The Effects of 6-OHDA Lesioning on Handedness in Rat Models of Parkinson's Disease

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Abstract

Parkinson's disease (PD) is a neurodegenerative disorder affecting the dopamine (DA) system. A common model used to imitate PD in rats is the 6-hydroxydopamine (6-OHDA) lesion model. This neurotoxin causes neuronal atrophy similar to that of late-stage PD, when 80% of DA neurons in the substantia nigra have died off. The present study aimed to see how the inclusion of handedness as a factor may affect lesion expression in 6-OHDA models of PD. The forepaw adjusting steps test (FAS) and the vibrissae elicited paw placement test were used to determine normal paw use for each rat. The vermicelli handling test and the grip strength racquet test (GSR) were used pre-lesioning to determine the subjects' innate dominant sides. The rats were randomly divided into four lesion groups based on their determined side dominance. All four tests were then reflected post-lesioning. FAS and vibrissae were used to examine motor deficits caused by the lesioning while GSR and vermicelli were conducted to examine any possible changes in paw preference or grip strength that lesioning could have cause. The present study determined that handedness is not a confound affecting expression of PD. Handedness, however, may have other applications in rat models of PD so this factor should not be ignored.

The Effects of Lesioning on Handedness in the 6-OHDA Rat Model of Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder caused by depletion of the neurotransmitter dopamine (DA). As a result, DA loss affects motor function. Some motor symptoms caused by PD are: akinesia, bradykinesia, tremor, gait disorders, postural instability, and falling (Bezard et al., 1997). In many cases, the onset of PD occurs unilaterally in the dominant hemisphere of the brain (Yust-Katz et al., 2008). This (and lateralization of the brain) explains why early symptoms are seen on the side of the body contralateral to the dominant hemisphere. As it progresses, the disorder becomes more bilateral and affects both sides of the body (Yust-Katz et al., 2008). It is possible that the motor symptoms could be overlooked if symptoms of the disorder are seen first in the dominant side. Even with deficits and neuron loss the dominant side may be stronger than the non-dominant side, which is why the disorder may be misdiagnosed until it progresses and becomes more bilateral.

Rats, like humans, exhibit side dominance. There is a population-wide right side preference in rats but female rats are more commonly left-side dominant (Tang & Verstynen, 2002). Handedness should be considered when studying rat models of PD, as the same effects seen in humans are likely seen in laboratory models. But this factor is still overlooked. Currently, rat models of PD are created by unilaterally lesioning the medial forebrain bundle (MFB) with 6-hydroxydopamine (6-OHDA) to cause DA neuronal atrophy (Chang et al., 1999). Because the volume of neurotoxin used to create bilateral lesions can be lethal, unilateral lesions are used. These lesions are beneficial because they allow researchers to compare results within subjects before and after lesioning. It is also easier to see asymmetry within subjects caused by the lesion. This study created DA lesioning in the MFB in the side ipsilateral or contralateral to the

dominant side of the body to see the effects of handedness on lesion expression. Tests were conducted to determine the subjects' prior handedness, to establish baseline behavioral norms, and -after lesioning- to test for motor deficits. It was expected that most of the subjects would show a left-side preference. It was also expected that the rats with lesioning contralateral to the dominant side would show the greatest motor deficits. This is because motor pathways in the dominant hemisphere are stronger and more numerous, so loss of these motor pathways would cause more deficits (Pool et al., 2014). This study sought to see how handedness factors into motor deficits in rat models of PD so more accurate rat models of PD can be used in the future.

Methods

Animals

In this study, 14 female Sprague-Dawley rats were used. These rats were bred on site at Binghamton University and were housed separately until postnatal day 21, when they were pair-housed in 42 cm x 25 cm x 21 cm cages. At the start of the experiment, rats weighed between 200 and 300 grams. There was free access to food and water. The rats were kept in the dark for 12 hours of the day and in the light for the other 12 hours, starting at 7 AM; the colony room temperature was kept at approximately 22°C. Animals in this experiment were treated humanely as mandated in the Institutional Animal Care and Use Committee of Binghamton University's guidelines as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals' guidelines.

Surgery

Seven weeks into the testing period, all subjects received a unilateral lesion in the MFB using 6-OHDA (12 μg in 4 μL total volume). According to Paxinos & Watson (2005), lesioning

occurred at the coordinates AP: -1.80mm, L: ±2.00 mm, V: -8.60 mm. The study was between subjects, so the hemisphere of the lesion was dependent upon what group the rat was randomly assigned to. Rats determined to be left-dominant were lesioned in the dominant or non-dominant hemisphere, while ambidextrous rats were lesioned in the left or right hemisphere. The analgesic used before and after the surgeries was 0.03 mg/kg of Buprenex (injected subcutaneously). An intraperitoneal injection (0.1 ml/kg) of a ketamine (83 mg/kg)/Xylazine (17 mg/kg) was used as the anesthetic in these surgeries. The rats were allowed 13 days to recover with access to diet gels to supplement and aid the recovery process.

Procedures

This experiment examined the effects of handedness on MFB lesion expression and symptomatology. Two tests were conducted to establish prior handedness and to see effects on behavior after lesioning. Testing began after 1 week of habituation to handling. All tests were conducted before surgery to establish a baseline and to establish each rat's dominant side. Tests were mirrored after surgery to determine lesion efficacy and to see the effects of lesioning on handedness.

Rats were first habituated to handling. Habituation and baseline began the second week of testing. Surgery occurred 7 weeks into the study, with 13 days allowed for recovery after surgery. Testing after the recovery period determined the effect of the lesion and if the lesion group the rats were assigned to (lesioning in dominant or non-dominant hemisphere; see Table 1) had any effect on motor deficits. The timeline followed in this study is outlined in Figure 1.

Forepaw adjusting steps test

The forepaw adjusting steps (FAS) test was modeled after Chang et al. (1999). This test was used to see the effects of lesioning on steps taken by a rat's forepaw when dragged across a

table. The equipment used included leather gloves, a table, and a timer. The rat was held above a table so only one forepaw had free range of motion. The rat was held at a 45° angle with the table with its weight being bore on the free forepaw. The tester then dragged the rat a distance of 90 cm in 10 seconds. Each trial consisted of dragging the rat laterally in both directions so steps are taken in the forehand (towards the body) and the backhand (away from the body) direction.

Each step taken by the rat was recorded. There were 10 trials total, 5 for each paw. There were 2 habituation days, 3 pre-surgery testing days, and 3 post-surgery testing days. Data was expressed as average percent intact steps.

Vibrissae elicited paw placement test.

The vibrissae elicited paw placement was used in this study to compare sensorimotor function before and after lesioning. The protocol followed was modeled after the procedures from Anstrom et al. (2007). This test was conducted by holding the rats off the table and restricting movement of the two hindpaws and one forepaw. The forepaw being tested was brought into contact with the table before recording data. This is done for the rat to become aware of the surface's location after being lifted. The tester then made circular motions with the rat so the vibrissae were perpendicular to the edge of table. The vibrissae ipsilateral to the forepaw being tested were brushed against the edge of the table 10 times. The number of paw reaches in response to the sensory input was recorded. This was done 3 times to each set of vibrissae for a total of 30 attempts per side.

Before surgery there were 3 days in testing days that followed 2 habituation days. The test was also conducted for 3 days post-surgery. Scores taken from this test represented only the successful attempts to reach the table with the forepaw ipsilateral to the vibrissae coming in

contact with the table. These scores help to see motor deficits and paw use asymmetry. Scores are represented as percent intact paw placements.

Grip strength racquet test.

The grip strength racquet test (GSR) conducted to determine handedness in this experiment was a novel way of examining grip strength and hand dominance in rats. The only equipment required for this test was a standard tennis racquet and a table. This test was conducted by first training rats to grasp to the strings of a tennis racquet with both forepaws. The tennis racquet was then lifted from the table, leaving 1 inch between the rat's hindpaws and the surface. Eventually the rat let go of the tennis racquet with one paw, and whichever paw remained gripped to the tennis racquet last was said to be dominant.

There was 1 day of habituation and 3 days of testing prior to surgery in which 5 trials were conducted. This test was also conducted post-surgery to determine if the lesion had an effect on paw preference. Data for this test is expressed as the amount of times each paw is determined to be the dominant paw.

Vermicelli handling test

The vermicelli handling test can also be used with handedness in rats, following protocol from Tennant et al. (2010). This test examined the effects of lesioning on paw preference. The materials necessary for this test were: a table, a camera, vermicelli pasta, and a mirror. The pasta pieces all had the same diameter and were cut to 6 cm lengths and marked every 1 cm. Markings on the pasta help make viewing and scoring the test easier for experimenters. A mirror was placed behind the rat during testing to see the rat from as many angles as possible.

The pasta was held in front of the rat so the rat could grasp it. If the rat did not take the piece of pasta from the tester's hand, it was placed on the floor of the testing container (a 21 cm

diameter Plexiglass cylinder or the rat's home cage). Typical handling involved a grasping paw (which initially grasped the pasta and was held lower on the pasta length to ensure the rat had control of the pasta) and a guiding paw (which was placed above the grasping paw on the pasta and was used to direct the pasta into the rat's mouth). It was considered atypical for the handling and guiding paws to switch to the other paw; other atypical results include: placing paws together when pasta was longer than 3 cm, failure to move paws symmetrically when pasta was shorter than 3 cm, pulling pasta with the mouth rather than the paws, failure of 1 paw to make contact with the pasta for an extended period of time, hunched posture, and dropping the pasta. Atypical results lead to failed trials and results were not recorded.

Due to the nature of testing, all procedures were recorded. Rats were habituated to this test with the cameras present as well. Video scoring was conducted to ensure accuracy of live scoring.

There were 3 pre-surgical testing days with 5 trials each day. One piece of pasta was given to the rat per each trial. The same protocol (3 testing days with 5 trials each day) were followed for testing after lesioning. Scores from this test are expressed in the number of times each paw is the grasping (dominant) or guiding (non-dominant) paw.

High performance liquid chromatography.

Once testing was completed, rats were sacrificed. The brain was then removed and the MFB was dissected. High performance liquid chromatography (HPLC-ED) was used to analyze the amount of DA present in the brain, as seen in Ostock et al. (2015). The striatum was homogenized in 0.1 M perchloric acid with 1% ethanol and 0.02% EDTA. This solution was spun in a centrifuge for 45 minutes. The liquid from the top of the solution was collected and

analyzed with HPLC-ED. This was used to verify the lesioning conducted by comparing standard DA concentrations to the DA concentration in this brain sample.

Statistical Analysis

Percent intact paw placements in this case were calculated by dividing the paw placements of the paw affected by the lesion by the placements of the paw unaffected and multiplying this quotient by 100 to form a percentage.

The statistics run on results from testing were 2-way mixed design ANOVAs and Fisher LSD post hoc tests to see results between subjects (result differences between groups depending on dominance) and within subjects (result differences before and after lesioning for individual rats). These tests were run on Statistica 13 with an alpha level of 0.05.

Results

The vibrissae elicited paw placement and FAS tests were conducted before and after lesioning to determine a baseline and lesion efficacy. There were four lesion groups: left side dominant rat with lesioning in the left hemisphere of the brain (non-dominant hemisphere lesioning; ND), left side dominant rat with lesioning in the right hemisphere (dominant hemisphere lesioning; Dt), ambidextrous rat with lesioning in the left hemisphere (AL), and ambidextrous rat with lesioning in the right hemisphere (AR). 2(timing) x 4(lesion type) mixed design ANOVAs with an alpha level of 0.05 were conducted using Statistica 13 to test for difference in paw use before and after surgery and to prove lesion efficacy. As seen in Figure 2, the ANOVA showed that there was no interaction between timing and lesion location for the vibrissae elicited paw placements test [$F(3,10)=.59874$, $p>0.05$]. However, there was a

significant effect of timing on vibrissae elicited paw placements [F (1,10)=35.309, $p<0.05$], as seen in the decrease in paw placements.

Percent intact values were also calculated before and after lesioning for FAS. A 2 (timing) x 4 (lesion type) mixed design ANOVA, which is shown in Figure 3, determined there was no significant main effect of lesion on paw use [F(3,10)=.93609, $p>0.05$], nor an interaction of timing and lesion on paw use [F(3,10)=2.6676, $p>0.05$]. There was a significant main effect of timing on paw use [F(1,10)=72.559,$p<0.05$], as seen in the decrease in percent intact across all groups. Fisher LSD post hoc testing showed a significant statistical difference within the Dt, ND, and AL groups before and after lesioning.

The vermicelli handling test and the grip strength racquet test were both used to establish if the subjects exhibited handedness and if so, what their side preference was. Figure 4 shows the determined side preferences for each rat and what lesion group they were randomly divided into.

The vermicelli handling test was used to determine paw preference in the rats. A 2(timing) x 4(lesion type) x 2(paw) mixed design ANOVA was run to determine if lesion location affected handedness. Figure 5 shows the significant main effect of lesion code on paw preference [F(3,10)=3.9740,$p<0.05$] and the interaction between timing and lesion code on paw preference [F(3,10)=4.1631,$p<0.05$]. Fisher LSD post hoc testing showed a significant difference before and after lesioning within the ND group as well as significant differences between AR and AL groups, Dt and AL groups, AR and ND groups, and Dt and ND groups.

The GSR test was used to determine paw preference by examining grip strength in rats. A 2(timing) x 4(lesion) mixed design ANOVA was run on data from this test. Figure 6 shows the significant main effect of lesion code [F (3,10)= 25.99, $p<0.05$]. There was also a significant

interaction between timing and lesion code [$F(3,10)= 4.03$, $p<0.05$]. There was no main effect of timing [$F(1,10)= 0.20$, $p>0.05$]. The Fisher post-hoc showed differences within the ND group before and after lesioning as well as significant differences post-lesioning between Dt and AL groups, Dt and ND groups, Dt and AR groups, and AL and ND groups.

Discussion

The goal of this study was to prove if handedness is a confounding variable to be considered in rat models of PD. It was hypothesized that unilateral 6-OHDA lesioning in the MFB would cause decreased use of the paw contralateral to the lesioned hemisphere.

The decrease in percent intact values from the FAS and the vibrissae elicited paw placement test proved that the lesions were effective. The lesion-affected paws were used less in all groups, meaning the lesions successfully created motor deficits similar to those in PD.

The vermicelli handling test and the grip strength racquet test (GSR) conducted before lesioning determined that rats can have side preferences, as seen in Güven (2003). The majority of the all-female rat sample in this study exhibited left side dominance (n=9), in accordance with previous research (Tang & Verstynen, 2002). Some rats (n=5) in the sample exhibited ambidexterity. Regardless of prior handedness, however, both groups with lesioning in the left hemisphere showed a decrease in left paw use. It was predicted that lesioning in the dominant hemisphere would lead to decreased use of the dominant paw (and vice versa for the ND group). This was supported by the GSR test. This test showed a switch in the paw that held on the longest. The vermicelli handling test results, however, refuted the hypothesis.

In the vermicelli handling test, an increase in dominant paw use was seen in the Dt group and a decrease in dominant paw use was seen in the ND group. The increased use of the lesion-

affected paw may indicate a neuroprotective effect due to high concentration of motor pathways in the dominant hemisphere (Nithianatharajah et al., 2009). If there are greater motor pathways in the dominant hemisphere, the injection of a toxin would cause neuronal atrophy but not at a level high enough to cause results as drastic as expected. Freezing and rigidity are common side effects of simulated PD models in rats (Duty & Jenner, 2011). However, the Vermicelli handling test failed to account for these symptoms. This freezing behavior may have caused false successful trials, causing testers to misjudge the test and state that a frozen or rigid paw was dominant. It is also possible this may not test fine motor movement. It may be feasible for a rat with a lesion-affected dominant paw to still use the dominant paw to grasp pasta, causing a misinterpretation of paw use.

Because the rats in this study were all female, future studies in this field should be conducted on male and female to account for possible sex differences in handedness (i.e. male rats have a population right preference, while female rats have a population left preference; Güven et al., 2003). It is also possible that the hemisphere of PD onset in humans could differ based on sex. Future samples should also be larger, as n=14 is a low value that may not accurately represent the rat population. Levodopa or other treatments for PD in humans should be used in future research to see how treatment and PD affect handedness in conjunction with one another, as these treatments could cause a reversal effect.

This research proved that handedness is not a confound because all rats performed worse after lesioning on FAS and vibrissae, which are tests that measure severity of PD lesioning. This does not mean, however, that handedness is not a factor to be considered in future studies of PD.

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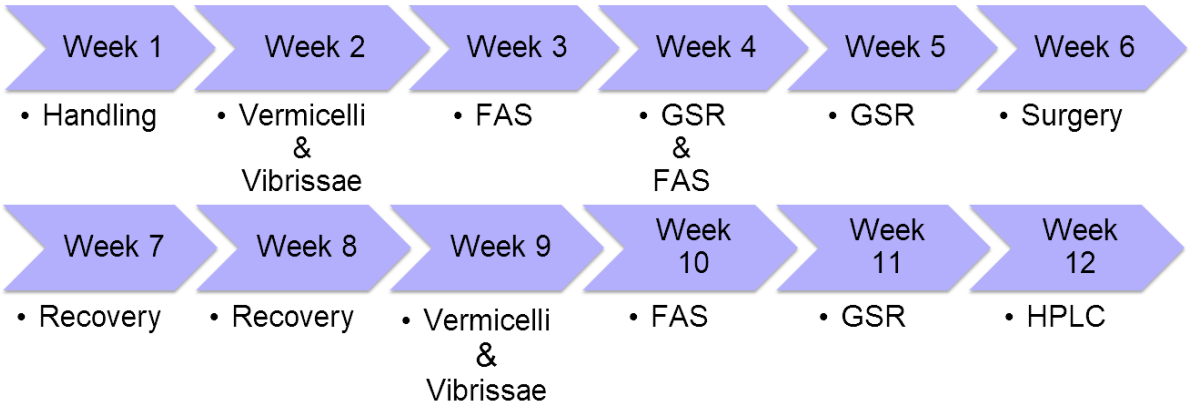

Figure 1. Timeline of procedures. Illustrated here is the timeline of procedures followed during this study. Rats were handled for 1 week prior to the 5 week habituation and baseline testing period. There were 13 days post-surgery. Testing continued for 4 weeks after lesioning, mirroring the pre-lesion testing order. Tests were conducted to either determine handedness or show the effect of lesioning. Rats were sacrificed upon completing testing and HPLC was conducted to verify lesion efficacy.

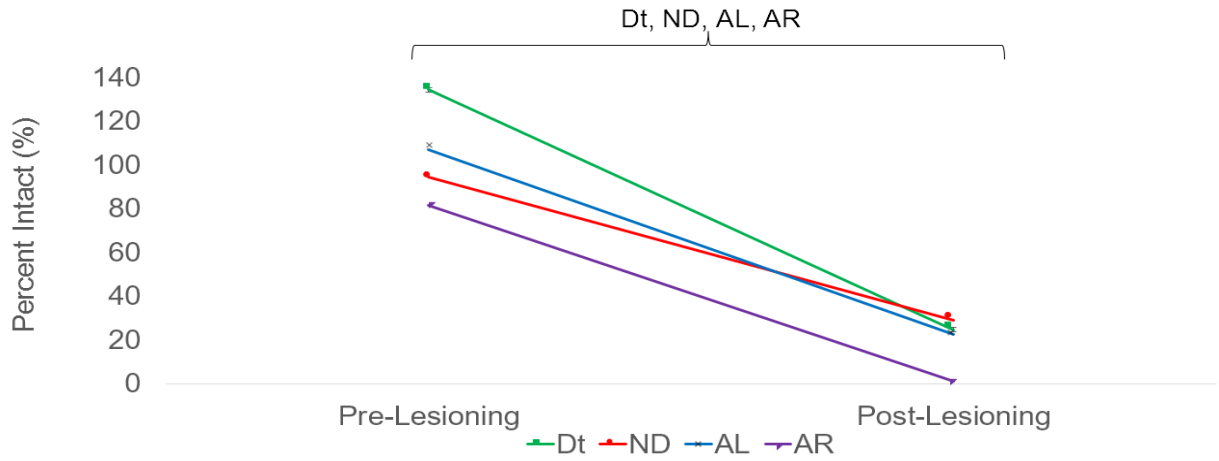

Figure 2. Vibrissae elicited paw placement average percent intact values before and after lesioning. Shown here is the average percent intact paw placements on the vibrissae elicited paw placement test across all testing days. Testing was conducted on female Sprague-Dawley rats (n=14) for 1 day of habituation, 2 days of baseline pre-lesioning, and 3 days post-lesioning. There were 4 lesion groups: left dominant rats with lesioning in the right hemisphere of the brain (Dt; n=4), left dominant rats with lesioning in the left hemisphere (ND; n=5), ambidextrous rats with lesioning in the left hemisphere (AL; n=3), ambidextrous rats with lesioning in the right hemisphere (AR; n=2). There was a significant main effect of lesioning on paw placement ability, causing a decrease in paw placements [$F(1,10)=35.309, p<.05$].

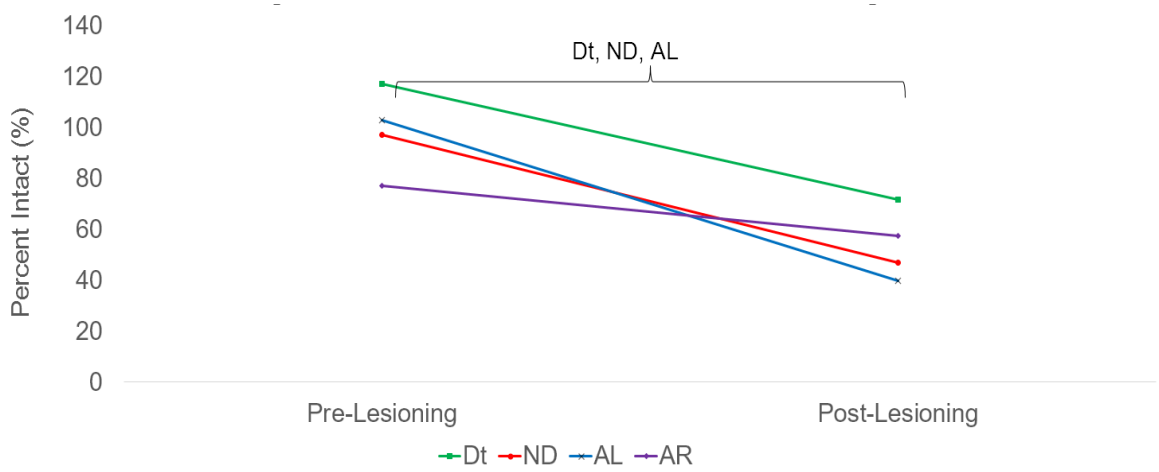

Figure 3. Forepaw adjusting steps average percent intact values before and after lesioning. The forepaw adjusting steps test was conducted on female Sprague-Dawley rats (n=14) to establish baseline paw use and determine lesion efficacy. There were 4 lesion groups: left dominant rats with lesioning in the right hemisphere of the brain (Dt; n=4), left dominant rats with lesioning in the right hemisphere (ND; n=5), ambidextrous rats with lesioning in the left hemisphere (AL; n=3), ambidextrous rats with lesioning in the right hemisphere (AR; n=2). There was a significant main effect of timing on paw use [$F(1,10)=72.559, p<.05$], as denoted by the bracket.

Overall Paw Dominance and Lesion Categories				
Rat Number	GSR	Vermicelli	Overall Dominance	Group
1	L	L	L	ND
2	L	A	L	Dt
3	A	L	L	ND
4	L	L	L	Dt
5	R	L	A	AR
6	L	L	L	ND
7	R	L	A	AL
8	L	R	A	AR
9	L	R	A	AL
10	L	R	A	AR
11	L	L	L	Dt
12	A	L	L	ND
13	A	L	L	Dt
14	A	L	L	ND

Figure 5. Paw Dominance and Lesion Categories. Shown above are the dominance assignments for each female Sprague Dawley rat (n=14) based on the grip strength racquet and vermicelli handling tests. After baseline handedness determination (L meaning left dominant, A meaning ambidextrous, R meaning right dominant), rats were further divided randomly into lesion groups: lesioning in the non-dominant hemisphere (ND; n=5), lesioning in the dominant hemisphere (Dt; n=4), ambidextrous rats with lesioning in the left hemisphere (AL; n=2), and ambidextrous rats with lesioning in the right hemisphere (AR; n=3).

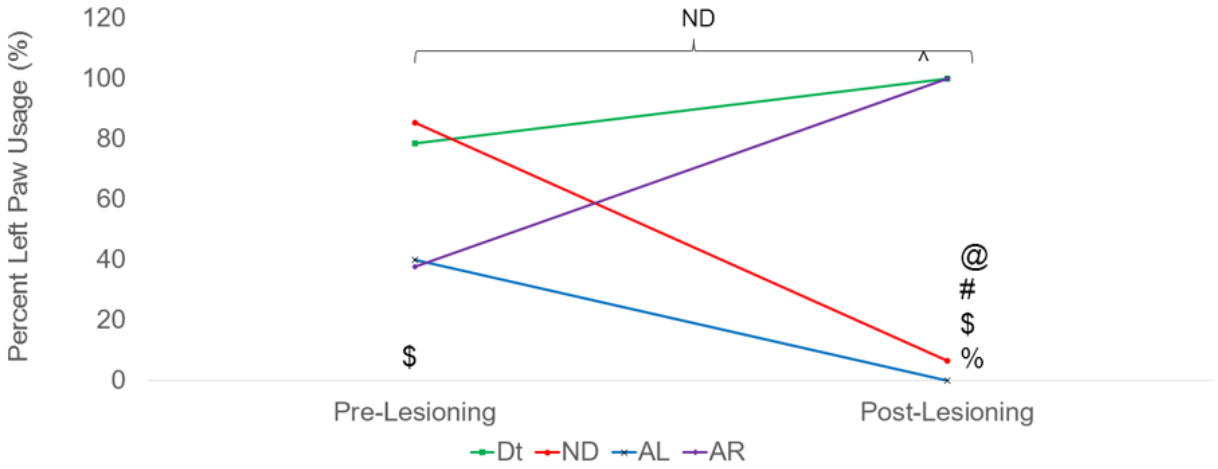

Figure 5. Vermicelli handling test average percent left paw usage before and after lesioning. The vermicelli handling test was conducted on female Sprague-Dawley rats (n=14) to establish baseline handedness and paw preference and handedness post-lesioning. There were 4 lesion groups: left dominant rats with lesioning in the right hemisphere (Dt; n=4), left dominant rats with lesioning in the left hemisphere of the brain (ND; n=5), ambidextrous rats with lesioning in the left hemisphere (AL; n=3), ambidextrous rats with lesioning in the right hemisphere (AR; n=2). There was a significant main effect of lesion type on percent left paw use [$F(3,10)=3.9740, p<.05$] and a significant interaction of timing and lesion code on left paw use [$F(3,10)=4.1631, p<.05$]. The symbols denote significant statistical differences between groups (@ denotes $p<0.05$ for AR vs AL, # denotes $p<0.05$ for Dt vs AL, $ denotes $p<0.05$ for AR vs ND, % denotes $p<0.05$ for Dt vs ND, ^ denotes $p<0.05$ for Dt vs AR, the bracket denotes within effects before and after lesioning).

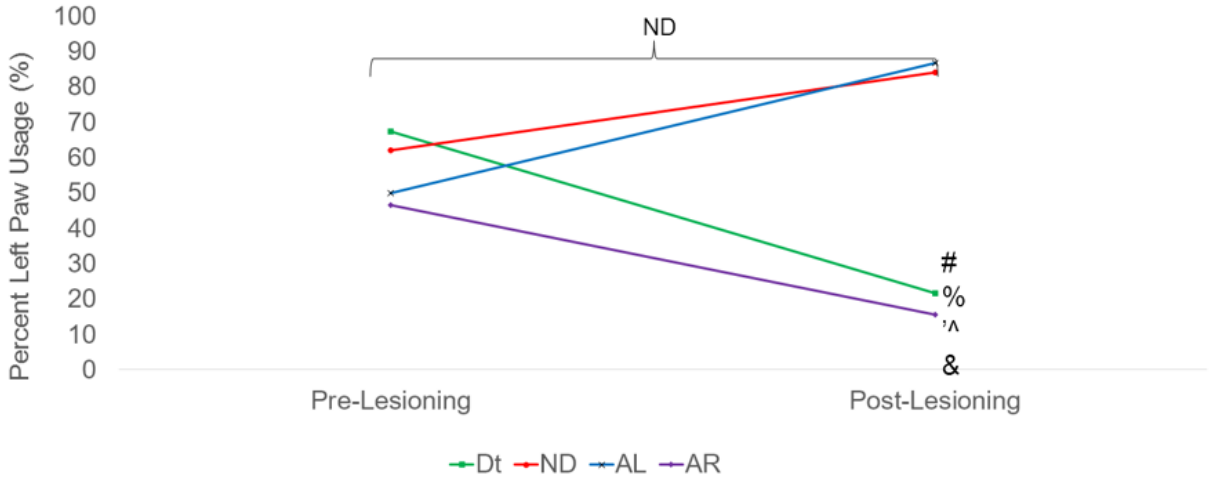

Figure 6. Grip strength racquet percent left paw use before and after lesioning. The grip strength racquet (GSR) test was conducted on female Sprague-Dawley rats (n=14) to determine baseline handedness by examining grip strength and to see changes in grip strength post-lesioning. The symbols denote significant statistical differences between groups (@ denotes $p<0.05$ for AR vs AL, # denotes $p<0.05$ for Dt vs AL, $ denotes $p<0.05$ for AR vs ND, % denotes $p<0.05$ for Dt vs ND, ^ denotes $p<0.05$ for Dt vs AR, & denotes $p<0.05$ AL vs ND, the bracket denotes $p<0.05$ within groups before and after lesioning).