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Epigenetic effects of learning and memory in the I-Ppo-I mouse

Balta, Ana-Maria
BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

EPIGENETIC EFFECTS OF LEARNING AND MEMORY IN THE I-PPO-I MOUSE

by

ANA-MARIA BALTA
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I would like to dedicate this work to my mother, Ofelia Balta, my father, Martin Balta, and my grandfather, Leon Farauanu. I owe my success to your love, support and dedication.
EPIGENETIC EFFECTS OF LEARNING AND MEMORY IN THE I-PPO-I MOUSE
ANA-MARIA BALTA
ABSTRACT

The epigenetics of the aging brain is a growing field of study that holds great promise for the discovery of mechanisms and potential treatments for neurodegenerative diseases. In this current study, a novel, accelerated aging murine model, the I-Ppol/Cre, or ICE (Inducible Changes in the Epigenome) mouse, is studied to test its potential for demonstrating the theory of the rearrangement of chromatin (RCM) as the main cause of aging, and in particular, the mechanism through which the brain ages. Immunohistochemistry and behavioral assays are utilized to determine whether there are morphological changes, inflammatory responses, and changes in learning and memory. Results showed a significant increase in microglia and astrocytes, markers of inflammation, in I-Ppol/Cre mice compared to their Cre controls. Long term memory performance was also significantly decreased in the I-Ppol/Cre mice, demonstrated through contextual fear conditioning (CFC) testing, and Morris Water Maze (MWM) testing. Results from this study are in support of the I-Ppol/Cre mouse as a model of accelerated aging of the brain, with deficits in learning and memory. Further studies are needed to further characterize this murine model of accelerated aging.
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LIST OF ABBREVIATIONS

CFF ................................................................. Contextual Fear Conditioning
ICE ............................................................... Inducible Changes in Epigenome
IHC .................................................................. Immunohistochemistry
DBS .................................................................. Double strand break
INTRODUCTION

Epigenetics is the inheritance of changes in the gene function without any changes in the DNA nucleotide sequence.¹ With many studies demonstrating the role of epigenetics on gene expression patterns, as a result of aging², epigenetics has become an emerging field that holds promise of discovery and innovation in the field of aging genetics. Original evidence came from studies of the budding yeast *Saccharomyces cerevisiae*, in which the sirtuin histone deacetylase Sir2 was found to stabilize repetitive loci and extend lifespan³. Further studies have found that in cases of DNA damage or in aged cells, the “Sir2 complex will relocalize to sites of genomic instability and break sites, and will cause the desilencing of genes that cause sterility, a characteristic of yeast aging.”⁴ Studies by Oberdoerffer et al. (2008) have shown that the mammalian ortholog of Sir2, SIRT1, “dissociates from diverse loci in response to DNA damage, and relocalizes to DNA breaks to promote repair, resulting in transcriptional changes that parallel those in the aging mouse brain.”⁵ The authors reported that the target of SIRT1, H1K26, becomes deacetylated upon binding of SIRT1, resulting in the silencing of gene transcription.⁵

This discovery led to the hypothesis that chromatin factors, in response to DNA breaks, redistribute on the genomic DNA and leads to aging.⁶ While it has traditionally been believed that aging is mostly due to an accumulation of DNA mutations, this hypothesis highlights the rearrangement of chromatin, and not
genomic mutations, as a main contributor to aging. This theory was named the “relocalization of chromatin modifiers” or “RCM” hypothesis.

In order to test the hypothesis that the rearrangement of chromatin is the main cause of aging, the ICE mouse (Inducible Changes in Epigenome) mouse, which carries a tamoxifen-inducible homing endonuclease, was developed. For this murine model, the enzyme I-PpoI from the slime mold Physarum polycephalum was introduced into a C57BL/6J adult tamoxifen-inducible Cre mouse for the first time. This slime mold was chosen by the Sinclair lab due to previous studies that had used the I-PpoI enzyme to study DSB repair in human cells. In the slime mold, I-PpoI acts as a homing endonuclease. The novel transgenic mouse allows for the induction of site-specific DNA breaks into the nuclear genome of a mouse in any tissue or cell type with tamoxifen treatment, and subsequent stop of DSB induction once tamoxifen treatment is stopped. I-PpoI catalyzes a double strand break within a 15-bp recognition site. Cre fuses with ERT2 and translocates into the nucleus in the presence of tamoxifen. Cre cuts out the STOP cassette located upstream of the I-PpoI gene to induce Ppo1 expression. I-PpoI then induces double strand breaks (DSBs). The I-PpoI cuts 19 sites, nearly all of which are non-coding or intronic, and therefore induces low/moderate damage. Without the presence of tamoxifen, stability and Ppo1 is inactive; CreER is inactive as it is confined to the cytoplasm, via binding to heat shock protein-90. Ppol travels into the nucleus and induces double strand
breaks only in the presence of tamoxifen. This is the first mouse created that allows cell-type and sequence-specific DNA damage in a mammal.

For the current study, when mice reached 4-6 months, they were fed tamoxifen chow for three weeks. This was the optimal protocol determined by the Sinclair lab. This age was chosen in order to avoid developmental problems. Treatment with tamoxifen turns on DBS induction system, and upon the completion of the treatment, the DBS induction is turned off. After treatment, the development of their phenotype is monitored. It was observed that after one month, there were subtle changes in the appearance of the mice, such as greying hair and hair loss. After four months, the phenotype progressed to greater hair loss around the eyes and the ears, which is typical of aged mice. By ten months, the phenotype progressed even further, with major differences in hair loss and hair color between tamoxifen treated, and tamoxifen untreated mice.

Using SA-beta-gal staining, epidermal changes due to senescence were seen. The ICE mice showed more focal SA-beta-gal staining, which could either correspond to melanocyte stem cells or the sebaceous glands.

Further testing was conducted in order to characterize the progression of the aging phenotype in the ICE mice. Metabolic testing showed lower respiratory exchange ratio (RER) in old mice compared to young mice, which indicated a preference for fat as the predominant food source. Younger mice have higher RERs, and a metabolism that prefers carbohydrates as primary fuel sources.
This change in RER also corresponded to significantly lower body weight, despite equal levels of food consumption. A significant difference in RER was also found between Cre and I-Ppol/Cre mice; Cre mice had significantly higher RER compared to I-Ppol/Cre mice. This indicates changes in metabolism similar to changes seen in wild type aged mice.

There were also many changes in the musculoskeletal system. Bone CT scans were taken of the femur, and significantly less cortical and trabecular bone were seen. Decreases in muscle mass were seen, as well as decreased muscle endurance in the ICE mice, observed with treadmill tests. There were also decreases in grip strength performance tests, further indicating lower muscle endurance in the ICE mice. Ambulation during light and dark cycles was also evidenced by behavior studies.

Having preliminary evidence of physiological changes in the ICE mice that resemble those found in typically aged mice, further investigations were made to observe any changes within the brain. Many studies have also shown a correlation between age and increases in memory problems, with effects of age particularly upon the functioning of the hippocampus. Inflammation is associated with a broad spectrum of neurodegenerative diseases, and is prevalent in aging. In order to investigate inflammation in the brains of the I- I-Ppol/Cre mice, immunohistochemistry was completed in the hippocampus using IBA1 and GFAP, for microglia and astrocytes markers, respectively. These stains were chosen given studies that have established microglia and astrocytes are
hallmarks of inflammation\textsuperscript{9,10} and age-related changes in the immune system of the brain.

Several behavioral assays were also completed to characterize changes in behavior, ambulation, learning and memory in these mice. In order to look at spatial navigation and reference memory, memories that are hippocampally-dependent,\textsuperscript{11}, a standard Morris Water Maze protocol was used. Contextual fear conditioning (CFC) was also performed to evaluate long-term memory. Open field tests were completed to note ambulation differences, and any potential anxiety-related behaviors.

Further investigation of the brain of the mouse can also highlight epigenetic effects on neurodegeneration. Cognitive deficits such as dementia have become major health problems, and yet the exact causes and a ways to prevent or reverse progression have not been discovered. A compilation of testing aimed to characterize the I-Ppol/Cre mouse, and its potential as a murine of aging was investigated in this thesis.
METHODS (research-based)/PUBLISHED STUDIES (lit-based)

Animals

Male C57BL/6J mice were purchased from the National Insitute on Aging Aged Rodent Colony. 10 young mice (6 months) and 10 old mice (10 months) were used as controls. Male Cre mice and I-Ppol mice were bred by the Sinclair lab. 8 Cre mice were used and 8 I-Ppol mice were used for experiments.

Mice were provided a standard purified mouse diet and water (ad libitum) with 24 hours access. Cages were cleaned on a weekly basis. All IACUC protocols were approved.

Immunohistological analysis was utilized in order to detect inflammatory response in the brain, and particularly in the hippocampus.

Brains of Ippo-1 mice at 15 month, their Cre controls at 15 months were extracted. Young mice (6 months) and old mice (24 months) were also utilized. These brains were fixed with 4% PFA for 24 hours, then stored in PBS until they were processed for paraffin embedding, according to standard procedures. Several slices were also requested to be stained with H&E for morphology analysis.

IHC Protocol

Deparaffinization: Slices were shaken vigorously in Xylene twice in xylene for 3 minutes each time. Next, the slices are placed in 50/50 xylene and
100% ethanol for 3 minutes. Following this step, the slices were transferred to 100% ethanol twice, for three minutes each time. Next, they were placed in 95% ethanol for three minutes, then 70% ethanol for three minutes, 50% ethanol for three minutes, and then rinsed with deionized water for one minute.

**Antigen retrieval:** Slices were placed in 1X working concentration of citrate buffer (Vector labs) inside of microwave vessels. These were placed in the microwave at a power of two. Once the solution began to boil, the slides remained for 20 minutes. A vigorous boil, and the drying of the slices was avoided. Once 20 minutes elapsed, the vessel was removed and allowed to cool for 30 minutes. Upon cooling, the slides were rinsed in deionized water three times, for 5 minutes each.

Next, the slides were placed in 3% hydrogen peroxide for 10 minutes. Afterwards, the slides were washed in dH2 two times for 5 minutes each. The slides were allowed to dry, and then lined with an ImmEdge™ Pen (Vector, H-4000).

**Immunohistochemical staining:** The slices were blocked with 3% BSA in PBST (0.025% Triton-X), pH 7.4, for 2 hours at room temperature. Primary antibody was applied, diluted with PBS in 1% BSA. NeuN was diluted 1:200, IBA1 was diluted 1:500 and GFAP was diluted 1:500. The antibody was incubated overnight at 4°C.
After incubation, the slides were rinsed twice, for five minutes each, in PBS (pH 7.4) with 0.025% Triton. Then, an enzyme-conjugated secondary antibody (dilution: 50 ul Ab in 10ml PBS with 1% BSA) was added to the slides. The slides were then incubated for one hour at room temperature.

After incubation, the slides were rinsed in PBS three times, for five minutes each. A 1:100 diluted solution of solution A and B was made from the VECTASTAIN® ABC Reagent (A+B) kit, and allowed to incubate for 25 minutes. Afterwards, the solution was applied to the slides, and incubated for 30 minutes. Afterwards, the slides were washed for 5 minutes, two times, in PBS. Lastly, sections were incubated with peroxidase substrate (DAB), 0.015% H2O2, until an adequate stain intensity developed, and subsequently rinsed. Section were dried, then coverslipped and sealed.

**Statistical Analysis**

Quantification of the slices was completed using MEtaMorph image analysis. The number of target for each antibody was determined by manually counting and dividing by the area occupied by targets. The investigator was blinded to the genotype. After quantification was completed, a T-test was run to determined differences between young and old mice IHC results in order to confirm that previous results were replicated. Then, a T-test was run between the I-PpoI mice and their Cre controls.
Behavioral experiments

Next, a variety of behavioral assays were run in order to determined differences in learning and memory of the mice. A Morris water maze, fear conditioning and open field test test were run for Ippo1 mice and their Cre controls, as well as young and old wildtype mice.

Fear Conditioning

The fear conditioning paradigm was carried out over the course of two days. Day one tested the baseline level of freezing. Mice were placed inside a standard CFC cage, and their standard freezing behavior was measured every ten seconds, for a total of three minutes.

Next, the freezing behavior was tested with a tone. After 180 seconds, a pulse of 0.5 mA was administered. After another 30 seconds, another 0.5 mA pulse was given. After the second pulse, the level of freezing was measured every 10 seconds, for a total of one minute.

On day two, the mice were placed back in the age for a total of three minutes, with freezing behavior noted every ten seconds. No shocks were administrated. This served to measure the memory of the shock, and the association of the shock with the particular context.

Analysis initially took place with initial freezing behavior of the mice. If certain mice did not react to shocks on day 1, determined by observation as well as outlier statistical testing, those mice were not used for day 2 testing. Day 2
freezing was compared between Cre and I-PpoI mice, and also between young and old mice, using a T-test.

Open Field

Mice were placed in standard open field equipment from Cleversys, with dimensions of 50 x 50 cm for a total of 15 minutes. Each mouse underwent one trial. A tracking system through a Clever Cys software was used to record the time, duration, velocity, and distance of the mouse while in the designated outer corners and walls of the cage. The cage was cleaned with 70% ethanol to minimize olfactory cues. Analysis looked at the percentage of the time spent in the center of the open field apparatus, versus the edges. A T-test was run between Cre and I-PpoI mice, and young and old mice.

Morris Water Maze

A 122 cm black plastic tank was used for the water maze. A metal platform 12 cm in diameter, painted white, was also used, with a flag attached for a visual trial. Several large, colorful visual cues were placed on each of the four sides surrounding the pool. The water was filled to cover 1-2 cm of the platform, and was made opaque using white tempura non-toxic powder paint. The CleverSys tracking software was utilized, with spaces designated for four quadrants, the area around the platform, and the platform.
The first day consisted of a spatial acquisition, in which a flag attached to the platform is made visible. The mice were placed in the desired start position in the maze, facing the tank wall. The animal was released at water level, and the computer tracking program began once the animal was released. The software timer stopped once the animal reached the platform. If the animal did not find the platform within a time limit of one minute, they were removed from the water, and placed on the platform for 15 seconds. Then, the next mouse was placed into the pool, with the same starting position. Four trials were performed for the spatial acquisition day, with a different starting quadrant each time. This longer inter-trial interval was chosen in order to enhance learning performance.

For days 2-5, the platform remained submerged under 1-2 cm of opaque water, but the visual cue of the flag was removed. Again, the mice would be placed in a different quadrant as its starting position, for each of the four trials. The mice would be given one minute to find the platform. The tracking system would measure the distance, velocity, latency to the platform, and the time spent in each of the quadrants and areas of the platform and around the platform. If the mouse did not reach the platform within the time limit of a minute, the mice would be taken out of the water and placed on the platform for a minute.

Day 6 consisted of a reference memory probe trial. The platform would be removed from the water, and the animals would be placed in a novel start position in the maze, facing the wall (180 degrees from the original platform position). This was used in order to ensure that the spatial preference is a
reflection of the memory of the goal location rather than for a specific swim path. The tracking software would measure the latency to the platform area and quadrants, the velocity, duration, and swimming distance. The target data was the latency to the platform area and the time spent in the target quadrant and platform area.

Statistical analysis of the Morris Water Maze results were conducted using Excel; and SPSS. The percentage duration in the target quadrant was calculated for each experimental group, for each of the training days and plotted of a group. A repeated measures ANOVA was conducted to find differences in duration between training days, and also to measure differences in training amongst the days, between the different groups. Averages of the latency to the target, and the number of bouts to the target were also made. These were also plotted for the experimental groups, and repeated measures ANOVA were made. Probe day analysis was made by averaging the number of bouts into the target quadrant and platform area. The duration percentage of each group in the target quadrant was also averaged. A T test was run between Cre and Ppo1 groups, as well as between young and old mice.
RESULTS

H&E Hippocampus: No significant morphology differences

Figure 1. An H&E stain was performed on sagittal sections of tissue, particularly sections that showed the hippocampus. Both Cre and I-Ppol/Cre were stained, along with samples from young (6 mo) and old (24 mo) mice. This was completed in order to assess gross morphological differences.
Increased Microglia (Iba1) in the ICE Mouse Hippocampus

Figure 2. IBA1 staining for microglia was performed on sagittal sections of tissue in the hippocampal area. Both Cre and I-Ppol/Cre were stained, along with samples from young (6 mo) and old (24 mo) mice. This was completed in order to assess microglia, markers of inflammation.
Increased Hippocampal Microglia In the ICE Mouse

Figure 3. Quantifications of IHC staining was completed using MetaMorph software. A T-test showed a significant difference in IBA1, microglia staining between young and old mice, as well as Cre and I-Ppol/Cre mice (p < 0.05).
Increased Astrocytes (GFAP) in the ICE Mouse Hippocampus

Figure 4. GFAP staining for astrocytes was performed on sagittal sections of tissue in the hippocampal area. Both Cre and I-Ppol/Cre were stained, along with samples from young (6 mo) and old (24 mo) mice. This was completed in order to assess astrocytes, markers of inflammation.
Figure 5. Quantification of IHC staining was completed using MetaMorph software. A T-test showed a significant difference in GFAP, astrocyte staining between young and old mice, as well as Cre and I-Ppol/Cre mice ($p < 0.05$).
Figure 6. Percentage of initial freezing was averaged across young and old mice on day 1 of CFC in order to measure shock sensitivity and response. A T-test revealed no significant differences between young and old mice.
Figure 7. Percentages of freezing on day two were averaged for each group on day two of CFC testing. A T-test revealed a significant difference between the amounts of freezing between young and old mice.
Figure 8. Percentage of initial freezing was averaged for Cre and I-Ppol/Cre groups for day 1 of CFC in order to measure shock sensitivity and response. A T-test revealed no significant differences between the two groups.
Figure 9. Percentages of freezing on day two were averaged for each group on day two of CFC testing. A T-test revealed a significant difference between the amounts of freezing between young and old mice.
Figure 10. Latency to the visible platform (in seconds) was averaged for each experimental group (Cre and I-Ppol/Cre). A T-test revealed no significant differences in latency between Cre and I-Ppol/Cre mice.
Figure 11. Average % duration in the target quadrant was averaged for the Cre group and the I-Ppol/Cre group for each of the four training days. A repeated measures ANOVA was used, and results showed no significant differences between the two groups for percentage duration in each quadrant across the training.
Figure 12. Latency (in seconds) was averaged for each group (Cre and I-PpoI/Cre) for each of the training days. A repeated measures ANOVA was used, and results showed a $p = 0.05$, suggesting a trending difference between the latency to the platform throughout the training days, amongst the two groups.
Figure 13. Post-training, probe day data was gathered for duration %. The duration % in the target quadrant was averaged for Cre mice, as well as for I-PpoI/Cre mice. A T-test indicated no significant differences between the percentage duration in the target quadrant between the two groups.
Figure 14. Post-training, probe day data was gathered for bouts into the target quadrant for the Cre mouse group, as well as for the I-Ppol/Cre group. A T-test revealed a significant difference in the bouts between the two experimental groups.
Figure 15. Post-training, probe day data was gathered for duration %. The duration % in the target quadrant was averaged for young mice, as well as for old mice. A T-test indicated no significant differences between the percentage duration in the target quadrant between the two groups.
Figure 16. Post-training, probe day data was gathered for bouts into the target quadrant for the young mouse group, as well as for the old group. A T-test revealed a significant difference in the bouts between the two experimental groups.
Figure 17. Open field data was gathered by tracking software that recording the time spent in each of the designated areas of the open field apparatus: wall, center, and around center. Percentages of the time spent in each of these areas were averaged for the mice in the particular experimental group.
Figure 18. Open field data was gathered by tracking software that recording the time spent in each of the designated areas of the open field apparatus: wall, center, and around center. Percentages of the time spent in each of these areas were averaged for the mice in the particular experimental group.
Figure 19. Open field data was gathered by tracking software that recording the time spent in each of the designated areas of the open field apparatus: wall, center, and around center. Percentages of the time spent in each of these areas were averaged for the mice in the particular experimental group.
Figure 20. Open field data was gathered by tracking software that recording the time spent in each of the designated areas of the open field apparatus: wall, center, and around center. Percentages of the time spent in each of these areas were averaged for the mice in the particular experimental group.
Figure 21. Tracking software recorded the distance traveled in the open field apparatus. Distance traveled (in mm) was averaged for young mice, and for old mice. A T-test revealed a significant difference in traveling distance between the two groups.
Figure 22. Tracking software recorded the distance traveled in the open field apparatus. Distance traveled (in mm) was averaged for Cre mice, and for I-PpoI/Cre mice. AT-test revealed no significant difference in traveling distance between the two groups.
Figure 23. Tracking software recorded the velocity (in mm/s) of the mice in the open field apparatus. Velocity was averaged for young mice, and for old mice. A T-test revealed a significant difference in traveling distance between the two groups.
Figure 24. Tracking software recorded the velocity (in mm/s) of the mice in the open field apparatus. Velocity was averaged for Cre mice, and for I-Ppol/Cre mice. A T-test revealed no significant difference in traveling distance between the two groups.

Immunohistochemistry work was completed on young and old mice in order to replicate the findings from previous studies, and to make note of similarities between experimental findings in old mice and those found with the I-Ppol/Cre mice. H&E staining showed no gross morphology differences in the brains; no gross differences were noted in the hippocampus, a particular area of interest due to the focus of the study on learning and memory.

IHC staining in slices of the hippocampus for microglia using IBA1, and astrocytes using GFAP, was quantified using MetaMorph software. T-test analysis between young and old mice showed significant differences in IBA1 and
GFAP, with older mice having significantly higher densities of microglia and astrocytes. T-test analysis was also done on results of Cre and I-Ppol/Cre IHC, showing significant differences between the groups for IBA1 and GFAP. I-Ppol/Cre had increased densities in both markers of inflammation.

Behavior studies also revealed differences between experimental groups. Contextual fear conditioning neither showed differences in initial freezing and shock response between young and old mice, nor between Cre and I-Ppol/Cre mice. Second day testing analysis with a t-test showed significant differences in levels of freezing between young and old mice. T-test of the second day CFC results between Cre and I-Ppol/Cre mice also showed significant differences. Old mice have lower levels of freezing on day two, and I-Ppol/Cre mice also show significantly less freezing on day two compared to their Cre controls.

Open field testing revealed differences in some factors of ambulation between young and old mice. T-test analysis of velocity for young and old mice revealed a significant difference, with old mice traveling at lower velocities. T-test analysis for distance traveled for young and old mice also showed significant differences, with old mice traveling less. T-test analysis of Cre and I-Ppol/Cre results for velocity showed no significant differences; there were also no differences in distance traveled for Cre and I-Ppol/Cre mice in the open field test.

Further analysis of the percentage of duration the three main areas of the open field (wall, center, and around center) revealed no significant differences between Cre and I-Ppol/Cre, or between young and old.
Morris water maze initial analysis was performed on visual platform results, to rule out any visual and physiological that would prevent the mice from learning the task. Upon using a T-test to compare the latencies to the platform between Cre and I-Ppol/Cre mice, no significant differences were found. Learning acquisition data for the four training days was averaged for each day, and plotted. A repeated measures ANOVA was conducted for the percentage of time spent in the target quadrant, one of the markers of learning. No significant differences were found between Cre and I-Ppol/Cre mice. A repeated measures ANOVA was also performed for the latency data for the Cre and I-Ppol/Cre groups. A p value of 0.05 revealed a trending, but not significantly different latency over time for the two groups.

Final probe day analysis was completed, focusing on the percentage duration in the target quadrant and the bouts into the target quadrant. A T-test on the percentage duration between Cre and I-Ppol/Cre mice revealed no significant differences, but a T-test on the number of bouts into the target quadrant for Cre and I-Ppol/Cre mice showed significant differences. It was also observed that there was a significant difference in the number of bouts into the target quadrant made by young and old mice, but not significant differences in the percentage of duration in the target quadrant.
DISCUSSION

Results from these experiments give further evidence to the I-Ppol/Cre mouse as a good model of accelerated aging and support for the chromatin rearrangement hypothesis of aging. IHC results showed no gross morphology differences in the brains of the Cre and I-Ppol/Cre mice, and no differences particularly in the hippocampal regions. There were significant increases in the density of microglia and astrocytes in the brains of I-Ppol/Cre mice, indicating increases in inflammation. This is consistent with findings of increases of inflammatory markers in the brains of old mice. Future studies should look at synaptic changes using a synaptotagmin staining. Further experiments should also include a Gamma-H2AX staining both in the hippocampal sections, and several other sections of the brain. This would look at areas that have experienced DNA damage, and that are therefore most affected by the I-Ppol.

Behavioral results also revealed several similarities in performance between I-Ppol/Cre mice, and the performance typically found in aged mice. Contextual fear conditioning experiments indicated significant differences in memory for the I-Ppol/Cre mice, which is consistent with findings of contextual memory deficits in aged mice. On day two, I-Ppol/Cre mice froze significantly less than Cre mice, indicating a lack of memory for the association between the context of the testing box, and the administration of a small shock. It is important to note that the possibility of lack of sensation for the shock was eliminated as a possible confounding variable-day one results showed no significant differences
in the freezing response to shock administration between the Cre and the I-Ppol/Cre mice.

Furthermore, the Morris Water Maze also suggested memory deficits. Probe day findings for bouts into the target area revealed significantly less bouts by I-Ppol/Cre mice compared to Cre mice. This was similar to findings in young and old wildtype mice: old mice had significantly lower bouts into the target area compared to young mice. These differences suggest a deficit in memory for the I-Ppol/Cre mice, just as older mice have memory deficits. Analysis of the percentage of duration in the target quadrant, however, did not reveal significant differences. Furthermore, aged mice tend to have a large variation in performance; I-Ppol/Cre mice also had large variation in performances between the mice, and this resulted in error bars that overlapped. Since some factors, such as the bouts, are indicative of deficits in memory, but other factors, such as duration in target quadrant, are not significantly different, further studies should be done. Future studies should include reversal training, and also include a probe day that is performed after more than 24 hours have passed, such as a week or two after. Adding these tests would make the experiments more precise, and add a higher level of difficulty that may help distinguish the memory problems the I-Ppol/Cre mice may have.

It is also important to note that visual deficits or physiological deficits did not affect results. The initial visual platform test performed by the experimental groups was meant to rule out any of these deficits that would interfere with
learning and affect latencies to the platform. There were no significant
differences in latency to the platform between Cre and I-Ppol/Cre mice. A future
direction could be to include a Barnes Maze test of memory. This would reduce
the shock of swimming in water, and would put less physical strain on the mice.
Open field tests showed no differences in velocity or in distance traveled. This
indicates that although other studies have shown decreases in muscle mass and
bone density, significant effects to basic ambulation have not been carried over.
This would also eliminate confounding factors of ambulation in a Barnes Maze
test.

Another interesting observation was of the learning acquisition results.
Duration percentage in the target quadrant did not show a significant difference
between the groups throughout the training days, but latency showed a strong
trend. Cre mice gradually decreased in latency time, but I-Ppol/Cre mice
performed worse with time, until the last day of testing. The I-Ppol/Cre show this
trend of decreasing performance and inhibited learning, but further testing should
be done. This trend could also be a result of fatigue. Future studies should
measure acquisition learning, in a task such as the Barnes Maze, which still
incorporates elements of spatial learning and navigation.

Lastly, open field analysis of percentage duration spent in certain areas of
the open field apparatus showed normal behaviors for both Cre and I-Ppol/Cre
mice, as previous studies have published. The results for the young and old
wildtype open field testing are also consistent with published studies that indicate
a preference for the wall and corner areas of open fields, and an avoidance of the center. Similar to wildtype mice, both Cre and I-Ppol/Cre mice spent the majority of their time in the wall area. This is also indicative that there are no other neurological dysfunctions of the mice, and that they do not exhibit any anxiety abnormalities.

Overall, many significant results indicate that the I-Ppol/Cre (ICE) mouse is a strong model of accelerated aging. It displays similar increases in inflammation of the brain-particularly in the hippocampal region. Behavior studies show no ambulation effects, no anxiety or neurological effects, but possible learning acquisition deficits and long-term memory problems. Behavioral assays should be run in order to solidify support for memory problems in the I-Ppol/Cre mouse, and to discover further deficits and mechanisms to these deficits in the brain.
REFERENCES


VITA

Ana-Maria Balta
Birth year: 1992
2701 Homestead Road, Apt 704
Chapel Hill, NC 27516
anamariabalta@gmail.com
828-226-4603

Education
Boston University School of Medicine, Boston, MA
M.S. in Anatomy and Neurobiology - Vesalius Program. (Expected September, 2016)

University of North Carolina at Chapel Hill, Chapel Hill, NC
B.S. Psychology, May, 2014
Business Essentials Program, August, 2013

Durham Technical Community College, Durham, NC
Emergency Medical Technician-Basic Certification, May, 2011

Honors and Awards
- Carolina Scholars Scholarship 2010-2014
  Full-tuition merit scholarship granted for academic achievement
- Carolina Research Scholar 2010-2014
- Honors Program 2010-2014
- Dean’s List May 2012, 2013

Research Experience
- Graduate Neuroscience Researcher, Harvard Medical School
  Boston, MA
  January, 2015-May 2016
  Dr. David Sinclair, Ph.D
  Worked on an independent project specifically looking at the epigenetic effects of aging upon the brain of a novel, inducible, accelerated-aging mouse model, termed ICE mice (Inducible Changes in Epigenome).
  Utilized a variety of behavioral assays testing the learning and memory of the mice, and also completed and interpreted immunohistochemistry experiments to understand morphological and pathological changes in the brains. RNA sequencing and ChIP sequencing assays were completed and analyzed for epigenetic changes. This resulted in authorship on an abstract, contributed to a current grant application and manuscript, and will result in a 50 page masters thesis.
Neuroscience and Pharmacology Research Assistant, UNC School of Medicine  
Chapel Hill, NC  
August, 2013-May, 2014  
Dr. Thomas Kash, Ph.D  
Investigated the neuronal substrates involved with alcohol and drug abuse induced behaviors. Specifically looked at estrogen receptors in the bed nucleus of the stria terminalis and the paraventricular nucleus of the thalamus, and their connection to binge drinking.

Behavioral Neuroscience Research Assistant, UNC Department of Psychology  
Chapel Hill, NC  
August, 2012-May, 2013  
Dr. Rita Fuchs Lokensgard, Ph.D  
Studied the involvement of hippocampal cannabinoid receptors in the reconsolidation of cocaine-associated contextual memories, and the involvement of alpha-7 nicotinic acetylcholine receptors in cocaine-paired, context-induced impulsive decision-making. Resulted in two papers.

Presentations

1st Place, Oral Presentation, 2013 Georgia Undergraduate Research in Psychology Conference
Invited Presenter, Psychology, University of North Carolina at Chapel Hill Conference 2013
Invited Presenter, Psychology, University of Virginia Conference 2013
Invited Presenter, Psychology, University of California at Los Angeles Conference 2013
Invited Presenter, Psychology, Kennesaw State University Conference 2013

Recent Conferences Attended:

Healthy Aging Therapeutics Symposium 2014, Harvard Medical School, Boston MA
Experimental Biology 2015, Boston, MA
Northeastern Regional Glenn Symposium 2015, University of Connecticut Health Center, Hartford, CT
Harvard/Glenn Foundation Symposium on Aging 2015, Harvard Medical School, Boston MA
Future of Alzheimer’s Disease, AFAR Regional Scientific Panel and Awards Luncheon 2015, Boston MA
Professional Experience

- **Gross Anatomy Teaching Assistant (TA)**
  Boston University School of Medicine, Boston, MA
  August 2015-September 2015
  Teaching assistant for the Medical Gross Anatomy course. Responsibilities included preparing dissections for bi-weekly labs on an assigned cadaver, assisting with student dissections during lab, and preparing to show and explain structures and their functions during lab times and extra tutorial sessions. In addition to dissection time, 40 hours of teaching were completed.

- **Resident Advisor (RA)**
  UNC Housing and Residential Life, Chapel Hill, NC
  August, 2012-May, 2013
  The RA position is a full-time undergraduate job in which the RA serves as an administrator in enforcing the policies of the Department of Housing, a peer advisor, crisis manager, and friend to residents. Duties include organizing programs and providing resources that facilitate academic exploration, responding to emergency situations, being an active and visible presence in the community, and working as a liaison between residents and housing administration.

- **Emergency Medical Technician Assistant Instructor**
  Durham Technical Community College, Durham NC
  Assisted course instructors in presenting and lecturing to students about the various aspects of emergency medical care, including medical and trauma emergencies, anatomy and physiology, patient assessments, pharmacology, and techniques in splinting, drug administration, and patient transport.

Volunteer

- **Medical Assistant at Western North Carolina Internal Medicine**
  Sylva, NC June-July, 2010-15
  Dr. Thomas Wolf, M.D.
  Volunteered during the summer as a medical assistant/lab technician. I brought back patients, took vitals (blood pressure, heart rate, temperature, pulse oximetry), and assisted with phlebotomies, urine testing and EKGs.