Time-course of Motor Deficits in a Rat Model of Parkinson's Disease

by

Rohit Abraham

A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of Bachelor of Science

with Honors in Neuroscience from the

University of Michigan

2012

Advisor: Dr. Joshua D. Berke

Mentor: Dr. Daniel K. Leventhal

Abstract

Parkinson's disease (PD) is characterized clinically by the impaired ability to execute smooth, controlled movements, and pathologically by a lack of striatal dopamine. However, the role that dopamine plays in the striatum remains poorly understood. Previous experiments using striatal dopamine receptor blockade or dopaminergic denervation have demonstrated progressive impairment in conditioned behaviors, suggesting a role for dopamine in motor learning. Other evidence, however, suggests that dopamine is critical for motivating acute motor performance. In this study, I trained rats to a high degree of accuracy in a choice reaction-time task and then performed focal dopamine denervation in dorsolateral ("sensorimotor") striatum. After dopaminergic lesioning, the rats initially performed the task at baseline efficiency. Consistent with a "learning" model of dopamine function, task performance became gradually impaired with task repetition. I then attempted to rescue task performance via systemic administration of levodopa. Reaction times dropped to baseline levels on the first day of levodopa administration, and they continued to shorten with each day of practice. These low reaction times persisted into the third week even without acute dopamine replacement. These results suggest that striatal dopamine has roles in motor learning *and* acute motor performance. Further investigation is required to understand how these functions interact in normal motor learning and control.

Keywords: dopamine, PD, extinction mimicry, LDR, 6-OHDA, striatum, RT, learning

Introduction

Parkinson's disease (PD) is a neurodegenerative syndrome characterized by bradykinesia, rigidity, postural instability, and resting tremor. The motor deficits of PD have been traced to the progressive death of dopaminergic neurons in the substantia nigra pars compacta (SNc), which primarily projects to the striatum. It is well established that the motor deficits of PD are related to insufficient dopamine levels in the striatum (Hornykiewicz, 2010), which is the primary input nucleus of the basal ganglia. Therefore, it was widely believed for years that the primary role of dopamine in the basal ganglia is to allow smooth and well-coordinated movement.

More recently, however, empirical evidence has led to new hypotheses for the role of dopamine in normal physiology. Dopamine neurons had been observed to fire transiently in response both to conditioned and unconditioned visual stimuli (Schultz, 1993). This and similar studies imply a "reward" function for striatal dopamine which may play a causal role in certain forms of learning (Schultz, 2002). That is, when an unexpected positive event occurs, dopamine pulses signal to the striatum that the actions leading up to that event are worth repeating in the future. These bursts of phasic dopamine follow both unanticipated rewards and reward-predicting stimuli (Schultz, 2002). It has been suggested that phasic pulses of dopamine release serve as a mechanism for synaptic modification, encoding a reward prediction error (RPE) in a reinforcement-learning model of the striatum (Glimcher, 2011; Oyama et al., 2010). Other theories suggest that, instead of "liking" or learning, dopamine causes "wanting" of rewards (Berridge, 2007). In this construct, dopamine levels should impact performance in the current trial; under "learning" models, dopamine levels during the current trial should impact future task performance.

A role for dopamine in motor learning provides an alternative explanation for the motor deficits observed in PD. Following dopamine depletion, rats are able to perform an operant task normally, at least initially (Dowd & Dunnett, 2004). Only with repeated trials does their performance deteriorate. It was later suggested that deficits in movement initiation and execution might be downstream of the reward signal from phasic dopamine release (Dowd and Dunnett, 2007). Intrastriatal dopamine-receptor blockade has supported this claim, showing impaired task execution in an experience-dependent manner (Stoetzner, 2011). Therefore, a lack of dopamine in the striatum could essentially extinguish motor programs in a process that has been described as "extinction mimicry" (Wise, 1982). This mechanism could not only account for the bradykinetic symptoms of PD, but also the sustained improvement in the motor function of PD patients observed long after a levodopa dose has been eliminated from the body, clinically known as the long-duration response (LDR, Anderson & Nutt, 2011). This effect is in contrast to the rapid improvement in motor function observed after a single levodopa dose in PD, known as the short-duration response (SDR).

Complementing these behavioral results are physiological data suggesting cellular and molecular mechanisms for dopamine in striatal synaptic plasticity. In particular, the so-called "3-factor rule" suggests that synaptic plasticity at corticostriatal synapses is regulated by dopamine levels (Shen et al., 2008). Striatal projection neurons can be subdivided into 2 types: D1 receptor-expressing neurons and D2 receptor-expressing neurons, which project via the direct pathway and indirect pathway, respectively (Albin et al., 1989). Since D2 receptors have a higher binding affinity for dopamine than D1 receptors, it follows that D1 receptors may respond to phasic dopamine increases, whereas D2 receptors may respond to phasic dopamine decreases. Under the reinforcement-learning model, increases in phasic dopamine release associated with

positive RPEs would alter the direct pathway via D1 receptors. Dopamine depletion in PD could oppositely play a role in mediating the enhancement of the indirect pathway, giving rise to the hypokinetic movement disorder. During a levodopa drug holiday, synaptic connections—possibly representing specific motor programs—may persist until gradually degraded by the extinction mimicry process (Beeler, 2011), due to dysregulated dopamine-mediated synaptic plasticity.

In the current study, I explored the relationship between motor parkinsonism and dopamine's role in reward processing by analyzing the development of motor impairments in dopamine-depleted rats. I first trained several rats to a high performance level on a lateralized choice task. These rats were then unilaterally dopamine-depleted in dorsolateral ("sensorimotor") striatum. In order to investigate the time-course of motor deficit development and recovery, they were subsequently re-tested on the choice task, with and without levodopa treatment. In addition to the operant tasks, spontaneous motor deficits were investigated using the limb-use asymmetry (LUA) test, a commonly used metric of motor performance in unilaterally dopamine-depleted rodents. I hypothesized that spontaneous motor deficits would develop quickly, but motor deficits in the operant task would only gradually emerge with practice. Furthermore, I anticipated that the time-course of recovery given levodopa administration would show a similar gradual improvement. Such results would be compelling evidence in favor of the extinction mimicry model of PD.

Method

Overview

Animals were trained for several weeks on a lateralized choice task, advancing through progressive stages contingent upon task performance. Baseline tests were conducted at the most

difficult level for five days before the animals were lesioned with 6-OHDA in dorsolateral striatum (Figure 1). LUA testing occurred once during baseline testing, every three days for two weeks after the lesion, and then continued daily through the remainder of the experimental period. Two weeks following the lesion, animals were re-tested on the choice task daily for seven days. Testing continued for the next seven days, but this time after systemic administration of levodopa. Finally, testing for the next seven days occurred in the absence of levodopa. Lesions were verified post-mortem by immunohistochemical staining for tyrosine hydroxylase (TH).

Animal Care. All procedures were approved by the University of Michigan's Committee on Use and Care of Animals. Twelve adult male Long-Evans rats (350-550g) were group-housed on a 12/12 hr reverse light/dark cycle. Testing occurred at approximately the same time daily 5-6 days/week for each animal for 1 hour, during the dark phase. Animals were food-restricted to 15 g of chow per day to motivate them for the food reward. Access to food was ad libitum for the first week after surgery. Weight was measured weekly to ensure that at least 90% of predicted free-feeding weight was maintained.

Behavioral Experiment

Operant Training and Testing. Training and test sessions occurred in an enclosed operant chamber, containing 5 horizontally adjacent nose-poking portals along a slightly curved wall (Figure 2). Each portal contained a yellow LED and IR sensor. The opposite wall housed a well that dispensed fruit-punch flavored sucrose pellets (45 mg each, obtained from TestDiet, Richmond, IN; Item # 5TUT) as a food reward.

The task first required a nose-poke into a central lit port with a hold time randomly chosen from a uniform distribution (750 to 1250 ms). Next, either a 1000 Hz or 4000 Hz auditory cue was given, which signaled that the rat should swiftly nose-poke the left or right

adjacent portal, respectively. Each correct trial was rewarded with a sucrose food pellet, followed by a variable timeout period (15-25 seconds); each incorrect trial went directly to the timeout period with no reward. During the training period, animals advanced 9 difficulty levels, which progressively shaped behavior by decreasing the length of time the rats had to respond. Rats were advanced when they were correct on more than 80% of trials with less than 10% invalid trials (poking the wrong port to initiate a trial or not waiting for the tone). Reaction time (RT) was defined as the interval between the cue and exiting the initial nose-port. Movement time (MT) was defined as the subsequent interval between exiting the initial nose-port and entering the adjacent nose-port. Accuracy was defined as the number of rewarded trials divided by the total number of completed trials (that is, trials in which a side-port was poked). RT, MT, and accuracy were calculated independently for trials cued for movement ipsilateral and contralateral to the lesions.

Dopaminergic Lesions. A commonly used neurotoxic model of PD involves 6-hydroxydopamine (6-OHDA) infusion into the nigrostriatal system. 6-OHDA enters neurons via catecholamine transporters, and is therefore selective for catecholaminergic neurons. Once inside the cells, it is oxidized to produce toxic free radicals, leading to cell death. The dopaminergic system may be lesioned by 6-OHDA infusion directly into dopaminergic nuclei (i.e. SNc), projection pathways (i.e. the medial forebrain bundle – mfb), or terminal fields of dopaminergic projections (i.e. the striatum).

In this experiment, 3 μL of 5.0 μg/μL 6-OHDA was infused into dorsolateral striatum. After the infusion, the cannula was left in place for 2 minutes to allow for diffusion of the drug. Half of the animals were randomly assigned to the left hemisphere and the other half to the right hemisphere. Infusion coordinates were A-P +0.5 mm and M-L +/- 3.5 mm relative to bregma,

and D-V 3.5 mm relative to the brain surface (Figure 3). An intraperitoneal (IP) injection of 25-mg/kg desipramine was given 0.5 h prior to infusion to prevent 6-OHDA uptake into noradrenergic neurons. Animals were given two weeks to recover from surgery and to allow their lesions to stabilize before beginning testing on the operant task.

Limb-Use Asymmetry (LUA) Testing. The LUA test, also commonly known as the cylinder test, takes advantage of a rodent's instinctive response to explore a novel environment. When placed in a clear plastic cylinder, a rodent explores by rearing up on its hind legs and using its forelimbs to support its body weight against the enclosing wall. Spontaneous forelimb use is evaluated by recording every independent instance of a weight-supporting wall touch—the paw must be completely stretched and all digits should be in contact with the glass in order to be counted (Cenci & Lundblad, 2007). It is expected that unilaterally dopamine-depleted rodents will execute fewer touches with the limb contralateral to their lesion. The LUA score was calculated as the proportion of contralateral touches out of the total number of touches; a score of 0.5 therefore indicates no limb use asymmetry. Each LUA test lasted five minutes and occurred every 3 days after the lesion for 2 weeks, then daily preceding testing in the operant task, as described below.

Drug Administration. Test sessions began 14 days after 6-OHDA infusion. Each session was preceded by an IP injection of saline or levodopa/benserazide 35 minutes prior to the task. Levodopa is a dopamine precursor that can cross the blood-brain barrier and is subsequently converted into dopamine via dopa-decarboxylase in dopaminergic neurons. Benserazide, a dopa-decarboxylase inhibitor that cannot cross the blood-brain barrier, is co-administered with levodopa to prevent unwanted levodopa conversion elsewhere in the body. This both preserves

levodopa so that it can enter the brain and prevents systemic side effects from the peripheral conversion of levodopa to dopamine.

The drug administration schedule consisted of three periods as follows: saline during the first week ("Saline 1"), levodopa/benserazide during the second week ("Levodopa"), and saline during the last week ("Saline 2"). 30 minutes after the injection, LUA testing occurred for 5 minutes, followed immediately by testing in the operant task.

Histology. After the experiments, each animal was deeply anesthetized and transcardially perfused with 4% para-formaldehyde (PFA). Their brains were stored in 4% PFA for a period of 18 - 24 hours before transfer to a 30% sucrose solution in 1x phosphate buffered saline (PBS) for cryopreservation. Brains were then sliced on a cryostat at 50 µm for both Nissl and TH staining. The final coordinates of the cannulae were confirmed by image analysis and mapping onto a standard rat brain atlas.

Statistical analysis. Behavioral analysis was performed using a general linear model, using SPSS Statistics 19 (IBM, Inc.) and MATLAB R2010b (The Mathworks, Inc.). The MT and RT data were standardized by subtraction of the average baseline performance of an individual animal from its performance on every test session. Two-factor ANOVA tests were utilized to assess differences in several behavioral responses for the choice task and LUA scores (factors: drug period and movement direction relative to lesion). Paired t-tests were utilized to assess differences between individual testing sessions.

<div align="center">

Results

</div>

Histology. TH straining confirmed that some animals had substantial lesions, while other animals had relatively weak lesions. The locations of the cannulae were consistently targeted in the dorsolateral striatum, confirmed by Nissl stained sections. Out of a total of fifteen tested

animals, six animals had weak lesions and were therefore excluded from analysis (see Appendix). All the animals included in the final analysis performed a similar number of valid trials throughout all three drug periods (Figure 4).

Saline 1 Period. The behavioral choice data were segregated into contralaterally and ipsilaterally directed trials relative to the lesion site. Throughout the Saline 1 period, contralateral behavior shows significant deficits in comparison to baseline performance. Specifically, contralateral RT gradually increases with practice (Figure 6). Indeed, there is no significant difference in contralateral RT between the first test session of Saline 1 when compared to average baseline performance; $t(8) = -0.8510$, $p = 0.4195$. A two-factor analysis of variance tested the effects both of drug period and movement direction relative to the lesion. Performance during Saline 1 sessions showed a significantly higher RT than baseline performance, when comparing effects of drug period [$F(7,56) = 4.597$, $p = 0.030$] and direction [$F(1,8) = 6.726$, $p = 0.032$]. Contralateral accuracy immediately declines and remains fairly stable (Figure 7). Two-factor ANOVA similarly showed a significantly lower accuracy than baseline performance, when comparing effects of drug period [$F(7,56) = 2.368$, $p = 0.034$] and direction [$F(1,8) = 5.331$, $p = 0.049$]. Ipsilateral and contralateral MT remained at similar levels during all periods of testing (Figure 5). The gradual learning-like effect observed in RT is suggestive of the extinction mimicry model of PD. An alternative interpretation, however, is that the dopaminergic lesions progressed during the testing period.

Limb-use Asymmetry (LUA). The LUA behavioral data were used to evaluate if the gradually increasing RT might be explained by continued progression of the dopaminergic loss. Within 6 days of infusion, the rats showed a strong ipsilateral limb-use preference (Figure 8). These data suggest that the lesions develop quickly and stabilize during the post-operative period.

The LUA scores remain strongly asymmetric until levodopa treatment, which gradually rescues the values back to baseline levels. These levels once again decline during the Saline 2 period. Single-factor ANOVA suggests a significant difference between 0.5—a perfectly symmetric score—compared to every subsequent test [$F(25,104) = 1.718$, $p = 0.031$]. These results suggest that the dopaminergic lesions were stable prior to post-lesion testing on the operant task, and support an extinction-like effect for the decline in task performance with repeated practice.

Levodopa Drug Period. The levodopa drug period showed a rapid, dramatic improvement, restoring performance back to baseline levels as measured by both accuracy and RT (Figures 6, 7). There was no significant difference between in contralateral RT between the first test session of the levodopa drug period and baseline performance; $t(8) = -1.1835$, $p = 0.2706$. Comparing baseline performance to the levodopa drug period, 2-factor ANOVA reveals a non-significant change in RT, considering factors of drug period [$F(7,56) = 0.798$, $p = 0.593$] and direction [$F(1,8) = 0.221$, $p = 0.651$]. The decrease in RT progresses throughout the levodopa drug period, with a significant difference between the first and last sessions of the drug period; $t(8) = 2.6445$, $p = 0.0295$. Accuracy shows a similar recovery back to baseline levels, considering factors of drug period [$F(7,56) = 0.798$, $p = 0.593$] and direction [$F(1,8) = 3.883$, $p = 0.084$]. This acute rescue in motor behavior is analogous to the SDR.

Saline 2 Period. Behavior during Saline 2 revealed a delayed, but gradual increase in RT and a corresponding decrease in contralateral accuracy (Figure 6, Figure 7). Comparing baseline performance to the Saline 2 period, 2-factor ANOVA reveals a non-significant change in RT, considering factors of drug period [$F(7,56) = 0.627$, $p = 0.601$] and direction [$F(1,8) = 0.014$, $p = 0.908$]. This non-significant change verifies recovery from the motor deficits experienced during the Saline 1 period. Accuracy shows a similarly sustained recovery back to baseline levels,

considering factors of drug period [$F_{(7,56)} = 0.680$, $p = 0.509$] and direction [$F_{(1,8)} = 0.836$, $p = 0.387$]. This learning effect can cautiously be interpreted as analogous to the LDR.

Discussion

There are 3 primary results from these experiments. First, I demonstrated that unilateral dopamine depletion in dorsolateral (sensorimotor) striatum is sufficient to impair performance on a conditioned choice RT task. Second, I found that—despite early deficits in spontaneous limb use—motor performance on a conditioned task declines in a gradual, experience-dependent manner. Third, acute motor performance on the conditioned choice task can be rescued to baseline levels by systemic dopamine replacement, though there was also an experience-dependent decrease in RT with repeated levodopa exposure. Together, these results suggest that dopamine in the dorsolateral striatum plays a role in both motor learning and acute motor performance.

The Role of Dopamine in Dorsolateral Striatum. Previous studies have demonstrated that unilateral 6-OHDA infusions across the entire striatum are sufficient to significantly impair motor performance in an instrumental learning task (Dowd & Dunnett, 2005). In order to further disentangle the role of dopamine in learning, the infusions in this experiment were targeted to produce focal lesions limited to dorsolateral striatum. I demonstrated that dopamine depletion in dorsolateral striatum alone is indeed sufficient to impair motor performance in a conditioned choice RT task.

The dorsolateral striatum seemed a likely lesion candidate to impair task performance based on its functional implications and projection patterns. Substantial evidence has long suggested that dorsal striatum is important in learning and memory (Devan & White, 1999), but recent findings have revealed that dorsolateral striatum is necessary for stimulus-response

learning and habit formation (Featherstone & McDonald, 2005). It participates in cortical-basal ganglia loops, principally receiving input from primary motor cortex and passing its output back to primary motor cortex via the substantia nigra, pars reticulata and thalamus (Haber et al., 2006). It follows that a lesion in this small structure is likely to disrupt critical motor outputs. Indeed, dopamine loss in clinical Parkinsonism occurs first and most prominently in dorsolateral striatum (McCallum et al., 2005).

Motor Performance Versus Motor Learning. Parameters of motor performance from the first test session after the dopaminergic lesion were nearly identical to baseline performance levels. The subsequent deficit in contralateral RT seems to be an experience-dependent learning effect. Previous unilateral 6-OHDA experiments have also demonstrated this progressive motor deficit, which closely mirrors the behavior of unlesioned control animals that simply stop receiving a food reward (Dowd & Dunnett, 2007). Recent findings in our laboratory also show similar gradual deficits, using a similar task under dopamine receptor blockade in dorsolateral striatum (Stoetzner, 2011). Furthermore, while task performance was largely restored to baseline performance on the first "levodopa" day, repeated levodopa doses further modified task performance. Finally, Together, these results are consistent with a role for dopamine in motor learning.

Acute Motor Performance. Systemic levodopa rescued choice task performance to baseline levels during the first "levodopa" session. This finding seemingly contradicts the reinforcement-learning model for striatal dopamine, due to the rapid improvement in both RT and accuracy from the very first day of levodopa administration. In fact, this acute effect closely resembles the clinical short-duration response (SDR), which is characterized by a rapid improvement in motor function that typically lasts approximately 3-4 hours after a single dose of

levodopa. The onset and offset of the SDR parallels the rise and fall of serum levodopa levels (Marin et al., 2007), supporting a role for dopamine in acute motor performance.

The incentive salience hypothesis, an alternative explanation for the role of striatal dopamine in the nigrostriatal circuit (Berridge, 2007), could account for this acute motor rescue. Dopamine is hypothesized to enhance the "wanting" component of motivation in a state-dependent manner. After repeated extinction trials in a self-administration study, conditioned behavior was acutely rescued by reinstating dopaminergic drug (Stretch & Gerber, 1973). This effect is similar to the SDR, and it can be reconciled with the extinction mimicry model through the state-dependent presence of dopamine. In this respect, the extinction mimicry model does function like true extinction: previously conditioned behavior can be reinstated quickly, simply by restoring the reward (Wise, 2009).

LDR as Extinction Mimicry. In both the Saline 1 and Saline 2 periods, there is a sustained level of motor performance after dopamine loss, followed by gradual deterioration. Lacking all dopamine in dorsal striatum, the PITx3 genetic knockout mouse model also shows sustained sensorimotor improvement after a levodopa drug holiday (Beeler, 2010). It was similarly demonstrated that while dopamine is necessary for maintenance of motor function, animals could still perform an instrumental task at the same level immediately before and after dopamine-depletion (Dowd & Dunnett, 2005). The sustained effect common to these experiments may be analogous to the LDR, clinically observed in PD patients, in which the motor benefits of dopamine persist long after the elimination half-life of levodopa.

The LDR remains a poorly understood phenomenon, but the effect appears similar to the extinction mimicry process—perhaps by the gradual extinction of overtrained motor behavior. In the present experiment, it is possible that the synaptic weights representing motor programs are

sculpted both by positive RPEs during the Levodopa drug period and by negative RPEs during the Saline 2 period. However, the ratio of negative to positive RPEs and the magnitude of their effects on synaptic plasticity could potentially explain the LDR. Positive RPEs typically have a longer lasting maintenance effect than the degradation effects of negative RPEs, based on bidirectional Hebbian plasticity (Shen et al., 2008). Therefore, relatively few positive RPEs could counter the effect of more negative RPEs. Synaptic connections relevant to the task performance therefore may persist into the Saline 2 period, slowing the degradation process.

Dopamine-replacement-mediated RPE signaling could provide a cellular mechanism for the LDR. Interestingly, D2 dopamine receptor agonists can induce an LDR comparable to levodopa (Nutt & Carter, 2000). Because they have a higher binding affinity for dopamine, D2 receptors are principally occupied by basal dopaminergic tone. In normal physiology, a negative RPE may be encoded by an acute decrease in dopamine, resulting in fewer occupied D2 receptors and activation of the indirect ("No-Go") pathway. However, the constitutively low levels of striatal dopamine in PD change this communication—pathologically vacant D2 receptors permanently activate the indirect ("No-Go") pathway instead, giving rise to the hypokinetic movement disorder. It follows, then, that D2-receptor agonist administration may protect against the detection of negative RPEs, thus creating the LDR. Dopamine agonist-induced impulse control disorders may share a similar mechanism, in which susceptible patients are unable to process negative consequences of their actions.

In summary, this study suggests a link between "extinction mimicry" described in animal models of dopamine depletion and the bradykinesia associated with Parkinson's disease. I have demonstrated that dopamine signaling in the dorsolateral striatum has roles in both reinforcement-learning and motor performance. These effects occur at different time-scales that

are analogous to the LDR and SDR, respectively. Further work is needed to more clearly dissect the roles of D1 (direct pathway) vs. D2 (indirect pathway) dopamine receptors in motor learning vs. motor performance. As the link between dopamine and the LDR becomes more clearly defined, revised therapeutic strategies can potentially maximize the clinical benefit of the LDR in PD patients.

References

Albin, R.L., Young, A.B., Penney, J.B. (1989). The functional anatomy of basal ganglia

disorders. *Trends in Neurosciences*, *12*, 366-375.

Anderson, E., & Nutt, J. (2011). The long-duration response to levodopa: phenomenology,

potential mechanisms and clinical implications. *Parkinsonism and Related Disorders*, *17*,

587-592.

Beeler, J, Cao, Z., Kheirbek, M., Ding, Y., Koranda, J., Murakim, M., Kang, U., Zhuang, X.

(2010). Dopamine-dependent motor learning: insight into levodopa's long-duration

response. *Annals of Neurology*, *67*, 639-647.

Beeler, J.A. (2011). Preservation of function in Parkinson's disease: What's learning got to do

with it?. *Brain Research*, *425*, 96-113.

Berridge, K.C. (2007). The debate over dopamine's role in reward: the case for incentive salience.

Psychopharmacology, *191*, 391-431.

Cenci, A., & Lundblad, M. (2007). Ratings of L-DOPA-induced dyskinesia in the unilateral 6-

OHDA lesion model of Parkinson's Disease in rats and mice. *Current Protocols in*

Neuroscience, *9*, 1-23.

Devan, B.D., & White, N.M. (1999). Parallel information processing in the dorsal striatum:

relation to hippocampal function. *The Journal of Neuroscience*, *19*, 2789-2798.

Dowd, E., & Dunnett, S.B. (2004). Deficits in a lateralized associative learning task in

dopamine-depleted rats with functional recovery by dopamine-rich transplants. *The*

European Journal of Neuroscience, *20*(7), 1953-1959.

Dowd, E., & Dunnett, S.B. (2005). Comparison of 6-hydroxydopamine-induced medial forebrain

    bundle and nigrostriatal lesions in a lateralized nose-poking task in rats. *Behavioral Brain*

    Research, *159*, 153-161.

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*, 428-

    432.

Glimcher, P.W. (2011). Understanding dopamine and reinforcement learning: the dopamine

    reward prediction hypothesis. *Proceedings of the National Academy of Sciences of the*

    United States of America, *108*, 15647-15654.

Hornykiewicz, O. (2010). A brief history of levodopa. *Journal of Neuroscience, 257*, S249-S252.

Marin, C., Aguilar, E., Mengod, G., Cortés, R., Obeso, J. (2007). Concomitant short- and long-

    duration response to levodopa in the 6-OHDA-lesioned rat: a behavioural and molecular

    study. *European Journal of Neuroscience*, *25*, 259-269.

McCallum, S., Parameswaran, N., Perez, X., Bao, S., McIntosh, J., Grady, S., Quik, M. (2006).

    Compensation in pre-synaptic dopaminergic function following nigrostriatal damage in

    primates. *Journal of Neurochemistry*, *96*, 960-972.

Nutt, J.G., & Carter, J.H. (2000). Apomorphine can sustain the long-duration response to L-

    DOPA in fluctuating PD. *Neurology*, *54*, 247-250.

Oyama, K., Hernadi, I., Iijima, T., Tsutsui, K. (2010). Reward prediction error coding in dorsal

    striatal neurons. *Journal of Neuroscience*, *30*, 11447-11457.

Schultz, W. Apicella, P., Ljungberg, T. (1993). Responses of monkey dopamine neurons to

    reward and conditioned stimuli during successive steps of learning a delayed response

    task. *The Journal of Neuroscience, 13*, 900-913.

Schultz, W. (2002). Getting formal with dopamine and reward. *Neuron*, *36*, 241-263.

Fiorillo, C.D., Newsome, W.T., Schultz, W. (2008). The temporal precision of reward prediction

in dopamine neurons. *Nature Neuroscience*, *11*, 966-973.

Shen, W., Flajolet, M., Greengard, P., Surmeier, D.J. (2008). Dichotomous dopaminergic control

of striatal synaptic plasticity. *Science*, *321*, 848-850.

Stoetzner, C.R. (2011). Corticostriatal plasticity, learning, and choice. *University of Michigan

Deep Blue Archive*.

Stretch, R., & Gerber, G. (1973). Drug-induced reinstatement of amphetamine self-

administration behavior in monkeys. *Canadian Journal of Psychology*, *27*, 168-177.

Wise, R.A. (1982). Neuroleptics and operant behavior: the anhedonia hypothesis. *Behavioral and

Brain Sciences*, *5*, 39–87.

Wise, R.A. (2009). Roles for nigrostriatal—not just mesocorticolimbic—dopamine in reward and

addiction. *Trends in Neurosciences*, *32*, 517-524.

Author Note

Experimental Design

6-OHDA infusion

	Baseline Choice Task	Post-Lesion Period (LUA only)	Choice Task Testing + LUA (IP saline)	Choice Task Testing + LUA (IP L-dopa)	Choice Task Testing + LUA (IP saline)
Train rats					
~ 2 months	5 days	14 days	7 days	7 days	7 days
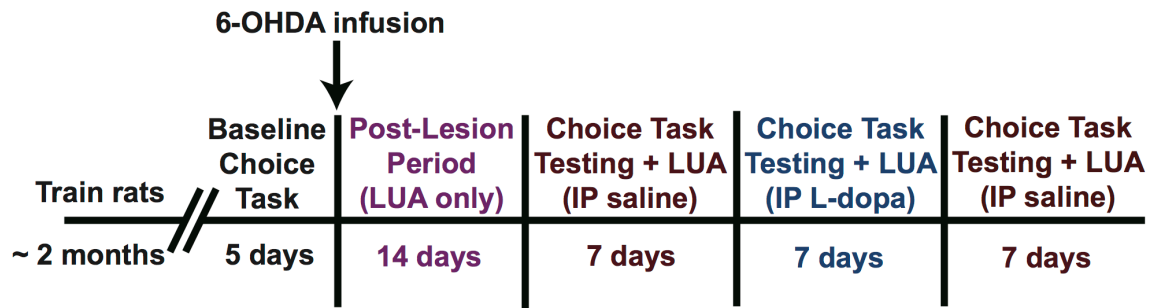
Figure 1. Timeline of Experimental Design for a Single Animal. Animals were trained to criterion (see text), and baseline performance was recorded for 5 days on the choice task. Animals were then given a unilateral infusion of 6-OHDA, lesioning the dorsolateral striatum. They were evaluated on the LUA test every 3 days for the first two weeks after lesioning. Beginning two weeks after these lesions, animals were re-tested on the choice task daily for 21 days after administration of either saline or levodopa/benserazide.
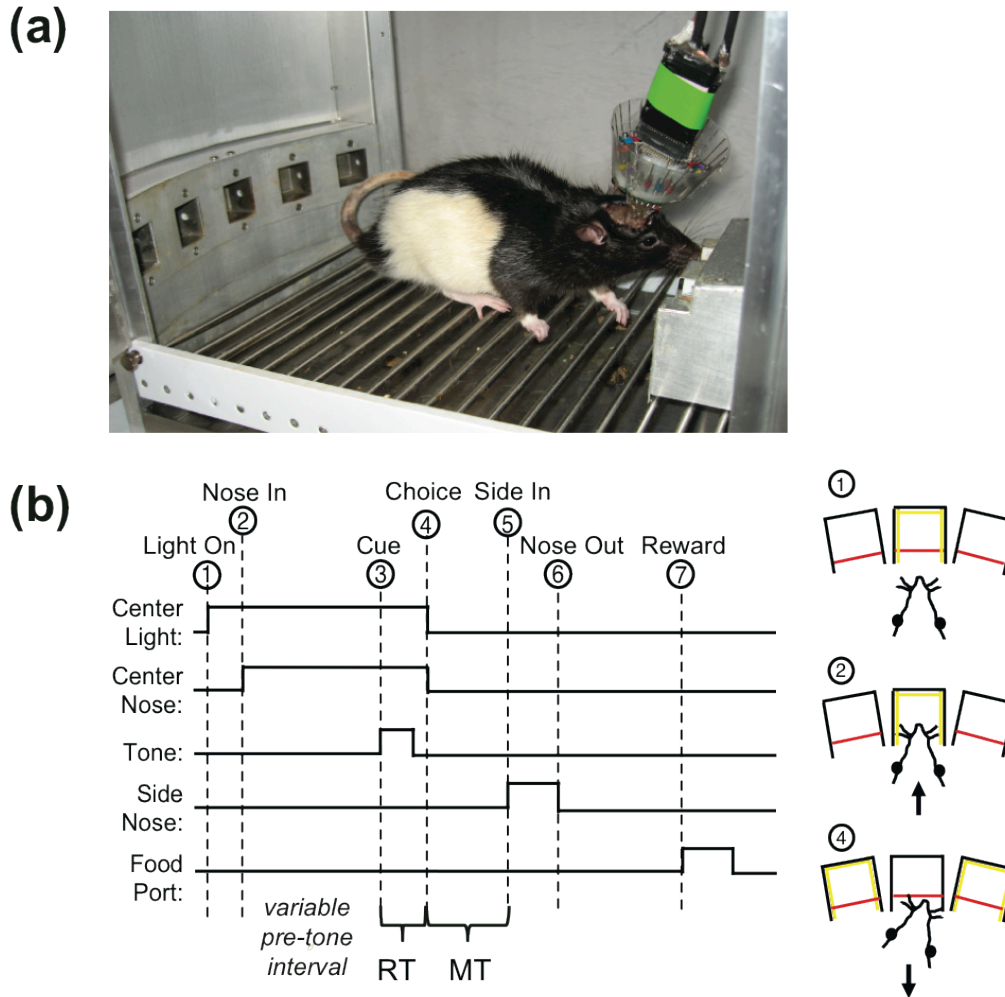
Figure 2. Choice task schematic, adapted from Gage et al, 2010. (a) Rat receiving reward for completing a correct trial in the operant chamber. (b) Schematic of the choice task. (1) One of the three center nose-poke ports lights up. (2) Rat pokes the lit port and holds for a variable interval. (3) 1 or 4 kHz pure tone instructs the animal to move left or right, respectively. (4) "Choice" occurs as rat pulls its nose out of the initially lit nose port. (5) Rat nose-pokes the correct adjacent port, as indicated by the cue tone pitch. (6) Rat exits the second port. (7) Rat receives sucrose pellet reward at the back of the operant chamber. RT = reaction time, MT = movement time.
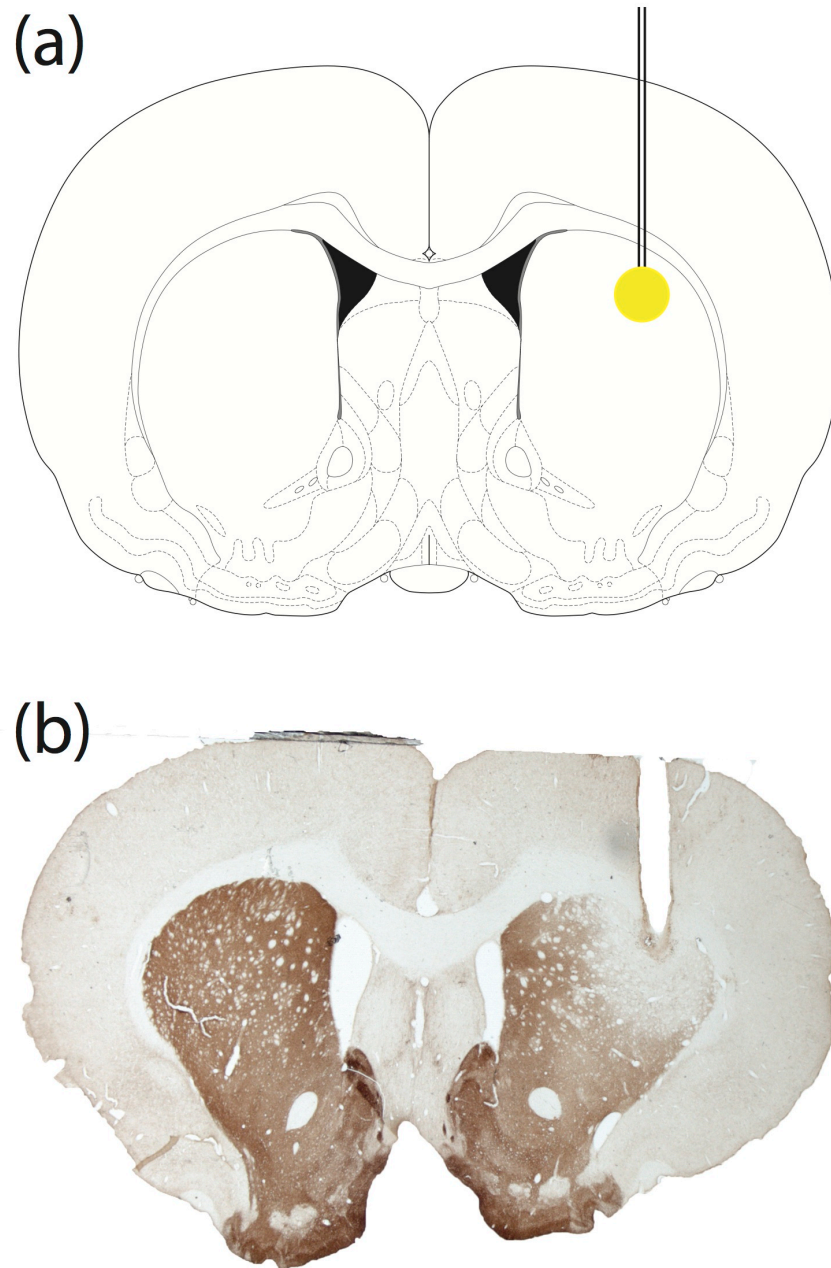
Figure 3. Dorsolateral striatal lesion. (a) Schematic illustrating the positioning of the cannula in the dorsolateral striatum. Figure provided by Dr. Daniel K. Leventhal. (b) Immunohistologic sample showing unilateral dopaminergic lesions via anti-tyrosine hydroxylase staining.
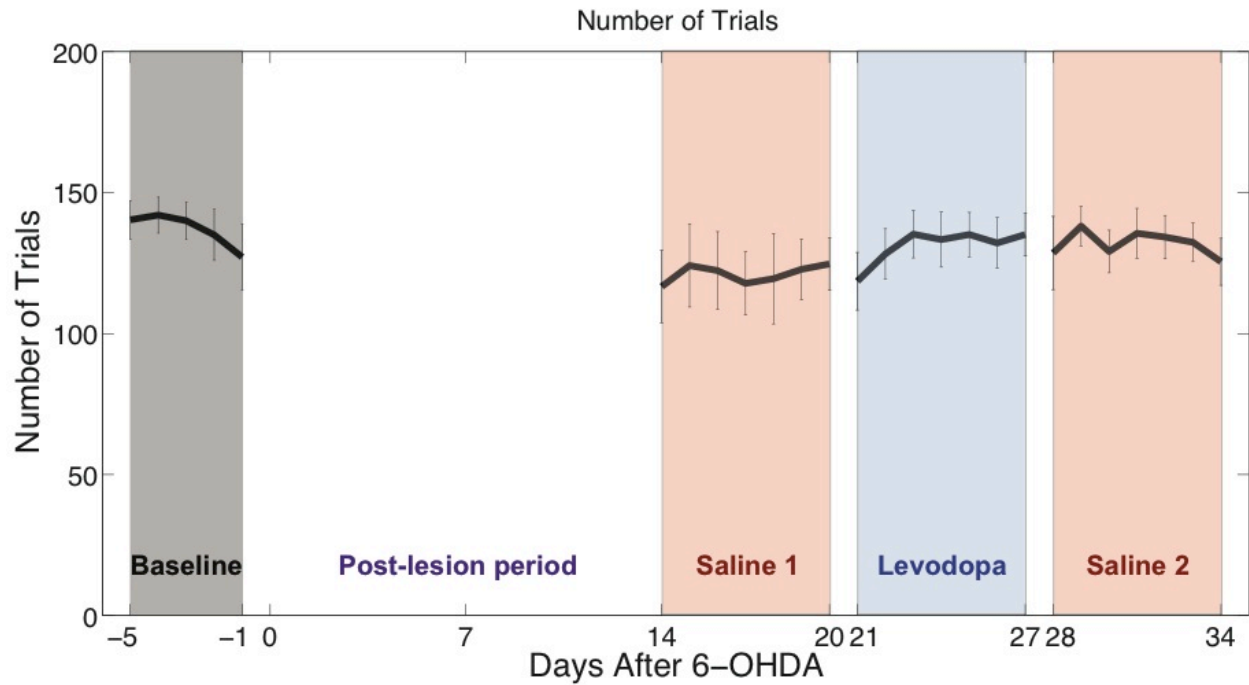
Figure 4. Mean Number of Trials per Session. The rats performed similar numbers of trials across experimental conditions. Baseline performance occurred between days -5 to -1. Saline 1 performance occurred between days 14 to 20. Levodopa performance occurred between days 21 to 27. Saline 2 performance occurred between days 28 to 34. Error bars indicate standard error in the mean.
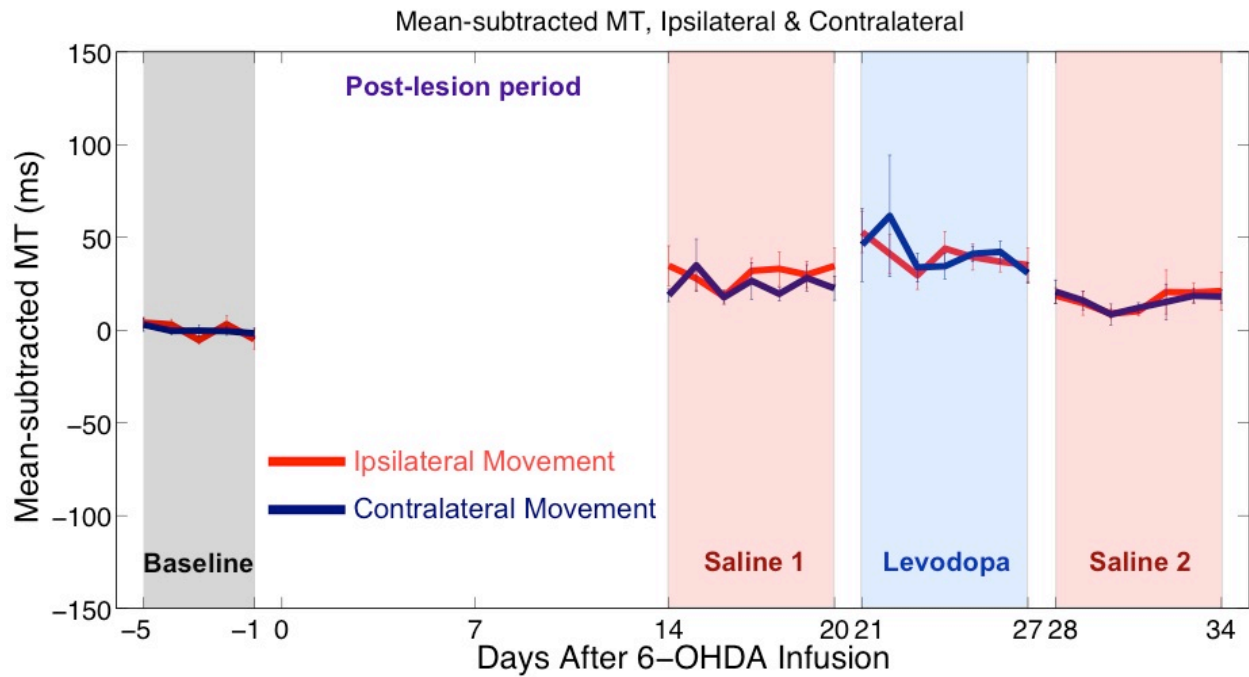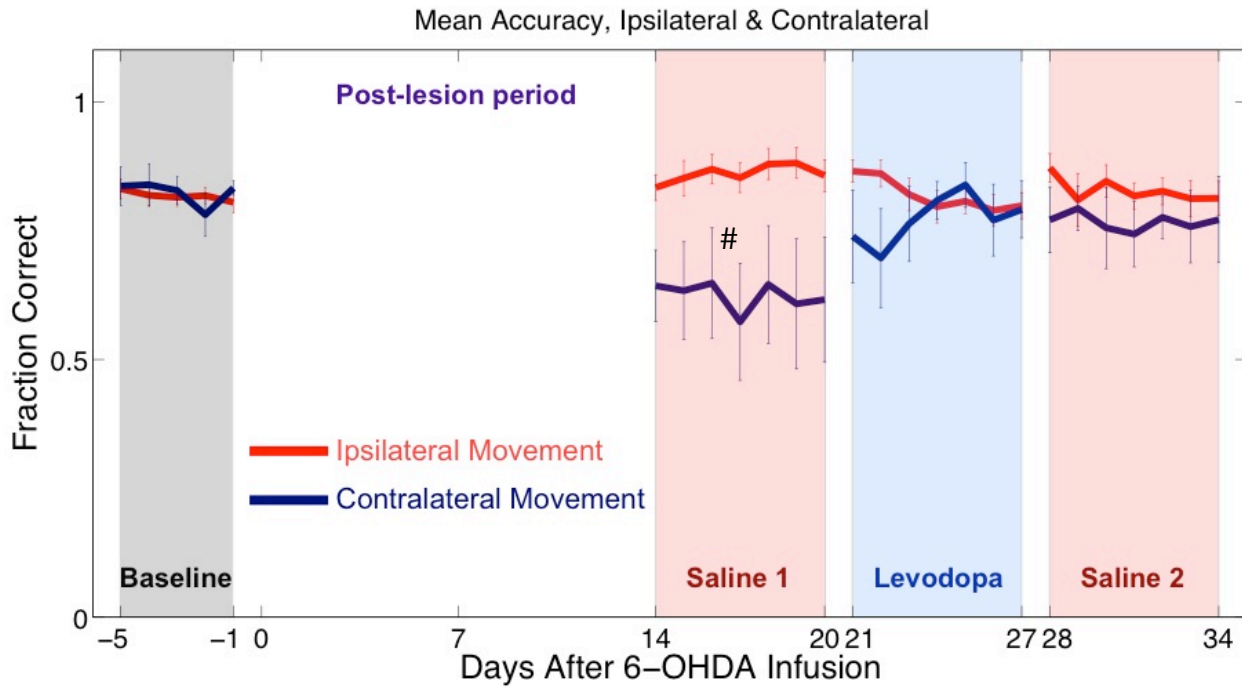
Figure 5. Mean-subtracted Movement Time (MT). Movements have been segregated by direction of movement relative to the lesioned hemisphere (ipsilateral vs. contralateral). The MT (ipsilateral and contralateral) data were standardized by mean-subtraction of the average baseline performance of an individual animal from its performance on every test session. Error bars indicate standard error in the mean.
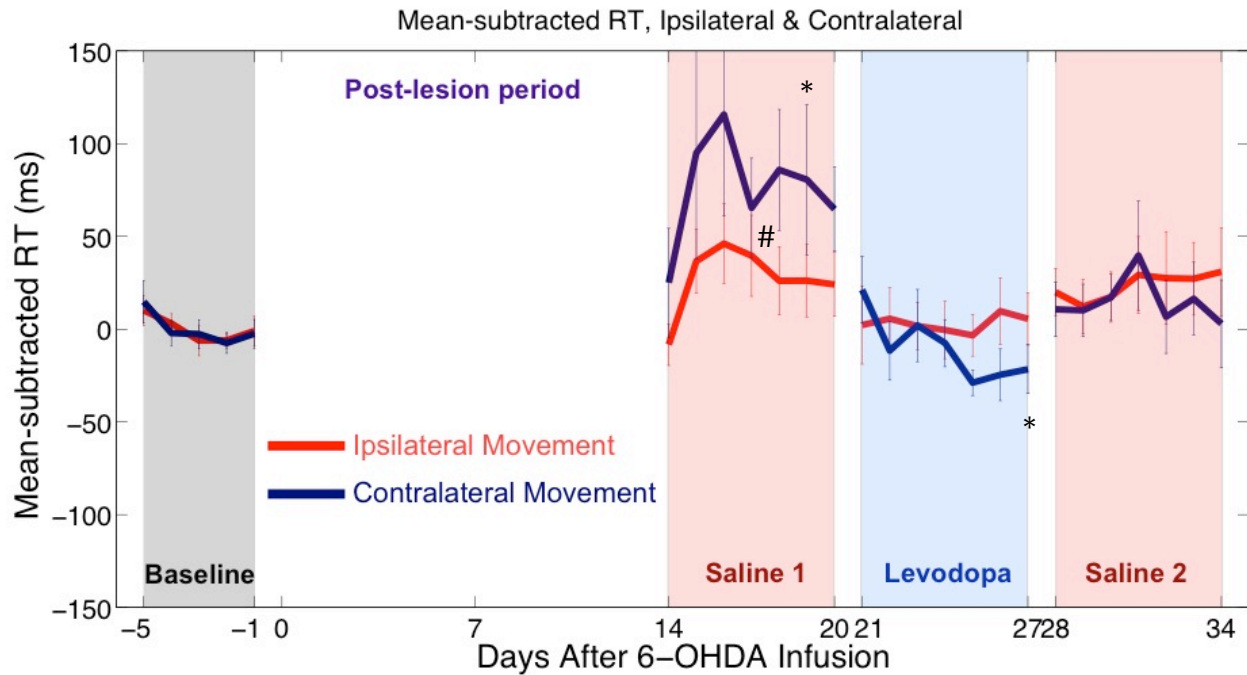
Figure 6. Mean-subtracted Reaction Time (RT). Movements have been segregated by direction of movement relative to the lesioned hemisphere (ipsilateral vs. contralateral). The RT (ipsilateral and contralateral) data were standardized by mean-subtraction of the average baseline performance of an individual animal from its performance on every test session. Error bars indicate standard error in the mean. Hash marks indicate a significant difference from baseline performance, considering both factors of drug period and movement direction. Asterisks indicate a significant difference between baseline performance and a particular testing session for contralateral movement.

Figure 6. Mean Accuracy. Movements have been segregated by direction of movement relative to the lesioned hemisphere (ipsilateral vs. contralateral). Error bars indicate standard error in the mean. Hash marks indicate a significant difference from baseline performance, considering both factors of drug period and movement direction.
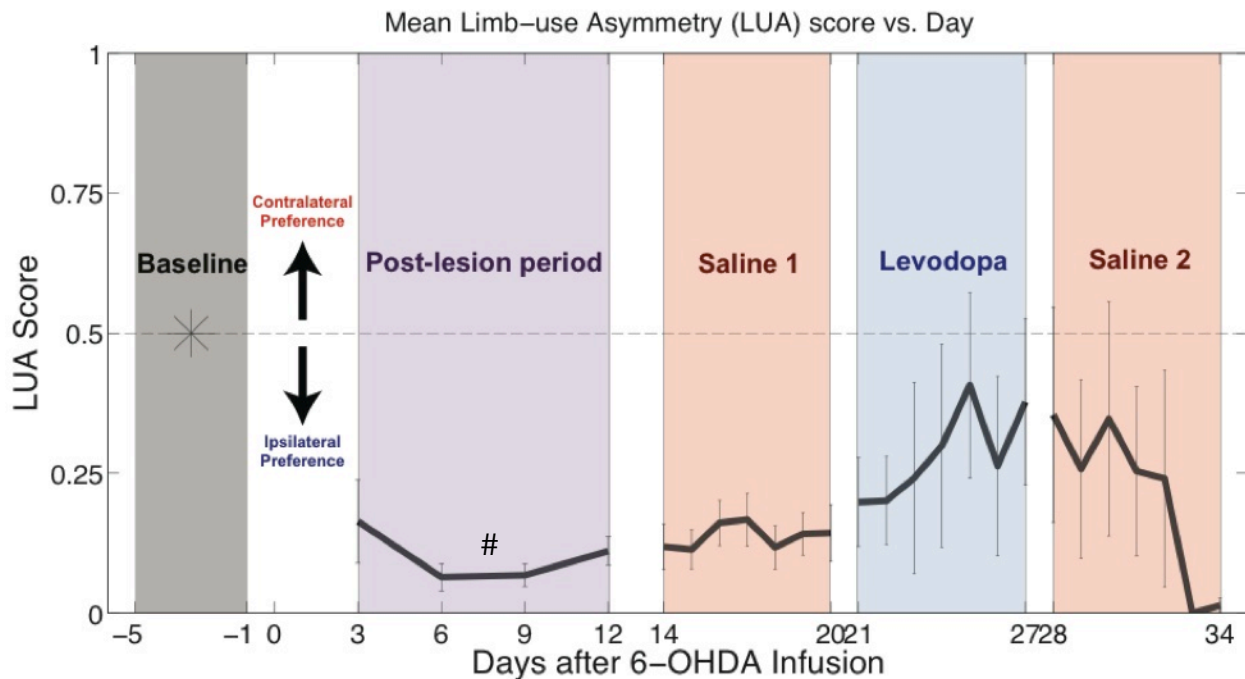
Figure 8. Mean Limb-use Asymmetry (LUA) scores. A score of 0.5 indicates completely symmetrical use of forelimbs, a score of 0 indicates a complete favor of the ipsilateral forelimb, and a score of 1 indicates complete favor of the contralateral forelimb. As expected, low asymmetry scores followed the unilateral 6-OHDA infusion (Day 0). These low post-lesion scores are relatively unaffected by levodopa treatment (Days 21-27). LUA scores finally begin to increase during the Saline 2 period (Days 28-34), which indicates that the lesion has begun to stabilize and that the rats may be learning to compensate for the lesion. Error bars indicate standard error in the mean. Hash marks indicate a significant difference between 0.5 (perfectly symmetric score) and every subsequent session, including all the post-lesion period and all three drug periods.
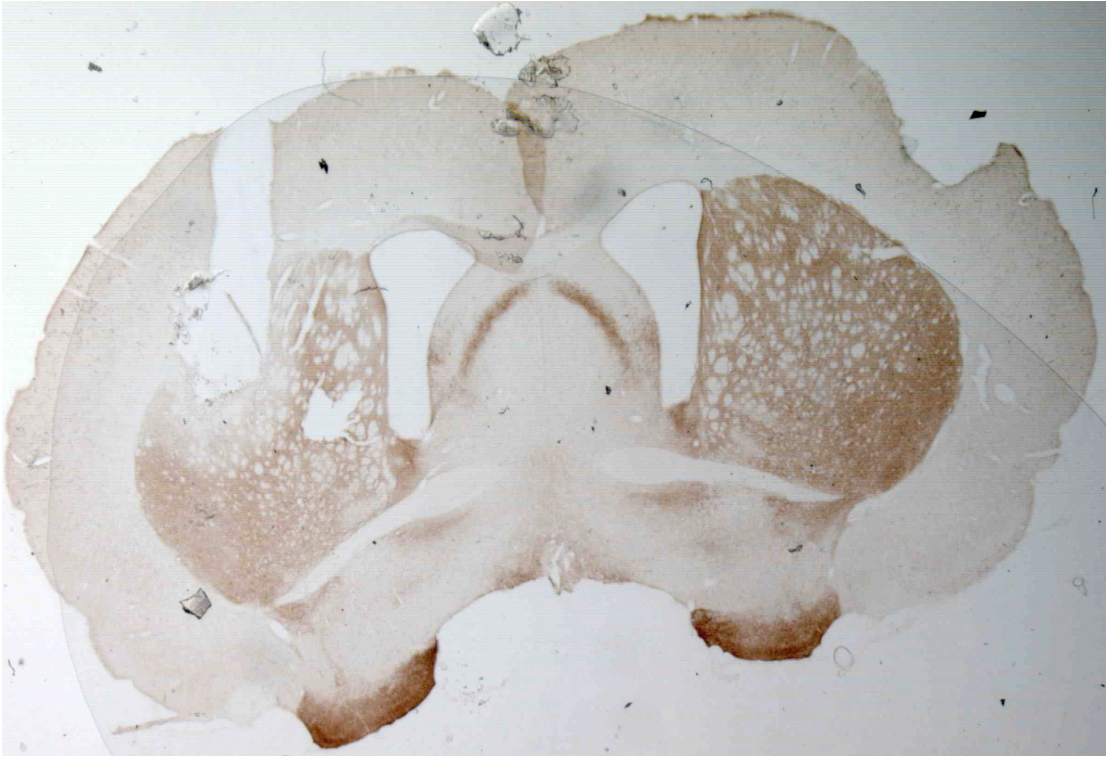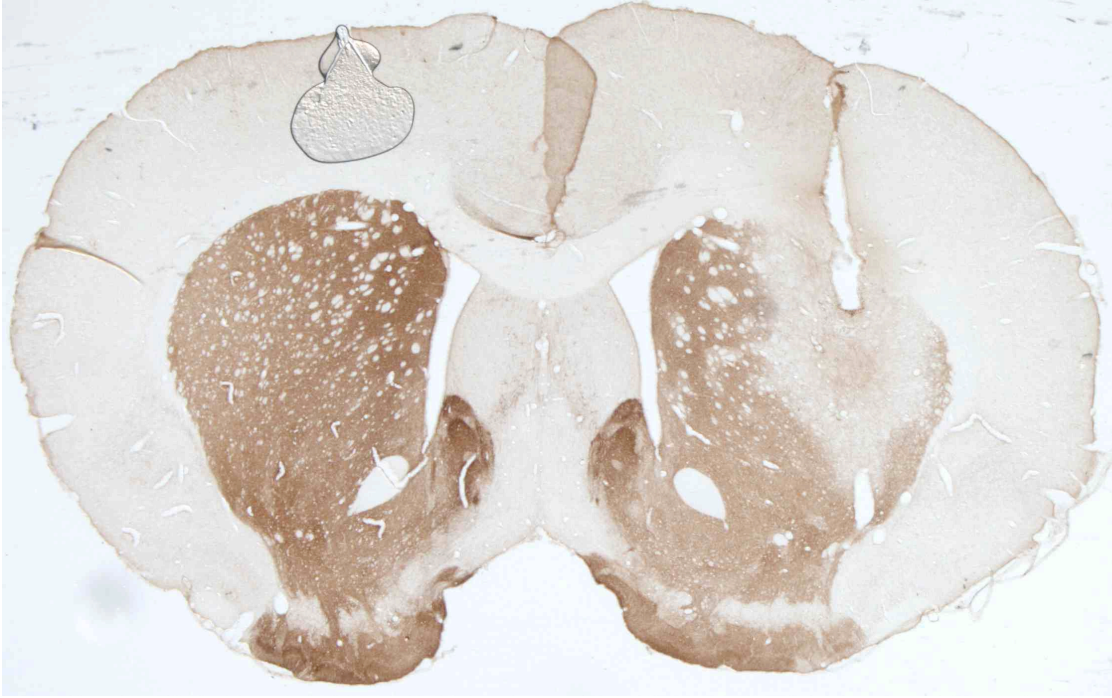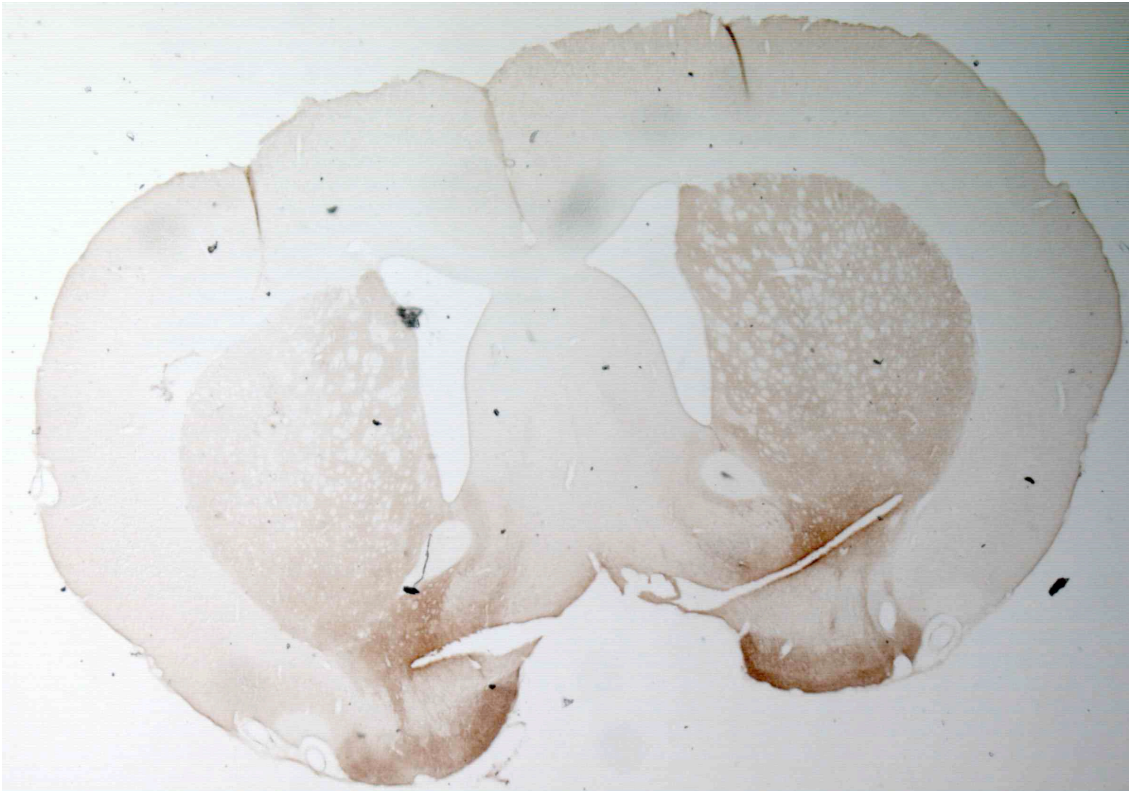
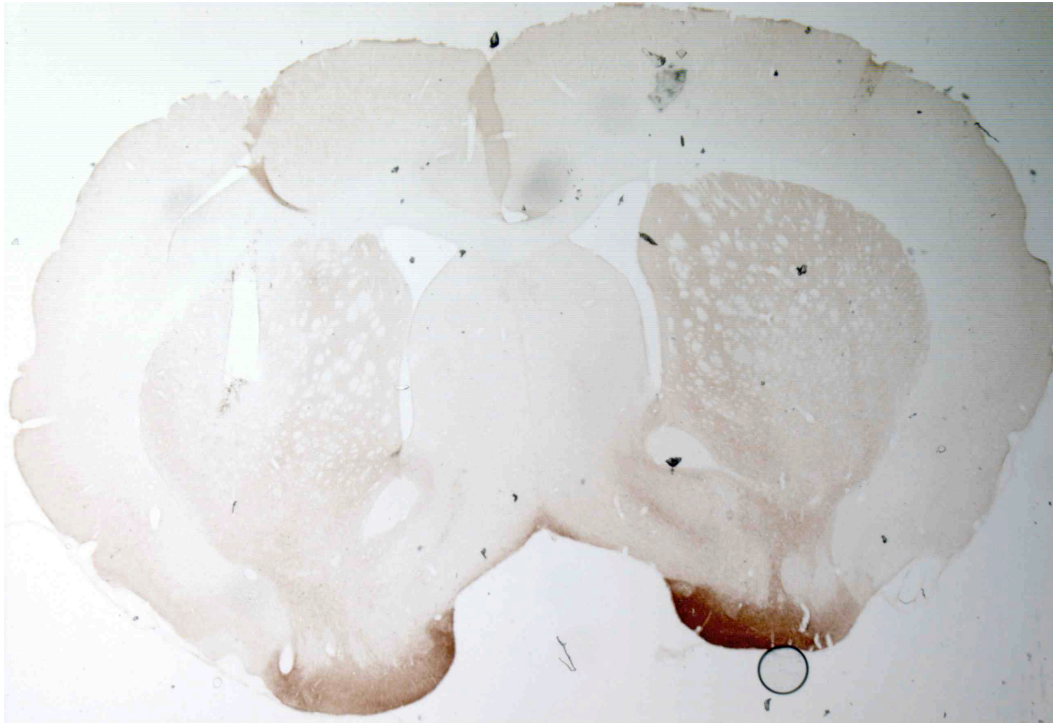*Appendix*

Anti-Tyrosine Hydroxylase Histology Images.

Animals with effective dorsolateral lesions of the striatum – Included in study
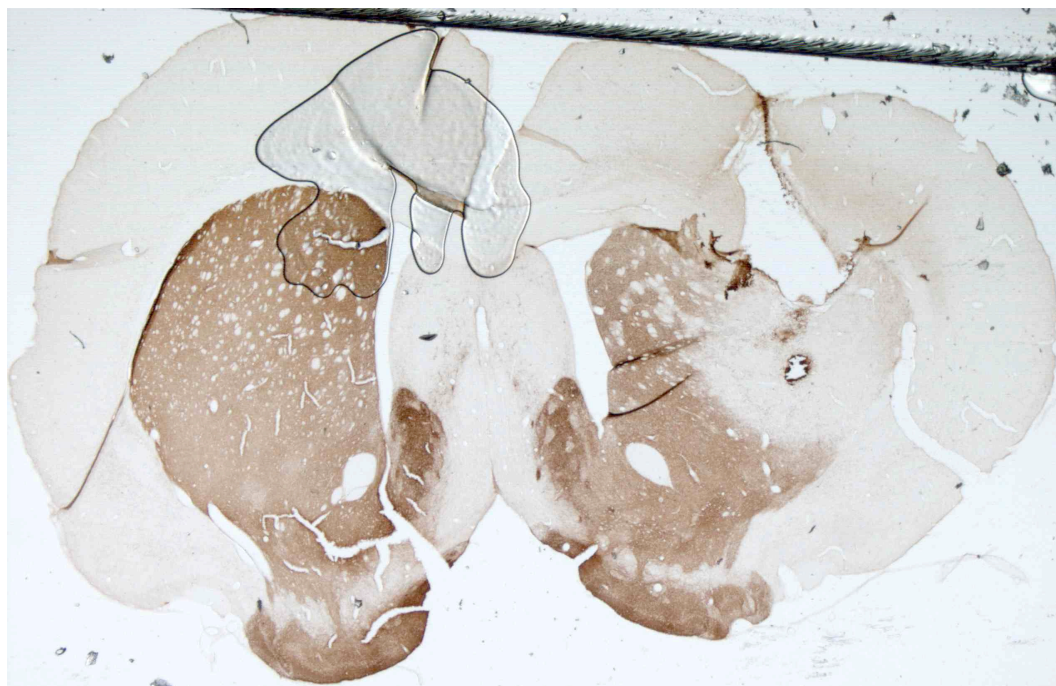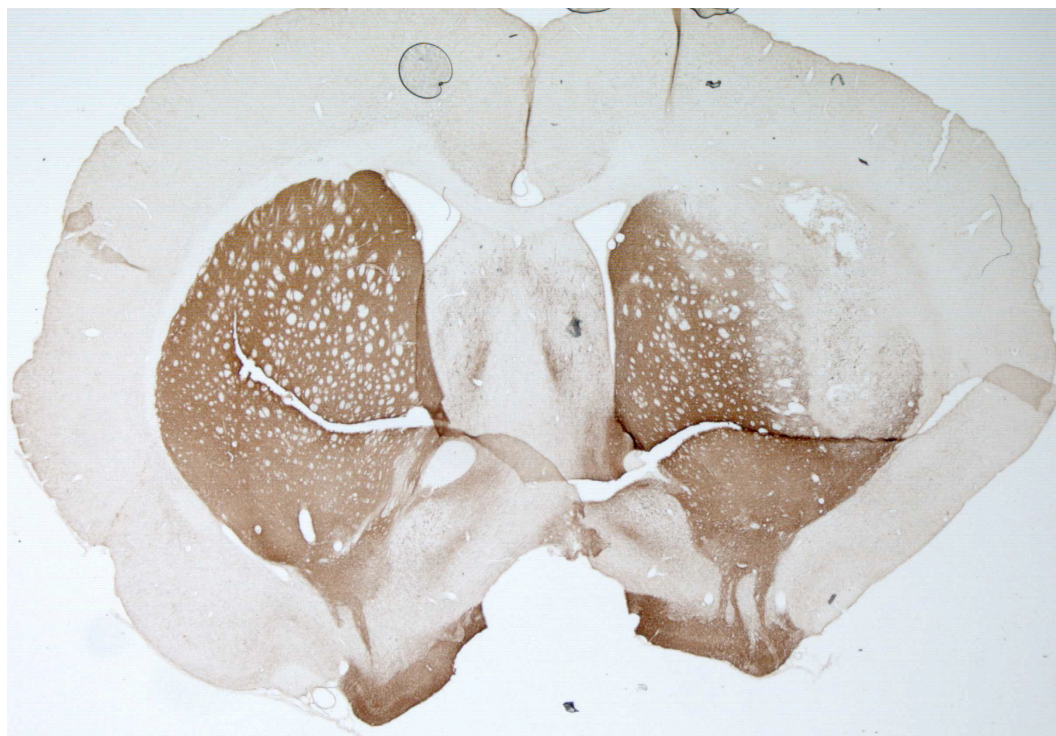
Animals with weak or inappropriate dorsolateral lesions of the striatum – Excluded