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Investigating Sex Differences in Cognitive Deficits Resulting from 24-hour Water Deprivation

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Abstract

Dehydration can negatively affect cognitive ability. Females, however, may be protected from impairments in cognitive performance associated with dehydration due to the influence estrogens have on fluid balance and cognition. Previous work in our lab demonstrated that water deprivation impaired performance in a novel object recognition task in females during times of low, but not high, estrogen levels, suggesting that estrogens protect memory recall during dehydration. However, rats in this experiment were only dehydrated in the testing phase. Whether dehydration during training impairs task performance is unclear. We, therefore, tested the hypothesis that dehydration during training impairs performance in a recognition task, but this effect will be mitigated in females with high estrogen levels. Male, diestrous (low estrogens) female, and proestrous/estrous (high estrogens) female rats were tested with a novel object recognition task. Twenty-four hours after a habituation trial, there was a training trial where rats were exposed to two identical objects in an open-field box. Subjects were trained in either a euhydrated state or in a 24-hour water deprived state. Immediately after training, water bottles were reintroduced. After 4 hours, animals underwent a testing trial where they were exposed to one of the original objects and a novel object and were allowed to investigate for 3 minutes. Females had higher activity levels than males ($p<0.05$), but there was no difference in activity between hydration states. Euhydrated rats spent more time exploring the novel object than the original object ($p<0.05$). Dehydrated diestrous females spent more time exploring the novel object, compared to the original object ($p<0.05$). Males and proestrous/estrous females showed no increase in time exploring the novel object than the original object. Estrogens did not appear to have a protective effect in female rats; in fact, high estrogen females and males were impaired after dehydration, while low estrogen females did not show any impairment after dehydration. This is opposite to what we predicted and suggests that males are cognitively impaired by dehydration and that in certain paradigms estrogens can enhance dehydration-induced cognitive impairments.

Introduction

Fluid balance in the body is maintained through various strategies involving both the central and peripheral nervous system, including changes in blood pressure, fluid intake, saline intake, and fluid excretion. Alteration in fluid balance can lead to dehydration. Osmotic, or intracellular dehydration, is caused by an increase in solutes in the extracellular space, thereby causing a loss of intracellular fluid through osmosis. In response to osmotic dehydration, water intake increases, while urination and saline intake are minimal. Hypovolemic, or extracellular dehydration, is caused by a loss of extracellular fluid. In response to hypovolemic dehydration, both fluid intake and saline intake are increased, but again urination is suppressed to conserve fluids. In cases of water deprivation, both osmotic and hypovolemic dehydration occur (Santollo & Daniels, 2015).

Estrogens play a protective role in fluid balance. In general, female rats drink less than male rats. Additionally, the fluid intake of intact female rats fluctuates in correspondence with the estrous cycle; There is about an 8-10% decrease in water intake during estrus. Similarly, there is also a decrease in saline intake during estrus. In ovariectomized rats, water intake increases. These changes can be attributed specifically to the hormone estradiol (Santollo & Daniels, 2015). Moreover, estrogen receptors are found in organs, such as the kidney and brain, that detect fluid balance. The hormones mediate both fluid regulation and protective measures when homeostasis is disturbed by osmotic changes or by volume changes. This is accomplished through estrogen mediate compensatory changes to cardiovascular, hormonal, or behavioral responses (Curtis, 2009). In cases of osmotic dehydration, estrogen does not appear to have a strong influence on drinking response; water intake in cases of osmotic dehydration does not vary across the estrous cycle. By contrast, estrogens play a much larger role in the response to hypovolemic dehydration; water intake during hypovolemic dehydration varies across the estrous cycle, with the lowest intake during estrus (Santollo & Daniels, 2015).

Maintaining proper fluid balance is critical for a number of physiological and psychological reasons. Of importance to this research, moderate and severe dehydration

has been shown to cause decreased cognitive function in human males and females. Humans show a decrease in cognitive abilities, including short term memory, and an increase in fatigue. In one study, Cian et al. dehydrated men by either exercise or heat exposure. Dehydrated subjects showed a decrease in performance several cognitive tasks. Response time for perceptual tasks increased, and short-term, but not long-term, memory was impaired in cases of 2% body water loss (Cian et al., 2001). Furthermore, there are sex differences in performance on cognitive tasks during dehydration. When both men and women underwent an exercise induced mild dehydration treatment, both groups reported increased thirst and negative mood. Both groups performed worse during cognitive tasks, but women were impaired to a lesser degree (D'anci et al., 2009). In a similar study, moderate dehydration was induced in men and women through 24-hour water deprivation. During subsequent cognitive tests, women showed prolonged reaction time while men did not (Szinnai et al., 2005). However, there have been inconsistencies in the literature over the extent of deficits caused by mild to moderate dehydration in humans. While Patel et al. reported a significant decrease in visual memory during moderate dehydration, Stechenfeld et al. found similar deficits in mild dehydration as well (Patel et al., 2007; Stachenfeld et al., 2018). Some studies found no relation between hydration status and cognitive performance. One study reported no change in short term memory or reaction time in men during moderate dehydration when compared to a hydrated control (Tomporowski et al., 2007). Similarly, Turner et al. found no difference in cognitive performance in dehydrated women and hydrated women (Turner et al., 2017). However, these inconsistencies may be due to the small sample sizes used, differences in method used for achieving dehydration, and the differences in cognitive tests (Adan 2012). Thus, an animal model that may aid in removing these discrepancies since they can be performed under more controlled conditions.

In rodents, dehydration resulting from 24-hour water deprivation has been shown to decrease cognitive performance in males. Faraco et al. used a Y maze to examine the effects of dehydration on spatial memory. Male mice were dehydrated for either 24 or 48 hours and allowed two arms of a Y maze during training and were allowed access to a novel third arm during the testing period. The mice were both trained and tested in a

dehydrated state. When tested with the Y maze, these mice spent less time and made fewer visits to the novel arm than the control animals, indicating that male mice have decreased spatial memory during dehydration (Faraco et al., 2014). While the study showed that dehydration decreased cognitive performance, several questions remain. First, the experiment only tested spatial memory. Whether dehydration affects other forms of cognition, such as object recognition, was not addressed. Second, since the mice were dehydrated during both training and testing, it is uncertain whether dehydration affects memory formation or retrieval. Finally, the experiment only tested male mice. Therefore, it is unclear whether sex influences cognitive performance during dehydration.

Previous work in our lab sought to answer these questions. Our previous study examined both osmotic and hypovolemic dehydration and its effect on memory with a 5-day novel object recognition paradigm. This paradigm consisted of one habituation day, three training days, and a testing day. The extended multiple training trials was targeted to enhance performance during testing trials. We tested males, high estrous females, and low estrous females to investigate possible sex differences, and rats were only dehydrated during the testing period. The study demonstrated that dehydration impaired performance in a novel object recognition task in male rats and female rats during times of low, but not high, estrogen levels, suggesting that sex does influence memory recall during dehydration. These effects were only seen in rats that underwent hypovolemic dehydration. Osmotic dehydration in rats did not show cognitive deficits (Santollo et al., 2019). Follow up studies repeated these experiments with ovariectomized females and castrated males. The loss of testicular hormones after castration did not affect cognitive performance. The loss of ovarian hormones after ovariectomy did result in decreased cognitive performance. This further supports the hypothesis that ovarian hormones, such as estrogens, may play a protective role in cognition during dehydration.

Estrogens also play a role in memory formation and retrieval, therefore it is not surprising that estrogens appeared to provide protection against dehydration-induced memory impairments. Estrogen influences hippocampal plasticity, neurogenesis, and long-term potentiation. Both short term exposure to and chronic treatment with

estradiol increases hippocampal cell proliferation in ovariectomized females (Galea et al., 2008). Administering high levels of estrogens to gonadectomized males also increased cell proliferation (Galea et al., 2008). This increase in proliferation can enhance spatial memory and long-term memory. Injection of estradiol directly into the hippocampus directly modulated multiple forms of learning and memory, including reference memory, working memory, spatial memory, and place preference. Estrogens may also influence the amygdala, prefrontal cortex, and nucleus accumbens (Galea et al., 2008). In addition, the hormone may be vital to both spatial and nonspatial memory. Estrogen has been shown to modulate many hippocampal dependent cognitive behavior (Spencer et al., 2008). Estradiol, in addition, influences several aspects of novel object memory by acting on reward centers. Following infusions of estradiol, ovariectomized rats had decreased accuracy on delayed non-matching to sample tasks, but novelty preference during a novel object preference test was increased (Gervais et al., 2016).

While our previous research demonstrated that sex and ovarian hormones can influence dehydration-induced memory impairments, many open questions remain. Whether the cognitive effects of estrogen effect memory consolidation or memory recall during dehydration is unclear. This project will further examine the protective effects of estrogens on cognitive performance after 24-hour water deprivation. We first sought to confirm the efficacy of a one day testing paradigm. Since rats underwent a novel object recognition test in a hydrated state after a dehydrated training period, a one day testing paradigm was needed to prevent unnecessary prolonged periods of dehydration in the subjects. We then examined the effects of estrogens on cognition during water deprivation. Male rats, and female rats in both high estrous and low estrous states were used to test the effects of both dehydration and estrogens on cognition in a novel object recognition test. We hypothesized that dehydration during training impairs performance in a recognition task, but this effect will be mitigated in females with high estrogen levels.

Methods

Animals – 60-80 day old age-matched male and female Sprague-Dawley rats (Charles Rivers Laboratory) were used. Animals were age-matched upon arrival into the facility. During the early parts of the light phase, animals were weighed and estrous cycle was monitored by vaginal cytology as previously described (Santollo et al., 2017). After vaginal cytology samples were collected, stage of estrous cycle was determined by the appearance of cells. Diestrus 1 was characterized by leukocytes and clusters of cornified cells, diestrus 2 was characterized by leukocytes and nucleated epithelial cells, proestrus was characterized by nucleated epithelial cells, and estrus was characterized by an abundance of cornified cells (Santollo et al., 2017). Cycle stage determinations corresponded to the previous 12-h dark phase and the subsequent 12-h light phase (Becker et al., 2005). Animals were pair housed in a standard shoebox until three days prior to behavioral tests. All animals had *ad libitum* access to standard rat chow and water prior to the commencement of behavioral tests. The colony room was maintained on a 12:12 h light dark cycle (lights off at 1900 h). All experimental protocols were approved by the Animal Care and Use Committee at the University of Kentucky, and the handling and care of individual animals was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

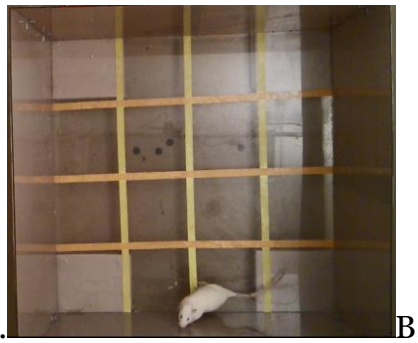
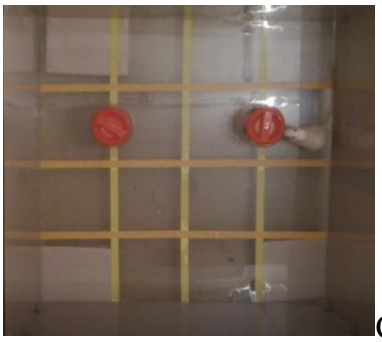
A.  B.  C. 

Figure 1. Open field set up for habituation (A), training (B), and testing (C) trials.

Experiment 1: Identify a one day novel object recognition paradigm that is effective in male and female rats. – A one day novel object recognition paradigm was performed in an open field measuring 75 cm (w) × 75 cm (l) × 45 cm (h). Grid lines on the open field divided it into 16 equal squares used to assess activity. All rats were habituated to the empty open-field box for a 10 min period on the day prior to testing (Figure 1A). The

following day consisted of a 3-min training task during which rats were exposed to two identical objects in the open-field box (Figure 1B). A four-hour interval period separated the training and testing task (Luine 2015). During the 3-min testing trial following the interval, subjects were exposed to one copy of the original object and a novel object (Figure 1C). Habituation and training occurred during the first half of the light cycle. Testing occurred in the later half of the light cycle. The objects used were either (A) a clear plastic cylindrical jar as the original object and a martini glass as the novel object, or (B) a green bulb shaped spray bottle as the original objects and a blue cylindrical spray bottle as the novel object. All objects were similar in height and width. Objects were secured to the floor of the open field with magnets to prevent them being knocked over or moved. Both male (n = 7) and female rats (n = 7) were used; estrus levels were not used for this experiment. All rats were kept in a euhydrated state.

Experiment 2: Does 24-hour water deprivation impair object recognition memory in male and female rats? – The same novel object recognition protocol in experiment one was followed, using the clear plastic cylindrical jar as the original object and a martini glass as the novel object. Male, diestrous (after low estrogen exposure) female, and proestrous/estrous (after high estrogen exposures) female rats were tested; each group consisted of 8 rats. After habituation, water bottles were removed for half of the subjects. After the training task the following day, all water bottles were weighed, and water was reintroduced to the water-deprived rats. After a 4-hour interval, water bottles were reweighed, and subjects were put back into the open field for a test trial.

NOR Scoring – Videos were taken of all habituation, training, and testing trials and were scored to record the overall activity and object exploration (seconds). Activity was defined as the number of boxes crossed. A box was considered crossed when both front paws had crossed over a grid line. Object exploration was defined as time spent interacting with the object (touching the object with the animal's head or front paws, licking, and sniffing the object). Touching of the object with hind paws or tail was not counted (Antunes & Biala, 2012). Videos were scored by at least two individuals blind to experimental conditions. There was an inter-scorer reliability of at least 90%.

Data Analysis – Data is presented as mean ± SEM. A significance value of $p < 0.05$ was used throughout. Intake, activity, and object exploration were analyzed with a 2 factor ANOVA (group X hydration) using the statistical software package Statistica with Fisher's post hoc tests used to follow up any significant findings.

Results

Experiment 1: Identify a one day novel object recognition paradigm that is effective in male and female rats.

For positive control, activity level was recorded. As expected, female rats had significantly higher levels of activity than males in the open field during test day (Figure 1, $p < 0.05$, data collapsed across testing groups).

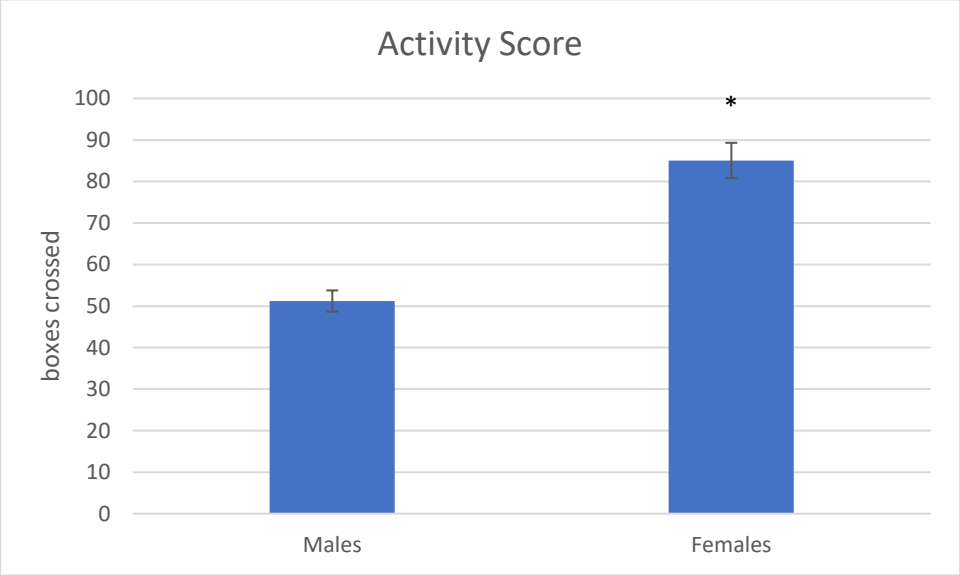


Figure 1. Activity level in the open field during training. Female rats had a significantly higher level of activity than male rats. *Greater than males, $p < 0.05$.

First, we used blue and green bottles as the objects in the novel object recognition test. When the blue and green bottles were used as objects, there was no significant increase in time spent by male rats with the novel object when compared to the original object (p = n.s.). Additionally, there was no significant increase in time spent with the novel object by female rats when compared to the original object (p = n.s.. ;Figure 2). Pilot

studies indicated that there were no differences in exploratory behavior or preference between the objects.

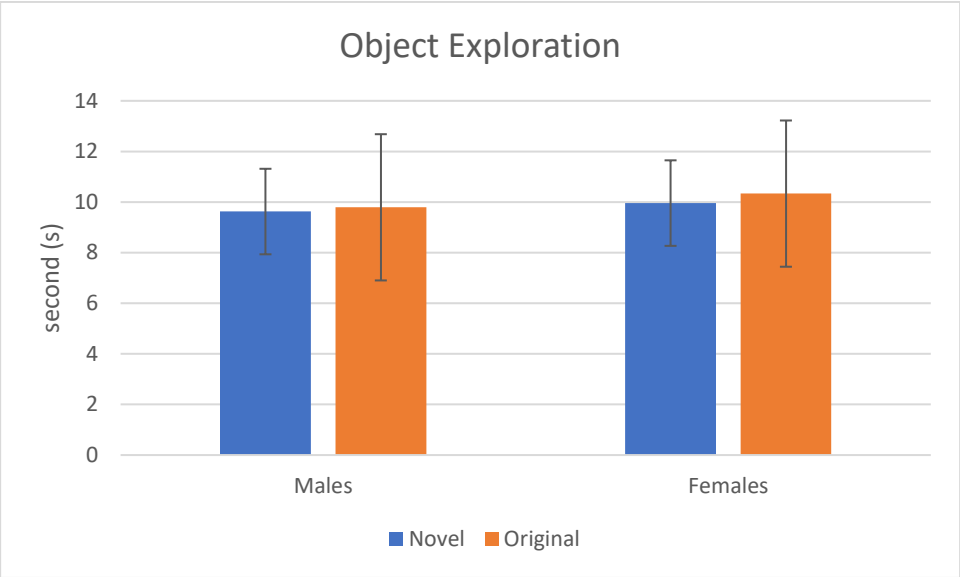


Figure 2. Time investigating original and novel objects when bottles were used. Regardless of sex, neither group spent more time exploring the novel object than the original object.

Next, we used a clear plastic cylindrical jar and a martinini glass as the objects in the novel object recognition task. When the clear plastic cylindrical jar was used as the original object and a martini glass as the novel object, there was a significant difference between object exploration of the novel and original object, regardless of sex (Figure 3). Male rats spent significantly more time investigating the novel object than the original object ($p < 0.05$). Similarly, female rats spent significantly more time investigating the novel object than the original object ($p < 0.05$).

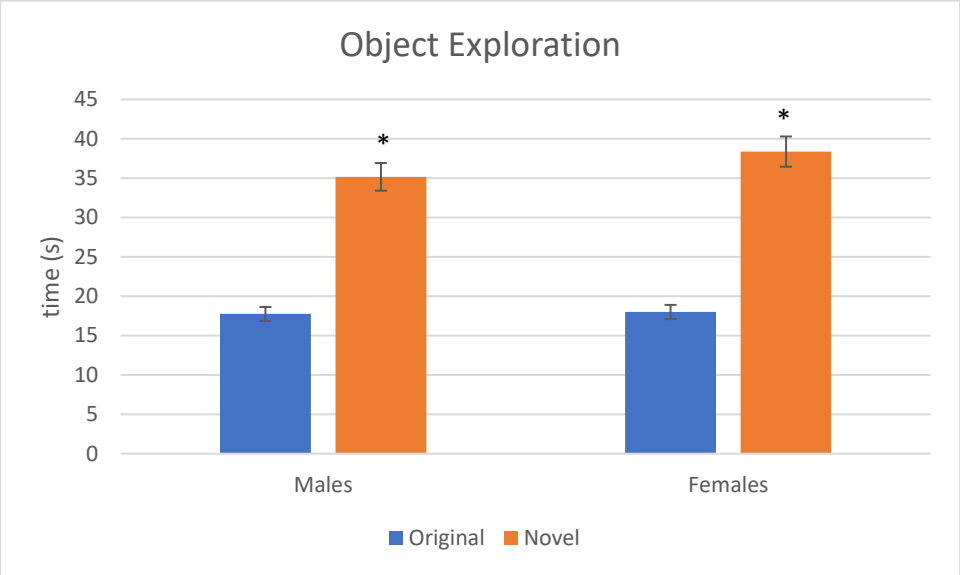


Figure 3. Time investigating original and novel objects when jar and glass were used, respectively. Regardless of sex, both groups spent more time exploring the novel object than the original object. *Greater than original object, $p < 0.05$.

Experiment 2: Does 24-hour water deprivation impair object recognition memory in male and female rats?

To confirm that water deprivation induced dehydration, intake was measured 4-hours after water was reintroduced. As expected, animals that underwent 24-hour water deprivation consumed significantly more water than control animals, regardless of sex or stage of estrous cycle $F(1,42) = 113.91$, $p < 0.001$(Figure 4). There was no effect of group, $F(2,42) = 2.58$, $p = 0.087$, nor an interaction between group and hydration, $F(2,42) = 1.58$, $p = 0.217$.

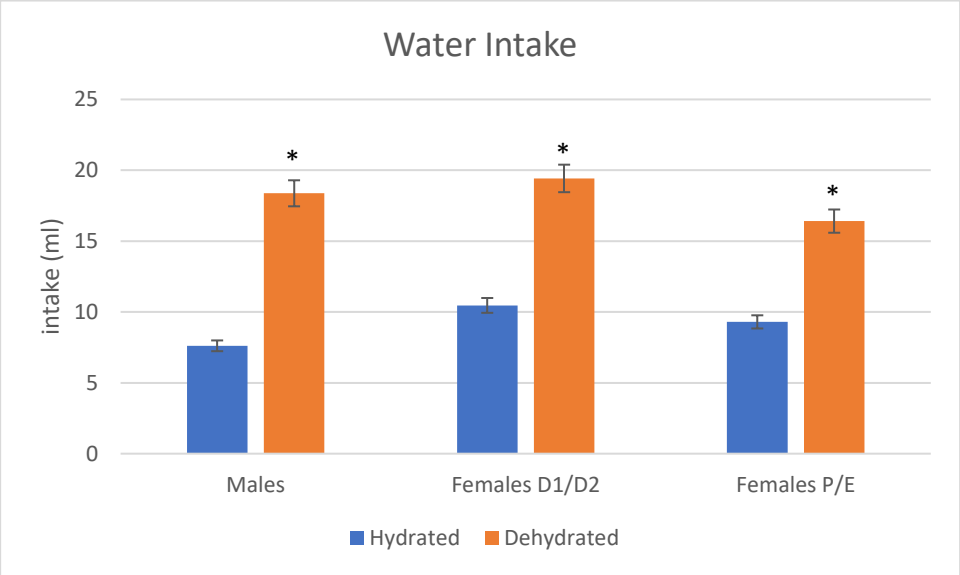


Figure 4. Water intake after novel object training. Regardless of sex or stage of estrous cycle, more water was consumed by dehydrated rats than euhydrated rats. *Greater than euhydrated control-group, $p < 0.05$

As a further positive control, activity level was measured to ensure that dehydration did not influence gross motor function and in turn impact object exploration during the testing trial. As expected, activity was influenced by group, $F(2,42) = 7.83$, $p = 0.001$. Both female groups showed higher levels of activity than males (Figure 5). There was no effect of hydration state, $F(1,42) = 0.056$, $p = 0.813$, nor an interaction between group and hydration, $F(2,42) = 0.063$, $p = 0.938$.

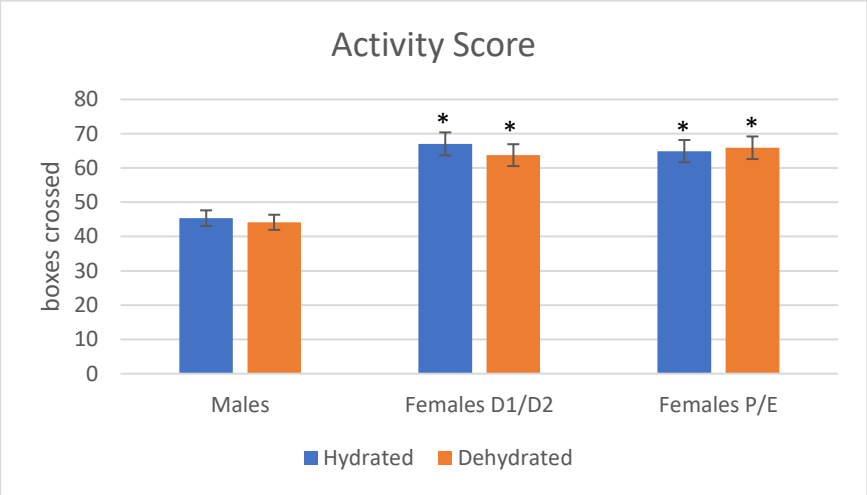


Figure 5. Activity level in the open field during testing. Regardless of hydration level, activity was higher in both groups of female rats compared to male rats. *Greater than males, $p < 0.05$.

Time spent exploring the objects was influenced by the object, $F(1,42) = 30.71$, $p < 0.001$. As excepted, time spent investigating the novel object was greater than time spent investigating the control object. There was no effect of Group, $F(2,42) = 1.58$, $p = 0.19$, nor any interactive effects of Group X Hydration, $F(2,42) = 0.14$, $p = 0.86$, Object X Group, $F(2,42) = 1.93$, $p = 0.15$, or Object X Hydration, $F(1,42) = 0.98$, $p = 0.32$. Furthermore, there was no effect of Object X Group X Hydration, $F(2,42) = 0.054$, $p = 0.94$, however, a priori we predicted there would be differences in time spent investigating the control and novel objects as a function of group and hydration status. A priori post hoc analysis revealed that all control animals spent significantly more time exploring the novel object than the than the original object ($p < 0.05$). Both dehydrated males and dehydrated proestrous/estrous females showed no significant difference between time spent exploring the novel object than time spent exploring the original object (p = n.s.). Surprisingly, diestrous females spent significantly more time exploring the novel object than the original object (Figure 6, $p < 0.05$).

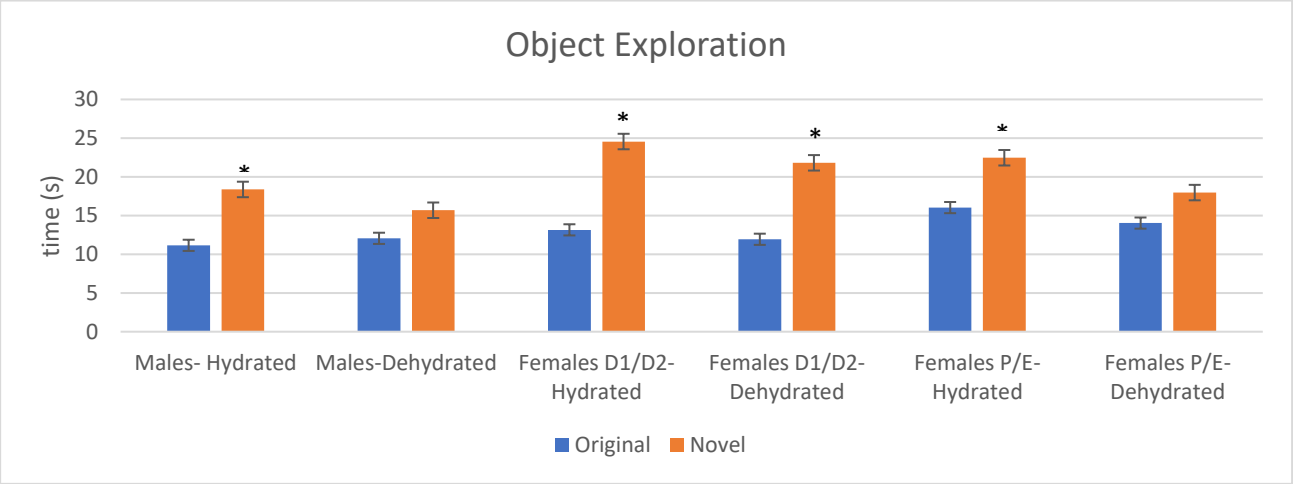


Figure 6. Time investigating original and novel objects. Regardless of sex or stage of estrous cycle, euhydrated rats spent more time exploring the novel object than the original object. Of the dehydrated groups, only D1/D2 females spent more time exploring the novel object than the original object. *Greater than original object, $p < 0.05$.

Discussion

This study sought to further examine the effects of dehydration on cognition and to elucidate the effects of sex on the cognitive effects of dehydration. This was done through a novel object recognition task in which rats were dehydrated during the training trial and hydrated during the test. Male, low estrous females, and high estrous females were used. Overall, this experiment demonstrated that dehydration negatively affected cognition in males and high estrous females, and that ovarian hormones may enhance the inhibitive effects of dehydration on cognition in some paradigms. This result was a surprise and opposite of what we predicted.

As expected, rats that underwent 24-hour water deprivation had a higher intake of water than the control. This served as a positive control, confirming that dehydration was indeed achieved through water deprivation. In the case of 24-hour water deprivation, both osmotic and hypovolemic dehydration was induced (Santollo & Daniels, 2015).

Females, regardless of hydration status, had higher activity levels than males. This was true of all trials in both experiments. This aligns with previous research which shows

that females generally have significantly higher levels of locomotion than males (Hyde & Jerussi, 1983). Within hormone groups, hydration status did not impact overall activity levels. This served as an additional positive control to ensure that object exploration was not itself was not influenced by dehydration.

In the first experiment, regardless of sex, there was no significant difference in the time spent with the novel object compared to the original when the blue bottle and the green bottle were used as the novel and original object. Thus, rats were unable to learn the novel object recognition task. The task may have been too difficult, either due to the objects used or the length of the time interval. There was a tendency of both male and female rats to spend slightly more time with the novel object than the original, suggesting some but not all rats may have been able to successfully discriminate between the novel and original object.

When the clear jar and the martini glass were used as the original and novel object, both male and female rats spent significantly more time exploring the novel object than the original object, indicating that both groups of rats were able to learn the task and successfully discriminate between the novel and original object. Thus, the two spray bottles used in the first trial were likely too similar in shape and thus too difficult for the rats to discriminate between. The jar and martini glass were much more different in shape and therefore easier to discriminate between.

In the second experiment, all hydrated hormone groups spent significantly more time interacting with the novel object than the original, indicating that the task was successfully learned. Dehydrated males and dehydrated proestrous/estrous females showed no significant increase in the amount of time spent with the novel object than the original, implicating both groups were cognitively impaired by dehydration. In contrast, diestrous females spent significantly more time interacting with the novel object, suggesting they were unimpaired by dehydration.

This study contrasts with our previous findings. When dehydrated during testing, male and diestrous female rats showed decreased cognitive performance, whereas proestrous/estrous females were affected to a lesser degree (Santollo et al., 2019). However, when dehydrated during the training phase, diestrous females showed less of

a cognitive deficit than proestrous/estrous females and males, both of which were cognitively impaired by the dehydration treatment. Additionally, our most recent study induced both osmotic and hypervolemic dehydration through water deprivation, whereas our previous study saw impairments only in cases of hypervolemic dehydration. This suggests that, although estrogens typically have protective effects on fluid balance and memory, some paradigms may, in fact, enhance dehydration-induced cognitive impairments. Further research should test the effects of different kinds of dehydration during training trials. Future studies should also compare previous results with the cognitive effects on subjects dehydrated during both training and testing (Faraco et al., 2014).

These results may have been impacted by the hormone grouping used (Becker et al., 2005). Estrogens peak during the proestrous phase of the estrous cycle. During the end of the estrous phase, estrogen levels have fallen significantly. Thus, since testing was done in the afternoon, animals in the estrous phase may have had low levels of estrogen. Similarly, animals in the second stage of the diestrous phase may begin to have higher levels of estrogens during the later part of the light phase. Therefore, repeating this experiment with different hormone groupings may elucidate this issue.

The differences in the results from our two studies are similar to the differences seen in human studies. Some studies in humans have reported that women performed worse than men on cognitive tasks during dehydration. Women showed both a significant decrease in both reaction time and number of errors (Adan, 2012). Further research could help us better understand the varying effects of ovarian hormones on both cognition and fluid balance.

References

Antunes, M., & Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cognitive Processing, 13*(2), 93-110.

Adan, A. (2012). Cognitive Performance and Dehydration. Journal of the American College of Nutrition, 31(2), 71-78.

Jill B. Becker, Arthur P. Arnold, Karen J. Berkley, Jeffrey D. Blaustein, Lisa A. Eckel, Elizabeth Hampson, James P. Herman, Sherry Marts, Wolfgang Sadee, Meir Steiner, Jane Taylor, Elizabeth Young. (2005). Strategies and Methods for Research on Sex Differences in Brain and Behavior. Endocrinology, 146(4), 1650-1673.

Cian, C., Barraud, Melin, & Raphel. (2001). Effects of fluid ingestion on cognitive function after heat stress or exercise-induced dehydration. International Journal of Psychophysiology, 42(3), 243-251.Curtis, K. (2009). Estrogen and the central control of body fluid balance. Physiology & Behavior, 97(2), 180-192.

D'Anci, K., Mahoney, C., Vibhakar, A., Kanter, J., & Taylor, H. (2009). Voluntary Dehydration and Cognitive Performance in Trained College Athletes. Perceptual and Motor Skills, 109(1), 251-269.

Faraco, G., Wijasa, T. S., Park, L., Moore, J., Anrather, J., & Iadecola, C. (2014). Water Deprivation Induces Neurovascular and Cognitive Dysfunction through Vasopressin-Induced Oxidative Stress. Journal of Cerebral Blood Flow & Metabolism, 34(5), 852–860.

Galea, Liisa A. M., Kristina A. Uban, Jonathan R. Epp, Susanne Brummelte, Cindy K. Barha, Wendy L. Wilson, Stephanie E. Lieblich, and Jodi L. Pawluski. 2008. Endocrine regulation of cognition and neuroplasticity: Our pursuit to unveil the complex interaction between hormones, the brain, and behaviour. Canadian Journal of Experimental Psychology 62, (4) (12): 247-60

Gervais, N., Hamel, L., Brake, W., & Mumby, D. (2016). Intra-perirhinal cortex administration of estradiol, but not an ERβ agonist, modulates object-recognition

memory in ovariectomized rats. Neurobiology of Learning and Memory, 133, 89-99.

Hyde, J., & Jerussi, T. (1983). Sexual dimorphism in rats with respect to locomotor activity and circling behavior. *Pharmacology, Biochemistry and Behavior, 18*(5), 725-729.

Luine, V. (2015). Recognition memory tasks in neuroendocrine research. Behavioural Brain Research, 285, 158-164.

Patel, A., Mihalik, J., Notebaert, A., Guskiewicz, K., & Prentice, W. (2007). Neuropsychological performance, postural stability, and symptoms after dehydration. Journal of Athletic Training, 42(1), 66-75.

Santollo, J., & Daniels, D. (2015). Control of fluid intake by estrogens in the female rat: Role of the hypothalamus. *Frontiers in Systems Neuroscience, 9*, 25.

Santollo, J., & Daniels, D. (2015). Multiple estrogen receptor subtypes influence ingestive behavior in female rodents. *Physiology & Behavior, 152*(Pt B), 431-437

Santollo, J., Myers, K., Rainer, I., & Edwards, A. (2019). Gonadal hormones in female rats protect against dehydration-induced memory impairments in the novel object recognition paradigm. Hormones and Behavior, 114, 104547.

Santollo, J., Torregrossa, A., & Daniels, D. (2017). Sex differences in the drinking response to angiotensin II (AngII): Effect of body weight. Hormones and Behavior, 93, 128-136.

Spencer, J., Waters, E., Romeo, R., Wood, G., Milner, T., & Mcewen, B. (2008). Uncovering the mechanisms of estrogen effects on hippocampal function. Frontiers in Neuroendocrinology, 29(2), 219-237.

Stachenfeld, N., Leone, C., Mitchell, E., Freese, E., & Harkness, L. (2018). Water intake reverses dehydration associated impaired executive function in healthy young women. Physiology & Behavior, 185, 103-111.

Szinnai, G., Schachinger, H., Arnaud, M., Linder, L., & Keller, U. (2005). Effect of water deprivation on cognitive-motor performance in healthy men and women. American Journal of Physiology, 58(1), R275-R280.

Tomporowski, Beasman, Ganio, & Cureton. (2007). Effects of Dehydration and Fluid Ingestion on Cognition. International Journal Of Sports Medicine, 28(10), 891-896.

Turner, J., Marsteller, D., Luxkaranayagam, A., Fletcher, J., & Stachenfeld, N. (2017). Mild exercise in female subjects impairs complex learning independent of hydration status and emotion. Physiology & Behavior, 180, 113-119.