Effects of G-protein-coupled receptor 30 agonist G-1 on spatial memory in adult female rats

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Abstract

As the expected lifespan of individuals in our society continues to rise, pathologies and diseases associated with aging are an increasing public health concern. Various therapies aimed at combating memory loss, particularly in post-menopausal women, have focused on activation of various estrogen receptors. In this study, I employed a rodent model to gain insight into potential receptor-specific therapies for treatment of spatial memory loss in post-menopausal women.

Ovarian hormones such as estradiol are known to have a complex relationship with spatial cognition. Despite many inconsistencies within the literature, estradiol can enhance spatial memory consolidation on a water maze task in female rodents when administered during a critical period immediately following training. While the rapid effects of estradiol on consolidation have been established, the mechanism responsible for them is still unknown. The primary objective of the current study was to determine if activation of the membrane bound G-protein-receptor 30 (GPR30) by estradiol is responsible for the enhanced consolidation of memory. Data were collected from forty adult, ovariectomized female rats, divided into three groups that were treated either with the GPR30-specific agonist G-1 immediately following training, G-1 two hours following training, or sesame oil vehicle immediately following training. Rats underwent training and retention trials on a water maze task with a fixed hidden escape platform. Latency to reach the platform and percent of pathlength spent in the quadrant containing the platform during the first retention trial were used as measures of successful consolidation of spatial memory. There was no significant difference in performance on the probe trials between rats treated with G-1 and sesame oil, suggesting that GPR30 activation is not sufficient to enhance consolidation of spatial memory. However, it is important to interpret these results against a backdrop of differences in experimental design which my have impacted the current results, such as differences between the existing literature and the current study including G-1 dose, type of vehicle, treatment administration regime, spatial cognition task, and the component of cognition studied.

Introduction

As the median age of our population increases, diseases and pathologies associated with aging become an increasingly relevant public health concern. Memory loss, for example, is a common symptom experienced by post-menopausal women (Devi, Hahn, Massimi, & Zhivotovskaya, 2005). One treatment of this and other menopausal changes involves administration of estrogen and progestin in the form of Menopausal Hormone Treatment (MHT). However, the risks of MHT can be high and often outweigh the potential benefits. Specifically, in many cases, treatment with estrogen and progestin causes a dangerous increase in risk of developing breast cancer (Rossouw, Anderson, Prentice, LaCroix, Kooperberg, Stefanick, Jackson, Beresford, Howard, Johnson, Kotchen, Ockene, & Writing Group for the Women's Health Initiative Investigators, 2002). The isolation of specific estrogen receptors involved in memory, such as GPR30, offers a novel method of treatment for memory loss. By targeting specific memory pathways, treatment could restore memory deficits while avoiding detrimental side effects that result from generalized treatment with estrogen and progestin.

Spatial memory is one component of cognition that can be impacted by the decrease in cognitive function experienced by many postmenopausal women. A drop-off in circulating estrogen level may contribute

to the cognitive decline associated with the onset of menopause in some women (Morrison, Brinton, Schmidt, & Gore, 2006). While the role of estrogens is clearly implicated in postmenopausal cognitive decline, the best method of treatment remains unclear. Although some studies indicate that hormone therapy can serve to protect aging women from postmenopausal cognitive decline (Sherwin & Henry, 2008), results of the large Women's Health Initiative Study (WHI) conducted by the National Institutes of Health indicated that treatment with estrogens could in fact increase an individual's risk of dementia (Daniel, 2013). Conflicting results such as these indicate an increase in the need for research into the complex relationship between estrogens and memory.

Introduction to Spatial Memory

Endogenous gonadal hormones are known to affect many behavioral functions in rodents and other mammals. One of the primary gonadal hormones in female rats, the estrogen estradiol, has been studied extensively in the context of reproductive behavior. However, estradiol circulates throughout the body and impacts many other behavioral processes including cognition (Dohanich, 2002). Learning and memory are particularly intriguing behaviors to study due to their varied and complex nature, manifesting in a variety of forms, including spatial and non-spatial, working, reference, and conditioning.

Spatial learning and memory rely on learning and remembering the relationships between surrounding cues and a site of reinforcement of a behavior (Dohanich, 2002). The ability to form these memories can be tested in rodents using many different tasks that depend on a variety of rewards and preferences. Some tasks involve the search for food rewards or escape from aversive stimuli while others rely simply on rodents' preference for novelty. Spatial memory is primarily dependent on the hippocampus and can be strongly impacted by circulating gonadal hormones (Hawley, Grissom, Martin, Halmos, Bart, & Dohanich, 2003; Daniel, 2006; Zurkovsky, 2006; Kim, Thompson, Hopkins, Kosslyn, & Squire, 2013).

Hormones and Spatial Memory

Female rats express enhanced memory on spatial tasks during proestrus when ovarian hormones are naturally elevated (Berry, McMahan, & Gallagher, 1997; Walf, Rhodes, & Frye, 2006). Treatment with 17β-estradiol has successfully reproduced this effect in ovariectomized female rats (Walf et al., 2006; Hammond, Mauk, Ninaci, Nelson, & Gibbs, 2009; Sandstrom & Williams, 2011). For example, 17β-estradiol can enhance hippocampal spatial memory on novelty-driven tasks such as the Y-maze task (Conrad, Jackson, Wieczorek, Baran, Harman, Wright, & Korol, 2004) and object location task (Gresack & Frick, 2006), food-driven tasks such as the radial arm maze task (Daniel, Fader, Spencer, & Dohanich, 1997), and escape-driven tasks such as the water maze task (Packard & Teather, 1997; Packard, 1998).

Exogenous 17β-estradiol has also been shown to have significant effects on hippocampal dendritic spine density in patterns that mimic the natural fluctuations in spine density that occur during the estrous cycle (Gould, Woolley, Frankfurt, & McEwen, 1990; González-Burgos, Alejandre-Gómez, & Cervantes, 2005). These structural fluctuations not only increase the density of synapses in CA1 pyramidal cells, but also enhance spatial memory (Woolley, Weiland, McEwen, & Schwartzkroin, 1997; Li, Brake, Romeo, Dunlop, Gordon, Buzescu, Magarinos, Allen, Greengard, Luine, & McEwen, 2004; Wu, Bryant, Dorsa, Adelman, & Maylie, 2013).

Memory: Acquisition and Consolidation

Memory formation is a multi-step process that involves elements of pre-training, training, consolidation, and retention (Dohanich, 2002). These steps are constantly interacting with one another and each plays a vital role in successful learning and memory. The *acquisition* component of cognition is the most difficult to isolate and study independently of other variables. The wide variety of methods of learning that can be employed by a learner during the acquisition stage makes it particularly susceptible to the effects of non-mnemonic factors, such as anxiety, activity, hunger, or thirst. Such non-mnemonic factors are also often affected by circulating hormones, and consequently, hormone treatments may not alter cognitive processes directly but rather affect non-mnemonic factors that then influence cognitive performance (Dohanich, 2002).

Despite the potentially significant confounding influence of non-mnemonic factors on acquisition,

studies involving pre-training administration of 17β-estradiol are the most common. Although there is strong evidence that the proper dose of 17β-estradiol can enhance spatial memory under certain conditions, these effects are highly labile (Dohanich, 2002). Therefore, studies indicating enhancement of acquisition through administration of 17β-estradiol must be interpreted against a backdrop of non-mnemonic factors such as anxiety that can affect performance on spatial tasks (Daniel et al., 1997; Holmes, 2002; Sinopoli, 2006).

The *consolidation* phase of memory takes place soon after training is complete, when information undergoes encoding as memory. This process involves the hippocampus transferring information from short-term memory to long-term memory (Winocur, 2013). In order to determine the underlying mechanisms at work during consolidation, treatments can be administered immediately following the completion of training. Packard (1998) conducted experiments on the effects of post-training 17β-estradiol administration on memory consolidation. Rats underwent eight training trials on a hidden platform water maze task and received post-training injections of 17β-estradiol either immediately following training or after a delay of two hours. Administration of 17β-estradiol immediately after training trials on the water maze task improved spatial memory in ovariectomized female rats; however, the delayed treatment did not (Packard & Teather, 1997; Packard, 1998). The time-dependent nature of this enhancement suggests that consolidation of learning occurs rapidly, within two hours following training. Thus, enhancement of consolidation occurs more quickly than would be expected if the effects of 17β-estradiol were mediated by intracellular estrogen receptors, indicating the putative involvement of some form of membrane estrogen receptor.

Estrogen Receptors: ERα, ERβ

Activation of the classical estrogen pathway involving intracellular estrogen receptors, ERα and ERβ is the most commonly studied with regards to spatial memory. The process involved in activating ERα and ERβ begins with estradiol binding to estrogen receptors located in the cell cytoplasm. The receptor complex then dimerizes and translocates from the cytoplasm to the cell nucleus. Once inside the nucleus, the complex binds with DNA, eventually resulting in RNA-dependent synthesis of proteins that can exert a multitude of effects on many different biological processes (Moss, Gu, & Wong, 1997). This classic transcriptional estrogen pathway is found throughout the body and its activation has been extensively studied in the context of learning and memory.

Administration of specific agonists that bind to ERα and ERβ receptors has been shown to improve acquisition of spatial memory on many different tasks (Hammond et al., 2009). However, because these receptors are intracellular and their genomic effects require hours or even days to appear (Moss et al. 1997, McEwen & Alves 1999), intracellular receptors cannot account for the more rapid effects of exogenous 17β-estradiol on consolidation as shown by Packard and others (Packard & Teather, 1997; Packard, 1998; Gresack & Frick, 2006).

Estrogen Receptors: GPR30

The rapid enhancement of spatial memory consolidation by estradiol implicates the involvement of a non-transcriptional membrane receptor. The putative estrogen receptor G protein-coupled receptor 30 (GPR30) is one such receptor that has recently been found to be involved in rapid cell signaling (Hasbi, O'Dowd, & George, 2005; Renvakar, Cimino, Sklar, Arterburn, & Prossnitz, 2005). GPR30 is positioned to mediate estrogen effects on spatial learning and memory due to its abundance in the hippocampus (Brailoiu, Dun, Brailoiu, Mizuo, Sklar, Oprea, Prossnitz, & Dun 2007; Hazell, Yao, Roper, Prossnitz, O'Carroll, & Lolait, 2009; Akama, Thompson, Milner, & McEwen, 2013). In ovariectomized rats, pre-training administration of the GPR30-specific agonist, G-1, restored spatial learning similar to effects seen after treatment with ERα and ERβ specific agonists (Hammond et al., 2009) and repeated short-term administration of GPR30 agonist G-1 enhanced retention on the Y-maze task (Hawley, Grissom, Moody, Dohanich, & Vasudevan, 2014). In addition, the GPR30-specific antagonist, G-15, impaired spatial learning when administered pre-training to intact female rats (Hammond, Nelson, Kline, & Gibbs, 2012). While there is increasing evidence for GPR30 mediation of spatial memory acquisition, there is a deficit in the knowledge regarding the role of GPR30 in spatial memory consolidation. As noted above, consolidation is a time dependent process that occurs immediately after training is complete,

implicating a rapidly-activated estrogen pathway. Due to the location of GPR30 in the cell membrane and the previously observed rapid effects of GPR30 activation, it is reasonable to suggest that GPR30 plays a role in mediating rapidly-signaling estrogen pathways. Therefore, post-training administration of the agonist G-1 to ovariectomized female rats might enhance consolidation of hippocampal-dependent spatial memory just as post-training administration of 17β-estradiol, as reported originally by Packard (1998).

This hypothesis was tested using a water maze procedure similar to that used by Packard (1998) to study memory consolidation after post-training administration of 17β-estradiol. We administered G-1 post-training either immediately after training trials or after a two-hour delay on the standard water maze task, and then conducted probe trials 24 hours after training to assess potential enhancement of spatial memory. We predicted that, in ovariectomized rats, post-training administration of the GPR30 agonist G-1 would enhance spatial memory consolidation, indicated by shorter escape latencies and higher percent of pathlength spent in the platform quadrant on probe trials administered 24 hours after training and G-1 treatment.

Methods

Subjects

Tulane University Institutional Animal Care and Use Committee approved all procedures in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (1996). Female Long Evans rats, divided into two waves (N=47; N_{w1}=23, N_{w2}=24) obtained from Harlan Inc. (Indianapolis, IN), arrived at approximately 65 days of age. Rats were housed in groups of two in clear plastic cages with free access to food and water in the Tulane University vivarium, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AALAC). The rats were kept on a 12:12 hour light-dark cycle with lights on at 07:00 hours. Rats were acclimated to the vivarium facilities for one week before undergoing surgery. All methods were carried out identically for both waves of rats.

Surgery

At approximately 72 days old, rats were ovariectomized under aseptic conditions by three experienced student researchers. The procedure was carried out under anesthesia induced by intraperitoneal injections of ketamine (100 mg/kg, Fort Doge Animal Health, Fort Dodge, IA) and xylazine (7 mg/kg, Miles Laboratories, Shawnee, KS). Bilateral dorsal ventral incisions were made approximately 5 cm from the most posterior point of the rib cage. Ovaries were removed, blood vessels were ligated, the muscle wall was closed with 4.0 silk sutures (Teleflex Medical, Kenosha, WI), and the skin was closed with titanium wound clips (Mercer Glasssware Inc., New York, NY). Post-surgical care included access to drinking water containing ibuprofen (25 mg/kg) for three days after surgery.

Testing and Treatment

Before testing began, one researcher handled rats for 60 seconds each day for seven consecutive days beginning one week after surgery to acclimate the rats to experimenters. Rats then underwent spatial learning and memory testing on the water maze task. The maze consisted of a white circular galvanized pool, 180 cm in diameter and 60 cm in depth, filled approximately 32 cm deep with water at 25°C made opaque by addition of non-toxic white tempera paint (Crayola). A 10-cm diameter movable Plexiglas escape platform was submerged approximately 2 cm below the surface of the water. Various fixed visual cues of two and three dimensions were located around the water maze to assist rats in learning their spatial locations. All trials were recorded by a video camera suspended above the maze and interfaced with a computerized tracking system (HVS Image™) that measures escape latency and pathlength to reach the platform and percentage of time spent in quadrants.

Rats underwent eight training trials starting from 4 different randomized entry points (N, S, E, W) with the submerged platform always in the same location (Fig. 1). For each trial, a rat was allowed 60 seconds to find the platform and 15 seconds on the platform to observe the extra-maze cues. If a rat was unable to find the platform in the time allotted, the researcher guided her there by hand. Following the last training trial, each rat received either a post-training intramuscular injection of sesame oil (0.1 ml, Sigma-Aldrich Co., St.

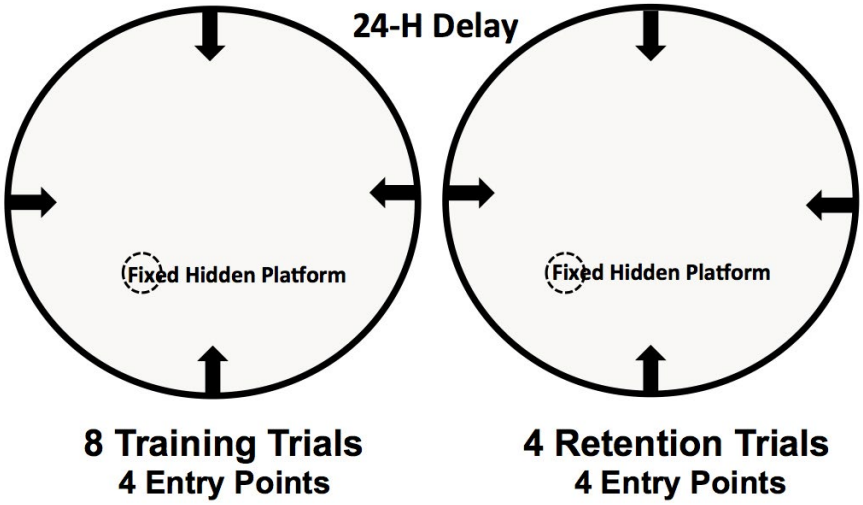

Figure 1. Layout of the water maze task

Louis, MO) or G-1 (50 μg/kg) suspended in sesame oil (0.1 ml) immediately following training, or an intramuscular injection of G-1 (50 μg/kg) in sesame oil (0.1 ml) two hours after the completion of training. Twenty-four hours later each rat underwent 4 probe trials with the escape platform in the same location. A team of six experienced student researchers conducted all testing identically.

Statistical Analyses

Analyses of variance (ANOVA) were conducted to determine differences in latency and percent of pathlength that was spent in the quadrant containing the platform (quadrant 1) during the first probe trial between conditions. Additional ANOVA tests were conducted on latency and percent of pathlength in quadrant 1 for the first probe trial each wave to determine if there were differences in group performance between waves. A repeated measures ANOVA was conducted to confirm the successful acquisition of the task during training trials by all groups. Statistical significance was indicated by $p \leq 0.05$, weak trends were indicated by $p \leq 0.3$. Rats that did not learn the task (indicated by outlier status for thigmotactic behavior on training trial eight), rats that demonstrated significant anxiety during training (indicated by repeated excessive squeaking during handling), and rats that had problematic injections were excluded from all analyses.

Results

Acquisition

Pathlengths to reach the platform over the eight training trials were analyzed to confirm that all groups learned the water maze task equally well, prior to treatment. Repeated measures ANOVA on latencies and pathlengths to reach the platform during the first probe trial revealed no significant differences in acquisition between the three treatment groups (Fig. 2; Fig. 3).

Comparison of Conditions

ANOVA tests on latencies and percent pathlengths in the target quadrant, quadrant 1, for the first probe trial were not significantly different between conditions (Fig 4. $F(2,26)=0.352$, $p=0.707$; Fig 5. $F(2,26)=0.703$, $p=0.504$). Exploratory analyses were conducted comparing conditions in a paired manner and weak trends were indicated by percent pathlengths in quadrant 1. Specifically, when immediate and delayed G1 injection groups were compared, a weak trend toward higher percent pathlength in quadrant 1 was revealed for rats administered G-1 immediately following training (Fig 5. line, $F(1,15)=1.394$, $p=0.256$).

Comparison of Waves

In order to address the possibility of differences between the two waves of rats that were tested three weeks

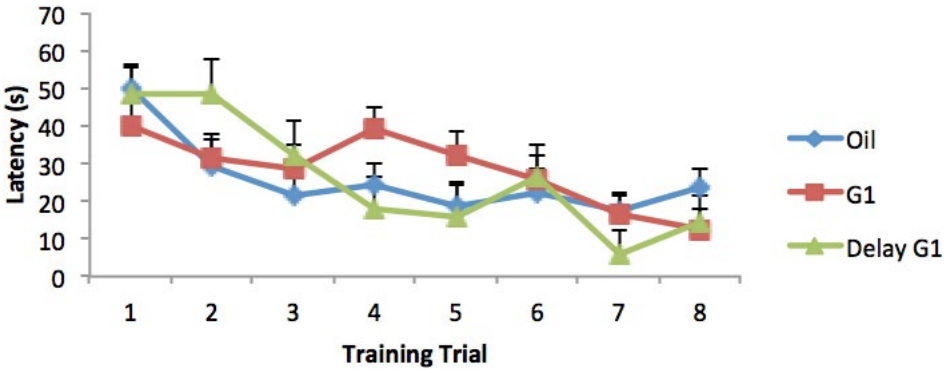

Figure 2. Latencies to reach the hidden platform over 8 training trials for all three treatment conditions decreased over time indicating that all groups learned the task.

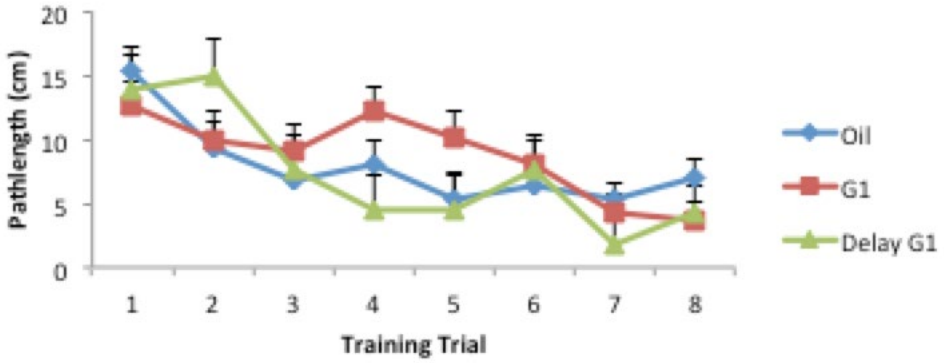

Figure 3. Pathlengths to reach the hidden platform over 8 training trials for all three conditions decreased over time indicating that all groups learned the task.

apart, ANOVA tests were run on latencies and percent pathlength in quadrant 1 by wave. Analysis of wave 1 showed trends toward shorter latencies and higher percent pathlengths in quadrant 1 for rats administered G-1 compared to rats administered sesame oil (Fig 6. $F(1,9)=1.802$, $p=0.212$; Fig 7. $F(1,9)=2.046$, $p=0.186$) that are not evident in the analysis of wave 2 (Fig 8. $F(1,8)=0.395$, $p=0.547$; Fig 9. $F(1,8)=0.931$, $p=0.363$).

Discussion

The results of this experiment did not support the hypothesis that administration of the GPR30 agonist G-1 immediately following training on the spatial memory water maze task would enhance performance on a probe trial after a 24-hour delay. Based on these results, G-1 does not affect spatial memory consolidation, indicating that the GPR30 receptor does not mediate the effect of estrogen on spatial memory consolidation. However, the current findings should be evaluated against a backdrop of differences in experimental design between this study and previous reports in the literature surrounding estradiol, GPR30, and the stages of learning and memory, which is discussed below.

Estrogen and Acquisition

There has been a history of inconsistencies and discrepancies in the literature regarding the effects of ovarian hormones on the acquisition of spatial memory tasks (Dohanich, 2002). For example, ovarian steroid hormones were reported to impair the acquisition or learning of spatial memory tasks, such as the water maze task, when training trials were administered over many days. In many reports, both gonadally-intact female rats, as well as ovariectomized rats treated throughout training with estradiol or estradiol and progesterone, displayed longer escape latencies and swim distances when learning to locate a fixed hidden platform compared to ovariectomized rats treated with an oil vehicle (e.g., Daniel, Roberts, & Dohanich, 1999;

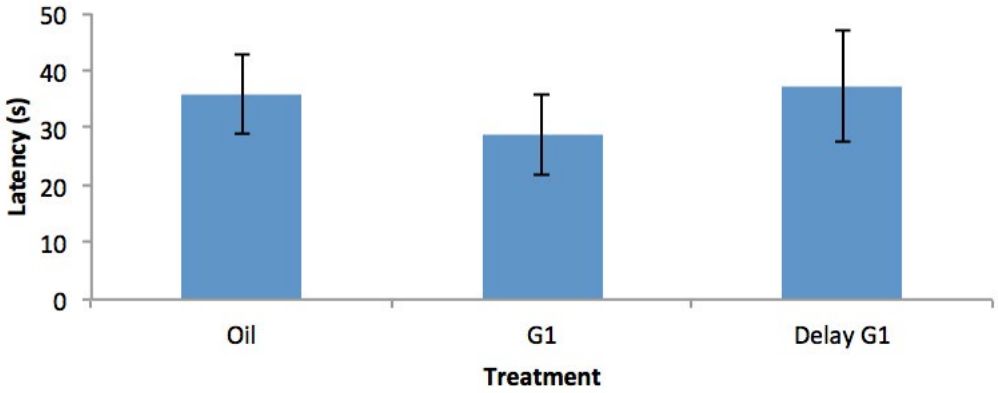

Figure 4. Mean latencies to reach the hidden platform during first probe trial administered 24 hours after training, by condition ($F(2,26)=0.352$, $p=0.707$).

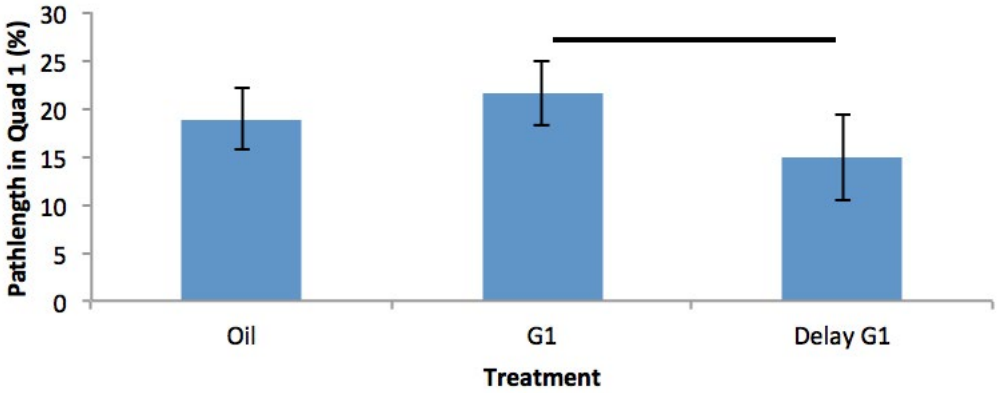

Figure 5. Mean percent of pathlength in quadrant 1 during the first probe trial by condition ($F(2,26)=0.703$, $p=0.504$). *Further analysis showed a weak trend toward higher percentage of pathlength spent in quadrant 1 for rats administered G-1 immediately after completion of training compared to rats administered G1 at 2 hours after completion of training* (line, $F(1,15)=1.394$, $p=0.256$).

Frye, 1995; Korol, 2004; Warren & Juraska, 1997). These results indicate that ovarian hormones can impair spatial learning of a hidden platform location in the standard water maze task. Circulating ovarian hormones also impaired performance during post-training probe trials when the platform was removed from the maze, indicating that these hormones also affected memory of the platform location (Daniel et al., 1999; Frye, 1995; Korol, 2004; Warren & Juraska, 1997). During proestrus, when 17β-estradiol levels are highest, gonadally-intact rats displayed the poorest acquisition and retention on the water maze task (Warren & Juraska, 1997). In stark contrast to reports of impairments on the standard water maze task, endogenous (Berry et al., 1997; Walf et al., 2006) and exogenous (Walf et al., 2006; Hammond et al., 2009) ovarian hormones have been reported to enhance spatial cognition on many different cognitive tasks, such as the T-maze, the radial arm maze, the Y-maze, and working- memory versions of the water maze task (reviewed by Dohanich, 2002). The varied effects of ovarian hormones on spatial learning acquisition indicate a highly complex relationship between ovarian steroids and cognitive functions.

Estrogen and Consolidation

A clever paradigm developed by McGaugh (McGaugh & Roozendaal, 2009) and adopted by Packard has been used to study the effects of various compounds, including estradiol, on the consolidation of memory. The current study of GPR30 and its potential effects on memory consolidation was based on Packard's successful enhancement of consolidation by post-training administration of estradiol (Packard & Teather, 1997; Packard, 1998). However, there are several important methodological differences between the reports by Packard and the current experiment.

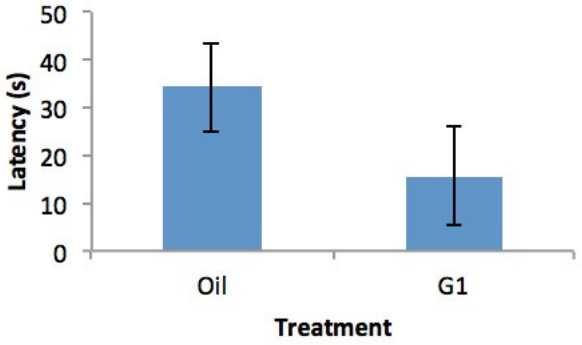

Figure 6. Mean latency to reach the hidden platform during the first probe trial for wave 1 showed a weak trend toward shorter latency in G-1 group ($F(1,9)=1.802$, $p=0.212$).

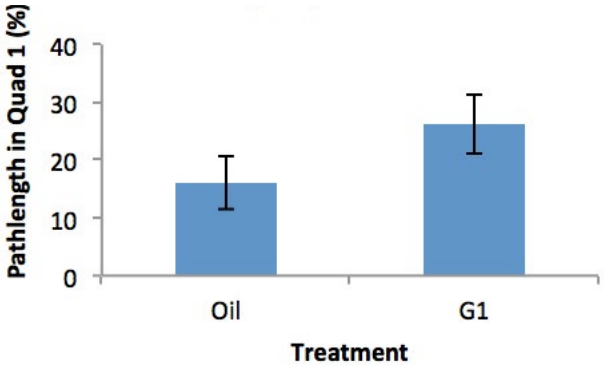

Figure 7. Mean percent of pathlength in quadrant 1 during the first probe trial for wave 1 showed trend toward higher percent pathlength in G-1 group ($F(1,9)=2.046$, $p=0.186$).

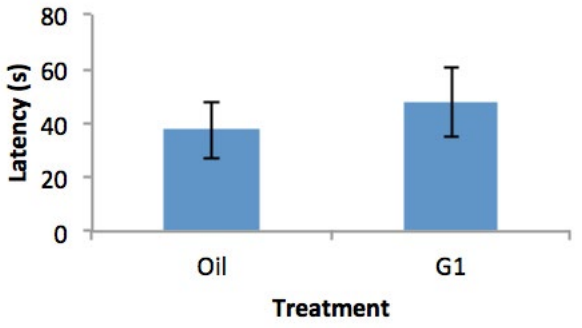

Figure 8. Mean latency to reach the hidden platform during the first probe trial for wave 2 showed no difference between conditions ($F(1,8)=0.395$, $p=0.547$).

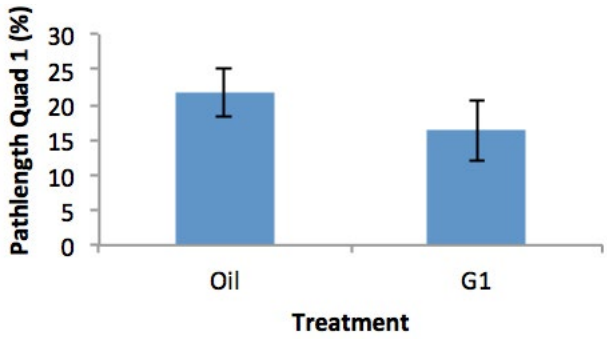

Figure 9. Mean percent of pathlength in quadrant 1 during the first probe trial for wave 2 showed no difference between conditions ($F(1,8)=0.931$, $p=0.363$).

For instance, Packard administered a water-soluble estradiol cyclodextrin inclusion complex providing a carrier system that allowed for rapid entry of estradiol into the brain during the time when memory of the platform location was consolidating (Pitha & Pitha, 1985; Taylor, Weiss, & Pitha, 1989). Furthermore, cyclodextrin vehicles promote rapid metabolism and clearance of hormones from the system. Therefore, the estradiol cyclodextrin complex is an optimal choice for use in studies of consolidation by facilitating rapid entry of the hormone into the brain and by promoting rapid clearance of the hormone, thus ensuring that any effects of estradiol are the product of enhanced consolidation as opposed to directly affecting performance on subsequent retention trials.

The inability of G-1 to affect consolidation in the current experiment could be due to the use of a sesame oil vehicle that reduced hormone availability and clearance. Hormones suspended in sesame oil allows for a create a slow release of the lipid-soluble hormone into circulation, followed by slow clearance from the body. Although some studies have found success suspending solutions of G-1 in DMSO, a rapidly-releasing vehicle analogous to the cyclodextrin complex used by Packard (Hammond et al., 2009), we decided against the use of DMSO because of many reports of various behavioral and toxic effects associated with *in vivo* use of this universal solvent (Castro, Hogan, Benson, Shehata, & Landauer, 1994; Kelava, Cavar, & Culo, 2011). The use of sesame oil solutions for assessing the rapid effects of drugs is debated within the literature because oil vehicles are employed traditionally to achieve slow release of compounds into the body (Banker, Siepmann, & Rhodes, 2002). As shown repeatedly by Packard, compounds administered after training trials are completed must access the brain within less than two hours in order to be able to achieve an effect on the ongoing consolidation process. Therefore, the use of an oil vehicle in the current experiment may have

reduced the rate of estradiol entry into the brain, thereby rendering G-1 ineffective.

Another important issue in the current experiment was identifying an effective dose of G-1. Previously, a dose of μg of G-1 proved to be effective at enhancing Y-maze retention on a spatial memory task in ovariectomized rats when administered at 48 and 24 hours before behavioral testing (Hawley et al., 2014). However, a dose of 25 μg of G-1 administered to a small number of rats in the first wave of the current experiment (N=7) failed to affect performance on retention trials, so a dose of 50 μg of G-1 was used in the remainder of the experiment. It is unclear whether this dose of 50 μg was still insufficient or possibly too high to enhance memory consolidation as predicted.

GPR30 and Acquisition

Hammond and Gibbs (2009) first established the involvement of GPR30 in spatial memory acquisition through administration of the GPR30 agonist G-1 and the GPR30 antagonist G-15 (Hammond et al., 2009; Hammond et al., 2012). There are, however, major differences between their experiment and the current study, specifically in the type of spatial task and administration regime of G-1. Hammond's results indicated that long-term treatment with GPR30 specific antagonist G-15 (10 μg/day) in intact rats effectively reduced the rate of acquisition to levels seen in ovariectomized rats and long-term treatment with G-1 (5 μg/day) restored the acquisition rates to those seen in intact rats (Hammond & Gibbs, 2011). Their results support the hypothesis that GPR30 activation improves learning on a spatial memory task, and implicate acetylcholine pathways as a neural mechanism underlying the effect of G1. However, key differences between this experiment and the current study in administration regime (long-term vs. acute), G-1 dose (5 μg/day vs. 50 μg), spatial task (DMP vs. water maze), and the stage of spatial cognition under study (acquisition vs. consolidation) make direct comparisons between these studies difficult.

GPR30 and Retention

While the reports by Hammond and Gibbs indicated the potential for GPR30 to mediate the effects of estradiol on acquisition of a spatial memory, our laboratory conducted experiments to study the potential role of GPR30 in the mediation of spatial recognition memory. A Y-maze task was adopted in which rats were given a 15-minute training session while they explored two arms of a Y-maze. After delays of 24 or 48 hours, rats were re-exposed to Y-maze for 5 minutes with access to all three arms. More entries into the arm that was previously blocked on training trials were indicative of intact spatial memory as rats typically seek out novelty. We found that administration of GPR30 agonist G-1 at 48 and 24 hours before behavioral testing enhanced spatial memory performance on of the Y-maze task (Hawley et al., 2014). Results of the study supported the involvement of GPR30 in spatial recognition memory on the novelty dependent Y-maze task. Repeated short-term administration of G-1 (25 μg/kg) was found to enhance retention of the Y-maze task just as short-term administration of 17β-estradiol did. Although the results indicated that GPR30 mediated spatial memory, the specific component of spatial cognition that was affected by GPR30 remained uncertain. It was unclear whether the enhancement of Y-maze performance was due to enhanced learning, retention, or consolidation. The current study was developed to continue this line of study by using Packard's post-training administration design to manipulate consolidation in order to determine if GPR30 agonist G-1 was acting via a consolidation mechanism in the previous study by Hawley et al. (2014). The results of the current study indicate that the enhancement of Y-maze performance through repeated administration of GPR30 agonist G-1 reported by Hawley was not due to an enhancement of spatial memory consolidation.

Wave differences

Interestingly, trends toward shorter latency and higher percent pathlength in the target quadrant were seen in rats treated with G-1 from the first wave, but not rats treated with G-1 from the second wave. This result indicates a discrepancy between the two waves of rats despite identical methods. The acquisition of spatial learning is a cognitively complex process that is susceptible to influences from many different factors, such as anxiety. The effect of anxiety on spatial memory is largely dependent on the source, level, and type of anxiety experienced by rats prior to or during testing (Dohanich, Korol, & Shors, 2009). There is clear evidence that individual trait anxiety can influence performance on spatial tasks, such as the water maze, with low anxiety rats outperforming rats with high trait

anxiety (Herrero, Sandi, & Vereno, 2006). Perhaps the second wave of rats tested in the current experiment possessed higher trait anxiety than rats tested in the first wave, which could have contributed to the trend toward better spatial memory displayed by the first wave of rats. While all rats from both waves were received from the same source at approximately the same age, it is possible that the rats from the second wave had higher trait anxiety, or were exposed to a significant stressor during transport or while housed in the vivarium. Indeed, four rats in the second wave expressed high levels of thigmotactic behavior during water maze testing indicated by swimming near the edge of the pool, a behavior proposed to reflect high anxiety (Bailey, 2005). While these individual rats were excluded from statistical analyses due to their outlier status, their anxiety could be indicative of a general trend for higher anxiety in the second wave of rats.

Summary and Future Directions

The results of the current study did not implicate G-protein-coupled receptor 30 as a mediator of the consolidation of spatial memory. The discrepancies between the results of this study and the existing literature on GPR30 can be attributed to a number of differences in experimental design including G-1 dose, type of vehicle, and administration regime, spatial cognition task, and aspect of cognition studied. This study does not support G-1 as a potential treatment for post-menopausal memory loss, though more experimentation is needed for conclusive determination of the potential therapeutic benefits of GPR30 activation.

Future experimentation on this topic could take many directions. In addition to testing varying doses of G-1 it would be worthwhile to administer G-1 via a more rapidly clearing vehicle. Additional experimentation could also incorporate multiple spatial memory tasks such as the Y-maze and the T-maze, in order to extend the role of GPR30 to different aspects of spatial cognition.

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