

***The effects of Estrogens on Spatial
and Environmental Novelty***

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Abstract:

The Dentate Gyrus (DG) is a part of the hippocampus, a region involved in learning and memory, especially the pattern separation processing and spatial discrimination. The underlying mechanism for learning and memory is an increase in synaptic plasticity called long-term potentiation (LTP). LTP means that stronger a communication of neurons results in better learning. -Estradiol (E2) is the main human estrogen. E2 modulates hippocampal function including the learning and memory. High expression of estrogen receptors in the DG suggests that E2 controls this region. Previous in vitro recordings from the laboratory show differences in the LTP magnitude in the DG. To evaluate the roles of E2 in the DG function in vivo, we employed two tasks that examine the pattern separation processing in form of response to environmental or metric novelty. Rats were ovariectomized. A week later, the rats were replaced with E2 (2 $\mu\text{g/day}$) for 4 days. Controls received oil. Animals were then tested in the environmental or metric novelty response. E2-replaced rats explored the arena significantly less after the environmental change compared to oil-injected controls as well as following the metric change. The data suggests that E2 affects DG function.

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Background and Introduction:

Estrogens play a modulatory role in learning and memory processing. Actions of estrogens involve a variety of effects, which include modulation of gene expression, regulation of neurotransmitter release, or direct interactions with neurotransmitter receptors (Veliskova et al., 2007). In terms of cognition development and memory, 17 β -Estradiol (E2) is a form of estrogen that has a vital role in brain function and cognition. E2 also regulates the strength of synaptic plasticity called long-term potentiation (LTP). LTP seems to be a substrate for learning and memory. E2 acts via estrogen receptors. These receptors include estrogen receptor-alpha, estrogen receptor-beta and a G-protein coupled estrogen receptor. The activation of these receptors leads to regulation of gene transcription or to acute effects, i.e., transmitter release (Kumar et al., 2015).

Undergoing ovariectomy means having one or both ovaries removed, and can be removed to treat endometriosis, breast cancer or lower the risk of ovarian cancer. 200,000 ovariectomies are performed in the US annually. Expected side effect of this procedure in a woman in reproductive age is premature surgical menopause, which has an adverse effects on the brain and other body structures, like the loss of bone density. Women that have had ovariectomies are at an increased risk of death (US study: Women who have ovaries removed face increased risk of death, *The Guardian*, 2009) Mice that have underwent an ovariectomy have shortened life-spans (Benedusi et al., 2015). It's expected that estrogen levels suddenly drop after the surgery which is why many women have estrogen replacement therapy to reverse these symptoms and replace the bodies natural estrogen. As a result of going through menopause or having an ovariectomy, levels of E2 could be altered beyond the appropriate level which could lead to cognitive

dysfunction. To reverse the impairment estrogen supplements should be given instantly after the hormone declination or menopause.

The function of E2 in the brain is also imperative as it facilitates the interaction between neurons in the hippocampus (Carpenter, S. (2001). Does estrogen protect memory? *American Psychological Association*, 32(1), 52. doi:10.1037/e300022003-032). The dentate gyrus and the CA3 are portions of the hippocampus that are involved in processing spatial information. However, their specific role in spatial information varies. The dentate gyrus (DG) is specialized for processing fine spatial discrimination, while CA3 processes large scale spatial information. Citing from the Hunsaker et al., 2008 paper (Discussion on CA3): CA3c is only involved in pattern separation processes when the animal is required to detect a metric change in object location. It must be noted, however, that dorsal CA3c lesions never caused effects as dramatic as those caused by dorsal DG lesions. For larger changes, such as for an overall environmental change, CA3c is not needed because the requirement for sparse encoding is not present (low spatial resolution is more than sufficient to distinguish a circle from a square environment). The dentate gyrus (DG) is the main structure in processing fine spatial discrimination and large scale spatial information. The CA3c is involved in assisting pattern separation processes (Hunsaker et al., 2008). Moreover, a minor change in the environment such as the movement of two objects would be correlated with both the DG and CA3; whereas a larger change in the geometry of the whole environment such as a circular environment to a square shaped environment would be processed by DG.

The dentate gyrus and CA3 communicate through mossy fibers which communicate by the unmyelinated axons of granule cells in the dentate gyrus acting to either excite or inhibit the

mossy cells of CA3. This interaction is necessary to process spatial information with high precision (Hunsaker et al., 2008). When a familiar environment is rearranged, spatial processing is demonstrated. This is demonstrated by re-exploring familiar objects. Through administration of E2 in ovariectomized animals, the dentate gyrus is inhibited. This plays a significant role on DG plasticity, which allows for the animals to spend more time on the incoming pattern. When there is a change in metric or environmental change, the animals injected with estrogen will be more interested in the object and explore it more than those injected with oil. The estrogen group will recognize the change in environment and the same objects from their previous trials. The change in the environment will be recognized by the oil group, but they will think the objects are new.

Statement of Purpose:

The goal of this study was determine the role of estrogen in the dentate gyrus and CA3 region.

This was examined by comparing the behaviors of an estrogen group and an oil group of ovariectomized rats to find out how estrogen rats behave differently when those regions of the brain are activated.

Materials and methods:

Subjects

Eighteen animals, adult female Sprague-Dawley rats from Taconic Farms weighing 175-225 grams served as subjects. All experiments were carried out during the light portion of the 12-h light-dark cycle, which animals were kept. All animals were provided with water and free-feeding after experimentation. All animal care and protocol conformed to NIH, IACUC and AAALAC standards, and veterinarian assessed health of all animals weekly.

Surgery and injections:

All animals were ovariectomized 10 days prior to experiment. All rats were anesthetized and maintained with isoflurane (5% (vol/vol) in 2 l/m oxygen). Following the surgery animals returned to their homecage. Seven days after the surgery was performed, animals were randomly assigned to the treatment or control groups and were injected with 0.1 milliliter of E2 (2 μ g/rat/day) or oil for four days.

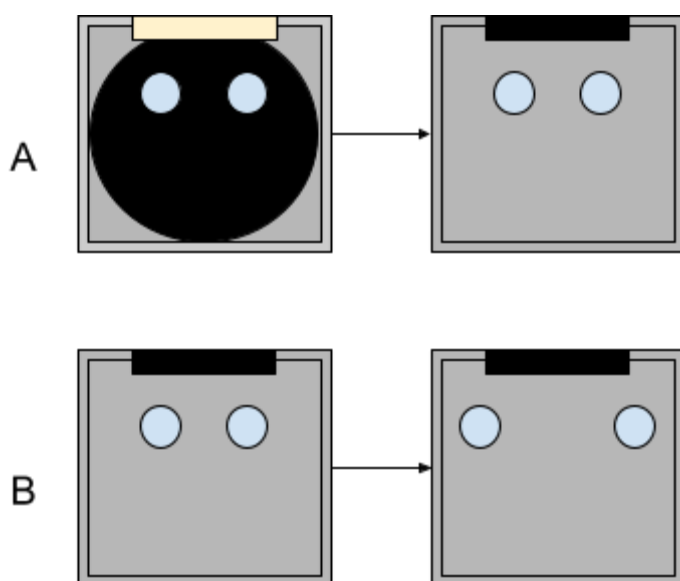


Fig 1. Behavioral apparatus. (A) Environmental change. Animals receive three 5 minute trials in the circled arena with objects 30 cm apart separated by 3 minute inter trial intervals. Circled arena is taken out and animals receive a single test trial on the squared arena with objects staying the same distance. (B) Metric change in object location. Only squared arena is used for this experiment. Animals receive three 5 minute trials with objects 30 cm apart, separated by 3 minute inter trial interval. Then, animal receive a single test trial in which objects are moved to 50 cm apart.

Behavioral methods:

Behavioral testing was performed the day after the last injection. In all cases, animals were recorded with an overhead camera Logitech webcam Pro 9000, and the data was recorded onto a computer program ANY-Maze. All animals were tested after the environment change test first and then after two hours were tested on the metric change experiment.

Experimental apparatus:

The apparatus for this experiment consist of a gray squared arena (78.7cm x 78.7cm) and a black circled arena (78.7 cm diameter) with walls 56.1cm tall. Circled arena was created by taping 4 cardboard together. Two cardboards that were closer to the object's were taped together at 6.35cm on each side, while the cardboard across from objects was taped 8.9cm on each side. In the center of the wall closer to the objects, a black cue card was placed for the gray squared arena. Similarly, a beige cue card was placed on the wall of the black circled arena. This is so that the rats have an object distance reference. The cue cards have dimensions of 6.35cm x 30cm. Objects are 13 cm tall and have a zone of entry around of 2.54cm radius. A 3x3 grid with squares

26cm x 26cm was drawn on the apparatus program to make zones of exploration for the animal. The circled arena was created by taping four cardboards together, each of the same proportions. A mark was made on the outside center of the circled arena at 39.37cm, so circle would be placed on the same position for all animal's trials.

Environmental change:

The environmental tests depends on the detection of change task. Animals were placed on the middle of the black circled arena with two objects 30 cm apart placed in quadrants I and III. Animals received three trials of five minutes for examination on the circled arena with the objects unchanged. There was a three minutes interval between trials. The box was wiped down and cleaned between each session with cleaning dilute to prevent any odors left by previous rat tested. After animals completed three trials of habituation, to the objects and environment, the circled arena was removed from the squared arena. The distance between the objects remained unchanged, and now animals were placed on the middle of the gray squared arena with objects at 30cm again. Animals were again given five minutes to explore the new environment.

Metric change:

The metric change test consisted of a change in the location of the objects. This test was performed two hours the after the first experiment and only the gray squared arena was used on this task. Animals were again placed on the middle of the gray squared arena with the two objects 30 cm apart placed on quadrants I and III. Animals received three trials of five minutes for examination on the circled arena with the objects unchanged separated by a three minute interval between the trials. The box was wiped down and cleaned between each session. This test was planned with this metric change from a smaller difference of 30 cm to a longer distance of

50 cm to avoid any disruption in behavior that could be caused by environmental change experiment tested previously. After animals completed three trials of habituation to the objects and environment, each object was moved 10 cm away from each other creating a new separation of 50cm between the objects. Then, the animals were given a five minute period to explore. The camera recorded the animals and the computer program collected data.

Results

Environmental Graphs

Figure 1

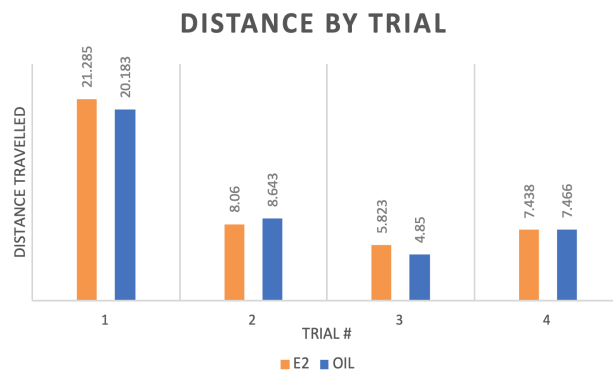


Figure 1 is the distance traveled by each group in each trial.

$p = 0.8610$

Figure 2

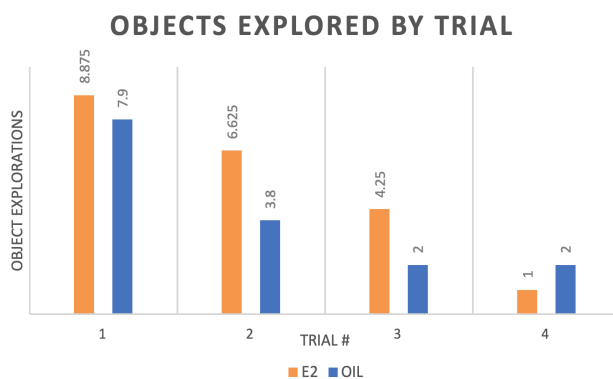


Figure 2 is the number of times the animals explored the objects on average each trial.

$p = 0.7450$

Figure 3

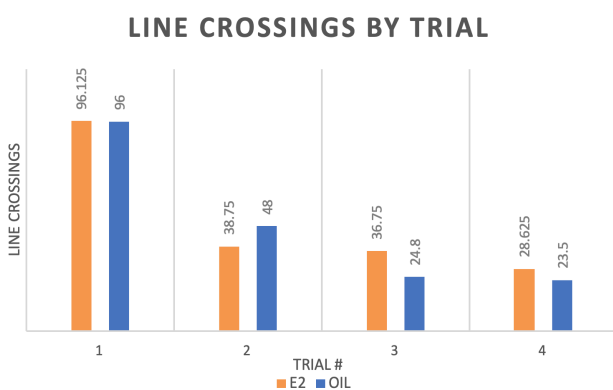


Figure 3 is the number of line crossings by each group in each trial.

$p = 0.8548$

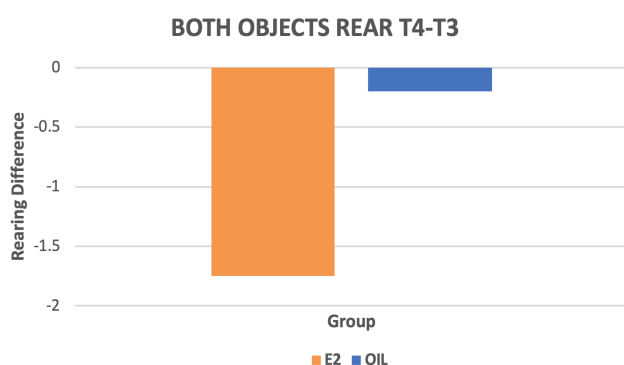
Figure 4

Figure 4 is the difference between trial 4 and trial 3 of the number of rearings of both objects.

$p = 0.0476$

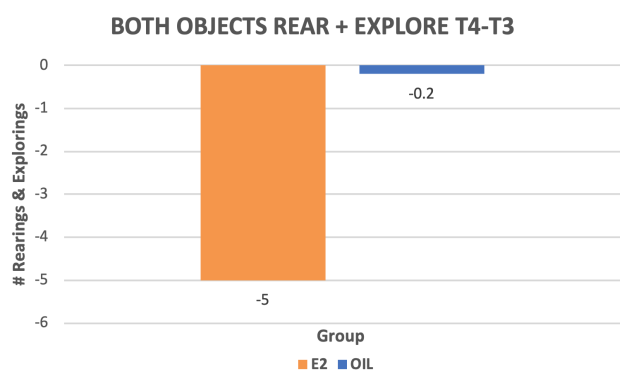
Figure 5

Figure 5 is the difference between trial 4 and trial 3 of the number of rearings and explorations of both objects.

$p = 0.0218$

Metric Graphs

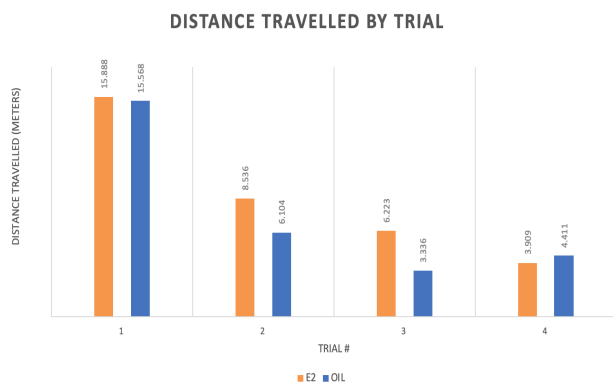
Figure 6

Figure 6 is the distance traveled by each group in each trial.

$p = 0.4210$

Figure 7

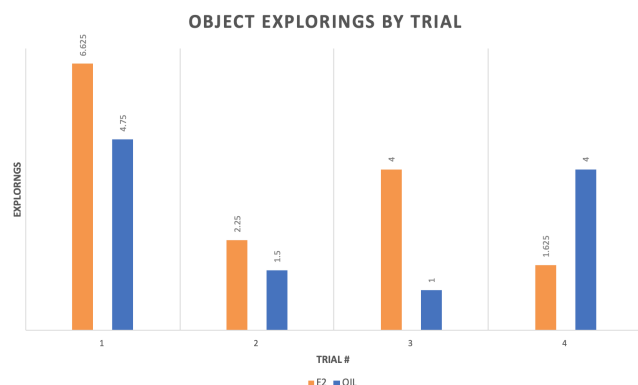


Figure 7 is the number of explorations on both of the objects in each trial.

$p = 0.2967$

Figure 8

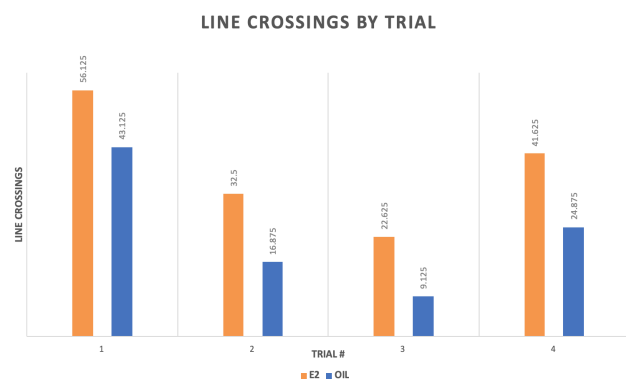


Figure 8 is the number of line crossings by each group in each trial.

$p = 0.0836$

Figure 9

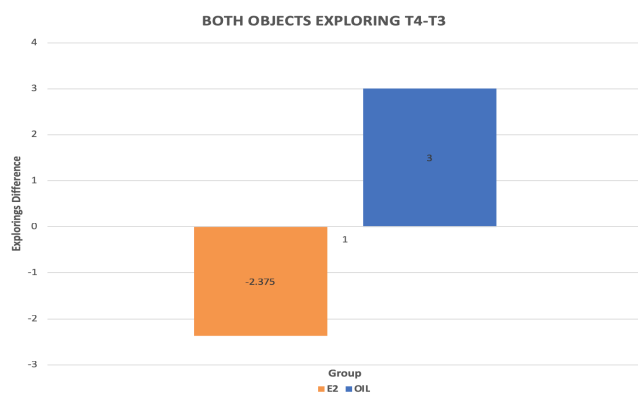


Figure 9 is the difference between trial 4 and trial 3 of the number of explorations of both objects.

$p = 0.0145$

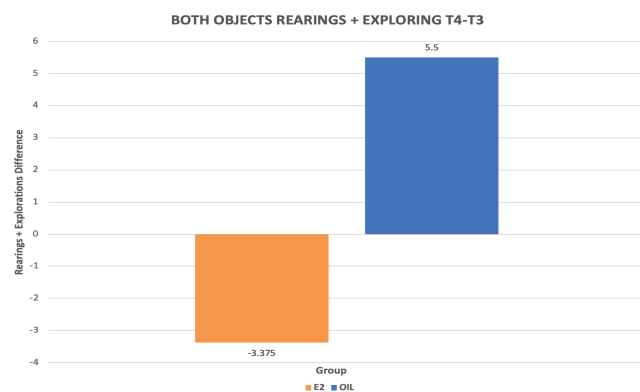
Figure 10

Figure 10 is the difference between trial 4 and trial 3 of the number of rearings and explorations of both objects.

$p = 0.0211$

Discussion:

Environmental Results:

Evaluation of distance traveled by this animal showed no statistical significance. This suggests the mobility of animals was not affected by hormonal status. There was no correlation between the rats and their treatment type on the distance they travelled, their activeness in exploring, and line crossings (Figure 1, 2 and 3).

We found a significant difference when the environment changed. Between the third and fourth trials, we found that estrogen group's exploring and rearing declined (Figures 4 and 5). This is indicated by the fact that the difference is negative, meaning the E2 group reared more in the third trial. Interestingly, the OIL group barely had a difference in rearing and exploring between trials (Figures 4 and 5).

Metric Results:

There was no correlation between the rats and their treatment type on the distance they travelled, their activeness in exploring, and line crossings (figures 6, 7 and 8).

We found a significant difference between the treatment and the rearing and exploring of the objects in the third and fourth trial. Based solely on exploring, between the third and fourth trials, the E2 group declined in explorations of the objects after they were moved whereas the OIL group increased in explorations of the objects. We found similar results when we factored both explorations and rearings of the objects. In this relationship, we found that the E2 group declined and the OIL group increased between the third and fourth trials. Both of these relationships were significant.

Conclusions:

From our data we found that the estrogen group most likely noticed both changes better because their rearings and explorations declined in both the metric and environmental tests (figure 4, figure 5, figure 9, figure 10). This is likely due to habituation within the environment each trial, and decreasing in rearings and explorations as a result of increasing familiarity. One interesting result in our findings was how the OIL group declined in rearings and explorations (barely) in the environmental test, and actually increased in rearings and explorations after the third trial suggesting that the metric test is more sensitive in ovariectomized rats compared to the change in the environment.

E2 treated animals showed deficits in exploration of the new environment as well as the new position of the objects. This suggests that the new information in these tasks may come in frequencies, which are filtered by the DG. The compromised DG filtering/gating in ovariectomized animals may have advantages in some tasks but disadvantages under other conditions, i.e., pathological processes.

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