EFFECTS OF SUPPLEMENTED ZINC ON LEARNING AND MEMORY IN TAU MICE (P301L/CAMKII) USING CONTEXTUAL AND CUED FEAR EXTINCTION

by

Carlos M. Hernandez

A Thesis
Submitted to the
Graduate Faculty
of
George Mason University
in Partial Fulfillment of
The Requirements for the Degree
of
Master of Arts
Psychology

Committee:

_____ Director

_____

_____

_____ Department Chairperson

_____ Dean, College of Humanities and Social Sciences

Date: _____ Summer Semester 2017
George Mason University
Fairfax, VA

Effects of Supplemented Zinc on Learning and Memory in Tau Mice (P310L/CaMKII) using Contextual and Cued Fear Extinction

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Arts at George Mason University

by

Carlos M. Hernandez
Bachelor of Arts
George Mason University, 2012
Associates of Arts
Northern Virginia Community College, 2009

Director: Jane Flinn, Associate Professor
Psychology

Summer Semester 2017
George Mason University
Fairfax, VA

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

EFFECTS OF SUPPLEMENTED ZINC ON LEARNING AND MEMORY IN TAU MICE (P310L/CAMKII) USING CONTEXTUAL AND CUED FEAR EXTINCTION

Carlos M. Hernandez, M.A.

George Mason University, 2017

Thesis Director

Neurofibrillary tangles (NFTs) are associated with behavioral disorders in Alzheimer's disease, Parkinson's disease, and in chronic traumatic encephalopathy (CTE). Research has shown that NFTs play a role in learning impairments and memory deterioration. Neurobehavioral abnormalities like these can be exacerbated by environmental factors such as zinc (Zn). Previous studies conducted in our lab have shown that excessive Zn led to increased freezing levels in animals during cued fear extinction (Railey et al., 2010). In another study that used tau mice, mice showed impaired retention in contextual fear conditioning (Hunsbergera et al., 2014).

Therefore, to observe the effects of both NFTs and Zn, mice were bred to possess the P301L tau mutation; consisting of both the human microtubule associated protein tau (huMAPT) mutation and the tTA transgene, with the CaMKII activator. The tau mice were subsequently raised on 10ppm supplemented zinc water. To test the animal's

learning and memory, cued and contextual fear conditioning will be conducted at 6 months of age. Since learning and memory is impaired in the tau mice it is hypothesized that they will perform poorly in fear extinction. It is also hypothesized that zinc supplemented mice will perform poorly in the experiment, as zinc has shown to effect fear extinction in mice.

INTRODUCTION

Molecular Pathology of Alzheimer's disease

Alzheimer's disease manifests itself by signs of mental deterioration; such as memory loss, decline of intellectual and social skills. At the cellular level the brain accumulates amyloid plaques and neurofibrillary tau tangles. In a healthy brain the tau protein stabilizes microtubules, which allows for neuronal connections to develop in the brain. However, in Alzheimer's disease tau protein acquires an abnormal amount of phosphate molecules, the tau around the microtubule hyperphosphorylates (Iqbal et al., 2010). This causes the tau threads to detach from the microtubule and allow the tau to clump together. This process creates neurofibrillary tangles (NFTs) and leads the neuron to collapse. In humans, NFTs begin to accumulate in the entorhinal cortex and spread out to neighboring brain regions, such as the hippocampus, and eventually to neocortical regions (Braak & Braak, 1991). The consequence of these events disrupts neuronal connections and eventually leads to memory disturbance.

Fear Conditioning and Extinction

The Alzheimer's Association (2015) states that patients affected with Alzheimer's disease (AD) have both a progressive loss of memory and a difficulty responding to their environment. Patients tend to feel lost in places that they would normally recognize, and

they forget recently learned information. Upon the onset of Alzheimer's disease, these behaviors can be exhibited as a decline in association learning, contextual and cued memory, all of which can be tested with fear conditioning (Curzon, Rustay, & Browman, 2009).

Fear conditioning allows researchers to study an animal's ability to learn and remember associations between a specific event and a cue or context in which the event occurs. This associative learning is a mechanism that provides the animal with the capacity to anticipate events, by pairing prior incidents with a cue or in specific context (Curzon et al., 2009). Apart from conditioning, fear extinction is used to study an animal's ability to learn new information about a situation or cue.

In cued fear conditioning, animals associate a conditioned stimulus (CS) such as a tone or light to an unconditioned stimulus (US) mild shock. The mild shock functions as an aversive stimulus that causes the animal to exhibit freezing behaviors. The animal will then associate the CS to the shock, thus leading the animal to exhibit freezing behaviors when only the CS is presented (Puzzo et al., 2014). The duration of the freezing behavior is recorded and used as an indicator of fear memory (Shoji et al., 2014). The advantage of this conditioning is that animals can instantly associate the cue to the shock and remember it for a long time; this allows researchers to test for retention (Puzzo et al., 2014). In cued fear extinction, the animal is repeatedly presented with the CS but without the mild foot shock. After multiple presentations of the CS the animal will exhibit less freezing behaviors, as it learns that the CS no longer precedes the aversive stimulus. The

extinction process involves the formation of new memories, rather than forgetting old information (Quirk et al., 2002).

Similarly, in contextual fear conditioning the animal associates the environment with a shock, and the freezing behavior is recorded. Therefore, when the animal is placed in the same environment in which it was given the mild shock, the animal should remember the environment and exhibit freezing behaviors. In contextual fear conditioning extinction, the freezing behavior decreases the longer the animal is exposed to the environment without the mild foot shock.

Fear conditioning on tau Mice

A common way to study tau pathology in Alzheimer's animal models is to use animals that express the P301L mutation. Development of NFT begins to accumulate between 4.5 months to 6.5 months (Lewis et al., 2000). P301L mice express abundant neuronal loss in the forebrain and tau pathology concentration in the CA1 region of the hippocampus, which is like human Alzheimer's (Ramsden et al., 2005). Additionally, there are NFTs observed in the piriform cortex, entorhinal cortex, basal ganglia and spinal cord (Lewis et al., 2000). Some studies constrict abnormal tau pathology only to the entorhinal cortex, and do not find cognitive deficits (Harris et al., 2012). To develop NFTs only in the entorhinal cortex the Tet-hTauP301L mouse must be bred with a neuropsin-tTA transactivator mouse, and have both neuropsin-tTA/tet-hTau genes. However, the absence of cognitive deficits in that study may be due to conducting only contextual fear condition and recall at 12 months of age. They did not test for contextual

fear extinction, cued recall, or cued extinction. Additionally, research that analyzes the progression of Alzheimer's should look at widespread accumulation of abnormal tau pathology to have a more accurate representation of the disease (Ramsden et al., 2005, Hunsberger et al., 2014).

In one study, tau expression was suppressed until 3 months of age by inserting a tetracycline-responsive element (TRE) into the genes that would normally bind to tTA (Hunsberger et al., 2014). The study conducted contextual and trace fear conditioning recall on the P301L mice. Trace fear conditioning is one method of performing cued fear conditioning. The primary difference is that in this case when the CS is presented, there is a short interval (usually 10 or 30 seconds) before the shock is delivered. Furthermore, trace conditioning is dependent on both the hippocampus and prefrontal cortex (Hunsberger et al., 2014). The researchers stated that testing with the P301L mice on trace fear conditioning had not been previously published, suggesting that more studies should be conducted in this area of research. Their results showed that P301L mice were significantly impaired in the retention of contextual memory, meaning that P301L mice expressed fewer freezing behaviors than control mice. With 30 second trace fear conditioning they reported that the P301L mice had fewer freezing behaviors than the control mice. These results suggest that development of NFTs, caused by the P301L gene expression, leads to problems with learning and retention of associations (Hunsberger et al., 2014). However, the researchers did not test for fear extinction. In another study, no cognitive deficits were found in 12-month old neuropsin-tTA/tet-hTau mice on contextual fear conditioning (Harris et al., 2012). However, the neuropsin-tTA/tet-hTau

mouse only has NFTs in the entorhinal cortex, unlike the P301L mouse that has NFTs all throughout the brain. They reported that the transgenic mice showed the same level of freezing behaviors as their non-transgenic control mice. The fact that their transgenic mice only developed NFTs in the entorhinal cortex makes it less relevant to the actual disease progression.

Few studies have examined fear conditioning and extinction in P301L mouse model for Alzheimer's disease. Even the studies previously conducted have variables not consistent with the actual disease progression. It is important to continue research in this area to understand the function NFTs have on learning and memory. Therefore, this study will test the P301L mouse model on cued and contextual fear extinction to understand how learning and extinguishing emotional memories are effected by NFTs. The mice will undergo an open field test after, as supplementary to fear conditioning and extinction.

Open Field Test on Tau Mice

Along with fear conditioning, it is recommended that an open field test be run. The open field test will provide information about whether freezing behaviors observed in fear extinction were due to motor impairments in the mouse or actual fear of the mild foot shock. In the open field test the animal is placed into a chamber or box, without any type of stimuli, and can explore for a certain amount of time. A camera above captures the activity and the software records and tracks various aspects of the animal's movements. These include but are not limited to: distance traveled, duration of activity, and speed.

Previous research has shown that P301L mice exhibited less freezing behavior than wildtype controls and suggested that P301L may have been hyperactive (Hunsberger et al., 2014). A study conducted by Stover et al (2015) stated that motor performances between transgenic animals and non-transgenic animals are an important consideration when interpreting behavioral tasks. In their study, they conducted fear conditioning on 3xTg-AD mice, and used the Barnes maze to examine motor performance. Therefore, by conducting open field we can see if the P301L mice move around in the chamber more than, or equal to, than the control mice. This test can help us better interpret the results from the fear conditioning assay by helping differentiate the reason for freezing behavior with additional behavioral data.

Trace Metal Zinc

Zinc is a trace metal essential for various molecular events, such as synaptic release, cell growth, and long-term potentiation (Yang et al., 2013). Zinc is prominently localized in synaptic vesicles--it regulates signaling between neurons by releasing zinc ions upon neuronal excitation.

The highest concentrations of zinc have been found in the hippocampus CA3 region, a region rich of neuronal connections involved in spatial and episodic memory. Within the CA3, zinc regulates the response of NMDA receptors. NMDA receptors are fundamental for synaptic plasticity and long-term potentiation, and thus play a role in learning new information. Thus, the interaction of zinc on the NMDA receptors in the CA3 plays a vital role in learning and memory (Yang et al., 2013). As studies have

shown that zinc deficiency leads to impairments in learning and memory (Yang et al., 2013, Bhatnagar, S. & Taneja, S., 2001).

Our lab focuses on supplementing trace metals in animal's diets and studying their subsequent behaviors. Results from our lab have shown that supplemented zinc leads to various memory impairments (Flinn et al., 2005, Railey et al., 2010). Railey et al used 10 ppm zinc supplemented water and conducted fear conditioning with Sprague-Dawley rats. Results showed that rats on zinc supplemented water had increased freezing levels in both cued and contextual extinction of fear conditioning, compared to rats on lab water. Railey et al. (2010) concluded that zinc supplemented animals had difficulty extinguishing fear to the cued and contextual associations. Studies should also focus on the effect of zinc supplementation on Alzheimer's mouse models. Some studies have already examined how learning and memory is impaired by the development of NFTs in the P301L mouse. It is also important to look at how zinc supplementation plays a role on the learning and memory of the P301L mouse.

PURPOSE

The studies discussed previously appear like this one. However, since our lab focuses on trace metals, we will supplement some of animals' diets with zinc and observe subsequent behaviors. A study conducted in our lab used rats on supplemented zinc diet and observed their behavior on fear extinction. Results showed no significant difference in acquisitions. However, with extinction, rats on zinc supplementation extinguished the fear association slower than the rats on control diet (Railey et al., 2010). Since these rats were not transgenic, we suggest the next step is to test fear conditioning and extinction with transgenic animals expressing the human tau protein, following dietary zinc supplementation. Such a study could be considered to have high face validity as tau tangles are seen in Alzheimer's disease and some older people are taking zinc supplements. Thus, we will use the P301L transgenic mice in our study to examine their ability to learn and retain information, as well as their ability to unlearn information. Since their gene expression leads to accumulation of tau, our results may have implications for disorders and conditions that involve abnormal tau accumulation such as AD. Additionally by supplementing zinc in the diet of the P301L mice, both transgenic and non-transgenic, we can examine how it affects their ability to learn and retain associations. Our results will then provide further insight into the effects of zinc on disorders and conditions of abnormal tau accumulation.

Hypotheses

1. As with results from previous studies, zinc should cause impairments in learning and memory. Therefore, we expect wildtype mice on supplemented zinc water to exhibit similar acquisition of fear conditioning but a slower fear extinction rate compared to wildtype mice on regular lab water.
2. Based on other studies, P301L mice should have impairments in learning and memory. We expect P301L mice on lab water to exhibit a significantly slower extinction rate during cued- and contextual- extinction trials compared to wildtype mice on lab water.
3. We expect P301L mice on zinc water to exhibit significantly slower extinction rates during cued- and contextual- extinction trials compared to wildtype mice on lab water. Additionally, we expect P301L mice on zinc water to have slower extinction rates during cued- and contextual- extinction trials compared to P301L mice on lab water.

METHODS

Breeding. Transgenic tau mice for will be purchased from Jackson Laboratory, FVB-Tg(tetO-MAPT*P301L)#Kha/JlwsJ (stock number 015815). These animals carry the mutant tau protein that will mimic NFTs found in human Alzheimer's disease. These mice will be bred with Tg(Camk2a-tTA)1Mmay/0 to activate and express the transgene in the offspring. Offspring that have both the human microtubule associated protein tau (huMAPT) mutation and the tTA transgene will be used as the transgenic animals. The offspring that do not have either transgene will be used as a control.

Housing. The animals will be housed in the Krasnow Institute Animal Facility at George Mason University. The light cycle of the housing room is on a 12-light/dark cycle. All cages include an igloo, an igloo with a running wheel, and a nyla bone. These serve as environmental enrichment for the animals, as this strain tends to be hyperactive and aggressive.

Zinc water. Zinc water will be prepared using a 1000ppm solution of Zn in a gallon of water to give a 10ppm of Zinc water solution. The solution will be buffered using sodium carbonate (NaCO3) to bring it to a pH of 7. Preparation will be completed on site and the date on which the water is made will be documented, as well as placed on the bottle. Preparation will be completed in accordance with prior studies (Railey et al., 2010; Railey et al., 2011). The water will be made and maintained in a polycarbonate

carboy. Water samples will be taken prior to the start of the study and tested for metal content using inductively coupled plasmaoptical emission spectroscopy and ion chromatography at the United States Geological Survey (USGS), Reston, VA. The zinc water will be provided to the animals via two plastic water bottles attached to the food supply within the cages. These water bottles are available through and will be provided by the Krasnow Institute. Mice will be approximately 8 weeks old when first given zinc water and this will continue throughout the experiment. Zinc water will be provided to half of the tau mice and half of the WT mice. To identify cages, cards will be placed in holders already on the cages. They will designate the number of mice in the cage, sex, and whether their water contains zinc or if it is lab water only. Furthermore, the water bottles within the cages will also be marked with an "X" if the water contains zinc. The bottles will be weighed every time the water is changed to account for how much water the mice are drinking. The water will be changed once the bottle is nearly empty and more is needed.

Cohorts. There will be a pilot study and three experimental cohorts of animals to undergo this paradigm. Each experimental cohort will be comprised of aged-matched animals assigned to one of the four experimental groups. Each cohort will have about 24 animals. All animals will undergo the same behavioral test as approved in the original protocol. This study is part of a larger study on the learning deficits in the P301L mouse. Therefore in addition to the fear conditioning experiment animals will be tested on nesting, circadian rhythm, novel object recognition, and Morris water maze. No new

animals will be added for fear conditioning or open field testing. These will be the same animals used in the other tests in the original IACUC.

Table 1: Experimental groups

	Tau Mice	Wildtype Mice
Zinc Water	16	22
Lab Water	16	22

Fear Conditioning

At six months of age, the mice will undergo contextual and cued fear conditioning. This will be the penultimate test in a battery of tests. Fear conditioning will begin approximately a week after Morris Water Maze.

On day one the mice will undergo conditioning. First, the mice will be removed from their home cage and placed into a transport cage with clean bedding. Two mice at a time will be brought into the behavior room where the fear conditioning boxes are set up. The boxes are two identical Plexiglas fear conditioning chambers (7 in x7 in x12 in) located inside sound attenuating boxes (Coulbourn Instruments). Each mouse will be placed into a separate box. The boxes are side by side. Since the sound attenuating boxes are thick and non-transparent, the animals cannot see or hear each other. The mice will acclimate in the fear conditioning box for 180-seconds. The interior of the chamber will be illuminated throughout the trial by a low emitting light of about 5 lux, located at the

top of the chamber. Then a tone (located at the top corner of the chamber, 75 decibels) will be switched on for 20 seconds to serve as the conditioned stimulus. The 75 decibels of the tone was determined by pilot testing and based on other studies. The tone will co-terminate with an unconditioned stimulus of a mild foot shock (0.5 mA, 2 seconds in duration). The tone/shock pairing will then be re-presented at 250 and 310 seconds. Once 60 seconds have elapsed from the last light/ shock, the animal will be taken out of the chamber, placed back into its transport cage and returned to their home cages. The freezing behavior of the animals will be recorded and tracked using FreezeScan software (Clever Sys, Inc.).

On day two the mice will undergo contextual fear extinction. Approximately 24 hours after conditioning, mice will be brought into the behavior room where the fear conditioning boxes are set up. The mice will then be placed in the same fear-conditioning chamber as before. The animal will be in the box for the same duration as before, 360 seconds. No tone nor shock will be presented during this test. Upon completion of the conditioning the animals will be returned to their home cages. The freezing behavior of the animals will be recorded and tracked using FreezeScan software (Clever Sys, Inc.). This will examine the animal's ability to remember the environment, or context, in which it received the aversive stimuli the previous day.

On day three mice will undergo cued fear extinction. Approximately 24 hours later mice will be brought into the same behavior room where the fear conditioning boxes

are set up. To avoid environmental similarities from the prior two days we will alter the behavior room, by having the lights off and only using a red light to illuminate the room. The fear-conditioning chamber will have a white Plexiglas that will cover the shock bars, along with some bedding on top of the Plexiglas. There will also be spatial cues on the sides of the chamber to change the context. Plexiglas will also be propped up vertically on the back outside of the chamber to give the appearance of a changed environment. The tone from day 1 will be presented again 25 times, every 60 seconds. However no mild foot shock will be administered. Upon completion of the conditioning the animals will be returned to their home cages. The freezing behavior of the animals will be recorded and tracked using FreezeScan software (Clever Sys, Inc.). This will test the animal's ability to retain association to the shock.

On day four the mice will undergo the second day of contextual fear extinction, which will be exactly like day two.Approximately 24 hours later mice will be placed in the same fear-conditioning chamber as in day two. No tone nor shock will be presented during this test. Upon completion of the conditioning the animals will be returned to their home cages. The freezing behavior of the animals will be recorded and tracked using FreezeScan software (Clever Sys, Inc.).

On day five the mice will undergo the second day of cued fear extinction, which will be exactly like day three. Approximately 24 hours later mice will be placed in the fear conditioning chamber with all the alterations done as on day three. The tones from

day 1 will be presented again 25 times, every 60 seconds. However, no shock will be administered. Upon completion of the conditioning the animals will be returned to their home cages. The freezing behavior of the animals will be recorded and tracked using FreezeScan software (Clever Sys, Inc.).

Open Field

Open field will be the final behavioral test to be conducted on the mice. It will begin approximately a week after fear conditioning. There will be four cohorts of animals to undergo this paradigm.

On testing day, mice will be transported from animal holding room via individual transport cages into the behavior room with the open field apparatus. Each animal will receive one 10-minute trial in the open field testing apparatus (blue plastic box, measuring approximately 20in X 20in X 20in; CleverSys, Inc.). TopScan analysis (CleverSys, Inc.) will be used to calculate total distance traveled, average speed, duration of inactivity and activity, and time spent in the center and in the surrounding areas of the arena. The testing boxes will be thoroughly cleaned with 70% ethanol in between animals to reduce odor cues. Following the completion of the task, animals will be returned to their group home cages**.**

ANALYSIS

A mixed Analysis of Variance (ANOVA) with repeated measures on which will be conducted to determine any significant differences in fear conditioning and extinction experiment. Freezing duration recorded from the FreezeScan software will be used to analyze the fear conditioning and extinction results. The freezing duration will be divided into 20 second bins; this will allow analysis of the 20-second duration in which the tone is presented. The higher the percentage obtained the longer the animal froze during the 20-second duration. The data will be analyzed individually by each day.

Day 1, acquisition, will be analyzed on its own to see if the mice associate the tone to the shock and exhibit freezing behaviors. The within-subject factor will be the three distinct times the tone is presented, and the between subject factors will be the genotype and water type given to the mice. Therefore, the analyses will be a 3 X 2 X 2 mixed ANOVA. The data points from all groups should plot an upward curve to indicate that the mice are freezing more after each pairing, this would indicate that all mice are learning the association regardless of their treatment groups.

From days two and four, contextual, the last 260 seconds will be analyzed to observe the duration of the freezing. The first 60 seconds, of the total 320 seconds, will be omitted as the animal habituates the environment. The within-subjects factor will be day two and day four, to compare the freezing percentage. By comparing the freezing

percentage from both days, we can observe the contextual extinction rate. The between subject factors will be the genotype and water type given to the mice. Therefore, the analyses will be a 2 X 2 X 2 mixed ANOVA. Percent freezing should decrease in the wild-type mice from day two to day four. To indicate a learned response that the context, or environment, is not be feared.

From days three and five, cued extinction, only the tone presentations will be analyzed. This will allow better analyses of the freezing behaviors and show a better extinction curve. Each day will provide 25 data points when the tone was presented. This data will be presented on a graph to show the trend. However, to interpret the interaction only five time points will be used, as 25 data points is not manageable. The five time points will be the 1^{st}, 6^{th}, 10^{th}, 16^{th}, and 21^{st} tone presentation. Therefore, the analyses will be a 5 X 2 X 2 mixed ANOVA. The data should show a decrease in freezing behaviors after multiple tone presentations. This is because the mice learn that the tone is not to be feared, since there is no shock delivered.

For the open field test a multivariate ANOVA will be conducted. The amount of time spent active, distance traveled, and speed, will be the dependent variable for each group. The fixed factors will be the four different experimental groups. Within the 10 minute trial the time inactive and duration of activity is measured in seconds. A threshold will be set to account for small movements, such as sniffing, head moving, and grooming in place; this threshold will differentiate between inactivity and actual movement across the arena. Distance traveled will be measured in centimeters. Speed will be calculated using distance traveled divided by duration of activity.

RESULTS

Fear Conditioning and Extinction

Fear Training

On day one, mice were presented three times with a 75-decibel tone that co-terminated with a two second 0.5 milliamp mild foot shock. During training, there were no significant differences in freezing percentage between the four groups ($F(3,72)=.645$, $p=.589$). A simple effects analysis shows that as each tone was presented the freezing percentage significantly increased ($F(2,144)=73.73$, $p<.001$)(Fig. 2).

Contextual Fear Extinction

On day two and four contextual fear extinction was performed. Mice were placed in the same chamber as in day one for 320 seconds. On first day of contextual fear extinction all mice exhibited freezing behavior averaging 53.04 ± 2.5%(Fig3B). On the second day of contextual fear extinction all mice exhibited lower freezing behavior averaging 38.59 ± 2.2%(Fig3C). There was a significant main effect from day two to day four ($F(1,72)= 38.03$, $p<.001$). However, no significant differences were seen between the animal groups ($F(3,72)=.764$, $p=.518$)(Fig3A).

Cued Fear Extinction

On day three and five cued fear extinction was performed. The mice were presented with the 75-decibel tones 25 times without the mild foot shock. To better analyze the extinction rate only five time points were selected from the second day of cued extinction, these are the 1st, 6th, 10th, 16th, and 21st tone presentations. There was a significant main effect over the series of tone presentation ($F(4,288)=38.73$, $p<.001$). However there was no significant difference between the animal groups ($F(3,72)=.269$, $p=.848$)(Fig.4A).

Open Field

Distance Traveled

Distance traveled was calculated in centimeters. There was a statistically significant difference between groups as determined by one-way ANOVA for distance traveled in the open field test ($F(2,72)=25.94$, $p<.001$) (Fig5A). A Bonferroni post-hoc test revealed that transgenic mice on lab water (4267.07 ± 1243.56cm) traveled significantly more than wildtype mice on lab water (2077.10 ± 820.03cm, $p<.001$) and wildtype on zinc water (2447.95 ± 757.35cm, $p<.001$). Transgenic mice on zinc water (4278.45 ± 1191.02cm) traveled significantly more than Wildtype mice on lab water (2077.10 ± 820.03cm, $p<.001$) and Wildtype mice on zinc water (2447.95 ± 757.35cm, $p<.001$).

Duration of Activity

Duration of activity was calculated in seconds, and determined as any movement that resulted in location change including small steps to running. If the mouse was grooming or sitting in place no activity was recorded. There was a statistically significant difference between groups as determined by one-way ANOVA for duration of activity in the open field test ($F(3,72)=5.96$, $p<.001$) (Fig 5B). A Bonferroni post-hoc test revealed that transgenic mice on lab water (540.53 ± 38.16sec) were more active than wildtype mice on zinc water (438.30 ± 154.05sec, $p=.007$). Transgenic mice on zinc water (551.52 ± 36.65sec) were more active than wildtype mice on zinc water (438.30 ± 154.05sec, $p=.002$).

Speed

Speed was calculated by dividing centimeters traveled and seconds of activity. There was a statistically significant difference between groups as determined by one-way ANOVA for speed ($F(3, 72)=6.26$, $p=.001$) (Fig5C). A Bonferroni post-hoc test revealed that transgenic mice on zinc water (9.39 ± 2.11cm/sec) were faster than wildtype mice on lab water (6.46 ± 1.41cm/sec, $p<.001$).

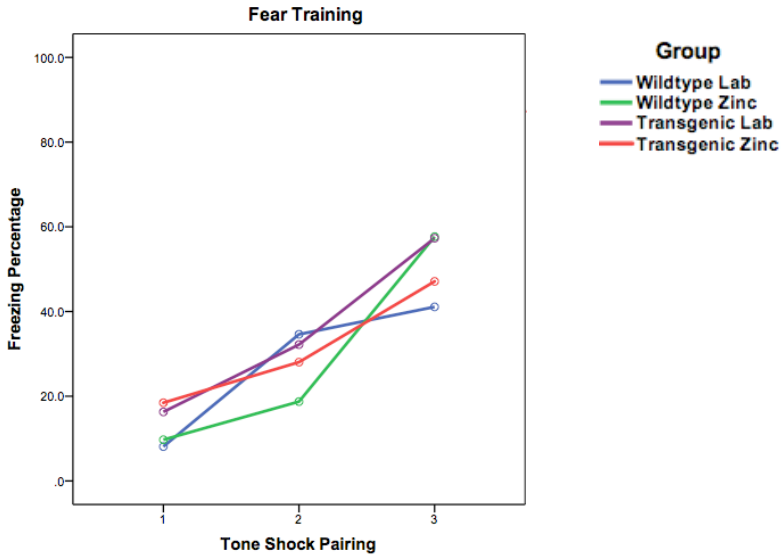

Figure 1: Fear Training

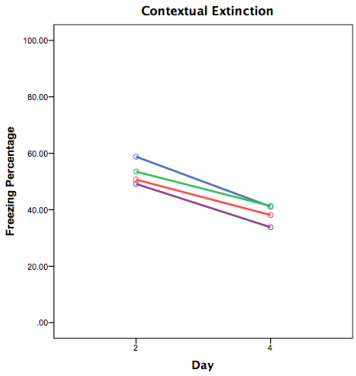

Figure 2: Contextual Extinction

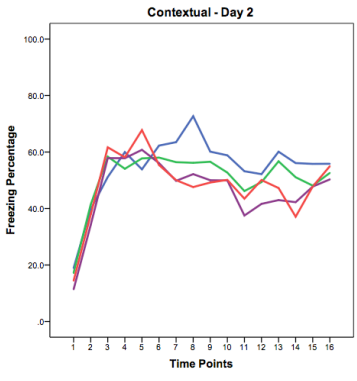

Figure 4: Contextual Extinction Day 2

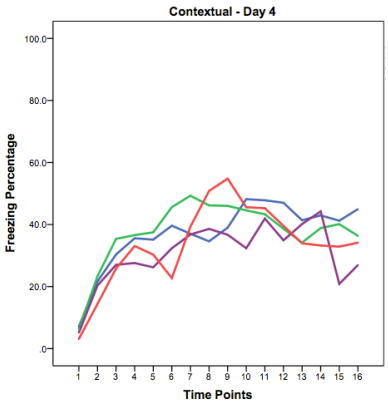

Figure 3: Contextual Extinction Day 4

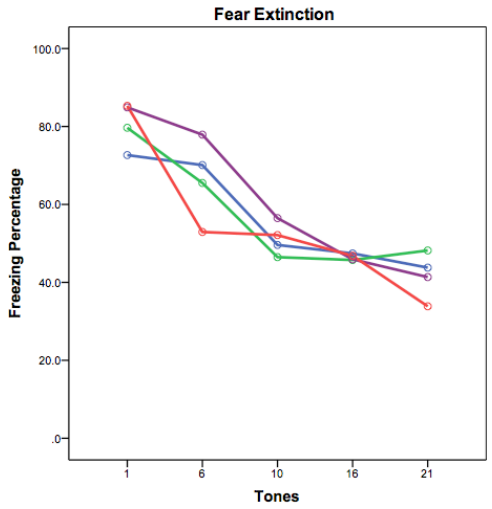

Figure 5: Fear Extinction

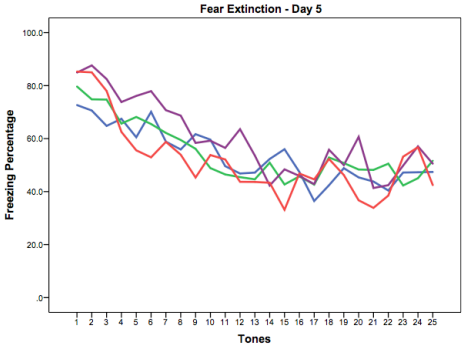

Figure 7: Fear Extinction Day 3

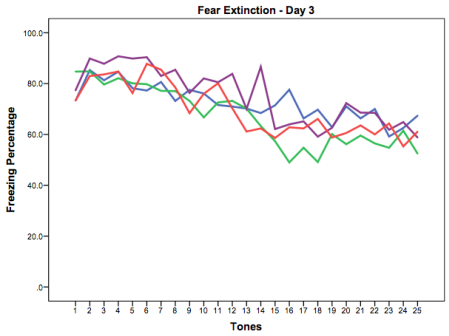

Figure 6: Fear Extinction Day 5

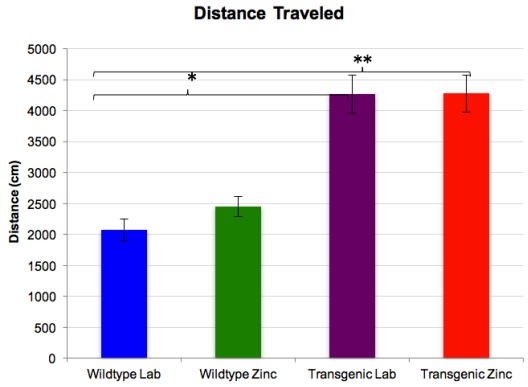

Figure 8: Total distance traveled in the open field

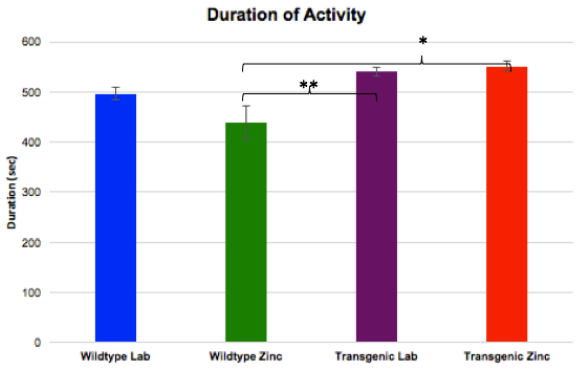

Figure 9: Duration of activity during the open field test

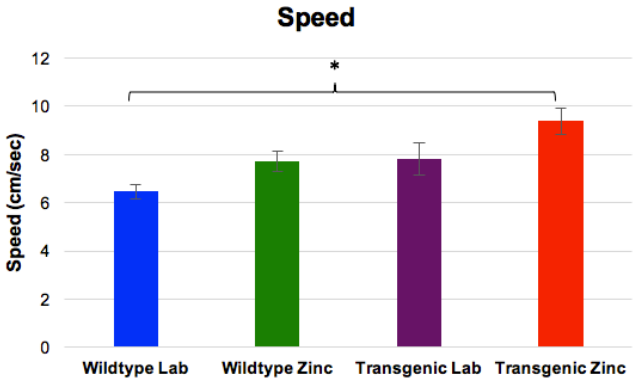

Figure 10: Speed of mice during movement

DISCUSSION

The tau mice were expected to have difficulty with fear extinction since their learning and memory were impaired due to neurofibrillary tangles (NFTs), however they had an extinction rate similar to the wildtype mice. As seen with Alzheimer's patients, they tend to forget the potential dangers of leaving the stove on or a cigarette burning. The Alzheimer's association has several safety recommendations for households with an Alzheimer's patient. They recommend removing electrical appliances from the bathroom to reduce the risk of electrical shock, secure power tools and guns, keep an eye out for cigarettes and such. These recommendations are made because patients tend to forget the potential dangers these items may cause. The fear extinction paradigm, with mice that develop NFTs, should have served to relate memories of associating certain cues to potential dangers. An animal's ability to associate unpleasant events with a stimulus is an essential behavior for survival. Therefore, it is a possibility that the specific brain regions, such as the amygdala, responsible for this function may be less susceptible to the neurofibrillary tangles present at 6 months of age. The similar rate of extinction in both transgenic and wildtype animals showed that there were no apparent impairments with learning and memory in this specific test. However, these same mice were tested on other behavioral tests including: nest building behavior, novel object recognition and Morris water maze. Results showed significant differences between groups. Therefore, NFTs

may have negative impact on certain behaviors. Such as what was observed in the open field test. The transgenic mice were hyperactive compared to the wildtype mice, as they were more active, faster, and traveled a greater distance within the maze. The results from the open field test support the notion that the P301L mouse tends to be hyperactive.

Mice on zinc water were also expected to perform poorly with the fear extinction test, as seen with other studies within our lab, but performed equally as the mice on lab water. The zinc levels in the water may not impact the animal's learning and memory in fear extinction to exhibit a significant difference between the animals on lab water. Furthermore, zinc water did not significantly influence activity and distance traveled in the open field test. Which may suggest that the neurofibrillary tangles had more of an effect on the behaviors studied in the open field test.

Compared to this study, other studies aged their mice for more months. It is possible that older P301L mice may exhibit greater learning and memory impairments in fear extinction. Which would allow for the NFTs to grow and spread to other brain regions. Previous studies with zinc supplementation in our lab used rats instead of mice, it is possible that zinc effects mice and rats differently. Or at least that zinc does not affect mice as much as it does it rats for fear extinction.

REFERENCES

Bhatnagar, S., Taneja, S. (2001). Zinc and cognitive development. *Journal of Nutrition Cambridge Journals*. Suppl 2:S139-45

Braak, H., Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* 82(4):239-59.

Curzon, P., Rustay, N.R., Browman, K.E. (2009) *Cued and Contextual Fear Conditioning for Rodent.* Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton (FL), Press/Taylor & Francis.

Flinn, J.M., Hunter, D., Linkous, D.H., Lanzirotti, A., Smith, L.N., Brightwell, J., & Jones, B.F. (2005). Enhanced zinc consumption causes memory deficits and increased brain levels of zinc. *Physiology & Behavior, 83*(5), 793 – 803.

Hunsberger, H. C., Rudy, C. C., Weitzner, D. S., Zhang, C., Tosto, D. E., Knowlan, K., Reed, M.N. (2014). Effect Size of Memory Deficits in Mice with Adult-Onset P301L Tau Expression. *Behavioural Brain Research*, *272*, 181–195.

Harris, J.A., Koyama, A., Maeda, S., Ho, K., Devidze, N. (2012) Human P301L-Mutant Tau Expression in Mouse Entorhinal-Hippocampal Network Causes Tau Aggregation and Presynaptic Pathology but No Cognitive Deficits. *PLoS ONE 7(9)*: e45881.

Iqbal, K., Liu, F., Gong, C.-X., & Grundke-Iqbal, I. (2010). Tau in Alzheimer Disease and Related Tauopathies. *Current Alzheimer Research*, *7*(8), 656–664.

Lewis, J., McGowan, E., Rockwood, J., Melrose, H., Nacharaju, P., Van Slegtenhorst, M., Gwinn Hardy, K., Paul Murphy, M., Baker, M., Yu, X., Duff, K., Hardy, J., Corral, A., Lin, W.L., Yen, S.H., Dickson, D.W., Davies, P., Hutton, M. (2001) Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat Genet*. 4:402-5.

Puzzo, D., Lee, L., Palmeri, A., Calabrese, G., Arancio, O. (2014). Behavioral assays with mouse Models of Alzheimer's disease: practical considerations and guidelines. *Biochem Pharmacol, 88 (4),* 240 - 467.

Quirk, G. J. (2002). Memory for Extinction of Conditioned Fear Is Long-lasting and Persists Following Spontaneous Recovery. *Learning & Memory*, *9*(6), 402–407.

Railey, A.M., Micheli, T.L., Wanschura, P.B., & Flinn, J.M. (2010). Alterations in fear response and spatial memory in pre- and post-natal zinc supplemented rats: Remediation by copper. *Physiology & Behavior, 100*(2), 95-100.

Railey, A.M., Groeber, C.M., & Flinn, J.M. (2011). The effect of metals on spatial memory in a transgenic mouse model of Alzheimer's disease. *Journal of Alzheimer's Disease*, *24*(2), 375-381.

Ramsden, M., Kotilinek, L., Forster, C., Paulson, J., McGowan, E., SantaCruz, K., Guimaraes, A., Yue, M., Lewis, J., Carlson, G. (2005). Age-dependent neurofibrillary tangle formation, neuron loss, and memory impairment in a mouse model of human tauopathy (P301L). *J.Neurosci.* 2005; 25: 10637–10647

Shoji, H., Takao, K., Hattori, S., Miyakawa, T. (2014). Contextual and Cued Fear Conditioning Test Using a Video Analyzing System in Mice. *Vis. Exp.* (85), e50871.

Stover, K.R., Mackenzie, A.C., Van Winssen, C.M., Brown, R.E. (2015). Early detection of cognitive deficits in the 3xTg-AD mouse model of Alzheimer's disease. *Behavioral Brain Research, 289*, 29-8

Wiltgen, B.J., M. Zhou, Y. Cai, J. Balaji, M.G. Karlsson, S.N. Parivash, W. Li, and A.J. Silva. (2010). The Hippocampus Plays a Selective Role in the Retrieval of Detailed Contextual Memories. *Curr Biol, 20(15),* 1336-1344.

Yang, Y., Jing, X.P., Zhang, S.P., Gu, R.X., Tang, F.X., Wang, X.L., Xiong, Y., Qiu, M., Sun, X.Y.,

Ke, D., Wang J.Z., Liu R. (2013). High dose zinc supplementation induces hippocampal zinc deficiency and memory impairment with inhibition of BDNF signaling. *PLoS One.* 8(1):e55384.

BIOGRAPHY

Carlos M. Hernandez graduated from Lake Braddock Secondary School, Burke, Virginia, in 2007. He received his Bachelor of Arts from George Mason University in 2012. He was employed by George Mason University as Animal Care Technician and received his Master of Arts in Psychology from George Mason University in 2017.