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Dopamine Regulation Of Disengagement In The Basal Ganglia Circuitry

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Dedication

Dedicated to My Family for all Their Love and Support

DOPAMINE REGULATION OF DISENGAGEMENT IN THE BASAL GANGLIA CIRCUITRY

by

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THESIS

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Abstract

Evidence suggests that dopamine (DA) is crucial for initiation and termination as well as sustained execution of movement. For the present study, it was hypothesized that DA plays a more important role in initiation and termination of movement than in its sustained production. To test these hypotheses, rats were trained to walk on a treadmill in a continuous and discontinuous (walk 30 secs/stop 15 seconds) fashion for one hour while striatal DA samples were collected using In Vivo Microdialysis (IVMCD). We predicted larger increases during discontinuous compared to continuous walking. It was found that brain dialysate levels of DA consistently increased from baseline to walking [$p<0.05$]. However, no significant difference was found in brain dialysate DA between continuous and discontinuous walking [$p>0.05$]. Similar to DA, its major metabolites DOPAC and HVA, and the serotonin metabolite 5-HIAA, increased during treadmill walking from resting state levels [$p<0.05$]. None of these metabolites showed a significant difference between continuous and discontinuous walking. The results of the present study are important because they demonstrate that 1) increases in movement-related changes in brain dialysate dopamine are detectable in contrast to many of the studies in the literature; and 2) the converging changes in DA and its metabolites might be used to monitor changes in functional utilization. In conclusion, a clear understanding for the modulatory role of DA in movement production is crucial for the development of biomedical interventions involving basal ganglia disease.

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Introduction

The Basal Ganglia Regulates Voluntary Movement

There are different motor systems that control movement in mammals. Activation of the muscles needed for locomotion depends on four different primary motor systems: the descending motor pathways, spinal motor circuits, cerebellum, and basal ganglia. Motor programs required to activate the different muscles are located in the spinal cord but are controlled by cortical and subcortical motor centers (Grillner et al., 2005; Duysens et al., 1998; Catsman-Berrevoets & Kuypers, 1976; Dum & Strick, 1991; Kamiyama et al., 2015). Many studies have explored the specific role each system plays in motor behavior. The descending motor pathways include neuronal projections from the cortex or brain stem to the motor neurons of the spinal motor circuits. These systems can regulate both reflexive and controlled movements. Interestingly, the cerebellum and basal ganglia do not project directly to the motor circuits of the spinal cord, which makes their role in motor regulation less intuitive. They are generally thought to have a regulatory effect on motor behavior but their specific roles are still vague.

The cerebellum is believed to be involved in control of fine movement (Gao et al., 1996) and the basal ganglia has been long associated with regulation of voluntary movement (Middleton & Strick, 2000). Despite the number of studies dedicated to understanding the function of the basal ganglia its specific role in movement regulation has remained elusive due to the complexity of the circuitry. A study involving decorticate dogs, cats, and rats showed they could still perform self-maintaining behaviors like eating, drinking, walking, running, and mating almost as well as normal animals (Bjursten et al., 1976; Bard & Macht, 1958; Bazzet & Penfield 1922; Bignall & Schramm, 1974). Decorticate animals had all of the neocortex removed but every other brain structure remained intact, including the basal ganglia. However, decorticate

animals could not make plans and execute sequences of movements to adapt to new situations. Therefore it has been theorized that the basal ganglia aids with the selection process of the motor systems needed to perform a desired behavior (Grillner et al., 2005; Jueptner & Weiller, 1998) rather than regulating the motivational intention behind them. Furthermore, pathologies emerging from the disruption of the basal ganglia circuitry lead to a spectrum of motor problems ranging from hyperkinesia (Hungtington's disease) to hypokinesia (Parkinson's disease), and even cognitive deficits (Middleton & Strick, 2000; DeLong, 1990; Groves, 1983).

Reviews on basal ganglia function have speculated that this circuitry generally contributes to the planning and execution of voluntary movements via a series of parallel loops (DeLong, 1990). However, more recent interpretations have challenged the idea that the basal ganglia controls movement in general (Middleton & Strick, 2000) and may instead play a role to mediate more specific qualities of movement such as initiation and termination of movement (Aceves et al., 2011; Grillner et al., 2005; DeLong, 1990), or goal-directed tasks (Takakusaki et al., 2004). Recent studies on the neurobiology and neurochemistry of the basal ganglia also support the idea that the basal ganglia plays a more specific role in modulation of initiation and termination of voluntary movements via the direct and indirect loops (Takakusaki et al., 2004; Grillner et al., 2005; Aceves et al., 2011).

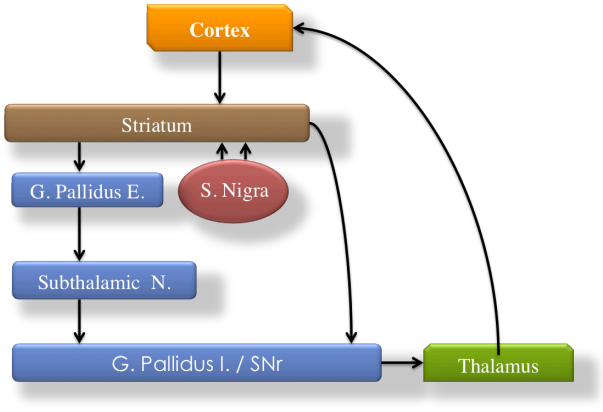

Figure 1. Basal Ganglia Circuit

Anatomy of the Basal Ganglia Circuitry

The basic organization of the basal ganglia circuit is shown in Figure 1. Distinct areas of the cortex send excitatory glutamatergic projections to the striatum (Berendse et al.,

1992; Donoghue & Herkenham, 1986; Künzle, 1975; Gerfen, 1984; Oka, 1980; Parthasarathy et al., 1992; Ragsdale & Graybiel, 1990). The striatum is also referred as the "input" nucleus of the circuit and it sends gamma-Aminobutyric acid (GABA) projections to the output nuclei. The output nuclei include the internal portion of the Globus Pallidus (GPi) and the Substantia Nigra pars Reticulata (SNr). The output nuclei in turn project an inhibitory GABAergic signal to the thalamus. The thalamus then sends an excitatory signal back to the cortex, thus completing the loop (Alexander & Crutcher, 1990).

The basal ganglia circuitry is composed of a direct and indirect pathway that have opposite effects on cortical activation. The direct pathway sends projections directly from the striatum to the output nuclei to ultimately disinhibit the thalamus and increase excitation of the cortex. The indirect pathway, on the other hand, sends projections from the striatum to the external portion of the globus pallidus (GPe) and the subthalamic nucleus (STN) before reaching the output nuclei. This pathway increases the inhibitory input on thalamus, which ultimately results in reduced excitation of the cortex (DeLong, 1990).

Initially the basal ganglia was solely described as a motor loop receiving input from motor and pre-motor cortex. Later studies showed that virtually all areas of cortex, including sensory and association cortices, also provided input to the basal ganglia circuitry (see Alexander et al., 1986 for review) and that each cortical area projects to discrete corresponding areas of the striatum (Gerfen, 1984; Künzle, 1975; Berendse 1992; Donoghue & Herkenham, 1986). For example, association cortex projects to the associative striatal territory (Parent, 1990). This evidence adds a new level of complexity to the understanding of this circuitry as it suggests that there are multiple parallel basal ganglia loops. The different basal ganglia loops have been subdivided into three main categories based on their function: motor, associative, and limbic.

More recent views on the nature and role of parallel basal ganglia loops propose that although the loops are activated independently they can function in an integrative way (Koob, 2009). Interestingly, regardless of the nature of the function being modulated, all basal ganglia (cortico-striatal) loops comprise the previously described direct and indirect pathways.

Multiple studies have speculated that activation of the motor basal ganglia direct and indirect pathways modulates seemingly opposite behaviors such as initiation and termination of movement (Aceves et al., 2011; Grillner et al., 2005; DeLong, 1990). The idea that the direct and indirect pathways modulate opposite behaviors has also been proposed for similar parallel loops that regulate drug addiction behaviors such as sensitization and resiliency to psychostimulants (Ferguson et al., 2011; Ferguson et al., 2013).

Dopamine Selectively Activates the Direct and Indirect Pathways

A compelling observation is that dopaminergic input from the substantia nigra to the striatum can selectively excite or inhibit the direct and indirect pathways respectively. The striatum is mostly comprised of medium spiny neurons (MSNs) that express the two main DA receptor subtypes: DR1 and DR2. DR1-expressing MSNs constitute the striato-nigral or direct pathway, and DR2-expressing MSNs comprise the striato-pallidal or indirect pathway (Gerfen et al., 1990). DA is believed to function as a comparator that regulates the excitatory and inhibitory input from the basal ganglia circuitry by modulating the activation of each pathway (Groves, 1983). Disruption of the dopaminergic input from substantia nigra to striatum due to neuronal death is the underlying neuropathology for the motor symptoms observed in Parkinson's disease, including problems initiating movement (DeLong, 1990). Dopamine's ability to selectively regulate the direct and indirect pathways makes it a viable candidate to control initiation and termination of movement mediated by the basal ganglia.

Inconsistent Reports of Dopamine Release in the Striatum During Continuous Locomotion in Rats

It is assumed that DA is correlated with motor behavior, therefore many studies that explore dopamine activity within the basal ganglia have used continuous locomotion as a tool to evoke DA release. However increases in functional DA release are not always observed when solely using continuous locomotion as a manipulation. Therefore, most studies have to rely on a combination of locomotor behavior and pharmacological manipulations to elicit detectable changes in DA activity. Six studies that report changes in DA release during continuous locomotion are inconsistent with each other (see Table 1). Two of these studies used treadmill walking and four studies used the Rotarod test as a locomotor task during microdialysis testing to measure striatal DA activity. Out of the six studies, two studies report significant increases in DA release relative to baseline, two studies do not report the statistical results for DA release, and two studies reported no significant increases in DA release.

Table 1. Increases in DA release in rats during continuous locomotion measured by microdialysis.

Article	Behavioral Design	DA Increase in Striatum
Castañeda et al., 1990	Treadmill Walking	Not significant
Damsma et al., 1992	Rotating Wheel	Not significant
Andersson et al., 2006	Rotarod Test	Not reported
Andersson et al., 2010	Rotarod Test	Not reported
Hattori et al., 1994	Treadmill Walking	Significant*
Bergquist et al., 2003	Rotarod Test	Significant*

These studies provide inconclusive results on the relationship between striatal DA release and locomotor activity. Some authors argue that DA within the striatum is important for maintaining locomotion, and others speculate that DA plays a more specific role within

movement. For example, some components of motor execution proposed to be mediated by striatal DA are: voluntary vs. reflexive movement, initiation vs. termination, and disengagement vs. engagement. The objective of this study is to explore the role of DA within the basal ganglia to regulate initiation and termination of movement. This theory is based on the role DA has in modulating the direct and indirect pathways to ultimately excite or inhibit the motor cortex.

Hypothesis: DA within the Basal Ganglia is Important for Initiation/Termination of Behavior

Despite the well-established association of DA with movement, there is a lack of understanding about the fundamental role of the basal ganglia to produce movement and how DA modulates this circuitry. A number of theoretical models have explicitly conjectured that DA plays a role in the initiation and termination of movement by modulating the direct and indirect pathways of the basal ganglia but have not been empirically tested (Groves, 1983; Surmeier et al., 2009; Wickens, 2009).

Yamamoto et al. (1982) indirectly attempted to answer this question by looking at striatal catecholamine (dopamine) release in rats engaged in reinforced intermittent circling behavior. In this study, circling behavior was reinforced by giving sugar water as a reward. Dopamine neurons from the substantia nigra have been shown to fire in response to a reward or reward-associated stimulus (Schultz, 1998). Therefore this natural reinforcer was likely a confound in evaluating whether catecholamine increase was due to the reinforcing effects of sugar water, the repeated initiation/termination of intermittent circling behavior, or the motor component of circling behavior.

Later, Schallert and Hall (1988) explored more explicitly the role of dopamine in engagement and disengagement from movement using a hemiparkinsonian model in rats. Behavioral testing was conducted after a unilateral 6-hydroxydopamine (6-OHDA) lesion to look

at motor, sensory and disengagement behavior. They designed an experiment to determine whether rats disengaged from consummatory behavior in response to tactile stimulation. Normal rats will immediately stop drinking to pay attention to the vibrissal stimulation ("disengagement"). Rats with a hemiparkinsonian lesion did not disengage when the stimulus was presented on the contralateral side of the lesion. When stimulation was presented on the ipsilateral side of the lesion rats displayed normal disengagement. Interestingly, if rats were not engaged in drinking water they responded normally to the tactile stimulus on either side. This evidence suggests that inability of rats to disengage was not a simple sensorimotor deficit. Disengagement behavior can be decomposed in three basic steps: 1) initiation of the *primary (i.e., first)* behavior 2) termination of the primary behavior and, 3) initiation of a new behavior (Posner et al., 1984). Based on the claim that DA is important for modulating disengagement, each of these steps (or all of them) can potentially be modulated by dopamine as well. Hence, there are two key limitations to this study: 1) the lack of DA measures collected concurrently with disengagement behavior and 2) delineating DA's contribution to each step of disengagement.

Based on the current literature, in this study we specifically explore the role of DA within the basal ganglia in modulating initiation and termination of a *primary* behavior. ***Our global hypothesis is that dopamine's ability to regulate the balance between excitatory and inhibitory neostriatal outflow (Groves, 1983) modulates the basal ganglia to produce initiation and termination of voluntary movement. Our working hypothesis is that DA release increases during intermittent walking that requires repeated initiation and termination of movement compared to continuous locomotion.*** To test this hypothesis this we measured DA release in

vivo using microdialysis while rats walked in a continuous or discontinuous (repeated start/stop) fashion on a treadmill.

Methods

Subjects

A total of 9 Wistar rats (5 male and 4 female) were used for the study. Rats were housed in a temperature-controlled environment on a reversed 12:12 h light/dark cycle. During microdialysis testing rats were housed for up to four days in a designated behavioral testing room. Food and water were available ad-lib throughout the course of the study, except during the brief (4 hours) experimental procedures, when they were removed from their home cage. All rats weighed between 340 and 430 g at the time of testing. All experimental procedures were done in accordance with IACUC regulations and approval.

Overview of the Protocol

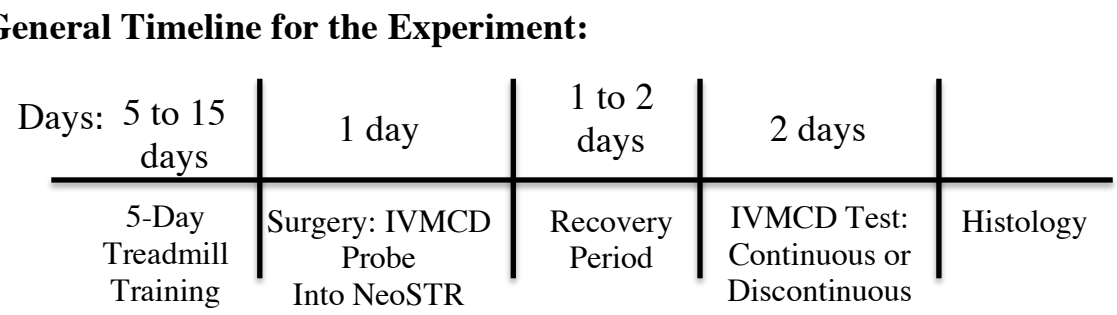

Figure 2. General Timeline for the Experiment

Rats were trained to walk on a treadmill until they were able to maintain a continuous steady pace for at least an hour. All rats were trained for a minimum of 5 days and a maximum of 15 days depending on individual performance. After rats met walking criteria (continuous walking for at least 1 hour at a minimum velocity of 7 m/min) they underwent stereotaxic surgery to implant a microdialysis probe unilaterally into the striatum. Rats recovered from surgery for one to two days before *in-vivo* microdialysis (IVMCD) testing. IVCMD testing was conducted for two consecutive days. Dialysate samples were collected using similar procedures across both

days: first, during baseline (a minimum of three 20-minute samples with <10% variability between each other); second, during treadmill walking for 1 hour (three 20-minute samples); and, third, only during the second day of testing, after amphetamine administration (one 20-minute sample). After testing was completed, rats were perfused using standard histological procedures and their brains were prepared to verify probe placement.

Experimental Design and Groups

A repeated measures design was employed with two factors, sample number (microdialysis sampling bins) and walking condition (continuous walking vs. discontinuous walking). All rats were tested for two consecutive days: continuous and discontinuous, one day they completed the continuous condition and on the other day the discontinuous. The order of the 2 conditions was counterbalanced across subjects to control for order effects.

Treadmill Training

On the first day of treadmill training, rats were handled for 5 minutes and then allowed to freely explore the treadmill for 20 minutes without any belt movement. Rats were then placed in individual lanes to initiate the training routine. Initial treadmill speed and duration was set at 5 m/min for 15 minutes for all rats. The speed on the treadmill and the duration of the training sessions was gradually increased according to individual performance for the following training days. Treadmill velocity during the training period never exceeded 15 m/min for any animal. Rats that met walking criteria (continuous walking for at least 1 hour at a minimum velocity of 7 m/min) before day 15 of training underwent stereotaxic surgery for microdialysis probe implant. A total of 9 rats that did not meet criteria after training day 15 were culled from the experiment. 2 rats successfully completed microdialysis testing but were removed from the overall data analysis due to incorrect probe placement at the ventricle, which resulted in null increases in

dopamine after a systemic injection of amphetamine given as a positive control for valid detection of extracellular DA.

Surgical Procedures - Dialysis Probe Implant

Within 3 days from the last training session, rats underwent stereotaxic surgery for unilateral microdialysis probe implant. Rats were anesthetized using the inhalational anesthetic Isoflurane. Using standard stereotaxic procedures (Castañeda et al., 1990), the microdialysis probe was implanted into the neostriatum in either the left or right hemisphere at the following coordinates: A + 0.5 mm, L +/-2.5 and V 7.0 mm from bregma (Paxinos & Watson, 1986). The animals were then placed into the test chamber and left overnight to recover for one or two days. Based on previous experience in our laboratory on time needed for rats to recover from surgery, we allowed rats that weighted less than 450g to recover for one day, and rats that weighed over 450 g to recover for two days. Ringers solution was pumped through the probe at 0.15 μl/min during this time. Testing began after the surgery recovery period; at least 18 h after probe implantation.

Construction of the Dialysis Probes and In Vitro Probe Recovery

Microdialysis probes with a 4 mm effective dialyzing length were built in accordance to Castañeda et al. (1989). In vitro testing prior to surgery was conducted to determine recovery values for DA, the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and the serotonin major metabolite 5-hydroxyindoleacetic acid (5-HIAA). Average recovery percentage for DA was 29.16 ± 0.78 , DOPAC 26.61 ± 1.47 , HVA 24.32 ± 0.76 , and 5-HIAA 24.66 ± 0.68.

In Vivo Microdialysis Procedures

During testing, pump speed was set at 1.5 µl/min 30 minutes prior to initiating baseline collections. A minimum of three consecutive 20-minute baseline samples that varied <10% from each other were collected. Samples were stored on ice for short periods, but all were assayed within 30 min of collection. After a stable baseline was collected, the animals were placed onto the conveyor belt where they walked for 1 h (continuously or discontinuously). Treadmill walking was defined as maintaining a steady but comfortable pace on the treadmill for a period of one hour. Rats walked uninterrupted during the continuous condition; during discontinuous walking rats walked for 30 sec with 15 sec rest intervals repeatedly throughout the one-hour phase. Time intervals for discontinuous walking were selected in an effort to optimize the repetitive pattern of initiating and terminating locomotion without disrupting overall walking behavior, which we found to be the case with shorter walk times in early phases of this study. During treadmill walking, three more 20-min samples were collected. At the end of testing day 1, animals were placed in the same holding environment and left there overnight. The same procedure from day 1 was repeated on the second testing using the other walking condition. At the end of the second test day, animals were returned to the holding boxes where they received an IP injection of 1.5 mg/kg of amphetamine (AMPH) to produce pharmacologically evoked DA overflow. Thus, one more 20-minute sample was collected after the AMPH injection as a positive control for working conditions of the animal preparation.

High Pressure Liquid Chromatography Coupled with Electrochemical Detection HPLC/EC

Reverse Phase Chromatography

HPLC methodology consists of two features for analysis, separation and detection, which are used to assay for brain DA and its major metabolites. A volume of 10-20 μl Dialysate was

injected into an HPLC system via a Rheodyne Model 7125 manual injector. The mobile-phase was optimized for DA detection.

Electrochemical Detection

A High Sensitivity Analytical Cell (ESA Model 5100A Coulochem Detector with a Model 5011 Analytical Cell) was used with the first electrode set to oxidize at +340 mV (500 nA sensitivity) and the second electrode was set to reduce at -340 mV (5 nA sensitivity). A Coulochem II detector (Model 5200A) was used to estimate unknown concentrations of analytes based on previously injected standards using linear regression analysis. The chromatographs were quantified using EZChrom SI software (Agilent Technologies).

Intracardial Perfusion and Histology

Within one week from the end of the experiment rats underwent standard intracardial perfusion to prepare the brain for histology and staining. After perfusion, 30 μm thick coronal slices of the brain were obtained 1 mm rostral and caudal of the implantation site. Brain slices were stained using cresyl violet and examined for accurate placement of the microdialysis probe with a projecting microscope. Figure 3 shows the placement of microdialysis probes in 7 out of the 9 animals that participated in the study. The two brains not shown were lost due to technical difficulties before brains could be harvested for histological examination.

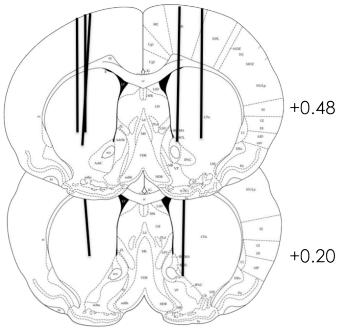

Figure 3. Histological analyses revealed that microdialysis probes were implanted within the striatum for six of the animals in which histology was conducted.

Data Analysis

Differences in baseline levels across conditions were explored using a two-way repeated measures ANOVA with two within-subjects factors: walking condition and baseline sample number. Second, a two-way

ANOVA with two within-subject factors: walking condition and DA sample during treadmill walking (mean baseline, 20-min, 40-min, 60-min) was conducted to assess changes in extracellular DA levels from baseline to treadmill walking. Post-hoc analyses were conducted using Sidak-Bonferroni test. The same statistical procedures were used to assess differences in baseline levels and changes during treadmill walking for the DA metabolites DOPAC and HVA, as well as the serotonin metabolite 5-HIAA.

A paired-samples t-test was conducted to analyze DA levels as a function of active walking time by comparing dialysate levels of DA based on the absolute time rats spent walking in both conditions. To test this, the average was calculated for the three samples collected during treadmill running for both the continuous and discontinuous test phases. Next, two thirds of the DA levels in the continuous condition were calculated to predict proportionate levels of 40 minutes of active walking, the time spent walking in the discontinuous phase. A paired-samples t-test was conducted to compare extracellular DA levels during active walking in the Discontinuous phase versus 60% of that calculated from the Continuous condition.

Results

Extracellular Dopamine Levels During Continuous and Discontinuous Treadmill Walking.

Figure 3 illustrates extracellular DA estimated by microdialysis during baseline and treadmill walking. First, the three baseline samples of DA collected just before the two conditions of treadmill walking were compared. There were no significant differences in basal levels between continuous and discontinuous walking [$F(1,8)=0.007$; p=ns]. There was a significant main effect of repeated sampling across the three 20-min dialysate samples collected during baseline [$F(2,16)=4.208$; $p<0.05$]; but pairwise post-hoc analyses across time points revealed no significant differences. There was no interaction across baseline sampling and walking condition [$F(2,16)=0.5222$; p=ns].Therefore, all three baseline samples were collapsed into a baseline average for continuous (21.05 ± 3.31 fmol/min) and discontinuous (20.80 ± 2.59 fmol/min) walking for use in subsequent analyses comparing baseline levels of extracellular DA against samples taken during walking.

A repeated measures two-way ANOVA was used to analyze the effects of treadmill walking on DA levels. No significant differences in extracellular DA were found between continuous vs. discontinuous treadmill walking [$F(1,8)=0.001$; p=ns]. A main effect over time was found for DA [$F(3,24)=21.15$; $p<0.001$]. Subsequent post-hoc analyses revealed significant differences from baseline vs. 20-, 40- and 60-min ($p<0.05$). The interaction between walking condition and repeated measures of extracellular DA was not significant [$F(3,24)=0.606$; p=ns].

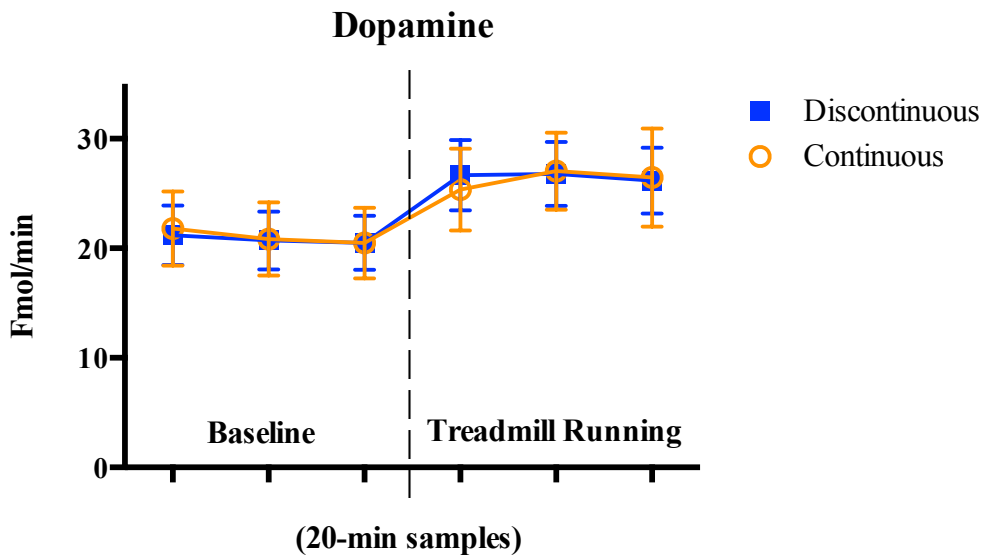

Figure 4. Extracellular DA levels during baseline and both treadmill walking conditions. There was a significant increase in extracellular DA across all samples collected during treadmill walking compared to the mean (±SEM) of baseline ($p<0.05$).

In an attempt to understand DA activity as a function of active walking duration, a separate t-test was conducted taking into account the overall walking time of each phase. While the total testing period was the same across both conditions (60 minutes), the ***total active walking*** time during continuous walking was 60 minutes but only 40 minutes during discontinuous walking. Therefore Figure 5 illustrates extracellular DA levels during discontinuous compared to two thirds of the continuous condition. There was a significant difference in the scores for discontinuous ($M=26.550$, $SD=9.020$) and continuous ($M=17.358$, $SD=7.627$) conditions; paired $t(8)=3.289$, $p < 0.05$.

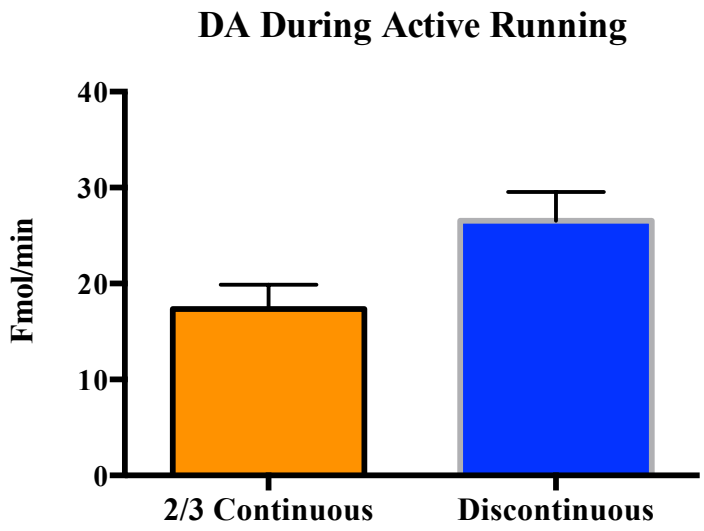

Figure 5. DA during active treadmill walking. When dialysate levels of DA were adjusted in the Continuous condition to factor out differences in the amount of walking compared to the Discontinuous condition, there was a significantly higher amount of DA overflow when repetitive initiation and termination of walking was present. ($p<0.05$)

Extracellular DOPAC Levels During Continuous and Discontinuous Treadmill Walking.

Figure 4 illustrates extracellular DOPAC estimated by microdialysis during baseline and treadmill walking. First, the three baseline samples of DOPAC collected just before the two conditions of treadmill walking were compared. There were no significant differences in basal levels between continuous and discontinuous walking [$F(1,8)=0.009$; p=ns]. No significant differences were found within repeated sampling across the three 20-min dialysate samples collected during baseline [$F(2,16)=0.087$; p=ns].There was no interaction across baseline sampling and walking condition [$F(2,16)=1.184$; p=ns].Therefore, all three baseline samples were collapsed into a baseline average for continuous (3984.97 ± 504.39 fmol/min) and discontinuous (4203.89 ± 511.53 fmol/min) walking for use in subsequent analyses comparing baseline levels of extracellular DA against samples taken during walking.

A repeated measures two-way ANOVA was used to analyze the effects of treadmill walking on DOPAC levels. No significant differences in extracellular DOPAC were found between continuous vs. discontinuous treadmill walking [$F(1,8)=0.027$; p=ns]. A main effect

over time was found for DOPAC [F(3,24)=31.83; p<0.001]. Subsequent post-hoc analyses revealed significant differences from baseline vs. 20-, 40- and 60-min (p<0.05). The interaction between walking condition and repeated measures of DOPAC was not significant [F(3,24)=1.060; p=ns].

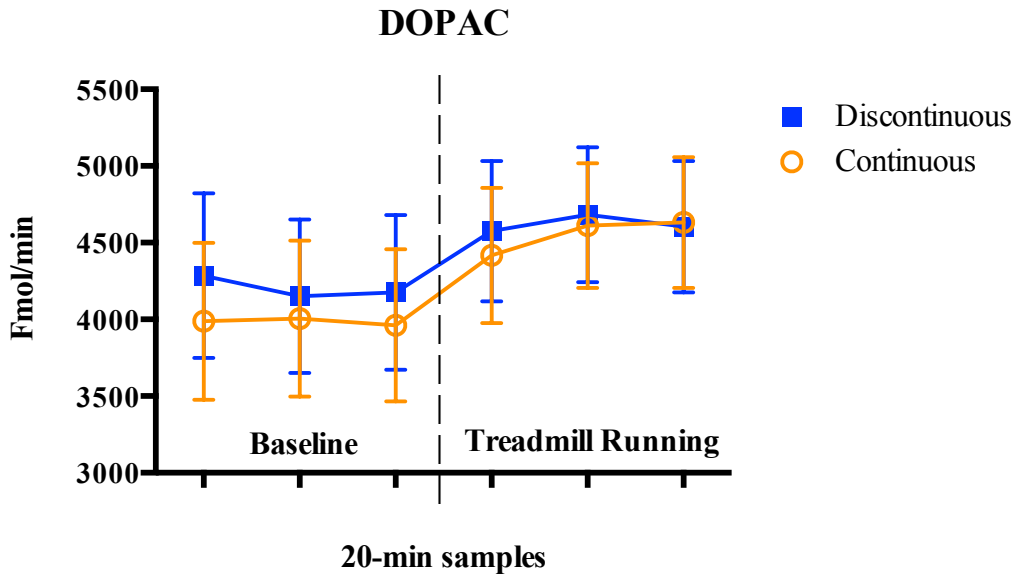

Figure 6. Extracellular DOPAC levels during baseline and both treadmill walking conditions. There was a significant increase in extracellular DOPAC across all samples collected during treadmill walking compared to the mean (±SEM) of baseline (p<0.05).

Extracellular HVA Levels During Continuous and Discontinuous Treadmill Walking

Figure 5 illustrates extracellular HVA estimated by microdialysis during baseline and treadmill walking. First, the three baseline samples of HVA collected just before the two conditions of treadmill walking were compared. There were no significant differences in basal levels between continuous and discontinuous walking [F(1,9)=0.209; p=ns]. No significant differences were found within repeated sampling across the three 20-min dialysate samples collected during baseline [F(2,16)=0.924; p=ns]. There was no interaction across baseline sampling and walking condition [F(2,16)=1.213; p=ns]. Therefore, all three baseline samples were collapsed into a baseline average for continuous (3197.43 ± 606.10 fmol/min) and

discontinuous (3665.95 ± 798.13 fmol/min) walking for use in subsequent analyses comparing baseline levels of extracellular DA against samples taken during walking.

A repeated measures two-way ANOVA was used to analyze the effects of treadmill walking on HVA levels. No significant differences in extracellular HVA were found between continuous vs. discontinuous treadmill walking [$F(1,8)=0.243$; p=ns]. A main effect over time was found for HVA [$F(3,24)=23.58$; $p<0.001$]. During discontinuous walking, subsequent post-hoc analysis revealed that significant differences from baseline vs. 20-min, 40-min and 60-min ($p<0.05$). During continuous walking, post-hoc analysis revealed significant differences between baseline vs. 40-min, 60-min during continuous walking ($p<0.05$). The interaction between walking condition and repeated measures of HVA was not significant [$F(3,24)=0.335$; p=ns].

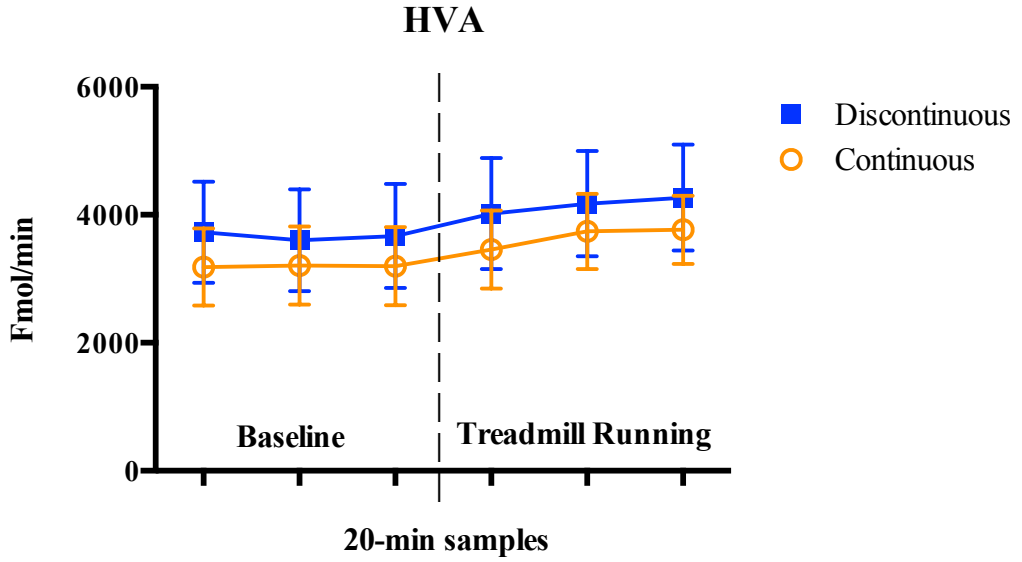

Figure 7. Extracellular HVA levels during baseline and both treadmill walking conditions. There was a significant increase in extracellular HVA across during the 40- and 60-min samples collected during treadmill walking for both conditions compared to the mean (±SEM) of baseline ($p<0.05$).

Extracellular 5-HIAA Levels During Continuous and Discontinuous Treadmill Walking.

Figure 6 illustrates extracellular 5-HIAA estimated by microdialysis during baseline and treadmill walking. First, the three baseline samples of 5-HIAA collected just before the two conditions of treadmill walking were compared. There were no significant differences in basal levels between continuous and discontinuous walking [$F(1,8)=0.492$; p=ns]. No significant differences were found within repeated sampling across the three 20-min dialysate samples collected during baseline [$F(2,16)=0.069$; p=ns]. There was no interaction across baseline sampling and walking condition [$F(2,16)=3.425$; p=ns]. Therefore, all three baseline samples were collapsed into a baseline average for continuous (1568.73 ± 65.93 fmol/min) and discontinuous (1713.43 ± 151.32 fmol/min) walking for use in subsequent analyses comparing baseline levels of extracellular DA against samples taken during walking.

A repeated measures two-way ANOVA was used to analyze the effects of treadmill walking on 5-HIAA levels. No significant differences in extracellular 5-HIAA were found between continuous vs. discontinuous treadmill walking [$F(1,8)=0.322$; p=ns]. A main effect over time was found for 5-HIAA [$F(3,24)=15.48$; $p<0.001$]. Subsequent post-hoc analyses revealed significant differences from baseline vs. 20-, 40- and 60-min ($p<0.05$). The interaction between walking condition and repeated measures of 5-HIAA was not significant [$F(3,24)=0.258$; p=ns].

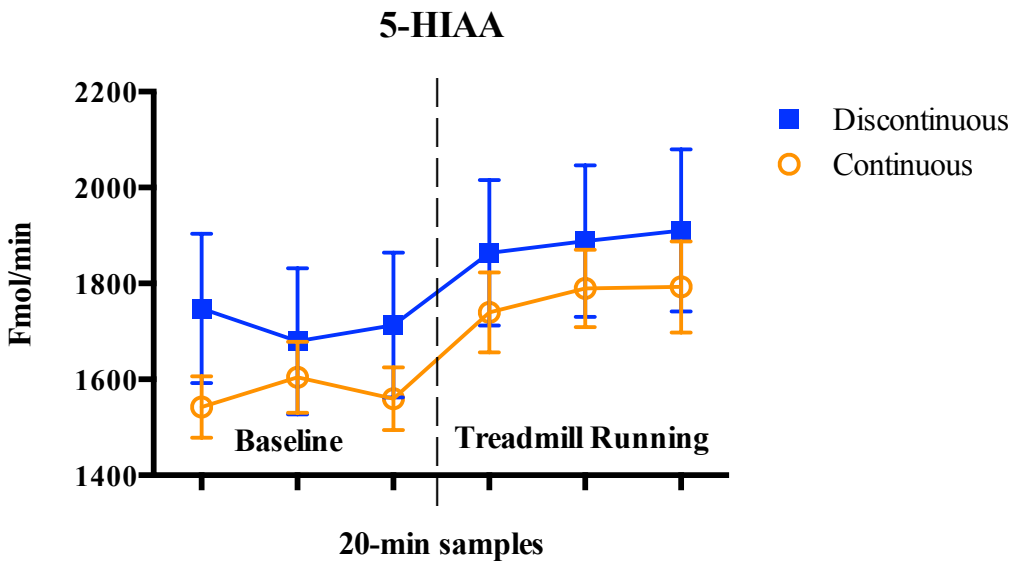

Figure 8. Extracellular DA levels during baseline and both treadmill walking conditions. There was a significant increase in extracellular DA across all samples collected during treadmill walking compared to the mean (±SEM) of baseline ($p<0.05$).

Discussion

The major finding of this report is that significant increases in extracellular DA levels related to treadmill walking can be measured using IVMCD. The initial hypothesis that a pattern of striatal DA overflow could be related to initiation and termination of movement was not supported. Specifically, it was predicted that greater DA utilization, reflected as increases in DA from microdialysate samples, would be observed during discontinuous walking compared to continuous walking. However, the present results found no significant difference in DA release between continuous vs. discontinuous walking. This study is significant because it provides a profile of neurochemical evidence that supports the idea that exocytotic mechanisms can be detected using IVMCD without reliance upon pharmacological manipulations. Similar to DA, its major metabolites DOPAC and HVA, and the serotonin metabolite 5-HIAA, increased during treadmill walking from resting state levels. The concomitant increases in extracellular DA and its metabolites suggest that IVMCD may be able to capture dynamic changes in presynaptic mechanisms such as release and reuptake in response to behavioral activation. There is a preponderance of evidence for a modulatory role of DA in movement and the reasons why such a function was not observed are discussed. A clear understanding for the role of DA in movement production is crucial for the development of biomedical interventions involving basal ganglia disease.

Role of DA in movement initiation

In humans, problems with initiation of movement are a disabling motor deficit typically seen in Parkinson's disease. Deficits in initiation and termination of movement can particularly be seen in gait disorders such as festination and freezing of gait. Surprisingly, these disabling

gait disorders still remain poorly understood (Iansek et al., 2006). The idea that DA is important for initiation of movement, however, is inconsistent throughout the literature.

If, in fact, the motor loop of the basal ganglia is important for initiation and termination of movement, then the present data suggest that a more complex experimental design is required to capture this function. The present findings are compelling that DA is involved in sustaining movement rather than in initiation or termination of movement. However, the literature about the functional nature of striatal DA in voluntary movement has been controversial for several decades (DeLong, 1990; Middleton and Strick, 2000; Takakusaki et al., 2004; Grillner et al., 2005; Aceves et al., 2011). Virtually all studies that have assessed this problem have done so by depleting striatal DA in combination with a battery of sensory, motor and cognitive tests to dissect the behaviors mediated by striatal DA neurons (Schallert & Hall, 1988; DeLong, 1990; Damsma et al., 1992; Cousins & Salamone, 1996; Brasted et al., 1997; Bromberg-Martin et al., 2010). In the unilateral 6-OHDA model, rats have been shown to display delayed initiation of movement on the contralateral side of the lesion in conditioned circling behavior (Dunnett & Bjorklund, 1983) in a bar-pressing paradigm (Cousins & Salamone, 1996), and in a nose-hole poke choice response task (Brasted et al., 1997). In contrast, movement execution remains intact once the behavior has been initiated. These movement initiation deficits are not simply due to sensorimotor neglect. Spirduso et al. (1985) found that rats with much smaller (11-24%) striatal DA lesions that spare sensorimotor ability still demonstrated movement initiation deficits in a reactive capacity task which requires rapid initiation of high speed movements. Therefore, dopamine seems likely to be important for initiation of movement.

Understanding the exact relationship between DA and movement remains a complex task given that in contrast, a number of studies have found support for the competing hypothesis that

DA is necessary for sustaining movement but not for initiating it (Döbrössy & Dunnet, 1997; Brasted et al., 1998). During a lateralized choice reaction task, overall movement execution (total time to respond) to a contralateral stimulus was increased but not reaction time (time to initiate movement). Conversely, when looking at DA activity as a function of continuous locomotion not every study finds significant increases in DA release as predicted if DA was critical for movement execution (Castañeda et al., 1990; Damsma et al., 1992). Clearly, more work needs to be done to understand the role of basal ganglia DA in mediating voluntary behavior. However, the ability to delineate such a function is likely to be limited because the technology for measuring DA and the kinematic analyses of behavior have not converged sufficiently to illuminate these complex relationships.

A significant challenge in this line of investigation is the operational distinction between initiation versus execution of movement. These concepts might be confounded by the use of different behavioral tasks in different studies. For example, when rats are placed in two conceptually similar reaction time tasks they display initiation deficits in a nose-poke paradigm but execution deficits in a bar-pressing task following unilateral DA depletion (Brasted, et al., 1998). These paradoxical results exemplify the contradictory evidence that mottles the literature about the role of DA in initiation and execution of movement. The challenge in the study of voluntary movement is that even within continuous locomotion there are smaller movement units performed in a chain sequence that constitute the "continuous" gait pattern (Takakusaki, 2013; Whishaw et al., 2010). Each of these units could be considered to have an initiation and termination cycle, creating a problem when trying to define which unit constitutes initiation versus execution of movement. To avoid the confounds created by complex behavioral actions, such as respondent behavior reliant upon sensorimotor integration and reinforcement effects, the

present study utilized a simple treadmill walking paradigm in a within-subjects experimental design. The only difference in the motor behavior between the two experimental manipulations was the repeated initiation and termination of treadmill walking in the discontinuous compared to the continuous condition.

Studies have found support for both the ideas that DA is important for initiating/terminating versus sustaining movement (Dunnett & Bjorklund, 1983; Döbrössy & Dunnet, 1997; Brasted, et al., 1998). In the present study, if striatal DA was important only for maintenance of locomotion then, lower DA levels would have been predicted during discontinuous walking compared to continuous locomotion when comparisons were made inclusive of the entire 60-min test period. In this way, the present data suggest striatal DA plays an important function for sustaining locomotion. There was a consistent and similar increase in extracellular DA levels during continuous versus discontinuous test phases. However, it is important to consider that during discontinuous walking rats repeatedly engaged in initiation and termination of movement unlike the sustained execution that took place during continuous walking. In other words, during the discontinuous phase, rats walked for only two-thirds, or 40 min, the amount of time as in the continuous phase, which was 60 min. Quite significantly, when differences in the absolute amount of time spent walking was adjusted in the measures of extracellular DA, these results strongly suggest that repetitive initiation and termination of locomotion produced significantly higher levels of DA overflow.

Therefore, viewed in this way, the corrected results support the hypothesis that DA is important for initiation and termination of movement. The present study provide compelling data, for the first time, that it will be important for future studies to design experiments that are adequately elegant to dissociate these two components of locomotion, its initiation and

termination compared to its sustained execution. In addition, it will be important to determine how the present findings generalize to other basal ganglia-mediated behaviors other than locomotion, especially as regards understanding the nature of behavioral decomposition during motor disorders.

The Behavioral Function of the Basal Ganglia is Not Simple

A compelling observation from previous studies is that many impart a reward component to evoke motor behavior. This poses a critical confound for testing the motor function of DA given that positive reinforcement has been extensively shown to increase DA activity (Schultz, 1998; Berridge & Robinson, 1998; Wise, 2005). However, DA within the limbic reward system has also been shown to be a system more complex than originally thought that has the capacity to signal "reward prediction error" and not just reward-paired cues (Schultz et al., 1997). This problem has been captured mainly by Dowd and Dunnett (2007) who have tried to understand the interaction of motor (habit) and limbic (reward) behavior when interpreting motor deficits in the 6-OHDA model. They propose that the movement deficits observed in the hemiparkinsonian rat model are derived from the loss of the dopaminergic rewarding signal associated with the task rather than deficits in motor abilities. The idea that DA is important for learning a motivated behavior has been proposed repeatedly throughout the literature (see Bromberg-Martin et al., 2010 for a review). Anatomical studies showing that the striatum is segregated into striosomes and matrisomes also support the idea that DA integrates movement and reward (Johnston et al., 1990). The striosomes receive inputs from limbic systems, while the matrisomes receive input from motor and premotor cortex (Haber, 2003). Although the idea proposed by Dowd and Dunnett (2007) that disruption of the dopaminergic rewarding signal is partially responsible for the loss of conditioned response after a unilateral 6-OHDA lesion is a compelling hypothesis,

other studies suggest otherwise. For example, using a mathematical model of operant conditioning that accounts for motor capacity, motivation, and short term memory (Mathematical Principles of Reinforcement; Killen, 1994), Avila et al. (2008) investigated the effects of 6-hydroxydopamine-induced DA depletions on break point performance. These authors concluded that a disruption in motor behavior, rather than upon motivation, played a significant role in performance deficits.

The present study attempted to mitigate the influence of a reinforcement factor by training animals to walk on a treadmill without a positive reinforcer (e.g., food reward). This does not completely eliminate the possibility of a negative reinforcer such as the discomfort of standing on a moving treadmill belt. However, negative reinforcers have been more frequently associated with decreases in DA levels (Mark et al., 1991; Lui et al., 2008; Roitman et al., 2008) rather than increases. How such a factor might influence the present results is not known.

An important aspect to consider about the experimental design of this study is whether the integration of different sensory and motor systems needed to produce normal gait pattern and modulate locomotion may mask any measurable changes in DA activation during initiation and termination of treadmill walking. Locomotor behavior involves sequential activation of different muscle groups needed for postural control (cortical and brain steam-derived pathways), synchronization based on proprioceptive and kinesthetic feedback (somatosensory, cerebellum) and intersegmental muscle coordination (spinal cord). Central pattern generators at brainstem and spinal cord levels are likely to coordinate gait rhythm that does not require input from higher motor systems and may decrease the need for basal ganglia modulation. It is beyond the scope of the present project to speculate about this complex integration. However, the present study does show a persistent increase of extracellular DA during treadmill walking. It is therefore

reasonable to speculate that future studies can use treadmill walking in combination with pharmacological manipulations targeting DA-modulating mechanisms to delve into the exact role of DA in locomotion.

Reliable Methodology to Measure DA Release.

One concern in the present study is the relatively long collection period necessary to collect microdialysate samples that limits temporal resolution by IVMCD. It is clear that a subsecond grain of analysis will be useful to understand the moment-by-moment utilization of DA needed to initiate movement in future studies. Future studies that examine the role of DA in the initiation or execution of movement will have to consider modifications in procedural or technical requirements. A possible solution to capture DA increase during initiation and termination of movement using microdialysis is to increase the frequency at which rats start/stop walking on the treadmill. A second alternative to consider for future studies is to use chronoamperometry, which allows readings of DA activity in milliseconds and might provide a more succinct profile of DA activity during initiation versus execution.

Despite the inconsistent literature reporting whether DA increases or not with continuous locomotion, the present study has demonstrated a reliable method to evoke functional DA release that does not rely on pharmacological agents. The significant contribution of the present study is to communicate that physiologically, rather than pharmacologically, induced changes can be observed with IVMCD. This method will serve as a tool to address DA-related questions in the future.

This study was important to conduct because a substantial number of studies use pharmacological manipulations to stimulate neurotransmitter overflow. However, pharmacologically induced DA overflow usually depends on mechanisms different than those

used for exocytosis. For example, amphetamine is a drug commonly used to produce DA overflow. Unfortunately, the mechanism of action by which amphetamine causes DA overflow involves reversing the function of the dopamine transporter (DAT), not exocytotic mechanisms that involve depolarizing inputs that yield calcium-dependent release of DA from storage vesicles. Interestingly, while amphetamine administration results in DA increase, the DA metabolites decrease since DA cannot undergo reuptake into the cell where the majority of neurotransmitter is degraded (Castañeda et al., 1990). In the present treadmill-walking study, the DA metabolites DOPAC and HVA levels increased, supporting the idea that increases in DA release should be followed by activation of downstream presynaptic mechanisms of reuptake and enzymatic degradation. Therefore, analyzing patterns of DA activity during neurologically relevant behavioral activity must include measures of DA metabolites to more fully understand how presynaptic mechanisms govern exocytosis.

Finally, this study also reports that the levels of the serotonin metabolite 5-HIAA significantly increased with treadmill walking. This finding supports the idea that DA as a lone mediator may be too simple for control of the initiation and termination of movement. Future studies may do well to expand the evaluation of other neurotransmitter systems within the basal ganglia, including serotonin.

Overall, the present work was able to demonstrate a reliable and consistent increase in functional DA release as a consequence of motor behavior without the potential confound derived from introducing a reward component. This study establishes a novel finding for the concurrent importance of DA in mediating initiation and termination, as well as sustained execution of locomotor behavior. Utilizing this technique will also allow the examination of DA activation in an exocytotic manner – rather than depending upon the prevalent use of drugs to

pharmacologically stimulate DA "release". For example, this technique will be useful to answer future questions about *functional* changes in DA release in models of Parkinson's disease and drug addiction.

References

Aceves, J. J., Rueda-Orozco, P. E., Hernandez-Martinez, R., Galarraga, E., & Bargas, J. (2011). Bidirectional plasticity in striatonigral synapses: a switch to balance direct and indirect basal ganglia pathways. *Learning & Memory*, *18*(12), 764-773.

Alexander, G. E., & Crutcher, M. D. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends in neurosciences*, *13*(7), 266-271.

Alexander, G. E., DeLong, M. R., & Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual review of neuroscience*, *9*(1), 357-381.

Andersson, D. R., Nissbrandt, H., & Bergquist, F. (2006). Partial depletion of dopamine in substantia nigra impairs motor performance without altering striatal dopamine neurotransmission. *European Journal of Neuroscience*, 24(2), 617-624.

Andersson, D. R., Björnsson, E., Bergquist, F., & Nissbrandt, H. (2010). Motor activity-induced dopamine release in the substantia nigra is regulated by muscarinic receptors. *Experimental neurology*, 221(1), 251-259.

Avila, I., Reilly, M. P., Sanabria, F., Posadas-Sánchez, D., Chavez, C. L., Banerjee, N., ... & Castaneda, E. (2009). Modeling operant behavior in the Parkinsonian rat. *Behavioural brain research*, *198*(2), 298-305.

Bard, P., & Macht, M. B. (1958). The behaviour of chronically decerebrate cats. In *Ciba Foundation Symposium-Neurological Basis of Behaviour*, 55-75.

Bazett, H. C., & Penfield, W. G. (1922). A Study of the Sherrington Decerebrate Animal in the Chronic as well as the Acute Condition. *Brain: A Journal of Neurology*.

Benloucif, S., & Galloway, M. P. (1991). Facilitation of dopamine release in vivo by serotonin agonists: studies with microdialysis. Eur J Pharmacol 200, 1–8.

Berendse, H. W., Graaf, Y. G. D., & Groenewegen, H. J. (1992). Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *Journal of Comparative Neurology*, *316*(3), 314-347.

Bergquist, F., Shahabi, H. N., & Nissbrandt, H. (2003). Somatodendritic dopamine release in rat substantia nigra influences motor performance on the accelerating rod. *Brain research,* 973(1), 81-91.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?. *Brain Research Reviews*, *28*(3), 309-369.

Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., & Greenamyre, J. T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature neuroscience*, *3*(12), 1301-1306.

Bignall, K. E., & Schramm, L. (1974). Behavior of chronically decerebrated kittens. *Experimental neurology*, *42*(3), 519-531.

Bjursten, L. M., Norrsell, K., & Norrsell, U. (1976). Behavioural repertory of cats without cerebral cortex from infancy. *Experimental brain research*, 25(2), 115-130.

Brasted, P. J., Humby, T., Dunnett, S. B., & Robbins, T. W. (1997). Unilateral lesions of the dorsal striatum in rats disrupt responding in egocentric space.*The Journal of neuroscience*, *17*(22), 8919-8926.

Brasted, P. J., Döbrössy, M. D., Robbins, T. W., & Dunnett, S. B. (1998). Striatal lesions produce distinctive impairments in reaction time performance in two different operant chambers. *Brain research bulletin*, *46*(6), 487-493.

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron*, *68*(5), 815-834.

Castañeda, E., Whishaw, I. Q., Lermer, L., Robinson T. E., (1990). Dopamine depletion in neonatal rats: effects on behavior and striatal dopamine release assessed by intracerebral microdialysis during adulthood. *Brain Research*, 508(1), 30-39.

Catsman-Berrevoets, C. E., & Kuypers, H. G. J. M. (1976). Cells of origin of cortical projections to dorsal column nuclei, spinal cord and bulbar medial reticular formation in the rhesus monkey. *Neuroscience letters*, 3(5), 245-252.

Cousins, M. S., & Salamone, J. D. (1996). Involvement of ventrolateral striatal dopamine in movement initiation and execution: a microdialysis and behavioral investigation. *Neuroscience*, *70*(4), 849-859.

Damsma, G., Pfaus, J. G., Wenkstern, D., Phillips, A. G., & Fibiger, H. C. (1992). Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion. *Behavioral neuroscience*, *106*(1), 181.

DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends in neurosciences*, 13(7), 281-285.

Döbrössy, M. D., & Dunnett, S. B. (1997). Unilateral striatal lesions impair response execution on a lateralised choice reaction time task. *Behavioural brain research*, *87*(2), 159-171.

Donoghue, J. P., & Herkenham, M. (1986). Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. *Brain research*, *365*(2), 397-403.

Dowd, E., & Dunnett, S. B. (2007). Movement without dopamine: striatal dopamine is required to maintain but not to perform learned actions.*Biochemical Society Transactions*, *35*(2), 428-432.

Dum, R. P., & Strick, P. L. (1991). The origin of corticospinal projections from the premotor areas in the frontal lobe. *The Journal of neuroscience*, *11*(3), 667-689.

Dunnett, S. B., & Björklund, A. (1983). Conditioned turning in rats: dopaminergic involvement in the initiation of movement rather than the movement itself.*Neuroscience letters*, *41*(1), 173-178.

Duysens, J., & Van de Crommert, H. W. (1998). Neural control of locomotion; Part 1: The central pattern generator from cats to humans. *Gait & posture*, *7*(2), 131-141.

Ferguson, S. M., Eskenazi, D., Ishikawa, M., Wanat, M. J., Phillips, P. E., Dong, Y., ... & Neumaier, J. F. (2011). Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nature neuroscience*,*14*(1), 22-24.

Ferguson, S. M., Phillips, P. E., Roth, B. L., Wess, J., & Neumaier, J. F. (2013). Direct-pathway striatal neurons regulate the retention of decision-making strategies. *The Journal of Neuroscience*, *33*(28), 11668-11676.

Gao, J. H., Parsons, L. M., Bower, J. M., Xiong, J., Li, J., & Fox, P. T. (1996). Cerebellum implicated in sensory acquisition and discrimination rather than motor control. *Science*, *272*(5261), 545-547.

Gerfen, C. R., (1984). The Neostriatal Mosaic: Compartmentalization of Corticostriatal Input and Striatonigral Output Systems. *Nature,* 311, (5985), 461–64.

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., & Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*(4986), 1429-1432.

Grillner, S., Hellgren, J., Menard, A., Saitoh, K., & Wikström, M. A. (2005). Mechanisms for selection of basic motor programs–roles for the striatum and pallidum. *Trends in neurosciences*, *28*(7), 364-370.

Goldman, S. M., Tanner, C. M., Oakes, D., Bhudhikanok, G. S., Gupta, A., & Langston, J. W. (2006). Head injury and Parkinson's disease risk in twins. *Annals of neurology*, *60*(1), 65-72.

Groves, P. M. (1983). A theory of the functional organization of the neostriatum and the neostriatal control of voluntary movement. *Brain Research Reviews,* 5(2), 109-132.

Haber, S. N. (2003). The primate basal ganglia: parallel and integrative networks. *Journal of chemical neuroanatomy*, *26*(4), 317-330.

Hall, S., & Schallert, T. (1988). Striatal dopamine and the interface between orienting and ingestive functions. *Physiology & behavior*, 44(4), 469-471.

Hattori, S., Naoi, M., & Nishino, H. (1994). Striatal dopamine turnover during treadmill running in the rat: relation to the speed of running. *Brain research bulletin*, *35*(1), 41-49.

Iansek, R., Huxham, F., & McGinley, J. (2006). The sequence effect and gait festination in Parkinson disease: contributors to freezing of gait?. *Movement Disorders*, *21*(9), 1419-1424.

Johnston, J. G., Gerfen, C. R., Haber, S. N., & van der Kooy, D. (1990). Mechanisms of striatal pattern formation: conservation of mammalian compartmentalization. *Developmental Brain Research*, *57*(1), 93-102.

Jueptner, M., & Weiller, C. (1998). A review of differences between basal ganglia and cerebellar control of movements as revealed by functional imaging studies. *Brain*, *121*(8), 1437-1449.

Kamiyama, T., Kameda, H., Murabe, N., Fukuda, S., Yoshioka, N., Mizukami, H., ... & Sakurai, M. (2015). Corticospinal Tract Development and Spinal Cord Innervation Differ between Cervical and Lumbar Targets. *The Journal of Neuroscience*, *35*(3), 1181-1191.

Killeen, P. R. (1994). Mathematical principles of reinforcement. *Behavioral and Brain Sciences*, *17*(01), 105-135.

Kish, S. J., Shannak, K., Rajput, A., Deck, J. H., & Hornykiewicz, O. (1992). Aging produces a specific pattern of striatal dopamine loss: implications for the etiology of idiopathic Parkinson's disease. *Journal of neurochemistry*, 58(2), 642-648.

Koob, G. F. (2009). Dynamics of neuronal circuits in addiction: reward, antireward, and emotional memory. *Pharmacopsychiatry*, 42(Suppl 1), S32.

Künzle, H. (1975). Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study inMacaca fascicularis. *Brain research*, *88*(2), 195-209.

Liu, Z. H., Shin, R., & Ikemoto, S. (2008). Dual role of medial A10 dopamine neurons in affective encoding. *Neuropsychopharmacology*, *33*(12), 3010-3020.

Mark, G. P., Blander, D. S., & Hoebel, B. G. (1991). A conditioned stimulus decreases extracellular dopamine in the nucleus accumbens after the development of a learned taste aversion. *Brain research*, *551*(1), 308-310.

Middleton, F. A., & Strick, P. L. (2000). Basal ganglia and cerebellar loops: motor and cognitive circuits. *Brain Research Reviews*, *31*(2), 236-250.

Oka, H. (1980). Organization of the cortico-caudate projections. *Experimental brain research*, *40*(2), 203-208.

Parent, A. (1990). Extrinsic connections of the basal ganglia. *Trends in neurosciences*, *13*(7), 254-258.

Parthasarathy, H. B., Schall, J. D., & Graybiel, A. M. (1992). Distributed but convergent ordering of corticostriatal projections: analysis of the frontal eye field and the supplementary eye field in the macaque monkey. *The Journal of neuroscience*, *12*(11), 4468-4488.

Posner, M. I., Walker, J. A., Friedrich, F. J., & Rafal, R. D. (1984). Effects of parietal injury on covert orienting of attention. *The Journal of Neuroscience*,*4*(7), 1863-1874.

Priyadarshi, A., Khuder, S. A., Schaub, E. A., & Shrivastava, S. (2000). A meta-analysis of Parkinson's disease and exposure to pesticides. *Neurotoxicology*,*21*(4), 435-440.

Ragsdale, C. W., & Graybiel, A. M. (1990). A simple ordering of neocortical areas established by the compartmental organization of their striatal projections. *Proceedings of the National Academy of Sciences*, *87*(16), 6196-6199.

Roitman, M. F., Wheeler, R. A., Wightman, R. M., & Carelli, R. M. (2008). Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. *Nature neuroscience*, *11*(12), 1376-1377.

Schallert, T., & Hall, S. (1988). 'Disengage'sensorimotor deficit following apparent recovery from unilateral dopamine depletion. *Behavioural brain research*, *30*(1), 15-24.

Semchuk, K. M., Love, E. J., & Lee, R. G. (1993). Parkinson's disease A test of the multifactorial etiologic hypothesis. *Neurology*, 43(6), 1173-1173.

Schultz, W., Dayan, P., & Montague, P. R. (1997). A neural substrate of prediction and reward. *Science*, *275*(5306), 1593-1599.

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of neurophysiology*, *80*(1), 1-27.

Spirduso, W. W., Gilliam, P. E., Schallert, T., Upchurch, M., Vaughn, D. M., & Wilcox, R. E. (1985). Reactive capacity: a sensitive behavioral marker of movement initiation and nigrostriatal dopamine function. *Brain research*, *335*(1), 45-54.

Surmeier, D. J., Plotkin, J., & Shen, W. (2009). Dopamine and synaptic plasticity in dorsal striatal circuits controlling action selection. *Current opinion in neurobiology*, *19*(6), 621-628.

Takakusaki, K., Saitoh, K., Harada, H., & Kashiwayanagi, M. (2004). Role of basal ganglia–brainstem pathways in the control of motor behaviors. *Neuroscience research,* 50(2), 137-151.

Takakusaki, K. (2013). Neurophysiology of gait: from the spinal cord to the frontal lobe. *Movement Disorders*, *28*(11), 1483-1491.

Whishaw, I. Q., Travis, S. G., Koppe, S. W., Sacrey, L. A., Gholamrezaei, G., & Gorny, B. (2010). Hand shaping in the rat: conserved release and collection vs. flexible manipulation in overground walking, ladder rung walking, cylinder exploration, and skilled reaching. *Behavioural brain research*, *206*(1), 21-31.

Wickens, J. R. (2009). Synaptic plasticity in the basal ganglia. *Behavioural brain research*, *199*(1), 119-128.

Wise, R. A. (2005). Forebrain substrates of reward and motivation. *Journal of Comparative Neurology*, *493*(1), 115-121.

Yamamoto, B. K., Lane, R. F., & Freed, C. R. (1982). Normal rats trained to circle show asymmetric caudate dopamine release. *Life sciences*, 30(25), 2155-2162.

Vita

Mabel Noemi Terminel was born in El Paso, TX but was raised in Cuidad Juarez, Mexico. She graduated from "El Chamizal" high school in May, 2006 and enter the El Paso Community College in January 2007. She obtained an Associate of Arts from the El Paso Community College in November, 2009. She entered the University of Texas at El Paso in January, 2010 where she pursued a degree in Psychology. As an undergraduate she worked in a legal psychology laboratory under the mentorship of Dr. James Wood. During this time she became interested in Neuroscience and started volunteering in the Laboratory of Behavioral and Neural Plasticity under the mentorship of Dr. Eddie Castañeda. Dr. Castañeda investigates changes in presynaptic mechanisms in drug addiction and Parkinson's disease. Mabel received her Bachelors of Science degree in Psychology in May 2012 and spend the following summer in the Summer Program In Survival, Ethics and Neuroscience (SPINES) at the Marine Biology Laboratory in Woods Hole, MA. She began working on her graduate studies in the Social, Cognition and Neuroscience program at the University of Texas at El Paso in August, 2012. As a graduate student she studied the role of dopamine within the basal ganglia in movement production. Her research interests also include the interaction of drug-induced neuroplasticity and sex-differences. During this time she has presented her work at local and national conferences, such as Society for Neuroscience. She also received the UT System LSAMP Bridge to the Doctorate Fellowship funded by the National Science Foundation. She received her Master of Arts in Experimental Psychology in July 2015 and is now entering the Neuroscience program at the University of Texas A&M where she will pursue her Ph.D.

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