2022

# Use of Forelimb Asymmetry in the Analysis of CNS Recovery From a Demyelination Event

Joseph C. Hinkle
*Wright State University*

# USE OF FORELIMB ASYMMETRY IN THE ANALYSIS OF CNS RECOVERY FROM A DEMYELINATION EVENT.

A thesis submitted in partial fulfillment of the
requirements for the degree of
Masters of Science

by

JOSEPH C. HINKLE

B.S.,Wright State University, 2019

2022

Wright State University

WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

November 4 2022

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Joseph C. Hinkle ENTITLED Use of forelimb asymmetry in the analysis of CNS recovery from a demyelination event BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

_____

Adrian M. Corbett, Ph.D.
Thesis Director

_____

Eric S. Bennett, Ph.D.
Chair, NCBP

Committee on Final Examination:

_____

Michael Matott, Ph.D.

_____

Christopher N Wyatt, PhD

_____

Adrian M. Corbett, Ph.D.

_____

Shu Schiller, Ph.D.
Interim Dean of the Graduate School

Abstract

Hinkle, Joseph C. M.S., Department of Neuroscience, Cell Biology, and Physiology, Wright State University, 2022. Use of forelimb asymmetry in the analysis of CNS recovery from a demyelination event.

Using forelimb asymmetry analysis along with Montoya Staircase methodology we tested how a fluoxetine simvastatin ascorbic acid drug cocktail would affect recovery from a demyelinating event in a rat model, with the hypothesis that if administered then it would help female recovery but hinder male recovery. It was found that a fluoxetine simvastatin and ascorbic acid drug cocktail did not significantly enhance recovery from a demyelination model injury in female rats, and that the same drug cocktail significantly slowed male rat recovery from the same type of injury. It was also apparent that the more effective methodology for investigating this model further would be the Montoya staircase. This was due to compensatory behavior masking the behavioral symptoms of white matter damage in forelimb asymmetry analysis.

Table of Contents

LIST OF FIGURES

## Introduction.

The goal of this experiment was to use forelimb asymmetry to analyze post injury recovery in a rat model after injection of a demyelinating agent near the corpus callosum. This demyelination was to simulate a Multiple sclerosis attack. Multiple sclerosis is an autoimmune disease that results from the immune system attacking the myelin sheaths in the central nervous system. The second goal of this experiment was to determine whether a combination of Compounds (consisting of fluoxetine, simvastatin and ascorbic acid) could assist in recovery following demyelination, and in addition would the recovery from demyelination vary between genders. Evidence shows that males tend to recover better from central nervous system injuries as testosterone has a neuroprotective effect. (1).

Our hypothesis based on the literature background and on prior work with this drug combination in ischemia models is that the female rats would have enhanced recovery when administered a regimen of Fluoxetine Simvastatin and Ascorbic acid, while the males would have reduced recovery with the same regimen

## Multiple Sclerosis

Multiple sclerosis (M.S) in humans is a disorder diagnosed via elimination; in other words, the diagnosis of Multiple Sclerosis is applied when no other disorder fits the symptoms. Features that can indicate M.S. as a possible diagnosis include a remission relapse pattern of the symptoms, as it is, other signs can vary but include headaches, partial blindness due to optic nerve demyelination, loss of proprioception, loss of ability to feel vibrations, pain and temperature loss and other symptoms

**Forelimb Asymmetry**.

Forelimb asymmetry (also known as the cylinder test) is a simple method of motor function analysis; in rats it utilizes normal rat exploratory behavior: when rats are in an environment with vertical surfaces they will rear up on their hind legs and using their whiskers and forelimbs to provide haptic feedback. (2) In a healthy rat the use of each limb should be around 50%. However in an injury model, the usage percentages of each limb can shift. This shift towards asymmetric use of limbs is the basis for forelimb asymmetry. Though some rats will have a dominant limb which is where baseline data and normalization come in. The fact that forelimb asymmetry uses exploratory behavior means that it does not require training of the animals before establishing a preinjury baseline. This leads to the advantage that every rat going through a procedure can be put through this test making large N values easier to obtain. It also has an advantage of being analogous to human behaviors that would be affected by brain injury, as the recording of which paw is used to support the rat's weight is like a human bracing a hand against something when getting up from a chair. This allows for the results to be more likely to translate from the animal model to a human subject. A downside of the forelimb asymmetry test is that it does not detect fine motor deficits as well as other tests such as the Montoya staircase test.

The use of forelimb asymmetry to test demyelination recovery is a novel use although forelimb asymmetry has been used to assess damage in stroke, Parkinson's models and traumatic brain injury(3, 4). The combination of drugs used in our study has previously been shown to help post stroke recovery via a mechanism of increased neurogenesis. Previous work in the lab also showed that differentiating between palm touches and fingertip touches showed significant difference in stroke recovery analysis.(5) Counting just the palms yielded a stronger correlation to the Montoya staircase results than if fingertip touches were included.

## Montoya staircase

The Montoya staircase involves a boxed enclosure for the rat, a removable double staircase food and a raised platform in the middle that the rat is on, the food is placed on the staircase in such a way that the rats must reach for it and the difficulty to reach the food increases with the lower steps.(6) The Montoya staircase, unlike the forelimb asymmetry does require prior training to use, however it also shows fine motor deficits in the form of measuring ability to grasp.

## Myelin

Myelin in nerves acts as a conduction speed aid allowing faster travel of the action potential down an axon by making a compromise between the ion cascade travel speed that is related to axon diameter, and the direct electrical conduction that is near light speed but has signal strength loss. This combined form is known as saltatory conduction, as the signal appears to jump from one gap in myelin to the next. Because in many cells the axon is covered in myelin other than the gaps, the sodium and potassium voltage gated channels necessary for the action potential effect are located only in and around the gap, also called a node of Ranvier. In a demyelinated axon, the conduction slows and can even have a conduction block due to delays in excitation of each successive node.(7)

Myelin is produced by two cell types depending on location: in the peripheral nervous system each interval of myelin is a single Schwann cell wrapping around the axon multiple times, so that a cross section of the myelin will look like the layers of phyllo dough in baklava. In the central nervous system, the myelin is made by cellular processes of the oligodendrocyte which unlike the Schwann cell is capable of producing multiple sheath segments on several axons.

## Microglia

Microglia are the primary immune cell of the central nervous system, which is considered generally immune privileged.(8) Microglia influence several processes of the central nervous system, including formation of synapses, maturation of neurons, clearing of cellular debris as well as inducing inflammation due to foreign bodies in the central nervous system(9, 10). Microglia have been implicated as being potential contributors to neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease Multiple Sclerosis, Amyotrophic Lateral Sclerosis, as well as being contributors to some of the wider spread damage of ischemic strokes.(9, 10) This is due to a phenomenon known as a reperfusion injury. This occurs because when a blood vessel in the brain is blocked the lack of blood flow causes an inflammatory response, the various inflammatory factors such as TNFα and some of the interleukin family build up in the blood being held back by the blockage these inflammatory factors can recruit immune cells from the bloodstream and loosen the blood brain barrier. When the blockage is removed the inflammatory signal rich blood rushes downstream and can cause widespread inflammation leading to widespread immune cell caused collateral damage. Another aspect of the reperfusion injury is relative hyperoxia compared to the hypoxic state caused by the blockage. Microglia conversely have also been implicated as being of potential benefit in those same diseases as microglia have multiple cellular behavioral modes, the M1 proinflammation state, and the M2 anti-inflammatory and damage repair state. (10-12) The M1 state therefore is the state that seems to cause the most damage in these disorders while activating the M2 state may be beneficial for recovery from said damage, thus drugs that can modulate this state change are worth investigating as potential treatments for neurodegenerative disorders. (11)

## Corpus Callosum

The corpus callosum, the major white matter tract that links the two hemispheres of the brain, is full of myelinated axons. This makes it a good place to focus a demyelinating agent such as lysolecithin. The corpus callosum is a good place to experimentally damage as you can assess recovery by comparing preinjury baselines to post injury lateralized behavior changes and then determine if and how quickly the behavioral patterns approach the pre-surgery baseline measures. Damage to the corpus callosum can cause motor function abnormalities both ipsi and contralateral to the injury(13, 14) In addition, the corpus callosum is one of the larger white matter tracts in the brain and is therefore a relatively large target to find, especially in small model organisms such as rats. In addition while not common Multiple sclerosis can present with behavioral changes in line with damage or severing of the corpus callosum such as alien hand syndrome.(15)

## Fluoxetine

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI). Traditionally SSRIs primary action has been to inhibit the transport proteins that retrieve serotonin from the synaptic cleft.(16). The serotonin remaining in the cleft would then lead to homeostatic mechanism desensitizing the post synaptic membrane. (17) SSRI's such as fluoxetine have been shown to increase neurogenesis when chronically administered. (18, 19), In addition fluoxetine has been shown to inhibit inflammation post injury: fluoxetine and other SSRI's have been shown to inhibit the inflammatory M1 mode of microglia, while increasing the anti-inflammatory and tissue regeneration encouraging M2 mode of the same microglia(11). There is evidence that fluoxetine does directly affect microglia via an inhibition of the NFκb pathway, which is a pathway that

causes downstream production of multiple pro inflammatory factors. These factors can include TNF-α, Nitric oxide and IL-1β(20). The mechanism by which fluoxetine inhibits the NFκb pathway seems to be that fluoxetine binds to Iκb-α. Iκb-α is a protein that in noninflammatory states along with Iκb-β and Iκb-ε complex with NFκb to keep the NFκb from crossing into the nucleus until an inflammatory signal is induced this allows inflammatory responses of cells to not be reliant on waiting for transcription and translation of NFκb. One part of the activation of NFκB is the ubiquitylation and subsequent protein degradation of Iκb-a and data suggests that fluoxetine by binding to Iκb-a prevents it from being ubiquitylated and therefore reduces NFκb activation. (21, 22) Additionally recent research has indicated that SSRIs antidepressant effects are in part due to immune system modulation and anti-inflammatory effects.(23)

**Simvastatin**

Simvastatin is also thought to induce neurogenesis after central nervous system injuries. (24) However there are some contradictory studies in this area, for example one study found that in prenatal development statins decreased neural progenitor cell expansion(25). Conversely another study found that statins increased progenitor survival in a viral encephalopathy disease state. (26) Simvastatin is in the drug class known as statins. The main function of statins is to reduce the production of cholesterol reducing blood LDL levels. Statins are also shown to reduce the inflammatory properties of various macrophage subtypes, including microglia. This is thought to occur through multiple mechanisms including the NFκB pathway (8, 27, 28). Statins were first isolated from an aspergillus species of fungus, though not the culinary aspergillus species used in sake and soy sauce/miso production. Later analogues were isolated from *Monascus ruber* which along with its species cousin *Monascus purpureus* are rice molds *Monascus purpureus* is in red yeast rice. Statins work via inhibiting the enzyme HMG-COA reductase, which is the starting point of cholesterol synthesis from acetyl-COA via conversion

into mevalonate. While cholesterol reduction was the original intended pathway for statins to work, the mevalonate pathway also leads indirectly to the production signaling pathways of various other inflammation related signals such as IL-6 and IL-8. (29, 30) Due to this, statins have potential to reduce even cholesterol unrelated inflammatory issues. (27, 28)

**Ascorbic Acid**

Ascorbic acid which is also known as Vitamin C is an acid that is used as a cofactor in collagen production enzymes: lack of this cofactor is the reason for the nutrient deficiency disease known as scurvy where connective tissues have issues because there is a lack of collagen production to compensate for normal collagen protein turn over. It is also an important antioxidant, reacting readily with reactive oxygen species. As myelin is mostly lipid, and that the Central nervous system is especially rich in unsaturated fats that are vulnerable to oxidative damage, there is evidence that part of the immune response in multiple sclerosis is due to oxidative stress changing the myelin sheath.(31) Reactive oxygen species are also associated with the recruitment of immune cells during inflammatory processes, and as such there are systems in place to deal with reactive oxygen species: so called antioxidants, several of which are enzymes but others include vitamins such as ascorbic acid(31, 32). It has also been found that the activity of enzymatic antioxidant participants such as superoxide dismutases and glutathione peroxidase are lower in Multiple sclerosis(31).This is one area though where rats may not be the best animal model for humans, as humans and other simians are incapable of synthesizing ascorbic acid via a de novo pathway, where rats are in fact capable of synthesizing it. (33) However humans can recycle oxidized ascorbic acid.(33-35).

.

## METHODS.

This study included 40 Sprague Dawley rats split evenly among genders: these rats were between 10-11 months old at the start of the study. The rats were kept in standard individual rat housing with wood-chip based bedding and a day night cycle mimicking equinox duration of day and night. They were also kept at an ambient temperature of approximately 74 degrees F.

### Demyelination Surgery

All but 4 rats (who were sham rats that failed Montoya staircase training), underwent a demyelinating procedure involving surgical injection of lysolecithin using stereotactic coordinates to make the injection occur in the right areas of the brain, directly below the Forelimb Motor Cortex. To prepare for the surgery the animals were first anesthetized in an isolation chamber using 5% isoflurane gas, then they were taken out and prepped, including shaving of the head and mounting in a stereotactic surgery device, using ear bars and a tooth hold to secure the head without trauma. The anesthesia was maintained using a nose cone sealed with a glove tied around the cone flesh interface that was supplied with 2.5 % isoflurane gas. An eye lube was applied to maintain moisture levels in the rats eyes during the surgery. The skin of the skull was then sanitized using a 1st coat Provo iodine, 2$^{nd}$ coat of 70% ethanol and third coat of Provo iodine. An incision was then made down the midline of the skull with a 0.25% bupivacaine solution as an analgesic applied to the site. A cotton swab was used to remove

blood from the site so the skull could be seen. Then the site bregma, an area where the bones of the skull fuse, was located, and marked with a fine tip permanent marker. A 0.7 mm drill bit was then attached to the stereotactic apparatus allowing for precise drilling relative in position to bregma. Two holes were drilled, with the mediolateral distance from bregma being .27 cm to the right from bregma in males and .25 cm to the right in females. The two holes had different anterior-posterior coordinates with one hole being 0 cm from bregma on the anterior posterior coordinates and the other being .15 cm anterior to bregma. The drill bit was removed and replaced with a Hamilton syringe filled with 3 microliters of 1% lysolecithin solution a concentration that is already present in the literature(36, 37). This was inserted to a depth of .29 cm in males and .27 cm in females. Once the desired depth was achieved the lysolecithin was injected at .1 microliters every 20 seconds until 1.5 microliters was added; the syringe was then moved to the second hole and this procedure was then repeated. After this the incision was sutured closed and provoiodine was applied to keep the wound clean. At this point the isoflurane was replaced with oxygen and the earbars and glove around the nose mask was removed. When the rats started stirring, they were put back in its cage over a heating pat until normal movement was observed, at which point the cage was moved back to the usual housing area. Acetaminophen was given to the rats the day of the surgery and the day after at a dosage of 200mg/kg administered in sugar cookie dough. Lysolecithin is a group of phospholipids that have had acyl groups removed. Lysolecithin works via several mechanisms to produce a demyelinating effect, including a surfactant effect disrupting cell membranes, and via affecting phospholipase signaling pathways to attract an immune response (38)

**Drug Delivery Post Surgery**

The experimental drug combo was administered via a 4 gram ball of sugar cookie dough, which acted as a vehicle control. The experimental Drug combination group (n=20, 50% male

50% female) received daily amounts of 5mg/kg fluoxetine, 1mg/kg simvastatin and 20mg/kg ascorbic acid. These drug doses were determined based on previous work of the lab and as doses equivalent to common doses of these drugs in clinical use. The control animals (N=16, 50% male and 50% female) had cookie dough balls with no drugs included. This food was all consumed voluntarily by the rats within approximately 5 minutes. Drugs were not given on day of euthanasia. Drug delivery was initiated 24 hours after surgery and continued for 30 days.

**Forelimb Asymmetry**

The rats were put in a clear cylinder with a mirrored right angle backing and allowed to explore for five minutes: this process was filmed for data collection. The four rats that had failed Montoya staircase training and hadn't undergone surgery were used as sham rats in the cylinder test as the cylinder test does not require prior training to work. In addition, to induce more exploratory activity, a maple extract was applied to the upper rim of the cylinder; this was to compensate for habituation and leads to more exploratory behavior to occur in a five-minute period than without. This compensated for one of the downsides of the forelimb asymmetry cylinder test, that the data point frequency can be slow if the rats are habituated to the environment and thus less motivated to explore it.(5) The result of a habituation occurrence would be a lower N of touches over a given timeframe. The videos were analyzed via windows media player using the playback enhancement menu and the spacebar to allow for better control of playback, allowing for ease of counting paw touches during playback. This was done while blinded to the experimental and control groups The analysis consisted of noting if the left paw vs right paw touched the wall of the cylinder and whether said touch was a palm supporting weight or a brush of the fingertips. This data was initially recorded via word documents with palm touches of left or right being represented by L or R respectively, the fingertip touches were represented by P and S for port and starboard. The number of each was then counted from the

word document using letter counting function of the word processor. These numbers were then transferred to the spreadsheet program Microsoft Excel, that contained the numbers of every run for numerical analysis and comparison. This data analysis included comparing both ipsilateral and contralateral function to baseline ratios. Both ipsilateral and contralateral functions were analyzed by counting of finger-tip touches, palm touches and total touches compared to the pre-surgery normal baseline. This normalization process divided the percent usage of each limb by the animal's pre-surgery usage. With this normalization analysis, if the end result was more than 1 that meant that the use of paws on that side of the rat was more than that seen during the pre-surgery baseline, as someone who has weakness in one side of the body might put more weight on the other side when doing a task such as getting out of a chair. If the analysis for paw use was shown to be less than one that showed that they were not using it to support weight as often.

This required a forelimb asymmetry test before any brain injury, to give a normal basal usage for each rat. This is to take into account individual rats preference to one side or another similar to human handedness. After this was done to establish a normal individual baseline for each rat, the rats underwent surgery to demyelinate the corpus callosum in several groups. This was done via an injection of lysolecithin below the forelimb motor cortex, and the forelimb asymmetry exploration test was repeated at 3 days post-surgery to establish the baseline deficit produced by the surgery. Each rat was also tested at 15 days post-surgery and 30 days post-surgery for each rat to assess functional recovery over time.

**Montoya Staircase**

In addition, the rats that were trained underwent a Montoya staircase(29) assessment that was analyzed and done by other lab members. Montoya staircase consisted of an apparatus

with a central beam where the rat would be placed and two trenches with seven increasingly lower platforms on either side, these platforms each had three 50 mg banana flavored sucrose pellets totaling 21 pellets for each forelimb. The rats were trained for 10 days before surgery, at least 15 minutes per day. The first three days the training occurred during the day part of their day night cycle to encourage them to come out, with these training tests unscored, and then also at night, when the tests were scored. Only the tests done during the dark part of the cycle were scored and analyzed for establishing baseline normal behavior. Training was done with an 85% of ad lib feeding condition in order to ensure the rats were not satiated enough to ignore the sugar pellets. The animals were fed each day after training was done. The scoring of the tests consisted of recording how many pellets were picked up with paws: the last three days of training were averaged to form a pre-injury baseline for each rat. Rats that did not pick up 9 pellets in each paw by the end of training were considered to have flunked the Montoya staircase training and as such were not used for this functional test. Post surgery tests were done on the rats on the day intervals 3-5, 15-17 and 30-32, with each interval consisting of one 15 minute test per day for the ranges given. During each interval an 85% ad lib food condition was implemented with full ad lib food being restored between intervals. The scoring for each interval was averaged together to get one data value per interval. After the data was collected and put in the excel spreadsheets, the data was then put through statistical analysis via the program Sigmastat and the data was put into graph form using Sigmaplot. The statistical method used to determine significance was a two way repeated measures analysis of variance test, known as a 2 way repeated measures ANOVA. In some of the data this was confirmed using a post hoc Holm Sidak test. A P value of less than .05 was considered to be a significant difference.

## RESULTS

The results for the male and female cylinder tests were put through a two-way repeated measures ANOVA statistical analysis and a post hoc Holm Sidak test for post-surgery days 3-30. Figure 1. shows the females in the forelimb asymmetry test when only palm touches were counted. The results were calculated into means of both the control group and the FSA (fluoxetine, simvastatin, ascorbic acid combination drugs) group on each day after surgery they were tested. As stated before, the results were normalized to the pre-surgery baseline for each subject: this was done by dividing the post-surgery results by the pre-surgery results. This normalization means that a value above 1 would show a heavier use of the limb in question post-surgery. Conversely a value below one shows less use of that limb post-surgery. Injury in the forelimb cortical region on the right side results in functional deficits in the left forelimb (contralateral paw injury). Injury in the forelimb corpus callosum region on the right side would result a bilateral injury state, thus deficits ipsilateral to the limb can be attributed to white matter damage.(39, 40) Recovery therefore would be shown as the post-surgery values of both ipsilateral and contralateral limbs approaching 1 (pre-surgery normal values).

In Figure 1, we see functional recovery in both the control and the experimental group analyzing the contralateral limb, with the control group going from a mean post-surgery day 3 normalized contralateral function of 0.849 with a std error of .0491 to a mean of 0.966 with a std error of .0686 on post-surgery day 30. The experimental drug group showed a mean post-surgery day 3 normalized contralateral function of .913 with a std error of .0567 which rose to a

mean of .996 with std error of .0411 on post-surgery day 30. Incidentally the mean of the normalized contralateral function for the control group on post-surgery day 15 was .999 with a std error of .0366 while the mean of the normalized contralateral function of the FSA group on post-surgery day 15 was 0.914 with a std error of .0341. The time factor showed significant differences (P value=.027) for both control and experimental drug groups. The variable of time after surgery produces an outcome that has a low probability of occurring due to random chance and therefore can be inferred to have an actual effect on the data set this is confirmed by the post-surgery day 3 to 30 post hoc test also being significant with a P value of.009. The ANOVA comparisons between the control and experimental drug groups and the drug interaction with time factor however are not significant, with P values of .912 and .206 respectively. This means that there is not a difference between the drug groups recovery and the control groups that cannot be ruled as more than just random variation.

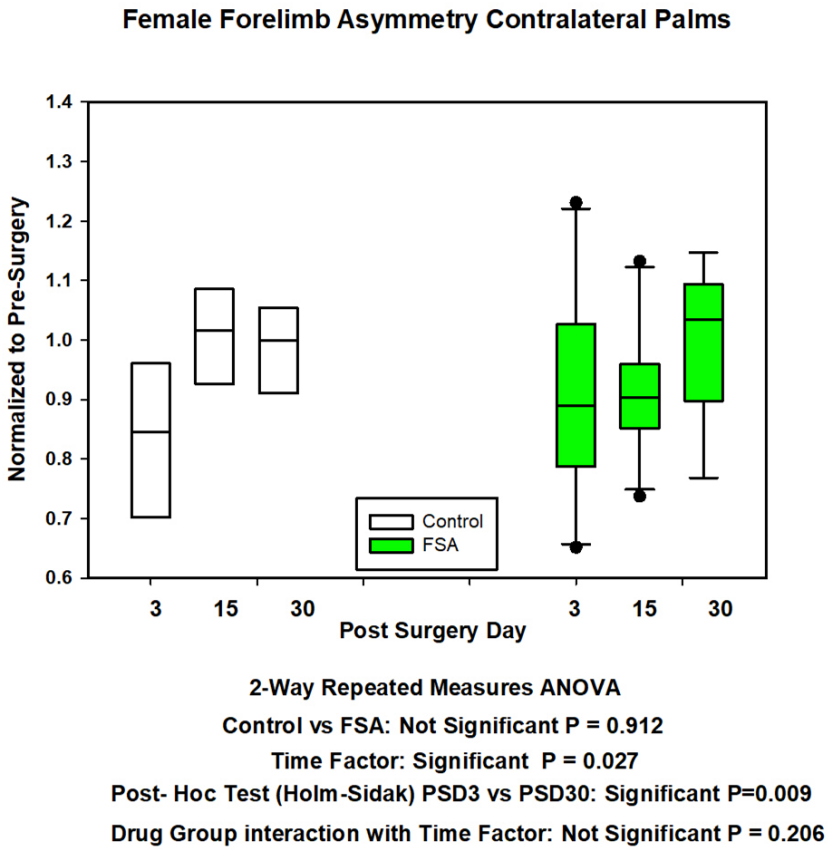


*Figure 1 Female rats, both control and experimental (N=19) for the palm measures of the limb contralateral to the induced white matter lesion. The only significance occurred between when post-surgery day 3 was compared with day 30. The graph is normalized to the presurgery baselines so 1.0 on the y axis is considered normal function. In post-surgery day thirty the FSA group was missing one datapoint from rat MS 21. The normalized values were then averaged and the std error of the average was taken to compare the control versus the experimental drug combo in a 2 way Repeated Measures ANOVA.*

Figure 2 is similar to Figure 1 except it counts fingertip touches in addition to the palm touches. Interestingly with the fingertip touches included the time factor is no longer significant. These are the same animals and the same runs, so the fingertips included removing significance might show that including the fingertip touches reduces the sensitivity of the test. For figure 2 the mean of normalized contralateral function on post surgery day 3 for the control was 0.874

with a standard error of 0.0314 and by the time post-surgery day 30 was recorded the mean normalized contralateral function was 0.953 with a std error of 0.0617. There were no statistical differences, as indicated by the higher P values. With the Repeated Measures ANOVA comparisons of Control vs FSA, Time factor, and drug group interaction with Time Factor all failing to reach significance, with P values of .869, .133, and .277 respectively

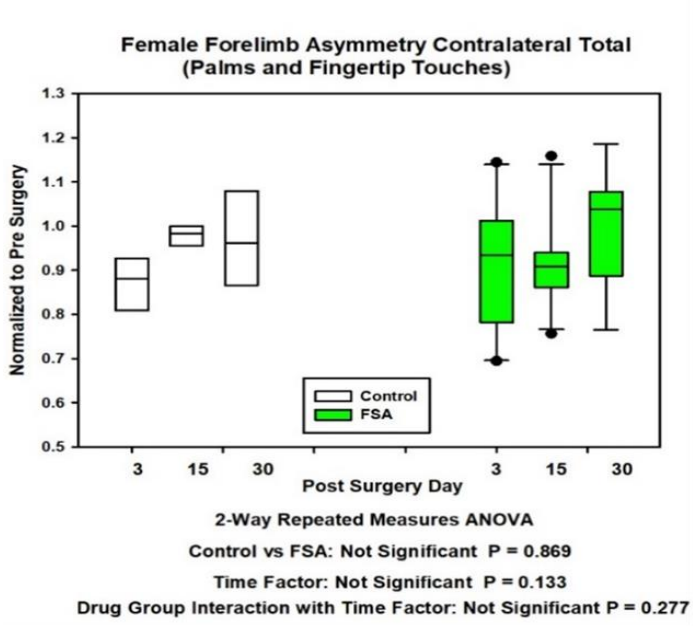


*Figure 2: Female forelimb analysis contralateral to the injury with both palm and fingertip touches. The normalized values were then averaged and the std error of the average was taken to compare the control versus the experimental. A 2 way Repeated Measures ANOVA was used for statistical analysis*

Figure 3 is similar to figure 1 in that it shows the what the data looks like when only palms are counted. However unlike figure 1, figure 3 shows the results for the forelimb ipsilateral to the site of the lesion. These were the same animals in the same sessions as figure 1 and 2 just with different analysis targets. The mean of the normalized ipsilateral function of the control group on post surgery day 3 was 1.147 with a std error of 0.0522. The mean of the normalized ipsilateral function of the FSA group on post surgery day 3 was 1.113 with a std error

of 0.0628. On post-surgery day 15 the mean of the normalized ipsilateral function for the control group was 1.006 with a std error of 0.0333. The mean of the normalized ipsilateral function of the FSA group on post-surgery day 15 was 1.092 with a std error of 0.0324. the mean of the normalized ipsilateral function for the control group on post-surgery day 30 was 1.007 with a std error of 0.0507. The post-surgery day 30 mean of normalized ipsilateral function for the FSA group was 1.020 with a std error of 0.0406. Like Figure 1, the Control vs. FSA, and Drug group interaction with time factor parts of the 2 way Repeated Measures ANOVA failed to reach significance with P values of 0.686 and 0.277 respectively, while the time factor and post-surgery day 3 vs post-surgery day 30 post hoc Holm Sidak tests were both significant with p values of 0.013 and 0.004 respectively.

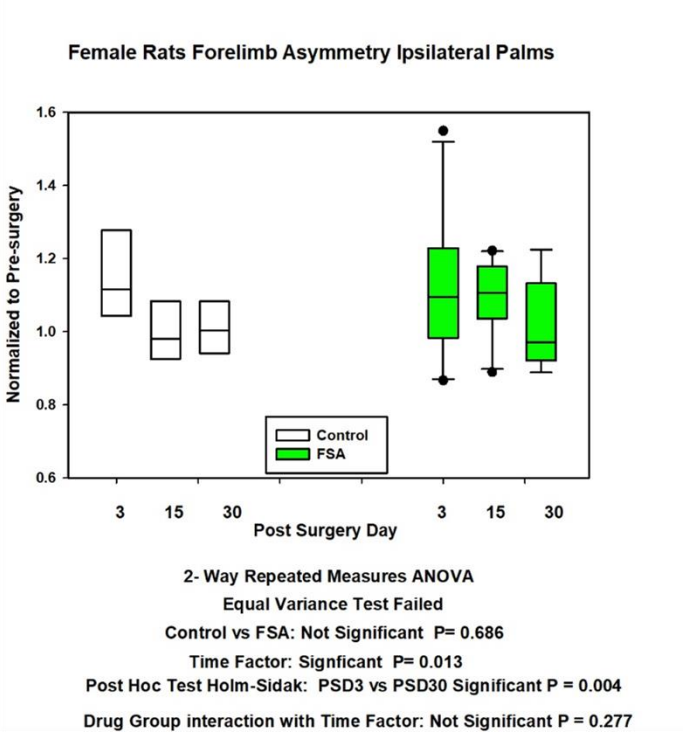


*Figure 3 female ipsilateral function. The normalized values were then averaged and the std error of the average was taken to compare the control versus the experimental drugs. A 2 way Repeated Measures ANOVA was used.*

Figure 4 is similar to figure 2 in that it shows palms and fingertip touches, but it shows the ipsilateral limb function (similar to Figure 3). In Figure 4 the post-surgery day 3 mean of the normalized ipsilateral limb function was 1.126 with a std. error of 0.0399 while the post-surgery day 3 mean of normalized ipsilateral limb function for the FSA group was 1.100 with a std. error of 0.0515. The post surgery day 15 mean of normalized ipsilateral limb function for the control group was 1.020 with a std. error of 0.0303. The post-surgery day 15 mean of normalized ipsilateral limb function for the FSA group was 1.089 with a std error of 0.0285. The post-surgery day 30 mean of ipsilateral limb function for the control group was 1.039 with a std. error of 0.0521 while the post-surgery day 30 mean of ipsilateral limb function for the FSA group is 1.002 with a std. error of 0.0449. Like figure 2 we see a failure to reach significance in all parts of the 2 way repeated measures ANOVA with the drug group interaction with time factor, Control vs. FSA, and time factor having p values of .314, .952 and .059 respectively. The time factor might have still been significant with a greater N value as it is close to the .05 default significant P Value threshold

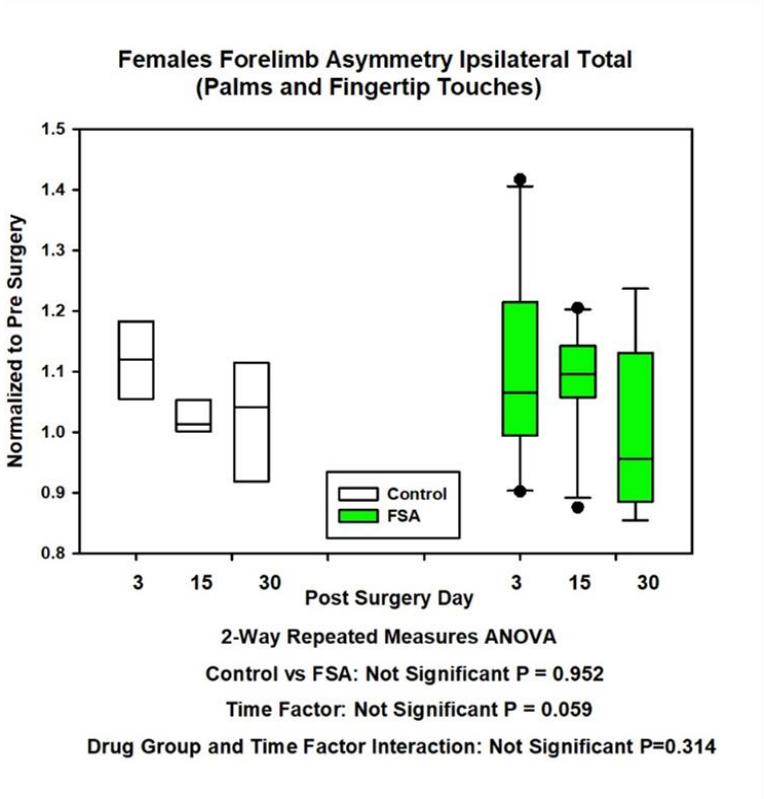


*Figure 4 female ipsilateral function values for each rat was normalized to its pre surgery baseline. The normalized values were then averaged and the std error of the average was taken to compare the control versus the experimental drug combination group. A 2 way repeated measures ANOVA was used for statistical analysis.*

.

Figure 5 shows the results of the contralateral palms touches for the male rats in this experiment. The n (N of control=5 N of experimental=4) of the male rats was rather low due to losing the videos of the pre-surgery cylinder test for many of them prior to analysis. Because these were the pre-surgery videos we had no data to normalize most of the male rats post-surgery results to. This led to the male rat section having a low statistical power of 0.05. This means that it would be folly to draw definitive conclusions from the male data, however the results of the male data may still be useful making a prediction on what would happen if the experiment was repeated with a greater statistical power. The post-surgery day 3 mean of normalized contralateral function for the control was 0.801 with a std. error of 0.111, while the

post-surgery day 3 mean of normalized contralateral function for the FSA group was 0.795 with a std. error of 0.0787. The post-surgery day 15 mean of the normalize contralateral function for the control group was 0.871 with a std. error of 0.0881, while the post-surgery day 15 mean of normalized contralateral function for the FSA group was 1.007 with a std. error of 0.0864. The post-surgery day 30 mean of normalized contralateral limb function for the control group was 0.854 with a std error of 0.214, while the FSA groups post-surgery day 30 mean of normalized contralateral limb function was 0.967 with a std. error of 0.0520. The p values in figure five are all very high, with the control vs FSA, time factor, and Drug group interaction with time factor having p values of .498, .291, and .707 respectively. None of these values approach the .05 p value default significance threshold. Due to the distance away from that threshold it is unlikely that a repeat of this experiment with greater statistical power for the males would achieve significance. We will however be using this data to perform a Power analysis for future experiments.

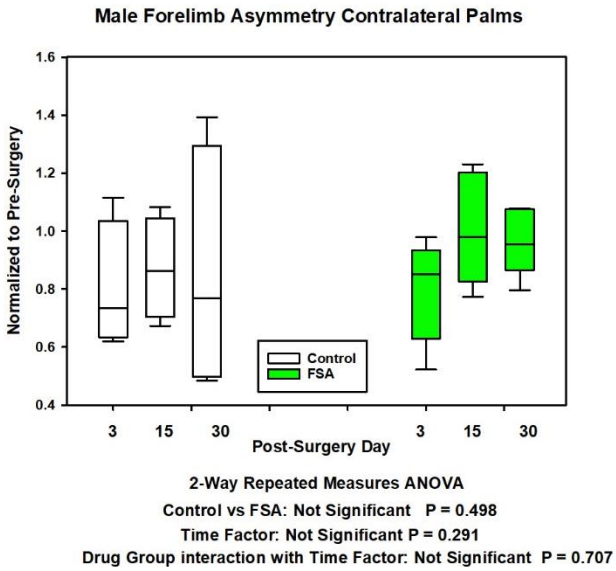


*Figure 5 male contralateral palms each individuals data post surgery was normalized to that individuals pre surgery baseline. Note that for the male rats we lost the pre-surgery data for 10 of the male rats: due to this we could not use their data as we did not have a baseline to normalize to. This means for the males the statistical power of the experiment is weak: N of control being 4 and N of the FSA group being 5. The normalized values of the males were then averaged with the experimental group they were a part of, and the std error of the mean was calculated. In order to compare the control groups data to the FSA group a two way repeated*

In figure 6 the data for the male contralateral total touches is shown. As with figure five the missing pre-surgery videos lowers the power as there were not many male rats we could normalize. The post-surgery day 3 mean of normalized contralateral function for the control group was 0.859 with a std. error of 0.0938, while the mean of normalized contralateral function for the FSA group on PSD 3 was 0.869 with a std error of 0.0484. The control post-surgery day 15 mean of the normalized contralateral function was 0.908 with a std error of 0.0731. while the FSA groups post-surgery day 15 mean of the normalized contralateral function was 0.990 with a

std error of 0.0628. The post-surgery day 30 mean of normalized contralateral function for the control was 0.873 with a std. error of 0.164. The post-surgery day 30 mean of normalized contralateral function of the FSA group was 0.967 with a std error of 0.0681. Again, though the statistical power of the experiment is low and the p values were 0.478, 0.555, and 0.845 for the control group vs FSA group, time factor, and drug group interaction with time factor tests respectively. None of these values are even close to the critical value of .05 meaning that it is unlikely that the results would differ in significance if repeated with a greater statistical power.

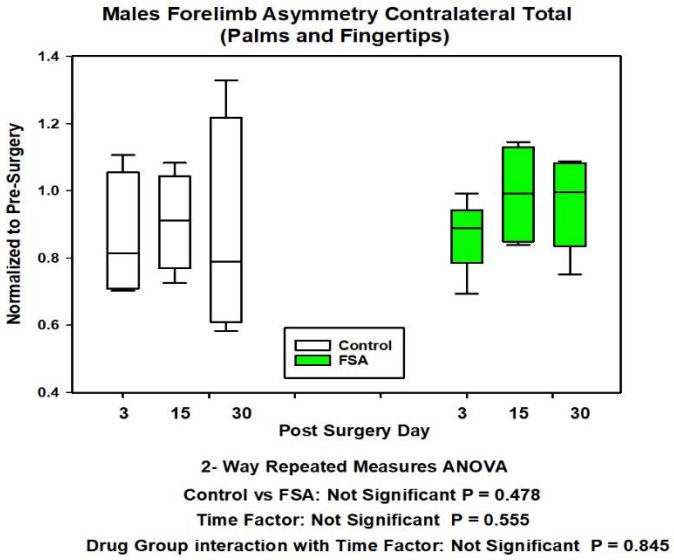


*Figure 6 male contralateral total, palms and fingertip touches included. The data of each individual was normalized to that individuals presurgery baseline, However as previously stated with the males we lost a good portion of the presurgery video files and therefore the males without a presurgery baseline had to be excluded from the data. The normalized values were then averaged in the experimental group they belonged to, either control or FSA and with the post surgery day that value was a part of. The std error of the mean was then calculated and 2-Way Repeated Measures ANOVA was used for statistical analysis*

Figure 7 shows the male ipsilateral palms touches in Forelimb Asymmetry test and as with Figures 5 and six the data set used was limited due to the loss of some critical video tapes. The mean of normalized ipsilateral function post-surgery day 3 for the control group was 1.233 with a std. error of .116, while the post-surgery day 3 mean of normalized ipsilateral function for the FSA group was 1.206 with a std error of 0.0738. The post-surgery day 15 mean of normalized ipsilateral function for the control group was 1.173 with a std error of 0.110, while the post-surgery day 15 mean of normalized contralateral function for the FSA group was 0.986 with a std error of 0.0899. On post-surgery day 30 the mean of normalized ipsilateral limb function in the control group was 1.260, with a std error of 0.265. while on post-surgery day 30 the mean of normalized ipsilateral limb function for the FSA group was 1.039 with a std error of 0.0604. None of the ANOVA test categories achieved significance, with Time Factor, Control Vs FSA and Drug group interaction with time factor having p values of 0.377, 0.335 and 0.575 respectively. As these values are all far from the .05 default significance threshold it is unlikely that repeating this assessment with higher statistical power would yield significance in its results

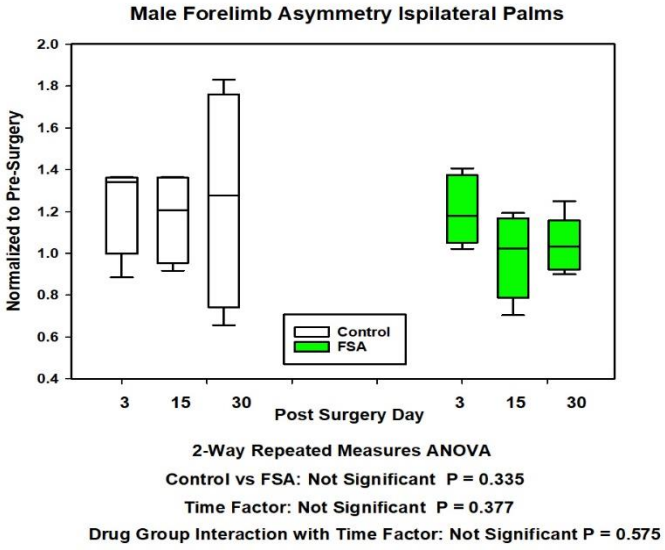


*Figure 7 Ipsilateral palms of the male rats: the data of each individual rat was normalized to its presurgery baseline if said baseline had been successful recorded; those that did not have an applicable baseline were discarded as without a baseline normalization could not occur. The normalized values were then averaged with their respective groups and the post surgery day group associated with the value. The standard error of the means was then calculated and the groups were compared using a two way repeated measures ANOVA*

Figure 8 shows the male ipsilateral total touches graph with fingertip touches counted in addition to palm touches in Forelimb Asymmetry. On post-surgery day 3 the mean of ipsilateral limb function for the control group was 1.151 with a std error of 0.0922, while the post-surgery day 3 mean of ipsilateral limb function for the FSA group was 1.135 with a std error of 0.0452. On post-surgery day 15 the mean of normalized ipsilateral function for the control was 1.107 with a std. error of 0.0783, while the mean of normalized ipsilateral function on post day 15 for the FSA group was 1.017 with a std error of 0.0721. On post-surgery day 30 the mean of the normalized ipsilateral function for control group was 1.187 with a std error of 0.192, while for

the FSA group on post-surgery day 30 the mean of normalized ipsilateral function was 1.053 with a std error of 0.0776. One interesting thing in the males from the forelimb asymmetry is the greater range of results on post-surgery day 30 in the control. The Drug group interaction with time factor, time factor, and Control vs FSA all failed to achieve statistical significance, with P values of 0.777, 0.614, and 0.441 respectively.

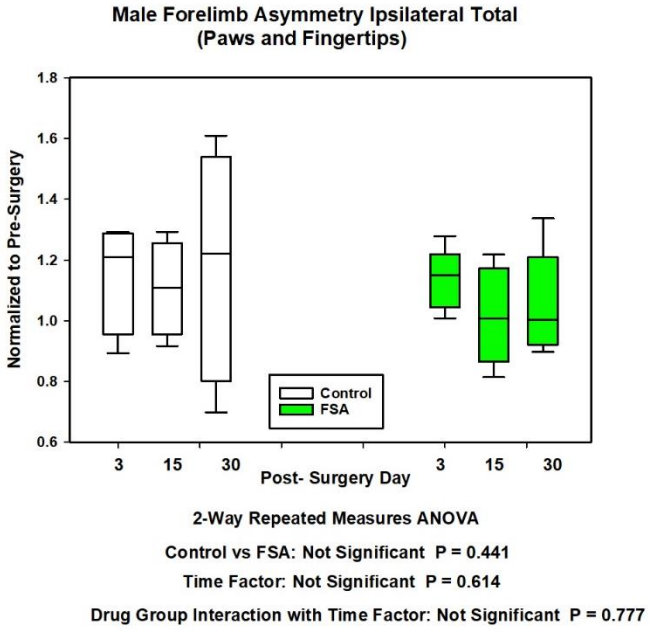


*Figure 8 male ipsilateral total. Each male rat had its values for each post surgery day normalized to the pre- surgery baseline; where no pre-surgery baseline data existed due to loss of files said individual was excluded from data analysis. The values for each day in each experimental group was averaged and the std error of each group and time mean was calculated. 2-way Repeated measures ANOVA was used for statistical analysis.*

Figure 9 shows the mean contralateral function of the female rats as measured by the Montoya staircase test. The mean contralateral function for the control group on post-surgery day 3 was 0.703 with a std error of 0.0590 while the FSA group on post-surgery day 3 had a

mean of normalized contralateral function of 0.500 with a std error of 0.0703. The control group on post-surgery day 15 had a mean of normalized contralateral function of 0.789 with a std error of 0.0563 while the FSA group on post-surgery day 15 had a mean of 0.731 with a std error of 0.0551. On post-surgery day 30 the control group had a mean of normalized contralateral function of 0.768 with a std error of 0.0935. In figure 9, significance for the time factor differences is achieved with a p value of 0.001. Significance is also seen between post-surgery day 3 and 15 as well as PSD 3 to 30 with p values of 0.001 and 0.002 respectively. This shows that the Montoya staircase test may have a greater sensitivity in detecting recovery in this model of M.S. However, there is no significance between the control data and the FSA group, or the drug group interaction with the time factor, p values of .248 and .117 respectively.

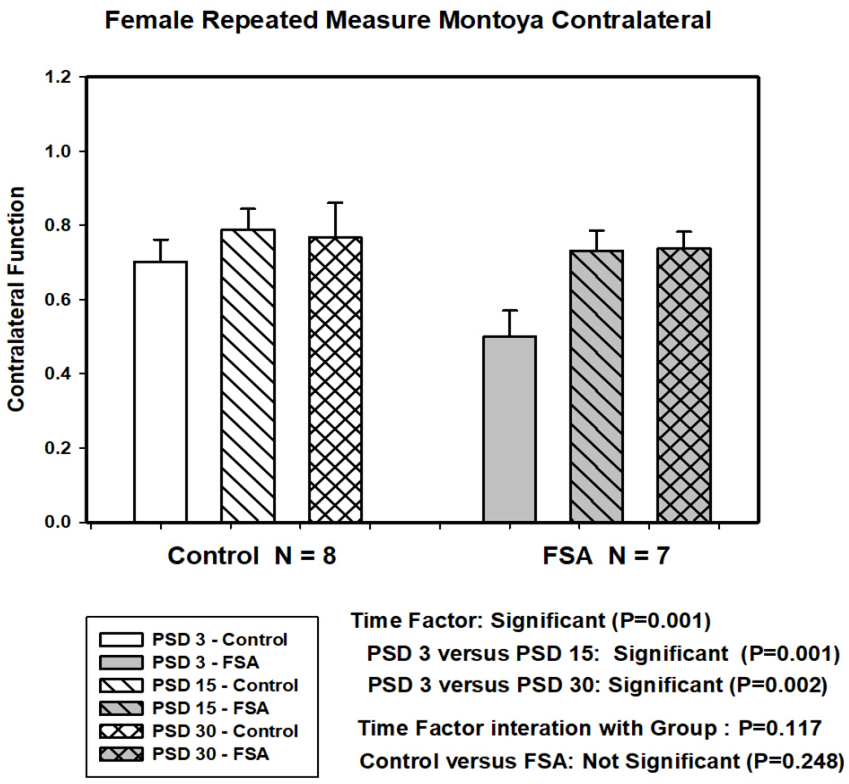


*Figure 9 Montoya staircase graph female rats contralateral to injury post surgery the rats were tested 3 times per individual once daily for the intervals post surgery day 3 to post surgery day 5, post surgery day 15 to post surgery day 17, and post surgery day 30 to post surgery day 32. The results of these intervals were averaged for each individual, normalized to baseline these values were then averaged to get a value for each interval in each experimental group. A two factor repeated measures ANOVA method was then used to compare the data values to each other N of control=8, N of experimental =7*

Figure 10 also shows the female rats results but with the mean ipsilateral function. It is a box and whisker plot as our lab is in the middle of transitioning to preferring box and whisker plots as more data is shown on the plot itself when compared to a bar graph. Again, we see significance differences in time, with an overall time factor P value of 0.038, and a significant time factor interacting with group difference whose overall p value is 0.049. The FSA group post-surgery day 3 vs post-surgery day 15 and FSA group post-surgery day 3 vs post- surgery day 30 both achieve significance, with p values of 0.003 and 0.006 respectively. However, again we see

there is no significant difference between the control and experimental group, p value of 0.112. The control group on post-surgery day 3 had a mean of normalized ipsilateral function of 0.786 with a std error 0.0293, while the post-surgery day 3 mean of normalized ipsilateral function for the FSA group was 0.643 with a std error of 0.0860. On post-surgery day 15 the control group had a mean of normalized ipsilateral function of 0.754 with a std error of 0.0591. The FSA group on post-surgery day 15 had a mean of normalized ipsilateral function of 0.823 with a std error of 0.0985. On post-surgery day 30 the control had a mean of ipsilateral function of 0.838 with a std error of 0.0593, while the FSA group had a mean of normalized ipsilateral function of 0.810 with a std error of 0.109.

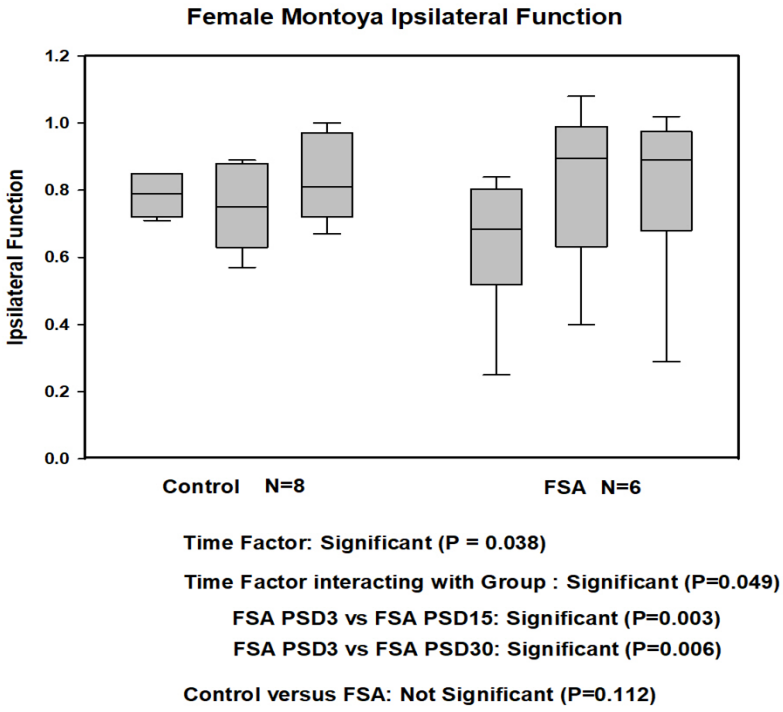


*Figure 10 Montoya Staircase female ipsilateral graph. Each rat was tested once daily in intervals of PSD 3-5, PSD 15-17, and PSD 30-32. The results of these three tests were averaged for each individual rat for each interval, the means of the individual rats were then normalized to the pre surgery baseline score. These normalized values were then averaged with the values belonging to the same interval and experimental group. Std error for each of these values was then calculated and the results were compared using a two way repeated measures ANOVA methodology. There is no significant difference between the control group and the FSA group in the female rats according to analysis of the Montoya staircase test N of control=8, N of experimental =7*

In figure 11 is where something interesting occurs: it shows the male contralateral function as measured by the Montoya staircase. The mean of normalized contralateral function for the control group on post-surgery day 3 was 0.838 with a std error of 0.0652. On post-surgery day 3 the mean of normalized contralateral function for the FSA group was 0.649 with a std error of 0.0726. The post-surgery day 15 control had a mean of normalized contralateral function of 1.002 with a std error of 0.0786, while the FSA group on post-surgery day 15 had a

mean of normalized contralateral function of 0.811 with a std error of 0.0472. On post-surgery day 30 the control group had a mean of normalized contralateral function of 1.089 with a std error of 0.103 while the FSA group on post-surgery day 30 had a mean of normalized contralateral function of 0.848 with a std error of 0.0489. This shows significance in between post-surgery day 3 and 15 and between PSD 3 and 30 ($P<.001$ in both cases). We also see significance in the difference between the control and the FSA group ($P=0.036$). In this case it appears that the recovery in the FSA group was slowed. This shows up on the Montoya but not the forelimb asymmetry.

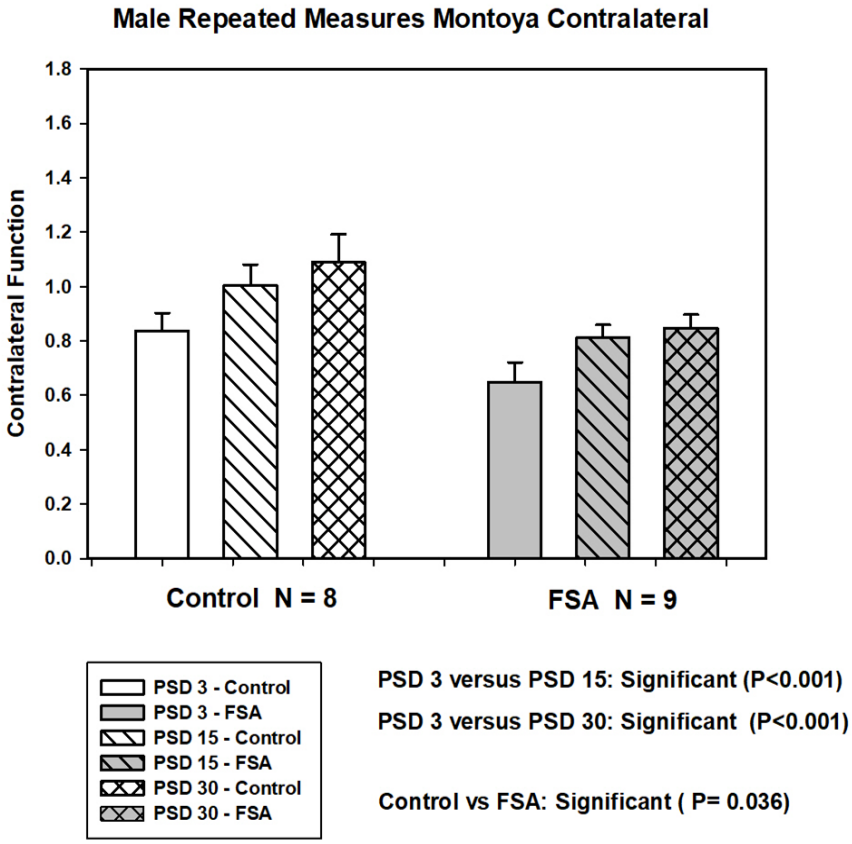


*Figure 11 male repeated measures contralateral graph. Each male rat was tested once daily for the test periods PSD 3-5, PSD 15-17, and PSD 30-32. The individual rats results were averaged for each interval period. The averaged values were then normalized to pre-surgery baseline data. The normalized values were then averaged across all male rats in a group for that interval. The std error was calculated for the resulting means, and the results were compared using a two-way repeated measures ANOVA test. Interestingly there is a significance for this test in both time and control vs FSA however the FSA group is significantly reduced in function: this points to an inference that the FSA treatment is in some way detrimental to the function required for the Montoya task. N of control=9 N of experimental=8*

Figure 12 shows the ipsilateral limb function as measured by the Montoya staircase test. On post-surgery day 3 the control group had a mean of normalized ipsilateral function of 0.739 with a std error of 0.0417 while the post-surgery day 3 mean of normalized ipsilateral function for the FSA group was 0.731 with a std error of 0.0533. On post-surgery day 15 the

mean of normalized ipsilateral function for the control group was 0.857 with a std error 0.0783. while the post-surgery day 15 mean of normalized ipsilateral function for the FSA group was 0.826 with a std error of 0.0534. The mean of normalized ipsilateral function for the control group on post-surgery day 30 was 1.036 with a std error of 0.0573 while the mean of normalized ipsilateral function of the FSA group for post-surgery day 30 was 0.823 with a std error of 0.0353. Interestingly while the change in function from PSD 3 to PSD 30 is significant statistically($P<0.001$) the change in function from PSD 3 to PSD 15 is not (p value=0.041) though only through a modified critical P value of .025 rather than the conventional .05 that is commonly used this is because a post hoc Holm-Sidak test showed the critical value of PSD 3 to PSD 15 as being .025. The control vs the FSA data is not significantly different with a P value of 0.139 though visually the graph does show a reduction in recovery from FSA treatment for the males; however as it is not significant, we cannot reasonably rule out that random chance caused the reduction in recovery compared to the control.

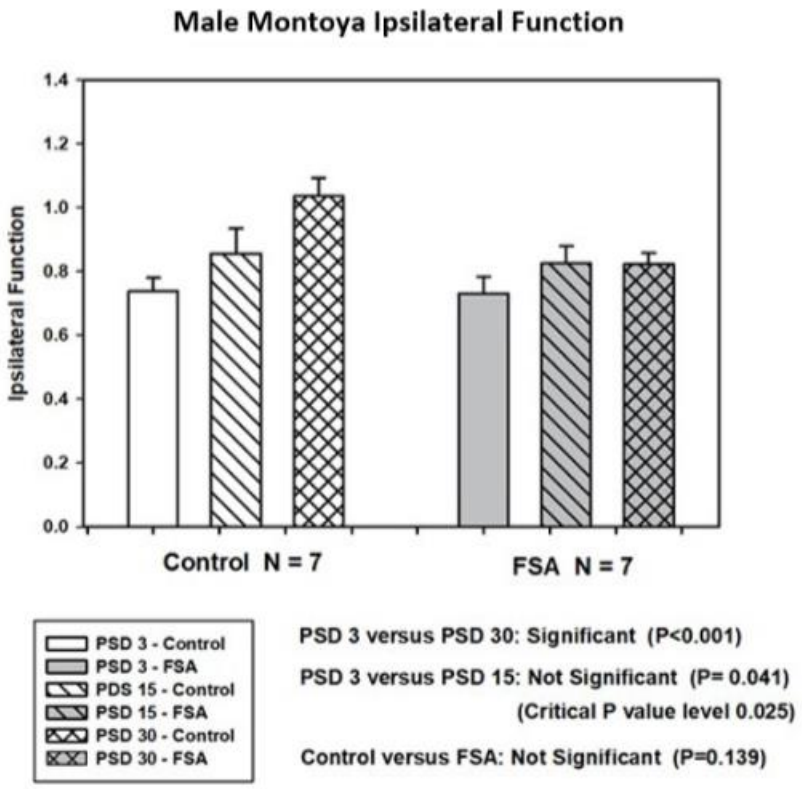


*Figure 12 Montoya ipsilateral function. Each male rat was tested once daily for the intervals PSD 3-5, PSD 15-17, and PSD 30-32. The individual rats results were averaged for each interval period. The averaged values were then normalized to presurgery baseline data. The normalized values were then averaged across all male rats in a group for that interval. The std error was calculated for the resulting means, and the results were compared using a two way repeated measures ANOVA test. The recovery from post surgery day 3 to post surgery day 30 is significant while recovery from post surgery day 3 to post surgery day 15 is not using an adjusted critical p value, of .025. the Control vs FSA is also not significantly different. This along with figure 11 indicates a more significant change in function over time for contralateral limb function over the ipsilateral side.*

## DISCUSSION

In the female contralateral function to injury, using the Montoya test there is a 29.7% mean deficit of function on post-surgery day 3 in the control group and a 50% mean deficit in the Fluoxetine Simvastatin and Ascorbic acid group on the same day. This deficit does show improvement over time: the mean deficit on post-surgery day 30 was 23.2% for the control group and a 26.1% deficit in the Fluoxetine Simvastatin and Ascorbic acid group, with time factor being significant to recovery P=0.001. The recovery was not significantly different between the control and fluoxetine, simvastatin, and ascorbic acid Drug groups with a p value of 0.248.

The female contralateral Forelimb asymmetry showed a mean deficit of 15.1 % on post surgery day three in the control group, a mean deficit of 0.01% on post surgery day 15 in the control group and a mean deficit of 0.4% on day 30 in the control group. In addition, in the Fluoxetine Simvastatin and Ascorbic acid group there was an 8.7% deficit on post-surgery day 3, an 8.6 % deficit on day 15 and a 0.4% deficit on day 30 post surgery. The functional differences over time were significant, p=0.027. For forelimb asymmetry the data was first converted into a ratio of the number of times one side was used over the sum of times that side was used plus the times the opposite side was used, for example $y = \frac{s}{(s+o)}$ where S is the limb in question and o is the opposite limb. For the post surgery days, y was further divided by the y value of said animals pre-surgery values. The end result of this is that a post-surgery Value of less than 1

indicates that said limb was used less than it was in baseline normal while a value greater than 1 indicated that the limb was used more than the presurgery normal baseline. This data suggests that female rats recover gross motor and weightbearing function regardless of the experimental variable. This is because forelimb asymmetry tests for gross motor function and ability to support an animals weight unlike the Montoya staircase test: the Montoya staircase test involves the reaching and grasping of an object, which is a test of fine motor control. This in part may be why compensatory behavior does not seem to be a large factor in the Montoya staircase data as those sort of fine motor tasks are not easily compensated for laterally whereas the forelimb asymmetry test being a procedure relying on analysis of how rats support their weight in a gross motor function test is a situation where the deficit of one side could lead to compensatory behavior and shifting of the weight support to the less injured side.

In the female ipsilateral Montoya test a deficit of function is observed on post-surgery day 3 with a deficit of 21.4% in the control group and a deficit of 35.7% in the Fluoxetine Simvastatin and Ascorbic acid Drug group. On post-surgery day 15 the control group deficit in function was 24.6% while the Fluoxetine Simvastatin and Ascorbic acid group had a deficit of 17.7%. On post-surgery day thirty the deficit in the control group was 16.2% while the deficit of the Fluoxetine Simvastatin and Ascorbic acid group was 19%; while there is some variation this does show an upwards trend. This is in contrast to the ipsilateral forelimb asymmetry which shows an increase in use, with usage 14.7 % above baseline in the post-surgery day 3 control, a usage of 11.3% above baseline in the Fluoxetine Simvastatin and Ascorbic acid Drug group. These above baseline measurements approached back to baseline as time went on with only a 0.7 % above baseline for the control group and a 2% above baseline for the Fluoxetine Simvastatin and Ascorbic acid group on post-surgery day 30. This is likely due to the forelimb asymmetry deficits being masked by compensatory behavior for the impairment of the contralateral limb which is

controlled by the brain hemisphere ipsilateral to the injection position. The move towards baseline indicates recovery as the move towards baseline would be evidence for a reduced need for compensatory behavior. This is evidence that the Montoya staircase methodology is a more sensitive measure of damage and recovery especially in cases where they damage is not purely unilateral as with Montoya we see damage from both sides where with forelimb asymmetry the side with lesser expected damage is masked by compensatory behavior

In the male contralateral Montoya results we see a mean deficit in function on post surgery day 3 of 16.2% in the control group and 35.1% in the experimental drug group. This is followed by a trend towards recovery in both groups, with post-surgery day 15 in the control being 0.2% above baseline, and the Fluoxetine Simvastatin and Ascorbic acid Drug group having a deficit of 18.9 %. This continues into post-surgery day 30 with the control being 8.9% above baseline, and with the deficit of the Fluoxetine Simvastatin and Ascorbic acid Drug group being 15.2%. The time factor P value of this group of data was less than 0.001 for both the time intervals of PSD 3 to PSD 15 and PSD 3 to PSD 30. Unlike the female test the male Montoya test does show significant difference between the control and the fluoxetine simvastatin and ascorbic acid Drug group.($p=0.036$): This difference is due to the Fluoxetine Simvastatin and Ascorbic acid Drug group has a reduced recovery rate compared to the control.

This significant hinderance in recovery is not seen in the male forelimb asymmetry contralateral palm test (fluoxetine simvastatin and ascorbic acid Drug group vs control group has a $P=0.498$) or the male forelimb asymmetry contralateral palms and fingertip test.(Fluoxetine Simvastatin and Ascorbic acid Drug Group vs control group has a $P=0.478$). While this could be a result of the low N values in the male forelimb Asymmetry tests, given that the female asymmetry data appeared to be less sensitive compared to the female Montoya data, and that the p values for the male asymmetry are above .15,I would hypothesize that if the experiment

was repeated to gain a higher N value then the male asymmetry tests would still exhibit a lack of sensitivity compared to the Male Montoya tests. The male contralateral asymmetry test had a normalized mean deficit of 19.9 % on post-surgery day 3 in the control group, and a 20.5% percent deficit in the Fluoxetine Simvastatin and Ascorbic acid Drug group on post surgery day. On post surgery day 15 the Fluoxetine Simvastatin and Ascorbic acid group had a .7 percent increase over baseline, while the control group had a 12.9 % deficit, on post-surgery day 30 the control group had a mean deficit of 14.6% while the Fluoxetine Simvastatin and Ascorbic acid Drug group had a mean deficit of 3.3%. While this may look initially like the males recovered more, the low data size plus the fact that the range of the post-surgery day 30 data(0.909) for the control group was around three times the range of all the other data sets, which had ranges around 0.4 to 0.5 except post-surgery day 30 for Fluoxetine Simvastatin and Ascorbic acid which had 0.283 for its range. Also, the post-surgery day 30 control data had a vastly lower min value of 0.484, while the post-surgery day 15 for Fluoxetine Simvastatin and Ascorbic acid Drug group has a much higher max compared to everything other than the aforementioned control PSD 30, leads me to believe that this trend in data is more attributable to the small sample size and the unreliability caused by said low sample size than by the Fluoxetine Simvastatin and Ascorbic acid Group itself. In addition, the values moving past 1 by a significant amount does not line up with the expected behavior of a recovery in forelimb asymmetry as due to the fact baseline activity would come out as 1, ideally if graphed as a line the data would behave as if the value of 1 was an asymptote.

The male ipsilateral Montoya data(figure 12) shows a deficit of 26.1% in the control group on post-surgery day 3 while the Fluoxetine Simvastatin and Ascorbic acid Drug group shows a deficit of 26.9% on the same day. On post-surgery day 15 the control group shows a deficit that has reduced to a 14.3% while the Fluoxetine Simvastatin and Ascorbic acid group had a 17.4%

deficit. On post-surgery day 30 the control group has slightly exceeded baseline at an excess of 3.6 %: although it exceeds baseline it does so by an amount that more likely due to natural variation than the injury causing better performance after recovery. However the Montoya data can be interpreted as the control group on average making a full recovery, or very close to full recovery by post-surgery day 30 in contrast the Fluoxetine Simvastatin and Ascorbic acid group on day 30 still had a deficit of 17.7%. The data does not show significance in control vs Fluoxetine Simvastatin and Ascorbic acid ($p= 0.139$) however it does show significant difference in function over time, with the PSD 3 vs PSD 30 $p<0.001$. The control vs Fluoxetine Simvastatin and Ascorbic acid p value could shift to significance with a higher n value as it is less than 0.15. Based on the means of each group at each time point, I would predict that if significance was reached with a greater N the data would show a significant reduced recovery in the Fluoxetine Simvastatin and Ascorbic acid Drug group, as the Fluoxetine Simvastatin and Ascorbic acid Drug groups recovery does show a pattern of slowing compared to the control, even if it was not a significant level of slowing with the size of the data set we were working with.

The male ipsilateral asymmetry palms touches had a post-surgery day 3 mean increase in use of 23.3% in the control and 20.6% in the fluoxetine simvastatin and ascorbic acid Drug group. On post-surgery day 15, the control increase in use had reduced to a 17.3 % while the Fluoxetine Simvastatin and Ascorbic acid actually had a deficit of 1.4%. On post-surgery day thirty the increase in use of was 26% in the control and a 3.9 % increase in use compared to baseline in the Fluoxetine Simvastatin and Ascorbic acid group.as with the contralateral male data this data might suffer from being a poor sample size of the population due to the loss of baseline data for a majority of the rats. There was not any significant difference between the fluoxetine simvastatin and ascorbic acid Drug group and control groups, with a p value of 0.335 and a drug group interaction with time factor p value of 0.575. While the ipsilateral asymmetry total had a

time factor p value of 0.614, a control group vs Fluoxetine Simvastatin and Ascorbic acid drug group p value of 0.441, and a drug group interaction with time factor p value of 0.777. With p values this high, even given the low statistical power we had with the male asymmetry tests it is unlikely that the asymmetry tests would yield statistically significant results if repeated with greater N values. The fact that the male Montoya ipsilateral was not quite significant might be due to the fact that any damage occurring to the contralateral brain hemisphere to the site of the intended injury was collateral. as motor control is located in the brain hemisphere contralateral to the periphery being tested.

Comparing the Montoya results to the analogous asymmetry results, the Montoya staircase test in all cases seems to be a more appropriate and sensitive test when testing this model of MS damage and recovery. Interestingly Ragas, Nagarajan and Corbett (28) found that in an ischemia model forelimb asymmetry was a less sensitive test for assessing damage if fingertip touches were included, however unlike this study they did not see a difference in forelimb asymmetry using only palms in comparison to the Montoya test. This along with the fact that forelimb analysis is a test of gross motor function while Montoya requires fine motor function leads me to postulate that the differences in this study and Ragas Nagarajan and Corbett(28), may be due to differences in how the injuries being modeled affect fine and gross motor function. Support for this idea may be found in the evidence that dyspraxia and apraxia type symptoms do have some correlation with callosal damage this additionally relates to the callosal demyelination being a good model for Multiple sclerosis as rarely multiple sclerosis manifests symptoms that are indicative of callosal damage such as alien hand syndrome(15, 41, 42) In addition multiple sclerosis often does involve damage to callosal axons(14) Additionally from comparing the Montoya data it is clear that female rats do not seem to be significantly affected by the fluoxetine simvastatin and ascorbic acid administration in terms of motor recovery, while

recovery for males seems to be adversely affected by fluoxetine simvastatin and ascorbic acid especially in the site of demyelination as evidenced by the slowing of recovery of peripheral contralateral function.. However, I feel that no conclusions about that can be definitively explored without histological analysis, as this data could also be explained by a lower amount of damage to be fixed on the hemisphere contralateral to the injection site. Said histological analysis was done by other members of the laboratory.

**Figure 13 Oligo1 staining of sham versus control**

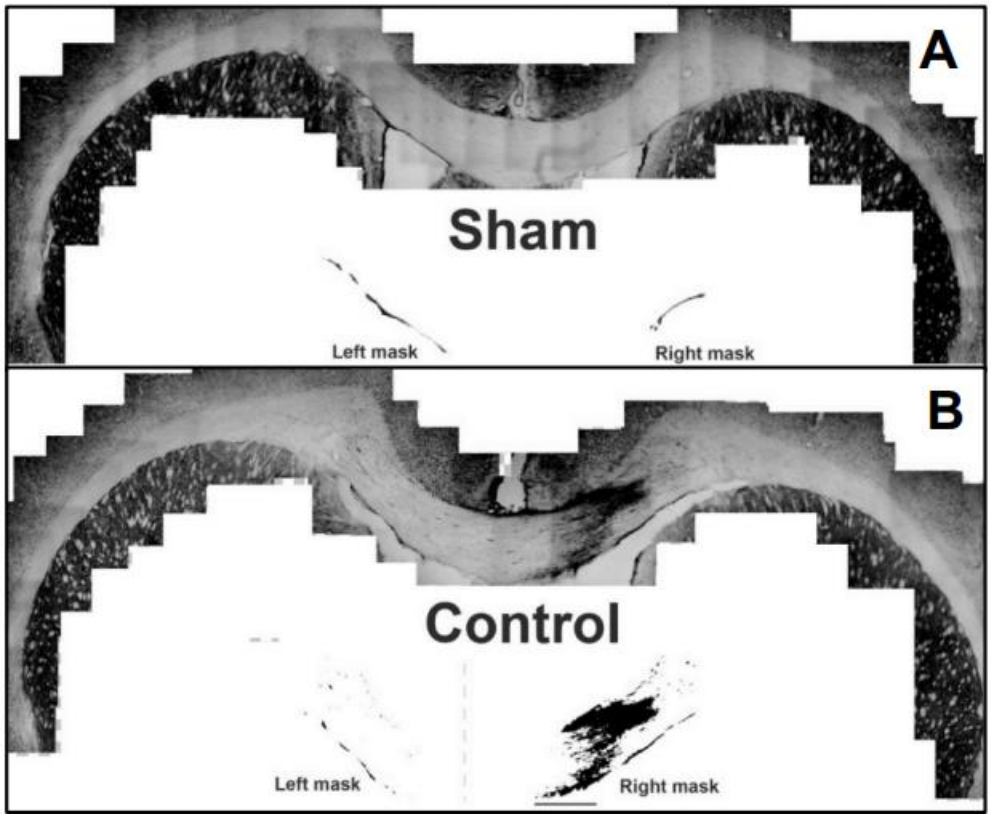


*Figure 13 oligo1 staining image the panel A is a sham rat which did not receive surgery while figure b is a control rat that received a surgery(43).*

**Figure 14 dcx staining of ventricles**

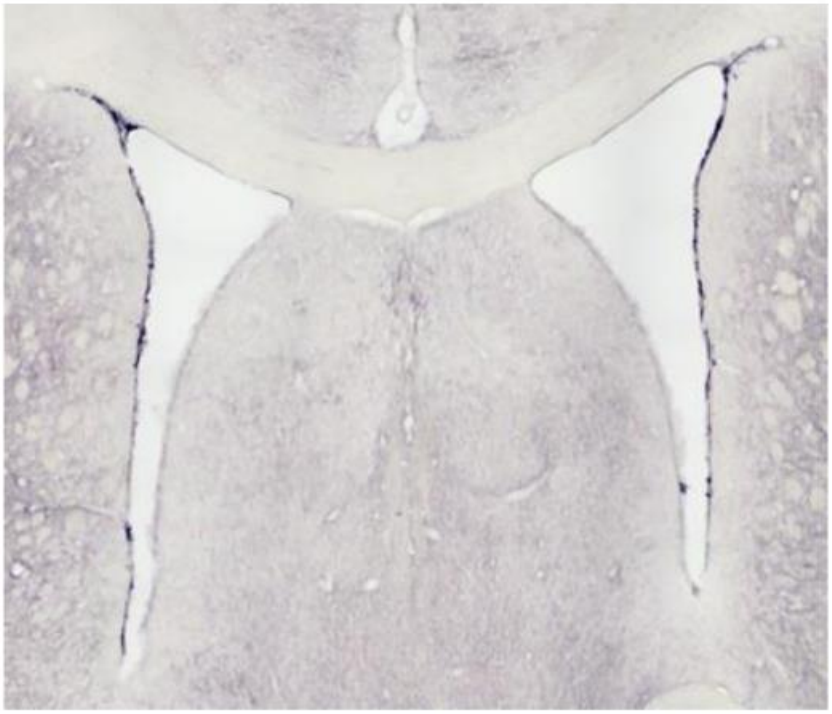

*Figure 14 is stitiched together from microscope images view of the ventricles with staining for dcx in dark purple.*

Figure 13 is from An Undergraduate senior capstone done in the lab, the increased staining in the right side on panel b shows that the lysolecithin injection was successful, this image is a representative of the group. Figure 14 is another histological image from the thesis of another member of the lab.(44) this shows the proliferation of progenitor cells including neurons and with a lesser density oligodendrocytes(45)

One possible explanation for difference in results based on gender involves the statin portion of the fluoxetine simvastatin and ascorbic acid, interfering with the cholesterol synthesis pathway by working against HMG-CoA reductase preventing the reduction of HMG-CoA into mevalonic acid. This may affect recovery because cholesterol is a precursor to the synthesis of testosterone which has some known neuroprotective effects and neurogenesis promoting effect(46-48). In addition there is a study that saw castration attenuate the rate of

remyelination in a lysolecithin demyelination model(49). While estrogen is also downstream of cholesterol the age of the rats, we used means that the female rats have reduced levels of estrogen in the first place, approximating a close to menopause state hormonally. Whether this is the case could be assessed by doing a testosterone assay at multiple points during treatment, having a Fluoxetine Simvastatin and Ascorbic acid Drug group a control group and repeating the Montoya staircase test to correlate recovery with the drug groups and with testosterone levels within those groups. In addition, a post euthanization assay for testosterone concentration could be done as well as histological staining for neurogenesis and oligodendrocyte growth indicators in the tissue. To further elucidate the individual effects of the components of the Fluoxetine Simvastatin and Ascorbic acid mix when it comes to male demyelination injury one could run trials with groups given each component individually and look at the testosterone results. One reason looking into what component of the fluoxetine simvastatin and ascorbic acid is contributing to the slowed recovery in males may have clinical importance as it shows situations where prescribing the components of the Fluoxetine Simvastatin and Ascorbic acid mix are contraindicated and alternatives should be attempted first.

Some other studies into drugs to attempt recovery of motor function in Multiple sclerosis models have been done previously. However, many of those seem to either be based solely on histological and electrophysiological data or behavioral methods like the Rotocylinder test, a test which involves having a rat try to stay on a rotating cylinder sort of like the lumberjack sport of log rolling except with only one direction of spin at a constant speed. which are not necessarily behavioral tests that model how the motor function affects of the injury would apply to normal human activities. (42, 50). Other studies use models where the region targeted for demyelination is the optic chiasm.(49) The literature does have much more analogous behavioral tests to this occurring in the realm of white matter damage caused by

ischemia(22, 39, 48) however extending the methodology to a model for multiple sclerosis seems novel in the literature.

It is also important to look at the laterality of affected behavior, previous studies in ischemic stroke models have shown that deficits to ipsilateral function are likely due to damage to white matter in and leading to the corpus callosum, as callosal damage causes bilateral deficits, while contralateral deficits are due to cortical damage(39, 40): this shows another reason that forelimb asymmetry is a less desirable test for this model when compared to the Montoya staircase. In the ipsilateral forelimb tests there is an increase in use, this increase in use is due to compensatory behavior; the issue is that this compensatory behavior masks any potential ipsilateral effects of white matter damage. The model of injecting lysolecithin into a white matter tract is supposed to model multiple sclerosis a myelin attacking autoimmune disease. This works due to lysolecithins such lysophosphatidyl choline being almost exclusively damaging to myelin and not other cellular components.(51) Myelinated axons are the primary component of white matter means that a test which masks white matter damage prevents the observation of pertinent data. As the main factor in Multiple sclerosis recovery would be remyelination of axons which could most readily be distinguished in the white matter tracts.

In summary the conclusions that can be reached from this experiment are as follows. One, the forelimb asymmetry test in this model is insufficient for assessing demyelination damage due to ipsilateral compensation for the cortical damage induced contralateral deficits acting to mask potential ipsilateral deficits which would be a better indicator of callosal myelin integrity. Two, in female rats from the Montoya results it can be concluded that the drug cocktail of fluoxetine, simvastatin, and ascorbic acid, does not significantly speed or hinder recovery compared to control. Three from the male Montoya data it can be inferred that something about the fluoxetine, simvastatin and ascorbic acid cocktail hinders male recovery

rate, though given the experiments done, the mechanism via which the cocktail hinders male rat recovery cannot be confirmed only speculated on and as a potential topic of further investigation.

## References


1.Białek M, Zaremba P, Borowicz KK, Czuczwar SJ. Neuroprotective role of testosterone in the nervous system. Polish journal of pharmacology. 2004;56(5):509-18. PubMed PMID: 15591638.
2.Whishaw IQ, Kolb B. The behavior of the laboratory rat : a handbook with tests. Oxford: Oxford University Press; 2004. 528 p. p.
3.Lee JH, Tigchelaar S, Liu J, Stammers AM, Streijger F, Tetzlaff W, Kwon BK. Lack of neuroprotective effects of simvastatin and minocycline in a model of cervical spinal cord injury. Exp Neurol. 2010;225(1):219-30. Epub 2010/07/06. doi: 10.1016/j.expneurol.2010.06.018. PubMed PMID: 20599974.
4.Sung CY, Chiang PK, Tsai CW, Yang FY. Low-Intensity Pulsed Ultrasound Enhances Neurotrophic Factors and Alleviates Neuroinflammation in a Rat Model of Parkinson's Disease. Cereb Cortex. 2021. Epub 2021/07/02. doi: 10.1093/cercor/bhab201. PubMed PMID: 34196669.
5.Ragas M, Nagarajan D, Corbett AM. Refining forelimb asymmetry analysis: Correlation with Montoya staircase contralateral function post-stroke. J Neurosci Methods. 2017;290:52-6. Epub 2017/07/26. doi: 10.1016/j.jneumeth.2017.07.021. PubMed PMID: 28739162.
6.Montoya CP, Campbell-Hope LJ, Pemberton KD, Dunnett SB. The "staircase test": a measure of independent forelimb reaching and grasping abilities in rats. J Neurosci Methods. 1991;36(2-3):219-28. Epub 1991/02/01. doi: 10.1016/0165-0270(91)90048-5. PubMed PMID: 2062117.
7.Rasminsky M, Sears TA. Internodal conduction in undissected demyelinated nerve fibres. The Journal of Physiology. 1972;227(2):323-50. doi: https://doi.org/10.1113/jphysiol.1972.sp010035.
8.Bagheri H, Ghasemi F, Barreto GE, Sathyapalan T, Jamialahmadi T, Sahebkar A. The effects of statins on microglial cells to protect against neurodegenerative disorders: A mechanistic review. Biofactors. 2020;46(3):309-25. Epub 2019/12/18. doi: 10.1002/biof.1597. PubMed PMID: 31846136.
9.Lee CY, Landreth GE. The role of microglia in amyloid clearance from the AD brain. Journal of Neural Transmission. 2010;117(8):949-60. doi: 10.1007/s00702-010-0433-4. PubMed PMID: 52760266.
10.Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. NATURE REVIEWS IMMUNOLOGY. 2011;11(11):775-87. doi: 10.1038/nri3086.
11.Su F, Yi H, Xu L, Zhang Z. Fluoxetine and S-citalopram inhibit M1 activation and promote M2 activation of microglia in vitro. Neuroscience. 2015;294:60-8. Epub 2015/02/26. doi: 10.1016/j.neuroscience.2015.02.028. PubMed PMID: 25711936.
12.Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 Macrophages and the Th1/Th2 Paradigm. The Journal of Immunology. 2000;164(12):6166. doi: 10.4049/jimmunol.164.12.6166.
13.Lenzi D, Conte A, Mainero C, Frasca V, Fubelli F, Totaro P, Caramia F, Inghilleri M, Pozzilli C, Pantano P. Effect of corpus callosum damage on ipsilateral motor activation in patients with multiple sclerosis: a functional and anatomical study. Human brain mapping. 2007;28(7):636-44. doi: 10.1002/hbm.20305. PubMed PMID: 17080438.

14.Russo AW, Stockel KE, Tobyne SM, Ngamsombat C, Brewer K, Nummenmaa A, Huang SY, Klawiter EC. Associations between corpus callosum damage, clinical disability, and surface-based homologous inter-hemispheric connectivity in multiple sclerosis. Brain Structure and Function. 2022. doi: 10.1007/s00429-022-02498-7.
15.Intermittent Alien Hand Syndrome and Callosal Apraxia in Multiple Sclerosis: Implications for Interhemispheric Communication. Hindawi Limited; 2014. p. 1-7.
16.Sommi RW, Crismon ML, Bowden CL. Fluoxetine: A Serotonin-specific, Second-generation Antidepressant. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 1987;7(1):1-14. doi: https://doi.org/10.1002/j.1875-9114.1987.tb03496.x.
17.Vidal R, Valdizán EM, Mostany R, Pazos A, Castro E. Long-term treatment with fluoxetine induces desensitization of 5-$HT_4$ receptor-dependent signalling and functionality in rat brain. Journal of Neurochemistry. 2009;110(3):1120-7. doi: 10.1111/j.1471-4159.2009.06210.x. PubMed PMID: 2010-03089-032.
18.Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci. 2000;20(24):9104-10. Epub 2000/01/11. PubMed PMID: 11124987; PMCID: PMC6773038.
19.Peng Q, Masuda N, Jiang M, Li Q, Zhao M, Ross CA, Duan W. The antidepressant sertraline improves the phenotype, promotes neurogenesis and increases BDNF levels in the R6/2 Huntington's disease mouse model. Experimental Neurology. 2008;210(1):154-63. doi: 10.1016/j.expneurol.2007.10.015. PubMed PMID: S0014488607003974.
20.Zhang F, Zhou H, Wilson BC, Shi J-S, Hong J-S, Gao H-M. Fluoxetine protects neurons against microglial activation-mediated neurotoxicity. Parkinsonism & related disorders. 2012;18 Suppl 1:S213-S7. doi: 10.1016/S1353-8020(11)70066-9. PubMed PMID: 22166439.
21.Scheidereit C. Docking IκB kinases. Nature. 1998;395(6699):225-6. doi: 10.1038/26121.
22.Tian M, Yang M, Li Z, Wang Y, Chen W, Yang L, Li Y, Yuan H. Fluoxetine suppresses inflammatory reaction in microglia under OGD/R challenge via modulation of NF-κB signaling. Bioscience Reports. 2019;39(4):BSR20181584. doi: 10.1042/BSR20181584.
23.Nazimek K, Strobel S, Bryniarski P, Kozlowski M, Filipczak-Bryniarska I, Bryniarski K. The role of macrophages in anti-inflammatory activity of antidepressant drugs. Immunobiology. 2017;222(6):823-30. doi: https://doi.org/10.1016/j.imbio.2016.07.001.
24.Chen J, Zhang ZG, Li Y, Wang Y, Wang L, Jiang H, Zhang C, Lu M, Katakowski M, Feldkamp CS, Chopp M. Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. Ann Neurol. 2003;53(6):743-51. Epub 2003/06/05. doi: 10.1002/ana.10555. PubMed PMID: 12783420.
25.Ross AC, Anthony CR, Serena JT, Alexis LF, Krystle AF, Jacob KW, Neerupma S, Suban B, James VP, Uma RC, Monaghan AP, Donald BD. Statins impact primary embryonic mouse neural stem cell survival, cell death, and fate through distinct mechanisms. PLoS ONE. 2018;13(5):e0196387-e. doi: 10.1371/journal.pone.0196387. PubMed PMID: edsdoj.fedb5c1811a044e9ab322d3d4ea1e739.
26.Wani MA, Mukherjee S, Mallick S, Akbar I, Basu A. Atorvastatin ameliorates viral burden and neural stem/ progenitor cell (NSPC) death in an experimental model of Japanese encephalitis. Journal of biosciences. 2020;45. PubMed PMID: 32515359.
27.Blanco-Colio LM, Tuñón J Fau - Martín-Ventura JL, Martín-Ventura Jl Fau - Egido J, Egido J. Anti-inflammatory and immunomodulatory effects of statins(0085-2538 (Print)).
28.Ruleva NY, Radyukhina NV, Zubkova ES, Filatova AY, Aref'eva TI. Inhibitors of 3-Hydroxy-3-Methylglutaryl Coenzyme a Reductase (Statins) Suppress Differentiation and Reduce LPS/IFNγ-Induced Cytokine Production in Human Monocyte/Macrophage Culture. Bulletin of

Experimental Biology & Medicine. 2020;170(2):236-40. doi: 10.1007/s10517-020-05042-x. PubMed PMID: 147410838.
29.Endo A. The origin of the statins. 2004. Atheroscler Suppl. 2004;5(3):125-30. Epub 2004/11/09. doi: 10.1016/j.atherosclerosissup.2004.08.033. PubMed PMID: 15531285.
30.Arefieva TI, Filatova AY, Potekhina AV, Shchinova AM. Immunotropic Effects and Proposed Mechanism of Action for 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Inhibitors (Statins). Biochemistry (Moscow). 2018;83(8):874-89. doi: 10.1134/S0006297918080023.
31.Tavassolifar MJ, Vodjgani M, Salehi Z, Izad M. The Influence of Reactive Oxygen Species in the Immune System and Pathogenesis of Multiple Sclerosis. Autoimmune Dis. 2020;2020:5793817. Epub 2020/08/14. doi: 10.1155/2020/5793817. PubMed PMID: 32789026; PMCID: PMC7334772.
32.Wong A, Dukic-Stefanovic S, Gasic-Milenkovic J, Schinzel R, Wiesinger H, Riederer P, Münch G. Anti-inflammatory antioxidants attenuate the expression of inducible nitric oxide synthase mediated by advanced glycation endproducts in murine microglia. The European journal of neuroscience. 2001;14(12):1961-7. doi: 10.1046/j.0953-816x.2001.01820.x. PubMed PMID: 11860491.
33.Montel-Hagen A, Kinet S, Manel N, Mongellaz C, Prohaska R, Battini JL, Delaunay J, Sitbon M, Taylor N. Erythrocyte Glut1 triggers dehydroascorbic acid uptake in mammals unable to synthesize vitamin C. Cell. 2008;132(6):1039-48. Epub 2008/03/25. doi: 10.1016/j.cell.2008.01.042. PubMed PMID: 18358815.
34.Frikke-Schmidt H, Tveden-Nyborg P, Lykkesfeldt J. l-dehydroascorbic acid can substitute l-ascorbic acid as dietary vitamin C source in guinea pigs. Redox Biology. 2016;7:8-13. doi: https://doi.org/10.1016/j.redox.2015.11.003.
35.May JM, Qu ZC, Whitesell RR. Ascorbic acid recycling enhances the antioxidant reserve of human erythrocytes. Biochemistry. 1995;34(39):12721-8. doi: 10.1021/bi00039a031. PubMed PMID: 7548025.
36.Keough MB, Jensen SK, Yong VW. Experimental demyelination and remyelination of murine spinal cord by focal injection of lysolecithin. Journal of visualized experiments : JoVE. 2015(97). doi: 10.3791/3564. (PMID: 22871843); Ann Neurol. 2012 Sep;72(3):419-32. (PMID: 23034914); Neurotherapeutics. 2013 Jan;10(1):44-54. (PMID: 23070731); Proc Natl Acad Sci U S A. 2013 Mar 5;110(10):4075-80. (PMID: 23431182); Neuron. 2013 Mar 6;77(5):873-85. (PMID: 23473318); Nat Neurosci. 2013 Sep;16(9):1211-8. (PMID: 23872599); Nat Med. 2014 Aug;20(8):954-60. (PMID: 24997607). Linking ISSN: 1940087X. Subset: MEDLINE; Date of Electronic Publication: 2015 Mar 26. ; Original Imprints: Publication: [Boston, Mass. : MYJoVE Corporation, 2006]- 10.3791/52679. PubMed PMID: 25867716.
37.Furusho M, Roulois AJ, Franklin RJM, Bansal R. Fibroblast growth factor signaling in oligodendrocyte-lineage cells facilitates recovery of chronically demyelinated lesions but is redundant in acute lesions. Glia. 2015;63(10):1714-28. doi: 10.1002/glia.22838. PubMed PMID: 2015-18317-001.
38.Sanabria-Castro A, Flores-Díaz M, Alape-Girón A. Biological models in multiple sclerosis. Journal of Neuroscience Research. 2020;98(3):491-508. doi: https://doi.org/10.1002/jnr.24528.
39.Allred RP, Cappellini CH, Jones TA. The "good" limb makes the "bad" limb worse: experience-dependent interhemispheric disruption of functional outcome after cortical infarcts in rats. Behavioral neuroscience. 2010;124(1):124-32. Epub 2010/02/10. doi: 10.1037/a0018457. PubMed PMID: 20141287; PMCID: PMC4888870.
40.Sulehria T, Corbett AM, Sharma N, Nagarajan D, Abushamma A, Gagle S, Johnson A. Increasing Progenitor Cell Proliferation in the Sub-Ventricular Zone: A Therapeutic Treatment for

Progressive Multiple Sclerosis? Recent Pat Drug Deliv Formul. 2020;14(3):233-41. Epub 2020/11/20. doi: 10.2174/1872211314999201117130123. PubMed PMID: 33208084.
41.Cimino-Knight AM, Gonzalez Rothi LJ, He Y, Heilman KM. Callosal ideomotor apraxia in Alzheimer's disease. JOURNAL OF CLINICAL AND EXPERIMENTAL NEUROPSYCHOLOGY. 2017;39(1):1-8. doi: 10.1080/13803395.2016.1180345. PubMed PMID: WOS:000389499700001.
42.Gazzaniga MS, Bogen JE, Sperry RW. Dyspraxia following division of the cerebral commissures. Arch Neurol. 1967;16(6):606-12. Epub 1967/06/01. doi: 10.1001/archneur.1967.00470240044005. PubMed PMID: 6026069.
43.Johnson T, Corbett A. Drug Combination Effect on Male Oligodendrocyte Proliferation Following Focal Demyelination. [senior capstone]. In press.
44.Webb CO. Effects of Fluoxetine/Simvastatin/Ascorbic Acid Combination Treatment on Neurogenesis and Functional Recovery in a Model of Multiple Sclerosis: Wright State University; 2021.
45.Boulanger JJ, Messier C. Doublecortin in Oligodendrocyte Precursor Cells in the Adult Mouse Brain. Frontiers in Neuroscience. 2017;11. doi: 10.3389/fnins.2017.00143.
46.Gurer B, Kertmen H, Kasim E, Yilmaz ER, Kanat BH, Sargon MF, Arikok AT, Erguder BI, Sekerci Z. Neuroprotective effects of testosterone on ischemia/reperfusion injury of the rabbit spinal cord. Injury. 2015;46(2):240-8. Epub 2014/12/04. doi: 10.1016/j.injury.2014.11.002. PubMed PMID: 25467821.
47.Spritzer MD, Roy EA. Testosterone and Adult Neurogenesis. Biomolecules. 2020;10(2). Epub 2020/02/08. doi: 10.3390/biom10020225. PubMed PMID: 32028656; PMCID: PMC7072323.
48.Corbett AM, Sieber S, Wyatt N, Lizzi J, Flannery T, Sibbit B, Sanghvi S. Increasing neurogenesis with fluoxetine, simvastatin and ascorbic Acid leads to functional recovery in ischemic stroke. Recent Pat Drug Deliv Formul. 2015;9(2):158-66. Epub 2015/01/24. doi: 10.2174/1872211309666150122102846. PubMed PMID: 25612744.
49.Sherafat M, Javan M, Mozafari S, Mirnajafi-Zadeh J, Motamedi F. Castration Attenuates Myelin Repair Following Lysolecithin Induced Demyelination in Rat Optic Chiasm: An Evaluation Using Visual Evoked Potential, Marker Genes Expression and Myelin Staining. Neurochemical Research. 2011;36(10):1887-95. doi: 10.1007/s11064-011-0510-6. PubMed PMID: 64846058.
50.Moore S, Khalaj AJ, Yoon J, Patel R, Hannsun G, Yoo T, Sasidhar M, Martinez-Torres L, Hayardeny L, Tiwari-Woodruff SK. Therapeutic laquinimod treatment decreases inflammation, initiates axon remyelination, and improves motor deficit in a mouse model of multiple sclerosis. Brain and behavior. 2013;3(6):664-82. doi: 10.1002/brb3.174. PubMed PMID: 24363970.
51.Hall SM. The Effect of Injections of Lysophosphatidyl Choline into White Matter of the Adult Mouse Spinal Cord. Journal of Cell Science. 1972;10(2):535-46. doi: 10.1242/jcs.10.2.535.

## Appendix A

*Table color key FSA stands for experimental drug combination*

| |
|---|
| FSA |
| control |
| sham |

*Table 1*

| Rat ID | Pre-stroke | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | R Palm | Right Tips | Right total | Left Palm | Left Tips | Left Total | Ispi Palms | Contr Palms | Ipsi Tot | Cont Tot |
| MS1 | 10 | 3 | 13 | 18 | 1 | 19 | 0.3571428571 | 0.6428571429 | 0.40625 | 0.59375 |
| MS2 | | | | | | | | | | |
| MS3 | | | | | | | | | | |
| MS4 | | | | | | | | | | |
| MS5 | | | | | | | | | | |
| MS6 | | | | | | | | | | |
| MS7 | | | | | | | | | | |
| MS8 | | | | | | | | | | |
| MS9 | | | | | | | | | | |
| MS10 | | | | | | | | | | |
| MS11 | | | | | | | | | | |
| MS12 | 24 | 1 | 25 | 21 | 2 | 23 | 0.5333333333 | 0.4666666667 | 0.5208333333 | 0.4791666667 |
| MS13 | 46 | 8 | 54 | 48 | 9 | 57 | 0.4893617021 | 0.5106382979 | 0.4864864865 | 0.5135135135 |
| MS14 | 29 | | 29 | 31 | | 31 | 0.4833333333 | 0.5166666667 | 0.4833333333 | 0.5166666667 |
| MS15 | 16 | 1 | 17 | 19 | 4 | 23 | 0.4571428571 | 0.5428571429 | 0.425 | 0.575 |
| MS16 | 101 | 10 | 111 | 86 | 15 | 101 | 0.5401069519 | 0.4598930481 | 0.5235849057 | 0.4764150943 |
| MS17 | 42 | 1 | 43 | 54 | 1 | 55 | 0.4375 | 0.5625 | 0.4387755102 | 0.5612244898 |
| MS18 | 30 | 9 | 39 | 22 | 3 | 25 | 0.5769230769 | 0.4230769231 | 0.609375 | 0.390625 |
| MS19 | 26 | | 26 | 26 | | 26 | 0.5 | 0.5 | 0.5 | 0.5 |
| MS20 | 35 | 3 | 38 | 26 | 2 | 28 | 0.5737704918 | 0.4262295082 | 0.5757575758 | 0.4242424242 |
| MS21 | 35 | 3 | 38 | 52 | 7 | 59 | 0.4022988506 | 0.5977011494 | 0.3917525773 | 0.6082474227 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| MS22 | 39 | 5 | 44 | 42 | 1 | 43 | 0.4814814815 | 0.5185185185 | 0.5057471264 | 0.4942528736 |
| MS23 | 46 | 1 | 47 | 51 | 4 | 55 | 0.4742268041 | 0.5257731959 | 0.4607843137 | 0.5392156863 |
| MS24 | 38 | 8 | 46 | 32 | 4 | 36 | 0.5428571429 | 0.4571428571 | 0.5609756098 | 0.4390243902 |
| MS25 | 42 | 14 | 56 | 58 | 3 | 61 | 0.42 | 0.58 | 0.4786324786 | 0.5213675214 |
| MS26 | 73 | | 73 | 63 | 4 | 67 | 0.5367647059 | 0.4632352941 | 0.5214285714 | 0.4785714286 |
| MS27 | 74 | | 74 | 70 | 2 | 72 | 0.5138888889 | 0.4861111111 | 0.5068493151 | 0.4931506849 |
| MS28 | 54 | 9 | 63 | 85 | 4 | 89 | 0.3884892086 | 0.6115107914 | 0.4144736842 | 0.5855263158 |
| MS29 | 114 | 18 | 132 | 104 | 8 | 112 | 0.5229357798 | 0.4770642202 | 0.5409836066 | 0.4590163934 |
| MS30 | 117 | 3 | 120 | 113 | 8 | 121 | 0.5086956522 | 0.4913043478 | 0.4979253112 | 0.5020746888 |
| MS31 | 72 | 1 | 73 | 55 | 15 | 70 | 0.5669291339 | 0.4330708661 | 0.5104895105 | 0.4895104895 |
| MS32 | 51 | 2 | 53 | 44 | 9 | 53 | 0.5368421053 | 0.4631578947 | 0.5 | 0.5 |
| MS33 | 33 | 4 | 37 | 26 | 5 | 31 | 0.5593220339 | 0.4406779661 | 0.5441176471 | 0.4558823529 |
| MS34 | 63 | 2 | 65 | 77 | 3 | 80 | 0.45 | 0.55 | 0.4482758621 | 0.5517241379 |
| MS35 | 86 | 3 | 89 | 79 | 3 | 82 | 0.5212121212 | 0.4787878788 | 0.5204678363 | 0.4795321637 |
| MS36 | 83 | 4 | 87 | 48 | 11 | 59 | 0.6335877863 | 0.3664122137 | 0.595890411 | 0.404109589 |
| MS37 | 108 | 3 | 111 | 108 | 4 | 112 | 0.5 | 0.5 | 0.4977578475 | 0.5022421525 |
| MS38 | 32 | | 32 | 21 | 4 | 25 | 0.6037735849 | 0.3962264151 | 0.5614035088 | 0.4385964912 |
| MS39 | 135 | 11 | 146 | 114 | 9 | 123 | 0.5421686747 | 0.4578313253 | 0.5427509294 | 0.4572490706 |
| MS40 | 50 | | 50 | 30 | 6 | 36 | 0.625 | 0.375 | 0.5813953488 | 0.4186046512 |

*This table shows the prestrike baseline measurements of the rats, the videos for analysis of MS 2-11 were lost and could not be found,*

*Table 2*

| Rat ID | Post Surgery Day 3 | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R Palm | Right Tips | Right total | Left Palm | Left Tips | Left Total | Ispi Palms | Contr Palms | Ipsi Tot | Cont Tot | Norm Ipsi P | Norm Contra P | Norm Ipsi Tot | Norm Contr Tot |
| MS1 | 20 | | 20 | 21 | 2 | 23 | 0.487804878 | 0.512195122 | 0.465116279 | 0.534883721 | 1.365853659 | 0.796747967 | 1.14490161 | 0.900856793 |
| MS2 | 51 | 1 | 52 | 36 | 11 | 47 | 0.586206897 | 0.413793103 | 0.525252525 | 0.474747475 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS3 | 126 | 1 | 127 | 100 | 1 | 101 | 0.557522124 | 0.442477876 | 0.557017544 | 0.442982456 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS4 | 36 | 1 | 37 | 13 | | 13 | 0.734693878 | 0.265306122 | 0.74 | 0.26 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS5 | 11 | | 11 | 11 | 1 | 12 | 0.5 | 0.5 | 0.478260870 | 0.521739130 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS6 | 18 | | 18 | 11 | | 11 | 0.620689655 | 0.379310345 | 0.620689655 | 0.379310345 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS7 | 55 | | 55 | 21 | 2 | 23 | 0.723684211 | 0.276315789 | 0.705128205 | 0.294871795 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS8 | 21 | 1 | 22 | 16 | 1 | 17 | 0.567567568 | 0.432432432 | 0.564102564 | 0.435897436 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS9 | 30 | 1 | 31 | 30 | 2 | 32 | 0.5 | 0.5 | 0.492063492 | 0.507936508 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MS 10 | 55 | | 55 | 26 | 4 | 30 | 0.679012345679 | 0.320987654321 | 0.647058823529 | 0.352941176471 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS 11 | 53 | 2 | 55 | 56 | 1 | 57 | 0.486238532110 | 0.513761467890 | 0.491071428571 | 0.508928571429 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS 12 | 59 | 8 | 67 | 24 | 10 | 34 | 0.710843373494 | 0.289156626506 | 0.663366336634 | 0.336633663366 | 1.332831325 | 0.619621342 | 1.273663366 | 0.702539819 |
| MS 13 | 52 | | 52 | 52 | 2 | 54 | 0.5 | 0.5 | 0.490566037736 | 0.509433962264 | 1.021739130 | 0.979166667 | 1.008385744 | 0.992055610 |
| MS 14 | 30 | | 30 | 16 | 2 | 18 | 0.652173913 | 0.347826087 | 0.625 | 0.375 | 1.349325337 | 0.673211781 | 1.293103448 | 0.725806451 |
| MS 15 | 21 | 1 | 22 | 18 | 5 | 23 | 0.538461538 | 0.461538461 | 0.488888888 | 0.511111111 | 1.177884615 | 0.850202429 | 1.150326797 | 0.888888888 |
| MS 16 | 79 | 0 | 79 | 25 | 14 | 39 | 0.759615384 | 0.240384615 | 0.669491525 | 0.330508474 | 1.406416603 | 0.522696778 | 1.278668499 | 0.693740560 |
| MS 17 | 27 | 2 | 29 | 19 | 9 | 28 | 0.586956521 | 0.413043478 | 0.508771929 | 0.491228070 | 1.341614907 | 0.734299516 | 1.159526724 | 0.875279106 |
| MS 18 | 43 | 3 | 46 | 47 | 1 | 48 | 0.477777777 | 0.522222222 | 0.489361702 | 0.510638297 | 0.828148148 | 1.234343434 | 0.803055100 | 1.307234043 |
| MS 19 | 23 | 2 | 25 | 29 | 2 | 31 | 0.442307692 | 0.557692307 | 0.446428571 | 0.553571428 | 0.884615384 | 1.115384615 | 0.892857142 | 1.107142857 |
| MS 20 | 18 | | 18 | 11 | | 11 | 0.620689655 | 0.379310344 | 0.620689655 | 0.379310344 | 1.081773399 | 0.889920424 | 1.078039927 | 0.894088607 |
| MS 21 | 35 | | 35 | 51 | 1 | 52 | 0.406976744 | 0.593023255 | 0.402298850 | 0.597701149 | 1.011627907 | 0.992173524 | 1.026920751 | 0.982661211 |
| MS 22 | 67 | 5 | 72 | 67 | 11 | 78 | 0.5 | 0.5 | 0.48 | 0.52 | 1.038461538 | 0.964285714 | 0.949090909 | 1.052093023 |
| MS 23 | 80 | 8 | 88 | 80 | | 80 | 0.5 | 0.5 | 0.523809523 | 0.476190476 | 1.054347826 | 0.950980392 | 1.136778116 | 0.883116831 |
| MS 24 | 25 | | 25 | 16 | 2 | 18 | 0.609756097 | 0.390243902 | 0.581395348 | 0.418604651 | 1.123234917 | 0.853658536 | 1.036400404 | 0.953488372 |
| MS 25 | 42 | 2 | 44 | 40 | | 40 | 0.512195122 | 0.487804878 | 0.523809523 | 0.476190476 | 1.219512195 | 0.841042893 | 1.094387755 | 0.913348946 |
| MS 26 | 88 | 1 | 89 | 52 | 5 | 57 | 0.628571428 | 0.371428571 | 0.609589041 | 0.390410958 | 1.171037182 | 0.801814059 | 1.169074873 | 0.815784093 |
| MS 27 | 62 | | 62 | 30 | 11 | 41 | 0.673913043 | 0.326086956 | 0.601941747 | 0.398058252 | 1.311398355 | 0.670807453 | 1.187614799 | 0.807173678 |
| MS 28 | 71 | 3 | 74 | 47 | 5 | 52 | 0.601694915 | 0.398305084 | 0.587301587 | 0.412698412 | 1.548807282 | 0.651345962 | 1.416981607 | 0.704833244 |
| MS 29 | 88 | 2 | 90 | 78 | 1 | 79 | 0.530120481 | 0.469879518 | 0.532544378 | 0.467455621 | 1.013739167 | 0.984939759 | 0.984400215 | 1.018385461 |
| MS 30 | 180 | 2 | 182 | 169 | 7 | 176 | 0.515759312 | 0.484240687 | 0.508379888 | 0.491620111 | 1.013885828 | 0.985622638 | 1.020996276 | 0.979177247 |
| MS 31 | 77 | 1 | 78 | 42 | 9 | 51 | 0.647058823 | 0.352941176 | 0.604651162 | 0.395348837 | 1.141339869 | 0.814973262 | 1.184453648 | 0.807641196 |
| MS 32 | 62 | | 62 | 30 | 3 | 33 | 0.673913043 | 0.326086956 | 0.652631578 | 0.347368421 | 1.255328218 | 0.704051383 | 1.305263158 | 0.694736842 |
| MS 33 | 26 | 1 | 27 | 14 | 4 | 18 | 0.65 | 0.35 | 0.6 | 0.4 | 1.162121212 | 0.794230769 | 1.102702702 | 0.877419354 |
| MS 34 | 53 | 5 | 58 | 31 | 8 | 39 | 0.630952381 | 0.369047619 | 0.597938144 | 0.402061855 | 1.402116402 | 0.670995670 | 1.333862014 | 0.728737134 |
| MS 35 | 68 | 7 | 75 | 54 | 2 | 56 | 0.557377049 | 0.442622950 | 0.572519084 | 0.427480916 | 1.069386199 | 0.924465568 | 1.100008577 | 0.891454054 |
| MS 36 | 56 | 1 | 57 | 46 | 3 | 49 | 0.549019607 | 0.450980392 | 0.537735849 | 0.462264150 | 0.866524923 | 1.230800654 | 0.902407286 | 1.143907899 |
| MS 37 | 54 | 2 | 56 | 43 | 5 | 48 | 0.556701030 | 0.443298969 | 0.538461538 | 0.461538461 | 1.113402062 | 0.886597938 | 1.081774082 | 0.918956044 |
| MS 38 | 49 | 1 | 50 | 35 | | 35 | 0.583333333 | 0.416666666 | 0.588235294 | 0.411764705 | 0.966145833 | 1.051587302 | 1.047794118 | 0.938823529 |
| MS 39 | 57 | 7 | 64 | 61 | 4 | 65 | 0.483050847 | 0.516949152 | 0.496124031 | 0.503875968 | 0.890960452 | 1.129125781 | 0.914091536 | 1.101972648 |
| MS 40 | 50 | 2 | 52 | 25 | 6 | 31 | 0.666666666 | 0.333333333 | 0.626506024 | 0.373493975 | 1.066666667 | 0.888888889 | 1.077590361 | 0.892235609 |

*This table shows the rats data on post surgery day 3, as well as the data as normalized to presurgery. the error divide by 0 is due to the aforementioned rats who did not have a baseline for comparison.*

*Table 3*

| Rat ID | Post Surgery Day 15 | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R Palm | Right Tips | Right total | Left Palm | Left Tips | Left Total | Ispi Palms | Contr Palms | Ipsi Tot | Cont Tot | Norm Ipsi P | Norm Contra P | Norm Ipsi Tot | Norm Contr Tot |
| MS1 | 20 | | 20 | 21 | 2 | 23 | 0.487804878 | 0.512195122 | 0.465116279 | 0.534883721 | 1.365853659 | 0.796747967 | 1.144901611 | 0.900856793 |
| MS2 | 51 | 1 | 52 | 36 | 11 | 47 | 0.586206897 | 0.413793103 | 0.525252525 | 0.474747475 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS3 | 122 | 3 | 125 | 139 | | 139 | 0.467432950 | 0.532567050 | 0.473484848 | 0.526515152 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS4 | 38 | 1 | 39 | 35 | | 35 | 0.520547945 | 0.479452055 | 0.527027027 | 0.472972973 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS5 | 17 | | 17 | 9 | 1 | 10 | 0.653846154 | 0.346153846 | 0.629629630 | 0.370370370 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS6 | | | 0 | | | 0 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS7 | 43 | 1 | 44 | 25 | 2 | 27 | 0.632352941 | 0.367647059 | 0.619718310 | 0.380281690 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS8 | 2 | 1 | 3 | 6 | | 6 | 0.25 | 0.75 | 0.333333333 | 0.666666667 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS9 | 60 | 1 | 61 | 61 | | 61 | 0.495867768 | 0.504132231 | 0.5 | 0.5 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS10 | 29 | 1 | 30 | 41 | 3 | 44 | 0.414285714 | 0.585714286 | 0.405405405 | 0.594594595 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS11 | 32 | | 32 | 37 | | 37 | 0.463768116 | 0.536231884 | 0.463768116 | 0.536231884 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS12 | 47 | 1 | 48 | 36 | 2 | 38 | 0.566265060 | 0.433734940 | 0.558139535 | 0.441860465 | 1.061746988 | 0.929432013 | 1.071627907 | 0.922143579 |
| MS13 | 52 | | 52 | 52 | 2 | 54 | 0.5 | 0.5 | 0.490566038 | 0.509433962 | 1.021739130 | 0.979166667 | 1.008385744 | 0.992055610 |
| MS14 | 30 | | 30 | 16 | 2 | 18 | 0.652173913 | 0.347826087 | 0.625 | 0.375 | 1.349325337 | 0.673211781 | 1.293103448 | 0.725806452 |
| MS15 | 70 | 3 | 73 | 64 | 4 | 68 | 0.522388059 | 0.477611940 | 0.517730496 | 0.482269504 | 1.142723881 | 0.879811469 | 1.218189403 | 0.838729571 |
| MS16 | 58 | 1 | 59 | 32 | 9 | 41 | 0.644444444 | 0.355555556 | 0.59 | 0.41 | 1.193179318 | 0.773126615 | 1.126846847 | 0.860594059 |
| MS17 | 4 | 1 | 5 | 9 | | 9 | 0.307692307 | 0.692307692 | 0.357142857 | 0.642857143 | 0.703296703 | 1.230769231 | 0.813953488 | 1.145454545 |
| MS18 | | | 0 | | | 0 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS19 | 11 | | 11 | 13 | | 13 | 0.458333333 | 0.541666667 | 0.458333333 | 0.541666667 | 0.916666667 | 1.083333333 | 0.916666667 | 1.083333333 |
| MS20 | 17 | 2 | 19 | 17 | | 17 | 0.5 | 0.5 | 0.527777778 | 0.472222222 | 0.871428571 | 1.173076923 | 0.916666667 | 1.113095238 |
| MS21 | 73 | 2 | 75 | 88 | | 88 | 0.453416149 | 0.546583851 | 0.460122699 | 0.539877301 | 1.127062999 | 0.914476827 | 1.174523733 | 0.887594841 |
| MS22 | 66 | 4 | 70 | 87 | 3 | 90 | 0.431372549 | 0.568627451 | 0.4375 | 0.5625 | 0.895927601 | 1.096638655 | 0.865056818 | 1.138081395 |
| MS23 | 102 | 4 | 106 | 120 | 4 | 124 | 0.459459459 | 0.540540541 | 0.460869565 | 0.539130435 | 0.968860164 | 1.028086911 | 1.000185014 | 0.999841897 |
| MS24 | 55 | 2 | 57 | 59 | | 59 | 0.482456140 | 0.517543860 | 0.491379310 | 0.508620690 | 0.888734995 | 1.132127193 | 0.875937031 | 1.158524904 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MS25 | 44 | | 44 | 43 | 2 | 45 | 0.505747126 | 0.494252873 | 0.494382022 | 0.505617977 | 1.204159825 | 0.852160126 | 1.032905297 | 0.969791858 |
| MS26 | 111 | 3 | 114 | 98 | 2 | 100 | 0.531100478 | 0.468899521 | 0.532710280 | 0.467289719 | 0.989447466 | 1.012227539 | 1.021636154 | 0.976426279 |
| MS27 | 79 | 1 | 80 | 52 | 2 | 54 | 0.603053435 | 0.396946564 | 0.597014925 | 0.402985074 | 1.173509387 | 0.816575690 | 1.177894312 | 0.817164179 |
| MS28 | 79 | 5 | 84 | 95 | | 95 | 0.454022988 | 0.545977011 | 0.469273743 | 0.530726256 | 1.168688804 | 0.892832953 | 1.132216015 | 0.906408883 |
| MS29 | 78 | | 78 | 71 | 1 | 72 | 0.523489932 | 0.476510067 | 0.52 | 0.48 | 1.001059696 | 0.998838409 | 0.961212121 | 1.045714286 |
| MS30 | 143 | 1 | 144 | 120 | 2 | 122 | 0.543726235 | 0.456273764 | 0.541353383 | 0.458646616 | 1.068863544 | 0.928698812 | 1.087218045 | 0.913502652 |
| MS31 | 74 | | 74 | 60 | 2 | 62 | 0.552238806 | 0.447761194 | 0.544117647 | 0.455882352 | 0.974087893 | 1.033921303 | 1.065874295 | 0.931302521 |
| MS32 | 72 | | 72 | 55 | 5 | 60 | 0.566929133 | 0.433070866 | 0.545454545 | 0.454545454 | 1.056044465 | 0.935039370 | 1.090909091 | 0.909090909 |
| MS33 | 104 | 3 | 107 | 73 | 14 | 87 | 0.587570621 | 0.412429378 | 0.551546391 | 0.448453608 | 1.050505051 | 0.935897435 | 1.013652882 | 0.983704689 |
| MS34 | 62 | 0 | 62 | 64 | 4 | 68 | 0.492063492 | 0.507936507 | 0.476923076 | 0.523076923 | 1.093474427 | 0.923520923 | 1.063905325 | 0.948076923 |
| MS35 | 105 | 1 | 106 | 74 | 1 | 75 | 0.586592178 | 0.413407821 | 0.585635359 | 0.414364640 | 1.125438483 | 0.863446715 | 1.125209501 | 0.864101873 |
| MS36 | 62 | 1 | 63 | 28 | 5 | 33 | 0.688888889 | 0.311111111 | 0.65625 | 0.34375 | 1.087282463 | 0.849074074 | 1.101293103 | 0.850635593 |
| MS37 | | | 0 | | | 0 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS38 | 39 | 2 | 41 | 32 | | 32 | 0.549295774 | 0.450704225 | 0.561643835 | 0.438356164 | 0.909771126 | 1.137491616 | 1.000428082 | 0.999452054 |
| MS39 | 104 | | 104 | 53 | 2 | 55 | 0.662420382 | 0.337579617 | 0.654088050 | 0.345911949 | 1.221797594 | 0.737344954 | 1.205134832 | 0.756506621 |
| MS40 | 43 | | 43 | 28 | 2 | 30 | 0.605633802 | 0.394366197 | 0.589041095 | 0.410958904 | 0.969014084 | 1.051643192 | 1.013150685 | 0.981735159 |

*This table shows the data for the rats at post surgery day 15 as well as the data of day 15 normalized to presurgery baseline.*

*Table 4*

| Rat ID | Post Surgery Day 30 | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R Palm | Right Tips | Right total | Left Palm | Left Tips | Left Total | Ispi Palms | Contr Palms | Ipsi Tot | Cont Tot | Norm Ipsi P | Norm Contra P | Norm Ipsi Tot | Norm Contr Tot | Isp P Rec | Contral Rec | Isp T Rec | Cont T Rec |
| MS1 | 17 | | 17 | 9 | | 9 | 0.653846154 | 0.346153846 | 0.653846154 | 0.346153846 | 1.830769231 | 0.538461538 | 1.609467456 | 0.582995951 | 0.464915572 | -0.258286429 | 0.464565846 | -0.317860841 |
| MS2 | 26 | 6 | 32 | 18 | 1 | 19 | 0.590909091 | 0.409090909 | 0.627450980 | 0.372549019 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |
| MS3 | 74 | 4 | 78 | 83 | 5 | 88 | 0.471337579 | 0.528662420 | 0.469879518 | 0.530120481 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |
| MS4 | 10 | 1 | 11 | 7 | | 7 | 0.588235294 | 0.411764705 | 0.611111111 | 0.388888889 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |
| MS5 | 3 | | 3 | 1 | | 1 | 0.75 | 0.25 | 0.75 | 0.25 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |
| MS6 | 27 | 4 | 31 | 21 | 3 | 24 | 0.5625 | 0.4375 | 0.563636363 | 0.436363636 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MS7 | 61 | 4 | 65 | 33 | 3 | 36 | 0.648936170212 | 0.351063829788 | 0.643564356436 | 0.356435643564 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |
| MS8 | 12 | | 12 | 22 | 3 | 25 | 0.352941176475 | 0.647058823525 | 0.324324324323 | 0.675675675677 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |
| MS9 | 45 | | 45 | 31 | 3 | 34 | 0.592105263152 | 0.407894736848 | 0.569620253202 | 0.430379746798 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |
| MS10 | 36 | | 36 | 30 | 1 | 31 | 0.545454545455 | 0.454545454545 | 0.537313432838 | 0.462686567162 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |
| MS11 | 27 | | 27 | 37 | 1 | 38 | 0.421875 | 0.578125 | 0.415384615384 | 0.584615384616 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | | | | |
| MS12 | 7 | 1 | 8 | 13 | 1 | 14 | 0.35 | 0.65 | 0.363636363636 | 0.636363636364 | 0.656255 | 1.392857143 | 0.698181812 | 1.328063241 | -0.676581325 | 0.773235800 | -0.575481548 | 0.625523421 |
| MS13 | 35 | 4 | 39 | 32 | 3 | 35 | 0.522388059701 | 0.477611940299 | 0.527027027 | 0.472972973 | 1.067488644 | 0.935323383 | 1.083333333 | 0.921052631 | 0.045749513 | -0.043843283 | 0.074989589 | -0.071002979 |
| MS14 | 9 | | 9 | 3 | 2 | 5 | 0.75 | 0.25 | 0.642857142 | 0.357142857 | 1.551724138 | 0.483870967 | 1.330049261 | 0.691244239 | 0.202398800 | -0.189340813 | 0.036945812 | -0.034562211 |
| MS15 | 25 | | 25 | 19 | | 19 | 0.568181818 | 0.431818181 | 0.568181818 | 0.431818181 | 1.242897727 | 0.795454545 | 1.336898396 | 0.750988142 | 0.065013111 | -0.054747883 | 0.186571598 | -0.137900746 |
| MS16 | 60 | 2 | 62 | 47 | 9 | 56 | 0.560747663 | 0.439252336 | 0.525423728 | 0.474576271 | 1.038215971 | 0.955118452 | 1.003511987 | 0.996140292 | -0.368200632 | 0.432421672 | -0.275156512 | 0.302399731 |
| MS17 | 24 | | 24 | 37 | | 37 | 0.393442623 | 0.606557377 | 0.393442623 | 0.606557377 | 0.899297423 | 1.078324226 | 0.896683187 | 1.080774963 | -0.442317482 | 0.344024709 | -0.262843536 | 0.205495855 |
| MS18 | 20 | 1 | 21 | 27 | | 27 | 0.425531914 | 0.574468085 | 0.4375 | 0.5625 | 0.737588652 | 1.357833656 | 0.717948717 | 1.44 | -0.090559495 | 0.123490221 | -0.085106382 | 0.132765957 |
| MS19 | 4 | 1 | 5 | 4 | | 4 | 0.5 | 0.5 | 0.555555556 | 0.444444444 | 1 | 1 | 1.111111111 | 0.888888889 | 0.115384615 | -0.115384615 | 0.218253968 | -0.218253968 |
| MS20 | 19 | | 19 | 16 | | 16 | 0.542857142 | 0.457142857 | 0.542857142 | 0.457142857 | 0.946122449 | 1.072527473 | 0.942857142 | 1.077551020 | -0.135650951 | 0.182607048 | -0.135182784 | 0.183462350 |
| MS21 | | | 0 | | | 0 | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| MS22 | 57 | 3 | 60 | 70 | | 70 | 0.448818897636 | 0.551181102364 | 0.461538461545 | 0.538461538455 | 0.932162325939 | 1.062992126 | 0.912587412 | 1.089445438 | -0.106299212 | 0.098706411 | -0.036503496 | 0.037352415 |
| MS23 | 99 | 6 | 105 | 108 | 2 | 110 | 0.478260869566 | 0.521739130434 | 0.488372093 | 0.511627907 | 1.008506616 | 0.992327365 | 1.059871351 | 0.948837209 | -0.045841209 | 0.041346973 | -0.076906764 | 0.065720326 |
| MS24 | 35 | | 35 | 37 | 1 | 38 | 0.486111111 | 0.513888889 | 0.479452054 | 0.520547945 | 0.895467836 | 1.124131944 | 0.854675402 | 1.185692542 | -0.227767080 | 0.270473407 | -0.181725002 | 0.232204169 |
| MS25 | 46 | 1 | 47 | 60 | 1 | 61 | 0.433962264 | 0.566037735 | 0.435185185 | 0.564814814 | 1.033243486 | 0.975927130 | 0.909226190 | 1.083333333 | -0.186268709 | 0.134842376 | -0.185161564 | 0.169984387 |
| MS26 | 70 | 1 | 71 | 61 | 1 | 62 | 0.534351145 | 0.465648855 | 0.533834586 | 0.466165413 | 0.995503503 | 1.005210227 | 1.023792358 | 0.974076983 | -0.175533678 | 0.203396167 | -0.145282515 | 0.158292890 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MS27 | 73 | 3 | 76 | 56 | 2 | 58 | 0.565891472 9 | 0.434108527 1 | 0.567164179 1 | 0.432835820 9 | 1.101194217 | 0.893023255 8 | 1.118999597 | 0.877694859 | -0.2102041374 | 0.2222158024 | -0.0686152026 5 | 0.0705211805 1 |
| MS28 | 70 | 3 | 73 | 81 | 2 | 83 | 0.463576158 9 | 0.536423841 1 | 0.467948717 9 | 0.532051282 1 | 1.193279372 | 0.877210751 9 | 1.129019129 | 0.908671852 5 | -0.3555279098 | 0.2258647897 | -0.2879624784 | 0.2038386083 |
| MS29 | 56 | 1 | 57 | 34 | | 34 | 0.622222222 2 | 0.377777777 8 | 0.626373626 4 | 0.373626373 6 | 1.189863548 | 0.791880341 9 | 1.157842158 | 0.813971742 5 | 0.1761243806 | -0.1930594172 | 0.1734419427 | -0.2044137181 |
| MS30 | 99 | 2 | 101 | 60 | 3 | 63 | 0.622641509 4 | 0.377358490 6 | 0.615853658 5 | 0.384146341 5 | 1.223949613 | 0.768074803 8 | 1.236839431 | 0.765117919 8 | 0.2101010302 | -0.2175478348 | 0.2158431553 | -0.2140593276 |
| MS31 | 79 | 3 | 82 | 78 | 8 | 86 | 0.503184713 4 | 0.496815286 6 | 0.488095238 1 | 0.511904761 9 | 0.887561925 | 1.147191662 | 0.956131767 8 | 1.045748299 | -0.2537779443 | 0.3322183998 | -0.2283218799 | 0.2381071033 |
| MS32 | 50 | 1 | 51 | 37 | 2 | 39 | 0.574712643 7 | 0.425287356 3 | 0.566666666 7 | 0.433333333 3 | 1.070544316 | 0.918234064 8 | 1.133333333 | 0.866666666 7 | -0.1847850585 | 0.2141826814 | -0.1719298246 | 0.1719298246 |
| MS33 | 68 | 10 | 78 | 28 | 3 | 31 | 0.708333333 3 | 0.291666666 7 | 0.715596330 3 | 0.284403669 7 | 1.266414141 | 0.661858974 4 | 1.315150012 | 0.623853211 | 0.1042929293 | -0.1323717949 | 0.2124473097 | -0.2535661438 |
| MS34 | 80 | 2 | 82 | 104 | 9 | 113 | 0.434782608 7 | 0.565217391 3 | 0.420512820 5 | 0.579487179 5 | 0.966183574 9 | 1.027667984 | 0.938067061 1 | 1.050320513 | -0.4359328272 | 0.3566723132 | -0.3957949531 | 0.3215833994 |
| MS35 | 66 | 2 | 68 | 68 | 4 | 72 | 0.492537313 4 | 0.507462686 6 | 0.485714285 7 | 0.514285714 3 | 0.944984380 4 | 1.059890421 | 0.933226324 2 | 1.072473868 | -0.1244018186 | 0.1354247645 | -0.1667822528 | 0.1810197622 |
| MS36 | 47 | 1 | 48 | 30 | 2 | 32 | 0.610389610 4 | 0.389610389 6 | 0.6 | 0.4 | 0.963386011 6 | 1.063311688 | 1.006896552 | 0.989830508 5 | 0.0968610883 6 | -0.1674889653 | 0.1044892648 | -0.1540773905 |
| MS37 | 51 | 2 | 53 | 52 | 1 | 53 | 0.495145631 1 | 0.504854368 9 | 0.5 | 0.5 | 0.990291262 1 | 1.009708738 | 1.004504505 | 0.995535714 3 | -0.1231107997 | 0.1231107997 | -0.0772695772 7 | 0.0765796703 3 |
| MS38 | 23 | 3 | 26 | 27 | 2 | 29 | 0.46 | 0.54 | 0.472727272 7 | 0.527272727 3 | 0.761875 | 1.362857143 | 0.842045454 5 | 1.202181818 | -0.2042708333 | 0.3112698413 | -0.2057486631 | 0.2633582888 |
| MS39 | 110 | 4 | 114 | 99 | 4 | 103 | 0.526315789 5 | 0.473684210 5 | 0.525345622 1 | 0.474654377 9 | 0.970760233 9 | 1.034626039 | 0.967931317 5 | 1.038065265 | 0.0797997819 4 | -0.0944997417 7 | 0.0538397808 8 | -0.0639073821 9 |
| MS40 | 39 | | 39 | 22 | | 22 | 0.639344262 3 | 0.360655737 7 | 0.639344262 3 | 0.360655737 7 | 1.022950082 | 0.961748633 9 | 1.099672131 | 0.861566484 5 | -0.0437158469 9 | 0.0728597449 9 | 0.0220817697 | -0.0306691245 9 |

*This table shows the post surgery day 30 data, normalized to baseline measures and the recovery by subtracting the normalized data for post surgery day 3 from the normalized data from post surgery day 30*

## Appendix B

**Two Way Repeated Measures ANOVA (One Factor Repetition)** Tuesday, May 18, 2021, 9:30:46 AM

**Data source:** Males Repeated Measures Ipsi in MS project.JNB

Balanced Design

Dependent Variable: Data

**Normality Test (Shapiro-Wilk)**Passed(P = 0.325)

**Equal Variance Test:** Failed(P < 0.050)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Factor B | 1 | 0.0738 | 0.0738 | 2.506 | 0.139 |
| Subject(Factor B) | 12 | 0.353 | 0.0294 | | |
| Factor A | 2 | 0.265 | 0.133 | 7.822 | 0.002 |
| Factor B x Factor A | 2 | 0.0885 | 0.0442 | 2.611 | 0.094 |
| Residual | 24 | 0.407 | 0.0169 | | |
| Total | 41 | 1.187 | 0.0290 | | |

The difference in the mean values among the different levels of Factor B is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Factor A. There is not a statistically significant difference (P = 0.139).

The difference in the mean values among the different levels of Factor A is greater than would be expected by chance after allowing for effects of differences in Factor B. There is a statistically significant difference (P = 0.002). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Factor B does not depend on what level of Factor A is present. There is not a statistically significant interaction between Factor B and Factor A. (P = 0.094)

Power of performed test with alpha = 0.0500: for Factor B : 0.194
Power of performed test with alpha = 0.0500: for Factor A : 0.894
Power of performed test with alpha = 0.0500: for Factor B x Factor A : 0.297

Least square means for Factor B :

| Group | Mean |
|---|---|
| Control | 0.877 |
| FSA | 0.793 |

Std Err of LS Mean = 0.0374

Least square means for Factor A :

| Group | Mean |
|---|---|
| PSD 3 | 0.735 |
| PSD 15 | 0.841 |
| PSD 30 | 0.929 |

Std Err of LS Mean = 0.0388

Least square means for Factor B x Factor A :

| **Group** | **Mean** |
|---|---|
| Control x PSD 3 | 0.739 |
| Control x PSD 15 | 0.857 |
| Control x PSD 30 | 1.036 |
| FSA x PSD 3 | 0.731 |
| FSA x PSD 15 | 0.826 |
| FSA x PSD 30 | 0.823 |

Std Err of LS Mean = 0.0549

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: **Factor A**

| **Comparison** | **Diff of Means** | **t** | **Unadjusted P** | **Critical Level** | **Significant?** |
|---|---|---|---|---|---|
| PSD 30 vs. PSD 3 | 0.194 | 3.949 | <0.001 | 0.017 | Yes |
| PSD 15 vs. PSD 3 | 0.106 | 2.163 | 0.041 | 0.025 | No |
| PSD 30 vs. PSD 15 | 0.0879 | 1.786 | 0.087 | 0.050 | No |