Behavioral treatment of rotational behavior in the rat model of Parkinson’s disease

Parkinson’s disease (PD) is a neurodegenerative disorder that mainly affects individuals 50 years and older. Degeneration is generally characterized by a loss of 80 percent or more of the dopaminergic neurons in the substantia nigra (SN) of the basal ganglia (BG), which is comprised of the SN, putamen, caudate nucleus, subthalamic nucleus, and globus pallidus that all work to control motor activity (1; 5). This degeneration leads to a decreased stimulation of dopamine (DA) receptors in the BG. The SN consists of the pars reticulata and the pars compacta, where the majority of the DA neurons are present. During the progression of PD, degeneration of dopaminergic neurons occurs in the SN along with an overall depletion of DA, leading to an under stimulation of the BG causing problems in both voluntary and involuntary movements (7). In post-mortem analyses it is evident that at least 50 to 70 percent of the DA neurons in the SN die (2).

The typical symptoms of PD include a resting tremor, rigidity of muscles and joints, postural problems, bradykinesia, and akinesia, which express in patients as problems with movement initiation and slowing of movement (2; 9; 7). Akinesia is thought to be caused by the disruption in the DA pathways from the SN pars compacta to other components of the BG (1). To treat these movement symptoms, doctors typically provide what is called a combination therapy, which consists of a mixture of L-DOPA and a DA receptor agonist. L-DOPA significantly reduces PD symptoms and the DA receptor agonist enhances the effects of DA in the synapse (7). However, in their review of L-DOPA, Marsden and Parkes outlined the negative side effects of long-term L-DOPA use, which include L-DOPA-induced dyskinesias (7). For this reason, researchers have moved away from this particular drug treatment to other more contemporary options.
For instance, Mohr et al. and Muller et al. provided a different approach to treating PD that is still being developed (9; 10). Mohr and colleagues set out to reduce PD symptoms through behavioral treatment. Participants followed relaxation training, training on personal motor problems, and training on social skills. Researchers successfully used positive and negative reinforcements to significantly improve these skills in the behavioral treatment group compared to the control group (9). In addition, Muller and collaborators applied positive reinforcement under a chain schedule in order to enhance posture in participants. After the 10 week experiment, subjects did not show a significant improvement in posture. However, researchers did find that subjects in the behavioral treatment group had a shorter latency when initiating voluntary movements compared to control (10). Although both studies administered L-DOPA and a DA agonist to all participants, they nonetheless support the effectiveness of a behavioral treatment and the suggestion of a shift to treatments that rely less heavily on drugs.

Today’s society is alarmingly dependent on drugs to cure and treat illnesses and disorders because it believes that they are fast and effective (3). However, there are other plausible treatments that could turn out to be beneficial to individuals and to society. Behavior modification techniques are used to acquire specific behavior, mainly by using contingency schedules to increase or decrease selected responses. These techniques have also been used to help people who suffer from traumatic brain injury (TBI) and schizophrenia (11; 15). Behavior modification is not only applicable to humans, but also to other animals, such as laboratory rodents.

A rat model of PD is achieved by chemically inducing a unilateral lesion of the SN (14; 4; 6), leading to a substantial loss of DA neurons similar to the depletion of DA neurons seen in humans with PD. Following this lesion, rats are administered direct or indirect DA agonists after
unilateral lesions to induce rotational behavior, which represents an antiparkisonian response (6; 4). Amphetamine and apomorphine are the most widely used DA agonists used to induce rotational behavior. These two drugs affect the same pathway but in two different ways.

Amphetamine is an indirect DA agonist, acting on the presynaptic neuron causing an increase in DA release and a decrease in DA re-uptake (12). The enhanced DA release stimulates the receptors on the postsynaptic neuron. This increase in activity is seen in the intact hemisphere because there are DA neurons at the non-lesioned side, typically resulting in rotation behavior ipsilateral to the lesion. On the other hand, apomorphine is a direct DA agonist, binding to the DA receptors. On the side of the lesion the receptors are supersensitive due to a chronic lack of DA. The binding between DA and these supersensitive receptors typically leads to a contralateral rotation (6; 4). Rotational behavior is widely used in the rodent model of PD as it has been demonstrated to be a reliable assay of large DA depletion in the SN.

Hypothesis

It was hypothesized that following the shaping (successive approximation) stage with constant reinforcement (CRF) schedule for rotational behavior, hemi-parkinsonian rats would show a higher number of correct rotations under the CRF with a low dose of d-amphetamine (0.56 mg/kg) when compared to the CRF or the higher dose of d-amphetamine (1.78 mg/kg) alone.

Methods

Subjects and design

Male Sprague-Dawley rats (N= 8, 100 days old) were housed individually with food and water ad libitum, had a 24 hour light-on schedule, and were fed labdiet P500 Prolab RMH 3000. Animals weighed between 380 grams (g) and 460g at the start of the experiment. They were
weighed each time they were handled ensuring a healthy weight. Each rat was trained to make full (360°) rotations. A repeated measures within-subject design was used where the number of rotations to the side of post-lesion amphetamine-induced bias was the dependent variable.

6-OHDA-induced PD

The rats were anesthetized using ketamine/xylazine, 70mg/kg and 6mg/kg respectively, (0.1mg/kg) injected intraperitoneally (i.p.) and placed in the stereotaxic apparatus. An incision was made along the medial line of each rat’s cranium. A small hole was drilled on the skull at -5.0mm anterior-posterior from bregma, 2.0mm lateral from the midline, and 8.0mm ventral from the surface of the brain. The mandible bar was set to -3.5mm. To create the lesion, 4µl of 6-OHDA (2.3mg/mL) was slowly injected with a microsyringe over the course of five minutes. The microsyringe was kept in place for an additional two minutes and was then slowly removed. Incisions were sutured and rats were returned to individual cages. To prevent infection, an antibiotic was administered daily for a week. Following recovery from surgery (10 days), d-amphetamine (1.78 mg/kg) was administered via i.p. injection in order to induce rotations, testing the effect of surgery.

Measurements

Before producing the unilateral lesion, rats were placed in a rotometer, a bowl shaped device to promote rotations with a harness, which attaches to the rat for recording rotations (See reference 14). Pre-lesion baseline was recorded for thirty minutes approximately five minutes following amphetamine administration. During this time, rats were placed in the rotometer and only full rotations emitted were recorded by the device and the observer. After lesion and recovery, post-lesion data were measured as described above and the rotational bias was noted.
(ipsilateral or contralateral to the lesion). Following post-lesion testing, rats were trained to rotate to the same side as the recorded post-lesion bias.

All rats were deprived of water for at least 24 hours (no longer than 35 hours) prior to training in order to establish a motivative operation. Rats were placed in the rotomor to begin successive approximation of behavior with positive reinforcement, a drop of water. This training was used in order to obtain full rotations to the same side of the previously recorded bias. Rats were reinforced for behavior that resembled the targeted final behavior more and more closely, starting with a slight turn and successively resulting in a full rotation. Training was implemented for 17-one hour sessions. Once the behavior was acquired, it was maintained under a CRF schedule. Following the 17 sessions, the number of rotations was tested under three conditions: CRF; CRF and low dose of d-amphetamine (0.56 mg/kg); and d-amphetamine (1.78 mg/kg). The rotometer was cleaned at the end of each session or test.

Histology

After the completion of the experiment, rats were be euthanized using 1cc Euthasol and perfused with formaldehyde (in 0.9% saline). Following removal and freezing of brain, 50 micronthick sections were taken using a sliding microtome and every fourth section was mounted on a frosted slide. A Nissl stain was done to view the density of cells present in the SN of both hemispheres. The SNs of both hemispheres were photographed using a Nikon Eclipse E600 microscope and a SPOT camera.

Statistical analysis

Two dependent-sample t-tests were done to determine the effect size of the lesion and the effect of the behavioral training on rotational behavior. Although all rotations were recorded, only the number of correct rotations was used for analysis. A correct rotation was defined as one
having the same direction as the bias established after surgery. This bias was demonstrated by a
difference of more than ten rotations to one side. A one-way within subject ANOVA was done
using SPSS. Mauchly’s test of sphericity was run to test for homogeneity. Alpha level was set at
p = 0.05. Rats that showed a reversed rotational bias before and after the 17 sessions were
excluded from the ANOVA test. A qualitative analysis was done of the histology.

Results

The 6-OHDA lesion was effective in producing biased rotations in all rats except one,
t(6) = -3.9, p < 0.05 (Table 1). The effect size of surgery on the number of correct rotations, as
measured by $r^2_{pb}$, was 0.71. The post-lesion amphetamine test demonstrated that seven of the
eight rats showed a counter clockwise preference while one rat, Rat 5, had a clockwise rotational
bias (Table 1). Six rats exhibited a preference seen as a response difference of 12 or more to one
side. Rat 4 had a difference of six and this difference was recorded as the bias, this data was
excluded from the pre and post lesion effect t-test. There was no significant difference in the
number of correct rotations before and after the 17 training sessions when administered
amphetamine (1.78mg/kg) alone, t(5) = -0.19, p > 0.05.

Figure 1 illustrates the acquisition and maintenance stages of rotational behavior for each
rat, along with results of the final test conditions. Although rats 2, 5, 6, and 7 began emitting
correct responses on different days, they reached a similar level by Day 17 (Figure 1). Under the
CRF test, these four rats maintained a similar level of responding to that acquired by Day 17,
while the other half had low levels of responding (Figure 1). When rats were tested under the
CRF and amphetamine (0.56mg/kg) condition, all but Rat 3 emitted a high number of correct
responses (Figure 1). This test condition provided the greatest number of correct rotations out of
the three test conditions. In the final amphetamine test (1.78mg/kg), four rats maintained their
original post-lesion bias (Rat 1, Rat 2, Rat 6, and Rat 8), two rats did not respond at high rates and did not have a strong preference to one side (Rat 4 and Rat 5), and two rats reversed their preference of rotation (Rat 3 and Rat 7).

Mauchly’s test of sphericity allowed for the assumption that there was homogeneity across test conditions, sig. = 0.99. There was a significant difference in the number of correct rotations produced by the different test conditions, F (2, 12) = 8.4, p < 0.05. It was found that the CRF with low amphetamine dose test (M = 64.4; SD = 26.7) led to significantly more correct rotations than either the CRF (M = 17.1; SD = 15.4) or the amphetamine (M = 31.4; SD = 20.3) tests alone (Figure 2). The CRF and amphetamine tests did not differ from one another, t(5, 10) = -1.24, p > 0.05 (Fisher’s t-test). The relationship between test conditions and correct rotations was low, eta^2 = 0.47. The right SN of Rat 1, Rat 2, Rat 3, Rat 5, Rat 6 and Rat 8 seemed to be less densely populated with cell bodies stained with thionine (a Nissl stain) (Figure 3, Rat 1 shown). There were no noticeable differences in the SNs of Rat 4. Also, Rat 2, Rat 6, and Rat 4 showed signs of necrosis in the right hemisphere (Not shown).

Discussion

Combination therapies using a DA agonist and a behavior modification program have led to successful improvements in the symptoms of PD patients (9; 10). The results of the present study could add to the body of research of therapies for PD. The combination of CRF and low dose (0.56mg/kg) of the psychomotor stimulant led to significantly more correct rotations than either the CRF schedule or the amphetamine (1.78mg/kg) alone, p < 0.05 (Figure 2). In addition, the rotational bias for each rat was expected to be ipsilateral to the lesion (clockwise or left); however, seven of eight rats showed a contralateral (counter clockwise or right) rotational bias.
(Table 1). The bias was considered to be an antiparkinsonian symptom regardless of direction because it implied that an imbalance in DA was present.

Interestingly, the qualitative analysis of the Nissl staining showed that though some rats showed a marginal decrease in cell density in the right SN, there was no noticeable difference in the other rats. It is possible that the targeted area for the 6-OHDA injection was missed decreasing the damage done to the DA system. Unlike the present study where Nissl staining was done, Ungerstedt used a fluorescent histochemical analysis to observe the DA neuron population remaining after lesion (13). It would have been useful to use a specific DA marker, such as an antibody, instead of a Nissl stain, as it is not selective for the different types of cells. Immunohistochemistry would provide a more detailed histology. In a future study, this selective histology could aid in further assessment of the degeneration in the SN and connecting nuclei.

Positive reinforcement has been studied in humans in the clinical setting (11; 9; 10; 15). Behavior modification has been shown to decrease or extinguish disruptive and aggressive behavior in schizophrenia and TBI patients more effectively than punishment (11; 15). In a case study, Watson and colleagues applied a differential reinforcement of lower rates schedule on a TBI patient and they successfully reduced the frequency of aggressive behavior. They were also able to decrease the dose of Clopixol, an antipsychotic drug, which the patient was taking (15). These findings suggest that behavioral interventions can, in some cases, be applied to modify behavior and as a secondary effect, decrease dependence on pharmaceutical treatments. The results of the present study are in agreement with human research (Figure 1; Figure 2). Behavior is controlled by contingencies and changes of behavior caused by shifts in these contingencies can lead to changes in brain chemistry. The lowered administration of Clopixol in the TBI
patient could be explained through these changes in contingencies, but further research is needed to support this suggestion.

The results provided by Watson and colleagues should be considered when making decisions of treatments for other disorders or diseases such as PD, as the research on long-term L-DOPA administration has suggested deleterious effects (15). For example, Marsden and Parkes reported that after long-term use of L-DOPA some patients begin to experience shorter time periods of the positive effects of the drug (the “on” period). Although the continued neurodegeneration is partially responsible for this trend, repeated use of L-DOPA may also play a role in inducing dyskinesia and other negative effects (7). In addition, in a study of short and long-term effects of L-DOPA on the 6-OHDA rat model of PD, researchers found that rats chronically treated with L-DOPA had smaller increases of DA than control rats when administered the drug. Melamed and Hefti concluded that the attenuation in increases of DA were partially due to the decrease in conversion of L-DOPA to DA (8). These findings suggest that as the positive effects of L-DOPA decrease, the doses must be increased to maintain constant DA levels.

In light of the research regarding the decreasing positive and increasing detrimental effects of long-term L-DOPA administration (7; 8; 4), it is important to consider other treatments. In the present study, a combination of a CRF schedule and low dose d-amphetamine (0.56mg/kg) administration led to the highest number of correct responses, that is to say, the most antiparkinsonian responses (Figure 2). These results are consistent with Mohr et al.’s and Muller et al.’s conclusions that behavior modification can improve motor and social skills and clinical PD symptoms (9; 10). It would not be ethical to completely remove L-DOPA from a PD patient; however, the results of the present study suggest that a lower dose could have a
sufficient stimulating effect in newly diagnosed PD patients. The behavior modification component could become the major player in treatment of this neurodegenerative disease.

More research is needed on the short-term and long-term effects of a combination treatment comprised of behavior modification techniques and low starting dose of L-DOPA. Of importance would be the dose-response curve of L-DOPA and the time course for readjusting the doses. It would also be of interest to see what brain anatomy and chemistry changes occur in response to the behavioral changes. Based on the results of the present study and the implications of the study by Watson et al. (15), if the negative effects of L-DOPA and the continued degeneration of DA neurons can be attenuated by changes in drug administration and in behavior, respectively, PD patients may have a longer life expectancy with a greater level of autonomy. Finally, though this study concentrated on PD, the effects of behavior modification are not limited to this neurodegenerative disease, as behavior modification techniques have already been successfully applied to individuals with schizophrenia and TBI (11; 15). Continued research on this type of treatment would be beneficial to society in helping it deviate from drug reliance.
Table 1. The number of amphetamine-induced (1.78mg/kg) rotations post-lesion per direction.

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<thead>
<tr>
<th>Rat</th>
<th>Left</th>
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<td>1</td>
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<td>2</td>
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<td>11</td>
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<td>8</td>
<td>32</td>
<td>1</td>
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Figure 1. The number of correct responses for all rats across the 17 training days and on the three testing conditions: CRF, CRF and low amphetamine (0.56mg/kg), and amphetamine (1.76mg/kg).
Figure 2. The mean number correct rotations post-training under each testing condition with standard error bars. Pre-training represents number of correct rotations following amphetamine (1.78mg/kg) administration.

Figure 3. Photomicrographs of Nissl stained sections of the left and right SN of Rat 1 (10X). On the left is the left SN, while on the right is the right. Dorsal-ventral (D-V) and lateral-medial (L-M) orientations are shown.
References


