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Evaluating Sex Differences in Procedural Learning and Memory Deficits Caused by 3-Nitropropionic Acid

by

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Abstract

Evaluating Sex Differences in Procedural Learning and Memory Deficits Caused by 3-Nitropropionic Acid

Kari M. Haines

The current study attempted to evaluate sex differences in procedural learning and memory deficits in mice using intraperitoneal (i.p.) injections of 3-nitropropionic acid (3NP). 3NP is a mitochondrial toxin that creates striatal lesions and mimics the cognitive and motor deficits seen in aged mice. This experiment compared two dosing regimens of 420 mg/kg 3NP and a saline control. Learning and memory was tested using the water-motivated Stone T-Maze (STM) and motor coordination was tested using the rotarod. Mice given 420 mg/kg committed significantly more errors than saline control mice in STM acquisition. Females committed significantly more errors than males in STM acquisition, but performed better on the rotarod. Future research should continue to develop an accelerated aging model using 3NP in order to study potential pharmaceutical and dietary interventions.

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Chapter One:
Introduction

Humans and animals are in a constant state of aging and, as a result, suffer from declines in cognitive and motor functioning. Animal models are often used to find possible interventions to slow, or reverse, these declines (e.g., Shukitt-Hale, Carey, Jenkins, Rabin, & Joseph, 2007). Current aging models include using older animals, animals on a high fat diet (Barry & Pistell, 2014, Unpublished manuscript), and animals with direct striatal lesions (Pistell et al., 2009). While effective tools to study the aging process, these current models are also time consuming, costly, and invasive. The current study attempts to create an improved accelerated aging model that will mimic the learning and memory impairments seen in aged animals using less invasive intraperitoneal (i.p.) injections of the mitochondrial toxin 3-nitropropionic acid (3NP). In addition, the present study explores possible sex differences of 3NP intoxication. Once this model is established, it can then be used to test the efficacy of dietary and pharmaceutical interventions for the common impairments associated with aging.

The Striatum and Previous Aging Models

The dorsal striatum, a structure in the basal ganglia, is composed of the caudate and the putamen and receives input from several important structures including the substantia nigra, thalamus, and cortex (Pinel, 2011). It has been implicated as an important brain region in habit formation as well as procedural learning and memory (Packard & Knowlton, 2002). Pistell et al. (2009) found that rats with kainic acid induced striatal lesions committed more errors in the 14-unit T-maze compared with controls. The authors concluded that while the striatum is not the only contributing brain structure in learning and memory, lesions to this area have a significant effect on maze acquisition.

McDonald and White (1993) demonstrated that there are multiple memory systems that can work independently of each other. Rats with dorsal striatal lesions performed in similar ways to controls on a spatial version of the eight-arm radial maze (win-shift task) while rats with hippocampal lesions showed significant acquisition impairment. The opposite was true when a habit formation version of the maze (win-stay task) was presented. These findings support that the hippocampus and striatum contribute to different functions of learning and memory. Previous aging models have been focused more on the hippocampus and the effects of aging on spatial memory (Rapp, Deroche, Mao, & Burwell, 2002; Wilson et al., 2004). Research has also examined improving the negative effects that aging has on spatial memory and on the hippocampus (Gage, Björklund, Stenevi, Dunnett, & Kelly, 1984). Evidence shows that there are at least two systems of learning and memory; therefore, accelerated aging models should also be focused on the striatum.

The interaction between acetylcholine and dopamine in the striatum is important for controlling motor function. Aging can affect this balance leading to impaired movement (Umegaki, Roth, & Ingram, 2008). 3NP is a mitochondrial toxin produced by the plant *indigofera* and the fungus *Arthrinium* and is often used as a model for Huntington's disease (HD) (Brouillet, Jacquard, Bizat & Blum, 2005). 3NP provides a useful tool to study cognitive and motor impairment, and possible interventions, because it produces select striatal lesions in the brain (Blum, Galas, Cuvelier, and Schiffmann, 2004).

3NP and Motor Tasks

Several studies have examined 3NP and its effects on motor skills. 3NP administration in rats has been shown to create a significant impairment in rotarod performance (Mehrotra & Sandhir, 2014) and decrease locomotor and rearing activity (Shetty, Hariharan, Shirole, and Japtop, 2015). Dopamine transporter knockout mice treated with a cumulative dose of 340 mg/kg 3NP were significantly impaired on the rotarod compared to wild type mice (Fernagut, Diguet, Jaber, Bioulac, & Tison, 2002). Elevated dopamine levels in these knockout mice could have led to increased toxicity of 3NP. These results suggest that 3NP disrupts the important role that dopamine within the striatum plays in motor functioning.

Fernagut, Diguet, Stefanova et al. (2002) were interested in the characteristics of motor impairments produced by 3NP treatment. Forty-two C57Bl/6J mice were given i.p. injections 12 h apart of either a cumulative dose of 340 mg/kg 3NP, 560 mg/kg 3NP, or saline over 7 days. Animals were tested at baseline pre-treatment on the rotarod, pole test, stride length, and open field spontaneous activity. They were then tested again at one and four weeks post-treatment. Results showed that animals treated with 340 mg/kg 3NP had a reduction in stride length and rearing activity at the first week of testing compared to both control animals and baseline scores. These deficits were no longer detected at 4 weeks post-treatment. No striatal lesion was observed, but there was a significant reduction in striatal volume. Animals treated with 560 mg/kg 3NP displayed a full range of motor disorders. There was a significant deficit compared to control animals and baseline performance on the rotarod and pole test as well as reduced stride length and rearing. A range of striatal lesions was observed, from almost complete destruction in

non-surviving animals to damage in the mid-striatum and anteroposterior striatum of survivors. This study demonstrates the importance of finding an appropriate dosing regimen that produces striatal lesions and motor deficits.

3NP as a Model for Huntington's Disease

Huntington's disease is a neurodegenerative disorder characterized by cognitive and motor impairment (Vonsattel & DiFiglia, 1998). 3NP has been universally accepted as an animal model of HD (Mehrotra & Sandhir, 2014) and therefore research surrounding its effects as a mitochondrial toxin on the striatum often focus on the cognitive and motor dysfunction seen in HD (see Brouillet et al., 2005 for a review). Mitochondrial dysfunctions have been linked to aging as well as part of the neurodegeneration in HD. The declines seen in aging animals are similar to those seen in animal models of HD, suggesting that 3NP could also be used to create a successful accelerated aging model.

Blum et al. (2004) used adult male Lewis rats to determine whether treatment with 3NP produces impairment of the nigro-striatal system as a model for HD. 3NP was delivered subcutaneously and rats were euthanized 5 days after treatment. Hematoxylin staining was used to reveal striatal lesions. They observed that 3NP produces oxidative-related changes and a loss of striatal dopamine terminals.

Research has also examined if genetics influence the effects of 3NP. Hickey and Morton (2000) compared transgenic mice carrying a mutated HD transgene (R6/2) with wild-type mice to see if resistance to the effects of 3NP may be age dependent. They concluded that R6/2 mice age 7 to 10 weeks old appeared more resistant to 3NP than wild-type mice based upon reduced frequency of striatal lesions. One possible

explanation they offer is that R6/2 mice have compensatory mechanisms that protect the striatum against toxic effects in these younger mice. Negative symptoms from the transgene become more apparent as the mice age and could help explain why HD symptoms appear later in a human's life. Another explanation is that the threshold for 3NP had not yet been reached before the mice had died.

To study possible interventions to combat the neuronal degeneration caused by oxidative stress from 3NP, Jadiswami et al. (2014) examined the protective effects of piroxicam. Animals were tested on neurological scoring, locomotor activity, beam walking, and hanging wire test in order to assess the effects of piroxicam against oxidative stress and behavioral impairments that is often seen in HD animal using 3NP. They noted that 3NP alone treated animals showed a significant reduction in body weight on the last day of treatment compared to the first day. 3NP created motor abnormalities in the neurological scoring, but piroxicam treated animals showed significant improvement when compared to 3NP only treated animals. There was a significant impairment in locomotor activity in animals treated only with 3NP compared with control mice, but again piroxicam reversed these impairments. Similar patterns were found on the beam walking and hanging wire test.

Jadiswami et al. (2014) explain that there are several models for HD but a benefit of the 3NP model is that it produces bilateral striatal lesions, causes a significant decrease in body weight, increases brain oxidative stress, and creates motor impairments. They provide evidence that piroxicam could protect against 3NP due to its antioxidant properties and demonstrates how intervention studies to combat the negative effects of 3NP are possible.

3NP and Maze Tasks

Lohmann and Riepe (2007) studied whether young mice treated with 3NP have impaired cognitive performance in old age. CD-1 female mice aged 4.5 and 9 month old were injected with a cumulative dose of 180 mg/kg of 3NP or saline and tested in a complex maze task. Mice were then aged to 22 months and tested in a mirrored complex maze task and an eight-arm radial maze task. The authors found that 4.5 month old control mice performed slightly better than 4.5 month mice treated with 3NP. They also found that 9 month old mice treated with 3NP were severely affected compared to control mice of the same age. In the mirrored-complex maze and the radial arm maze they discovered that mice treated with 3NP at 4.5 months showed significantly poorer performance than control mice. The results from this study suggest that 3NP may be more toxic to middle-aged animals and that it may advance aging through mitochondrial oxidative damage. The use of spatial as well as non-spatial tasks suggests that multiple brain regions may be impaired, including the hippocampus and striatum.

Similar results have been observed in maze tasks using rats and 3NP. Shetty et al. (2015) found that rats treated with 3NP had an increase in transfer latency in the elevated plus maze task and an increase in escape latency in the Morris water maze task. Mehrotra and Sandhir (2014) also revealed that 3NP treated rats took significantly longer in the plus maze than controls and had impaired performance in the T-maze task. There is evidence that 3NP has an adverse effect on performance in complex maze tasks.

The Stone T-Maze

Pistell et al. (2012) reported that maze tasks demonstrate deficits in learning and memory in rats and mice. The 14-unit T-maze was originally designed for use in rats and

required them to learn the correct sequence of left and right turns to reach a goal box. In early development of the maze, food deprivation was used as a motivator; in subsequent years, foot shock avoidance was utilized (Pistell et al., 2012). Pistell and Ingram (2010) developed a version of the maze to study the effects of aging in mice and found that escape from water was the best motivator. The water-motivated stone T-maze (STM) offers researchers a more reliable tool to measure learning and memory in mice when compared to other tasks such as the Morris water maze. The Morris water maze has been shown to be an effective tool in studying learning and memory in rats, but it has been difficult to find reliable results with mice. It is unclear if this is due to a lack of swimming capability or if the mice are not using the spatial cues required for that task to be effective (Pistell et al., 2012). The STM allows researchers to study the cognitive declines associated with aging as aged mice have impaired performance, independent of possible motor declines.

The STM lends itself to studying the effects of aging on the striatum because it does not rely on spatial cues for learning. It instead focuses on egocentric movement and habit formation used in procedural learning and memory. Another benefit of the STM is that motivation is equal across age groups (Pistell et al., 2012).

Sex Differences

Research using rodent subjects often focuses on males. Beery and Zucker (2011) found that 8 out of 10 biological fields surveyed demonstrated a male bias in animal studies in 2009. Use of only one sex in a sample limits scientific potential. Wetherington (2007) states that not testing sex differences could also lead to drawing the wrong conclusions as opposite effects for females and males have been reported.

Females and males have differences in brain structure and functioning (Cahill, 2005). Previous unpublished data in our lab demonstrate no difference between males and females in STM acquisition; however, some research has linked sex differences in learning and memory (McCarthy, Arnold, Ball, Blaustein, & Vries, 2012), and specifically it has been suggested that estrogen could impair egocentric learning (Korol, 2004). Sex differences in mitochondrial function and antioxidant enzymes have been reported (Demarest & McCarthy, 2015). Specifically, estrogen has been shown to be protective against oxidative stress (Simpkins et al., 2005). In the striatum, estrogen has been shown to moderate dopamine function (Korol, 2004) and release (Becker, 1999) and sex differences have been seen in striatum organization (Becker, 1999).

Nishino et al. (1998) focused on sex differences 3NP intoxication has on the lateral striatal artery in rats. They found notable differences between males and females in incidence of motor symptoms and striatal damage. They concluded that estrogen protected against the negative effects of 3NP while testosterone intensified the effects. These results demonstrate the importance of examining potential sex differences of 3NP treatment in mice. The goal of creating an accelerated aging model is to examine possible interventions, and it is important to know if any differences seen between males and females are the result of the intervention or the aging model.

The Current Study

3NP has been shown to create cognitive and motor deficits in rodents. The current study tested these cognitive and motor debilities in the STM and on the rotarod with the goal of creating an accelerated aging model in mice. While many studies have shown that 3NP creates a striatal lesion in the brain and impairs performance in behavioral tasks,

there has been little consistency about the dosing regimen used throughout the literature. My study attempted to create a standardized methodology for 3NP intoxication, and the appropriate dosing to disrupt performance in the STM. Based on previous studies our lab has conducted, two injection schedules of escalating cumulative dose 420 mg/kg 3NP were tested in the current study.

I hypothesized that mice treated with 3NP will perform worse in the STM as measured by errors when compared to control mice. Specifically, mice treated with 3NP should have significantly more errors than control mice in the third trial block. Mice treated with 3NP will have impaired performance on the rotarod when compared with control mice. These deficits may be more apparent in the higher 3NP dose group and, based on the protective effects of estrogen, male mice are expected to be more affected by 3NP than female mice.

Chapter Two:

Method

Animals

Subjects included 52 male and 44 female (n = 96) C57Bl/6 mice, aged 6-7 months old. All mice were bred at Towson University. The breeder mice were acquired from Charles River Laboratories. Mice were randomly assigned to either one of two 3NP treatment groups or a saline control group. Litters and dams were distributed across dosing groups. Animals were group housed (3-4/cage) where they had *ad libitum* access to standard food and water. The room the mice were housed in was kept at 70-72°F with a 12-hour light/dark cycle where lights came on at 7:00am. All procedures were approved by the Institutional Animal Care and Use Committee at Towson University (Appendix A).

3NP Intoxication

Injection schedules were chosen to expand on previous methodological studies our laboratory has conducted (Ardinger, Haines, & Pistell, 2016, Unpublished data). Both treatment groups and the saline control group received injections twice a day 12 hours apart in increasing doses over 7 days. Group 1 (Male, n = 12; Female, n = 12) was given a total of 13 i.p. injections (6 injections of 20 mg/kg, 6 injections of 40 mg/kg, and a final single injection of 60 mg/kg) resulting in a cumulative dose of 420 mg/kg 3NP. Group 2 (Male, n = 12; Female, n = 12) were going to receive a total of 14 i.p. injections (5 injections of 20 mg/kg, 5 injections of 40 m/kg, and 4 injections of 60 mg/kg) resulting in a cumulative dose of 540 mg/kg. Mice in Group 2 did not receive that cumulative dose due to a high mortality rate in this group. The last two planned injections were not given

resulting in a cumulative dose of 420 mg/kg 3NP. Although Group 1 and Group 2 did receive the same cumulative dose, the dosing schedule was different and therefore they were analyzed as two separate groups. A saline control group (Male, n = 12; Female, n = 12) was given a total of 13 injections following the same treatment schedule as Group 1. For a comprehensive breakdown of groups by treatment and sex see Table 1.

Apparatus

Water-Motivated Stone T-Maze (STM). Mice were evaluated in the STM 5 days post-treatment. The STM used was developed by Pistell and Ingram (2010). The STM is made of black acrylic and was placed in a steel pan that is filled with water maintained at 20-22°C that mice must wade but not swim through. A clear acrylic ceiling prevents the mice from rearing. A start box was used at the beginning of each trial that was also constructed of clear acrylic and consists of a locked top that opens for the loading of mice and a sliding front that is lifted for mice to enter the maze. A sliding plunger at the back of the start box can be used to gently push mice into the maze if needed.

Five guillotine doors placed throughout the maze are lowered behind the mouse to prevent it from backtracking. To avoid spatial cues that could help with navigation, there are false guillotine doors present at other T-junctions in the maze. At the end of maze, a goal box made of black acrylic was placed and allowed mice a place to escape from the water. The ceiling of the goal box was removable to gain access to the mice. A picture of the maze is shown in Figure 1 below.

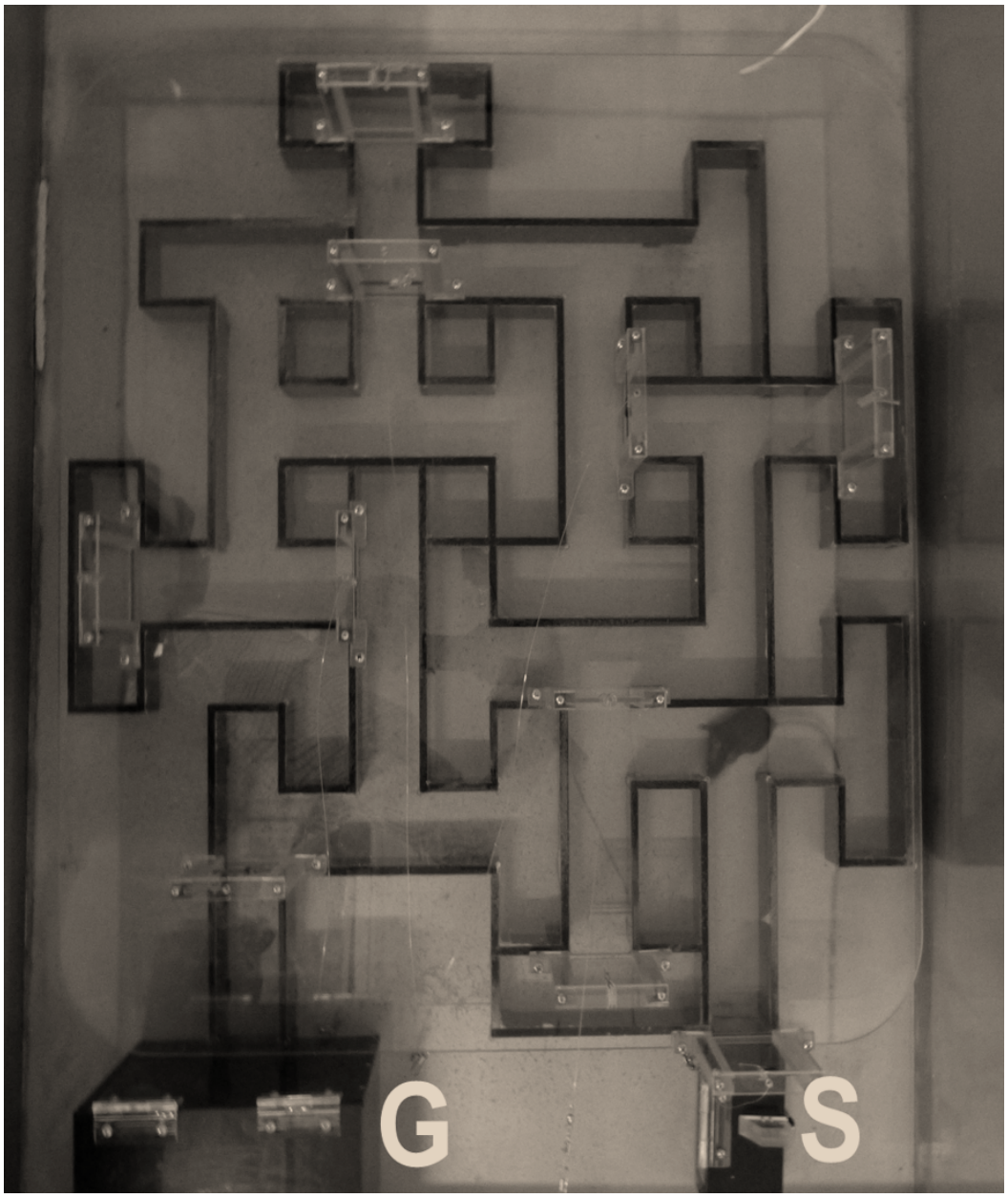

Figure 1. The water-motivated Stone T-maze, where “S” represents the start box and “G” represents the goal box.

Rotarod. The rotarod (Med-Associates, St. Albans, VT) is a rotating rod that gradually accelerates from 4 rpm to 40 rpm over 5 minutes. The average latency for mice to fall across the three trials was used as a test of motor function.

Procedure

Mice were weighed before the start of each injection day. All treatments were administered via i.p. injection, and the side of injection was alternated for the mouse's comfort. 3NP was dissolved in sterile saline at the start of each new dose and stored in the freezer. All mice were given a five-day recovery period before being tested in the STM.

One day prior to being tested in the STM (6-8 mice/day) mice received straight run training. The straight run is an alley made of the same materials as the STM in which the start box is placed at one end and the goal box is placed at the other. This training allowed mice to learn that moving forward through the water results in them reaching the dry, dark goal box. Mice had to complete the straight run in less than 15 seconds within 7 of the first 9 trials to be tested the following day in the STM. All mice met this requirement.

Mice were tested using 15 acquisition trials in the maze in a single day such that all mice ran trial 1 before mouse 1 started trial 2. Mice were randomly assigned to testing days so that treatment groups were unknown to the researcher. The measures used for learning and memory were latency and errors in the maze. An error was defined when a mouse's head entered an incorrect turn. Latency was calculated by the amount of time it took a mouse to reach the goal box from the start box. Each measure was tracked by the researcher, but was also video recorded using Any-Maze software (Stoelting; Wood Dale,

IL). If a mouse did not reach the goal box within five minutes it resulted in a failed trial. A mouse with three failed trials was taken out of initial acquisition testing. Retention testing was completed one week following acquisition using three additional trials in the STM. Only mice that completed acquisition testing were included in retention testing.

One day following maze acquisition testing, mice were tested on the rotarod. Mice were placed on the still rod facing the wall to minimize distraction. The rotarod ran for 60 seconds on the first trial, before acceleration was initiated, to acclimate mice to the task. Mice were then given three trials with approximately a 30-minute inter-trial interval (ITI). Trials were terminated at 300s and the average latency to fall across all three trials was used.

After all experimental testing was completed, mice were perfused and their brains were collected for histological analysis. Histology will not be discussed in this manuscript.

Chapter Three:

Results

STM Acquisition

Results for STM acquisition errors were analyzed using a 2 (Sex: Female vs. Male) x 3 (Dose: Saline vs. Group 1 vs. Group 2) x 3 (Trial Block: Block 1 vs. Block 2 vs. Block 3) mixed methods ANOVA. The 15 trials were collapsed into 3 trial blocks for ease of interpretation. A total of 63 mice were used for this analysis, where mice were missing from either death during treatment or failure to complete acquisition trials. Mauchly's test of sphericity was violated therefore corrected Greenhouse-Geisser statistics are reported. Results of this analysis show a main effect of trial block, $F(2,101.56) = 74.78$, $p < .05$, $\eta^2_p = .57$ [90% CI: .46, .64], Power = 1.00, where mice were committing less errors in the STM as they progressed through the trial blocks as shown in Figure 2 and Table 3.

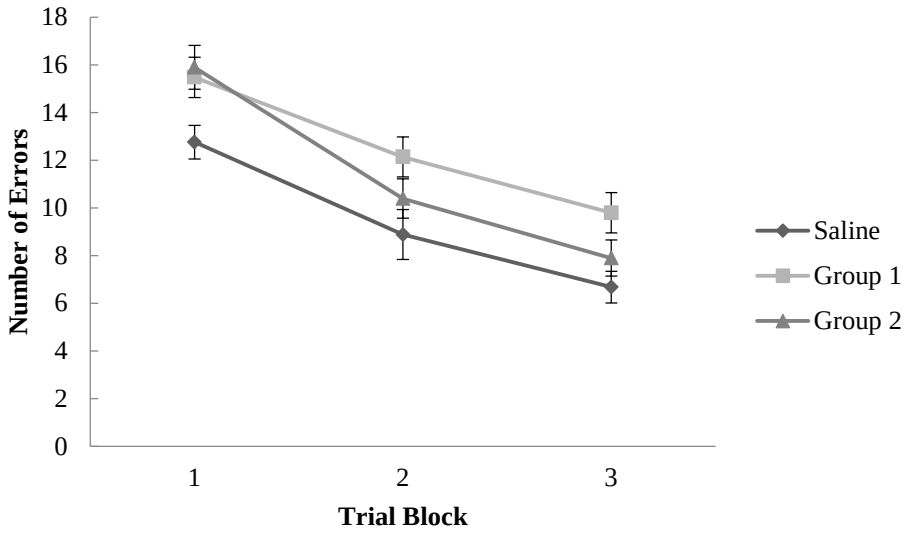

Figure 2. All treatment groups committed fewer errors at each trial block with Group 1 committing significantly more errors than saline controls in STM acquisition.

A main effect of dose was found, $F(1,57) = 7.27$, $p < .05$, $\eta^2_p = .20$ [90% CI: .05, .33], Power = .92. A Scheffe's post-hoc analysis revealed a significant difference between Group 1 and saline controls. Group 1 committed significantly more errors than controls at the third trial block, $t(42) = -2.92$, $p < .05$, $d = 0.89$ [95% CI: -.09, 1.90]. There was no significant difference between these groups at trial 1, $t(42) = .99$, $p > .05$, $d = 0.30$ [95% CI: -1.20, 1.82]. No other significant differences were found for dose. There was also a main effect of sex found, $F(1,57) = 5.60$, $p < .05$, $\eta^2_p = .09$ [90% CI: .01, .22], Power = .64, with females committing significantly more errors in the STM than males (Figure 3).There were no significant interaction effects between trial block and dose or trial block and sex.

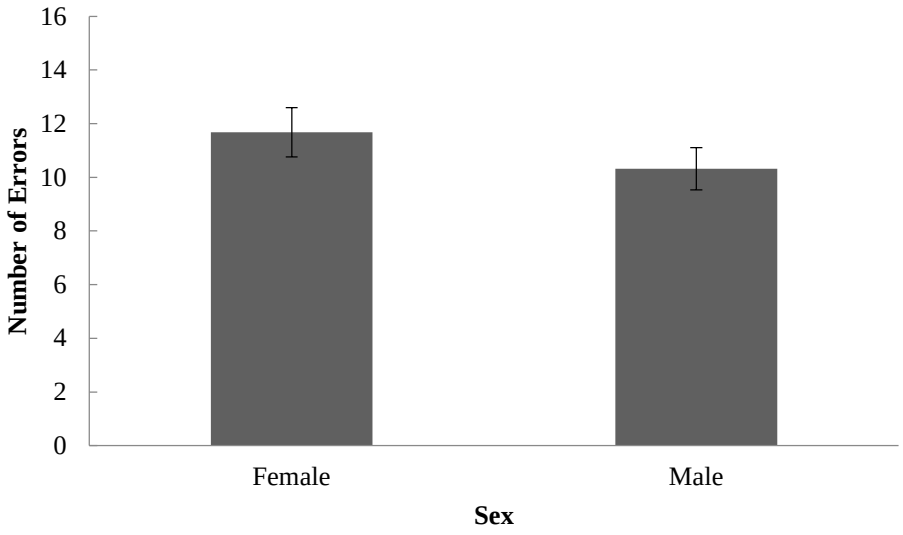

Figure 3. Main effect of sex in STM acquisition with females committing significantly more errors than males.

Results for acquisition trial latency were also analyzed using a 2 (Sex: Female vs. Male) x 3 (Dose: Saline vs. Group 1 vs. Group 2) x 3 (Trial Block: Block 1 vs. Block 2 vs. Block 3) mixed methods ANOVA. The corrected Greenhouse-Geisser statistic was

used because Mauchly's test of sphericity was violated. Results indicate there was no main effect of trial block, but there was an interaction effect of trial block and sex, $F(2,99.12) = 3.914$, $p < .05$, $\eta^2_p = .06$ [90% CI: .01, .14], Power = .65. Female mice decreased their time at each trial block while male mice decreased their time from trial block 1 to trial block 2 but their time increased from trial block 2 to trial block 3 (Figure 4). There were no other significant effects found (Table 4).

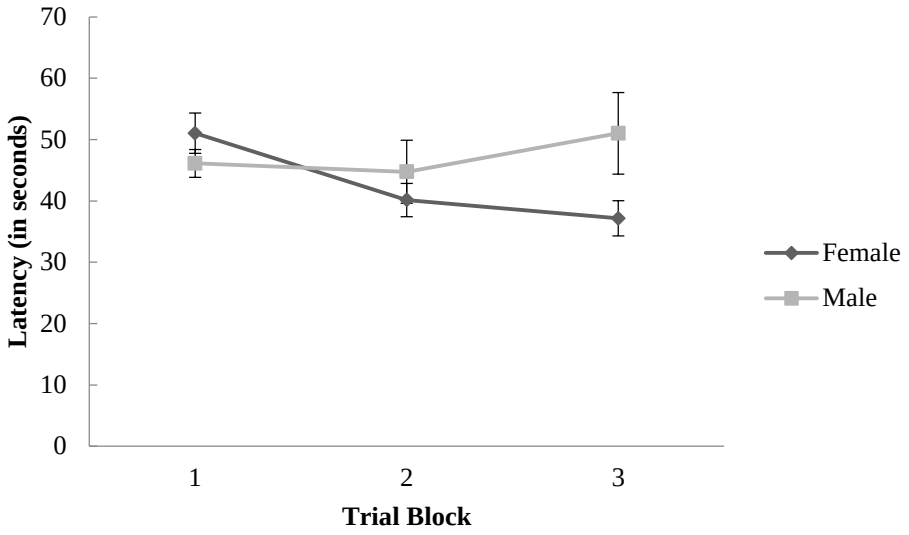

Figure 4. Females decreased their latency at each trial block while males increased their latency at trial block 3.

STM Retention

Results for retention trials were analyzed using two separate 2 (Sex: Female vs. Male) x 3 (Dose: Saline vs. Group 1 vs. Group 2) x 2 (Trial: Acquisition vs. Retention) mixed methods ANOVA's for errors and latency. The two trials compared were the last acquisition trial (trial 15) and the first retention trial. These trials were chosen for analysis because mice should know the STM task the best on the last acquisition trial, and the first trial of retention testing may be the best indicator of how well a mouse remembers the

task. Therefore, a comparison of these two trials offers an analysis of the mouse's recall of this task.

An analysis of errors indicated a main effect of trial, $F(1,57) = 14.48$, $p < .05$, $\eta^2_p = .20$ [90% CI: .01, .23], Power = .96. Mice committed significantly more errors in the first trial of retention compared to the last trial of acquisition (Figure 5, Table 5). There were no other main or interaction effects found.

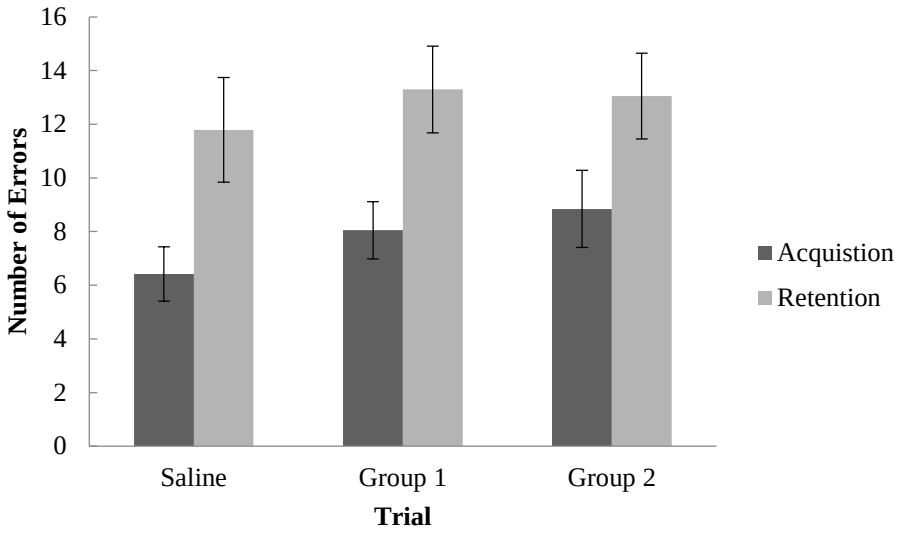

Figure 5. Mice committed significantly more errors in the first trial of retention compared to the last trial of acquisition.

Results for latency show a main effect of trial, $F(1,57) = 6.29$, $p < .05$, $\eta^2_p = .10$ [90% CI: .00, .26], Power = .67. Mice took more time to complete the maze in the last trial of acquisition than the first trial of retention (Figure 6, Table 6).

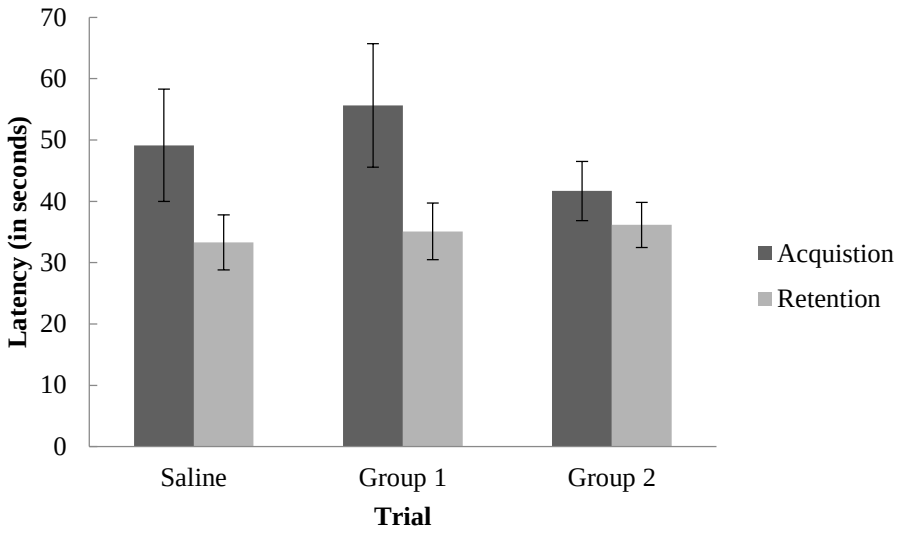

Figure 6. Mice took significantly less time to complete the first trial of retention compared to the last trial of acquisition.

Figure 7 shows a significant interaction effect of trial and sex, $F(1,57) = 4.87$, $p < .05$, $\eta^2_p = .08$ [90% CI: .00, .20], Power = .58. Follow-up simple effects tests indicated males (M = 60.88, *SD* = 47.79) took significantly more time to complete acquisition trial 15 than females (*M* = 36.65, *SD* = 22.21), $t(61) = -2.57$, $p < .05$, $d = 0.66$ [95% CI: -9.76, 8.45], but took the same time in retention trial 1. There was also a significant main effect for sex, $F(1,57) = 4.29$, $p < .05$, $\eta^2_p = .07$ [90% CI: .00, .19], Power = .53, in which males took significantly longer than females collapsed across trials. No other significant interaction or main effects were found.

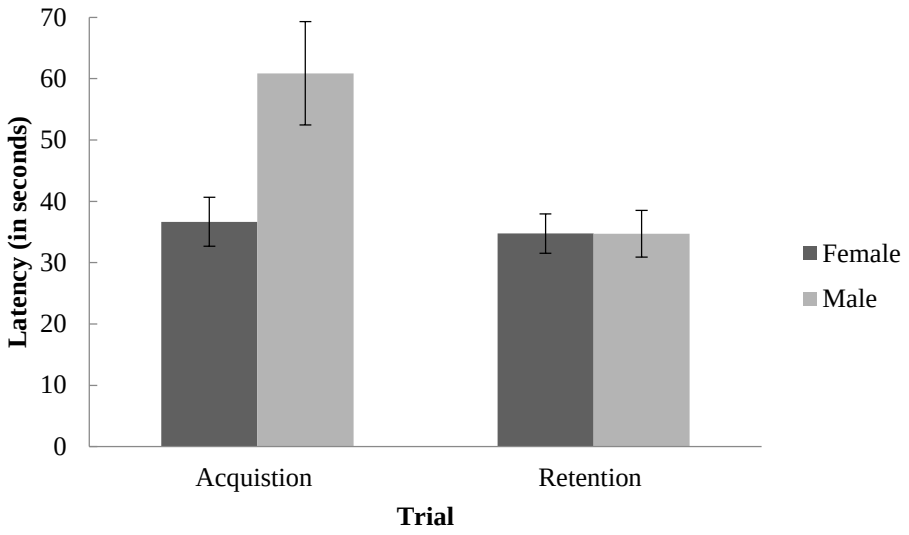

Figure 7. Males took significantly longer than females to complete the last trial of acquisition but took the same time in the first trial of retention.

Rotarod Latency

Rotarod latency was analyzed using a 2 (Sex: Female vs. Male) x 3 (Dose: Saline vs. Group 1 vs. Group 2) between-subjects ANOVA in which latency to fall across trials was averaged. A total of 76 mice were used for this analysis. A main effect of sex was found, $F(1,70) = 4.87$, $p < .05$, $\eta^2_p = .07$ [90% CI: .00, .17], Power = .56. Female mice ($M = 205.1$, $SD = 95.97$) were able to stay on the rotarod significantly longer than male mice ($M = 160.76$, $SD = 93.29$) which is shown in figure 8. No other significant effects were found (Figure 9, Table 7).

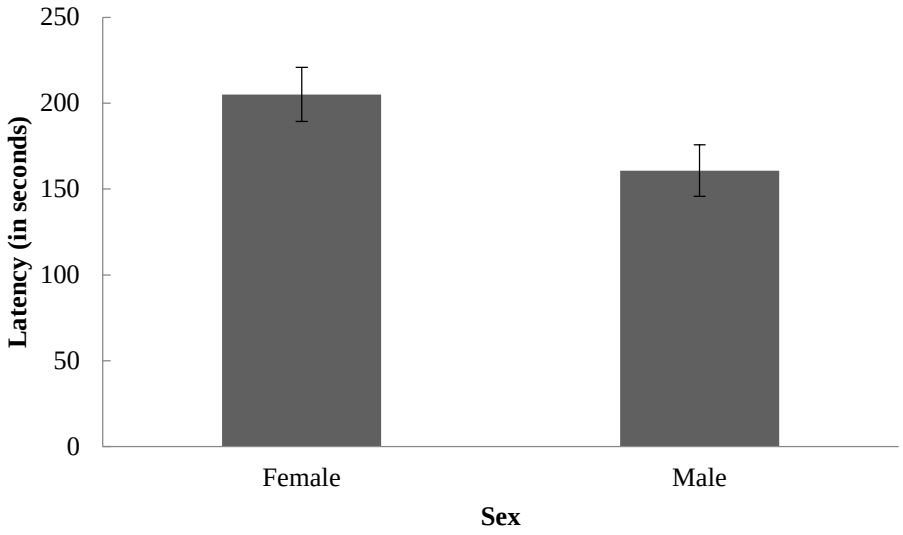

Figure 8. Females stayed on the rotarod significantly longer than males.

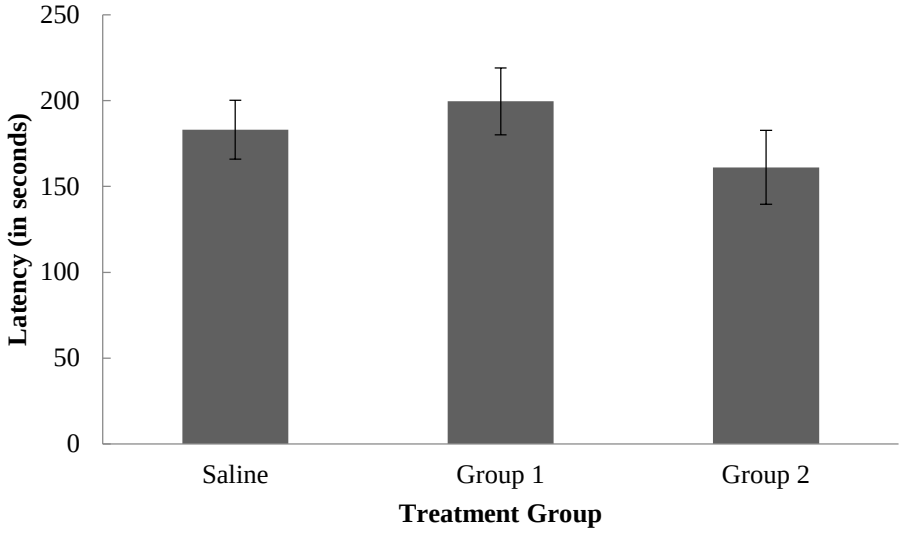

Figure 9. There were no significant effects of dose for average rotarod latency.

Chapter Four:

Discussion

Review of Findings

The findings of this study partially support the hypothesis that mice will perform worse in the STM as measured by errors when compared to control mice. Group 1 had significantly more errors than the saline controls at all three trial blocks. These results should be interpreted with caution, as Group 1 had significantly more errors than the saline controls at trial block 1. During this time, the groups should have a similar amount of errors. A lack of significant interaction effect suggests that the two groups learned the maze at the same rate, but with Group 1 committing more errors overall than saline controls. It was hypothesized that group differences would be more apparent in Group 2, but there were no significant differences between this group and saline controls in the STM. Group 2 received the same dose of 3NP as Group 1, but on a more rapid dosing schedule. Group 2 had a higher mortality rate than saline controls which may have caused the most affected mice to die before they were able to be tested.

No significant effects of dose were found on the rotarod which does not support the hypothesis that mice treated with 3NP will have impaired performance on tasks which measure motor function. This finding may distinguish this dosing regimen as a cognitive impairment model instead of a model of HD. An accelerated aging model that creates procedural learning and memory deficits without motor deficits would still allow for the study of possible interventions for impairments seen in aging.

It was hypothesized that male mice would perform worse than female mice due to the protective effects of estrogen. This hypothesis was not supported in the STM but was

supported on the rotarod. Female mice had more errors than male mice in STM acquisition. This difference was statistically significant, but may not be practically significant as there was only approximately one error difference between the two groups. There was no significant interaction effect between sex and trail block suggesting that males and females learned the maze at the same rate. Both sexes also performed similar in retention testing. Female mice, however, outperformed male mice on the rotarod. 3NP may impact females' learning and memory to a greater degree than males. Additionally, it has a greater impact on males' motor coordination. There was also a higher mortality rate of 3NP treated males compared to females, but no significant differences were found.

Limitations and Future Research

The current study found that 3NP affects females and males differently. However, estrous cycle for female mice and estrogen levels were not determined. Future research should examine estrogen levels in female mice to evaluate if estrogen is protective against 3NP.

The accelerated aging model in this study focused on procedural learning and memory deficits associated with aging. Although creating striatal lesions may mimic the cognitive and motor declines seen in aged animals, it does not encompass the entire aging process in rodents or humans. For example, older human women will be post-menopausal which will affect their hormone levels and may affect their response to certain pharmaceutical interventions. These changes would not be seen in a young mouse treated with 3NP.

A limitation in the current study was high attrition in 3NP-treated groups. In both cohorts, several mice died during injections or failed out of STM acquisition testing. This

contributed to low power and greater variance among groups. Future research should consider starting with larger sample sizes to allow for possible attrition to occur and to reach sufficient power.

Another limitation is that our lab is unable to complete histological procedures at this time. Histology would place this behavioral data into biological context of the brain. It would reveal if a lesion was being created or if there was a reduction in striatal volume. Histology could help direct future studies in adjusting the dose or dosing schedule of 3NP.

Future research should reduce the recovery period post-intoxication. Fernagut, Diguet, Stefanova et al. (2002) found that motor disorders caused by 3NP peaked 1-5 days following the final injection and there was rapid recovery after 1 week. This suggests that rotarod testing should take place soon after the last injection to see heightened motor deficits. Future studies should also examine possible age differences on the effects of 3NP. Minn et al. (2008) examined the mechanisms behind possible age-dependent vulnerability, but did not test behavior. Lohmann & Reipe (2007) found that 9 month old mice were severely affected by 3NP. In contrast, 4.5 month old mice were only slightly affected. If middle-aged mice are more sensitive to 3NP than young mice, it could be beneficial to use slightly older mice in an aging model.

The goal of creating an accelerated aging model is to look at possible interventions to combat cognitive and motor declines. Jamwal & Kumar (2016) found spermidine to be an effective intervention against the toxic effects of 3NP in rats. Future studies could also look at dietary intervention such as the protective effects of olive oil

(Tasset et al., 2011). Antidepressants have also been used as a pharmaceutical intervention for 3NP damage (Kumar, Kalonia, & Kumar, 2011).

Conclusion

The current study attempted to create an accelerated aging model that would mimic the cognitive and motor impairments seen in aged animals using two different doses of 3NP in female and male mice. Treatment Group 1 committed more errors in STM acquisition than saline controls. Female mice had more errors in STM acquisition than males, but outperformed them on the rotarod task.

Future research should continue to examine sex differences, as the current study found female and male mice react differently to 3NP. Creating an accelerated aging model using 3NP would allow for a less invasive, less time consuming, and more cost-effective model to study possible intervention effects to the cognitive and motor declines seen in aged animals. 3NP also allows researchers to examine the striatal-dependent aspects of learning and memory.

Table 1. Number of animals across treatment groups

	Treatment Group 1	**Treatment Group 2**	**Saline**
First Cohort			
Males	12	12	12
Females	12	12	12
Second Cohort			
Males	6	8	2
Females	3	3	2

Table 2. Animal mortalities across treatment groups

	Treatment Group 1	Treatment Group 2	Saline
First Cohort			
Males	3	6	0
Females	1	3	0
Second Cohort			
Males	1	3	0
Females	2	1	0

Table 3. Errors committed by mice during STM acquisition testing.

	Female			Male		
	Saline	**Group 1**	**Group 2**	**Saline**	**Group 1**	**Group 2**
Trial Block 1						
M	12.93	17.42	17.60	12.58	13.88	14.00
SD	3.81	4.57	4.29	3.25	2.04	2.72
Trial Block 2						
M	8.08	13.07	12.68	9.68	13.07	12.68
SD	4.14	2.98	3.06	6.06	2.98	3.06
Trial Block 3						
M	6.28	10.62	8.60	7.08	8.99	7.11
SD	2.83	3.22	3.32	3.68	4.00	3.28

Table 4. Latency of mice during STM acquisition testing (in seconds).

	Female			Male		
	Saline	**Group 1**	**Group 2**	**Saline**	**Group 1**	**Group 2**
Trial Block 1						
M	43.98	54.00	56.82	43.93	43.95	51.67
SD	19.09	15.03	18.97	15.56	10.67	10.45
Trial Block 2						
M	31.70	48.21	43.04	42.73	55.91	33.78
SD	14.62	13.94	12.76	31.40	35.33	5.51
Trial Block 3						
M	32.38	46.78	32.24	43.60	66.93	41.24
SD	14.21	20.17	10.54	20.10	57.25	18.12

Table 5. Errors committed by mice during STM during retention testing.

	Female			Male		
	Saline	**Group 1**	**Group 2**	**Saline**	**Group 1**	**Group 2**
Acquisition Trial 15						
M	5.67	6.78	8.40	7.42	9.09	9.33
SD	4.92	3.19	5.10	4.81	5.72	7.65
Retention Trial 1						
M	12.58	14.89	15.30	11.00	12.00	10.56
SD	10.04	7.77	7.30	9.36	6.84	6.00

Table 6. Latency of mice during STM retention testing (in seconds).

	Female			Male		
	Saline	**Group 1**	**Group 2**	**Saline**	**Group 1**	**Group 2**
Acquisition Trial 15						
M	37.25	38.44	34.30	61.00	69.73	49.89
SD	29.58	20.72	13.55	55.11	55.02	25.42
Retention Trial 1						
M	31.42	37.11	36.60	35.17	33.45	35.67
SD	18.75	19.18	16.90	25.69	22.69	13.11

Table 7. Latency of mice during rotarod testing.

	Female			Male		
	Saline	**Group 1**	**Group 2**	**Saline**	**Group 1**	**Group 2**
Trial 1						
M	157.43	216.33	200.55	144.21	143.64	74.91
SD	94.03	108.74	104.29	113.14	112.15	79.01
Trial 2						
M	211.50	217.58	218.73	162.29	189.21	130.09
SD	102.95	114.62	105.82	101.06	120.70	119.91
Trial 3						
M	227.07	196.42	204.27	195.86	238.79	138.00
SD	103.19	128.91	117.58	100.96	106.85	113.69
Average						
M	198.67	210.11	207.85	167.45	190.55	114.33
SD	93.41	106.27	96.42	88.46	95.72	85.50

Appendix

Appendix A

TOWSON UNIVERSITY

December 18, 2015

Office of Sponsored Programs and Research

Towson University
8000 York Road
Towson, MD 21252-0001

t. 410 704-2236
f. 410 704-4494

To: Paul Pistell, PhD

From: Towson University Institutional Animal Care and Use Committee
Louis DeTolla, Chairperson

RE: IACUC PROTOCOL# 12182015PP-02
Assessment of nutriceutical and pharmacological Interventions to ameliorate experimentally-induced or natural age-related declines in memory and motor function

This is to certify that the Institutional Animal Care and Use Committee has reviewed your protocol and granted FULL APPROVAL. The approval date for this protocol is December 18, 2015

Your protocol is approved for a period of 3 years; an annual report must be submitted to the IACUC six weeks before each anniversary date of the protocol.

Please note your protocol will expire December 17, 2018. If you need to extend the protocol beyond this date, you must submit an Animal Care and Use form at least three months prior to the expiration.

If you have any questions, please do not hesitate to contact the OSPR Compliance Administrator by phone (410.704.2236) or email (OSPR@towson.edu).

Louis DeTolla, VMD, MS, PhD, DACLAM
Chair and Veterinarian, Towson IACUC

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May 2017 (expected) MD	**M.A. in Experimental Psychology**	Towson University	Towson,
GPA: 3.868	Thesis Advisor: Dr. Paul Pistell		
	Thesis: Evaluating Sex Differences in Procedural Learning and Memory Deficits Caused by 3-Nitropropionic Acid		
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Research/Internship Experience:

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2016 – Present *Oak Ridge Institute for Science and Education (ORISE) Fellow*
US Army Medical Research Institute of Chemical Defense (USAMRICD)
Aberdeen Proving Ground – Edgewood, MD
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Projects: (1) Pediatric susceptibility of rats to nerve agent-induced seizures and effectiveness of anticonvulsant treatments (2) Amelioration of soman-induced neuropathology in rats with NAAG-related compounds (3) Investigation of individual differences in mouse strains in response to nerve agent exposure (4) Rat and guinea pig models of nerve agent intoxication to evaluate delayed treatment with novel anticonvulsants
Skills: Project coordination, rodent surgery (EEG lead implantation), anesthesia monitoring, aseptic technique, suturing, post-operative care, EEG interpretation (use of Spike2 computer software), perfusion, brain extraction, injections, animal handling and observation, drug preparation (dose calculations, syringe preparation), rat pup weaning and sexing, histopathology evaluation, data analysis, abstract writing and poster presentation

2013 (Winter) – Present *Research Assistant*
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Baltimore, MD
Evaluation of midazolam and diazepam to treat nerve agent-induced seizures in pediatric and adult rats
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Edgewood, MD
Evaluation of anticonvulsant treatments for nerve agent-induced seizures in pediatric and adult rats
Matson, L.M., **Haines, K**., Ardginer, C., Dunn, E., McCarren, H., Miller-Smith, S., McDonough, J.

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Ardinger C, **Haines K**, Pistell P
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Teaching Experience:

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