THE EFFECT OF MEDROXYPROGESTERONE ACETATE ON LEARNING AND MEMORY IN OVARY-INTACT MICE

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THE EFFECT OF MEDROXYPROGESTERONE ACETATE ON LEARNING AND MEMORY IN OVARY INTACT MICE

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ABSTRACT

Medroxyprogesterone Acetate (MPA) is birth control that is used for contraception and hormone therapy (Seven et al., 2014). MPA is considered to be an ideal birth control option for adolescents because of its convenient administration and fast onset (Rickert et al., 2006). While there are studies that indicate detriments in spatial memory in ovariectomized or older rats following administration of MPA (Braden et al., 2011), there is limited research investigating the effects of MPA in young ovary-intact mice. The goal of this project was to assess if the effects of progestin previously described are apparent in an ovary-intact population. Behavioral assays measured anxiety-like behavior, spatial memory, and activities of daily living. Despite no significant findings in these measures, this study provides a stepping point for investigating this compound/birth control in a population of ovary-intact animals.

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INTRODUCTION

Medroxyprogesterone Acetate (MPA) is an injectable form of birth control that is used for contraception and hormone therapy (Seven et al., 2014). MPA contains synthetic progesterone, progestin. The research on progestin has not explored female mice at the beginning stages of sexual maturity; rather, focusing on models that have been ovariectomized and rats mimicking hormone therapy used during menopause. Research has been conducted on MPA in combination with endogenous progesterone or with equine estrogen (Frye, Walf, & Paris, 2010). MPA evaluated alone has only been done in ovariectomized rodents rather than ovary-intact rodents. MPA's effect on cognitive function, specifically in the hippocampus, has not been studied at the beginning stage of sexual maturity. Depo-Provera has been available for contraceptive-use and hormone therapy since 1992, yet the implications on the brain have hardly been studied. Little research has been done in assessing the detriments to memory in conjunction with progestin use, making more insight imperative.

Importance and Use of Birth Control

Birth control can be used for the prevention of acne, regulating or halting menstrual cycles, reducing pain, regulating blood flow, and for contraceptive purposes (Spencer, Bonnema & McNamara, 2009). In many countries, there has been an increased use of birth control (Sámano et al., 2019). There is a negative social stigma attached to young girls becoming pregnant and there is a financial cost associated with a pregnant child that can hinder the family unit's economic bearings (Klepinger, Lundberg & Plotnick, 1999).

Additionally, pregnant young girls are unlikely to succeed academically and face an increased high school dropout rate than their non-pregnant counterparts (Klepinger, Lundberg & Plotnick, 1999).

However, there is little research investigating the effect of contraceptives on health in young girls (Chandra-Mouli et al., 2017). Depo-Provera, a contraceptive containing MPA, has been examined as an ideal form of birth control for adolescent girls due to its convenience and fast onset (Rickert et al., 2006). While Depo-Provera seems to be an advantageous tool for adolescent girls, there is little research on how MPA affects the brain; specifically in regard to learning and memory.

Medroxyprogesterone Acetate

Depo-Provera is commonly prescribed for contraception or hormone therapy in women (Westhoff, 2003). Depo-Provera does not contain estrogen; rather, progestin is the only hormonal ingredient. Progestin is the exogenous, not naturally produced, form of progesterone which is a sex hormone essential for menstruation and pregnancy. Some women may have complications with estrogen infused with other contraceptives such as, a transdermal patch, intravaginal ring, or various oral contraceptives, making the singular hormonal ingredient of Depo-Provera, progestin, more accommodating (Spencer, Bonnema & McNamara, 2009). Some women who are breastfeeding fall under these circumstances. Depo-Provera is administered intramuscularly or subcutaneously every three months through the deltoid or gluteal muscle or under the skin at the upper arm (Spencer et al., 2009). This three-month administration regimen is appealing for many women in comparison to daily oral contraceptives (Spencer et al., 2009). Women report amenorrhea, or loss of menstrual

cycle, after the second dose. Additionally, after ten months of cessation, fertility may resume. While Depo-Provera does not protect against sexually transmitted diseases or infections, the chances of becoming pregnant while adhering to proper administration is 0.3% (Spencer et. al., 2009).

Side Effects

There are several potential negative side-effects/risks from taking Depo-Provera. A common risk associated with Depo-Provera is the loss of bone mineral density which some studies have shown lead to a reversal after cessation of use (American College of Obstetricians and Gynecologists [ACOG], 2014). However, health professionals suggest that women only use Depo-Provera for up to two years to reduce the risk of fractures. Weight gain is often associated with contraceptive use and depression can also be exacerbated with the use of hormonal contraceptives (ACOG, 2014). As previously mentioned, amenorrhea is common with Depo-Provera making detecting pregnancy difficult. The association between memory impairment and progestin has only begun to be investigated. Additionally, MPA has been shown to have implications on anxiety and impact quality of life (Wershler, 2004).

Involvement of the Brain

There are numerous regions of the brain associated with memory; however, the hippocampus is one of the most prominent, being an essential component to long-term and spatial memory (Shrager, Bayley, Bontempi, Hopkins & Squire, 2007). The progestin in MPA appears to negatively affect the hippocampus (Ciriza, Carrero, Frye & Garcia-Segura, 2006), a brain structure critical in learning and memory; most notably, spatial memory. Spatial memory in the hippocampus works by exciting neurons according to environmental

cues (Shrager et. al., 2007). The cells that initiate the action potential of the neurons in response to spatial memory comprise several areas of the brain, however, they are more concentrated in and around the hippocampus (Kentros, 2006). Research on Depo-Provera, a synthetic progestin, on ovary-intact young female mice has not been done. However, research has investigated this paradigm in ovariectomized rats and found impairment in cognitive tasks (Braden et. al., 2007; Frye, Koonce & Walf, 2013).

Hippocampal-dependent memory has been assessed with progestin in combination with other estrogen, oestradiol, and progesterone derivatives utilized in hormone treatments (Chan et. al., 2014; Chislom & Juraska, 2012; Lewis et. al., 2008). These combinations of hormones describe the synergistic effects of several hormone treatments. Indeed, progestin's effects on learning and memory have not been assessed in young female rodents and necessitates investigation.

Previous Research

The research methods of the available studies are limited as well, with many tests being repeated in each study. In rodent models, tests that evaluate spatial memory include: the Barnes maze, Morris water maze (MWM), and the radial arm maze. Current literature assessing spatial memory and progestin utilize the radial arm maze and the MWM (Bimonte et al., 2000; Braden et al., 2011). These mazes use visual cues which allow the rodents to locate a hidden platform (Bimonte, et al., 2000; Morris et. al., 1982) and assess an animal's ability to learn its location over a period of time. Additional research assessing progestin's effects on memory utilizing biological testing is limited and inconsistent as well. Other measures outside of memory have not been examined. Activities of daily living have not

been previously studied with the effects of MPA and is therefore, warranted.

Based on the available research, a few inferences can be drawn pertaining to the effects of progestin on the hippocampus. MPA has been found to work against estrogen's neuroprotective and memory functioning (Song et al., 2020). In particular, this antagonistic effect caused by MPA is concentrated in the hippocampus. (Nilsen & Brinton, 2002). These findings support Depo-Provera's role in cognitive decline in the hippocampus and hippocampal-dependent tasks.

Purpose/Hypothesis

The goal of this project was to assess if the effects of progestin previously described are apparent in an ovary-intact population. Behavioral assays used measured hippocampal-dependent memory (Morris Water Maze) (Morris, Garrud, Rawlins & O'Keefe, 1982) anxiety (Elevated Zero Maze and Open Field), and activities of daily living (Burrowing and Nesting). The main hypothesis was that female mice given MPA would do worse than those not receiving MPA in each behavioral test including the open-field test, elevated zero maze, MWM, and in activities of daily living. Additionally, it was hypothesized that those mice that began with MPA and were switched to vehicle injections would have worse learning and memory deficits than the control mice. In the present study, the goal was to administer MPA to ovary-intact mice starting roughly during adolescence through young adulthood and assess learning and memory.

METHODS

Subjects/Housing

Forty-eight adolescent female mice (C57BL/6J), aged 21 days, were purchased from the Jackson Laboratory (Jax, Bar Harbor, ME). Upon arrival at the psychology lab vivarium, the animals were group housed three to a cage in an Animal Care Systems Optirat© rodent caging system and provided *ad libitum* access to food and water. The lab was maintained on a 12-hour light/dark cycle. The mice were ear punched at 4 weeks old for identification purposes. Animals were routinely handled twice a week so that they became used to human touch.

This study was done utilizing two cohorts separated by two weeks. The use of two cohorts allowed researchers to avoid running 48 mice at a time during behavioral testing, eliminating the need to run behavioral tests for an extended time. There was a total of 48 mice being used in this study, however, two mice died prior to behavioral testing. In each cohort, there was 24 mice. Following the acclimation period, animals were randomly assigned to one of four treatment groups: control (n=11), MPA (n=12), MPA Cessation (n=12), and MPA Delayed (n=11). (*as described below*). The same schedule and testing were done with each cohort; with the exception of the Morris Water Maze for the second cohort. Due to the University's Coronavirus (Covid-19) response and a SMART video tracking software malfunction, MWM data was unable to be collected for the second cohort.

Drug Conditions

When mice were 6 weeks old, each treatment group began to receive weekly subcutaneous injections of their respective drug/vehicle for 6 weeks. The weights of each

mouse was collected to ensure that each mouse received the correct dosage. The back of the neck was pulled with two fingers creating a space for the injection to occur. The dosage utilized in this study follows previous research that has been shown to have anti-ovulatory effects (Braden et. al, 2011; Bhowmik and Mukherjea, 1988). The control group received 6 weeks of continuous vehicle injections (0.4 ml Sesame Oil + 0.02 ml DMSO/ kg). The MPA group received 6 weeks of continuous MPA injections (3.5mg MPA + 0.4ml Sesame Oil + 0.02 ml DMSO/ kg). The MPA Cessation group received 3 weeks of MPA injections, followed by 3 weeks of vehicle injections. The MPA Delayed group received 3 weeks of vehicle injections, followed by 3 weeks of MPA injections. Sesame oil, DMSO, and MPA were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Body Weight

The mice were weighed the day before injections, at the same time, in grams. This ensured that each mouse was given the correct dosage according to their weight. This value was converted to kg for purposes of injections. Additionally, body weight was collected over the length of the experiment (9 weeks) to determine if MPA had an impact on weight gain as birth control has been shown to impact weight (Lange, 2015).

Behavioral Measures

Open-Field Test

The open field test is a measure of innate exploratory behavior and is commonly used as a "control" assay for other behavioral tests that involve activity. The open field test can also measure anxiety-like behavior in mice by measuring how much time is spent in the surround or edges of the box compared to the time spent in the center of the box. The mouse

was placed into a novel white box measuring 45cm x 45cm without any type of extraneous stimuli and was allowed to explore for 5 minutes. Animals were gently placed in the apparatus, facing a wall, and activity was recorded over a 5-minute period using the SMART video tracking system (Panlab, Harvard Apparatus). Data collected included: latency (s) to first enter the center of the OF, time (s) spent in the surround and center, and distance travelled throughout the single trial. The open field box was thoroughly cleaned with 70% ethanol between each mouse to reduce olfactory cues.

Elevated Zero Maze

The elevated zero maze (EZM) is a test that measures anxiety and risk-taking behavior. The apparatus is an "O" shaped platform that is raised above the ground and is divided into two sections with walls surrounding the edges and two open sections with no surrounding walls. The EZM has two open portions and two closed portions; in addition, the EZM does not have the middle intersection, forcing the mouse to either be in an open or a closed section at any given time. Being in a portion of the maze was operationally defined as having all 4 paws being in that area. Each animal was given a single trial that began with the mouse being placed in a closed portion of the maze facing inward. Once the animal was placed, data was collected for one, 5-minute trial. SMART video tracking software (Panlab, Harvard Apparatus) recorded the 5-minute trial allowing the researcher to measure the time (s) spent in each section of the maze (open v. closed), the total number of entries into each section, and the latency to first enter an open arm. In addition, the number of head dips that the mice made were recorded. Head dips were counted when the mouse looked over the edge of the open arm. The maze was wiped down with 70% ethanol to reduce olfactory cues

between animals upon completion of each animal's single trial.

Morris Water Maze

The Morris Water Maze (MWM) is commonly used in the literature assessing MPA and spatial memory (Braden et al., 2011; Lewis et al., 2008). The MWM task is used to assess spatial memory in rodents and relies on the hippocampus. Animals learn to locate a clear platform hidden just below water (~ 5-10mm) to allow them to escape the water. MWM testing took place over 6 consecutive days with 4 trials per day, except for days 5 and 6 which consisted of only 1 and 2 trials, respectively. The MWM tub was filled with opaque water (water made opaque through the addition of non-toxic white paint) to keep the animals from seeing the platform. Visual cues were black and white images located around the tub, which was surrounded by a white curtain (approximately 12 inches away from the tub). Each mouse was placed in the tub facing the wall. The submerged escape platform was in a fixed location for the trials but where the animal started changed for each trial, forcing them to use the cues around the pool to recall the where the platform was located. The mice were given 60 seconds to find the hidden platform while being tracked by an overhead camera (using the SMART Video tracking system (Panlab), Harvard Apparatus). Distance traveled in inches, latency to find the platform, and percent time spent in the target quadrant were measured. In addition, thigmotaxicity was measured, a measurement of how much time the mouse spent in the outer edges of the pool, which is an indicator of anxiety-like behavior. At the end of the 60 second trial, if a mouse failed to find the platform, the mouse was gently guided to the platform where it remained for 10 seconds before being removed. Upon completion of the trial, the animal was dried with a towel and placed under a heating lamp for 45 seconds

before beginning the next trial (45 second intertrial interval). Once the animal had completed all 4 trials, it was finished for the day.

On days 2 and 4, the last trial was a probe trial; this consisted of the platform being lowered so that the animal was unable to climb onto it. The mice were given 60 seconds before the platform was raised and they were guided to it. The purpose of the probe trial was to measure the amount of time the animal spent searching in the target quadrant where the platform was located; in addition, crosses over where the platform would normally be was measured. The more time spent in the target quadrant suggests that the animal remembers where the platform would normally be.

Day 5 consisted of a single trial which was a probe trial. This trial was used to assess long-term memory of the platform's location; additionally, platform crosses was recorded.

Day 6 consisted of 2 trials using a visual platform to ensure there were no visual abnormalities. This involved moving the platform to a different quadrant and raising the platform above the water with a large blue Lego block to attract visual attention in a different quadrant than it normally was placed in. This was to assess whether mice could see the platform and rule out any visual deficits.

Activities of Daily Living

Burrowing

Burrowing is an innate behavior in mice that provides shelter (Deacon, 2006). The burrowing test is relatively easy to set up and allows a quick assessment of a behavior which may point toward neurological deficits, specifically deficits in activities of daily living. This assessment was done at 5PM, so that a preliminary assessment of burrowing

was made an hour before lights go out in the facility (7PM). A hollow tube (PVC pipe) with one end closed was filled with 250 grams of pea-gravel (small rocks) and weighed after a mouse had interacted with it for 2 hours and was weighed again the following morning. In order to perform the burrowing assay, each mouse was individually housed for a period of less than 24 hours. The next morning, the tubes were weighed again. No additional stimuli were added to the cage.

Nesting

Nesting is a simple measure of activities of daily living in mice. For nesting, the cage was filled with corn cob bedding (enough to cover the bottom of the cage) and 3.5 grams of shredded white paper were scattered around the bottom. Nests were scored on a 1-5 scale by trained raters who were uninformed to treatment condition with the following scale:

- 1-the shredded paper appears to be untouched
- 2-there was some attempt to build a nest, but the majority of paper is still scattered throughout the cage
- 3-a nest was somewhat constructed using a majority of the paper but there still shredding remains around the cage
- 4-a nest was constructed with only minimal paper not incorporated
- 5-all of the paper has been used in the building of the nest.

Raters scored the nests after all testing and euthanasia was completed and inter-rater reliability was measured. Raters were shown pictures of nests to train them on how to recognize nest scores; each rater rated nests individually. Rating was accomplished by showing raters pictures of the nests that were built and then the raters scored each nest

individually. The raters did not come into contact with the mice while they were in the nesting assay. Animals were separately housed in cages for the duration of the nesting experiment. They had to be housed separately so that nest building ability could be accurately assessed. After nesting was complete, animals were placed back into their home cage.

Statistical Analysis

After the data were collected, each behavioral test was analyzed separately. A mixed ANOVA was run for body weight; time was the within-subject factor and treatment group the between-subject factor. For the open-field test, elevated zero maze, and activities of daily living (burrowing and nesting), a one-way ANOVA was conducted (factor being drug administration with 4 levels). Morris Water Maze data was analyzed using a mixed ANOVA with experimental groups (between-subjects variable) and days (within-subject variable) as the factors. All data were analyzed through the use of SPSS software.

RESULTS

Body Weight

A mixed ANOVA was conducted weekly body weight to assess differences between treatment groups. There was a significant within-subject effect on weekly body weight, F (4.06, 170.38) = 364.49, $p < .01$. These results indicate that as time increased, body weight increased. There was no difference in weight between treatment groups. **(Figure 1).**

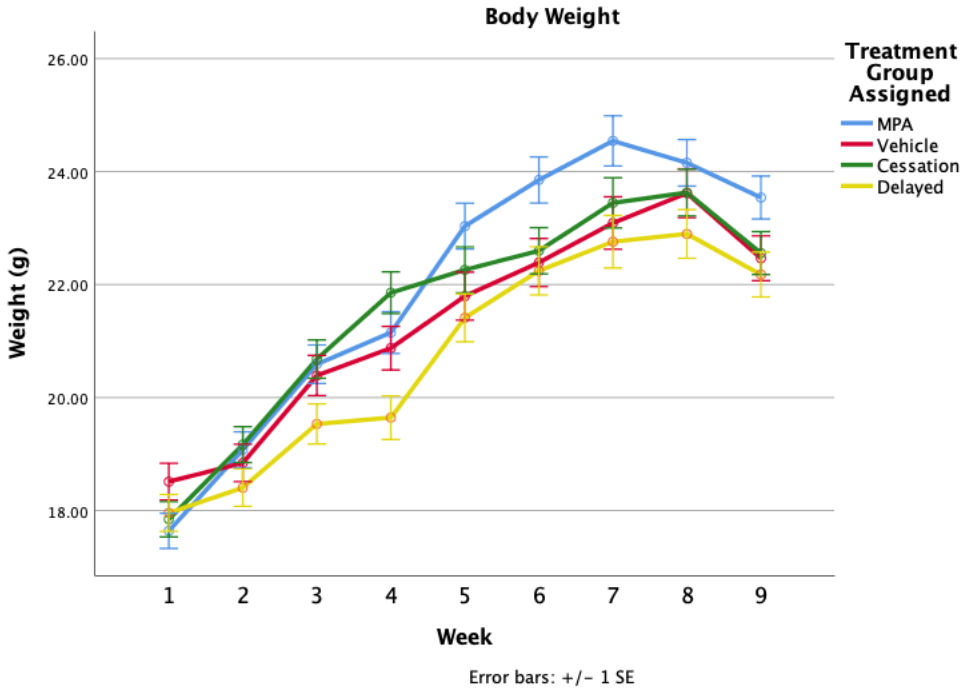

Figure 1. The mice in all treatment groups gained a significant amount of weight over nine weeks.

Open Field Test

Latency (s) to first enter the center of the OF

There was no significant difference in treatment group on latency to enter the center of the open field, F (3,42) = .32, $p = .81$.

Time spent in the surround and center

There was no significant difference in either percent time spent in the surround, F (3, 42) = .59, p = .69 or the center, F (3, 42) = .54, p = .66 between treatment conditions.

Distance travelled throughout the single trial

There was no significant difference between treatment groups in the total distance traveled in the open field task, F (3,42) = .83, p =.49.

Elevated Zero Maze

Latency to the Open Arm

There was no significant difference between treatment groups in the latency to enter an open arm, F (3,42) = .58, p = .63.

Time Spent in the Open Arm

There was no significant difference between treatment groups in the time spent in the open arms, F (3,42) = 2.32, p = .09. However, mice given MPA or those that received MPA during the last half of doses spent, on average, more time in the open arm **(Table 1).**

Treatment Group	*Mean*	*Standard Error*	*Lower Bound*	*Upper Bound*
MPA	8.348	1.393	5.538	11.159
Vehicle	5.187	1.455	2.252	8.123
Cessation	4.224	1.393	1.413	7.035
Delayed	8.400	1.455	5.465	11.336

Table 1. The mice in the MPA and MPA Delayed treatment group spent more time in the open arm of the EZM compared to the vehicle and MPA cessation treatment group.

Number of Entries into a New Arm

There was no significant difference between treatment groups in the number of entries into a new arm, F (3,42) = 1.96, p = .14.

Head Dips

There was no significant difference between treatment groups in the amount of head dips, F (3,42) = 2.26, p = .10.

Morris Water Maze

A mixed ANOVA was run for all variables assessed in the MWM.

Thigmotaxicity

There was a significant within-subjects effect days on thigmotaxicity, F (2.001, 36.022) = 55.48, p < .01. These results indicate that as the days progressed, the mice spent significantly less time around the border of the pool, signaling that the mice showed less anxiety over time. There was no significant difference between treatment groups **(Figure 2).**

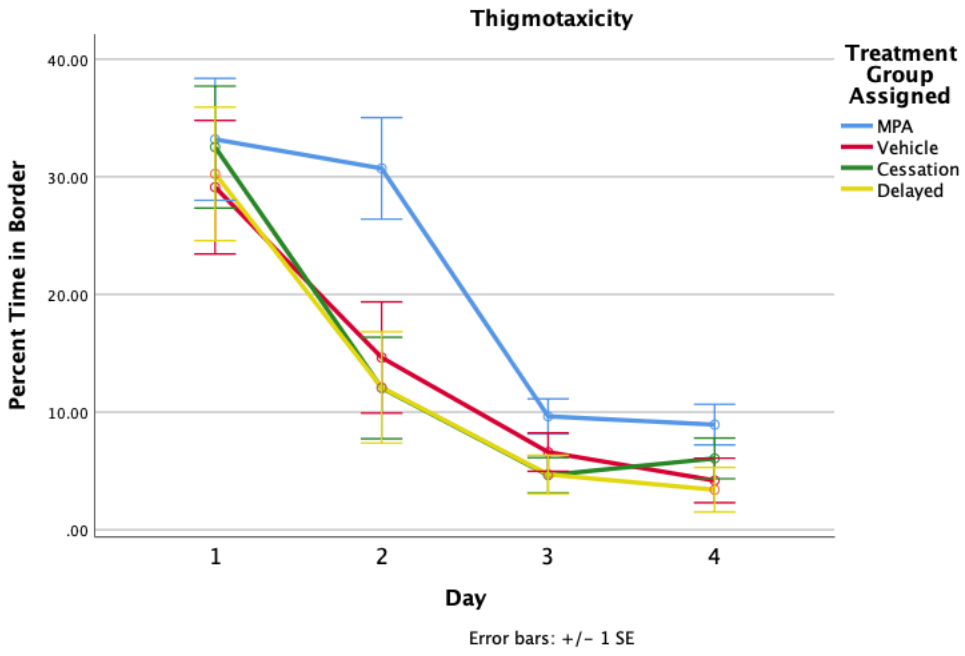

Figure 2. Thigmotaxicity is the percent time spent in the surround in the tub, which is indicative of anxiety-like behavior.

Time Spent in Target Quadrant

There was a significant within-subjects effect for percent time spent in the target quadrant, F (1.81, 5.44) = 65.097, $p < .05$. As the days progressed, the mice spent significantly more percent time in the target quadrant, showing that the mice were retaining the spatial information directing the mice to the target quadrant. There was no significant difference between treatment groups **(Figure 3).**

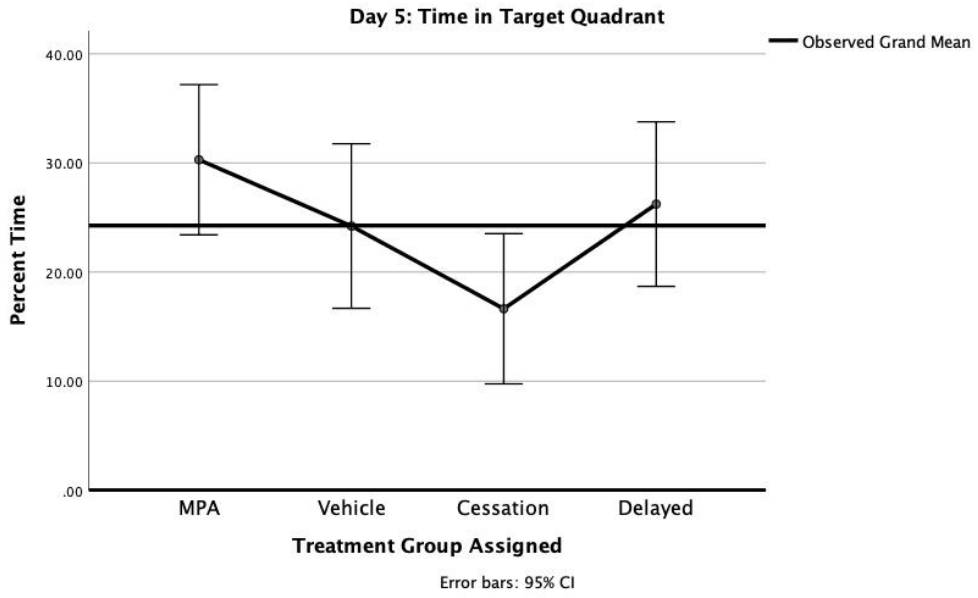

Figure 3. Time spent in the target quadrant, the quadrant where the platform is located, on day five (probe day) by treatment group.

Target Crosses

There was no significant difference between treatment groups for target crosses on day five, F (3,18) = .62, p = .61.

Latency to Target

There was a significant within-subjects effect on latency to reach the target platform, F (2, 36) = 5.63, $p < .01$. These results indicate that as the days progressed, the mice

decreased the time spent finding the target, signaling that the mice learned over time. **(Figure 4).** There was no significant difference between treatment groups for latency to reach the target.

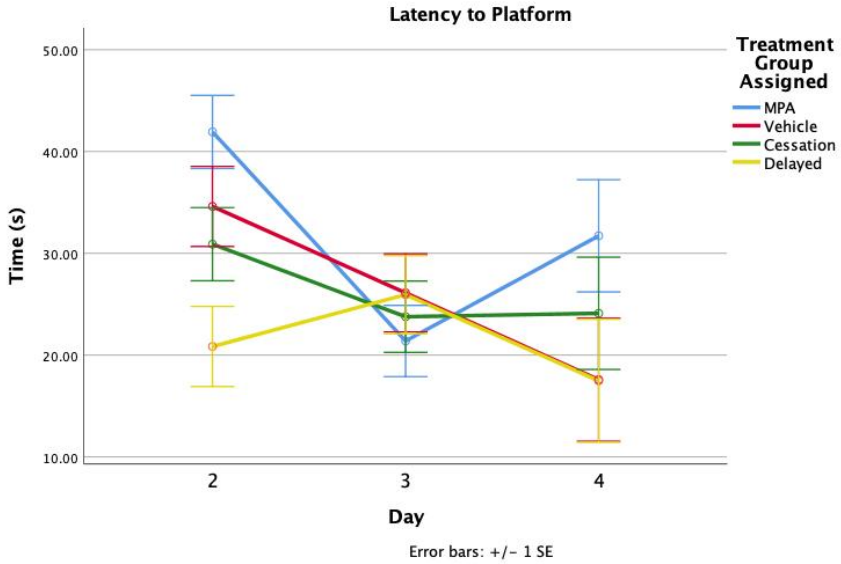

Figure 4. The time it took for the mice to find the platform on days two, three, and four by treatment group.

Assessing Visual Abnormalities

There were no differences between groups to locate the platform during the visual cue day indicating that there were no visual abnormalities, F (3, 18) = .196, p= .898

Activities of Daily Living

Burrowing Behavior

There was no significant difference between treatment groups in the amount of pea gravel burrowed at 2hrs, F (3,42) = .66, p = .58 nor overnight, F (3, 41) = 1.34, p = .27.

Nesting

There was no significant difference between treatment groups for nests built, F (3,42) = .144, p = .93. All mice built highly rated nests, regardless of their treatment group **(Figure**

5). Cronbach's alpha was run to determine if there was agreement between the raters; there was an acceptable agreement between the two raters, $\alpha = .76$.

Figure 5., *Nesting.* Mouse 16 from the MPA treatment group was given a score of "4" was given to this mouse's nest, while Mouse 7 from the Delayed treatment group was given a score of "3".

DISCUSSION

The purpose of this study was to examine the influence of Depo-Provera on weight gain, anxiety, learning, and activities of daily living in ovary-intact mice during adolescence. The study did not find any effect of Depo-Provera on any of the behavioral assays administered nor on weight gain. The current study's findings did not match previous findings in other studies that assessed MPA. However, these studies utilized MPA either in conjunction with other hormones, were administered at a later age, or were given in ovariectomized rodents.

Experimental Methods

Body Weights

There were no significant differences in body weights amongst treatment groups, which does not match previous findings in human studies (Oshodi et al., 2019). In humans, females on Depo-Provera have experienced an increase in body mass, however, with the use of Depo-Provera an increase of dietary intake is also apparent (Lange et al., 2015). In this study, food was given ad libitum and the amount of food consumed was not measured. Perhaps in this study, the mice consumed food in equal portions. Researchers should consider dietary intake when measuring the effects of MPA or other forms of birth control in rodents.

Open Field Test and Elevated Zero Maze

The results from the open field test and the elevated zero maze did not support the predicted hypotheses. There have not been previous studies assessing MPA's effect on anxiety-like behavior. The current study indicated that MPA did not impact anxiety-like behavior. This aligns with studies done in humans assessing the influence of Depo-Provera

and anxiety in women who were not predisposed to anxiety disorders (Hall et al., 2015; Westhoff et al., 1998). Additionally, the mice were handled frequently as a form of habituation and while receiving weekly injections; this may have resulted in a decrease in stress and in turn translated into a decrease in anxiety-like behavior. Similar to the open field test, the elevated mero maze assesses anxiety-like behavior. The current study did not find an effect of MPA on risk-taking behavior and anxiety-like behavior, which did not support the hypothesis.

Morris Water Maze

The Morris Water Maze results did not support the hypothesis. In the MWM, the only significant results were found in thigmotaxicity and latency to target demonstrating the effect of learning over days, however, there was not any significant difference between treatment groups. The results from this study did not align with previous findings regarding the use of MPA. However, in a study assessing oral contraceptives and verbal learning in humans, oral contraceptives actually aided in learning (Pletzer & Kerschbaum, 2014), so it is possible that birth control, including MPA, can aid in cognitive tasks. Additionally, other studies predominately used ovariectomized (Braden et al., 2011) or middle-aged rats (Chisholm & Juraska, 2011) rather than ovary-intact animals. Other studies found that MPA use alone impairs hippocampal-dependent tasks such as the Radial Arm Maze (Braden et al., 2011). No significant differences in the MWM could have occurred due to the low sample size or the duration of MPA administration. In this study, however, there were trending data. These data perhaps may have been significant if the sample size were to have increased by utilizing the second cohort as intended.

Activities of Daily Living

There were no significant differences found amongst any of the groups, indicating that innate behavior was not impacted by MPA. There have not been previous studies utilizing activities of daily living corresponding with the use of MPA. It would be ideal to utilize activities of daily living in rodents that are ovariectomized or in later stages of life, especially to assess MPA use in later life or in cases of neurodegenerative disease such as Alzheimer's disease. In later life, females' estrogen levels diminish, affecting the neuroprotective effects associated with estrogen (Nilsen & Brinton, 2002), which is believed to contribute to the disproportionately higher rates of Alzheimer's disease in women than men (Pike, 2017). MPA is often utilized in conjunction with other hormones for hormone treatment to treat menopause symptoms, so it would be vital to see if MPA has an impact on activities of daily living in an older population with a decreased level of estrogen.

Limitations

Limitations in this study predominately affected the Morris Water Maze results. Specifically, in the first day of the first cohort of the Morris Water Maze, thigmotaxicity and latency to the platform could not be included for analysis due to these variables not being recorded. This was due to a computer error. Another limitation in this study was the Morris Water Maze data from the second cohort was not included for analysis due to a malfunction in the SMART system's tracking along with restricted access to campus due to the Coronavirus (Covid-19) pandemic. The Morris Water Maze was the only hippocampal-dependent test assessing spatial memory.

Future Directions

The analysis of brain regions such as the hippocampus and the amygdala could further describe the relationship between MPA, learning, and anxiety. While this study did not find negative effects of MPA on behavior, brain regions may be affected prior to influencing behavior.

Future studies could lengthen the timeline of injections to ensure drug efficacy and to clarify the effects of long-term use. Additionally, different time points could be assessed. For example, MPA could be administered at the beginning of sexual maturity, as done in this study, in mid-adulthood, and during late adulthood.

Further investigation is needed across different age populations and across different time points regarding the contraceptive use of MPA. These studies should conduct more behavioral tests to capture a more in-depth analysis. For example, in this study, the Morris Water Maze was the only learning and memory assessment given. Other studies could include the Radial Arm Maze or Barnes Maze to assess spatial memory. In regard to body weight, the amount of food consumed could be an important variable to consider; additionally, body composition could be measured as well. Body composition analysis, examining the proportion of skeletal, muscular, or adipose tissue, would determine how MPA is impacting the body. Additionally, measuring particular hormone levels such as cortisol would aid in establishing whether a relationship exists between anxiety and MPA.

CONCLUSION

Birth control has many uses. Depo-Provera is prescribed to many patients for various reasons, and there has been an uptake in adolescent girls being prescribed Depo-Provera. However, with an increase of use during a developmental time period, research needs to be done to investigate the impact it has on learning and memory. The findings in the current study did not find any impact of MPA on weight gain, anxiety, or learning and memory. These results did not align with previous studies examining MPA perhaps due to increase rodent handling throughout the weekly injections and a low sample size in the MWM. More research is needed in this area, so that women can make a more informative decision about extended or acute use of Depo Provera.

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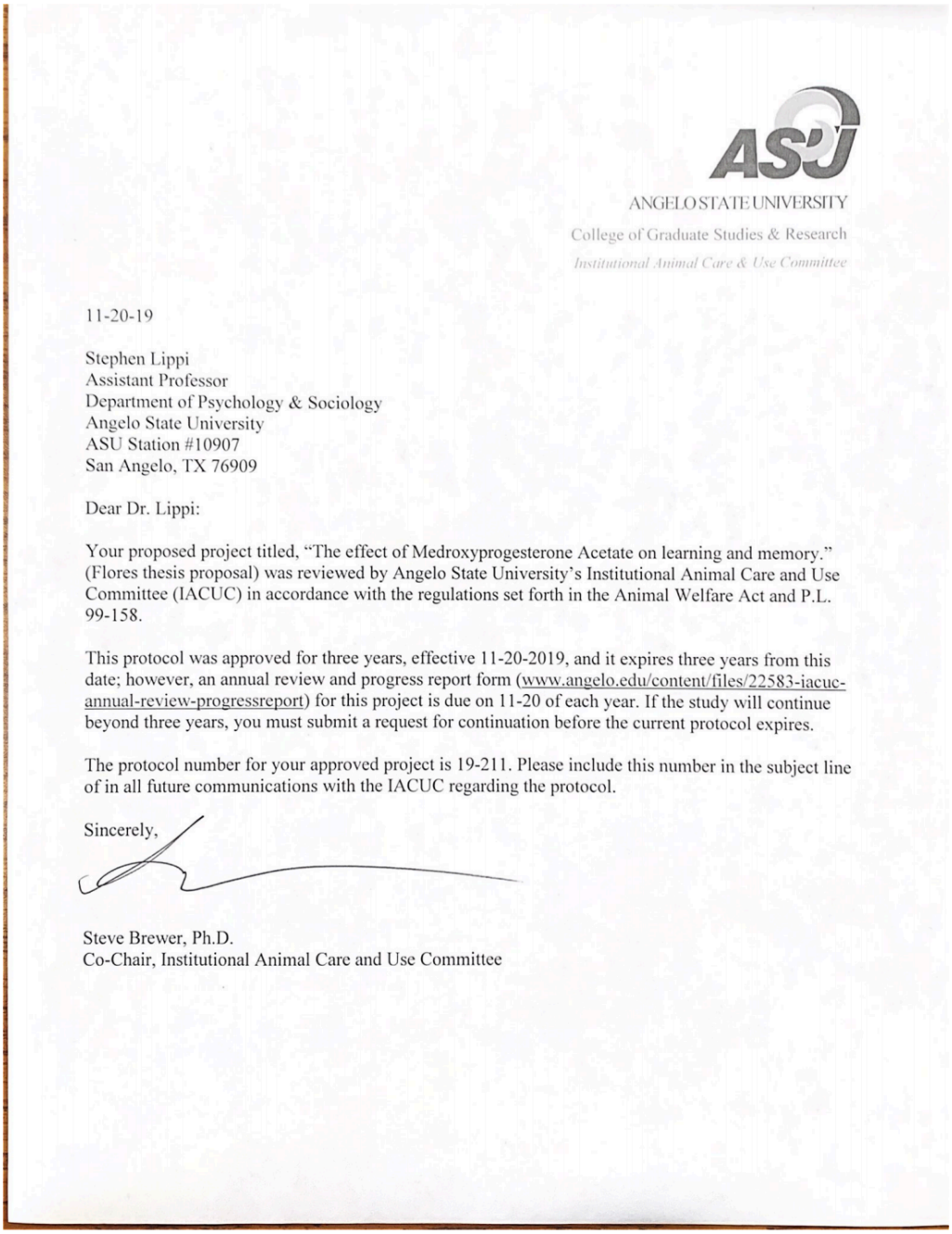
APPENDIX A

IACUC APPROVAL



ANGELO STATE UNIVERSITY
College of Graduate Studies & Research
Institutional Animal Care & Use Committee

11-20-19

Stephen Lippi
Assistant Professor
Department of Psychology & Sociology
Angelo State University
ASU Station #10907
San Angelo, TX 76909

Dear Dr. Lippi:

Your proposed project titled, "The effect of Medroxyprogesterone Acetate on learning and memory." (Flores thesis proposal) was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective 11-20-2019, and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on 11-20 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 19-211. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Steve Brewer, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee

BIOGRAPHY

Alyssa Marie Flores graduated from Angelo State University with a Bachelor of Science degree in 2018. She double majored in kinesiology and psychology. Alyssa Marie Flores was a member of Sigma Kappa and held several leadership positions. She is also a member of Psi Chi, a psychology honor society, and Order of Omega, a Greek honor society that only takes the top 3% of Greeks. Alyssa Marie Flores worked at Angelo State University as a registration assistant, lab assistant, and a graduate assistant in the Office of Title IX Compliance. Alyssa Marie Flores has presented at three conferences and worked on nine research projects and a publication in the past two years. Alyssa Marie Flores is continuing her education at New Mexico State University in the Master of Arts Clinical Mental Health Counseling program.