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Alleviation of the Negative Effects of Restraint Stress on Cognitive Learning and Retention in Rats by Estrogen and Estrogen + Progesterone

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Alleviation of the Negative Effects of Restraint Stress on Cognitive Learning and Retention in Rats by Estrogen and Estrogen + Progesterone

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Abstract

Although the positive effects of female hormones on central nervous system function have been well documented, the actions of estrogen and progesterone on the known negative effect of stress on learning and retention have been examined in only a limited number of studies. This study used ovariectomized rats, injected with pharmacological dosages of estrogen or estrogen plus progesterone. The animals were placed in restraints to apply non-escapable stress and then were tested in a Morris water maze to evaluate cognitive learning and memory. Rats were injected, stressed and run through the maze until the maze was learned. The hypothesis was that estrogen and estrogen plus progesterone would help alleviate stress thereby increasing cognitive learning and retention. The stressed rats receiving estrogen + progesterone had a significantly increased retention compared to stressed rats. No statistically significant effects of female hormones on the alleviation of stress on learning were found.
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Introduction

The effect of estrogen on cognitive learning has been studied extensively in the last decade. Using ovariectomized rats and giving the rats a constant dosage of estrogen Pan et al., (2000) showed that estrogen helped increase cognitive learning. It has also been shown that the effect of non-escapable stress impairs cognitive learning in rats (Shors et al., 1992).

Many studies have addressed effects of estrogen on memory. Wickelgren (1997) examined the molecular and cellular effects of estrogen on memory and demonstrated that estrogen can help improve retention along with learning in mammals. Sandstrom and Williams (2001) found that estrogen and progesterone modulated and increased retention time in rats and concluded that the effect of estrogen and progesterone acted as a positive factor on retention. Spatial reference can also be enhanced by the estrus cycle. Frick and Berger-Sweeney (2001) found that increased levels of ovarian hormone affect the function of the hippocampus and neocortex. With the increased hormone levels spatial memory was also enhanced.

Although there has been extensive research on estrogen and progesterone and their effects on learning and retention, little literature deals with the specific effect of estrogen and progesterone on cognitive learning within a stressful environment. Estrogen and progesterone has been found to alleviate the effect of stress on the immune system. Smart (2001) found that estrogen and progesterone altered the immune response to non-escapable stress as measured on white blood cell differentials in Mus musculus.

My research monitored the effects of high doses of estrogen and progesterone and a non-escapable stressor on learning and retention of memory. Based on the current
literature, I hypothesized that estrogen and progesterone may alleviate stress and help with cognitive learning and memory retention. In addition, I hypothesized that estrogen would have a greater effect than when combined with progesterone.
Learning and Memory Retention

Although there’s no universally accepted definition of learning, most researchers would agree that learning is a reaction to a stimulus (Montpellier et al., 1970). Mourad defined learning as a specific alteration in a reaction as a result of repetition to a particular stimulus in a given situation (Montpellier et al., 1970). With learning there is a trend toward improvement of performance due to the practice or recurring stimuli (Hovland 1951). Spatial learning and orientation are the most common types of learning that are used on animals for observation of phenomena of acquisition and learning. Spatial learning is widely used because it is one of the better methods for monitoring the learning progress because the spatial cues are left in the same area making orientation easier for the subject to learn. The maze is more of a discrimination situation in which there are many alternative responses, and each of these alternatives is an absolute spatial localization. The reaction of the organism is established by a repetition of the same stimulating situation (Montpellier et al., 1970).

Memory retention is defined as the “persistence” of a particular performance over a lapse of time where the performance or stimulus have not been performed (Brogden, 1951). Memory retention is the ability of the organism to retain or remember the stimuli that were once performed. Retention is the difference in proficiency after a lapse of time from the acquisition learned earlier. The lapse of time between the retest and the original acquisition is usually greater than the intervals between trials of the acquisition. During the lapse of time the performance is not performed, but the variables may still be controlled (Brogden, 1951).
**Glucocorticoids**

Glucocorticoids are hormones that are released by the adrenal cortex; they include cortisol, cortisone, and corticosterone. The main stimulant of the release of glucocorticoids from the adrenal cortex is adrenal corticotropic hormone (ACTH) released by the adenohypophysis (Randall *et al.*, 2002). Glucocorticoids have many functions from gluconeogenesis in the liver to the stress response of an organism.

Glucocorticoids play very important roles in restraint response. When stress occurs, there is an increase in the glucocorticoids that are released by the adrenal cortex, and a corticosteroid response is initiated (Sheridan *et al.*, 1994). Kelley (1985) observed a three-fold increase in corticosterone production when an organism was placed under chronic stress.

Glucocorticoid cells also show typical characteristics of steroidogenesis. These cells have mitochondria with vesicular and tubular cristae, smooth endoplasmic reticulum, granular endoplasmic reticulum, Golgi areas, and lipid globules. These cells are surrounded by a network of sinusoidal blood spaces and respond to secretagogues, ACTH being the most prominent one, with an increase in steroid production and release. Although long-term stimulation by ACTH causes the production and release of sex steroids (Balm, 1999), sex steroids are mainly produced and released by gonadotropic hormones.
**Estrogen and Progesterone**

Estrogen and progesterone are steroid sex hormones that are released from the ovaries in females and are also released in very small amounts from the adrenal cortex in both sexes (Randall *et al.*, 2002). Estrogen and progesterone are not used in the establishment of the reproductive tract but are used later in life in the reproductive cycle. Estrogen is used for the development of the uterus, ovary, vagina, and breasts. Estrogen also acts as an agent in a negative and positive feedback system during the reproductive cycle. Estrogen also causes growth spurts in bones and secondary sex characteristics in females (Randall *et al.*, 2002). Progesterone maintains uterine secretion and acts as part of a negative feedback system controlling the levels of follicular stimulating hormone (FSH) and luteinizing hormone (LH) (Randall *et al.*, 2002).

The human menstrual cycle consists of three significant stages that together average about 28 days in length. The first stage, the follicular stage of the cycle, begins with menses. During this stage the levels of estrogen and progesterone are low. Low levels of estrogen and progesterone cause levels of LH and FSH to increase (Randall *et al.*, 2002). FSH stimulates the follicles while LH stimulates the theca cells around the follicles. Theca cells secrete androgen into the granulosa cells, which then release estrogen. The increase in estrogen causes the development of the endometrium and also causes the cervical plug to become less viscous (Randall *et al.*, 2002). When estrogen is released it acts as part of a feedback system. When the estrogen increases it feeds back to the pituitary and hypothalamus and inhibits the release of LH and FSH. LH and FSH are accumulated but are not released during this part of the reproductive cycle. Also, the
estrogen increase causes the granulosa cells to secrete inhibin, which inhibits the release of FSH directly by the anterior pituitary gland.

The luteal phase is the second phase of the menstrual cycle and occurs after ovulation. Ovulation usually occurs around day 14, midway through the menstrual cycle. During ovulation, estrogen levels continue to increase for 1 to 2 days and this causes a surge in LH (Randall et al., 2002). The surge in LH allows the primary oocyte to finish meiosis I and triggers the rupture of the mature follicle by stimulating the granulosa cells around the follicle. LH also stimulates the transformation of remaining cells into the corpus luteum. The corpus luteum releases estrogen and progesterone. The increase in estrogen and progesterone initiates secretion in the uterus. Progesterone causes the cervical plug to become more viscous and also promotes the secretion of the endometrial fluid and increased vascularization of the endometrium. Estrogen plus progesterone inhibit the release of LH and FSH. The corpus luteum also produces inhibin, which decreases the levels of FSH (Randall et al., 2002).

Although estrogen is thought to affect the brain in many different sites, the area of the brain important to cognition and memory that is affected by estrogen is the hippocampus (Winn, 2001). The hippocampus has estrogen receptors and is the site of spatial learning for the environment. The limbic system is also thought to be correlated with the mechanisms of cognition, and is also affected by estrogen (Winn, 2001). Estrogen boosts the chemical functions of the brain cells, which spurs their growth and helps to keep them alive by avoiding toxins (Wickelgren, 1997). Estrogen was shown to help stimulate nerve cell growth in developing embryos affecting the cognition areas of the brain (Wickelgren, 1997). Wickelgren (1997) also found that estrogen might be
involved in learning and memory because it may help to build and maintain the synapses. Estrogen affects the basal forebrain cells and this activity causes an increase in the enzyme choline acetyltransferase (ChAT). ChAT makes acetylcholine, which is the chemical that communicates between some nerve cells (Wickelgren, 1997).

**Stress**

The definition of stress has not been agreed on in the fields of biology and psychology. Selye (1950) defined stress as being a “non specific response” of an organism to a demand made upon it. The stressor that induces or causes the stress, involves an internal or external challenge that disrupts the physiological equilibrium of an organism (Ramsey, 1982). Stress is known to be subjective, as what may be stressful for one person may not be very stressful for another (Friedman et al., 1996). Stress must be perceived by the brain, affecting some of the body’s homeostatic systems and the endocrine system. Two types of stress come into play: “fight or flight” and chronic stress. “Fight or flight” is a response to stress in which blood is redistributed and the body’s metabolism produces glucose (Cannon, 1914). The production of glucose is used to provide energy to the muscles, which allow an organism to either fight the stressor, or to flee the stressor (Cannon, 1914). During “fight or flight”, the adrenal medullary response is involved by releasing catecholamines: epinephrine and norepinephrine (Cannon, 1914).

Chronic or non-escapable stress occurs when one is unable to avoid the pressure caused by the stressor and has been associated with release of glucocorticoids. Mammals respond to stress by the stimulation of the hypothalamic pituitary adrenal axis, producing adrenal cortical secretions and an increase in glucocorticoids (Sheridan et al., 1994).
When chronic stress is encountered the hypothalamus is stimulated causing the release of corticotropic releasing hormone to the anterior pituitary initiating adrenocorticotropic hormone (ACTH) release. ACTH, when released, stimulates the adrenal cortex, initiating the release of glucocorticoids. The increase in glucocorticoids affects carbohydrate metabolism by increasing or initiating gluconeogenesis. Gluconeogenesis produces new glucose that can be used by many of the body’s organs as a source of fuel. The glucose production along with the metabolism change helps to cope with the stress that the body perceives (Randall et al., 2002).
Materials and Methods

Animals

Long Evans female rats (160-180 grams) were obtained from Charles River Laboratories in Massachusetts and were 40 to 60 days old. The animals were housed in a secure room that had a 12:12 photoperiod, and a constant temperature of 70°F. The rats were kept two to a cage and had free access to tap water and food.

Ovariectomy

Both ovaries were removed from each rat (D'Amour et al., 1965). Each rat was then given an injection of 0.07 ml of the antibiotic, baytril. The rats were allowed ten days to recover before the learning protocol was started.

Hormone Protocol

Rats were divided randomly into four groups with six rats in each group. The control group received vehicle (sesame oil) and did not undergo any stress. The next three groups were all stressed. The second group also received vehicle. The third group received estradiol. The final group received estradiol plus progesterone. The hormones, B-estradiol (1,3,4,[10]-estratriene-3,17 B diol) and progesterone (4-Pregnene-3,20-dione), obtained from Sigma, were dissolved in sesame oil. A priming dose of estrogen (5µg/100 g wt) was given 48 hours before the pharmacological dose. The pharmacological dosage of estrogen (100 µg/100 g wt) and the progesterone dosage (5000 µg/100 g wt) were given two days in advance of the learning protocol and were continued daily until the end of the experiment (Samuel et al., 1996). Subcutaneous injections were administered behind the shoulders of the rats, alternating sides each day.
**Restraint Procedure**

Restraint was used as a means of stressing the experimental groups. Restraints were made from two-inch diameter, thin-walled PVC pipe. Each restraint was made to be approximately eight to nine inches in length. Five holes were drilled on each side of the pipe. The holes were placed one and a half inches apart to allow air to circulate freely within the restraint. The ends of the restraints were closed using two and one half-inch styrofoam balls secured to the PVC pipe with duct tape. The rats were left in the restraint for one hour before they were run through the Morris water maze as described by Stefanski and Engler (1998).

**Learning Protocol**

The protocol for construction of the maze was modified from Morris (1981). The Morris water maze consisted of a round plastic swimming pool that was 183 cm in diameter and 38 cm deep. The platform, a glass beaker 13 cm in diameter and 17 cm tall, was placed 23 cm away from the edge of the pool and directly across from where the rats were released. The pool was filled to a depth of about 18.5 cm, so that the platform was approximately 1.5 cm beneath the water. The water was kept at room temperature and was made opaque by using non-toxic, water-soluble paint to prevent the rats from seeing the platform. The placement of the maze, platform and spatial cues in the surrounding environment were kept constant, as was the point of release of the rat in the Morris water maze. The time the rat took to reach the platform was recorded for each run. The rat was judged to have reached the platform when she placed her front paws on it. If the rat did not reach the platform within two minutes, she was placed on top of the platform and left there for 25 seconds. If the rat did reach the platform before the time was up, she was
still left on the platform for 25 seconds. Rats were run once a day until they all reached a time of 10 seconds or less, which in this experiment took 26 trials or days. Ten seconds was used because this was the time when each rat’s trial time leveled off. The experiment ended when every rat could run the maze under ten seconds for four consecutive days.

**Retention Procedure**

During the retention part of the experiment the injections and the stress by restraint were administered for seven days, but the rats were not tested in the Morris water maze. On the eighth day, the rats were placed in the Morris water maze again (Brogden, 1964). They were repeatedly tested until the trial time was below ten seconds for all rats.

**Statistical Analysis**

Statistical data for each rat were plotted in Excel to produce a learning curve along with the retention curves. Excel was also used to find the regression line for each individual rat for the number of trials needed to learn the maze. The statistical significance was tested using an ANOVA post hoc test in SPSS.
Results

A statistically significant difference was found in the retention rats given estrogen plus progesterone compared to the retention rats that were stressed alone. The mean difference was significant at the 0.05 level (Table 1). The rest of the experimental groups showed no significant difference when compared with each other (Table 1). There seems to be a difference between the average retention time of the groups (Figure 1). Although there wasn’t a significant difference, when looking at the individual trial times (Appendix B) it is apparent that the female hormones are having an effect on alleviation of stress. This shows that there may be a possible correlation between estrogen and the alleviation of stress.

Although estrogen plus progesterone had a statistically significant effect on retention, no statistical significance could be seen when comparing the average number of trials required to learn the maze (Figure 2). However, figure 3 suggests that there may be a difference in the rate of learning when one examines the daily timed trials. Although statistical analysis of the regression curve of the learning times shows no difference between the groups (Table 2 and 3), observation of the raw data indicates that there may be a difference, which is not evident statistically because of the small sample number along with the infrequency of the trials (Appendix 1). It appears evident that two of the experimental groups receiving female hormones decreased their learning time per trial compared to the stress group and the control group (Figure 3).

Examination of the individual trial times (Appendix 1) demonstrates that outliers existed in the data between the stress groups and the estrogen plus progesterone groups.
These outliers skewed the statistical analysis. Repetition of this experiment using a larger number of animals in each experimental group and frequent trials is warranted.
Table 1. Multiple comparisons of a post hoc test, showing the significance between all of the experimental groups for memory retention. The mean difference is significant at the 0.05 level for the bold faced numbers.

<table>
<thead>
<tr>
<th>Group (I)</th>
<th>Group (J)</th>
<th>*Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Stress</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>Estrogen</td>
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</tr>
<tr>
<td></td>
<td>Estrogen + Progesterone</td>
<td>0.737</td>
</tr>
<tr>
<td>Stress</td>
<td>Control</td>
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</tr>
<tr>
<td></td>
<td>Estrogen</td>
<td>0.523</td>
</tr>
<tr>
<td></td>
<td>Estrogen + Progesterone</td>
<td><strong>0.022</strong></td>
</tr>
<tr>
<td>Estrogen</td>
<td>Control</td>
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</tr>
<tr>
<td></td>
<td>Stress</td>
<td>0.523</td>
</tr>
<tr>
<td></td>
<td>Estrogen + Progesterone</td>
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</tr>
<tr>
<td>Estrogen + Prog</td>
<td>Control</td>
<td>0.737</td>
</tr>
<tr>
<td></td>
<td>Stress</td>
<td><strong>0.022</strong></td>
</tr>
<tr>
<td></td>
<td>Estrogen</td>
<td>0.305</td>
</tr>
</tbody>
</table>

*Standard errors for all tests were 3.25.

Table 2. Multiple comparisons of a post hoc test, showing the significance between all of the experimental groups for cognitive learning.

<table>
<thead>
<tr>
<th>Group (I)</th>
<th>Group (J)</th>
<th>*Significance</th>
</tr>
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<tbody>
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<td>Control</td>
<td>Stress</td>
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<tr>
<td></td>
<td>Estrogen</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>Estrogen + Progesterone</td>
<td>0.992</td>
</tr>
<tr>
<td>Stress</td>
<td>Control</td>
<td>0.837</td>
</tr>
<tr>
<td></td>
<td>Estrogen</td>
<td>0.239</td>
</tr>
<tr>
<td></td>
<td>Estrogen + Progesterone</td>
<td>0.685</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Control</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>Stress</td>
<td>0.239</td>
</tr>
<tr>
<td></td>
<td>Estrogen + Progesterone</td>
<td>0.837</td>
</tr>
<tr>
<td>Estrogen + Prog</td>
<td>Control</td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>Stress</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>Estrogen</td>
<td>0.837</td>
</tr>
</tbody>
</table>

*Standard errors for all tests were 1.794
Table 3. Multiple comparisons of a post hoc test, showing the significance of the slope between all of the experimental groups for cognitive learning.

<table>
<thead>
<tr>
<th>Group (I)</th>
<th>Group (J)</th>
<th>*Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Stress</td>
<td>0.837</td>
</tr>
<tr>
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<td>Estrogen</td>
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<td></td>
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</tr>
<tr>
<td>Stress</td>
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<td></td>
<td>Estrogen</td>
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<tr>
<td>Estrogen</td>
<td>Control</td>
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<td></td>
<td>Stress</td>
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<td>Estrogen + Progesterone</td>
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<tr>
<td>Estrogen+ Progesterone</td>
<td>Control</td>
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<tr>
<td></td>
<td>Stress</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>Estrogen</td>
<td>0.837</td>
</tr>
</tbody>
</table>

*Standard errors for all tests were 1.794
Average Retention Time

Figure 1. Comparison of average retention times for control and experimental groups.
Figure 2. Comparison of the number of trials needed by each group to learn the Morris water maze. Error bars show mean, +/- 1.0 standard deviation. Note overlap of bars and no statistical difference between groups.
Figure 3. Comparison of average daily trial runs in the Morris water maze for various groups.
Discussion and Conclusion

Many studies have examined the link between the effect of estrogen on neural tissue and learning. Estrogen increases the amount of an enzyme that is needed to produce acetylcholine (Fackelmann, 1995). Acetylcholine is a neurotransmitter that has a major role in memory. With the enhancement of the acetylcholine neurotransmitter, it is thought that estrogen helps enhance verbal memory. The higher the concentration of estrogen in the blood, the higher the tests scores were for memory in rats (Fackelmann, 1995). In one study scientists depleted rats of estrogen by removing their ovaries and noticed a lower activity of some hippocampal cells. This finding suggested that estrogen-induced dendritic changes do, in fact, affect neuron function by linking them to a molecule that plays a very important role in cognition: NMDA (Wickelgren, 1997). NMDA is a membrane protein that detects incoming signals for the neurotransmitter glutamate; rats treated with estrogen had a 30% increase of the NMDA in hippocampal neurons (Wickelgren, 1997). In the same study, the hippocampal cells were stimulated, but only the NMDA receptors were active. Neurons that were treated with estrogen responded with larger currents than did the control rats. Yaffe et al. (2001) studied postmenopausal women in which one group was given raloxifene, which is an estrogen-receptor modulator, and the second group was given a placebo as a control. Cognitive tests were given every year for three years. Testing was double blinded, meaning that the testing personal were not aware of the treatment groups. Women on raloxifene had a lower risk of cognitive decline (Yaffe et al., 2001). Kampen and Sherwin (2001) studied the effects of estrogen on men who were given high dosages of estrogen, and found that
estrogen enhanced the visual memory in healthy young men. Learning is also affected by estrogen, as learning is a process of memory. It has been found that estrogen enhances cognitive learning and rats injected with estrogen learned place-oriented tasks more quickly than did rats with no estrogen treatments (Kotulack and Van, 2002).

Frick and Sweeny (2001) also discovered that estrogen increases the spatial reference memory and the neurochemistry in intact female mice. Female rats that were given estrogen had an increased spatial memory compared to the control rats.

Estrogen has also been known to enhance memory retention. In one study, adult women were given estrogen treatments. Fackelmann (1995) found that the estrogen users performed better than their peers when a memory test was administered. Sandstrom and Williams (2001) found that injecting ovariectomized rats with estrogen 72 hours or 48 hours before a memory retention test greatly enhanced the retention time of the rats. In the same study they found that progesterone also maintained the retention time. Keenan (2001) performed a study on women who were receiving hormone replacement therapy. She found that women who did not receive hormone replacement therapy were sometimes impaired in recognizing words that were once previously learned, and performed worse on tests for working memory. Women with the hormone replacement therapy performed better on the working memory tests along with the word testing (Keenan, 2001). Alzheimer’s patients, who were given estrogen for 12 months prior to testing, had significant improvements in their memory and cognitive function when compared to the control group (Powledge, 1997).

Stress also plays a major role in learning and memory. Stress is known to impair subsequent learning. Shores et al. (1992) placed rats in inescapable shock or stress and
found that the stress impaired the learning, which is sometimes referred to as “learned helplessness”. Estrogen has also been shown to have an effect on stress. Smart (2001) researched the effects of estrogen on stress in the immune system and found that when estrogen was injected into the mice, the WBC count increased in neutrophils which help to counteract the effects of stress.

Estrogen has thus been shown to enhance memory retention and learning as well as to alleviate a negative stressor. I proposed that estrogen and estrogen + progesterone could alleviate the negative effect of stress on learning and memory. Although my data gave only limited support to my hypothesis, the trends visible in the raw data indicate that repetition of the experiments using larger sample groups might provide statistically significant results. We have only begun to scratch the surface of the effects of estrogen on the influence of stress.
Literature Cited


Appendix A:
Learning curves for each experimental rat in the Morris water maze.
A. Learning curve for rat A of the control group in the Morris water maze.

B. Learning curve for rat B of the control group in the Morris water maze.
C. Learning curve for rat C of the control group in the Morris water maze.

D. Learning curve for rat D of the control group in the Morris water maze.
E. Learning curve for rat E of the control group in the Morris water maze.

F. Learning curve for rat F of the control group in the Morris water maze.
G. Learning curve for rat G of the stress group in the Morris water maze.

H. Learning curve for rat H of the stress group in the Morris water maze.
I. Learning curve for rat I of the stress group in the Morris water maze.

J. Learning curve for rat J of the stress group in the Morris water maze.
K. Learning curve for rat K of the stress group in the Morris water maze.

L. Learning curve for rat L of the stress group in the Morris water maze.
M. Learning curve for rat M of the stress and estrogen group in the Morris water maze.

N. Learning curve for rat N of the stress and estrogen group in the Morris water maze.
O. Learning curve for rat O of the stress and estrogen group in the Morris water maze.

P. Learning curve for rat P of the stress and estrogen group in the Morris water maze.
Q. Learning curve for rat Q of the stress and estrogen group in the Morris water maze.

R. Learning curve for rat R of the stress and estrogen group in the Morris water maze.
S. Learning curve for rat S of the stress and estrogen plus progesterone group in the Morris water maze.

T. Learning curve for rat T of the stress and estrogen plus progesterone group in the Morris water maze.
U. Learning curve for rat U of the stress and estrogen plus progesterone group in the Morris water maze.

V. Learning curve for rat V of the stress and estrogen plus progesterone group in the Morris water maze.
W. Learning curve for rat W of the stress and estrogen plus progesterone group in the Morris water maze.

X. Learning curve for rat X of the stress and estrogen plus progesterone group in the Morris water maze.
Appendix B:
Memory retention for each experimental rat in the Morris water maze.
A. Retention curve for rat A of the control group in the Morris water maze.

B. Retention curve for rat B of the control group in the Morris water maze.
C. Retention curve for rat C of the control group in the Morris water maze.

D. Retention Curve for rat D of the control group in the Morris water maze.
E. Retention curve for rat E of the control group in the Morris water maze.

F. Retention curve for rat F of the control group in the Morris water maze.
G. Retention curve for rat G of the stress group in the Morris water maze.

H. Retention curve for rat H of the stress group in the Morris water maze.
I. Retention curve for rat I of the stress group in the Morris water maze.

J. Retention curve for rat J of the stress group in the Morris water maze.
K. Retention curve for rat K of the stress group in the Morris water maze.

L. Retention curve for rat L of the stress group in the Morris water maze.
M. Retention curve for rat M of the stress and estrogen group in the Morris water maze.

N. Retention curve for rat N of the stress and estrogen group in the Morris water maze.
O. Retention curve for rat O of the stress and estrogen group in the Morris water maze.

![Graph](image)

P. Retention curve for rat P of the stress and estrogen group in the Morris water maze.

![Graph](image)
Q. Retention curve for rat Q of the stress and estrogen group in the Morris water maze.

R. Retention curve for rat R of the stress and estrogen group in the Morris water maze.
S. Retention curve for rat S of the stress and estrogen plus progesterone group in the Morris water maze.

T. Retention curve for rat T of the stress and estrogen plus progesterone group in the Morris water maze.
U. Retention curve for rat U of the stress and estrogen plus progesterone group in the Morris water maze.

V. Retention curve for rat V of the stress and estrogen plus progesterone group in the Morris water maze.
W. Retention curve for rat W of the stress and estrogen plus progesterone group in the Morris water maze.

X. Retention curve for rat X of the stress and estrogen plus progesterone group in the Morris water maze.
Appendix C:
Regression line for the number of trials to learn the Morris water maze for each rat.
A. Regression line for rat A of the control group in the Morris water maze.

\[ y = -4.3647x + 87.679 \]
\[ R^2 = 0.9372 \]

B. Regression line for rat B of the control group in the Morris water maze.

\[ y = -3.4579x + 61.958 \]
\[ R^2 = 0.4466 \]
C. Regression line for rat C of the control group in the Morris water maze.  

\[ y = -5.2574x + 106.38 \]  
\[ R^2 = 0.3783 \]  

D. Regression Line for rat D of the control group in the Morris water maze.  

\[ y = -8.4489x + 137.54 \]  
\[ R^2 = 0.7986 \]
E. Regression line for rat E of the control group in the Morris water maze.

\[ y = -5.5464x + 83.905 \]

\[ R^2 = 0.3314 \]

F. Regression line for rat F of the control group in the Morris water maze.

\[ y = -0.7802x + 29.615 \]

\[ R^2 = 0.0325 \]
G. Regression line for rat G of the stress group in the Morris water maze.

H. Regression line for rat H of the stress group in the Morris water maze.
I. Regression line for rat I of the stress group in the Morris water maze.

\[ y = -3.8652x + 65.081 \]
\[ R^2 = 0.3772 \]

J. Regression line for rat J of the stress group in the Morris water maze.

\[ y = -2.4534x + 46.811 \]
\[ R^2 = 0.3602 \]
K. Regression line for rat K of the stress group in the Morris water maze.

\[ y = -3.568x + 66.895 \]
\[ R^2 = 0.3725 \]

L. Regression line for rat L of the stress group in the Morris water maze.

\[ y = -5.3143x + 108.9 \]
\[ R^2 = 0.648 \]
M. Regression line for rat M of the stress and estrogen group in the Morris water maze.

\[ y = -3.5143x + 67.6 \]
\[ R^2 = 0.3332 \]

N. Regression line for rat N of the stress and estrogen group in the Morris water maze.

\[ y = -3.1813x + 47.038 \]
\[ R^2 = 0.1544 \]
O. Regression line for rat O of the stress and estrogen group in the Morris water maze.

\[ y = -9.3162x + 96.455 \]
\[ R^2 = 0.5527 \]

- Rat O
- Linear (Rat O)

Trial

P. Regression line for rat P of the stress and estrogen group in the Morris water maze.

\[ y = -3.1679x + 44.543 \]
\[ R^2 = 0.2354 \]

- Rat P
- Linear (Rat P)

Trial
Q. Regression line for rat Q of the stress and estrogen group in the Morris water maze.

\[ y = -3.8374x + 59.352 \]
\[ R^2 = 0.2193 \]

R. Regression line for rat R of the stress and estrogen group in the Morris water maze.

\[ y = -4.1201x + 70.375 \]
\[ R^2 = 0.3795 \]
S. Regression line for rat S of the stress and estrogen plus progesterone group in the Morris water maze.

\[ y = -2.2074x + 47.45 \]
\[ R^2 = 0.137 \]

T. Regression line for rat T of the stress and estrogen plus progesterone group in the Morris water maze.

\[ y = -2.2794x + 41.397 \]
\[ R^2 = 0.1967 \]
U. Regression line for rat U of the stress and estrogen plus progesterone group in the Morris water maze.

\[ y = 0.16x + 64.164 \]
\[ R^2 = 0.279 \]

V. Regression line for rat V of the stress and estrogen plus progesterone group in the Morris water maze.

\[ y = -2.3015x + 50.125 \]
\[ R^2 = 0.275 \]
W. Regression line for rat W of the stress and estrogen plus progesterone group in the Morris water maze.

\[ y = -3.4301x + 67.716 \]
\[ R^2 = 0.5147 \]

X. Regression line for rat X of the stress and estrogen plus progesterone group in the Morris water maze.

\[ y = -3.9759x + 74.447 \]
\[ R^2 = 0.4311 \]