

Computational modelling identifies key determinants of subregion-specific dopamine dynamics in the striatum

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The conclusions of this work are based on **valuable** simulations of a detailed model of striatal dopamine dynamics. Establishing that a lower dopamine uptake rate can lead to a 'tonic' level of dopamine in the ventral but not dorsal striatum, and that dopamine concentration changes at short delays can be tracked by D1 but not D2 receptor activation, is of value and will be of interest to dopamine aficionados. However, the simulations are **incomplete**, providing only partial support for the key claims. Several things can be done to strengthen the conclusions, including, for example, but not exclusively, a demonstration of how the results would change as a function of changes in D2 affinity.

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Abstract

Striatal dopamine (DA) release regulates reward-related learning and behavioural activation and is believed to consist of a short-lived *phasic* and continuous *tonic* component. Here, we build a three-dimensional model of extracellular DA dynamics in dorsal (DS) and ventral striatum (VS) that include tens of thousands of DA release sites. The model predicts rapid dynamics in DS with little-to-no basal DA, and much slower dynamics in the VS enabling build-up of a *tonic* DA level. These regional differences do not primarily reflect different release phenomena but rather arise from differential dopamine transporter (DAT) activity. Interestingly, our simulations posit prevalent nanoclustering of DAT as a possible regulator of this activity. Receptor binding simulations show that D1 receptor occupancy follows extracellular DA concentration with millisecond delay, while D2 receptors integrate DA signal

over seconds. Summarized, our model distills recent experimental observations into a computational framework that challenge prevailing paradigms of striatal DA signalling.

Introduction

Striatal dopamine (DA) release is essential for regulating reward-related learning, incentive motivation, and motor function (Berke, 2018 [DOI](#); Klaus et al, 2019 [DOI](#)). DA exerts these roles over a broad range of time scales, yet DA primarily operates as a volume transmitter that targets metabotropic receptors located within a micrometre range from the sites of release (Agnati et al, 1995 [DOI](#); Borroto-Escuela et al, 2018 [DOI](#); Cragg & Rice, 2004 [DOI](#); Gonon et al, 2000 [DOI](#); Sulzer et al, 2016 [DOI](#)). The temporal and spatial dynamics of DA release in striatum, however, remain a highly contested topic. Classically, DA release has been divided into *tonic* release, driven by pacemaker-like spontaneous firing, and *phasic* release from coordinated bursts of firing across neurons (Niv et al, 2007 [DOI](#); Schultz, 2007 [DOI](#); Sulzer et al., 2016 [DOI](#)). However, this sharp distinction in release modes, as well as the existence of a basal DA level has recently been challenged (Berke, 2018 [DOI](#); Ejdrup et al, 2023 [DOI](#); Jorgensen et al, 2023 [DOI](#); Liu et al, 2021 [DOI](#); Sippy & Tritsch, 2023 [DOI](#)).

The picture is further complicated by major regional differences across striatal sub domains. These include differences in Ca^{2+} -channel and nicotinic acetylcholine receptor (nAChR) expression profiles on DA terminals, as well differential regulation and expression of the DA transporter (DAT) (Brown et al, 2011 [DOI](#); Cardozo & Bean, 1995 [DOI](#); Kearney et al, 2023 [DOI](#); Richards & Zahniser, 2009 [DOI](#); Threlfell et al, 2010 [DOI](#)). In addition, we and others have found remarkable differences in extracellular DA release dynamics between the dorsal (DS) and ventral striatum (VS) (Jorgensen et al, 2023 [DOI](#); Mohebi et al, 2024 [DOI](#); Salinas et al, 2023 [DOI](#)). Fibre photometry recordings in the DS in mice using the DA sensor dLight1.3b during self-paced exploratory activity showed a rapidly fluctuating signal, whereas we observed up to minutes-long DA dynamics in VS that correlated with behavioural output (Jorgensen et al., 2023 [DOI](#)). Concurrent measurements of extracellular DA by microdialysis and fibre photometry have furthermore corroborated the lack of tonic levels of DA in DS while supported its presence in VS (Ejdrup et al., 2023 [DOI](#); Jorgensen et al., 2023 [DOI](#)). Despite these reported differences in striatal DA dynamics, electrophysiological recordings suggest that DA neurons from the primary innervators of DS, substantia nigra par compacta (SNc) and VS, ventral tegmental area (VTA), have remarkably similar firing patterns at rest (Dodson et al, 2016 [DOI](#)). We therefore set out to better understand the fundamental principles governing extracellular DA dynamics by constructing a new computational model of the striatal DA system.

Extracellular DA dynamics have been modelled before; either one-dimensionally or with a primary focus on single release events or post-synaptic receptor binding (Beyene et al, 2017 [DOI](#); Dreyer et al, 2010 [DOI](#); Dreyer & Hounsgaard, 2013 [DOI](#); Dreyer et al, 2016 [DOI](#); Venton et al, 2003 [DOI](#); Wiencke et al, 2020 [DOI](#)). Here, we present a three-dimensional model of tens of thousands of release sites, focused on larger-scale signalling and based on experimentally observed biological parameters. The model faithfully replicates experimentally observed results as well as the difference in DA dynamics between DS and VS. Importantly, it offers compelling evidence that these differences do not primarily reflect different release phenomena but rather arise from differential expression and possibly nanoscale localization of the DA transporter (DAT).

Results

Construction of a model of DA dynamics in the striatum

We constructed a novel model of DA release using experimentally determined parameters from DS, including release, uptake, and cytoarchitecture (Doucet et al, 1986 [DOI](#); Dreyer et al., 2010 [DOI](#); Dreyer & Hounsgaard, 2013 [DOI](#); Liu et al., 2021 [DOI](#); Olson et al, 1972 [DOI](#); Sulzer et al., 2016 [DOI](#)). DA release sites on axons projecting from the midbrain were randomly simulated as uniformly distributed discrete points in a three-dimensional space (**Figure 1A** [DOI](#)). The release events themselves were simulated as point source events (Cragg & Rice, 2004 [DOI](#)) driven by action potentials (AP). We then modelled DA release for each voxel in the simulation containing a release site as function of three key parameters; firing rate, release probability and quantal size:

(1)

$$\text{release}_{n,t} = \text{Poisson}(f_{\text{rate}} \, dt)_n P(R_{\%})_t Q \, dt$$

where $\text{Poisson}(f_{\text{rate}} \, dt)_n$ is a Poisson distribution of action potentials (AP) for the given neuron (n) with the firing rate f_{rate} , $P(R_{\%})_t$ is the probability of release at the individual terminal (t) for each AP, while Q is the number of DA molecules released per event (dopaminergic quantal size) and dt the time step. Changing f_{rate} can be used to model both pacemaker firing, typically reported at 2-10 Hz, and burst firing, which can exceed 20 Hz (Sulzer et al., 2016 [DOI](#)).

DA reuptake in the striatum is almost exclusively mediated by the DA transporter (DAT) (Jones et al, 1998 [DOI](#)), which is widely distributed along DA axons and varicosities (Block et al, 2015 [DOI](#); Eriksen et al, 2010 [DOI](#); Eriksen et al, 2009 [DOI](#)). As reuptake follows concentration-dependent Michaelis-Menten kinetics (Nicholson, 1995 [DOI](#)), we simulated uptake as follows:

(2)

$$\text{uptake} = \frac{V_{\text{max}} [\text{DA}]}{K_m + [\text{DA}]} \, dt$$

where [DA] is the concentration of DA for each voxel in the model, V_{max} is the maximal uptake capacity in the region and K_m is the concentration of DA at which half of V_{max} is reached.

The spatial distribution of released DA is a complex interplay between release, uptake and diffusion. Diffusion in an open 3D space can be simulated for each voxel with a Laplacian operator:

(3)

$$\text{diffusion} = \frac{\partial \text{DA}_{x,y,z,t}}{\partial t} = D_a \, dt \left(\frac{\partial^2 \text{DA}_{x,y,z,t}}{\partial x^2} + \frac{\partial^2 \text{DA}_{x,y,z,t}}{\partial y^2} + \frac{\partial^2 \text{DA}_{x,y,z,t}}{\partial z^2} \right)$$

where D_a is a corrected diffusion coefficient and dt is the timestep. As the extracellular space of the striatum is tortuous, we modified the conventional diffusion coefficient D to an apparent diffusion coefficient (D_a) to correct for the tortuosity (λ) of the striatum (Cragg & Rice, 2004 [DOI](#); Nicholson, 1985 [DOI](#)):

(4)

$$D_a = \frac{D}{\lambda^2}$$

Combining [equation 1](#) through 4, we model DA changes in each voxel with a single conceptual equation:

(5)

$$\frac{d\text{DA}}{dt} = \text{release} - \text{uptake} + \text{diffusion}$$

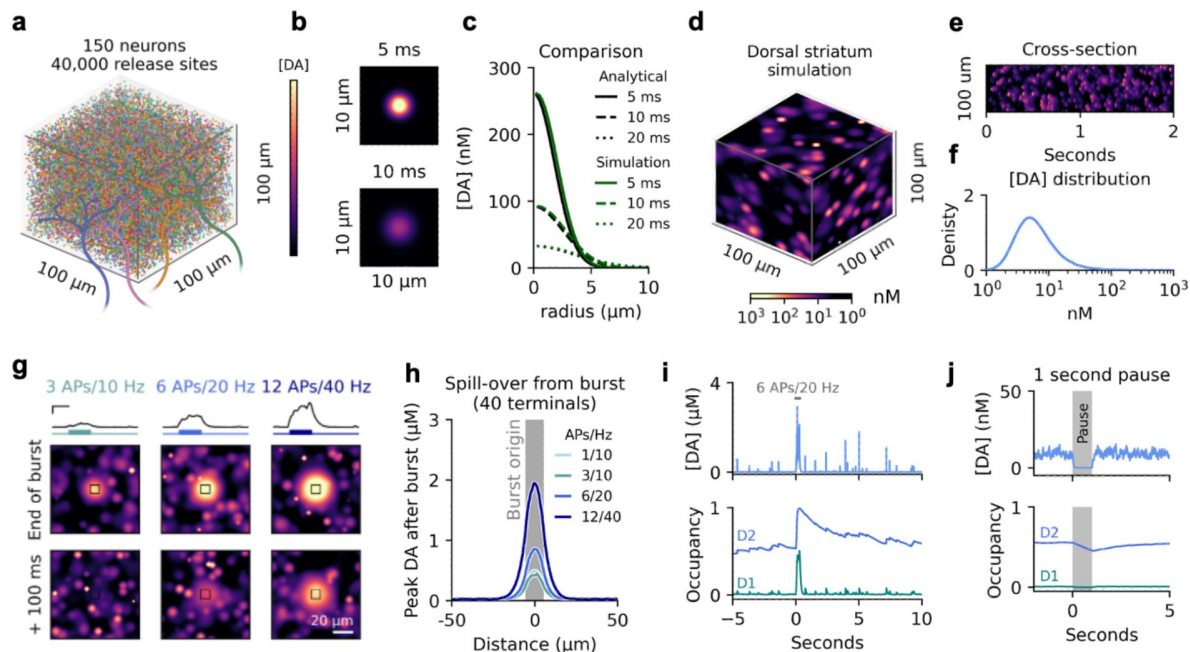


Figure 1.

Large-scale 3D model of the dorsal striatum.

(a) Self-enveloped simulation space of $100^3 \mu\text{m}^3$ with approximately 40,000 release sites from 150 neurons. Colours of individual release sites are not matched to neurons. (b) Simulation of a single release event after 5 ms and 10 ms. Colour-coded by DA concentration. (c) Comparison of analytical solution and simulation of diffusion after a single release event at three different time points. (d) Representative snapshot of steady state DA dynamics at 4 Hz tonic firing with parameters mirroring the dorsal striatum. (e) Cross-section of temporal dynamics for data shown in (d). (f) Histogram of DA concentrations ([DA]) across the entire space in (d). (g) DA release during three burst activity scenarios for all release sites in a $10 \times 10 \times 10 \mu\text{m}$ cube (black boxes) and spill-over into the surrounding space. Burst simulated as increase in firing rate on top of continued tonic firing of the surrounding space. Traces on top are average DA concentrations for the marked cubes, with bursts schematized by coloured lines below. First image row is at the end of the burst, second row another 100 ms after. Scale bars for traces are 200 ms and 500 nM. Scale bar for the images is $20 \mu\text{m}$. (h) Peak DA concentration reached at different distances from area with phasic activity for the three firing scenarios. (i) Top: representative [DA] trace $1 \mu\text{m}$ away from a release site during pacemaker and burst activity. Bottom: Occupancy of D1Rs and D2Rs for same site. (j) Effect of complete pause in firing for 1 second on both average [DA] and D1R and D2R occupation.

We first compared our 3D model of DA dynamics to the analytical solution of a single release event (Cragg & Rice, 2004 [↗](#); Gonon et al., 2000 [↗](#)). To do this, we simulated a quantal event of 3000 DA molecules and calculated DA concentrations across space at three separate time points (**Figure 1B** [↗](#), C). The analytic solution and our model predicted almost identical results. Slight differences were introduced as the analytical solution assumes linear uptake from DAT while our model incorporates non-linear Michaelis-Menten kinetics. These differences, however, were almost negligible. The main difference between the two models lies in scalability across both space and time. Summarized, the model enables a dynamic incorporation of the surrounding DA concentration, release events and uptake, and can be scaled to cover DA dynamics of a large 3D space, whose size and granularity is only limited by computing power (see Code Availability Section for the Python code with numerical implementations of the equations listed and scripts to run the simulations and plot the main figures).

We also tested the validity of our model, by examining the response to electrical stimulation. Importantly, our model faithfully mirrored DA release seen with fast-scan cyclic voltammetry (FSCV) recordings upon direct stimulation of striatal slices when we corrected for kinetics of the typical FSCV recording setup (Figure S1b, c and discussion in Supplementary Information) (Atcherley et al, 2015 [↗](#); Brimblecombe et al, 2019 [↗](#); Stuber et al, 2010 [↗](#); Xie et al, 2020 [↗](#)).

Simulating large scale DA dynamics of the dorsal striatum

To better understand the extracellular DA dynamics that arise from the balance between dopaminergic pacemaker activity and uptake, we simulated DA dynamics in the DS generated by pacemaker activity (4 Hz) of 150 neurons in the midbrain in a $100 \times 100 \times 100 \mu\text{m}$ space (**Figure 1D** [↗](#), see parameters in **Table 1** [↗](#)). Our simulations yielded a pattern of partially segregated DA hot spots with large fractions of the simulated space devoid of DA, suggesting that release events in DS only elevate DA in the immediate surroundings with DAT-dependent clearance preventing a larger spread in space (**Figure 1D** [↗](#)). This was also illustrated by a cross-section in time (**Figure 1E** [↗](#)). In line with our recent in vivo microdialysis experiments, the average DA concentration in the simulations during pacemaker activity was approximately 10 nM (Ejdrup et al., 2023 [↗](#)). Further, when we calculated the average concentration of a larger area across time, which fibre photometry conceivably does, the results resembled a tonic DA concentration (Figure S1A). However, our model predicted a spatial distribution that is highly heterogeneous and devoid of pervasive resting or tonic DA levels (**Figure 1D-F** [↗](#)).

To ensure our simulations were performed within a sufficiently large space to yield consistent results, we tested different sizes of the simulated area and found a diameter of $50 \mu\text{m}$ to faithfully mimic the results of larger simulations (Figure S2A, B). Additionally, we tested our simulations at different granularity (0.1, 0.5, 1 and $2 \mu\text{m}$). The finer the spatial grain, the higher detail close to a release event; however, at a spatial granularity of $1 \mu\text{m}$, [DA] deviated by <2% across most percentiles and only by >1 nM above the 99.5th percentile (Figure S2C-F), leading us to use this voxel size for our simulations.

Burst firing and receptor occupancy

DA cell bodies are known to fire short bursts of APs, which is a phenomenon strongly linked to reward-prediction error and learning (Schultz, 2007 [↗](#)). To gain insights into accompanying extracellular DA dynamics, we simulated three different firing scenarios for a group of terminals within a $10 \times 10 \times 10 \mu\text{m}$ field encompassing roughly 40 release sites from the randomly simulated 150 neurons: 3 pulses at 10 Hz, 6 pulses at 20 Hz and 12 pulses at 40 Hz (**Figure 1G** [↗](#) – burst properties matched to be the same duration). Unsurprisingly, peak DA concentration was reached at the end of the bursts (**Figure 1G** [↗](#)). The 3 APs/10 Hz bursting scenario generated no significant spill-over of DA outside the region of activity, whereas the 6 APs/20 Hz and 12 APs/40 Hz bursting scenarios markedly overwhelmed uptake (**Figure 1G** [↗](#)). The relationship between firing rate and the sphere of influence by DA became further evident when plotting maximal concentration of the

Variable	Abbreviation	Value	Reference
Firing rate		4 Hz	(Paladini et al, 2003)
Release probability		6%	(Dreyer et al., 2010)
DA molecules per vesicle		3000	(Klaus et al., 2019)
Diffusion coefficient		$763 \mu\text{m}^2 \text{s}^{-1}$	(Nicholson, 1995)
Tortuosity		1.54	(Rice & Nicholson, 1991)
Vmax		$6.0 \mu\text{m s}^{-1}$	See Table S2
Km		210 nM	(Hovde et al., 2019)
Active terminal density	-	$0.04 \mu\text{m}^{-3}$	(Liu et al., 2021)
Extracellular volume fraction		0.21	(Rice & Nicholson, 1991)
Number of neurons in simulation space	-	150	(Matsuda et al., 2009)

Table 1.

List of variables used in simulation of the dorsal striatum.

surrounding space (**Figure 1H**) and the volume of space with a DA concentration above 100 nM (**Figure S1D**). We found that the 3 APs/10 Hz stimulation produced DA responses that largely resembled that of a single pulse. In both cases, DA was mostly cleared after 100 ms and the volume exposed to greater than 100 nM was similar (**Figure 1G** and **Figure S1D**). In contrast, the high bursting activities caused a frequency-dependent spill-over, where the areas exposed to a DA concentration above 100 nM were 10 and 30 times larger than the terminal origin for 6 APs/20 Hz and 12 APs/40 Hz, respectively. Even after 100 ms, a considerable amount of DA remained in the 12 APs/40 Hz scenario (**Figure 1G**).

To understand how these DA dynamics could affect the postsynaptic response, we modelled receptor binding. D1 receptors (-Rs) were assumed to have a half maximal effective concentration (EC_{50}) of 1000 nM and we extrapolated the reverse rate constant (K_{off}) to 19.5 s^{-1} based on a linear fit of the recently characterized DA receptor-based sensors (**Figure S1E**) ([Labouesse & Patriarchi, 2021](#)). We set the EC_{50} of D2Rs to 7 nM and K_{off} to 0.2 s^{-1} based on the DA sensor kinetic fit (**Figure S1E**), which matches a recent binding study ([Agren et al, 2021](#)). To determine how these receptors would respond to our predicted DA dynamics, we simulated pacemaker activity at 4 Hz with an added burst of 6 APs/20 Hz. **Figure 1I** shows a representative trace of DA concentration and occupancy of the D1R and D2R within 1 μm of a release site. During pacemaker activity, D1R showed an occupancy close to 0, whereas D2R occupancy was approximately 0.65 (**Figure 1I**). Both D1Rs and D2Rs occupancies were largely invariant to individual release events caused by pacemaker activity. However, upon coordinated burst firing, the occupancy rapidly increased (**Figure 1I**). D1R receptor occupancy closely tracked extracellular DA with a delay of only ~ 50 ms (**Figure S1F**). In contrast, it took at least 5 seconds before the burst-induced increase in D2R occupancy had declined to baseline levels (**Figure 1I**), and, perhaps more surprisingly, the D2R occupancy only fell from 0.55 to 0.45 when simulating a full second pause in firing due to the slow off kinetics (**Figure 1J**).

These simulations suggest that the dopaminergic architecture of the DS limits DA overflow during physiologically relevant bursting activity. Further, DA receptors had a temporally mostly uniform response to DA release caused by pacemaker activity with D1R occupancy responding rapidly to both onset and offset extracellular DA concentrations following bursts, while D2R showed seconds-long delays in offset.

Ventral striatum maintains pervasive DA tone

Mounting evidence points to considerable differences in DA dynamics across striatal subregions ([Jorgensen et al., 2023](#); [Mohebi et al., 2024](#)), which might reflect differences in the cytoarchitectural and/or molecular dopaminergic make up. In line with this, most studies report lower dopaminergic density in the VS than in DS regardless of methodological modality with a median value of $\sim 90\%$ in VS relative to DS (**Table S1**). Further, DAT-mediated uptake capacity is reported to be lower in VS with a median capacity at $\sim 30\%$ of DS (**Table S2**). Consistently, we observed a clear dorsoventral gradient for DAT expression when analysing immunostainings in striatal mouse brain slices from a previous publication ([Sorensen et al, 2021](#)) (**Figure S3A, B**). By contrast, the VMAT2 staining only decreased slightly from VS to DS (**Figure S3A-C**).

We simulated DA release during pacemaker activity in both DS and VS. DS values were set as previously described ($25\text{ }\mu\text{m}^3$ per terminal, uptake capacity of $6.0\text{ }\mu\text{M s}^{-1}$), but for VS we reduced the terminal density to 90% ($27.8\text{ }\mu\text{m}^3$ per terminal) and DAT uptake capacity to 33% ($2.0\text{ }\mu\text{M s}^{-1}$) (**Tables S1 and S2**). The remaining parameters were kept identical. With these two differences, our simulations revealed markedly different spatiotemporal DA distributions during pacemaker activity. While DS formed segregated domains with low DA concentrations in the inter-domain space (**Figure 2A**), DA diffused further throughout the simulated space in VS, before being cleared by DAT. This gave rise to what may be considered a tonic DA level with hotspots of higher DA concentrations (**Figure 2A**, B).

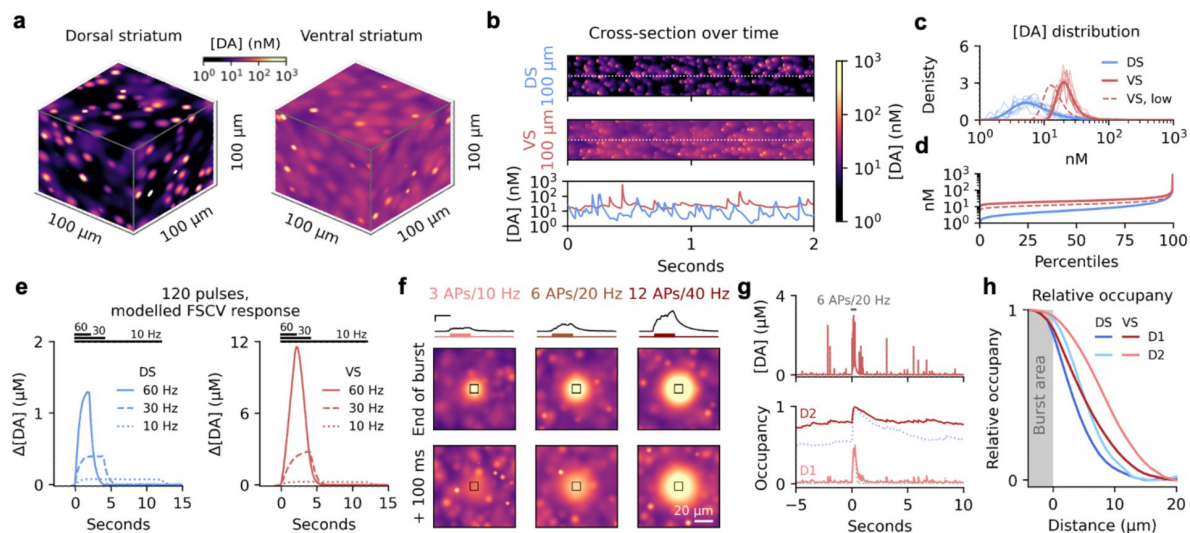


Figure 2.

Regional difference in uptake greatly impact DA dynamics.

(a) Representative snapshots of steady state dynamics at 4 Hz tonic firing with parameters mirroring the dorsal (left) and ventral striatum (right). (b) Cross-section of temporal dynamics for data shown in a. Bottom row show concentrations of the dashed lines in the top panels. (c) Normalized density of DA concentration of simulations in (a). Thick lines are for the entire space, thin lines are across time for five randomly sampled locations. Dashed red line is for simulation of the ventral striatum with lowest reported innervation density in the literature. (d) Same data as in (c), but for concentration percentiles. Note that even the lowest percentiles of VS were above 10 nM in [DA]. (e) Convoluted model response (Figure S1c) to mimic FSCV measurements mirroring the experimentally tested stimulation paradigm in (May & Wightman, 1989) for the dorsal (left) and ventral striatum (right) (f) DA release during three burst activity scenarios for all release sites in a $10 \times 10 \times 10 \mu\text{m}$ cube (black boxes) and spill-over into the surrounding space. Burst simulated as increase in firing rate on top of continued tonic firing of the surrounding space. Traces on top are average DA concentrations for the marked cubes, with bursts schematized by coloured lines below. First image row is at the end of the burst, second row another 100 ms after. Scale bars for traces are 200 ms and 500 nM. Scale bar for the images is $20 \mu\text{m}$. (g) Top: representative [DA] trace $1 \mu\text{m}$ away from a release site during pacemaker and burst activity. Bottom: Occupancy of D1Rs and D2Rs for same site. Occupancy data from corresponding DS simulation on Figure 1k shown in as dotted line. (h) Peak occupancy at different distances from the area bursting normalized to maximal and minimum occupancy.

We compared our model of the two regions with existing experimental data. In an earlier study by May and Wightman, 120 stimulus pulses were delivered in the medial forebrain bundle (MFB) at either 10, 30 or 60 Hz and DA responses were recorded by FSCV in both caudate-putamen (CPu) and nucleus accumbens (NAc) (May & Wightman, 1989). To mirror this, we simulated 120 action potentials at similar frequencies (10, 30, and 60 Hz) at 6% release probability and ran the result through convolution, as in Figure S1B and C, to generate an FSCV read-out (Figure 2E). Since May and Wightman reported no significant difference in DA released per electrically delivered pulse ($[DA]_p$) between VS and DS, we applied equal quantal size and R_0 for DS and VS in our simulations, while uptake capacity in VS was kept to a third of DS and terminal density was set to 90% as specified above. Importantly, our simulated FSCV data closely resembled the earlier findings, with VS reaching considerably higher DA levels for all three stimulation frequencies (Figure 2E). This regional difference presumably arises from differences in DAT capacity between DS and VS, as the lower terminal density in VS would have the opposite effect (see below) and the remaining parameters were held identical.

To compare with our results for DS, we tested how VS responded during simulated burst activity. Using firing patterns identical to the DS simulations (Figure 1G), we found a larger spill-over of DA into the surrounding areas in VS (Figure 2F and Figure S3D, E). Significant amounts of extracellular DA also remained 100 ms after the physiologically relevant 6APs/20Hz firing stimulus. At the receptor level, D1R occupancy in VS showed a similar response to that in DS during the burst (Figure 1I and 2G). By contrast, D2R occupancy during pacemaker activity was higher in VS than DS (~0.8 versus ~0.65 in DS), in accordance with the higher prevailing basal DA concentration. Additionally, the larger DA overflow in VS after a burst caused a higher relative increase in receptor occupancy further away from the area actively bursting than compared to DS (Figure 2H). A pause in firing had the same effect on D2R as in DS (Figure S3F).

Testing sensitivity of model parameters

To test the robustness of the results, we performed simulations across wide ranges of the variable key parameters on which the model is based. First, we varied the number of varicosities actively releasing DA by setting the varicosity density to one site per $9 \mu\text{m}^3$ (Doucet et al., 1986) and simulating 4 Hz pacemaker activity with the release fraction ranging from 5% to 100% (reported values range from 20% to virtually all) (Ducrot et al., 2021; Liu et al., 2021; Liu et al., 2018; Pereira et al., 2016) (Figure 3A). As the fraction of active sites increased, DA concentrations increased at both the median level (50th percentile), which we consider a measure of tonic or baseline DA levels, and at peak levels (99.5th percentile) in both DS and VS (Figure 3B, see Figure S4A for schematic of tonic and peak DA). We then used the 99.5th/50th percentile ratio as a measure of the focality of the DA distribution (i.e. hotspot DA relative to baseline DA). Quantifying this across the percentage of active terminals showed that the focality of the DA distribution dropped as the active fraction increased in both regions (Figure 3C). However, the percentage of active sites in VS needed to drop to 5% to reach a relative distribution resembling the DS at a full 100% active sites, underscoring a marked difference in the spatial confinement of DA signals in VS and DS.

The predicted total DA content of a vesicle and the fraction of content released per fusion event is reported to range from 1,000 to 30,000 molecules (Garris et al., 1994; Pothos et al., 1998; Staal et al., 2004; Sulzer & Pothos, 2000) (Figure 3D). As expected, tonic and peak concentrations increased in both DS and VS as quantal size was increased (Figure 3E). Also as expected, the focality of the DA distributions dropped for both regions as quantal size increased (Figure 3F). The relative difference in tonic DA, however, remained persistently higher in VS and even increased as quantal size increased, indicating a tendency for VS to maintain basal levels of DA regardless of release content (Figure 3G). The higher end of quantal sizes, however, resulted in median concentrations far beyond what is typically reported (Figure 3E) (Sulzer et al., 2016). We observed largely similar pattern when changing either release probability or firing rate (Figure S4B-G).

DAT activity is governed by two parameters: K_m and V_{max} (Kristensen et al, 2011 [\[1\]](#)). To mimic competitive inhibition of DAT by e.g., cocaine, we ran a simulation across various K_m values (**Figure 3H** [\[1\]](#)) showing that increasing K_m caused a linear increase in DA levels, consistent with DAT uptake rate responding almost linearly to increases in [DA] below K_m (**Figure 3I** [\[1\]](#)). Of note, most microdialysis studies have reported that cocaine increases [DA] to the same degree in both DS and VS; however, these quantifications are usually derived as a ratio of the absolute baseline level (Carboni et al, 2001 [\[1\]](#); Maisonneuve & Glick, 1992 [\[1\]](#)). If we divide our simulations of increasing K_m with the basal levels estimated in **Figure 3A-C** [\[1\]](#) a similar response for DS and VS is found (**Figure 5A**). Further, we observe a convergence on a two-fold difference in the absolute values at both tonic and peak levels, which matches reports from earlier FSCV studies (**Figure S5B**) (Wu et al, 2001 [\[1\]](#)). This regionally differential response to cocaine matches our observations in a previous biosensor-based study (Jorgensen et al., 2023 [\[1\]](#)).

Finally, we changed V_{max} by $\pm 50\%$ in both regions and observed a smaller change in for tonic level in DS (11 nM) than in VS (38 nM) (**Figure 3K** [\[1\]](#)). This suggests modulation of V_{max} has higher impact in VS than DS. Further, the cusp-like behaviour of V_{max} in VS was independent of both Q and $R_{\%}$ within values typically reported in the literature (**Figure S5C, D**). In contrast to the changes in tonic levels, the relative effect V_{max} had on peak levels was much more modest (**Figure 3L** [\[1\]](#)).

Changes to uptake rate may be mediated by DAT internalization pathways but to our knowledge there is not in vivo evidence of differential release-uptake balances between animals that could lead to varying tonic DA levels across animals. We therefore reanalysed data from our previously published comparison of fibre photometry and microdialysis and found evidence of natural variations in the release-uptake balance of the mice (**Figure 5F, G**), which may underlie different tonic levels of DA in the striatum between animals.

Simulations of DAT nanoclustering

Our simulations highlight DAT V_{max} as an effective regulator of extracellular DA levels in VS (**Figure 3K** [\[1\]](#)). Internalization of DAT can serve as a mechanism for this control but is a relatively slow process operating on the order of minutes (Kristensen et al., 2011 [\[1\]](#)).

Interestingly, our recent studies have provided evidence that DAT move laterally in the plasma membrane and transition from a clustered to an unclustered nanoscale distribution in response to excitatory drive and other inputs (Lycas et al, 2022 [\[1\]](#); Rahbek-Clemmensen et al, 2017 [\[1\]](#)). This led us to hypothesize that DAT nanoclustering serve as a mechanism for regulating DAT activity on a faster time scale. We speculated that dense nanoclusters of DAT would produce domains of low [DA] due to uptake overpowering diffusion (**Figure 4A** [\[1\]](#)). As uptake rate is concentration dependent, this would reduce uptake efficiency (**Figure 4B** [\[1\]](#)). To address this hypothesis, we simulated a single ellipsoid varicosity of 1.5 μm in length and 800 nm thick with surrounding extracellular space (Ducrot et al., 2021 [\[1\]](#)). The surface was unfolded to a square of equal area (**Figure 4D** [\[1\]](#)), and as 9-16.4% of terminals in the striatum are estimated to be dopaminergic (Hokfelt, 1968 [\[1\]](#); Tennyson et al, 1974 [\[1\]](#)), we set the volume of the surrounding space to seven times the varicosity volume. On the surface of the varicosity, we randomly distributed eight DAT nanoclusters (**Figure 4C** [\[1\]](#)) and ran simulations of how DAT clustering density influenced the DA clearance from the surrounding space. The observed DA concentration in the space surrounding the varicosity shown in **Figure 4C** [\[1\]](#) is illustrated by the cross-section shown in **Figure 4D** [\[1\]](#). Mean DA uptake capacity of the entire space was kept constant at 4 $\mu\text{M s}^{-1}$ (between the values observed for DS and VS) throughout the simulations, representing a constant amount of DAT molecules on the surface of the varicosity. We only changed the fraction of the surface of the varicosity that was uptake competent by altering the cluster size from small clusters of high density to large clusters of lower density. We ran simulations of eight clusters at 20, 40, 80 or 160 nm in diameter to mirror experimentally observed cluster sizes on DA varicosities, as well as a scenario with DAT fully dispersed (**Figure 4E** [\[1\]](#)) (Lycas et al., 2022 [\[1\]](#); Rahbek-Clemmensen et al., 2017 [\[1\]](#)). We then set the extracellular space to a [DA] of 100 nM and simulated uptake for the four

cluster sizes and an unclustered distribution of DAT. As shown in **Figure 4F**, the distribution of DAT into clusters of increasing density resulted in progressively slower DA clearance. Thus, the unclustered scenario reduced [DA] to 10 nM in just above 200 ms, whereas the 20 nm clusters took ~400 ms. As cluster size increased and DAT density decreased, clearance time approached that of the fully unclustered scenario (**Figure 4G**). The resulting hyper-local low-[DA] environment that came from dense DAT clusters was apparent when we plotted the [DA] at the centre of a cluster compared to the mean [DA] of the extracellular space (**Figure 4H**). For the 20 nm cluster-scenario, the clearance was almost entirely limited by how quickly DA diffused to the nanocluster, as the local [DA] dropped to near zero. We originally hypothesized the effect would mainly be due to a depression in [DA] at the cluster centre, but a concentration profile of the 80 nm cluster-scenario revealed the entirety of the cluster was enveloped in a low-[DA] environment (**Figure 4I**).

Summarized, the data supports that dense DAT nanoclusters produce domains of low [DA] as uptake outcompetes diffusion. With transporter uptake being concentration dependent, uptake efficiency is reduced. The simulations therefore posit DAT nanoclustering as an efficient way to regulate the extracellular levels of tonic DA, particularly in VS.

DAT clusters more in the ventral striatum

As our simulations suggest that nanodomain clustering is a way of regulating uptake, and this may be particularly efficient at controlling extracellular DA in VS, we hypothesized nanoscale clustering would be more prominent in VS. To investigate this, we reanalysed data from a previous publication (Sorensen et al., 2021), where we acquired super-resolution images of coronally sliced striatal sections from mice stained for DAT. For the present study, images were split in DS and VS based on where in the slices they were taken (**Figure 4J**). DA terminals were identified by vesicular monoamine transporter 2 (VMAT2) expression, and qualitatively DAT appeared more clustered in VS than DS (**Figure 4K**). To quantify this, we applied the clustering algorithm Density-Based Spatial Clustering of Applications with Noise (DBSCAN) (**Figure 4L**). This confirmed a regional difference with more DAT localized to mean cluster size of 80 nm in VS compared to DS (**Figure 4M**), and we observed a similar regional difference across the range of sizes typically reported (**Figure 4N**) (Lycas et al., 2022; Rahbek-Clemmensen et al., 2017) supporting the conclusion that DAT nanoclustering is more prevalent in VS than DS.

Discussion

We developed a three-dimensional, finite-difference computational model to investigate spatiotemporal DA dynamics of striatal subregions in detail. Leveraging prior experimental information on regional differences in dopaminergic innervation density and DAT uptake capacity, our model predicts important differences in dopaminergic dynamics between DS and VS. Strikingly, our simulations suggest that large areas of the DS are effectively devoid of a basal level of DA at pacemaker activity, whereas VS maintain a more homogenous tonic-like basal DA concentration with only small changes in uptake activity powerfully regulating the extracellular DA tone. Furthermore, we modelled receptor binding kinetics and found that D1R binding faithfully followed recently described rapid DA dynamics of the striatum (Ejdrup et al., 2023; Jorgensen et al., 2023; Markowitz et al., 2023), while D2R, with an off-rate of ~5 seconds, appeared better suited for detecting background tone, ramps and integrating prolonged activity (Howe et al., 2013; Jorgensen et al., 2023). Collectively, these observations have important implications for our understanding of striatal function in behaviour, including decoding of inputs from the prefrontal cortex (PFC) and limbic system as well as the influential phasic-tonic model of dopaminergic signalling (Grace et al., 2007; Niv et al., 2007; Schultz, 2007).

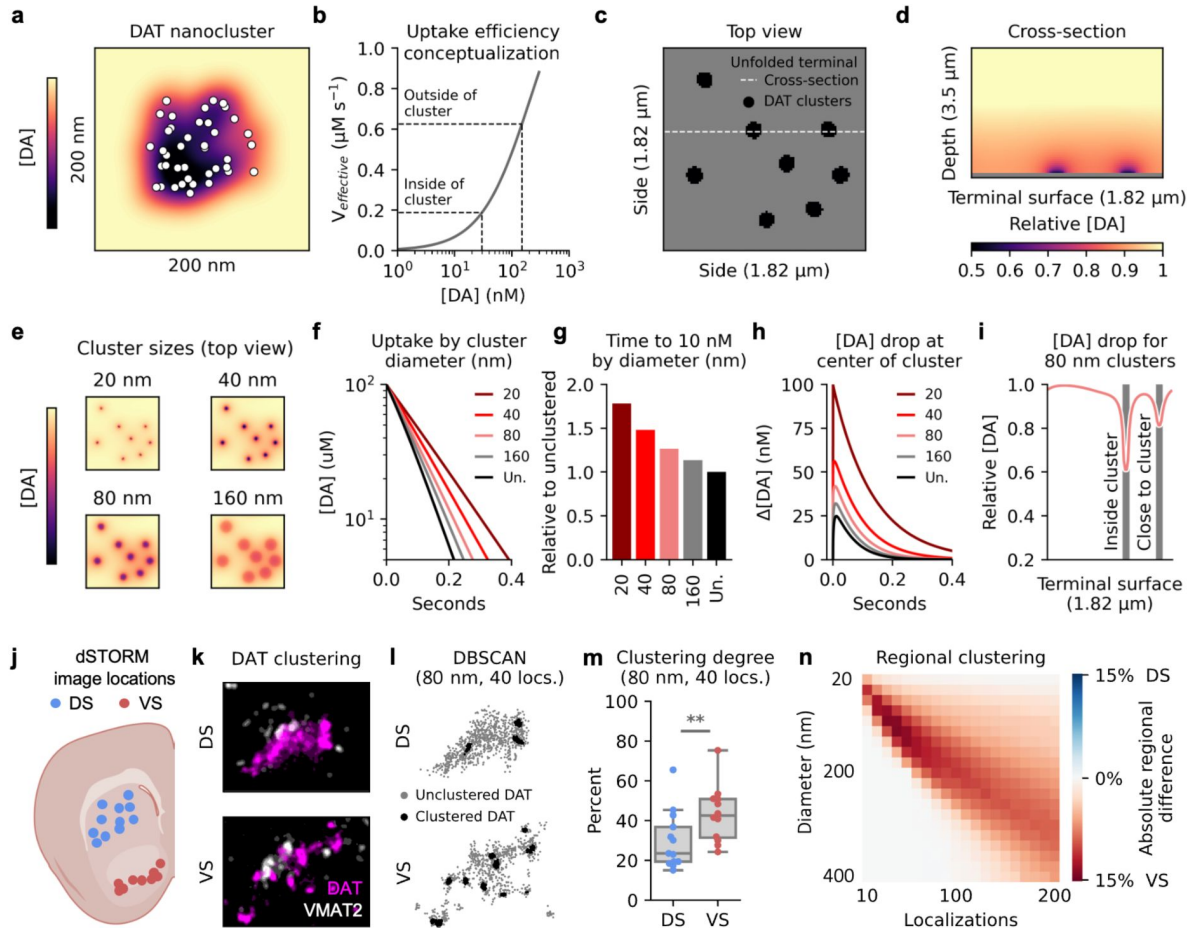


Figure 4.

DAT nanoclustering reduces uptake and shows regional variation.

(a) Schematic of dense DA cluster. White dots represent individual DAT molecules, and colour gradient the surrounding DA concentration. (b) Effective transport rate dependent on local concentration. (c) Top view of unfolded DA varicosity. Black shapes denote clusters of DAT. Dashed white line indicates placement of cross-section shown in (d). (d) Cross section showing DA concentration in space surrounding varicosity unfolded in c. Grey line at the bottom is surface of the varicosity. Colour-coded for DA concentration. (e) Top view from (c), but colour coded for DA concentration immediately above membrane surface at different DAT cluster sizes. (f) Mean [DA] over time for different DAT cluster sizes and unclustered (Un.). (g) Time to reach a mean DA concentration of 10 nM for different DAT cluster sizes and unclustered (Un.). (h) Difference between DA concentration at the centre of clusters and mean concentration throughout the simulation for different cluster sizes and unclustered (Un.). (i) Concentrations across a cross section of a surface with 80 nm diameter clusters. Shaded areas highlight cluster locations. (j) Location of images of the dorsal (DS) and ventral striatum (VS) in striatal slices from mice as imaged in (Sorensen et al., 2021) with direct stochastic optical reconstruction microscopy (dSTORM). (k) Two representative DA varicosities from DS and VS with VMAT2 in white and DAT in magenta. Images are $1.5 \times 2 \mu\text{m}$. (l) Individual DAT localizations from images in (k) coloured by clustering. Black indicates localization identified as clustered based on DBSCAN with parameters 80 nm diameter and 40 localizations. Grey indicates unclustered localizations. (m) Quantification of clustering across all images in (j) with parameters in (l). Welch's two-sample t-test, $P = 0.012^*$, $n = 12$ (DS) and 13 (VS). (n) Absolute difference in percentage of clustering as assessed with DBSCAN across a range of parameters. VS has a higher propensity to cluster across cluster sizes typically reported for DAT clusters.

It has been assumed for long that there are tonic levels of DA in the striatum (Niv et al., 2007 [↗](#); Schultz, 2007 [↗](#); Sulzer et al., 2016 [↗](#)), although the phenomenon has no clear definition (1). Our simulation of DA dynamics in DS during pacemaker activity showed no evidence for a homogenous extracellular distribution. Rather, elevated [DA] was transiently present around release sites during pacemaker activity with the remaining space mostly depleted of DA. The absence of a general tonic DA level in DS predicted by our model directly supports the notion that DA release sites in DS establish distinct and only partially overlapping DA domains rather than diffuse, tonic DA levels (see Liu et al., 2021 [↗](#) (Liu et al., 2021 [↗](#))). This conclusion aligns with recent data where we found that DA concentrations measured by microdialysis correlates with the average of rapid activity recorded with fibre photometry rather than a baseline DA tone (Ejdrup et al., 2023 [↗](#)).

In contrast to the DS, our model predicted VS to hold a considerable basal level of DA even in spaces without an immediately adjacent release site. This is conceivably what most refer to as tonic DA. In this study, we quantified tonic DA as the median concentration of the entire space (50th percentile), which appeared significantly higher in VS than DS because of the lower VS uptake capacity. Importantly, this matches the results of our direct in vivo comparison of DS and VS in freely moving mice (Jorgensen et al., 2023 [↗](#)). To challenge our model, we performed our simulations across a wide range of parameters. Only changes to $V_{\max}$ for uptake generated differential responses in the two regions. With release and uptake parameters at values from the literature, VS was at a critical point where minor changes to uptake significantly impacted the tonic levels without any major effect on peak concentrations.

Contrary to our observations, some previous microdialysis experiments have suggested higher basal DA levels in the DS compared to VS (Kuczenski & Segal, 1992 [↗](#); Shen et al., 2004 [↗](#) have reported 2-4 times higher [DA] in DS compared to VS (Kuczenski & Segal, 1992 [↗](#); Shen et al., 2004 [↗](#)). However, there are disparate observations in the literature (e.g. Carboni et al., 2001 [↗](#) reported 20% higher [DA] in VS (Carboni et al., 2001 [↗](#))). Moreover, it is important to note that regional comparisons in microdialysis might be confounded by the considerably higher uptake rate in DS. This will increase the extraction fraction and possibly lead to a significant overestimation of the extracellular concentration as compared to a region with lower uptake rate, such as the VS (Chefer et al., 2009 [↗](#)).

Our three-dimensional simulations highlight DAT-mediated reuptake as a key mechanism governing striatal DA dynamics and as a key mediator of regional-specific DA dynamics. A physiologically relevant way to regulate uptake capacity is moving DAT to-and-from the plasma membrane. Indeed, DAT is subject to such regulation and some of these mechanisms may even be exclusive to the ventral region, including protein kinase C-induced DAT internalization and Vav2 regulation of DAT surface expression (Fagan et al., 2020 [↗](#); Zhu et al., 2015 [↗](#)). Chemogenetic G_q -coupled DREADD activation of DA neurons also result in differential DAT trafficking in the two regions (Fagan et al., 2020 [↗](#); Kearney et al., 2023 [↗](#)). The findings position DAT regulation as an excellent candidate for changing tonic DA levels in VS, which has been proposed to selectively attenuate afferent drive from the PFC through D2R activation (Grace et al., 2007 [↗](#)). Recent studies of D2R expressing spiny projection neurons (SPNs) in the VS also suggest that the receptor is not fully saturated under basal firing (Lee et al., 2020 [↗](#)), matching both our simulations of receptor binding and the notion that tonic DA can be manipulated to alter D2R activation.

If changing uptake capacity is to have a behavioural relevance on a fast timescale, a mechanism to regulate DAT function faster than internalization must exist. Importantly, the transporter does not only move to-and-from the surface, but also laterally in the plasma membrane. We have reported that DAT forms nanoclusters in the plasma membrane that dynamically reshape based on excitatory and inhibitory input (Lycas et al., 2022 [↗](#); Rahbek-Clemmensen et al., 2017 [↗](#)). Moreover, we have previously shown that cocaine, which both competitively inhibits DAT and reorganizes the transporter nanodomains (Lycas et al., 2022 [↗](#)), changes the DA signal of the DS to

dynamics akin to the VS (Jorgensen et al., 2023). Importantly, our simulations showed that nanoclustering may be an effective way to sequester DAT in a dense domain where uptake overpowers diffusion and as a result brings down effective uptake speed through microdomain DA depletion. This is in line with evidence that these DAT nanoclusters are enriched in phosphatidylinositol-4,5-bisphosphate (PIP2), and that metabolism of PIP2 decreases uptake rate of DAT (Lycas et al., 2022; Carvelli, 2002 #2339). We also found that the nanoclustering phenomenon was considerably more prevalent in VS than in DS. Taken together, these data point to DAT nanoclustering as a way to shape tonic levels of DA in the striatum – particularly in the VS.

Our incorporation of receptor binding was inspired by previous data (Dreyer et al., 2010; Dreyer & Hounsgaard, 2013; Hunger et al., 2020). Dreyer & colleagues assumed instantaneous equilibrium between extracellular DA and receptor occupation while Hunger & colleagues based their kinetics on early pharmacological studies (Burt et al., 1976; Maeno, 1982; Nishikori et al., 1980; Sano et al., 1979). However, we based our receptor kinetics on newer pharmacological experiments in live cells (Agren et al., 2021) and properties of the recently developed DA receptor-based biosensors (Labouesse & Patriarchi, 2021). Indeed, these sensors are mutated receptors but only on the intracellular domains with no changes of the binding site (Labouesse & Patriarchi, 2021). Overall, they exhibit kinetics that are well aligned with both modelled and experimentally reported extracellular DA dynamics using non-biosensor-based methods (Atcherley et al., 2015; Gonon et al., 2000; Venton et al., 2002).

The presented simulations suggested that receptor binding was largely invariant to single release events during pacemaker activity, while bursts of activity rapidly changed occupancy. Both D1R and D2R immediately responded to burst onset; however, while D1R occupancy rapidly declined to zero within approximately 50 ms, the slow D2R kinetics resulted in an occupancy decline over ~5 seconds, returning to the baseline maintained by tonic firing. This means that D1R is better suited to discriminate inputs in rapid succession and allow for postsynaptic decoding of the fast-paced in vivo dynamics described in particularly for DS (Ejdrup et al., 2023; Jorgensen et al., 2023; Markowitz et al., 2023). By contrast, our analysis shows that D2Rs integrate DA signals over several seconds. As D1R occupancy is negligible during pacemaker activity and D2R kinetics are too slow to pick up rapid changes in DA concentration, our simulations moreover suggest pauses in firing of less than 1 second is not an effective way of signalling for the striatal dopaminergic system.

In conclusion, we have developed a three-dimensional model for DA release dynamics and receptor binding that integrates a wealth of experimentally determined parameters and generates responses to electrical and pharmacological input that fits robustly with literature observations. The model offers an important theoretical framework and a predictive tool that can serve as the basis for future experimental endeavours and help guide the interpretation of new as well as older empirical findings on DA signalling dynamics under both physiological conditions and in disease.

Methods

Three-dimensional finite difference model

DA release sites in the striatum were simulated at a density of one site per $25 \mu\text{m}^3$ in DS and one site per $27.8 \mu\text{m}^3$ in VS (except for **Figure 4b and c**). As a single medium spiny neuron in the striatum is estimated to be influenced by axons from 95 to 194 dopaminergic neurons (Matsuda et al., 2009), we simulated DA release sites as originating from 150 separate cell bodies. These were set to fire independently from each other at 4 Hz generated by a Poisson distribution. We set the release probability in response to an action potential at the individual terminal levels to 6%

(Dreyer et al., 2010 [DOI](#)), and if a release occurred the DA concentration of the voxel was elevated by the equivalent of 3000 DA molecules as per Equation 1 [DOI](#). The volume of each voxel was corrected for an extracellular volume fraction of 0.21 (Rice & Nicholson, 1991 [DOI](#)).

We did not incorporate paired-pulse depression in our model as most in vivo FSCV studies have not reported this phenomenon (Chergui et al, 1994 [DOI](#); Garriss & Wightman, 1994 [DOI](#); Hamid et al, 2016 [DOI](#); May & Wightman, 1989 [DOI](#)). In addition, we could explain the gradual blunt of the response during prolonged stimulation (Figure 2E [DOI](#)) as the result of an equilibrium between release and uptake rather than a depression in release. Newer in vivo biosensor-based studies observe the same “blunting” phenomenon during stimulation trains (Mohebi et al, 2023 [DOI](#)). A low initial release probability that scales well with frequency has also been reported for VMAT2-positive release sites in dopaminergic cultures where the axons are not severed from their cell bodies as in acute striatal slices (Silm et al, 2019 [DOI](#)). Note that we did not explicitly model autoreceptor inhibition at pacemaker activity. However, this value should be implicitly reflected in the in vivo estimates we base our model on.

When simulating bursts or trains of stimulations, action potentials were added as described for each simulation, but release probability was kept the same unless otherwise noted. Uptake was evenly distributed throughout the simulation space with a $V_{\max}$ at $6 \mu\text{m s}^{-1}$ for DS and $2 \mu\text{m s}^{-1}$ for VS (see Table S2) and a K_m at 210 nM (Hovde et al, 2019 [DOI](#)) and calculated for each voxel as per Equation 2 [DOI](#). For each time step, dt , diffusion calculated with a finite difference implementation of the Laplacian operator described in Equation 3 [DOI](#) using a diffusion coefficient corrected for tortuosity at $321.7 \mu\text{m}^2 \text{s}^{-1}$. Periodic boundaries were used to avoid edge issues with a simulation size of $50 \times 50 \times 50 \mu\text{m}$ unless otherwise noted. The Python-based implementation of the model can be found as described in the Code and Data Availability section.

Immunohistochemistry analysis

Analysed widefield images were obtained from (Sorensen et al., 2021 [DOI](#)). The mouse striatum slices were stained for either DAT or VMAT2 (see Sørensen et al., 2021 for slicing, fixation, staining and imaging protocol (Sorensen et al., 2021 [DOI](#)). Fluorescence intensities were extracted in a single dorsoventral line parallel to the midline through the horizontally centre of the anterior commissure using ImageJ v1.53q. for each slice. Data was vertically centred around the anterior commissure. No background subtraction was performed.

FSCV modelling

FSCV recordings were modelled by convoluting a simulated DA response to a pseudo FSCV trace. The impulse function used was as described in (Venton et al., 2002 [DOI](#)). In brief, the impulse function was defined as $IF_{FSCV} = e^{-(t+1)(k_{-1}t_s+k_{-2}t_0)}$, where t is time, t_s is scan time, t_0 is oxidation time, and k_{-1} and k_{-2} desorption constants. T_0 was set to 100 ms as reported in (May & Wightman, 1989 [DOI](#)) and t_0 to 4 ms, which is half of the typically reported symmetrical waveform length. The desorption constants ($k_{-1} = 1.2 \text{ s}^{-1}$ and $k_{-2} = 12 \text{ s}^{-1}$) were taken directly from (Venton et al., 2002 [DOI](#)).

Nanocluster uptake modelling

Nanoclustering of DAT was simulated in Python. An ellipsoid-shaped varicosity of $1.5 \mu\text{m}$ in length and 800 nm in width was modelled as a flat, square surface with a width of $1.8 \mu\text{m}$ to match the total surface area (Lycas et al., 2022 [DOI](#); Rahbek-Clemmensen et al., 2017 [DOI](#)). As 9-16.4% of terminals in the striatum are estimated to be dopaminergic (Hokfelt, 1968 [DOI](#); Tennyson et al., 1974 [DOI](#)), we set the height of the simulation space to $3.52 \mu\text{m}$ to generate an extracellular volume seven times the size of the varicosity. Axons and cell bodies are likely a non-negligible part of the striatal volume but omitted in this analysis due to a lack of information in the literature (however, decreasing the share of striatal space taken up by DA varicosities works in the direction of our hypothesis). Spatial granularity was set to 20 nm , and we randomly placed eight non-overlapping

locations for clusters on the simulated varicosity. Uptake capacity per voxel for each clustering scenario was set so the mean uptake of the entire space was $4 \mu\text{m s}^{-1}$, which resulted in clusters with increased DAT density with decreasing cluster size. We set the initial DA concentration of the entire space to 100 nM and ran the simulation with a time step of 1.875e^{-7} s to avoid numerical instability. Diffusion corrected for tortuosity was set to $321.7 \mu\text{m}^2 \text{s}^{-1}$ and calculated with a Laplacian operation as described in Equation 3. Periodic boundaries were used to account for the varicosity being circular and avoid issues at the edge of the simulation space. The full implementation in Python can be found as described in the Code and Data Availability section.

Super-resolution clustering analysis

Super-resolved fluorescence microscopy dual color images from (Sorensen et al., 2021) of striatal brain slices from mice were analyzed based on their position in the dorsal or ventral regions (Figure 4J). Localizations were fitted using DAOSTORM (Babcock et al, 2012) on the originally acquired direct stochastic optical reconstruction microscopy (dSTORM) videos with the following settings: background sigma of 8, maximum likelihood estimation as the fitting error model, 20 peak identification iterations, an initial sigma estimate of 1.5 and a threshold adjusted to each imaging session. Each image was analysed for percentage of localizations within a cluster using density-based spatial clustering of applications with noise (DBSCAN) (Ester et al, 1996) using the Python package Scikit-learn 0.22 (Pedregosa et al, 2021). To uncover broader trends, we scanned across a range of parameters: radius was run from 10 nm to 200 nm and number of localizations from 10 to 200. For Figure 4M a diameter of 80 nm was chosen to match the size reported in (Rahbek-Clemmensen et al., 2017).

Statistical Analysis

The statistical analyses performed can be found in the legends of each figure. Statistical analyses were performed using open-source python packages SciPy v1.5.2 and NumPy v1.18.1. Boxplots show the 25th and 75th percentile range and whiskers indicate up to 1.5 times the interquartile range. The remaining data points are plotted as individual outliers.

Data availability

Numerical implementation and code to run the simulations are available at: <https://github.com/GetherLab/striatal-dopamine-modelling>. All experimental data are from Ejdrup & Wellbourne-Wood et al., 2023 or Sorensen et al., 2021. Corresponding authors on these papers should be consulted for data availability.

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Additional information

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Author contributions

ALE, FH and UG conceptualized the study. ALE built the model with help from JKD. ALE ran the simulations. MDL and SHJ provided technical input on experimental data. ALE drafted the manuscript. ALE, FH and UG wrote the final version of the manuscript with comments and suggestions from JKD, TWR and JWD. All authors critically evaluated the manuscript. FH and UG supervised the project.

Additional files

Supplementary materials [↗](#)

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Reviewer #1 (Public review):

Ejdrup, Gether, and colleagues present a sophisticated simulation of dopamine (DA) dynamics based on a substantial volume of striatum with many DA release sites. The key observation is that a reduced DA uptake rate in the ventral striatum (VS) compared to the dorsal striatum (DS) can produce an appreciable "tonic" level of DA in VS and not DS. In both areas they find that a large proportion of D2 receptors are occupied at "baseline"; this proportion increases with simulated DA cell phasic bursts but has little sensitivity to simulated DA cell pauses.

They also examine, in a separate model, the effects of clustering dopamine transporters (DAT) into nanoclusters and say this may be a way of regulating tonic DA levels in VS. I found this work of interest and I think it will be useful to the community. At the same time, there are a number of weaknesses that should be addressed, and the authors need to more carefully explain how their conclusions are distinct from those based on prior models.

(1) The conclusion that even an unrealistically long (1s) and complete pause in DA firing has little effect on DA receptor occupancy is potentially important. The ability to respond to DA pauses has been thought to be a key reason why D2 receptors (may) have high affinity. This simulation instead finds evidence that DA pauses may be useless. This result should be highlighted in the abstract and discussed more.

(2) The claim of "DAT nanoclustering as a way to shape tonic levels of DA" is not very well supported at present. None of the panels in Figure 4 simply show mean steady-state extracellular DA as a function of clustering. Perhaps mean DA is not the relevant measure, but then the authors need to better define what is and why. This issue may be linked to the fact that DAT clustering is modeled separately (Figure 4) to the main model of DA dynamics (Figures 1-3) which per the Methods assumes even distribution of uptake. Presumably, this is because the spatial resolution of the main model is too coarse to incorporate DAT nanoclusters, but it is still a limitation. As it stands it is convincing (but too obvious) that DAT clustering will increase DA away from clusters, while decreasing it near clusters. I.e. clustering increases heterogeneity, but how this could be relevant to striatal function is not made clear, especially given the different spatial scales of the models.

(3) I question how reasonable the "12/40" simulated burst firing condition is, since to my knowledge this is well outside the range of firing patterns actually observed for dopamine cells. It would be better to base key results on more realistic values (in particular, fewer action potentials than 12).

(4) There is a need to better explain why "focality" is important, and justify the measure used.

(5) Line 191: "D1 receptors (-Rs) were assumed to have a half maximal effective concentration (EC50) of 1000 nM"

The assumptions about receptor EC50s are critical to this work and need to be better justified. It would also be good to show what happens if these EC50 numbers are changed by an order of magnitude up or down.

(6) Line 459: "we based our receptor kinetics on newer pharmacological experiments in live cells (Agren et al., 2021) and properties of the recently developed DA receptor-based biosensors (Labouesse & Patriarchi, 2021). Indeed, these sensors are mutated receptors but only on the intracellular domains with no changes of the binding site (Labouesse & Patriarchi, 2021)"

This argument is diminished by the observation that different sensors based on the same binding site have different affinities (e.g. in Patriarchi et al. 2018, dLight1.1 has Kd of 330nM while dLight1.3b has Kd of 1600nM).

(7) Estimates of Vmax for DA uptake are entirely based on prior fast-scan voltammetry studies (Table S2). But FSCV likely produces distorted measures of uptake rate due to the kinetics of DA adsorption and release on the carbon fiber surface.

(8) It is assumed that tortuosity is the same in DS and VS - is this a safe assumption?

(9) More discussion is needed about how the conclusions derived from this more elaborate model of DA dynamics are the same, and different, to conclusions drawn from prior relevant models (including those cited, e.g. from Hunger et al. 2020, etc).

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Reviewer #2 (Public review):

The work presents a model of dopamine release, diffusion, and reuptake in a small (100 micrometer² maximum) volume of striatum. This extends previous work by this group and others by comparing dopamine dynamics in the dorsal and ventral striatum and by using a model of immediate dopamine-receptor activation inferred from recent dopamine sensor data. From their simulations, the authors report two main conclusions. The first is that the dorsal striatum does not appear to have a sustained, relatively uniform concentration of dopamine driven by the constant 4Hz firing of dopamine neurons; rather that constant firing appears to create hotspots of dopamine. By contrast, the lower density of release sites and lower rate of reuptake in the ventral striatum creates a sustained concentration of dopamine. The second main conclusion is that D1 receptor (D1R) activation is able to track dopamine concentration changes at short delays but D2 receptor activation cannot.

The simulations of the dorsal striatum will be of interest to dopamine aficionados as they throw some doubt on the classic model of "tonic" and "phasic" dopamine actions, further show the disconnect between dopamine neuron firing and consequent release, and thus raise issues for the reward-prediction error theory of dopamine.

There is some careful work here checking the dependence of results on the spatial volume and its discretisation. The simulations of dopamine concentration are checked over a range of values for key parameters. The model is good, the simulations are well done, and the evidence for robust differences between dorsal and ventral striatum dopamine concentration is good.

However, the main weakness here is that neither of the main conclusions is strongly evidenced as yet. The claim that the dorsal striatum has no "tonic" dopamine concentration is based on the single example simulation of Figure 1 not the extensive simulations over a range of parameters. Some of those later simulations seem to show that the dorsal striatum can have a "tonic" dopamine concentration, though the measurement of this is indirect. It is not clear why the reader should believe the example simulation over those in the robustness checks, for example by identifying which range of parameter values is more realistic.

The claim that D1Rs can track rapid changes in dopamine is not well supported. It is based on a single simulation in Figure 1 (DS) and 2 (VS) by visual inspection of simulated dopamine concentration traces - and even then it is unclear that D1Rs actually track dynamics because they clearly do not track rapid changes in dopamine that are almost as large as those driven by bursts (cf Figure 1i). The claim also depends on two things that are poorly explained. First, the model of binding here is missing from the text. It seems to be a simple bound-fraction model, simulating a single D1 or D2 receptor. It is unclear whether more complex models would show the same thing. Second, crucial to the receptor model here is the inference that D1 receptor unbinding is rapid; but this inference is made based on the kinetics of dopamine sensors and is superficially explained - it is unclear why sensor kinetics should let us extrapolate to receptor kinetics, and unclear how safe is the extrapolation of the linear regression by an order of magnitude to get the D1 unbinding rate.

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Author response:**eLife Assessment**

The conclusions of this work are based on valuable simulations of a detailed model of striatal dopamine dynamics. Establishing that a lower dopamine uptake rate can lead to

a 'tonic' level of dopamine in the ventral but not dorsal striatum, and that dopamine concentration changes at short delays can be tracked by D1 but not D2 receptor activation, is of value and will be of interest to dopamine aficionados. However, the simulations are incomplete, providing only partial support for the key claims. Several things can be done to strengthen the conclusions, including, for example, but not exclusively, a demonstration of how the results would change as a function of changes in D2 affinity.

We sincerely thank the Editors and Reviewers for their insightful comments on our manuscript. We are pleased that our simulations are recognized as interesting, sophisticated and valuable. Moreover, we fully agree that many of the findings will be of particular interest to dopamine aficionados. While we maintain that our simulations provide a solid basis for the key claims, we acknowledge that the conclusions can be further strengthened by the revisions suggested below.

Reviewer #1 (Public review):

Ejdrup, Gether, and colleagues present a sophisticated simulation of dopamine (DA) dynamics based on a substantial volume of striatum with many DA release sites. The key observation is that a reduced DA uptake rate in the ventral striatum (VS) compared to the dorsal striatum (DS) can produce an appreciable "tonic" level of DA in VS and not DS. In both areas they find that a large proportion of D2 receptors are occupied at "baseline"; this proportion increases with simulated DA cell phasic bursts but has little sensitivity to simulated DA cell pauses. They also examine, in a separate model, the effects of clustering dopamine transporters (DAT) into nanoclusters and say this may be a way of regulating tonic DA levels in VS. I found this work of interest and I think it will be useful to the community. At the same time, there are a number of weaknesses that should be addressed, and the authors need to more carefully explain how their conclusions are distinct from those based on prior models.

(1) The conclusion that even an unrealistically long (1s) and complete pause in DA firing has little effect on DA receptor occupancy is potentially important. The ability to respond to DA pauses has been thought to be a key reason why D2 receptors (may) have high affinity. This simulation instead finds evidence that DA pauses may be useless. This result should be highlighted in the abstract and discussed more.

We appreciate that the reviewer finds our work interesting and useful to the community. However, we acknowledge that in the revised version we need to better describe how our conclusions are different from those reached based on previous models.

We will also carry out new simulations across a range of D2R affinities to assess how this will affect the finding that even a long pause in DA firing has little effect on D2 receptor occupancy. As also suggested, the results will be highlighted and further discussed.

(2) The claim of "DAT nanoclustering as a way to shape tonic levels of DA" is not very well supported at present. None of the panels in Figure 4 simply show mean steady-state extracellular DA as a function of clustering. Perhaps mean DA is not the relevant measure, but then the authors need to better define what is and why. This issue may be linked to the fact that DAT clustering is modeled separately (Figure 4) to the main model of DA dynamics (Figures 1-3) which per the Methods assumes even distribution of uptake. Presumably, this is because the spatial resolution of the main model is too coarse to incorporate DAT nanoclusters, but it is still a limitation.

We will improve our definitions and descriptions relating to nanoclustering of DAT in the revised version of the manuscript. We fully agree that the spatial resolution of the main

model is a limitation and, ideally, that the nanoclustering should be combined with the large-scale release simulations. Unfortunately, this would require many orders of magnitude more computational power than currently available.

As it stands it is convincing (but too obvious) that DAT clustering will increase DA away from clusters, while decreasing it near clusters. I.e. clustering increases heterogeneity, but how this could be relevant to striatal function is not made clear, especially given the different spatial scales of the models.

Thank you for raising this important point. While it is true that DAT clustering increases heterogeneity in DA distribution at the microscopic level, the diffusion rate is, in most circumstances, too fast to permit concentration differences on a spatial scale relevant for nearby receptors. Accordingly, we propose that the primary effect of DAT nanoclustering is to decrease the overall uptake capacity, which in turn increases overall extracellular DA concentrations. Thus, homogeneous changes in extracellular DA concentrations can arise from regulating heterogeneous DAT distribution. An exception to this would be the circumstance where the receptor is located directly next to a dense cluster – i.e. within nanometers. In such cases, local DA availability may be more directly influenced by clustering effects. This will be further discussed in the revised manuscript.

(3) I question how reasonable the "12/40" simulated burst firing condition is, since to my knowledge this is well outside the range of firing patterns actually observed for dopamine cells. It would be better to base key results on more realistic values (in particular, fewer action potentials than 12).

We fully agree that this typically is outside the physiological range. The values are included to showcase what extreme situations would look like.

(4) There is a need to better explain why "focality" is important, and justify the measure used.

We will expand on the intention of this measure in the revised manuscript. Thank you for pointing out this lack of clarification.

(5) Line 191: "D1 receptors (-Rs) were assumed to have a half maximal effective concentration (EC50) of 1000 nM" The assumptions about receptor EC50s are critical to this work and need to be better justified. It would also be good to show what happens if these EC50 numbers are changed by an order of magnitude up or down.

We agree that these assumptions are critical. Simulations on effective off-rates across a range of EC50 values will be included in the revised version.

(6) Line 459: "we based our receptor kinetics on newer pharmacological experiments in live cells (Agren et al., 2021) and properties of the recently developed DA receptor-based biosensors (Labouesse & Patriarchi, 2021). Indeed, these sensors are mutated receptors but only on the intracellular domains with no changes of the binding site (Labouesse & Patriarchi, 2021)"

This argument is diminished by the observation that different sensors based on the same binding site have different affinities (e.g. in Patriarchi et al. 2018, dLight1.1 has Kd of 330nM while dLight1.3b has Kd of 1600nM).

We sincerely thank the reviewer for highlighting this important point. We fully recognize the fundamental importance of absolute and relative DA receptor kinetics for modeling DA

actions and acknowledge that differences in affinity estimates from sensor-based measurements highlight the inherent uncertainty in selecting receptor kinetics parameters. While we have based our modeling decisions on what we believe to be the most relevant available data, we acknowledge that the choice of receptor kinetics is a topic of ongoing debate. Importantly, we are making our model available to the research community, allowing others to test their own estimates of receptor kinetics and assess their impact on the model's behavior. In our revised manuscript, we will further discuss the rationale behind our parameter choices, including: Our selection of a K_d value of 1000 nM for D1R (based on the observed affinities for D1R sensors) and an extrapolated K_{off} of 19.5 s^{-1} (Labouesse & Patriarchi, 2021). Our use of a K_d value of 7 nM and an extrapolated K_{off} of 0.2 s^{-1} for D2R, consistent with recent binding studies (Ågren et al., 2021).

(7) Estimates of V_{max} for DA uptake are entirely based on prior fast-scan voltammetry studies (Table S2). But FSCV likely produces distorted measures of uptake rate due to the kinetics of DA adsorption and release on the carbon fiber surface.

We fully agree that this is a limitation of FSCV. However, most of the cited papers attempt to correct for this by way of fitting the output to a multi-parameter model for DA kinetics. If newer literature brings the V_{max} values estimated into question, we have made the model publicly available to rerun the simulations with new parameters.

(8) It is assumed that tortuosity is the same in DS and VS - is this a safe assumption?

The original paper cited does not specify which region the values are measured in. However, a separate paper estimates the rat cerebellum has a comparable tortuosity index (Nicholson and Phillips, J Physiol. (1981)), suggesting it may be a rather uniform value across brain regions.

(9) More discussion is needed about how the conclusions derived from this more elaborate model of DA dynamics are the same, and different, to conclusions drawn from prior relevant models (including those cited, e.g. from Hunger et al. 2020, etc).

As part of our revision, we will expand the current discussion of our finding in the context of previous models in the manuscript

Reviewer #2 (Public review):

The work presents a model of dopamine release, diffusion, and reuptake in a small (100 micrometer^2 maximum) volume of striatum. This extends previous work by this group and others by comparing dopamine dynamics in the dorsal and ventral striatum and by using a model of immediate dopamine-receptor activation inferred from recent dopamine sensor data. From their simulations, the authors report two main conclusions. The first is that the dorsal striatum does not appear to have a sustained, relatively uniform concentration of dopamine driven by the constant 4Hz firing of dopamine neurons; rather that constant firing appears to create hotspots of dopamine. By contrast, the lower density of release sites and lower rate of reuptake in the ventral striatum creates a sustained concentration of dopamine. The second main conclusion is that D1 receptor (D1R) activation is able to track dopamine concentration changes at short delays but D2 receptor activation cannot.

The simulations of the dorsal striatum will be of interest to dopamine aficionados as they throw some doubt on the classic model of "tonic" and "phasic" dopamine actions, further show the disconnect between dopamine neuron firing and consequent release, and thus raise issues for the reward-prediction error theory of dopamine.

There is some careful work here checking the dependence of results on the spatial volume and its discretisation. The simulations of dopamine concentration are checked over a range of values for key parameters. The model is good, the simulations are well done, and the evidence for robust differences between dorsal and ventral striatum dopamine concentration is good.

However, the main weakness here is that neither of the main conclusions is strongly evidenced as yet. The claim that the dorsal striatum has no "tonic" dopamine concentration is based on the single example simulation of Figure 1 not the extensive simulations over a range of parameters. Some of those later simulations seem to show that the dorsal striatum can have a "tonic" dopamine concentration, though the measurement of this is indirect. It is not clear why the reader should believe the example simulation over those in the robustness checks, for example by identifying which range of parameter values is more realistic.

We appreciate that the reviewer finds our work interesting and carefully performed. The reviewer is correct that DA dynamics, including the presence and level of tonic DA, are parameter-dependent in both the dorsal striatum (DS) and ventral striatum (VS). Indeed, our simulations across a broad range of biological parameters were intended to help readers understand how such variation would impact the model's outcomes, particularly since many of the parameters remain contested. Naturally, altering these parameters results in changes to the observed dynamics. However, to derive possible conclusions, we selected a subset of parameters that we believe best reflect the physiological conditions, as elaborated in the manuscript. This is eventually required in computational modelling of biological systems. In response to the reviewer's comment, we will place greater emphasis on clarifying which parameter regimes produce a "tonic" versus "non-tonic" DA state in the DS. Additionally, we will underscore that the distinction between tonic and non-tonic states is not a binary outcome but a parameter-dependent continuum—one that our model now allows researchers to explore systematically. Finally, we will highlight how our simulations across parameter space not only capture this continuum but also identify the regimes that produce the most heterogeneous DA signaling, both within and across striatal regions.

The claim that D1Rs can track rapid changes in dopamine is not well supported. It is based on a single simulation in Figure 1 (DS) and 2 (VS) by visual inspection of simulated dopamine concentration traces - and even then it is unclear that D1Rs actually track dynamics because they clearly do not track rapid changes in dopamine that are almost as large as those driven by bursts (cf Figure 1i).

We would like to draw the attention also to Fig. S1, where the claim that D1R track rapid changes is supported in more depth. According to this figure, upon coordinated burst firing, the D1R occupancy rapidly increased as diffusion no longer equilibrated the extracellular concentrations on a timescale faster than the receptors – and D1R receptor occupancy closely tracked extracellular DA with a delay on the order of tens of milliseconds. Note that the brief increases in [DA] from uncoordinated stochastic release events from tonic firing in Fig. 1i are too brief to drive D1 signaling, as the DA concentration diffuses into the remaining extracellular space on a timescale of 1-5 ms. This is faster than the receptors response rate, and does not lead to any downstream signaling according to our simulations. This means D1 kinetics are rapid enough to track coordinated signaling on a ~50 ms timescale and slower, but not fast enough to respond to individual release events from tonic activity. In our revised manuscript we will expand the discussion of this topic to provide greater clarity.

The claim also depends on two things that are poorly explained. First, the model of binding here is missing from the text. It seems to be a simple bound-fraction model,

simulating a single D1 or D2 receptor. It is unclear whether more complex models would show the same thing.

We realize that this is not made clear in the methods and, accordingly, we will update the method section to elaborate on how we model receptor binding. The model simulates occupied fraction of D1R and D2R in every single voxel of the simulation space.

Second, crucial to the receptor model here is the inference that D1 receptor unbinding is rapid; but this inference is made based on the kinetics of dopamine sensors and is superficially explained - it is unclear why sensor kinetics should let us extrapolate to receptor kinetics, and unclear how safe is the extrapolation of the linear regression by an order of magnitude to get the D1 unbinding rate.

We chose to use the sensors because it was possible to estimate precise affinities/off-rates from the fluorescent measurements. Although there might some variation in affinities that could be attributable to the mutations introduced in the sensors, the data clearly separated D1R and D2R with a D1R affinity of ~1000 nM and a D2R affinity of ~7 nM (Labouesse & Patriarchi, 2021) consistent with earlier predictions of receptor affinities. From our assessment of the literature we found that this was the most reasonable way to estimate affinities and thereby off-rates. Importantly, the model has been made publicly available, so should new measurements arise, the simulations can be rerun with tweaks to the input parameters.

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