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Parkinson's Disease in the Mouse: Valid and Invalid Models for Future Use

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RESEARCH / CREATIVE PROJECT ABSTRACT / EXECUTIVE SUMMARY
FINAL REPORT FORM

Title of Project
Parkinson's Disease in the Mouse: Valid and Invalid Models for Future Use

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Abstract
"Parkinson's Disease (PD) is a neurodegenerative disorder affecting approximately 1% of elderly Americans that results from progressive loss of dopaminergic neurons. Common motor symptoms of PD include resting tremor, rigidity, bradykinesia or akinesia and postural instability" (Zesiewicz et al., 2009). "Almost all patients with PD have non-motor and neuropsychiatric features, including sleep disturbances, compulsive and impulsive behaviors, autonomic dysfunction and psychosis" (Fernandez, 2012). In the years to come, as the average age increases the amount of PD patients will also increase due to the late onset of most PD cases. As the amount of PD cases caused by environmental factors decreases, the amount of genetic PD research may increase significantly. There are several different gene mutations that are believed to cause a form of inherited PD. "Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common genetic cause of Parkinson's disease" (Li et al., 2009). In this study we evaluated the appropriateness of the FVB/N-Tg(LRRK2*R1441G)135Cjli/J as a mouse model compared to the symptoms commonly seen in human PD LRRK2 patients and to those of the common reserpine induced model of PD.

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The end product of this project in electronic format has been submitted to the Provost/Vice President for Academic Affairs via the Office of Grants & Sponsored Projects Officer (Maxwell 161, npeterson@winona.edu).

Student Signature ____________________ Date ____________

Faculty Sponsor Signature ____________________ Date ____________

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Matthew A. Weber and Jennifer A. Aeling

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Abstract

"Parkinson's Disease (PD) is a neurodegenerative disorder affecting approximately 1% of elderly Americans that results from progressive loss of dopaminergic neurons. Common motor symptoms of PD include resting tremor, rigidity, bradykinesia or akinesia and postural instability" (Zesiewicz et al., 2009). "Almost all patients with PD have non-motor and neuropsychiatric features, including sleep disturbances, compulsive and impulsive behaviors, autonomic dysfunction and psychosis" (Fernandez, 2012). In the years to come, as the average age increases the amount of PD patients will also increase due to the late onset of most PD cases. As the amount of PD cases caused by environmental factors decreases, the amount of genetic PD research may increase significantly. There are several different gene mutations that are believed to cause a form of inherited PD. "Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common genetic cause of Parkinson's disease" (Li et al., 2009). In this study we evaluated the appropriateness of the FVB/N-Tg(LRRK2*R1441G)135Cjli/J as a mouse model compared to the symptoms commonly seen in human PD LRRK2 patients and to those of the common reserpine induced model of PD.

Introduction

Parkinson's disease is the second most common neurodegenerative disease in humans (Lichtenbery et al., 2011). It is a disease that causes degeneration of dopamine producing neurons in the substantia nigra pars compacta and ventral tegmental areas of the brain (Lichtenbery, et al., 2011). The subsequent behavioral deficits include hyperkinesia, resting tremors, bradyskinesia, postural instability, and muscle rigidity. L-dopa is commonly used to treat PD but is not without severe side effects (Kuoppamaki et al., 2007). L-dopa causes a nonselective increase in dopaminergic activity at all five dopamine receptors, and will cause severe side effects after several years of use. The side effects include uncontrollable movements similar to that of Huntington's disease and severely debilitating hallucinations.

Parkinson's disease can be found sporadically in the population, usually caused by environmental, industrial or drug toxicity. The reserpine form of PD commonly used in mice is supposed to model this form of sporadic PD. Reserpine acts as an "irreversible inhibitor of the vesicular monoamine transporter 2 (VMAT-2)" (Fernandes et al., 2012). To test the usefulness of reserpine as a mouse model of Parkinson's disease, three aspects of human Parkinson's disease were compared to the mouse model. Behaviorally, the characteristics of human Parkinson's disease that were examined were gait patterns, motor activation, and posture which were then compared to the animal model induced by reserpine. Pharmacologically, carbidopa and L-dopa was administered to determine recovery from symptoms. Anatomically, a tyrosine hydroxylase staining assay was performed on 40 µm sections to determine loss of dopaminergic neurons. Data was collected on the behavior, pharmacology, and anatomical aspects of this model to determine if reserpine does indeed induce a valid model of human PD.

The genetic mutation in the LRRK2 gene is the most common form of familial Parkinson's disease and is modeled by the FVB/N-Tg(LRRK2*R1441G)135Cjli/J (LRRK2*R1441G) strain of laboratory mice. "A mutation in the leucine-rich repeat kinase 2 (LRRK2) is the most common genetic cause of Parkinson's disease" (Yanping et al., 2009). To test the usefulness of this genetic model of PD, the three factors discussed above were also examined here with the exception that the open-field was added to the behavior testing to measure overall activity levels. The pharmacological and anatomical factors were examined identically to the reserpine model.

This research not only examines two separate models of PD but also a tyrosine hydroxylase assay to determine the anatomical deficits of these models. In previous research, there was no

comprehensive tyrosine hydroxylase assay available for use. To determine proper pathology and dopamine depletion in the brain when modeling PD in animals, an anti-tyrosine hydroxylase stain should be used. Using this type of stain allows for analysis of the affected areas and accurate pathology diagnosis. To stain tyrosine hydroxylase positive neurons located in the substantia nigra pars compacta, 40 µm sagittal sections of both control and experimental reserpine brains were stained with an immunohistochemical assay using a rabbit polyclonal anti-tyrosine hydroxylase antibody. Initially, the assay described was modeled after Varcin and colleagues (2011). This research was extremely helpful, but most steps were not fully described or were unnecessary. This required the development of a new protocol. The full protocol was developed and validated using materials obtained from several different sources (e.g., Varcin et al., 2011), Vector Laboratories Inc. and EMD Millipore Company.

Materials and Methods

Subjects

Thirty-four male C57BL/6J mice were bred at Winona State University from breeders purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were coded so that the experimenter was unaware of their treatment condition (reserpine or placebo-control).

Seventeen (eight male and nine female) LRRK2*R1441G mice and 17 (seven male and ten female) control mice were bred at Winona State University from breeders purchased from Jackson Laboratory (Bar Harbor, ME). The subjects were allowed to age to 12 months without any type of testing. Mice were coded so that the experimenter was unaware of their genetic condition (carrier or non-carrier control). Eight (five male and three female) LRRK2*R1441G mice and eight (five male and three female) control mice were allowed to age until the end of their natural lifespan or the development of Parkinsonism.

Animals were housed in groups of four to eight animals and maintained on a reverse 12/12 hour light/dark cycle in a temperature and humidity controlled vivarium, with continual access to food and water. In the reserpine model, behavioral testing occurred 24 to 48 hours after the reserpine injection or 30 minutes after the L-Dopa injection. All subjects were tested between six and eight weeks from birth. In the LRRK2*R1441G model initial testing began at 12 months of age. All procedures followed NIH guidelines for the care and use of laboratory animals, and were approved by the Winona State University Animal Care and Use Committee.

Behavioral Testing

There were four separate behavioral tests that were used to measure motor and behavioral activity in the LRRK2*R1441G mice. The first three behavior tests were also used in the reserpine condition but the fourth test was only implemented for the LRRK2*R1441G. All behavior tests were conducted during the dark phase of the light/dark cycle. The first three behavior tests were conducted 24 hours before the fourth behavior test. The first test was used to examine the posture of each subject. A digital photograph was taken of each subject and the subject's posture was rated as "Abnormal" or "Normal". The second test was used to measure the motor activation of the subject. This test used a bar test similar to the one described by Tsung-Hsun et al. (2011) which measured the amount of time it took for the subject to remove both front paws from the suspended bar. The third test was used to measure the common shuffling gait that is seen in PD patients. Each subject had their paws placed in a water soluble die and allowed to walk over a sheet of white paper (7.5 cm x 90 cm). The subjects were allowed two minutes to walk the entire length of paper and the time it took to complete was recorded. The distances between each paw were measured. Walking patterns were

examined to determine if the subjects maintained a normal walking pattern of the stereotypic shuffling gait seen in PD patients. Only for the LRRK2*R1441G, 24 hours after the shuffling gait test was concluded, the subjects were recorded with a digital video recorded and allowed to freely roam in an open-field apparatus (43.2 cm x 43.2 cm) for five minutes. The open-field was conducted in the light and several different variables were measured including amount of movement, rearing, quadrant crossings, and if a resting tremor was observed.

Pharmacological Testing

The two most widely used drugs to treat PD in humans were tested for effectiveness in the subjects to further validate each model. Levodopa (L-dopa) is the most common treatment for relieving the symptoms of PD. Carbidopa must be used concurrently with L-dopa to prevent conversion into dopamine prematurely by the peripheral nervous system before L-dopa passes through the blood-brain-barrier. Fifty minutes before behavioral testing, carbidopa was injected i.p. at 12.5 mg/kg and 30 minutes before behavioral testing, L-dopa was injected i.p. at 50 mg/kg. If the symptoms of PD were reduced when examining the behavior, then this result was considered to be pharmacologic evidence in support of the validation of the PD model.

Anatomical Testing

Immunohistochemical

After the completion of all motor and behavior tests, the subjects were sacrificed and each brain was removed. For the LRRK2*R1441G mice, samples of the tail, ear and blood was also taken. The brains were fixed in formalin for a minimum of 14 days and thin-sectioned at 40 µm. The sections were then stained using a ploYclonal anti-tyrosine hydroxylase stain obtained from Vector Laboratories Inc. and EMD Millipore Company. This stained was used to determine a distinct pathology of PD, loss of dopaminergic neurons within the substantia nigra pars compacta, by showing the amount of tyrosine hydroxylase positive neurons within the area. The full immunohistochemical staining process is described below.

Materials

All necessary materials were obtained from Vector Laboratories, Inc., EMD Millipore Company and Invitrogen of Life Technologies. The only material obtained from EMD Millipore Company was the rabbit polyclonal anti-tyrosine hydroxylase antibody (Cat. # AB152). Critical materials were also received from Vector Laboratories, Inc. which included the Vectastain® ABC Kit (PK-4001) and DAB Substrate Kit (SK-4100). Normal goat serum and Tris-saline was obtained from Invitrogen.

Day One

The staining assay began with three washes in Tris-saline for five minutes each. Note: Tris-saline concentration was held constant throughout protocol at 0.01 M TrisHCl at a pH of 7.4. A permeabilizing treatment was then applied to the slides. Slides were incubated at 37°C in 0.1% Trypsin for 60 minutes. The slides were then washed twice, five minutes each, in Tris-saline. The slides were quenched in endogenous peroxidase for 30 minutes in 3% H_2O_2. Once again, the slides were washed in Tris-saline two times at five minutes each. Next, a normal goat serum, diluted 20% in Tris-saline, was used for 45 minutes to block non-specific binding. Approximately, for the next 14 hours (exact time was 13 hours and 56 minutes), the tissues were incubated in the rabbit polyclonal anti-tyrosine hydroxylase antibody at room temperature. The rabbit polyclonal anti-tyrosine hydroxylase antibody was diluted 1:1000 in Tris-saline along with 2% normal goat serum and 0.3% Triton X-100. Times varied depending on the

dilution of the rabbit polyclonal anti-tyrosine hydroxylase antibody and also the temperature at which the tissues were incubated.

Day Two

Day two of the staining protocol began with two washes in Tris-Saline for five minutes each. For 60 minutes the tissues were incubated in the biotinylated secondary antibody at 37°C. The biotinylated secondary antibody was diluted 1:200 in Tris-Saline and 2% normal goat serum. Slides were washed twice in Tris-Saline for five minutes each. During the incubation in the biotinylated secondary antibody, a horseradish peroxidase conjugate was mixed for 30 minutes before application. The horseradish peroxidase conjugate was one mL Reagent A (Avidin DH) and one mL Reagent B (Biotinylated Horseradish peroxidase H) provided in the Vectastain® ABC Kit diluted in 100 mL of 0.01% PBS. The horseradish peroxidase conjugate was applied for 60 minutes. After the application of the horseradish peroxidase conjugate, the slides were washed in Tris-saline twice for five minutes each. The tissues were then rinsed in an acetate buffer solution for five minutes which consisted of 8.2 grams of sodium acetate and 300 µL glacial acetic acid diluted in 1 L distilled water. The next step consisted of the application of the 3,3'-diaminobenzidine which was mixed from the components from the DAB substrate kit. The solution consisted of two drops of the buffer stock solution, four drops of the DAB stock solution and two drops of a 3% hydrogen peroxide solution diluted and mixed well in 5 mL distilled water. Average of two to three drops were applied to each section of tissue. There were two more washes though Tris-saline at five minutes each and optimal staining was determined through light microscopy. If proper staining is not obtained, the DAB substrate would need to be applied again along with more washes through Tris-saline. If optimal staining is determined, then the tissues can be dehydrated through alcohol. At five minutes each, the tissues were dehydrated through 70%, 95% and 100% alcohol. Finally, the slides were cleared with xylene for just over an hour. Slides were then coverslipped and digitally photographed.

PCR

Specific to the LRRK2*R1441G subjects, genotyping via polymerase chain reaction (PCR) and gel electrophoresis was completed to determine if the subjects did indeed have the correct mutation of the LRRK2 gene using standard methods recommended by the distributor of this mouse model (www.JAX.org).

Results

Tyrosine Hydroxylase Assay

The rabbit polyclonal anti-tyrosine hydroxylase antibody stained the proper structures of the brain (see Figures 1 and 2). Figures 1 and 2 show the staining of the substantia nigra pars compacta. Figure 3 shows that the cerebral cortex (negative control) appropriately was absent of staining. The rabbit polyclonal anti-tyrosine hydroxylase antibody efficiently and effectively penetrated the soma of the tyrosine hydroxylase positive neurons (see Figure 4). The rabbit polyclonal anti-tyrosine hydroxylase antibody correctly stained axon and dendrite projections (see Figure 5). Figure 6 shows the staining of all neurons within in substantia nigra pars compacta using a standard cresyl violet stain. Comparison of the cresyl violet stained neurons to those stained for anti-tyrosine hydroxylase correctly reveals that only a subset of neurons in the substantia nigra stain are dopaminergic.

Reserpine Model

Reserpine significantly altered the shuffling gait distance (cm) $t(25)= 5.25323$, $p < .05$. Subjects in the control group had significantly longer gait distances than subjects in the reserpine only group. Reserpine significantly altered the shuffling gait time (s) $t(25)= -4.507621$, $p < .05$. Subjects in the reserpine only group had significantly longer times than subjects in the control group. Reserpine significantly altered the bar time (s) $t(25)= -2.641695$, $p < .05$ (see Figure 8). Subjects in the reserpine only group had significantly longer times than subjects in the control group.

Combined treatment with reserpine, carbidopa and L-dopa (RC(12.5)L(50)) significantly altered the shuffling gait distance (cm) $t(18)= 7.701276$, $p < .05$ (see Figure 6). Subjects in the control group had significantly longer gait distance than subjects in the RC(12.5)L(50) group. RC(12.5)L(50) significantly altered the shuffling gait time (s) $t(18)= -8.213235$, $p < .05$. Subjects in the RC(12.5)L(50) group had significantly longer times than subjects in the control group. RC(12.5)L(50) significantly altered the bar time (s) $t(18)= -31.01461$, $p < .05$. Subjects in the RC(12.5)L(50) group had significantly longer times than subjects in the control group.

RC(12.5)L(50) did not ameliorate the impairments in shuffling gait distance (cm) $t(19)= -1.441261$, $p > .05$. Subjects in the reserpine only group did not have significantly longer gait distances than subjects in the RC(12.5)L(50) group. RC(12.5)L(50) also did not alter the shuffling gait time (s) $t(19)= 0.4508894$, $p > .05$ (see Figure 7). Subjects in the RC(12.5)L(50) group did not have significantly longer times than subjects in the reserpine only group. RC(12.5)L(50) significantly altered the bar time (s) $t(19)= 3.497898$, $p < .05$. Subjects in the RC(12.5)L(50) group had significantly longer times than subjects in the reserpine only group.

Reserpine significantly affected the posture $\chi^2 = 21.950$, $p < .05$ (see Figure 1) compared to the control group. RC(12.5)L(50) significantly affected the posture $\chi^2 = 25.898$, $p < .05$ compared to the control group. Reserpine did not significantly affect posture $\chi^2 = 2.677$, $p < .05$ compared to the RC(12.5)L(50).

Reserpine did not alter the average color intensity of the polyclonal anti-tyrosine hydroxylase stain $t(17)= 0.1149269$, $p > .05$ (see Figure 2). Subjects in the reserpine only group did not have significantly less tyrosine hydroxylase positive neurons than subjects in the control group.

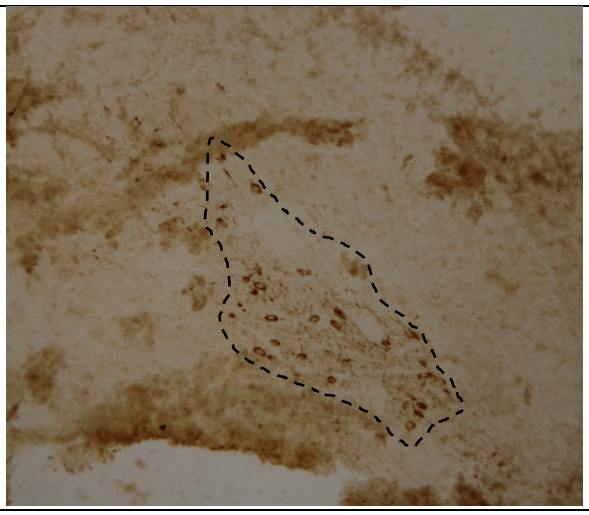


Figure 1. 40 µm sagittal section of the substantia nigra pars compacta in a C57BL/6J mouse at 10x power.

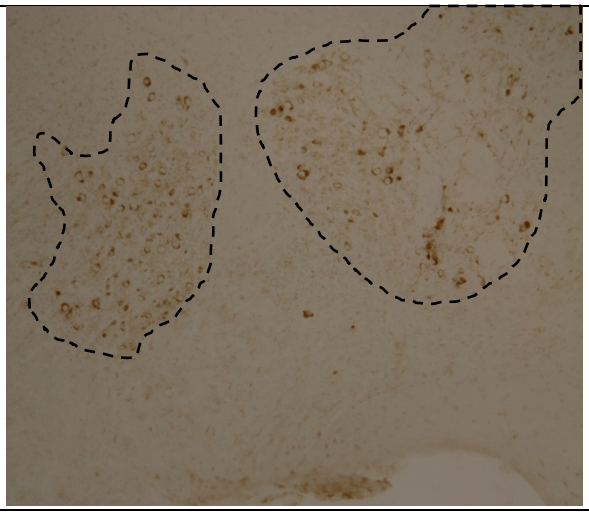


Figure 2. 40 µm sagittal section of the substantia nigra pars compacta in a C57BL/6J mouse at 10x power.

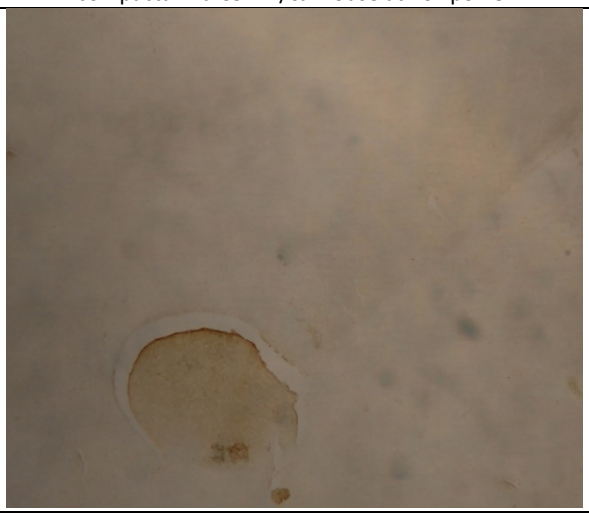


Figure 3. 40 µm sagittal section of the cerebral cortex in a C57BL/6J mouse at 4x power.

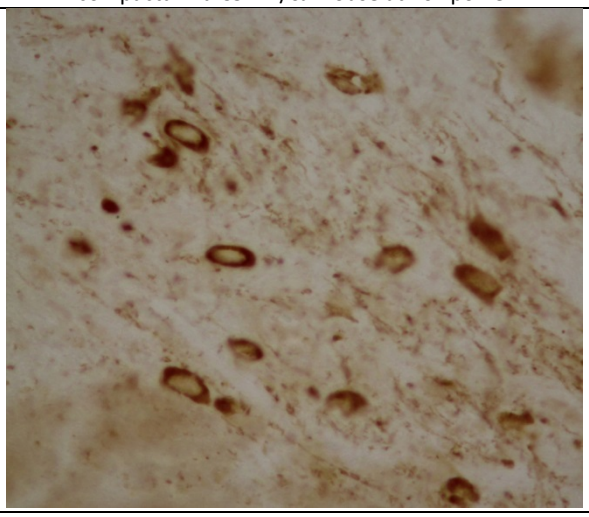


Figure 4. 40 µm sagittal section of the substantia nigra pars compacta in a C57BL/6J mouse at 40x power.

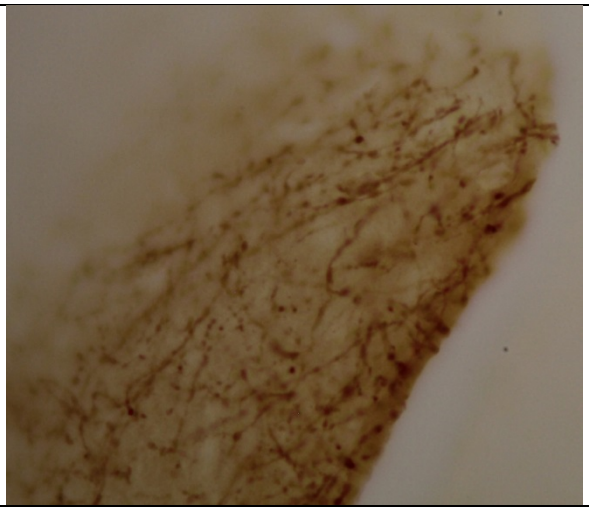


Figure 5. 40 µm sagittal section of the substantia nigra pars compacta in a C57BL/6J mouse at 40x power.

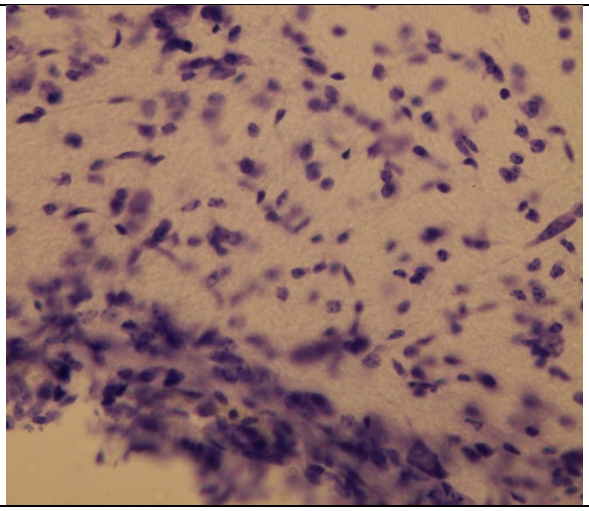


Figure 6. 40 µm sagittal section of the substantia nigra pars compacta in a C57BL/6J mouse with cresyl violet at 40x power.

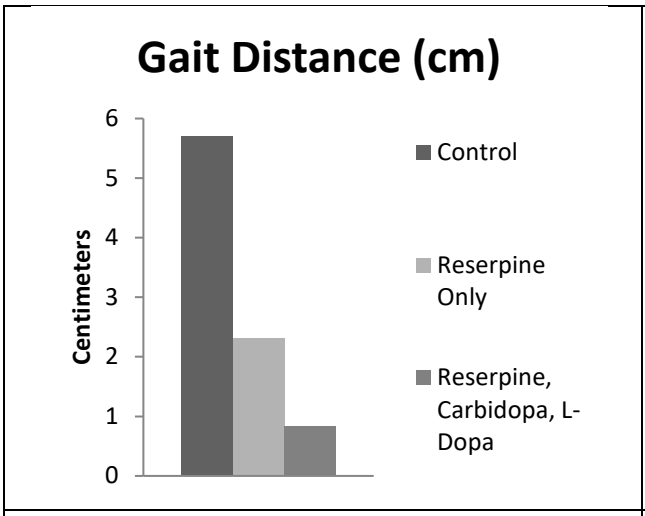


Figure 3. *Mean gait distances in centimeters.*

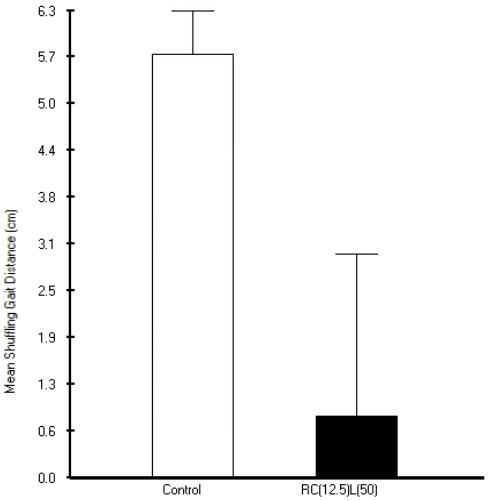


Figure 6. *Control vs. RC(12.5)L(50) gait distance.*

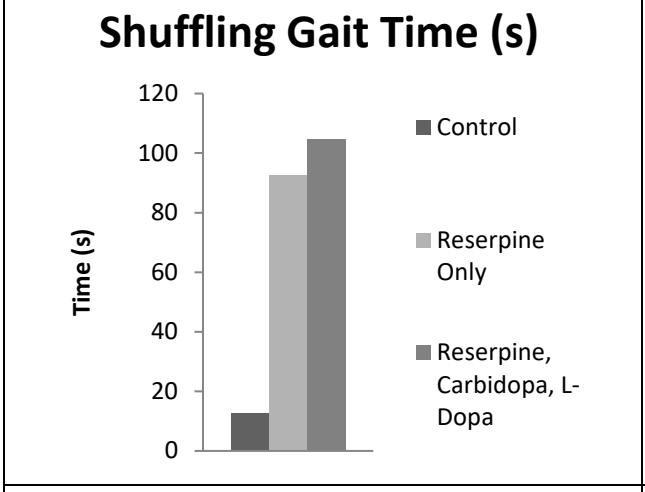


Figure 4. *Mean gait time in seconds.*

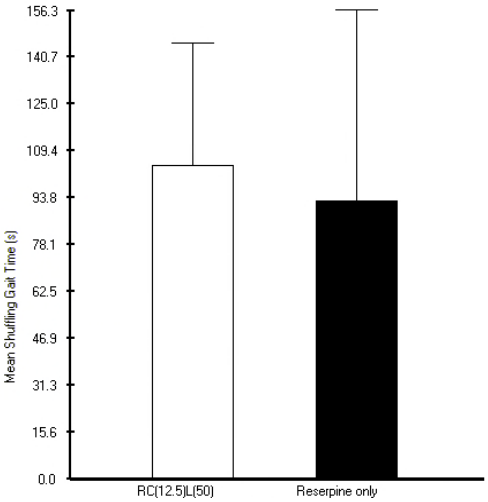


Figure 7. *RC(12.5)L(50) vs. reserpine gait time.*

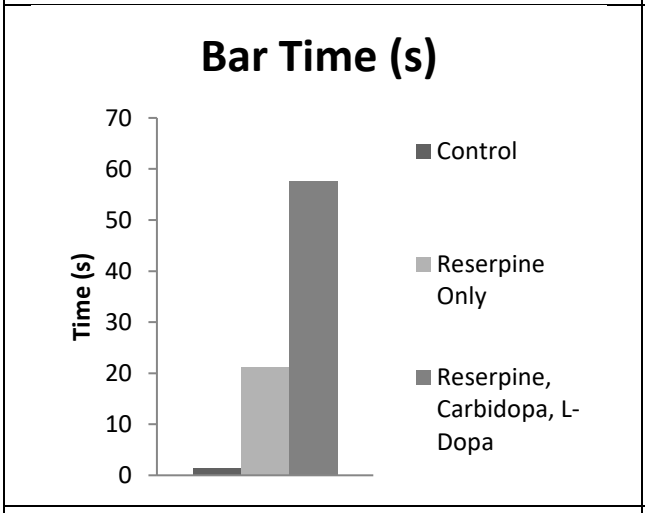


Figure 5. *Mean bar time in seconds.*

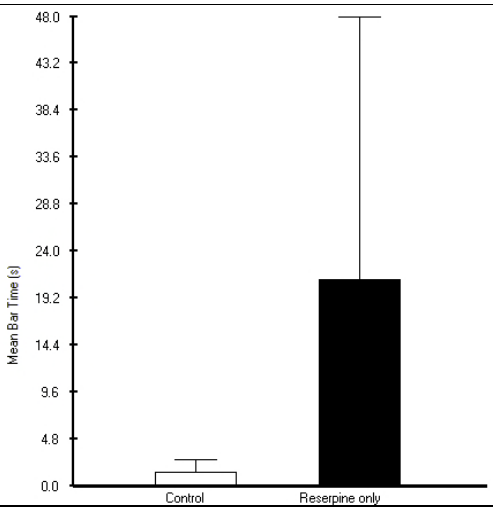
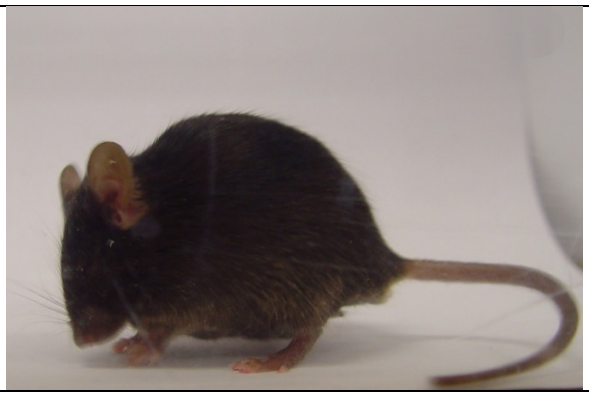
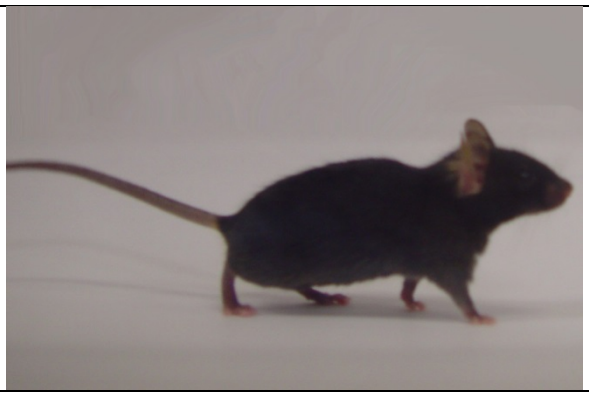


Figure 8. *Control vs. reserpine bar time.*



C57BL/6J Reserpine Injection



C57BL/6J Control

Figure 9. *Posture of C57BL/6J reserpine and C57BL/6J control mice.*

LRRK2*R1441G Model

Currently, the part of the study that is examining the validity of the LRRK2*R1441G is still in progress. Behavioral evaluations at 10 months, 12 months and 18 months have failed to detect any behavioral indications of parkinsonism that are statistically different from controls. Because the study design requires the continued maintenance of the subjects until symptoms develop we are continuing to maintain the colony. The maximum lifespan of the laboratory mouse is 18 to 24 months. It is expected that this portion of the study will end within the next six months.

Discussion

Tyrosine Hydroxylase Assay

This study has shown that the steps taken throughout the staining process are effective for showing tyrosine positive neurons in the substantia nigra pars compacta. The rabbit polyclonal anti-tyrosine hydroxylase antibody has been previously shown to be effective in doing so; not only in the substantia nigra, but also other structures within the brain. Without a comprehensive assay to follow, it is exceptionally difficult for independent researchers to do this process themselves. In the article published by Varcin et al. (2011), the process of staining is extremely helpful but not fully complete. This is similar to the protocols received from both EMD Millipore Company and Vector Laboratories Inc.

For example, as explain by Varcin and colleagues, the sections were incubated "with rabbit polyclonal anti-TH antibody at room temperature overnight". As described in the protocol obtained by Millipore, the incubation times at room temperature range from two to eighteen hours. The protocol described in Vector Laboratories describes incubating sections for 30 minutes at an unknown temperature. Two protocols describe different thicknesses at which the tissue should be sectioned, also. Varcin et al. sectioned at 50 µm and Millipore ranges from 15 to 30 µm. At different thicknesses and temperatures, times of incubation may vary drastically. The information provided by all three protocols ranges significantly, and because of this range, it is difficult for one to determine what may or may not actually work. This study has found that at 40 µm, approximately 14 hours is a suitable time for incubation in the primary antibody.

Another example of the range in information is the application of the DAB substrate, 3'3-diaminobenzidine. Varcin et al., describes it as simply as "3'3-diaminobenzidine was applied until staining was optimal as determined by light microscopy". The protocol obtained from Millipore

described this step as incubating the tissue at room temperature for two to thirty minutes in 50 mg diaminobenzidine per 100 mL PBS with 33 µL of 30% H_2O_2. The protocol that was received from the company, Vector Laboratories, who provided the DAB substrate kit described the process that was explained in the methods section. The difference between this study and the protocol is that the 3'3-diaminobenzidine was applied through a droplet form and not incubation as describe by Vector Laboratories. This study found that a two to three droplet average provides optimal staining at 40 µm. If the tissues were incubated in the 3'3-diaminobenzidine for two to ten minutes, a likely possibility would be that the stain would not have worked.

There are several key features that need to be examined when determining the suitable staining of tyrosine hydroxylase positive neurons. These factors include which brain structures were stained, neuronal penetration (soma, axon, dendrites), number of neurons stained compared to cresyl violet, and artifact levels. The brain structures that were stained are critical to determine if the rabbit polyclonal anti-tyrosine hydroxylase antibody actually stained tyrosine hydroxylase positive neurons. If large areas of the cerebral cortex had been stained, then we would be forced to conclude that this protocol does not work. Since areas like the cerebral cortex had not been stained though and areas like the substantia nigra pars compacta and basal ganglia were stained; we can determine that the antibody was selective to the areas that contain tyrosine hydroxylase. Accurate neuronal penetration is also key to determining effective staining. As shown by Figure 4, the antibody was able to penetrate the soma, effectively showing which neurons were tyrosine hydroxylase positive. Comparing this to Figure 6 (substantia nigra pars compacta stained with cresyl violet) it is quite clear that the number of cells stained is quite different. If the antibody had stained the same amount of cells as the cresyl violet, the antibody would not be effective in staining only those neurons that contain tyrosine hydroxylase. Looking at Figure 4 also shows accurate neuronal penetration with the staining of axons and dendrites within the substantia nigra pars compacta. Finally, artifact levels can make or break the decision to use a protocol. If there are large of amounts of artifact as shown by Figure 1, then the dilution of the primary antibody or even the amount of the DAB substrate may need to be examined. Even though there was strong soma penetration shown in Figures 1, 2, and 4, the amount of artifact may lead to a skewed analysis of how well the stain actually did to identify tyrosine hydroxylase positive neurons. Overall, the rabbit polyclonal anti-tyrosine hydroxylase antibody works as a stain to determine tyrosine hydroxylase positive neurons. This fact is nothing new, but we have shown that using this protocol, 40 µm tissue sections will have proper tyrosine hydroxylase positive neuron placement, strong penetration of somas, and can be used to determine the pathology of an animal model of Parkinson's disease.

Reserpine Model

The results of this study show that the use of reserpine as a model of Parkinson's disease should be reconsidered. While reserpine was found to produce gait disturbances (see Figures 3 and 6), decreased motor activation (see Figure 5 and 8), and postural deficits (see Figure 9) similar to the human disease, the anatomical characteristics do not match the human disease (see Figure 1, 2, and 4). Specifically there was no evidence of cell loss in the substantia nigra.

Anatomically, reserpine is not useful because there were no significant changes between the analysis of control and reserpine treated mouse brains. Specifically, there was not a significant loss of dopaminergic neurons. Reserpine does not cause the death of neurons (see Figure 1, 2, and 4). It only reduces the amount of dopamine available for neurotransmission. This is very different from human Parkinson's where symptoms begin to manifest when more than 50% of the dopaminergic neurons have died. To be considered anatomically valid, we would expect to see a significant difference in the amount of tyrosine hydroxylase positive neurons between the control and reserpine subjects.

These data clearly show that the reserpine model is not pharmacologically valid. Combined treatment with Carbidopa and L-dopa in human Parkinsonism works on the neurotransmitter level allowing more L-dopa to be available for conversion into dopamine. This allows the still living neurons to flood the system with dopamine, compensating for the neuronal loss in Parkinson's disease. Testing between the reserpine only and reserpine with carbidopa and L-dopa failed to produce significant improvements in the severity of behavioral symptoms. This finding shows that a treatment that has proven useful in humans with Parkinson's disease does not reverse reserpine induced Parkinsonism. This means that screening new drugs in this model could lead researchers to falsely reject drugs with potential therapeutic properties.

When looking at reserpine as a full model of human Parkinson's disease, we are forced to conclude that it should not be used. Even though there are significant behavioral and postural differences induced by reserpine, the use of reserpine as a model of Parkinson's disease is not justified in this study as it fails to produce the anatomical deficits associated with the disease and is not reversible with the current antiparkinsonism drugs.

LRRK2*R1441G

The original developer of the LRRK2*R1441G mouse model reported that deficits appear by 10 months of age (Li et al, 2009). At the time of this report we had tested animals as old as 18 months and have yet to find any symptoms of Parkinsonism. After starting the study we received a communication from Jackson Labs (the distributor of the line) indicating that no one had been able to validate the model. This communication caused us to look for possible factors. We believe we may have discovered a possible reason. Recently it was reported that in an in vitro culture of cells from the LRRK2*R1441G mouse the administration of a lipopolysaccharide (LPS) (an agent released by microglia during neuroinflammation) triggers cell death in dopaminergic neurons from the substantia nigra (Gillardon et al, 2012). This would mean that in persons with this gene a trigger capable of producing neuroinflammation (e.g., a bacterial infection targeting the brain) could be the cause of their parkinsonism. This would be an exciting breakthrough if confirmed in the complete model. For these reasons, we have now divided our colony into two groups. The first group will continue on untreated until they live out a natural lifespan or until they become symptomatic. The second group was given LPS over a seven-day period to model the consequences of neuroinflammation. These animals are currently being tracked along with the untreated group. Hopefully, within the next few months, we will be able to say whether or not the disease will present without the use of LPS and whether or not the LPS actually works to induce the disease.

We are grateful for the opportunity to complete this project and wish to thank the selection committee for the award. This project would not have been possible without the financial support provided by this grant. This opportunity was vital to our career development as the experience allowed us to demonstrate our knowledge of the field and research abilities to prospective graduate schools. Both of us were admitted to doctoral programs and we will begin our graduate studies in the fall of 2013.

Presentations Based on this Work: Portions of this work was presented at the 2012 annual meeting of the annual Midbrains Undergraudate Neuroscience Conference of the Midwest.

Aeling, J. A., Weber, M . A. and Finnesgard, M. (2012). Reserpine treatment in mice is an incomplete model of parkinson's disease. Presented at the 6th Annual MidBrains Undergraduate Neuroscience Conference of the Midwest. Carleton College, Northfield, MN. **Faculty Sponsor: R. Deyo**

Weber, M . A. and Aeling, J. A. (2012). A polyclonal anti-tyrosine hydroxylase assay to determine dopaminergic neurons. Presented at the 6th Annual MidBrains Undergraduate Neuroscience Conference of the Midwest. Carleton College, Northfield, MN. **Faculty Sponsor: R. Deyo**

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