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Dopamine Spillover after Quantal Release: Rethinking Dopamine Transmission in the Nigrostriatal Pathway

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Abstract

The predominance of dopamine (DA) receptors at extrasynaptic versus synaptic sites implies that dopamine signaling is by diffusion-based volume transmission. In this review, we compare characteristics that regulate extracellular DA behavior in substantia nigra pars compacta (SNc) and striatum, including regional differences in structure (a 40% greater extracellular volume fraction in SNc *vs.* striatum) and in dynamic DA uptake (a 200-fold greater DA uptake rate in striatum *vs.* SNc). Furthermore, we test the assumption of diffusion-based volume transmission for SNc and striatum by modeling dynamic DA behavior after quantal release using region-specific parameters for diffusion and uptake at 37 °C. Our model shows that DA uptake does not affect peak DA concentration within 1 μ m of a release site in either SNc or striatum because of the slow kinetics of DATs *versus* diffusion. Rather, diffusion and dilution are the dominant factors governing DA concentration after quantal release. In SNc, limited DAT efficacy is reflected in a lack of influence of uptake on either amplitude or time course of DA transients after quantal release up to 10 μ m from a release site. In striatum, the lack of effect of the DAT within 1 μ m of a release site means that perisynaptic DATs do not “gate” synaptic spillover. This contrasts with the conventional view of DA synapses, in which DATs efficiently recycle DA by re-uptake into the releasing axon terminal. However, the model also shows that a primary effect of striatal uptake is to curtail DA lifetime after release. In both SNc and striatum, effective DA radius after quantal release is ~2 μ m for activation of low-affinity DA receptors and 7–8 μ m for high-affinity receptors; the corresponding spheres of influence would encompass tens to thousands of synapses. Thus, the primary mode of intercellular communication by DA, regardless of region, is volume transmission.

Keywords

diffusion; dopamine transporter; quantal release; somatodendritic; striatum; substantia nigra; synapse; synaptic spillover; synaptic transmission; volume transmission

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Introduction

The nigrostriatal DA pathway extends from the substantia nigra pars compacta (SNc) to the dorsal striatum via the median forebrain bundle. Somatodendritic release of DA in the SNc, as well as axonal DA release in the striatum are necessary for basal ganglia-mediated motor behaviors (Robertson and Robertson, 1989; Timmerman and Abercrombie, 1996; Bergquist et al., 2003). Transporters for DA (DATs) are expressed exclusively by DA neurons and are found extrasynaptically on DA axons in striatum and on DA somata and dendrites in midbrain (Ciliax et al., 1995; Nirenberg et al., 1996; Hersch et al., 1997). Moreover, DA receptors are also predominantly extrasynaptic (Sesack et al., 1994; Yung et al., 1995; Hersch et al., 1995; Khan et al., 1998). This implies that intercellular communication by DA *requires* diffusion-based volume transmission (Fuxe and Agnati, 1991), which is most simply defined as “a functionally significant association of release and receptor sites via extrasynaptic diffusion” (Rice, 2000).

One would expect, therefore, that extracellular DA concentration ($[DA]_o$) and lifetime after release would be regulated by diffusion, as well as uptake. This is indeed the case, although the relative contribution of each process differs between striatum and SNc. In striatum, $[DA]_o$ is often considered to be “uptake-limited”, with strong local regulation by the DAT (Stamford et al., 1988; Giros et al., 1996; Floresco et al., 2003), whereas in SNc, the influence of uptake is much less (Cragg et al., 1997), so that diffusion-based volume transmission is expected to dominate (Rice, 2000; Cragg et al., 2001). Recently, however, both of these basic expectations have been challenged. Evidence from physiological recording in midbrain DA neurons suggests limited diffusional contributions to DA transmission in the SNc (Beckstead et al., 2004; Beckstead et al., 2007), whereas modeling of quantal DA release from DA axons in striatum indicates minimal regulation of synaptic spillover by the DAT (Cragg and Rice, 2004).

In this review, we extend our previously published model of quantal DA release in striatum (Cragg and Rice, 2004) to include somatodendritic release in SNc. Other models that have been used to estimate the sphere of influence of released DA have been limited by the single distance examined (Gonon, 1997; Cragg et al., 2001) or by the evaluation of a population response in which DA diffusion, and thus diffusion distance, is irrelevant (Garris et al., 1994; Sulzer and Pothos, 2000). The model used here is based on experimentally determined parameters for diffusion and uptake in SNc and striatum and permits comparison of the duration and sphere of influence of $[DA]_o$ after single-vesicle release in both regions. Our findings confirm the comparatively limited role of uptake in regulation of DA behavior in SNc *versus* striatum and call for the revision of the current concept of synaptic DA release regulation, in which the DAT “gates” DA overflow by reuptake into the releasing presynaptic terminal.

The diffusion model and parameters for quantal DA release

Theoretical concentration-time profiles for quantal DA release at 37 °C were generated using the expression for diffusion from an instantaneous point source (Nicholson, 1985; Cragg et al., 2001) using the program VOLTORO by Dr. C. Nicholson (NYU School of Medicine, USA):

$$C(r, t) = \frac{UC_f}{\alpha(4D^*t\pi)^{3/2}} \exp\left(\frac{-r^2}{4D^*t}\right) \exp\left(-k't\right). \quad (\text{Eq. 1})$$

Quantal DA release was assumed to be instantaneous from a vesicle of volume U with a filling concentration of C_f , such that quantal size, $Q = UC_f$. Diffusion distance, r , was varied from 1–20 μm .

The structural parameters that govern diffusion are the extracellular volume fraction (α) and the tortuosity (λ) of extracellular diffusion paths (Nicholson and Sykova, 1998). Experimentally derived values for α , λ , k' , C_f and U for SNc and striatum are given in Table 1. Macroscopic diffusion parameters have been shown to be appropriate to describe microscopic structure, including that surrounding a synapse (Rusakov and Kullmann, 1998). These parameters are assumed to be temperature independent. On the other hand, the diffusion coefficient, D , for DA is temperature dependent; the value used for D at 37 °C was $7.63 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (Table 1), calculated from D at 32 °C (Nicholson, 1995), assuming a 2% increase per °C.

In experimental studies of DA diffusion and uptake in the SNc, we found that the linear uptake term k' for specific DA uptake was sufficient to fit DA diffusion curves in this region (Cragg et al., 2001). To model nigral DA behavior at 37 °C, we increased the k' value for SNc in accordance with the temperature-dependence of DA uptake evaluated in striatum (Cragg and Rice, 2004). Similarly, the k' for non-specific DA uptake, determined from measurements of the diffusion of tetramethylammonium (TMA^+) in midbrain (Cragg et al., 2001), was temperature-corrected to 37 °C (Table 1), and was used for “diffusion only” curves generated for both SNc and striatum.

Linear uptake was also used to model DA behavior in striatum (Cragg and Rice, 2004). The relationship between Michaelis-Menten uptake rate and concentration becomes linear when $[\text{DA}]_0$ is substantially below K_m , i.e. $C \ll 0.21 \text{ } \mu\text{M}$ (Ross, 1991). Then, the uptake rate, $V = V_{\text{max}}C/(C+K_m)$, simplifies to first order kinetics with respect to C , such that $V = V_{\text{max}}C/K_m = k' C$, where $k' = V_{\text{max}}/K_m$. Use of linear uptake for striatum in this model is justified because $[\text{DA}]_0$ remains in the nanomolar range following release of a single vesicle, except at distances very near to the site of release (Cragg and Rice, 2004). At those sites, the use of linear uptake overestimates uptake when $[\text{DA}]_0 \geq K_m$, because uptake rate increases linearly with increasing concentration ($V = k' C$), rather than approaching an asymptotic value ($V = V_{\text{max}}$). However, this overestimation only strengthens the case for the limited DAT-dependent regulation of peak $[\text{DA}]_0$ after quantal release described below.

A further assumption of our model is that DA vesicle fusion at a DA synapse (or varicosity) (Descarries et al., 1996) in striatum or on a DA cell body or dendrite in SNc acts as an instantaneous point source (Cragg and Rice, 2004). The validity of this assumption for SNc is a matter of debate, given the uncertainty of whether release is mediated *exclusively* by conventional vesicle fusion (Cragg et al., 1997; Chen and Rice, 2001; Falkenburger et al., 2001). Nonetheless, quantal release from DA cells in the SNc *has* been recorded in acute midbrain slices (Jaffe et al., 1998). We used the reported quantal size (Q) of 14,000 DA molecules for simulations of DA behavior in SNc (Table 1), unless otherwise stated.

Because the extracellular volume fraction of midbrain is 40% larger than that in striatum (Rice and Nicholson, 1991; Cragg et al., 2001), the apparent source strength is 40% lower in SNc than in striatum. For initial comparisons of extracellular DA behavior in SNc and striatum, we normalized source strength between regions by a proportional decrease in striatal Q to 9,800 molecules (Table 1). The resultant values of C_f (350 mM, SNc; 250 mM, striatum) are within a range consistent with those determined experimentally for DA in cultured neurons (Pothos et al., 1998).

DA behavior after quantal release in SNc and striatum

In the SNc, relatively low DAT activity compared to that in striatum leads to the surprising result that peak $[\text{DA}]_0$ is minimally affected by uptake even 20 μm from the site of release (Cragg et al., 2001) (Fig. 1, lower panel). By contrast, in striatum, where k' is nearly 200-fold greater (Table 1), an effect of uptake on peak $[\text{DA}]_0$ is seen by $r = 2 \text{ } \mu\text{m}$ (Fig. 1, lower panel).

Notably, however, whether SNc- or striatum-specific DA uptake is included or not, the dominant influence on $[DA]_o$ is the distance from a release site (note the order-of-magnitude decrease in concentration at each successive r in Fig. 1), as diffusing DA is diluted into an increasing volume defined by $4/3\pi r^3$ (Cragg and Rice, 2004). Overall, therefore, diffusion and dilution are the main factors that govern absolute $[DA]_o$ after quantal release in both SNc and striatum.

Do perisynaptic DATs in striatum modulate synaptic concentration and “gate” DA spillover?—A striking finding from our simulations of DA behavior in striatum is the complete lack of uptake on peak $[DA]_o$ within 1 μm of a release site (Cragg and Rice, 2004) (Fig. 1, lower panel). Given that the cross-synaptic diameter of a striatal DA synapse is 0.3–0.6 μm (Pickel et al., 1981; Groves et al., 1994; Descarries et al., 1996), this means that DA must necessarily spill over from a synapse or other release site, with its maximum concentration unmodulated by uptake. We tested the hypothesis that this occurs because the rate of diffusion exceeds that of DA uptake near a release site ($r < 5 \mu\text{m}$; see Fig. 1) by “slowing” DA diffusion by doubling of the tortuosity factor, λ , which decreases the apparent diffusion coefficient, D^* , by a factor of four ($D^* = D/\lambda^2$) (Cragg and Rice, 2004). The theoretical slowing of DA diffusion allows more time for DAT mediated uptake and led to a slight decrease in peak $[DA]_o$, even 1 μm from the release site. This result confirms that DA uptake normally loses the kinetic competition against diffusion in mediating DA clearance within a synapse. Thus, perisynaptic DATs neither modulate maximum synaptic DA concentration nor gate initial DA spillover (Cragg and Rice, 2004).

How far does DA diffuse?—The primary role of diffusion in governing $[DA]_o$ at all distances from a release site, whether in SNc or striatum, leads naturally to the question of how far DA can diffuse. In the absence of uptake (or metabolism) and with sufficient time, DA molecules can diffuse from a release site to the physical boundaries of the brain, i.e., a ventricle or the subarachnoid space. However, the better question is how far from a release site will DA have physiological consequences? This obviously depends on the concentration-sensitivity of DA receptors within a potential sphere of influence. Specifically, the EC_{50} values for activation of D_1 -like and D_2 -like receptors *in vitro* are on the order of 1 μM for low-affinity states and 10 nM for high-affinity states (Richfield et al., 1989; Neve and Neve, 1997). In striatum, the majority of D_1 -like receptors are suggested to be in a low-affinity state, whereas D_2 -like receptors are predominantly high affinity (Richfield et al., 1989). Assuming these relative affinities also hold true for SNc, the *maximum* “effective radius” can be defined as the distance within which release of one quantum of DA reaches high affinity D_2 receptors at ≥ 10 nM above baseline (Cragg and Rice, 2004).

With this constraint and using the normalized Q values in Table 1, the maximum effective radius for spillover in SNc and in striatum would be 8.2 μm , in the absence of uptake (diffusion only) (Fig. 2). Unsurprisingly, given limited DA uptake in SNc, including DAT-mediated uptake in the simulation has no effect on maximum effective radius in SNc (Fig. 2, upper panel). However, in striatum, uptake would decrease the maximum effective radius to 7.0 μm (Cragg and Rice, 2004). Although the absolute difference in r seems negligible, the r^3 -dependence of spherical volume means that the sphere of influence of DA, and therefore the number of receptors activated, would be 40% smaller with normal uptake than under conditions of DAT inhibition.

The effective radius for DA acting at low-affinity, predominantly D_1 -like receptors is much smaller than for high-affinity receptors. For receptors requiring 1 μM DA for activation, the maximum effective radius would be $< 2 \mu\text{m}$ in SNc or striatum (Fig. 2). At this distance from the release site, there is little influence of the DAT on peak $[DA]_o$ and consequently little influence on the sphere of influence in either SNc or striatum (Fig. 2). Consequently, these

data indicate that DAT inhibition will have a greater influence on the activation of high-affinity DA receptors than on low-affinity receptors.

Influence of the DAT on the active lifetime of DA transients—Thus far, we have considered the influence of the DAT on peak $[DA]_o$ and effective radius of DA transients. A third parameter of importance in DA transmission is active lifetime, i.e., the time DA is available at sufficient concentration to activate DA receptors. The maximal active lifetime is the time during which $[DA]_o$ remains above the EC_{50} for high affinity DA receptors, 10 nM, at a given site after quantal release (Cragg and Rice, 2004). This is measured as the difference between the time at which $[DA]_o$ first exceeds 10 nM (onset) and when it falls below 10 nM (offset) (Fig. 3, inset). In SNc, active lifetime is longest at $r \leq 1 \mu m$, with a maximum lifetime of 100 ms for a single Q , then decreases progressively as r approaches the limit of the effective radius (Fig. 3, upper panel). This pattern is the same whether SNc-specific DA uptake is present or not. In striatum, a similar lifetime pattern is seen in the absence of uptake (Fig. 3, lower panel). However, under conditions of normal striatal DAT activity, uptake curtails the duration of striatal $[DA]_o$ transients within the effective radius by 50%. Thus, a major role of the DAT in striatum, but not in SNc, is to limit DA lifetime after release. Through this mechanism, the DAT can readily influence DA transmission.

Quantal size and the sphere of influence in SNc and striatum

The sphere of influence of DA after quantal release can be calculated from the maximum effective radius for a given set of conditions. We determined the sphere of influence for activation of high-affinity (e.g., D_2 receptors) and low-affinity DA receptors (e.g., D_1 receptors), based on the effective radii for EC_{50} values of 10 nM and 1 μM , respectively, for a range of Q in SNc and striatum (Fig. 4). The range of Q examined was based on available literature indicating that the number of DA molecules per vesicle can vary from 1,000 to 14,000 (Garris et al., 1994; Pothos et al., 1998; Jaffe et al., 1998), reflecting the plasticity of the volume and quantal content of catecholamine vesicles (Pothos et al., 1998; Colliver et al., 2000), as well as methodological differences in assessing Q . We did not normalize Q between SNc and striatum for this comparison, but rather used identical Q values for both regions to gain insight into the role of α , as well as the DAT, in the regulation of $[DA]_o$ after quantal release.

Our model shows that in SNc, the sphere of influence is *directly proportional* to the number of molecules released, whether specific DAT-mediated uptake is active or inhibited (Fig. 4A). For example, a seven-fold larger Q (14,000 vs. 2,000) will have a seven-fold larger sphere of influence. A similar linear dependence on Q also occurs in striatum in the absence of uptake (Fig. 4C), although the resulting spheres would be roughly 40% larger than those in SNc for equal Q , because of the smaller striatal volume fraction, α (compare Figs. 4A,B and 4C,D for “diffusion only”).

Normal DA uptake in striatum constrains the sphere of influence for activation of high-affinity DA receptors across a range of Q when compared to the spheres seen when clearance is by diffusion only (Fig. 4C). Importantly, the influence of the DAT increases as Q increases, because larger, longer-lasting $[DA]_o$ transients allow longer times for uptake to remove DA (Cragg and Rice, 2004). For example, uptake decreases the sphere of influence by ~20% for $Q = 2,000$ DA molecules, but by ~40% for $Q = 14,000$ (Fig. 4C). Strikingly, the spheres of influence for activation of low-affinity DA receptors are largely unaffected by uptake, regardless of Q , in both SNc (Fig. 4B) and striatum (Fig. 4D). Again, this reflects the rapid clearance of DA by diffusion and the consequently minimal influence of the DAT at $r < 2 \mu m$ within which $[DA]_o$ is $\geq 1 \mu M$.

The minimal effect of DA uptake on the spheres defined by activation of low-affinity DA receptors coupled with the smaller α of striatum *versus* SNc means that the absolute tissue

volume affected for a given Q is larger in striatum than in SNc. In contrast, the spheres of influence for DA acting at high-affinity DA receptors in the presence of region-specific uptake are surprisingly similar between SNc and striatum after the release of equal Q (compare Figs. 4A and 4C for “diffusion + uptake”). This similarity reflects the opposing influences of uptake and α in each region: the larger α of SNc offsets the lower k' , whereas the higher k' of striatum is balanced by the smaller α . Intriguingly, the relative effects of these two parameters on sphere of influence vary with Q . For small, fast transients generated by the release of small Q , α dominates over uptake, such that for $Q = 2,000$ the sphere of influence is *smaller* in SNc than in striatum, whereas for larger transients larger Q , uptake dominates, such that for $Q = 14,000$ the sphere is *larger* in SNc than in striatum.

How many synapses are within the sphere of DA influence in striatum and SNc?

—Anatomical data suggest that there are on average 0.05 DA synapses per μm^3 or 1 DA synapse per $20 \mu\text{m}^3$ in the striatum (Doucet et al., 1986), although recent estimations suggest that the density might be twice as high as that (Arbuthnott and Wickens, 2007). Even assuming a density of only 1 DA synapse per $20 \mu\text{m}^3$, when the average intersynaptic distance can be calculated to be $3.5 \mu\text{m}$, synaptic “spillover” to neighboring synapses within these spheres of influence will readily occur, with gradients of efficacy that depend on receptor sensitivity (Cragg and Rice, 2004). How many synapses will be affected? In striatum, the spheres of influence for $Q = 2,000$ – $14,000$ DA molecules acting at high-affinity receptors would contain ~20–100 DA synapses under condition of normal DAT activity (Cragg and Rice, 2004). Spillover of DA to neighboring synapses will therefore necessarily occur after single-vesicle release. However, the sphere defined by $r \leq 2 \mu\text{m}$ for low affinity DA receptors would affect 0–1.5 DA synapses (Cragg and Rice, 2004).

Comparable calculations cannot be made for SNc, however, given the absence of axo-dendritic DA synapses and only few dendro-dendritic synapses in that region (Wilson et al., 1977). Moreover, DA receptors are expressed peri- and pre-synaptically at non-DA synapses, as well as on neuronal soma in both SNc and striatum. A better sense of DA influence might therefore come from considering the number of receptors within a given sphere. However, such evaluation is hampered at present by a lack of quantitative data about receptor number for high and low affinity DA receptors in striatum or SNc. A practical alternative approach is to consider the total population of synapses (DA and non-DA) potentially available for dopaminergic regulation. Total synaptic density in striatum is ~1 synapse per μm^3 (Pickel et al., 1981). Given the consistency of this synaptic density with that in other CNS regions, e.g., the CA1 region of hippocampus (Rusakov et al., 1999), we used this value to assess the influence of DA in SNc, as well as striatum. With normal uptake, released DA would encounter ~300 to 2,500 synapses within the spheres defined by $[\text{DA}]_0 \geq 10 \text{ nM}$ for $Q = 2,000$ – $14,000$ molecules in both regions (Fig. 4A,C). By contrast, the number of synapses in the spheres defined by $[\text{DA}]_0 \geq 1 \mu\text{M}$ is nearly 200-fold lower, with only ~5 to 35 synapses encountered (Fig. 4B,D). Notably, the minimal influence of uptake on DA transients in these smaller spheres unmasks the effect of the larger α of SNc *versus* striatum, with a lower number of synapses encountered in SNc than in striatum for all Q .

One final point to consider for both SNc and striatum is the active lifetimes of DA within these spheres of influence. Our model indicates that over the quantal range examined, the active lifetime during which $[\text{DA}]_0$ exceeds a minimum of 10 nM would be 10 to 100 ms. Notably, the duration of extracellular DA availability does not need to be shorter than this since the effector responses of DA receptors, which are G-protein coupled, range from 100 to 5000 ms (Cragg and Greenfield, 1997; Robinson and Caron, 1997; Gonon, 1997; Benoit-Marand et al., 2001; Phillips et al., 2002).

Temporal and spatial summation of DA quanta—In striatum, the greater effect of the DAT on larger, longer-lasting $[DA]_o$ transients after the release of larger Q (Fig. 4C), would also hold true for the release of multiple vesicles, from either single or multiple sites. The temporal and spatial summation of the resultant transients determine net $[DA]_o$ in the striatum and would therefore be expected to be strongly regulated by the DAT. Such a role is indeed consistent with the increases in basal $[DA]_o$ known to occur after uptake blockade (Cragg et al., 1997; Bradberry et al., 2000) or in DAT knockout mice (Giros et al., 1996). Importantly, the relative roles of the DAT on striatal $[DA]_o$ during tonic and phasic activity of midbrain DA neurons have also been determined experimentally (Floresco et al., 2003). As predicted by our model, inhibition of the DAT was found to cause a greater increase in $[DA]_o$ during enhanced burst firing of DA neurons, which leads to quantal summation, than during a simple increase in asynchronous tonic activity in these cells (Floresco et al., 2003).

Summary and implications for DA transmission in SNc and striatum

Our model of DA behavior after quantal release indicates that uptake does not limit initial DA overflow from a release site in either striatum or midbrain. This contrasts completely with regulation of the prototypical fast synaptic transmitter, glutamate (Rusakov and Kullmann, 1998; Barbour, 2001; Cragg and Rice, 2004). Moreover, diffusion, rather than uptake, is the key factor that regulates dynamic DA behavior, not only in the SNc, as predicted, but also in dorsal striatum in which regulation of $[DA]_o$ has been characterized as “uptake limited.” Nevertheless, this process constrains dopaminergic transmission in space and time: a DA transient produced by release of a single Q will have a maximum effective radius of 7–8 μm for activation of high-affinity DA receptors and an active lifetime of 10–100 ms. Thus, our model shows that diffusion and dilution, especially when aided by uptake, regulate dopaminergic transmission to a greater extent than sometimes imagined for signaling via volume transmission.

Volume transmission in the SNc—Evidence for DA volume transmission in the SNc (Fig. 5) includes the predominance of extrasynaptic DA receptors (Cameron and Williams, 1993; Sesack et al., 1994; Yung et al., 1995), the virtual absence of DA synapses (Wilson et al., 1977), and a limited influence of DA uptake on either exogenously diffusing (Cragg et al., 2001) or endogenously released DA (Cragg et al., 1997; Chen and Rice, 2001). Moreover, as discussed here and elsewhere (Rice, 2000; Cragg et al., 2001), the release of a single vesicle of DA could modulate the excitability of tens to thousands of synapses within distances a few micrometers of a release site, in both SNc and striatum. This broad influence of released DA meets the definition of volume transmission given in the Introduction, which is independent of the source of release, i.e., from synaptic or non-synaptic sites.

Recently, the view that DA acts by volume transmission in the SNc has been challenged, based on electrophysiological monitoring of D_2 -receptor mediated inhibitory “postsynaptic” currents (IPSCs) in DA neurons (Beckstead et al., 2004; Beckstead et al., 2007). In these studies, local electrical stimulation in the presence of a cocktail of glutamate and GABA receptor antagonists evoked a robust IPSC in most DA neurons examined. Beckstead, Williams and colleagues have argued against DA volume transmission in the SNc (Beckstead et al., 2004; Beckstead et al., 2007) based on their observation that the time course of DA-dependent IPSCs is unaltered under conditions that increased the amplitude of the IPSCs, including by increasing stimulation intensity (Fig. 6A,B) or increasing quantal content with L -DOPA. Their interpretation is based on the premise that, “If super-threshold concentrations of dopamine were typically traveling micrometers to reach target D_2 receptors then increasing release would have prolonged the time course of the synaptic current” (Beckstead et al., 2007). There are two flaws in this premise, however. First, there are few dendrodendritic synapses in the SN (Wilson et al., 1977) that could mediate synaptic DA transmission; moreover, no *synaptic* D_2 receptors have

been identified in midbrain DA neurons (Sesack et al., 1994; Yung et al., 1995; Khan et al., 1998). Second, the authors' premise does not take into account the time course of diffusion of DA from a release site to its site of action. As shown in this review, the time to peak and duration of $[DA]_o$ transients after quantal release are much shorter than those of recorded IPSCs. At a distance of 5 μm from a release site, the resulting $[DA]_o$ transient would reach a maximum ~ 10 ms after release, whereas the observed peak IPSC does not occur until ~ 500 ms after the stimulus (Fig. 6B; $[DA]_o$ data from Fig. 1 superimposed on IPSC records from Beckstead et al. 2004). Even 20 μm from a release site, peak $[DA]_o$ would occur only 250 ms after release, which is still half the time of the IPSC peak (Fig. 6B). Thus, the IPSC data lack the temporal resolution to provide insight into the role of volume transmission on DA communication on the SN.

The rate-limiting step in IPSC generation is activation of D_2 -receptor linked GIRK (G-protein coupled inward rectifier K^+) channels, not DA diffusion (Beckstead et al., 2004). The IPSC data reported are consistent with previous studies of the time course of D_2 -autoreceptor mediated inhibition of DA release in striatum, in which autoinhibition begins 50-100 ms after DA single-pulse evoked DA release, then persists for up to 5 s afterwards, which greatly exceeds the lifetime of the DA transient that initiated the autoreceptor response (Phillips et al., 2002). It is unsurprising, therefore, that the time course of DA IPSCs would be independent of the amplitude of rapid $[DA]_o$ transients that initiate D_2 receptor-linked GIRK channel opening. Indeed, the dependence of IPSC amplitude on stimulation intensity supports a role for DA volume transmission, rather than arguing against it. Local stimulation activates not just the recorded neuron, but also surrounding cells and dendrites, such that the resulting increase in $[DA]_o$ is a population response. As stimulus intensity is increased, the resulting increase in current spread will lead to action potential generation and somatodendritic DA release from increasingly distant neurons. The dependence of D_2 -autoreceptor mediated current amplitude on stimulus intensity (Fig. 6A) is therefore consistent with increases in $[DA]_o$ detected by the recorded cell as the summation of DA transients from both local and increasingly distant sources, reflecting volume transmission.

Given the likely contribution of distant release sites, and therefore multiple vesicles, to initiating $[DA]_o$ transient, it is also unsurprising that one condition that did increase IPSC time course in DA neurons was DAT inhibition by cocaine (Beckstead et al., 2004; Beckstead et al., 2007) (Fig. 6C). This response is consistent with previous studies of the effect of DAT inhibition on experimental DA diffusion profiles in the SNc recorded 100 μm from a pressure ejection pipette (Cragg et al., 2001) (Fig. 6D). The proportionately greater effect of DAT inhibition on the IPSC records than on DA diffusion curves reflects the primary contribution of uptake to DA clearance after release by electrical stimulation, in which a locally homogeneous increase in $[DA]_o$ is predominantly cleared by uptake rather than diffusion (Garris et al., 1994). As Beckstead et al. (2004) conclude, their effect of cocaine confirms "the crucial role of uptake transporters in limiting the time dopamine is in the extracellular space," and is therefore available to mediate volume transmission.

A new model of the DA synapse—The conventional representation of a striatal DA synapse shows vesicular release with reuptake of release DA into the presynaptic terminal by perisynaptic DATs, which restricts synaptic spillover of DA and regulates synaptic DA concentration (Fig. 7, left panel). However, our kinetic analysis of DA diffusion and uptake after quantal release shows that synaptic DA spillover cannot be gated by perisynaptic DATs, because the kinetics of DA transport cannot compete with the rapid escape of DA away from a release site by diffusion (Cragg and Rice, 2004). This further means that perisynaptic DATs can do little to alter the concentration of DA *within* a striatal synapse. It is therefore time for a significant revision of the depiction of a DA synapse that incorporates these inconvenient facts (Fig. 7, right panel).

Two main factors contribute to the dominance of diffusion over DAT-mediated uptake. The first is the rapidity of diffusion over micrometer distances and the low DAT cycle rate (2-5 DA molecules per sec per transporter at 37 °C) (Povlock and Schenk, 1997; Prasad and Amara, 2001). The second, less obvious factor is that DAT expression is limited to DA neurons, including DA axons in striatum (Nirenberg et al., 1996). Immediately after release, therefore, a DA molecule diffusing in three-dimensional space will be subject to transporter activity only if it encounters the presynaptic membrane of the releasing DA axon (Fig. 7, right panel). This geometric organization is analogous to that of the retina, in which DA is released from DAT-expressing amacrine cells oriented in a single plane in the inner plexiform layer: once a DA molecule escapes this plane, its behavior is governed only by diffusion (Witkovsky et al., 1993; Rice, 2000). Diffusing DA can readily interact with predominantly extrasynaptic DA receptors (Sesack et al., 1994; Yung et al., 1995; Hersch et al., 1995; Khan et al., 1998) (Fig. 7, right panel), until it reaches the boundary set by receptor sensitivities (e.g., Fig. 2).

The perisynaptic landscape of a glutamate synapse is very different from that for DA synapses (Cragg and Rice, 2004). Glutamate transporters are localized primarily on non-glutamatergic cells, including astrocytic processes that can ensheath a glutamate synapse, as well as post-synaptic neurons (Seal and Amara, 1999; Danbolt, 2001). Moreover, glutamate transport kinetics exceed those of the DAT by an order of magnitude, with transport-cycle rates of over 35 glutamate molecules per sec per transporter at 37 °C (EAAT2) (Wadiche et al., 1995). Most glutamatergic communication is mediated by fast-responding (1-2 ms), ionotropic glutamate receptors located immediately postsynaptically, with slower modulation via metabotropic receptors found near the perimeter of the synapse (Ottersen and Landsend, 1997) and possible additional contributions from extrasynaptic receptors (Galvan et al., 2006). Although there is experimental evidence to support glutamate spillover (Rusakov and Kullmann, 1998; Rusakov et al., 1999), released glutamate is typically constrained by uptake to remain perisynaptic, thus preserving synaptic independence (e.g. Barbour, 2001).

At the present time, DA synapses are most commonly illustrated as though they were glutamate synapses (Fig. 7, left panel), despite marked differences between the structural and functional anatomy of these transmitter release sites. To understand dopaminergic transmission requires that the classic representation of the DA synapse be updated to reflect the functional microanatomy of these synapses, which, unlike glutamate synapses, are designed for overflow and volume transmission (Fig. 7, right panel).

Conclusions—The slow kinetics and limited distribution of the DAT leads to predominant regulation of DA behavior by diffusion in both SNc and striatum. Following quantal release, nigrostriatal DA can readily spill out from release sites and act within effective radii of several micrometers. Thus, diffusion-based volume transmission is the predominant mode of DA signaling after both somatodendritic and synaptic release.

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Abbreviations

DA	dopamine
DAT	dopamine transporter
[DA] _o	extracellular DA concentration

SNC substantia nigra pars compacta

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Figure 1. DA diffusion following quantal release in SNc and striatum: distance and uptake

Theoretical curves for DA diffusion at 37 °C were generated using Eq. 1 with region-specific parameters for α , λ , k' and normalized Q (Table 1). Curves show dynamic changes in $[\text{DA}]_0$ at diffusion distances, r , of 1-20 μm in SNc (**upper panel**) and striatum (**lower panel**), following release of a single DA vesicle (*arrows*). The effect of DA uptake is indicated by diffusion curves generated with only non-specific uptake ($k' = 0.007 \text{ s}^{-1}$; diffusion only, black lines) vs. region-specific uptake (Table 1; diffusion + uptake, red lines). Scale bars indicate variations in concentration and time across distance. Striatal data are modified from Cragg and Rice, 2004.

Figure 2. Effective radius for DA after quantal release in SNc and striatum

(A) Peak $[DA]_0$ vs. diffusion distance, r , from Fig. 1. The maximum radius at which peak $[DA]_0$ reaches 10 nM (e.g., the concentration required for activation of high affinity D_2 DA receptors, $D_2\text{Rs}$) was 8.2 μm in SNc or in striatum for diffusion only (non-specific k') and in SNc with region-specific uptake with normalized Q to compensate for the larger α of SNc (Table 1). In striatum, region-specific uptake decreases the effective radius to 7.0 μm , resulting in a 40% smaller sphere of influence than for diffusion only. The maximum radius at which peak $[DA]_0$ reaches 1 μM (e.g., for activation of low affinity D_1 DA receptors, $D_1\text{Rs}$) is $\sim 2 \mu\text{m}$ in both SNc and striatum. Given the dominance of diffusion over the DAT at this distance from a release site, the sphere of influence for low affinity DA receptors is unaltered by DAT-mediated uptake. Striatal data are modified from Cragg and Rice, 2004.

Figure 3. Effect of the DAT on active DA lifetime in SNc and striatum

Onset and offset times at varying r after quantal release in (A) SNc and (B) striatum, defined as the times at which a change in $[\text{DA}]_o$ reaches (onset) and then falls below (offset) 10 nM. The inset in (A) indicates these time points for a theoretical DA diffusion curve at $r = 5 \mu\text{m}$ in SNc. The data were calculated using the normalized values for Q in Table 1. The effect of uptake on onset and offset times in (A) SNc and (B) striatum was also assessed; compare diffusion only (gray lines) with diffusion + region-specific DA uptake (red lines). In SNc, DAT-mediated uptake does not alter active lifetime. In striatum, however, uptake curtails offset time, leading to a 50% decrease in active lifetime at all distances within the effective radius. Although the maximum effective radius in SNc is unaltered by the DAT, region-specific uptake

in striatum limits the effective radius for activation of high-affinity DA receptors (as also seen in Fig. 2). Striatal data are modified from Cragg and Rice, 2004.

SNc

A ≥ 10 nM, e.g. D₂Rs **B** $\geq 1 \mu\text{M}$, e.g. D₁Rs

Striatum

C ≥ 10 nM, e.g. D₂Rs **D** $\geq 1 \mu\text{M}$, e.g. D₁Rs

Figure 4. The sphere of influence of DA after quantal release in striatum and SNc depends on quantal size (Q) and DA receptor affinity

Sphere of DA influence, including number of synapses encountered vs. quantal size, Q , in (A,B) SNc and (C,D) striatum, with (diffusion + uptake) and without (diffusion only) region-specific DA uptake for activation of high-affinity (≥ 10 nM) and low affinity ($\geq 1 \mu\text{M}$) DA receptors. **A,B**) In SNc, the sphere of influence of DA activation of either high-affinity (e.g., D₂ receptors, D₂Rs,) or low-affinity (e.g., D₁ receptors, D₁Rs) was not affected by uptake, regardless of Q . **C,D**) In striatum, however, the sphere of influence for (C) high-affinity receptors was increasingly constrained by uptake as Q increased, consistent with a greater effect of the DAT on larger, longer-lasting DA transients. **D**) The sphere for low-affinity

receptors was minimally affected by DAT-dependent uptake. In anatomical terms, the number of synapses contained in spheres defined by the $[DA]_o$ required to activate high-affinity receptors (**A,C**) in either SNc or striatum is 300-2,500, whereas only 5-35 synapses would be encompassed within the sphere defined by the $[DA]_o$ required to activate low-affinity receptors (**B,D**). Panel A is modified from Cragg and Rice, 2004.

Figure 5. Volume transmission of DA in the SNc

Schematic representation of the influence of somatodendritically released DA from one DA neuron (black) diffusing to activate DA receptors (D₂ autoreceptors) in the releasing cell, as well as on the soma and dendrites of neighboring DA neurons (gray). The representations of DA receptors and DATs in this diagram do not reflect actual densities; the DA neuron is modified from a drawing by Fallon et al., 1978.

Figure 6. Comparison of the time course of IPSCs and DA diffusion in the SNc

A) Increasing current amplitude of D_2 -autoreceptor-mediated IPSCs evoked in a midbrain DA neuron by local stimulation with increasing the stimulus intensity (modified from Beckstead et al., 2004). **B)** The time course of the IPSCs is unaffected by increasing current amplitude, as seen when the current records in (A) were normalized to their maxima (black lines) (modified from Beckstead et al., 2004). Superimposed on these IPSCs are normalized DA diffusion curves (red lines) modeled for $r = 5$ and $r = 20$ μm from a release site in SNc (from Fig. 1). This comparison shows that the time course of DA transients is much faster than receptor-mediated signaling. **C)** Increased time course and amplitude of evoked IPSCs in a midbrain DA neuron when the DAT is inhibited by cocaine (modified from Beckstead et al., 2004). **D)** Increased

time course and amplitude of experimentally monitored DA diffusion curves in the SNc when the DAT is inhibited by GBR 12909 ($r = 100 \mu\text{m}$) (modified from Cragg et al., 2001).

Conventional dopamine synapse

Updated dopamine synapse

Figure 7. Volume transmission of DA in the striatum

A) Schematic representation of a conventional DA synapse on the neck of a dendritic spine on a striatal medium spiny neuron. Glutamatergic input forms synapses on the heads of these spines. Synaptically released DA is often depicted as being constrained within a synapse by perisynaptic DATs, with primary activation of subsynaptic DA receptors, like the regulation of glutamate (Glu) at a glutamatergic synapse. The extrasynaptic localization of DATs and DA receptors, as well as the rapid time course of DA diffusion away from a synapse compared to DAT-mediated uptake make this “conventional” model synapse inaccurate and outdated. **B)** Schematic representation of an updated DA synapse that shows ready DA spillover, unconstrained by DATs. The cloud of released DA, diffusing in three dimensions, encounters predominantly extrasynaptic DA autoreceptors on DA axons and heteroreceptors on neighboring cells, including medium spiny neurons. Released DA will be taken up only when it encounters DATs on DA axons. The representations of DA receptors and DATs in **A** and **B** do not reflect actual densities.

Table 1

Experimental Data used to Model DA Behavior in Striatum and SNc

Parameter	Value	Reference
Striatum		
α	0.21	Rice and Nicholson, 1991
λ	1.54	Rice and Nicholson, 1991
k' for DA	20 s^{-1}	$V_{\max}/K_m = (4.1 \text{ } \mu\text{M s}^{-1})/(0.21 \text{ } \mu\text{M})$ (Ross 1991)
k' for non-specific uptake	0.007 s^{-1}	derived from Cragg et al., 2001
Q	9,800 molecules	derived from Jaffe et al., 1998; Cragg et al., 2001
U	$6.5 \times 10^{-20} \text{ L}$	Nirenberg et al., 1997
C_f	250 mM	derived from Jaffe et al., 1998; Nirenberg et al., 1997; Cragg et al., 2001
$D (37^\circ\text{C})$	$7.63 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$	derived from Nicholson, 1995
SNc		
α	0.30	Cragg et al., 2001
λ	1.58	Cragg et al., 2001
k' for DA	0.12 s^{-1}	derived from Cragg et al., 2001
k' for non-specific uptake	0.007 s^{-1}	derived from Cragg et al., 2001
Q	14,000 molecules	Jaffe et al., 1998
U	$6.5 \times 10^{-20} \text{ L}$	Nirenberg et al., 1997
C_f	350 mM	derived from Jaffe et al., 1998; Nirenberg et al., 1997
$D (37^\circ\text{C})$	$7.63 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$	derived from Nicholson, 1995

α , extracellular volume fraction; λ , tortuosity factor; k' , linear uptake term; Q , number of DA molecules per vesicle; U , vesicle volume; C_f , intravesicular DA concentration; D , DA diffusion coefficient.