15]. D1-like receptors are coupled to $G_{\alpha s}$ or $G_{\alpha olf}$ to activate adenylate cyclase and to increase excitability. By contrast, D2-like receptors couple to $G_{\alpha i}$ or G_o, reducing adenylate cyclase activity and having an overall inhibitory effect[108–110].

In the striatum, D1 receptors are threefold to fivefold more abundant than D2 receptors; the expression of other dopamine receptors is generally low[108,111–113]. The presence of D1 or D2 receptors defines MSN subtypes (as D1-MSNs or D2-MSNs, respectively), and D2 receptors are also expressed on dopamine axons[12,114,115] (FIG. 5). The distinct receptor localization on MSN subtypes forms the foundation of striatal dopamine regulation and defines functions of the classic model of direct and indirect pathways[1,114–117]. Approximately half of MSNs predominantly express D1 receptors and form the direct pathway, facilitating movement and reinforcement learning. The other half mostly express D2 receptors and give rise to the indirect pathway, which generally inhibits the same functions[25,114,117–121].

Receptor organization. The subcellular distribution of dopamine receptors remains largely unknown. However, their opposing effects on cell excitability, their cell type-specific distribution and their distinct behavioural functions as revealed by pharmacological manipulations

Direct and indirect pathways
The prominent output circuits of the striatum that originate from separate medium spiny neuron populations and project to distinct target areas.



may suggest an organization for selective activation. A simple solution would be that a single dopamine axon specifically targets nearby receptors on a specific MSN. Dopamine reuptake mechanisms could be organized locally to ensure that dopamine spread is limited. The targeting of a specific MSN by a dopamine axon and the presence of mechanisms to limit dopamine spread would be similar to synapses (FIG. 1), for which the defining features are receptor clustering within tens of nanometres of release sites and limited transmitter spread past the boundaries of the synaptic cleft[62,64] — fundamentally different from classical models of volume transmission.

However, most morphological studies are not easily compatible with such a synapse-like organization of dopamine transmission. Ultrastructural analyses

◀ Fig. 5 | **Dopamine receptor organization and activation.** A working model of dopamine receptor organization with an overview (panel **a**) and zoom-in on a varicosity that makes a synapse-like contact (panel **b**). Dopamine is released from non-synaptic and synaptic varicosities. The main dopamine receptors are segregated over two subtypes of medium spiny neurons (MSNs): those expressing dopamine type 1 (D1) receptors (D1-MSNs) and those expressing D2 receptors (D2-MSNs). Both MSN types sense dopamine release from non-synaptic varicosities (panel **a**, left), and can receive synaptic-like inputs from dopamine axons with appositions between dopamine varicosities and GABAergic postsynaptic assemblies (panel **a**, right, and panel **b**). Dopamine receptors are widely distributed on MSNs and may be present in clusters. Importantly, currently available tools have not found dopamine receptors in the postsynaptic specializations (panel **b**). Instead, these specializations may contain gephyrin and other proteins typically found at GABAergic synapses[13]. Individual vesicle-fusion events may activate both D1 receptors and D2 receptors, and nearby receptors are more likely to be activated by dopamine than those farther away. The exact organization of dopamine receptors relative to release sites is not known but may strongly impact dopamine functions.

have revealed that most dopamine varicosities are not apposed to postsynaptic cells and densities[42–44]. Although antibody-labelling often suffers from nonspecific signals, studies with several different dopamine receptor antibodies consistently suggest a distributed localization. Light and immunoelectron microscopy have revealed that D1 receptors are broadly localized on MSNs, with somatic, dendritic shaft and dendritic spine localizations, and that they sometimes seem to be clustered[13,14,122]. Similarly, D2 receptors appear broadly distributed on D2-MSNs, with some studies suggesting an enhanced presence on distal dendrites[12–14]. A recent immunoelectron microscopy study focused on the subset of dopamine varicosities that make synapse-like contacts[13]. There, D1 and D2 receptors were not commonly found in the apposed postsynaptic membrane but were distributed perisynaptically (within 100 nm of the edges of the synapse-like apposition) or extrasynaptically (beyond 100 nm of the synapse-like contact). Together, these morphological studies suggest that dopamine receptors are at least partially clustered on MSNs, but they fail to detect a synapse-like apposition for most receptors (FIG. 5). As such, the term 'dopamine synapse' should not be used at this point to describe the general striatal dopamine signalling architecture.

Receptor activation. Receptor activation is a dynamic process, relying on ligand availability and receptor-binding properties. An early observation was that D2 receptors exhibit a high-affinity state (with a dissociation constant (K_d) of approximately 25 nM), whereas D1 receptors are mainly in a low-affinity state (with a K_d of approximately 1 μM)[112]. This has led to a model in which tonic dopamine levels (about 2–20 nM) and phasic dopamine release respectively activate D2 receptors and D1 receptors. The necessary release–receptor distances were estimated based on these affinities and corresponding models were proposed[123–125]. However, the experimental conditions during affinity measurements may have confounded the initial results. GTP that is present in MSNs substantially reduces the affinity of D2 receptors to levels similar to those of D1 receptors[112], and the models proposed on the basis of the estimated affinities have since been challenged[113,126]. Most studies in brain slices find that the half-maximal effective dopamine concentration for activation of the two receptor types is similar and in the micromolar range, suggesting that both dopamine receptors are mainly in a low-affinity state in vivo[16,127–129].

A single vesicular release event generates a spreading sphere of dopamine with a steep concentration gradient surrounding this point source, and the degree of receptor activation depends on release–receptor distances and on dopamine dwell times[22,123,130]. In contrast to synapses, where cleft organization leads to transmitter concentrations above 1 mM (REF.[64]), dopamine is free to diffuse in the extracellular space and is quickly diluted upon release. If nanomolar dopamine is sufficient for receptor activation and a binding equilibrium is immediately reached, a release event might be able to recruit dopamine receptors as far as a few micrometres away. However, dopamine transients from a single vesicle are brief (by about 3 ms, they are diluted to less than 200 nM)[113,123], dopamine receptor activation necessitates micromolar dopamine levels[16,127,128] and dopamine receptors probably have relatively slow binding kinetics[131,132]. Therefore, it is improbable that receptors at micrometre distances from release sites are efficiently activated by a single vesicular release event. Instead, the effective distance for receptors to detect quantal release is probably less than 1 μm (REFS[22,113,123,130–132]).

An additional important consideration is the relationship between diffusion and dopamine reuptake via the dopamine transporter (DAT). Experimental data and modelling suggest that diffusion dominates dopamine levels over distances of several micrometres from release sites and that DAT effects are likely negligible at short distances from secretory events[105,123,133]. As described above, single release events generate a small receptor activation zone, probably in the sub-micrometre range, and are therefore probably exempt from substantial DAT regulation; however, DAT activity may determine the degree of 'crosstalk' between adjacent release sites and thus control the spatiotemporal characteristics of phasic signalling[22,123].

Electrophysiological studies of D2 receptors further contrast affinity-based models. They have established that D2 receptor signalling relies on nearby low-affinity receptors that are rapidly activated. D2-IPSCs can be measured as GIRK2 currents in midbrain dopamine neurons and are triggered by somatodendritic release[95]. Notably, fluorescently tagged endogenous D2 receptors are at least partially clustered on midbrain dopamine neurons[134]. Somatodendritic transmission occurs in a rapid and localized manner[95,135,136], contrary to the classical view of tonic D2 receptor signalling. The resulting D2-IPSCs are readily evoked by a single stimulus and do not require repetitive stimulation, they have relatively fast kinetics, with a lag of approximately 50 ms after stimulation, and they necessitate high dopamine concentrations[95,135,136]. Such D2-IPSCs can be recorded even in the absence of action potentials, suggesting efficient receptor activation through the exocytosis of single vesicles (that is, quantal release events)[48]. Together, these studies reveal that, in the midbrain, release sites and receptors are organized such that individual secretory events effectively mediate somatodendritic transmission. Notably, many of these features are shared by striatal

Dissociation constant
An equilibrium constant that specifies the tendency of an agent to separate from its target; a high dissociation constant reflects a low binding affinity.

Dwell times
The period of time for which a ligand is bound to its target; prolonged dopamine dwell times generally result in enhanced dopamine receptor signalling.

D2 receptor transmission when it is monitored via the expression of GIRK2 channels[16]. Most importantly, strong stimulation enhances D2-IPSCs over repetitive optogenetic stimulation, and D2 receptors are only saturated by approximately 100 µM dopamine, meaning that striatal D2 receptors respond to phasic release[16].

Altogether, studies on release and receptors predict that there is a steep dopamine gradient originating from a point source, that receptors within approximately 1 µm of a release event are rapidly activated, and that dopamine dynamics and receptor states strongly influence receptor activation. Given the low density of active release sites resulting from the low active-zone density and release site depression (FIGS 3,4), large striatal areas are likely out of the reach of individual vesicle-fusion events (FIG. 5), and receptors in these farther-away areas require dopamine spread and pooling from multiple sites for activation, as proposed in the domain-overlap model.

A framework for dopamine signalling

Building on the new knowledge of dopamine release and reception, we herein propose a micrometre-scale framework for dopamine transmission. We first present a new model — the domain-overlap model — and then discuss how it relates to existing models of dopamine signalling.

The domain-overlap model. Baseline dopamine signalling is often considered to rely on uniform, steady-state dopamine concentrations[101,102,137], a view that arose from sampling large volumes at low frequency, for example, with microdialysis or voltammetry (FIG. 2). As discussed above, many dopamine release sites are depleted during tonic firing. As a consequence, uncoordinated tonic dopamine neuron activity leads to release from only a small and changing subset of release sites that are sparsely distributed in the striatum, effectively generating small 'dopamine domains' (FIGS 3,4,6a). Upon release, dopamine is diluted to sub-micromolar concentrations at micrometre distances within a matter of milliseconds[50,123,138], and overlap with dopamine from adjacent domains is unlikely. As dopamine dwell times around these sparse events are short, release during tonic firing cannot maintain steady-state receptor activation[113] but instead transiently activates a changing small subset of receptors (FIG. 6a). This dynamic process may appear as steady-state signalling in measurements with low spatiotemporal resolution.

By contrast, groups of dopamine neurons are activated synchronously by reward consumption or movement initiation[6,20,21,139]. This simultaneous activity leads to phasic release and overlap of dopamine domains from multiple sites. As a result, more-homogeneous dopamine levels and receptor activation beyond the individual, micrometre-sized signalling domains are likely (FIG. 6b). According to this domain-overlap model, understanding whether there is differential dopamine receptor distribution at 'long', micrometre distances from release sites is critical, as this would determine which receptors are activated during tonic firing versus during phasic firing. A dynamic activation mechanism, combined with distinct distributions of receptor subtypes at multi-micrometre scales, might contribute to pathway selection and underlie the diverse roles of dopamine.

Relationship to previous models. Whereas dopamine release and diffusion occur within a few milliseconds, dopamine receptors, like other GPCRs, require approximately 100 ms or more to signal, limiting signalling speeds[16,17,80]. Thus, the high *P* observed in striatal axons of dopamine neurons may not be necessary for rapid signalling but instead generates fast, synchronous release needed for phasic signalling via the activation of receptors beyond the individual signalling domains. Such simultaneous release would transiently overwhelm the DAT, lead to overall enhanced dopamine levels and increase dopamine dwell times to reach the activation thresholds of receptors residing farther away[16,22,123,137]. This domain-overlap model reconciles features of D2-IPSCs with morphological studies[13,16,95]. As long as receptors are reached within milliseconds by dopamine levels sufficient for their activation, they may not need to be precisely apposed to release sites; indeed, localization within hundreds of nanometres may be sufficient for activation (FIGS 3,5). DAT blockade enhances D2 receptor activation during phasic release and may do so because dopamine travels over longer distances and hence reaches farther-away D2 receptors in areas where DAT effectively modulates dopamine levels and dwell times[13,16,136]. Rapid release with sharp increases in extracellular neuromodulator concentration, followed by diffusion over distance, might represent a universal mechanism for modulatory systems in the brain.

D1-MSNs and D2-MSNs define the direct and indirect pathways[114,117,118], respectively, with the direct pathway facilitating movement and reinforcement learning and the indirect pathway inhibiting these functions[25,116,119–121]. In a simplified view, the role of dopamine in these pathways is twofold[6,20,21,25]. For movement control, dopamine modulates moment-by-moment activity in the striatum to mediate action selection. For learning, it couples active ensembles of neurons to induce long-term synaptic changes and to enhance select neural trajectories during task execution. Dopamine activates the direct pathway and inhibits the indirect pathway, changing the net output and triggering synaptic plasticity in this circuitry[25,114,115,140,141]. We propose the reconsideration of affinity-based models in which extrasynaptic D2 receptors preferably sense baseline dopamine, and D1 receptors mostly respond to high-amplitude phasic dopamine at synaptic-like structures[106,112,125,142]. This model assumes a higher affinity of D2 receptors, does not consider receptor activation kinetics and concludes that D2 receptors are widespread. However, as outlined throughout this Review, many findings challenge affinity-based models. D2 receptors probably operate in a low-affinity state in vivo and are not efficiently activated by background dopamine levels (that is, levels within 2–20 nM). Instead, they reliably detect phasic release, saturate only at 100 µM dopamine[16] and are markedly clustered in the midbrain[134]. The kinetics, affinity, abundance and distribution of D1 and D2 receptors make both receptors suited for tonic and phasic signalling[13,113,126,131,132]. Furthermore, tonic release does

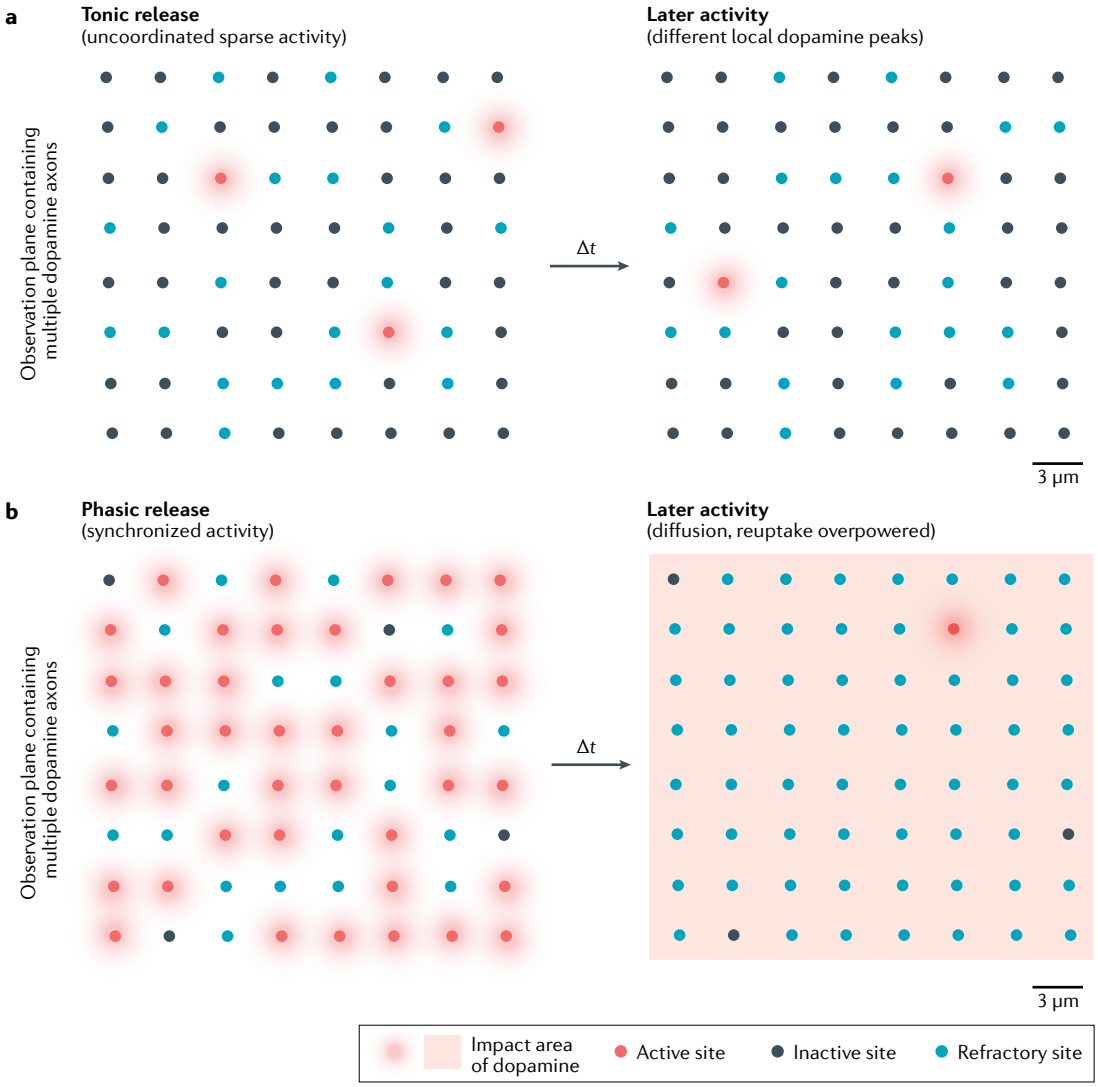


Fig. 6 | **The domain-overlap model.** A model of dopamine signalling domains during tonic and phasic release. In a given area of the striatum, tonic release (panel **a**) generates short-lived dopamine peaks that are confined to a small domain and only recruit proximal receptors (left). After a short time interval (Δ*t*), a distinct set of release sites is active and targets a different subset of receptors (right). The synchrony of phasic release across many release sites in a given area (panel **b**) generates substantial crosstalk between these signalling domains. After a brief interval, dopamine spreads and dopamine levels increase beyond the micrometre-sized domains of active sites and transiently overwhelm the dopamine transporter (DAT). This leads to augmented dopamine dwell times and to the activation of more receptors that reside farther away from release sites. This domain-overlap model may form a basis for the recruitment of small variable subsets of receptors during tonic activity (arising from small dopamine domains) and of larger numbers of distant receptors during phasic activity (arising from overlap of dopamine domains). The regional heterogeneity in release-site distribution within the complex dopamine axonal arbor may influence receptor activation domains, and co-release could further shape the signalling of dopamine neurons. The differential distribution of distinct dopamine receptors or cell types at micrometre-scale distances may lead to distinct pathway modulation during tonic and phasic release, respectively.

not produce steady-state dopamine levels. Instead, the domain-overlap model contends that tonic release is a dynamic process that transiently activates nearby receptors, and that both D1 receptors and D2 receptors can be activated if they are close enough (FIG. 5). Finally, the domain-overlap model implies that phasic release recruits receptors that are farther away, and that the degree of activation is determined not by distinct receptor affinities but by release–receptor topographies and dopamine dwell times.

Receptor clustering at micrometre-scale distances is well suited to explain the modulation of striatal pathways by dopamine. Although some of the receptor clusters may be in a near-synaptic organization, they do not need to be. Instead, receptor clustering is needed for the compartmentalized and robust induction of intracellular signalling and, according to the domain-overlap model, release–receptor distances that vary at a micrometre scale determine activation during tonic or phasic release (FIG. 6). Early studies probably overestimated the

density of functional dopamine release sites because varicosities rather than active zones per se were counted as the relevant sites (FIG. 3), and because the notion that spontaneous firing depresses most sites was not considered (FIG. 4). This resulted in models in which release modes account for homogeneously low concentrations of 'tonic' dopamine and sharp, brief, local increases in 'phasic' dopamine. We propose that switches between firing modes can enable the select activation of receptor clusters by generating separated or overlapping signalling domains, where the relevant parameter is not how much dopamine levels increase at release sites, but how far dopamine spreads. Whereas isolated release events activate only nearby receptors, synchronized firing across dopamine neuron populations recruits additional receptors, owing to the crosstalk of active release sites (FIG. 6). If D1 and D2 receptors are distributed equally around the release sites, phasic dopamine release will not change their ratio of recruitment. Conversely, if the distances to release sites are different between receptor subtypes, during phasic release, the distal receptors will be relatively more preferred. The organization between dopamine release sites and its receptors not only allows a single release site to recruit multiple surrounding receptors, but may also enable receptors to receive input from distinct subsets of dopamine axons. There are currently no data on release-site distribution along a single axon, but heterogeneity in the distribution of release sites along the extensively arborized axon may further affect dopamine signalling. Ultimately, the relative organization of dopamine release sites and of distinct receptor subtypes remains an open question, but a higher-order architecture at micrometre scales might be exceptionally well suited to control the distinct output neuron populations via switches in firing modes.

Conclusions and outlook

With the domain-overlap model, we propose that dopamine signalling has evolved to control striatal output through a specialized architecture with relevant release–receptor assemblies at micrometre scales. This is different from synaptic transmission, and embodies a form of volume transmission that is more refined than the often-proposed diffuse organization.

A key feature of dopamine signalling is that tonic firing and synchronized burst firing encode distinct functions. The mesoscale signalling structure of the domain-overlap model permits differential coding. Tonic release activates variable subsets of nearby receptors through secretion from sparse sites. Burst firing triggers synchronized release from a population of neurons, which leads to overlap of the dopamine signalling domains (FIG. 6) and recruitment of additional distant receptors. The domain-overlap model, relying on fast release and diffusion to activate distant receptors, may represent a universal mechanism for volume transmission in the brain. Cholinergic transmission, for example, shares key morphological features, high *P*, and pacemaker and burst-firing modes with dopamine transmission[44,143].

Future work should aim at testing this model and at mechanistically dissecting four important points. First, a precise understanding of receptor distributions at micrometre distances from release sites is essential, including their clustering, their positioning relative to the secretory hotspots and their activation profiles during firing. Second, most varicosities may release little or no dopamine in response to action potentials[17,18,41]. What is the benefit of having them? Material storage in the extensive axonal arbor, a reservoir that can be activated during structural plasticity and a source for action potential-independent dopamine release are possibilities that should be investigated. Third, dopamine signalling in the striatum relies on extensive local regulation[8] and a remarkable feature is the cholinergic interneuron-dependent triggering of striatal dopamine release[17,84–87]. It will be important to assess how these regulatory mechanisms are embedded in the signalling architecture we describe here. Finally, at fast synapses, cell adhesion proteins are thought to provide critical signals for nanometre-scale release–receptor apposition[62,64]. How a micrometre-scale architecture between release sites and receptors can be set up and modulated is unclear.

Similar to other areas in neuroscience, we are at an exciting stage in understanding dopamine as new technology is developed to drive progress. Important recent advances include the development of multiple dopamine sensors[22,79,83,144], the generation of genetic resources for the inactivation or slowing down of action potential-triggered dopamine release[17,40,41], and advances in super-resolution microscopy that enable studies of the protein composition of brain circuits at high resolution over large volumes[145,146]. We expect that these tools will enable mechanistic insight into dopamine signalling networks with unprecedented precision and hope that this progress will contribute to the understanding of principles of neuromodulation in general.

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