As one of the most important neurotransmitters in the central nervous system (CNS), dopamine (DA) contributes to many critical functions such as reward mechanisms, learning and motor control. Any kind of abnormality in the DA system could lead to disorders including schizophrenia, Parkinson's disease, addiction and Alzheimer's disease. By introducing artificial electrical stimulation *in vivo* at the rat's medial forebrain bundle (mfb), evoked DA signals can be measured at a carbon fiber microelectrode with fast scan cyclic voltammetry (FSCV) in the dorsal striatum. Previously, our lab discovered that DA kinetics are highly heterogeneous for measurements in the dorsal striatum. The responses were widely categorized into slow and fast sites. Hypothetically, the slow responses result from autoinhibition, which exists prior to the stimulus, presumably due to the occupation of pre-synaptic D2 autoreceptors by basal DA in the extracellular space.[1] Fast responses have minimal autoinhibitory effect because the DA can be observed within 200 ms while it can require more than 2 s for the slow sites.

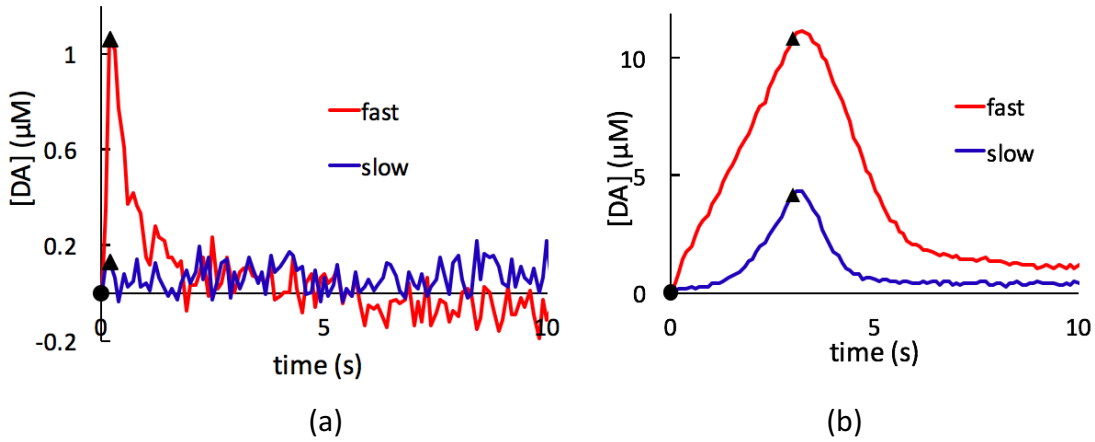


Figure 1. (a) The difference between fast and slow sites are that after giving a 0.2 s electrical stimulation at 60 Hz, there is an evoked response in fast site but not in slow site. **(b)** While introducing a 3 s stimulation at 60 Hz, there is an initial "lag" in slow sites where there shows no delay in fast sites. N=10 rats, each rat is measured with brand new carbonfiber electrode.

Previously, the Diffusion Gap (DG) model was the reigning explanation of the heterogeneous kinetics of DA. This model proposes that there is a physical gap between the DA terminal and the recording site[2]. However, there are several problems with this model. For example, if there is a response delay at the onset of the stimulus due to the gap, the fall of the response should have the same delay when the stimulus ends, however this is not always observed. In addition, raclopride, a D2 antagonist, can eliminate the delay in the onset of the DA signal observed in a slow site. This would not be possible if there is a physical gap between the recording site and the DA terminals.

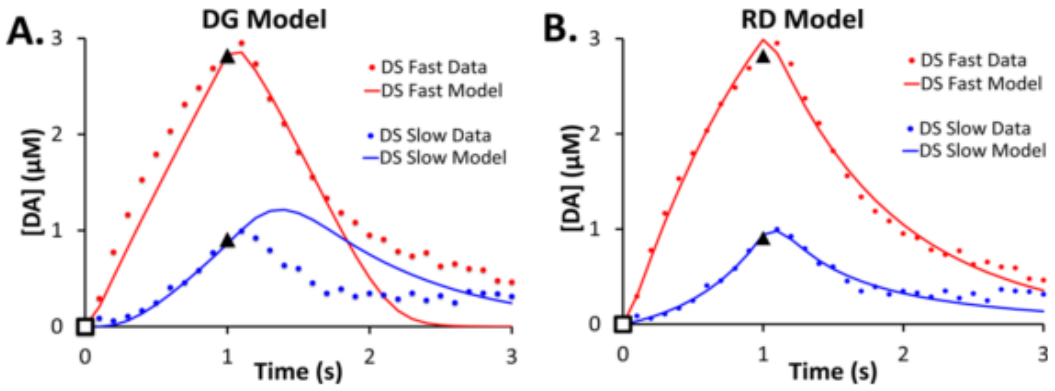


Figure 2. Fits of the DG (A) and RD (B) models for averaged responses for fast and slow domains of the dorsal striatum.

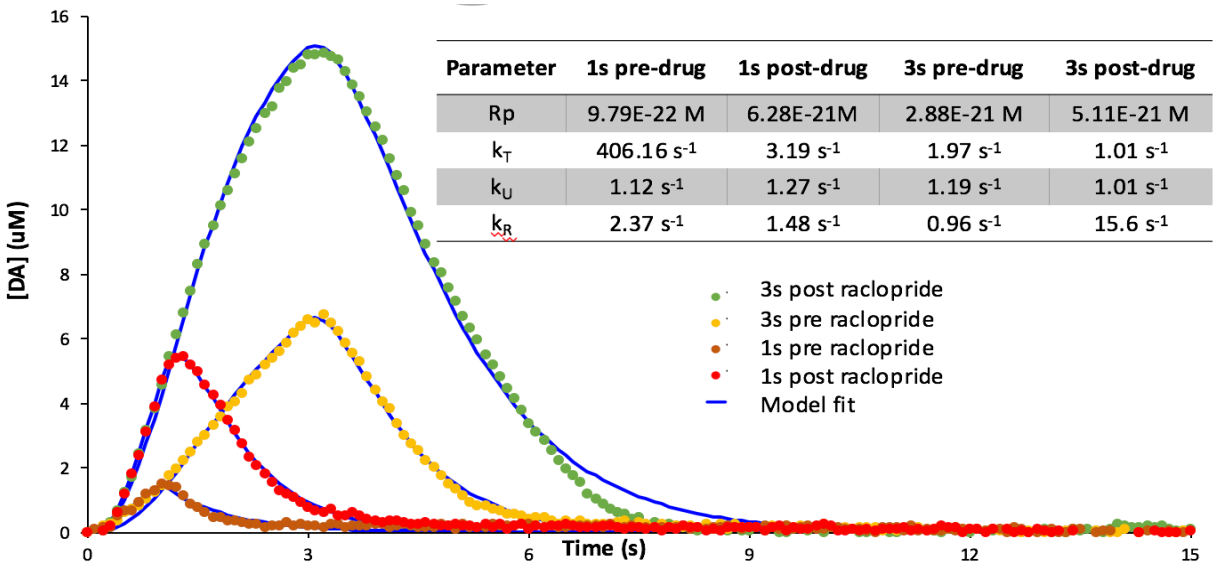
To address this problem, a new model was introduced by our lab based on the concept of restricted diffusion (RD)[3]. It suggests that DA is first released to an inner compartment then transported to an outer compartment. DA is detected in the outer compartment by FSCV and then cleared by re-uptake. The delay between the stimulus and the electrode's response is represented by k_T, which is a first-order term dominated by mass transport. The uptake from the outer compartment back to the vesicles is represented by a first-order term k_U, and the release term is represented by Rp which represents amount of DA released per pulse. A fourth parameter k_R was introduced as an exponential release modulator because the DA release is affected by autoinhibition[4] (equation 1 & 2). The RD model is based on mathematical calculations to examine experimental data and investigate the dopaminergic system (figure 3). To further refine the RD model, a study was designed to explore different stimuli pulses which are super-physiological to force evoked release and examine how these responses are seen in the dorsal striatum after the rats are given raclopride. Modeling responses obtained with high-pulse stimuli, the RD model reveals new information about the kinetics of the slow kinetic domains[3].

$$\frac{dDA_{ic}}{dt} = R_p \cdot f \cdot (1 - e^{-k_R t}) - DA_{ic} \cdot k_T$$

Equation 1. DA concentration change in the inner compartment space over time.

$$\frac{d[DA]_{oc}}{dt} = \frac{DA_{ic} \cdot k_T}{V_{oc}} - [DA]_{oc} \cdot k_U$$

Equation 2. DA concentration change in the outer compartment space over time.



Parameter	1s pre-drug	1s post-drug	3s pre-drug	3s post-drug
Rp	9.79E-22 M	6.28E-21M	2.88E-21 M	5.11E-21 M
k_T	406.16 s^{-1}	3.19 s^{-1}	1.97 s^{-1}	1.01 s^{-1}
k_U	1.12 s^{-1}	1.27 s^{-1}	1.19 s^{-1}	1.01 s^{-1}
k_R	2.37 s^{-1}	1.48 s^{-1}	0.96 s^{-1}	15.6 s^{-1}

Figure 3. Responses from 1 s and 3 s stimulations can be modeled with a single set of parameters using the 4 parameter version of the restricted diffusion model. Stimuli frequency = 60Hz. Slow sites in n=5 rats.

However, the RD model was not sufficient to describe the responses of longer stimulation length. Although post-raclopride responses can be depicted by the 4-parameter model, pre-drug, 600 pulses responses cannot be portrayed presumably due to two components of the dopamine release. While the mfb is being stimulated the entire time, the vesicles release DA for the duration, but the dopamine concentration increases to a certain degree then starts to decrease until the stimulus ends (unlike the 1 second and 3 seconds evoked responses).

To investigate whether introducing a fifth parameter, k_{R2}, to the RD model is appropriate, rats were treated with raclopride to reduce the autoinhibition effect. 600 pulses at 60 Hz were applied, and pre-drug and post-drug responses were modeled (figure 4). Fits are very good, and all the parameters remained almost unchanged while the k_{R2} of the post-raclopride model increased for more than tenfold. This indicates that a fifth parameter is essential for describing longer stimulation responses, and the original RD model was not capable of creating such good fits without the k_{R2} term (equation 3).

As autoreceptors function in both fast and slow domains, it seems that the domains are different in autoinhibitory tone which derives from different basal DA concentration required to activate the D2 receptors. Due to this effect, future experiments will be focused on investigating the fast sites kinetics and whether the refined RD model can portray the responses. Moreover, lower frequency (i.e. 30 Hz, 15 Hz) stimulations will be applied to both slow sites and fast sites and the responses will be modeled. By studying the physiological sources of DA kinetics, we can investigate the responses similar of human neurons since the mammalian neuron fires at around 20 Hz[5]. Nomifensine (a DA uptake inhibitor) might need to be applied to the lower frequency responses in order to increase the signal amplitude since the signal to noise ratio decreases as the frequency decreases.

$$\frac{dDA_{ic}}{dt} = R_p \cdot f \cdot (e^{-k_{R1}t} - e^{-k_{R2}t}) - DA_{ic} \cdot k_T$$

Equation3. The 5-parameter RD model.

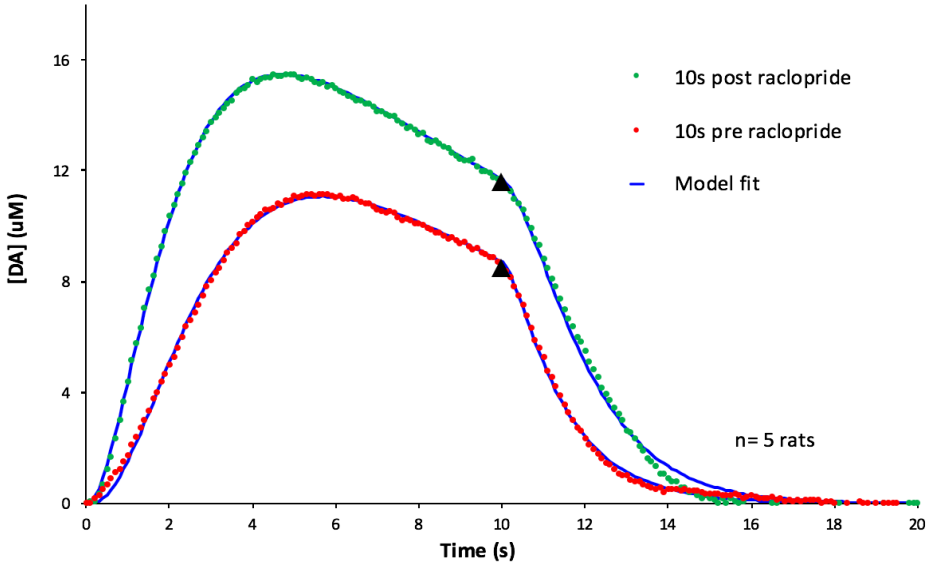


Figure 4. Data from slow sites of n=5 rats. 600 pulses at 60 Hz were applied. Triangles represent when the stimuli end. The data was fit with the 5-parameter RD model.

(1) Moquin, K. F.; Michael, A. C. Tonic Autoinhibition Contributes to the Heterogeneity of Evoked Dopamine Release in the Rat Striatum. *J. Neurochem.* **2009**, *110* (5), 1491–1501.

(2) Wightman, R. M.; Amatorh, C.; Engstrom, R. C.; Hale, P. D.; Kristensen, E. W.; Kuhr, W. G.; May, L. J. Real-Time Characterization of Dopamine Overflow and Uptake in the Rat Striatum. *Neuroscience* **1988**, *25* (2), 513–523.

(3) Walters, S. H.; Taylor, I. M.; Shu, Z.; Michael, A. C. A Novel Restricted Diffusion Model of Evoked Dopamine. *ACS Chem. Neurosci.* **2014**, *5* (9), 776–783.

(4) Walters, S. H.; Robbins, E. M.; Michael, A. C. Modeling the Kinetic Diversity of Dopamine in the Dorsal Striatum. *ACS Chem. Neurosci.* **2015**, *6* (8), 1468–1475.

(5) Cragg, S. J.; Rice, M. E. DAncing Past the DAT at a DA Synapse. *Trends Neurosci.* **2004**, *27* (5), 270–277.