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Environmental- and Ethanol-Induced Effects on Reference and Short-Term Memory in the Rat

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by

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Abstract

Environmental enrichment (EE) is a combination of complex physical and social stimulation beyond that which would be received in standard or isolated laboratory housing. Continuous enrichment paradigms have been shown, among other influences, to increase neurogenesis and dendritic branching, and enhance learning and memory. Recently, the preventative effects of enrichment have been considered, specifically relating to drugs of abuse (Solinas, Thiret, Chauvet, & Jaber 2010; Stairs & Bardo 2009). This series of studies examined the effects of daily EE on reference memory and short-term memory, as assessed in the radial arm water maze (RAWM) and Morris water maze (MWM). Sprague-Dawley rats (N=18) were exposed to either EE or isolation for 4 hours/day for 4 weeks prior to and during the experiments. Animals were first trained and tested with non-spatial cues located at the entrance of the maze alleys in the RAWM (Experiment 1); but, they were unable to successfully learn the task. In Experiment 2, distal spatial cues were added to the maze. The rats learned the task, as evident in their reduced rates of errors. They were then trained to consume a 10% ethanol-Polycose gel (Rowland, Nasrallah, Robertson, 2005) and subsequently tested in the RAWM. Ethanol negatively affected reference memory in both treatment groups, but only disrupted short-term memory in isolated rats. EE may have protected against harmful ethanol effects on memory. In Experiment 3, reference and short-term memory were evaluated in a hidden platform and a moving platform paradigm, respectively, of the MWM. Enrichment significantly enhanced learning in the hidden platform paradigm. The short-term memory paradigm failed as a measure of short-term memory; however, due to the enriched rats' unexpected development of a search strategy that did not depend on short-term memory. Ethanol consumption adversely affected enriched rats' performance. This may be because ethanol can disrupt strategy use, affecting enriched rats. Isolated rats did not appear to use an alternate strategy regardless of ethanol consumption. Overall, a small enrichment effect on learning and memory was observed, which may be task dependent. Whereas ethanol negatively affected memory, EE appears to have protected against some detrimental ethanol effects on short-term memory.

Environmental and Ethanol-Induced Effects on Reference and Short-Term Memory in the Rat

Environmental enrichment has been implicated in experimental protocols for decades. Donald Hebb was the first to propose the use of a stimulating environment to influence behavior in the late 1940s (van Praag, Kempermann, & Gage, 2000) after discovering that rats taken home as pets developed more advanced cognitive abilities than those housed in the laboratory (Simpson & Kelly, 2011). Since then, using environmental enrichment as a testable scientific variable has become the topic of many journal articles. Enrichment is commonly studied as a treatment to enhance brain plasticity subsequent to a physical or chemical insult. Recently, the preventative effects of enrichment have been considered, specifically relating to drugs of abuse such as amphetamine, opioids, and ethanol (Solinas, Thiret, Chauvet, & Jaber, 2010; Stairs & Bardo, 2009).

In a broad definition, environmental enrichment (EE) is a combination of complex physical, cognitive, and social stimulation beyond that which would be received in standard or isolated laboratory housing (Bennett, McRae, Levy, & Frick, 2006; Simpson & Kelly, 2011; van Praag et al., 2000). Enrichment requires an interaction of multiple factors, not just one aspect, to be most effective.

Typical enrichment environments consist of large cages, sometimes with multiple levels, containing a variety of toys, balls, houses, tunnels, novel foods, running wheels, and platforms. Stimuli are varied regularly (daily, weekly, monthly, etc.) to retain novelty. Each cage can contain multiple animals. Depending on the variables being studied, enrichment can be introduced at various points throughout the lifespan (e.g., to observe developmental or aging effects). The duration can also vary. Many studies use continuous, 24-hour enrichment

paradigms, while others restrict EE to daily doses of just a few hours (Simpson & Kelly, 2011). Protocols can last weeks, months, or even years (1 week-2 month average) before and during various behavioral and neurological testing.

Behavioral testing used to study the effects of environmental enrichment have measured exploratory behavior (Widman & Rosellini, 1990), motor activity (Simpson & Kelly, 2011), spatial learning and memory (Bennett et al., 2006; Bindu, Rekha, & Kutty, 2005; Clements & Wainwright, 2006; Frick & Fernandez, 2003; Lambert, Fernandez, & Frick, 2005; Pereira, Arteni, Petersen, de Rocha, Achaval, & Netto, 2007), and recognition memory (Bruel-Jungerman, Laroche, & Rampon, 2005; Rampon, Tang, Goodhouse, Shimizu, Kyin, & Tsien, 2000) in male and female rats and mice.

In these and other studies, enrichment has been shown to reduce anxiety symptoms as measured by decreased locomotor activity, increased exploration, and reduced rearing in the open field task, induce an antidepressant-like effect in the forced swim task as measured by an increased swimming rate and reduced immobility time (Brenes, Padilla, & Fornaguera, 2009; Solinas et al., 2010), increase object exploration and recognition (Simpson & Kelly, 2011), reduce latency to the platform and thigmotaxis in the Morris water maze (e.g., Harburger, Lambert, & Frick, 2007; Harris, D'Eath, & Healy, 2009; Schrijver, Bahr, Weiss, & Würbel, 2002; Speisman et al., 2013), and reduce short-term and reference memory errors in the radial arm maze, Hebb-Williams maze, and radial arm water maze (e.g., Bennett et al., 2006; Sampedro-Piquero, Zancada-Menendez, Begega, Rubio, & Arias, 2013). We will focus on short-term, daily environmental enrichment and its behavioral and neurological effects on learning and memory.

Mazes have played an integral role in the understanding of animal learning and memory. Many commonly used mazes have been designed to observe particular strategic learning and problem solving behaviors of various animal species. To effectively navigate a maze, an animal must maintain knowledge of its current location and the location or locations providing food, escape, or some other reward (Golob & Taube, 2002; Hodges, 1996). By tweaking and altering maze protocols, researchers have been able to investigate how an animal uses spatial and non-spatial information to guide its behavior and later use its memory to demonstrate knowledge of the learned tactic. Spatial memory refers to the portion of memory responsible for encoding, storing, and retrieving information about the relative arrangement and location of objects in space (Paul, Magda, & Abel, 2009). Non-spatial memory is memory for any information or events that does not require a spatial component. Both short- and long-term (or reference), spatial and non-spatial learning and memory can be specifically accessed through use of mazes such as the radial arm maze, Morris water maze, and radial arm water maze. Reference memory refers to memory for information that remains constant over test days (or test sessions) and is not altered by performance over trials (Gresack & Frick, 2003).

Short-term memory is memory for information that changes over time. In an experiment, for example, this information is manipulated so that it changes across trials (see below for example). Information stored in short-term memory remains there for a brief duration before it decays and is lost unless the memory is maintained by rehearsal. When evidence demonstrates that short-term memories are updated or manipulated (rather than simply held as-is in a store), the short-term memory is more appropriately called working memory (Hyde, Hoplight, & Deneberg, 1998; Molnár, Boha, Czigler, & Gaal, 2010). In animal studies, however, short-term memory and working memory are often treated as synonyms. Although experimental methods

that investigate working memory in rodents are available, the large majority of studies actually investigate short-term memory because evidence of the updating and manipulation of memory in a short-term store are not provided (see Matzel & Kolata, 2010).

The radial arm maze (RAM) consists of several alleyways, or arms, (typically 6 to 8), equally spaced and radiating from a central platform (Bindu, Rekha, & Kutty, 2005; Gresack & Frick, 2003). In a working memory protocol, each arm is baited with food pellets. Rats are released into the center and, utilizing spatial extra-maze cues (such as distal visual-spatial information) or non-spatial intra-maze cues (such as varying textures or odors), must retrieve the food from all eight arms without revisiting previously entered arms. Thus, on each trial the arms that are baited have changed relative to the previous trial. To perform optimally, the animals must avoid the arms that were visited on previous trials. Any reentry into an already visited arm is considered a short-term memory error. In a protocol assessing short-term and reference memory simultaneously, only half of the arms are baited with food pellets every day. Because the same arms are baited each day, reference memory depends on information that is consistent across days and not altered by performance across trials; that is, about which arms are baited rather what arm was recently visited on a previous trial. Again, utilizing extra- or intra-maze cues, rats must retrieve the pellets without revisiting a previously baited arm (short-term memory error) or entering arms that were never baited in the past (reference memory error).

Hyde, Hopight, and Denenberg (1998) describe some of the common disadvantages of the spatial radial arm maze including odor trails and stress due to food or water deprivation to increase motivation. Odor trails can be caused by the animals themselves, or the food reward at the end of each arm. Using the odors, the animal is able to determine which arms have the reward without utilizing spatial cues. In order to motivate the animals to search for rewards, food

or water deprivation is commonly employed, with food or water being the reward at the end of each arm. This deprivation can induce stress in the animal, leading to alterations in performance.

The Morris water maze (MWM) is an aversively motivated task that uses negative reinforcement (escape from the water) as a motivating factor. It is comprised of a large circular pool filled with water (Morris, 1984). The pool is conceptually divided into quadrants (Pereira, Arteni, Petersen, da Rocha, Achaval, & Netto, 2007). A spatial reference memory protocol is traditionally utilized when employing this maze. Here, a platform is submerged approximately 2 cm below the surface of the water in one quadrant, which remains constant across trials (Bindu et al., 2005). Rats are released from various starting points around the pool and must swim to find the platform, which remains in the same spot each day, using spatial extra-maze cues to guide them. Escape latency is the most common scale of assessment, however additional measures include swim path distance, swim speed, and proximity to the platform location. Memory for the location of the escape platform is assessed in a single probe trial, where the platform is removed from the pool. Time spent in the quadrant previously containing the platform and platform location crossings are common scales of assessment during probe trials.

In a short-term memory protocol, the platform changes positions each day. The animals are given two trials. The first trial allows the animal to locate the platform for the day. The second trial tests the memory for the platform location (Vorhees & Williams, 2006). Protocols to assess non-spatial memory use a visible or cued platform. The animals are readily able to identify where the platform location is due to the intra-maze cue. The MWM requires animals to use a 'win-stay' strategy, in that animals must return to the previous location where they have found the platform (Wishaw & Pasztor, 2000).

The radial arm water maze (RAWM) was designed to combine the advantages of the land radial arm maze and the Morris water maze without many of the common disadvantages associated with the two. In this design, 6-8 alleyways, or arms, radiate from a central area, consistent with a traditional radial arm maze, and are sunken into a large pool of water, coherent with a traditional Morris water maze. Platforms are placed approximately 2 cm below the water at the end of the arms to allow for escape. Short-term and reference memory protocols are akin to those of the RAM and are able to be examined simultaneously using the RAWM (Berchold, Castello, & Cotman, 2010; Clements & Wainwright, 2006; Gresack & Frick, 2003; Hyde et al., 1998). In this task, only half of the arms are baited with escape platforms. After a platform is found, it is removed for the rest of the block. Using spatial or non-spatial cues, animals must remember which arms have never been baited (reference memory) and which arms were previously baited but no longer contain a platform (short-term memory). As trials progress, the short-term memory load increases. This requires a 'win-shift' strategy, in that after an animal 'wins' (finding an escape platform), it must 'shift' away from arm choices previously rewarded (Clements & Wainwright 2006).

The motivation to successfully complete the RAWM task is the negative reinforcement of escaping the water by platforms submerged at the end of specific arms (Gresack & Frick, 2003). The water eliminates the odor trail from previous animals running the maze. An added advantage is that errors of wrongful arm entries can be scored instead of relying on escape latency timing and proximity to the escape platform as in the MWM (Berchtold, Castello, & Cotman, 2010).

Many studies utilizing a daily enrichment protocol have observed its effects following experimenter-induced brain injuries. Will, Rosenzweig, Bennett, Hebert, and Morimoto (1977) studied the effect of environmental enrichment on learning as assessed by the Hebb-Williams

maze in male rats. Will and colleagues exposed rats to either bilateral occipital cortical lesions, shown to impair learning and problem solving, or a sham operation. In two experiments, animals were exposed to enrichment for 24 hours/day, 2 hours/day, or isolated as controls. Results revealed that the daily enrichment period produced effects comparable to those of continuous enrichment. Both enrichment groups had significantly lower error rates in the Hebb-Williams maze as compared to isolated controls. The effects of enrichment were more apparent in the group exposed to lesions than in either the sham operation or isolated control groups. This may be due to several reasons; however, the authors were hesitant to conclude that EE is actually more beneficial after lesions due to lack of literature support.

Short-term and reference memory following disruptive brain lesions and daily environmental enrichment has also been examined. Bindu, Rekha, and Kutty (2005) studied the effects of post-lesion enrichment on performance in the RAM and MWM. The authors exposed male rats to ventral subiculum lesions, known to impair spatial learning and memory, or sham operations and subjected them to either enriched or standard housing conditions. Enrichment took place for 6-hours/day for 10 days. Large cages equipped with tunnels, toys, and platforms of varied size and textures housed 8-10 rats during enrichment sessions. Stimuli were varied daily. Behavioral testing utilized the radial arm maze to evaluate spatial short-term and reference memory and the MWM to evaluate spatial reference memory. The authors found that rats exposed to lesions and EE performed comparable to sham operated control rats for both short-term and reference memory tests in the radial arm maze. Enrichment significantly recovered deficits due to lesions. Performance in the MWM was not as striking. Groups with lesions displayed impaired acquisition and retention as compared to control groups. Bindu and

colleagues (2005) concluded that behavioral recovery of learning and memory following bilateral subicular lesions may be task dependent.

Pereira, Arteni, Petersen, da Rocha, Achaval and Netto (2007) have found discrepant results in the effect of daily enrichment on MWM performance. Pereira and colleagues (2007), however, studied these effects following neonatal hypoxia-ischemia, rather than subicular lesions (Bindu et al., 2005), confounding comparison between studies. Hypoxia-ischemia (HI) is an event that damages brain tissue due to diminished blood oxygenation and reduced cerebral blood flow. This has previously been shown to negatively affect spatial learning and memory in the water maze, inhibitory avoidance, and shock avoidance. Following insult, male rats, in groups of 7-10, were exposed to enrichment for 1-hour/day for 9 weeks. The environment involved a large cage with three levels containing ramps, toys, tunnels, and running wheels that were changed weekly. Reference and short-term memory was evaluated in the MWM. Results showed significantly shortened latency to the platform during testing and a greater number of platform location crossings during the probe trial in HI rats exposed to EE as compared to post-lesion standard housed rats in the reference memory protocol. The short-term memory protocol displayed no significant difference between enriched HI rats and controls in latency and platform crossings. Overall, the study concluded that short term exposure to enrichment reversed the performance deficits in reference memory even when introduced as long as 2 weeks post-insult.

Finally, daily enrichment effects have also been studied in aging. Two studies in particular have observed inconsistent results when assessing spatial short-term and reference memory in aged female (Frick & Fernandez, 2003) and male mice (Bennett et al., 2006) in the Morris water maze. Frick and Fernandez (2003) exposed aged female mice to enrichment for 3-hours/day for 23 days. The environment consisted of a large bin containing plastic dwellings,

toys, tubes, running wheels, and a climbing apparatus, which were changed daily. Mice were subsequently tested in a spatial (hidden platform) version and a non-spatial (cued platform) version of the MWM measuring reference memory. In the spatial task, aged control mice had greater swim distances and latency to the platform as compared to aged enriched mice, who performed similarly to young controls. Enrichment was shown to significantly improve acquisition of the task and development of a spatial bias in the probe trial. There was no effect of enrichment in the non-spatial version, which may suggest a specific effect on spatial reference memory.

Bennett et al. (2006) described disparate results when testing aged male mice. Bennett and colleagues provided continuous 24-hour enrichment or daily enrichment for 3-hours/day for 6 weeks prior and 4 weeks during testing (a total of 10 weeks). The environment was identical to that of Frick and Fernandez (2003). Animals were subsequently tested in a MWM task measuring spatial reference memory, a cued water maze task to measure non-spatial reference memory, and a RAWM simultaneously measuring spatial short-term and reference memory. Briefly, results illustrated that daily enrichment had little beneficial effect on spatial reference memory, which was surprising considering that only two weeks of the same treatment improved spatial reference memory in the aged female mice (Frick & Fernandez, 2003). This group was impaired compared to young controls. In some instances, aged daily enriched mice performed worse than aged controls, specifically in the non-spatial MWM. Aged mice that were continuously enriched displayed consistently shorter swim speed and latency to reach the platform than aged daily enriched mice. They also made fewer reference memory errors than any group in the RAWM. Overall, authors conclude that EE may have been more beneficial to reference memory than short-term memory, but this can depend on task difficulty. They also

offer some reasons as to why the daily enrichment did not provide benefits, citing the amount of time in EE, the time of day EE was offered, and quarrels over territory marking in males.

Studies assessing the neurological effects of environmental enrichment have predominantly focused on the hippocampal formation in the medial temporal lobe, including the dentate gyrus, entorhinal cortex, subiculum, basal forebrain, and the hippocampus, due to their strong involvement in learning and memory (García-Moreno & Cimadevilla, 2012). As previously mentioned, many of these studies have been based on the reversal of cognitive deficits associated with normal aging, stroke, lesions, and diseases that affect the hippocampal locality such as Alzheimer's and epilepsy (Simpson & Kelly, 2011; van Praag et al., 2000). Neurological enhancement due to enrichment has been observed with much support despite differences across studies. Some foci of enhancement include plasticity & neurogenesis, neurotransmitters, and synapse/ dendrite augmentation.

Neurogenesis and proliferation are defined as the processes of generating, multiplying, and integrating new neurons in the brain (Beaquis, Roig, De Nicola, & Saravaia, 2010). The plasticity of these neurons allows them to adapt and modify themselves in the wake of new experiences. Enrichment has been shown to promote neurogenesis, proliferation, cell survival, and plasticity in the dentate gyrus and hippocampus of normal and lesioned rats (Beaquis et al., 2010; Bindu, Alladi, Mansooralikhan, Srikumar, Raju, & Kutty, 2007; Bruel-Jungerman et al., 2005; van Praag et al., 2000; Will et al., 1977). In one study (Bruel-Jungerman et al., 2005) newborn cells in the dentate gyrus were increased by almost 70% and were specifically beneficial to memory consolidation. These enhancements have also been shown to improve latency to the platform and overall performance in the MWM. Some of this increased plasticity in the hippocampus is hypothesized to be due to increased long term potentiation (Simpson &

Kelly, 2011). Long term potentiation (LTP) is widely considered one of the major underlying mechanisms of learning and memory by means of increased signal transmission between neurons.

Some of the key players in promoting LTP and plasticity are neurotransmitters such as glutamate, gamma-aminobutyric (GABA), serotonin 1A, and acetylcholine, as well as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and N-methyl-D-aspartic acid (NMDA) receptor agonists (Rampon et al., 2000; Simpson & Kelly, 2011). Enrichment has been shown to enhance LTP in the hippocampus by increasing AMPA and NMDA receptor binding. Increasing monoamines, such as serotonin and noradrenaline, and affecting choline uptake in the hippocampus has also been shown to positively influence learning and plasticity (van Praag et al., 2000).

Rampon and colleagues (2000) produced CA1-specific NMDA receptor 1 subunit-knockout (CA1-KO) mice to observe the NMDA receptor's dependence on non-spatial memory formation. Damage to the CA1 sub-region of the hippocampus leads to declarative memory deficits in humans. Results show that enrichment provided for just 3 hours/day for 2 months was enough to significantly rescue these non-spatial memory deficits as assessed through a novel object recognition task, fear conditioning task, cued conditioning task, and an olfactory dependent food preference task.

Synaptic and dendritic density, length, and plasticity in the hippocampal formation regions aids in the ability to transmit information via neurotransmitters and neurotrophic factors. The interaction of these mechanisms points to enhanced learning and memory. Bindu and colleagues (2005; 2007) found a complete reversal of dendritic atrophy and spinal density damage caused by subicular lesions after short term environmental enrichment. The authors

hypothesize that increased presence of spines augments synaptic efficacy and enhances the excitability of the learning process network. Reviews by van Praag et al. (2000) and Simpson and Kelly (2011) reveal that enrichment also increases higher order dendritic branching, brain weight/size, spine density, and synaptogenesis in the cortex (Will et al., 1977) and hippocampus (specifically CA1 and CA3 regions).

Alcohol

Alcohol is a major part of American culture, often used for celebrations, relaxing, relief, meeting new people, and most notoriously college fraternity parties. Though there are safe habits of consumption, alcohol is the third leading lifestyle-related cause of death in the US. Alcohol can also have serious detrimental effects on health, contributing to psychiatric, cardiovascular, gastrointestinal, and liver problems (Center for Disease Control and Prevention). Neurologically, alcohol can disrupt intellectual functioning, cognitive flexibility, executive functioning, learning, and memory (Cacace, Plescia, La Barera, & Cannizzaro, 2011).

Reviews of the effects of alcohol on memory have observed that alcohol does not affect the brain as globally as previously thought, but rather affects specific areas and systems. Although it affects almost every main neurotransmission system, alcohol notably potentiates the inhibitory effects of GABA, acts as an NMDA antagonist, suppresses hippocampal pyramidal cell activity, reduces spatial specificity of hippocampal place-cells, inhibits long-term potentiation (LTP), and decreases levels of glutamate (Matthews, Ilgen, White, & Best, 1999; Silvers, Tokunaga, Berry, White, & Matthews, 2003; White, Matthews, & Best, 2000).Interestingly, alcohol appears to effect similar processes that environmental enrichment also influences but in an opposite manner, including effects on NMDA receptors, hippocampal cell activity and LTP.

Cognitive processes, including spatial learning and memory, mediated by the hippocampus and its associated structures (e.g., afferent connections to the septum, ventral tegmental area, and raphe nuclei) may be particularly sensitive to the harmful effects of alcohol (Chin et al., 2011; Matthews, Simson, & Best, 1995; White et al., 2000). The amnesic effects of alcohol appear strikingly similar to those of hippocampal lesions, suggesting that the hippocampus plays an important role in alcohol induced memory disruption (Berry & Matthews, 2004; Ryabinin, 1988; White et al., 2000). Behaviorally, these effects cause long-term and short-term memory disruptions in humans, including episodic memory impairments and blackouts (Molnár et al., 2010; Silvers et al., 2003). In animals, alcohol can have differential disruptive effects on acquisition and performance of short-term and reference memory tasks depending on the dose and route of administration used, as well as the age, species, and strain of animal utilized (Cacace et al., 2011; Molnár et al., 2010; White et al., 2000).

Several studies point to detrimental effects of acute ethanol administration on spatial memory performance as assessed by the MWM. Acheson, Ross and Swartzwelder (2001) administered either a low (0.5 g/kg) dose or a high (2.5 g/kg) dose of ethanol to adolescent and adult rats prior to daily training in a spatial version and a non-spatial version of the MWM. A probe test was given the following day. The low dose of ethanol did not impair spatial memory performance; rather, it facilitated acquisition during the initial training phase as suggested by lower average escape latency comparable to that of controls. Rats given the high dose of ethanol were unable to successfully learn the spatial water maze task, and therefore had no spatial memory of the target location during the probe trial. Performance on the non-spatial, cued platform water maze task was not significantly affected after controlling for baseline

performance. These results indicate that acquisition of a spatial task is markedly impaired by acute high doses of ethanol, though performance on a non-spatial task did not suffer.

Berry and Matthews (2004) found similar results using C57BL/6J mice. Mice were trained in a spatial version of the MWM. The next day they were given one of three doses (1.25 g/kg, 1.75 g/kg or 2.25 g/kg) of ethanol or a dose (1.75 g/kg) of saline 30 minutes prior to a probe trial. The same mice were then trained in a non-spatial, cued version of the Morris water maze. The day after training was complete, appropriate doses of ethanol or saline were administered 30 minutes prior to a probe trial. All mice were able to successfully learn both versions of the maze. Spatial memory was significantly impaired at the two highest doses of ethanol (1.75 and 2.25 g/kg) as noted by increased latency to reach the escape platform. Non-spatial memory was impaired only at the highest dose of ethanol administered, 2.25 g/kg, evidenced in higher latency to reach the escape platform. In a second study performed by the authors, the same procedure was administered to a new set of naive mice. However, in this study, path length (to measure spatial memory) and swim speed (to assess motor performance) were recorded in addition to latency to the platform. Results demonstrated that spatial memory, but not motor performance, was impaired in the spatial version of the maze. In the non-spatial version, ethanol impaired swim speed but not path length, indicting a motor impairment, rather than a memory impairment as the underlying factor. The authors concluded that alcohol selectively impairs hippocampal-mediated spatial memory.

In accordance with the two previously mentioned studies, other work examining the effect of alcohol on spatial and non-spatial memory have found that alcohol impairs spatial memory performance (e.g., increasing latency to the platform & swim path in the MWM) but spares, and sometimes even facilitates (e.g., Acheson et al., 2001; Matthews et al., 1999), non-

spatial memory (Cain, Finlayson, Boon, & Beiko, 2002; Matthews & Silvers, 2004; Matthews et al., 1995; Matthews et al., 2002; Molnár et al., 2010; White et al., 2000). These effects appear to be dose-dependent, with higher doses interfering with spatial learning and memory processes more than lower doses (Chin et al., 2011; Devenport & Merriman, 1983; García-Moreno & Cimadevilla, 2012; Novier, Van Skike, Chin, Diaz-Granados, & Matthews, 2012). Additionally, alcohol can decrease stress response and anxiety behaviors at low doses, increase locomotor activity and exploratory behavior (Cacace et al., 2011), and impair strategy use in maze testing (Cain et al., 2002; Devenport & Merriman, 1983; Matthews et al., 1995) in animals.

Ribiero de Carvalho and colleagues (2010) studied the effect of environmental enrichment on ethanol consumption and preference in female spontaneously hypertensive rats, a rodent model of attention deficit hyperactivity disorder shown to display sensitivity to abused drugs. Animals reared in EE displayed decreased novelty-seeking behavior, as measured through exploration and locomotor activity in a novel open field environment. Enrichment also reduced ethanol consumption in a free-choice paradigm and did not produce a conditioned place preference for either dose of ethanol (0.5 or 1.2 g/kg).

Others have also studied the effect of rearing condition on ethanol response. Deehan, Cain and Keifer (2007) measured operant responding for ethanol in a 30 or 60 minute operant responding session. Authors found that male rats raised in isolated conditions responded significantly more for 10% ethanol during both access sessions as compared to rats raised in enriched conditions. When given a preference test (ethanol vs. water), enriched and socialized rats responded to both at very low rates. Isolated rats, however, displayed a clear preference for ethanol, even when responses during an initial sucrose training were not significantly different between any of the groups. Sucrose training is an approach employed by the authors to

encourage operant responding to ethanol. Sucrose is first introduced in the operant chamber instead of ethanol. Successive amount of ethanol are added daily to habituate rats to the taste. These results suggest that there may be explanations other than novelty-seeking at work.

A series of studies by Rockman and colleagues (Rockman & Borowski, 1986; Rockman & Gibson, 1992; Rockman, Hall, & Markert, 1988) have found discordant effects of enrichment and isolation on voluntary ethanol consumption. In two of these studies (Rockman & Borowski, 1986; Rockman et al., 1988), authors exposed rats to 90 days of continuous enrichment or isolation. During this time, rats were exposed to increasing percentages of ethanol in a solution. Animals were subsequently tested in voluntary ethanol intake. Authors observed that enriched rats displayed higher ethanol consumption than isolated rats. However, when Rockman and Gibson (1992) assessed the effect of 60 days of continuous enrichment or isolation, they found that the groups did not significantly differ in voluntary ethanol consumption. Authors concluded that timing and duration of environmental exposure impact the amount of ethanol voluntarily consumed.

Deatherage (1972) observed results opposite to those of studies performed by Rockman (Rockman & Borowski, 1986; Rockman & Gibson, 1992; Rockman et al., 1988). In his study, rats were housed in groups of six or individually and given free-access to water, 10% ethanol solution, or 20% ethanol solution for 30 days. The mean ethanol intake was determined for each group daily. On average, rats that were individually housed consumed more 20% ethanol than socially housed rats. There were no significant differences in 10% ethanol or water conditions.

Traditional studies assessing the effects of alcohol in animals have used forced- and free-choice bottle paradigms, usually requiring training and food/water deprivation. In these tasks, ethanol is diluted to make a solution that is presented alongside water or another non-alcoholic

beverage to see how much the animal drinks of each. Recent studies have utilized an ethanol-Polycose gel matrix (Nasrallah, Yang, & Bernstein, 2009; Peris et al., 2006; Rowland, Nasrallah, & Robertson, 2005). This method of consumption does not require training or deprivation to promote intake and produces brain ethanol levels comparable to those of voluntary ethanol drinking (Peris et al., 2006). This paradigm is also more realistic and analogous to human alcohol consumption. Customary ethanol studies in animals have used water as a vehicle, whereas humans typically drink alcohol in vehicles containing carbohydrates and macronutrients (Rowland et al., 2005). Using Polycose and gelatin as a vehicle introduces those nutrients to animals while consuming alcohol.

In addition to studying how environmental enrichment affects recovery of brain lesions and aging, its effect in preventing and treating substance abuse has also been investigated. Stairs and Bardo (2009) describe the neurobehavioral effects of environmental enrichment in drugs of abuse. Several studies reviewed have shown the EE increased sensitization of locomotor activity, as measured in an open field, in response to acute administration of stimulant drugs, such as amphetamine and cocaine. Rats reared in EE also show a greater acquisition of a conditioned place preference (CPP) and increased sensitization to the rewarding (antinociceptive) and aversive properties of opiates administered prior to CPP conditioning. A conditioned place preference paradigm is a model used to evaluate the rewarding and aversive effects of drugs. The design consists of a three-chamber box in which the animal learns to associate a certain chamber of the box with drug treatment. The preference ratio for the environment paired with drugs relative to the environment associated with no drug is calculated by recording time spent in each chamber. Alternatively, Solinas, Chauvet, Thiret, El Rawas, and Jaber (2008) report that mice housed in enriched environments for one month subsequent to

cocaine-induced CPP decreased sensitization and reinstatement of CPP. Similarly, environmental enrichment has been shown to decrease stimulant self-administration at low doses (Solinas et al., 2010; Stairs & Bardo, 2009).

One explanation for the discrepancy between conclusions drawn from CPP and self-administration studies may be learning (Solinas et al., 2010). CPP procedures rely on learning and memory, which is typically enhanced following enrichment. These effects may facilitate learning of the drug-environment relationship. Another explanation is that EE may reduce impulsivity, a trait shown to predict drug abuse (Stairs & Bardo, 2009) and novelty-seeking behavior (Ribeiro de Carvalho, Pandolfo, Pamplona & Takahashi, 2010).

Ethanol administration has also been found to impair the effective use of strategies within a maze (Cain et al., 2002).

Maze Behavior

There is a vast amount of strategies that animals can use to effectively navigate and solve mazes. Several approaches will be outlined here. Both humans and rodents can use allocentric or egocentric strategies to orient themselves and learn the path between themselves and the goal (Rubio, Begega, Méndez, Méndez-López, & Arias, 2012). Allocentric (or locale) learning involves creating a spatial map of the environment and commonly implicates two sub-strategies, direction learning and place learning. In direction learning, the animal learns to navigate in a particular direction (e.g., move towards the west). In place learning, the animal uses the configuration of distal, extra-maze cues to navigate to the same relative location within the maze. Egocentric learning is based on an internal point of reference and guided by proprioceptive and vestibular clues. The position of objects is relative to the individual rat (D'Hooge & Deyn, 2001;

O'Keefe & Nadel, 1978; Olton & Samuelson, 1976; Restle, 1957; Wishaw & Pasztor, 2000). The nature and efficiency to which a strategy is assumed is task dependent.

Tolman, Ritchie, & Kalish (1946) contend that place learning is more natural for rodents than response learning. In his 1948 work, Tolman introduces the cognitive mapping theory, which states that a temporary map is constructed that determines what response an animal will emit. This map includes routes, paths, and spatial relationships within the environment. The theory predicts that rats have a bias to use and process spatial information, tasks that can be learned utilizing spatial information will be learned faster than non-spatial tasks, and that animals will preferentially use spatial information when both types of cues are available (Hebb, 1938; Tolman, 1948).

Strategies can also be more paradigm specific. Mazes assessing spatial and non-spatial learning and memory typically evoke many kinds of search strategies. The structural characteristics of the maze, as well as the type of information available, influence which type of strategy will be used (Olton, 1979; Rubio et al., 2012). Olton (1982) describes five approaches for implementing a win-shift strategy in mazes such as the RAM. Utilizing a *distance minimizing* tactic, animals would travel the most direct route between goal locations. In a *central-place search*, the animal would venture out to the goal, but return to the central location between trips. Applying a *trail following* approach, animals follow specific routes to the goal. In the case of mazes, the arms or alleyways serve as trails. A sequence of successive arm choices due to trail following is referred to as chaining or serial searching (Harrison, Reiserer, Tomarken, & McDonald, 2006). A *random search* tactic follows unpredictable routes to the goal. Finally, an animal would search in a *thigmotaxic* manner if it searched by traveling along the walls of a maze arena. In general, when trails are available, animals will predominantly choose a trail

following method of searching, followed by distance minimizing (Olton, 1982). This may be because this is a species-typical strategy in rodent life. Following trails in the wild promotes reliable location of shelter, other rats within a group, and food (Telle, 1966). An animal-centered view of maze behavior suggests that animals can perform laboratory tasks most readily when the apparatus is tuned to elicit natural behaviors, such as foraging in a RAM (Hoffman, Timberlake, Leffel, & Gont, 1999).

Garthe, Behr, & Kempermann (2009) describe several search strategies seen in their analysis of the spatial reference memory protocol in the MWM, a win-stay task. Some tactics, such *thigmotaxis* and a *random search* seen in win-shift tasks, can also be used to navigate the MWM. *Scanning*, as opposed to thigmotaxis, refers to a strategy where the animal spends most of its time traveling around the inner most part of the pool. *Chaining* in the MWM refers to the tendency of an animal to spend the majority of its time circling a fixed distance from the pool wall in order to locate the platform. A *direct* or *focal search* is most efficient. This occurs when the animal travels a direct path to the platform location and searches in a particular location. Janus (2004) found that in reference memory paradigms of the MWM, animals tend to use scanning and chaining search strategies at first, but quickly switch to more direct strategies.

Studies have concluded that rats can use several strategies simultaneously during acquisition and performance of a task. They may display behaviors reinforced during training, but can also employ unconditioned search behaviors (Whishaw & Mittleman, 1986). Additionally, animals may use multiple cues, such as visual, olfactory, tactual, or kinesthetic stimuli. The extent to which animals use each cue and learn a specific strategy depends on how relevant the cues are to successfully guiding behavior and the proportion of relevant cues among the total number of cues (Restle, 1957). Spatial cues tend to be preferred when both intra- and

extra-maze cues are available, though near perfect performance can be attained using just one type of cue (Harrison et al., 2006; Olton & Samuelson, 1976; Restle, 1957).

Current study

The current study combines various aspects of the cited literature as well as original ideas to examine the effect of daily environmental enrichment on short-term memory, reference memory, and alcohol effects as assessed through the RAWM and MWM. We hypothesize that enrichment will facilitate short-term and reference memory in the RAWM and MWM. We also hypothesize that enrichment will alter responsiveness to the effects of ethanol on performance. If the finding that environmental enrichment has some protective effects against deficits produced from brain damage extends to deficits due to drugs and alcohol, then enrichment may reduce the detrimental effects of alcohol on short-term and reference memory.

In the first of three experiments, non-spatial short-term and reference memory were assessed simultaneously in the RAWM.

Experiment 1

Method

Subjects. Eighteen Sprague Dawley rats were used in the study. Animals were obtained from Harlan Laboratories, Inc. (Indianapolis, Indiana) and were approximately six weeks old at the start of the experiment. Rats were kept on a 12:12 light/dark cycle (lights on at 0800 hours) with food and water access *ad libitum*. Training and testing took place during the dark cycle (maze testing began approximately at 2300 hours). Animals were double housed in translucent standard shoebox cages. Seton Hall University's Institutional Animal Care and Use Committee (IACUC) approval was obtained prior to beginning any experimentation.

Apparatus. ***Environmental exposure.*** Daily environmental enrichment took place in two large cages measuring 76 cm x 46 cm x 91.5 cm (n=4 and n=5 in each cage). Cages had three levels with ramps leading up to each level (Martin's Cages, Inc., Model R-695). Wooden toys, houses, paper tubes, a running wheel, and novel rat treats provided cognitive stimulation. Toys were changed 1-2 times per week to sustain novelty. Daily isolation took place in standard shoebox cages (1 rat per cage) covered on four sides with black construction paper. Food and water was available *ad libitum* during environmental exposure. Rats were exposed to environmental conditions for 4 hours during the dark cycle prior to maze testing.

Radial arm water maze. A radial arm water maze (RAWM) was used to evaluate short-term and reference memory. The RAWM consisted of a 6-arm radial maze constructed within a large black tub (see Figure 1). The round tub had a total diameter of 130 cm and a depth of 52.5 cm. Twelve pieces of Plexiglas measuring 45 cm x 61 cm were inserted into the tub to form six "pie" shaped slices, forming the six arms of the maze. Each arm measures 25.5 cm wide and 61 cm in length; the center area has a diameter of 48 cm. Escape platforms, measuring 12.5 cm in

diameter and 19 cm tall, were submerged approximately 2 cm beneath the surface of the water at the end of three arms (arms B, E, & F in Figure 1). Escape platforms were consistently placed in the same arms each day to give rats the opportunity to develop a reference memory for their location. Tactile and olfactory cues marked the entrance of baited arms. Strips of white gauze measuring approximately 5 cm x 7.5 cm were affixed to either side of each arm, approximately 2.5 cm inset. Gauze in the baited arms was scented with 1 mL of McCormick brand vanilla (arm A), coconut (arm E), or mint (arm F) extract and was applied prior to the start of testing each day. Tactile cues consisted of fishing line strung across the entrance of the arm, immediately behind the gauze. Five pieces of fishing line hung down approximately 10 cm and grazed the surface of the water to touch the animal when it entered a baited arm. To eliminate extra-maze cues, three white opaque shower curtain liners were hung around the maze and the animals were tested under dim red light illumination. The tub holding the RAWM was rotated 90° clockwise each day of testing to control for any extra-maze cues the animals may have been utilizing.

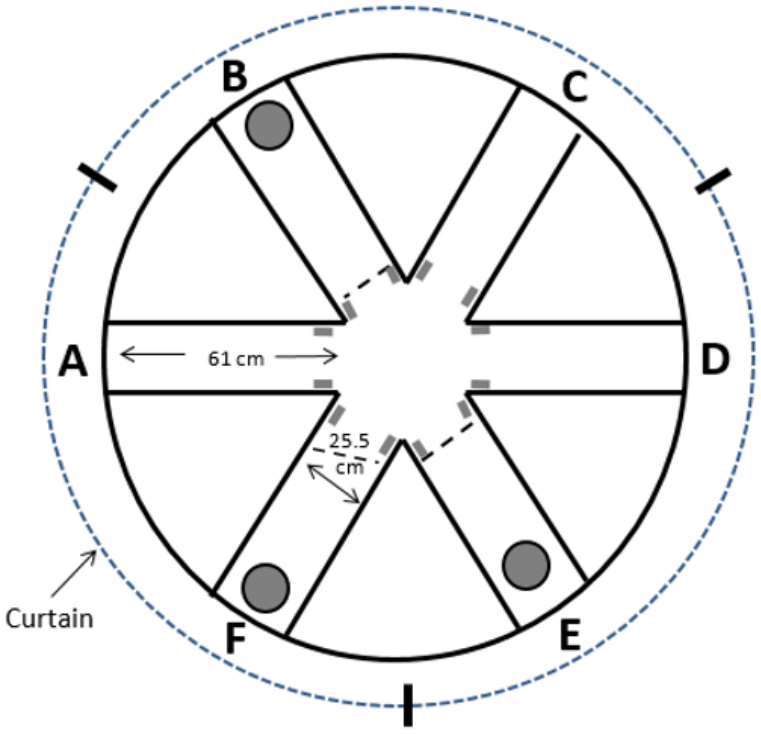

Figure 1. Schematic of the radial arm water maze with tactile/olfactory cues. Dotted lines across arms indicate hanging fishing line. Grey boxes at the entrance to each arm indicate where gauze was placed. Grey circles at the end of an arm indicates where an escape platform was located. Arms containing escape platforms were scented via extract applied to the gauze. Solid black lines crossing the curtain indicate where the curtain opened to be used as a starting position.

Procedure. ***Non-spatial RAWM training.*** Initial training in the RAWM was included to adapt the animals to the swimming requirement of the task and to familiarize the animals with the 6 arms and the availability of escape platforms in some of the arms. Initial training lasted 4 days (6 blocks, 3 trials = 1 block). One block of training was performed on day 1 to introduce the rats to the maze; two blocks of trials were performed on Days 2 and 3 of training to allow for additional training of the rats; one block of training was performed on day 4 to begin preparation of acquisition testing. The animals were run in 3 squads of 6 animals each. A squad was transported to the RAWM room and placed individually in stainless steel holding cages. The rats

were then tested one at a time. There were three starting positions that were utilized (see Fig 1), with each rat being placed in the maze from each starting position throughout the block. Starting positions were pseudorandomized by block. Rats were given 90 s to find an arm with an escape platform. Animals remained on the platform for 5-10 s before being placed back into their holding cage for approximately 50 s, for an inter-trial interval (ITI) of approximately 1 minute. If the platform was not found within 90 s, the experimenter guided the rat to the nearest platform and followed the same ITI procedure. An opaque white plastic sheet was attached to block entry into that arm in order to prevent a rat from returning to the same arm and forcing the rat to swim to, and therefore experience, all three arms containing escape platforms. Reference memory errors (RME) and repeated reference memory errors (rRME) were recorded. A reference memory error is defined as the first entry into an arm never containing a platform (i.e., arms A, C & D). A repeated reference memory error is defined as the second and subsequent entries into reference memory arms across the block. Entry into an arm is defined as when the rat's body (minus the tail) passed the gauze within the arm.

***Testing*.** The rats were next tested for acquisition of the RAWM for 5 days (5 blocks, 1 block/day). (Acquisition testing was delayed by 1 week due to experimenter illness). The procedure was identical to initial training except that once a platform was found it was removed for subsequent trials rather than blocking the entrance to the arm. Rats were still able to access this arm for the remaining trials. Reference memory errors, repeated reference memory errors, and short-term memory errors (WME) were recorded. A short-term memory error is defined as a re-entry into an arm previously containing an escape platform.

Results and Discussion

Non-spatial RAWM training. A 2x4 [environment (between-subjects factor) x days (within-subjects factor)] mixed-design analysis of variance (ANOVA) was used to analyze RME and rRME for the training data. Figure 2 depicts the average RME by group for the training period. Error bars on graphs represent standard error of the mean for all graphs. Evidence of learning that escape platforms are located in specific arms would be reflected in performance as a decrease in the RME error rate over days. It was hypothesized that enriched rats would improve performance at a quicker rate than isolated rats. However, results revealed no significant main effect of days [$F(3, 48)=1.53$, $p=.22$, $\eta^2_{partial}= .09$] or environment [$F(1, 16)=2.01$, $p=.18$, $\eta^2_{partial}= .11$], as well as no days x environment interaction [$F(3, 48)=.43$, $p=.73$, $\eta^2_{partial}= .03$]. Both enriched and isolated rats did not improve across days, but rather displayed relatively high RME rates across training.

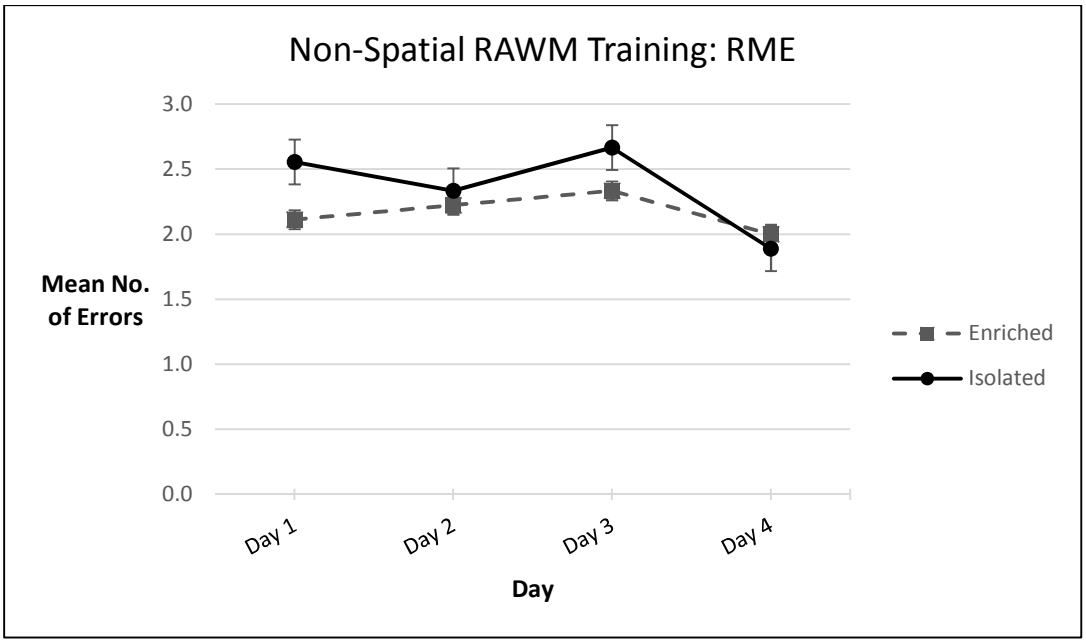

Figure 2. Average reference memory errors in non-spatial RAWM training.

Figure 3 displays the rRME for the training period for enriched and isolated rats. As with RME, a decline in error rates across days indicates improved performance. A 2x4 [environment (between-subjects factor) x days (within-subjects factor)] mixed-design ANOVA was used to analyze the data. There was no statistically significant main effect of days [$F(3, 48)=1.54$, $p=.14$, $\eta^2_{partial}= .11$] or environment [$F(1, 16)=.21$, $p=.65$, $\eta^2_{partial}=.01$]. Additionally, there was no statistically significant days x environment interaction [$F(3, 48)=1.25$, $p=.30$, $\eta^2_{partial}=.07$].

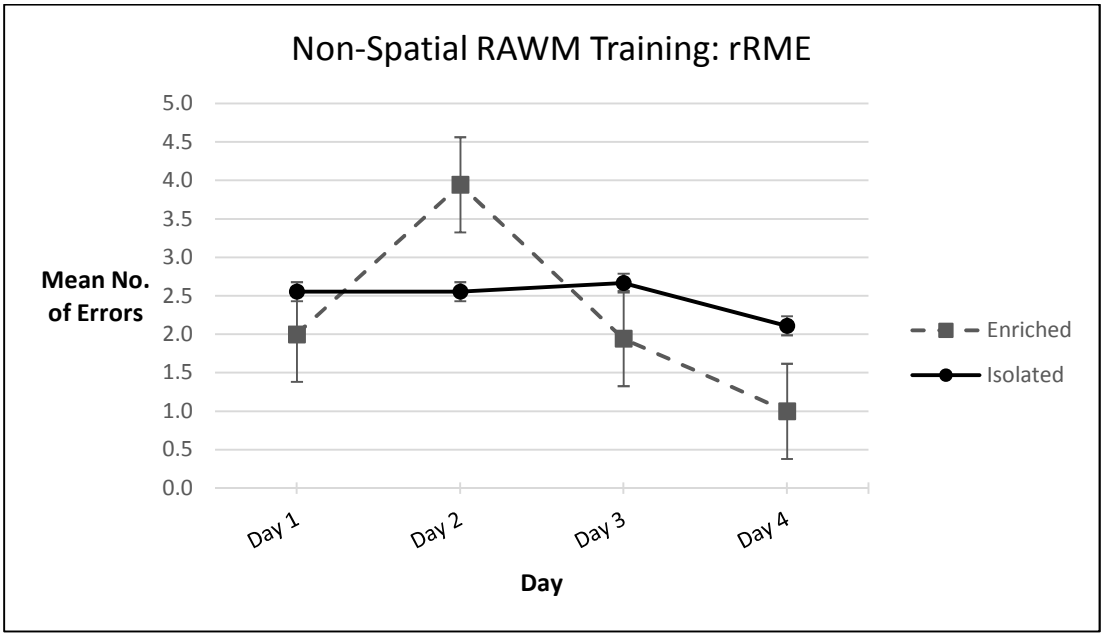

Figure 3. Average repeated reference memory error during non-spatial RAWM training. Data for day 2 and day 3 represent the average of two blocks of trials. One block of trials was performed on day 1 and day 4.

Testing. The acquisition data are shown in Figures 4, 5, and 6 (RME, rRME, and WME respectively). A separate 2x5 (environment x days) mixed-design ANOVA was used to analyze each type of error in the testing data. Environment was the between-groups factor; days was the within-groups factor. Figure 4 displays the RME performance for both groups during acquisition. The 2x5 mixed-design ANOVA revealed a main effect of days [$F(4, 64)=2.36$, $p=.05$,

$\eta^2_{partial}$=.14]. This, however, indicates an increase in error rates, suggesting poorer performance across days. Both groups of rats displayed an initial decrease in RME from day 1 to day 2. A paired samples t-test revealed a statistically trending decrease in errors for the enriched group [M_{D1}=2.56, SD_{D1}=.88; M_{D2}=1.56, SD_{D2}=1.13; t(8)=2.12, p=.07, d=.99]. Additionally, both groups displayed an increase in error rates on subsequent days. Paired samples t-tests were used to analyze these differences. Enriched rats' increase in errors from day 3 to day 5 was trending towards significance [M_{D3}=1.56, SD_{D3}=1.13; M_{D5}=2.56, SD_{D5}=.73; t(8)=-2.27, p=.05, d=-1.05] indicating a gradual worsening of performance. Isolated rats also demonstrated a significant increase in errors, on average, from day 2 to day 4 [M_{D2}=1.67, SD_{D2}=1; M_{D4}=2.44, SD_{D4}=.88; t(8)=-2.8, p=.02, d=-.82], confirming that they performed significantly worse across days. There was no main effect of environment [F(1, 16)=.01, p=.92, $\eta^2_{partial}$<.01] nor a days x environment interaction [F(4, 64)=.76, p=.56, $\eta^2_{partial}$=.05].

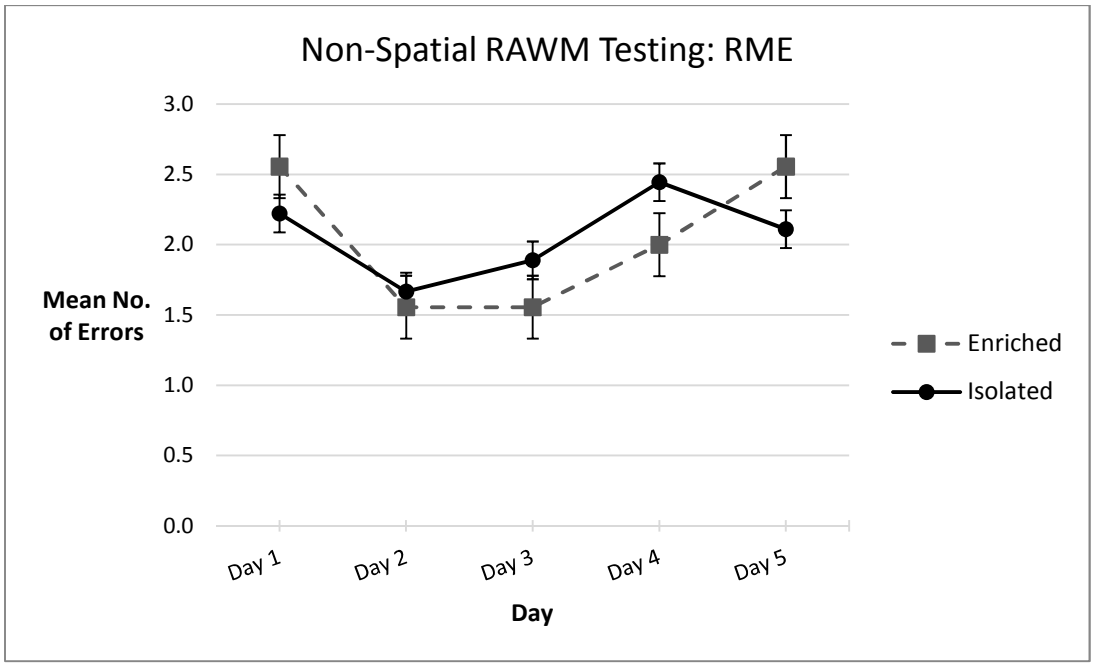

Figure 4. Average reference memory errors for during non-spatial RAWM acquisition.

Figure 5 shows rRME performance across acquisition days. Performance in this domain was not consistent. This is confirmed by a 2x5 mixed-design ANOVA, which revealed no statistically significant main effect of days [$F(1, 4)=1.77$, $p=.16$, $\eta^2_{partial}= .10$] or environment [$F(1, 16)=.17$, $p=.69$, $\eta^2_{partial}= .01$]. Additionally, there was no statistically significant days x environment interaction [$F(4, 64)=.49$, $p=.74$, $\eta^2_{partial}= .03$].

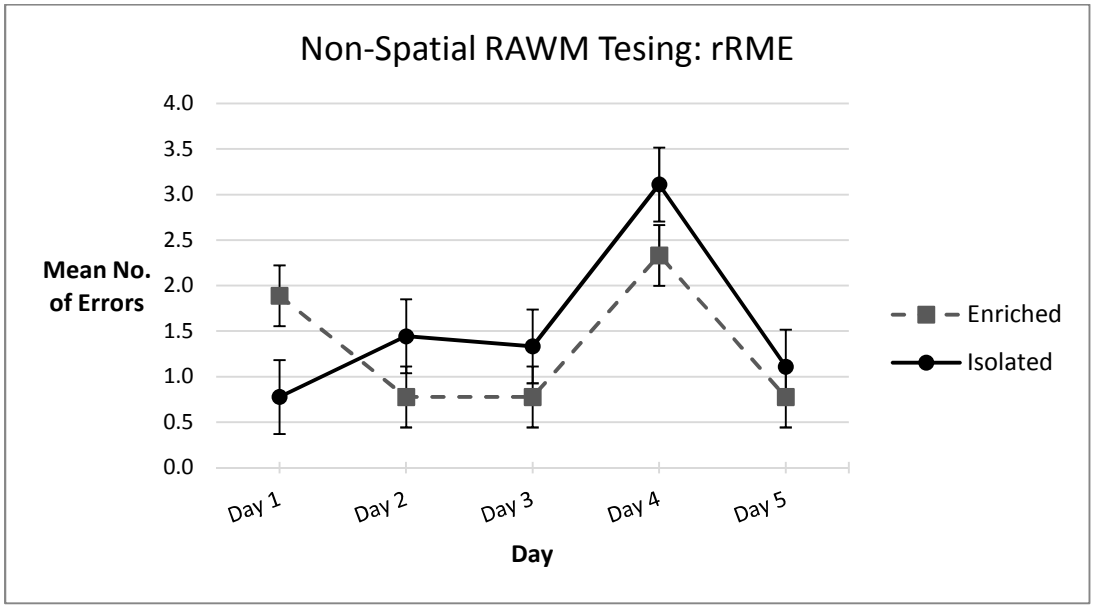

Figure 5. Average repeated reference memory errors for each group during non-spatial RAWM acquisition.

Figure 6 displays WME performance during acquisition. Analysis of the data reveals a trend towards a main effect of days in WME [$F(4, 64)=2.14$, $p=.09$, $\eta^2_{partial}= .12$]; however, a look at the graph illustrates that this trend demonstrates an increase in error rates across days, particularly from days 2-4, performance opposite that of our hypothesis. There was no statistically significant main effect of environment [$F(1, 16)=.45$, $p=.51$, $\eta^2_{partial}= .03$] or a days x environment interaction [$F(4, 64)=1.38$, $p=.25$, $\eta^2_{partial}= .08$].

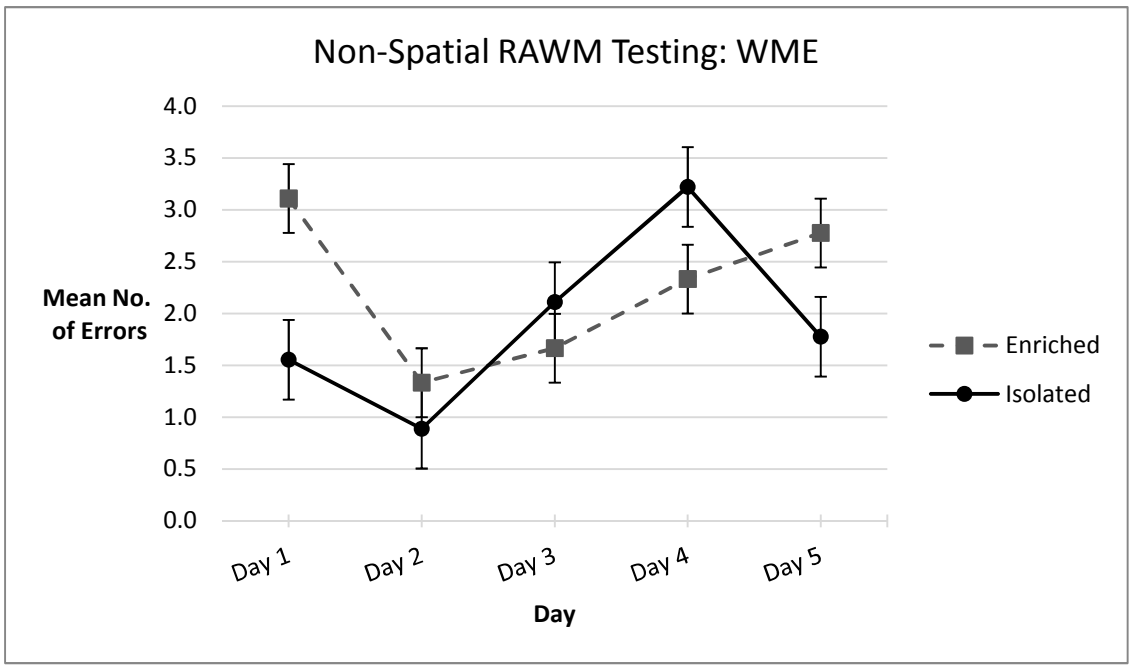

Figure 6. Average short-term memory errors for each group during non-spatial RAWM acquisition.

Results indicate that the rats showed some change in performance over days, but overall, this change did not reach conventional levels of statistical significance. Rats were not able to learn the maze to maximum stable performance. Both groups of rats displayed inconsistent performance in both training and acquisition. The lack of literature regarding non-spatial RAWM performance, as well as the current data, may point to one reason why performance was not as expected. It is possible that this type of maze may be more difficult for rats to learn and perform. This may be because it is not in a rat's natural, instinctual, behavior pattern to perform in this manner. According to various conditioning theories, reinforcement of a particular behavior should strengthen that behavior; however, we often come across instances where animals perform behaviors outside of those of which they were conditioned. Breland and Breland (1961) describe this phenomenon in animals as instinctual drift. This means that although an animal may have learned a conditioned behavior, its performance gradually drifts towards more instinctual behavior patterns. In a way, evolution may not have prepared these animals to behave

in the way we would expect them to. Although we are reinforcing ideal behaviors (e.g., removal from the water once a platform is found), it is possible that the rat's biological predispositions and evolutionary history are not in agreement, and more ecologically relevant patterns of behavior emerge (Hoffman et al., 1999). This is in contrast to typical laboratory expectations.

Smell is most closely related to obtaining food than any other sense (Vincent, 1970). The use of olfactory and tactile cues, cues which rats may typically use to find food, may have evoked behaviors typically seen in a behavior subsystem of feeding, a concept introduced by Timberlake (2001). Additionally, rats are nocturnal creatures, implying that their prime feeding time is at night. Training and testing rats under dim red light may have elicited a general search mode, which rats would typically use as part of a feeding subsystem. This would produce behaviors such as traveling, locomotion, investigating, sniffing, and scanning (Timberlake, 2001). Instead of carrying out feeding behaviors, rats in this experiment were swimming, and therefore unable to produce behaviors that would assist in the collection of food. Ultimately, this may have interfered with learning the maze.

Though behavior in the maze was generally inconsistent, a comparison of rRME of enriched rats during training (figure 3) and acquisition (figure 6) shows that rRME rates during acquisition were mostly at or below 1.5 errors, whereas during training error rates only reached that level once. This indicates that although they could not perform consistently, enriched rats showed evidence of learning. Nevertheless, the task was still extremely difficult for them and none of the animals was able to learn the maze to criterion.

Additional evidence of learning may be seen when analyzing effect sizes. Assuming Cohen's (1992) conventions, effect sizes for the effect of days produced by the mixed-design ANOVAs examining RME ($\eta^2_{partial}$=.09) and rRME ($\eta^2_{partial}$=.11) during training and RME

($\eta^2_{partial}$=.14) during testing reflect medium-large effects. This suggests that more statistical power may be necessary to reach statistical significance.

Experiment 2

In order to provide another strategy for rats to learn the RAWM, we provided visual spatial cues in experiment 2. These types of cues are more typically used in RAWM procedures and therefore, should provide opportunities for additional strategy use by the rats. Due to ethanol and environmental enrichment's opposing effects on memory (previously discussed), a phase observing the effect of ethanol consumption on spatial RAWM maze behavior was also introduced.

Method

Subjects. Subjects were the same rats used in experiment 1. Animals were approximately 11 weeks old at the start of this experiment and were maintained as in Experiment 1 throughout training and baseline testing. During ethanol gel consumption training, animals were placed on a restricted food schedule which lasted through the end of the study (5 weeks total). Rats were given unrestricted access to food for 1 hour per day after testing for that day was complete. Water was available *ad libitum*. All procedures took place during the light cycle (approximately 1100-1300 hours).

Apparatus. Enrichment and isolation cages were the same cages used in experiment 1. Rats were exposed to their environments after access to food was finished. No food was available during this time; however water was available *ad libitum*. The radial arm water maze was the same maze used in experiment 1, but modifications were made to allow testing with the presence of spatial extra-maze cues. For this experiment, one of the shower curtains was removed, leaving one shower curtain on each side of the maze (see Figure 7). Visual spatial cues were affixed to each shower curtain. Four sets of stripes were taped to the south curtain; four circles were taped to the north curtain. An example of the visual cues is shown in Figure 8. Each

set of cues measured 43.2 cm x 55.9 cm. Tactile and olfactory cues remained in the maze and were the same cues used in experiment 1. Escape platforms were placed in the same arms each day (arms B, E, & F). The tub containing the RAWM was not rotated as in the previous experiment and remained in the same place across all training and testing so that the rats had the opportunity to use the fixed spatial extra-maze cues in addition to the olfactory and tactile intra-maze cues to locate the alleys with the escape platforms.

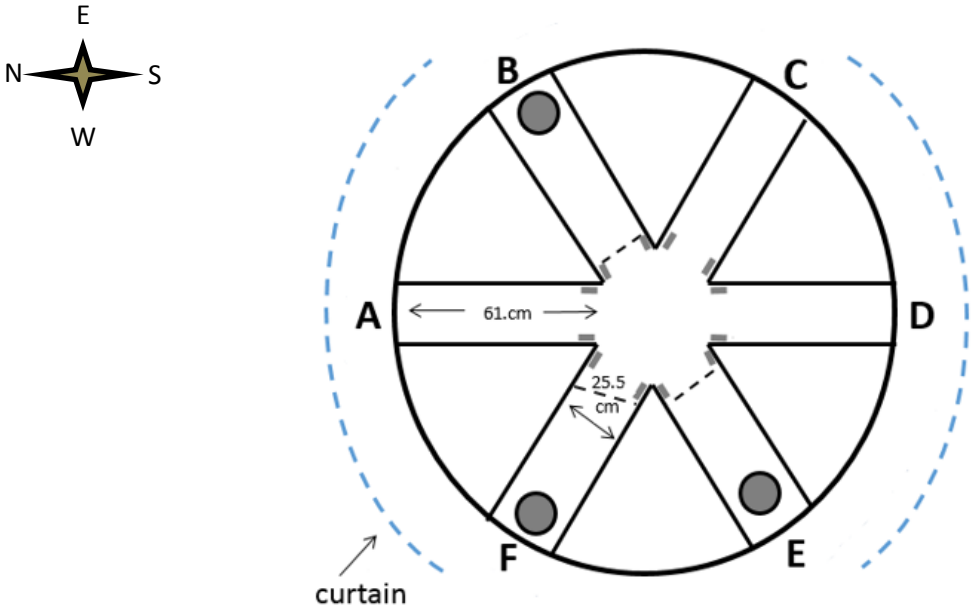

Figure 7. Schematic of the radial arm water maze. Dotted lines across arms indicate hanging fishing line. Grey boxes at the entrance to each arm indicate where gauze was placed. Grey circles at the end of an arm indicate where an escape platform was located. Arms containing escape platforms were scented via extract applied to the gauze. Visual spatial cues (see Figure 8) were affixed to the center of the each curtain.

Figure 8. Visual spatial cues attached to the shower curtains surrounding the RAWM. Stripes were attached to the south curtain; circles were attached to the north curtain.

Ethanol (10%) -Polycose gel. Polycose is a glucose polymer (oligosaccharide) used commonly by human as a source of carbohydrates for those with increased caloric needs. Polycose powder can be prepared directly in foods and beverages due to its relatively tasteless nature to humans (Abbott Laboratories, 2013). But Polycose is very tasty to rats (Sclafani, 1987) and therefore it is commonly used in rodent experiments as a palatable agent. We used a Polycose solution in gelatin form, akin to that of (Rowland et al., 2005), as a vehicle for ethanol consumption. Rats were first introduced to the Polycose gel without any ethanol in order to develop a taste preference for it. Ethanol was added after the rats showed stable, elevated consumption rates. To prepare the gel without ethanol, water was boiled and unflavored gelatin powder was added (Knox; 3 g/100 ml). Polycose was then added (10% by weight) and the solution was mixed until all powder was dissolved. The solution was then poured into mini 1 3/4" Clay Pots (Michaels Stores), covered with plastic wrap, and allowed to cool. Each flower pot was previously altered to contain a screw through the bottom of the pot so that it could be affixed to the floor of the test cage. When preparing the ethanol gel, a 10% ethanol solution was used as the base. The ethanol was heated until it was almost boiling, to avoid evaporation of ethanol. Polycose and gelatin powder were then added and the solution was mixed until powder dissolved. Again, it was poured into small clay flower pots, covered with plastic wrap and allowed to cool. The gels were prepared daily to limit evaporation of ethanol and gelatin.

Procedure. All rats were given initial training and baseline testing in the modified RAWM, followed by several weeks of Polycose gel consumption training before the impact of ethanol-gel consumption on RAWM performance could be assessed.

Spatial RAWM training. Training in the modified spatial version of the RAWM lasted 8 days (11 blocks) and the procedure was identical to experiment 1, with previously visited arms with escape platforms blocked on subsequent trials. Three days of training allowed the animals 2 blocks of trials for additional practice in the maze. Error rates were averaged across blocks each day for analysis. Reference memory errors and rRME were recorded.

Baseline RAWM testing. Baseline testing lasted 10 days (10 blocks, 1 block/day). The testing procedure was identical to that of experiment 1 with RME, rRME, and WME recorded as the dependent variables with previously visited arms with escape platforms no longer blocked as in training.

10% ethanol-Polycose gel consumption training. Training of Polycose gel consumption lasted 22 days. Although RAWM testing was suspended for the duration of gel consumption training, environmental exposure was maintained throughout all phases of the study. Each day all rats were transported to their holding cages in the RAWM room. The screw attached to the pot was threaded through the grid wire floor of the holding cage and held in place by a clothes pin. This allowed the pot to remain upright and minimized spillage, even when the rats tried to manipulate the pots. Some spilling of the gel did occur early in the procedure; however, the amount spilled was negligible (< 3 g) and decreased over time. Rats were given access to the gel for 30 minutes. Pots were weighed before and after consumption. During the first 12 days Polycose were provided in the pots. For the remaining 10 days ethanol was added to the Polycose gel. After 6 days of consuming the ethanol-Polycose gel the rats were placed on food deprivation (I hour per day) due to decreasing levels of gel consumption.

RAWM performance after ethanol-Polycose gel consumption. All rats received 4 days (4 blocks) of re-training in the RAWM to remind them of the procedure. Testing lasted 5 days (5

blocks). Rats were brought to the RAWM testing room and placed in holding cages. All rats were given access to ethanol-Polycose gel for 30 minutes and subsequently tested in the RAWM. Gel access was staggered by 5 minutes across rats in order to test rats immediately following consumption. The clay pots were weighed before and after testing to determine consumption.

Results and Discussion

Spatial RAWM training. Figure 9 depicts the RME for training in the spatial RAWM. As in experiment 1, it was hypothesized that learning where the escape platforms were located would be reflected in performance as decreased error rates across days. We also hypothesized that enriched rats would display significantly lower error rates than isolated rats. As the graph shows, both groups of rats displayed lower error rates, on average, across days. This was verified by performing a 2x8 [environment (between-subjects factor) x days (within-subjects factor)] mixed-design ANOVA. A main effect of days was found [$F(7, 112)=11.50$, $p<.001$, $\eta^2_{partial}=.42$] indicating that both groups of rats performed significantly better over time. The decrease in error rates across days suggests that the rats did learn where the escape platforms were located. Beginning on day 3, the enriched rats produced lower error rates, on average, than the isolated rats; however, the groups did not significantly differ from each other, as no main effect of environment was found [$F(1, 16)=2.58$, $p=.13$, $\eta^2_{partial}=.14$] nor did environment interact significantly with days [$F(7, 112)=1.40$, $p=.21$, $\eta^2_{partial}=.08$].

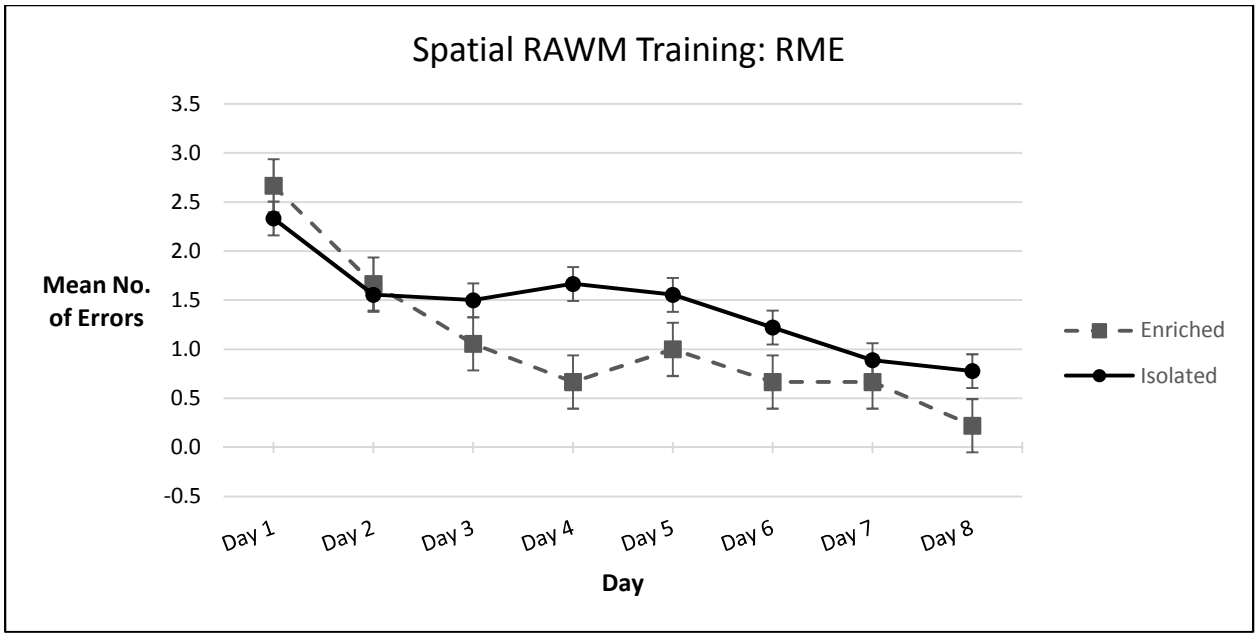

Figure 9. Reference memory errors across 8 days of spatial RAWM training. Two blocks of training were provided on days 2, 3 and 7; data points represent the average error rate per day.

Figure 10 illustrates the rRME during spatial RAWM training. The graph illustrates a similar pattern to RME, displaying decreased error rates across days for both groups. A 2x8 [environment (between-subjects factor) x days (within-subjects factor)] mixed-design ANOVA reveals a main effect of days [$F(7, 112)=7.72$, $p<.001$, $\eta^2_{partial}=.33$] suggesting that rats performed significantly better over time. This indicates that once the rats entered an arm that never contained a platform, they were less likely to enter that arm again during the training block. There was no main effect of environment [$F(1,7)=0.87$, $p=.37$, $\eta^2_{partial}=.05$], nor an environment x days interaction [$F(7, 112)=.07$, $p=.39$, $\eta^2_{partial}=.06$].

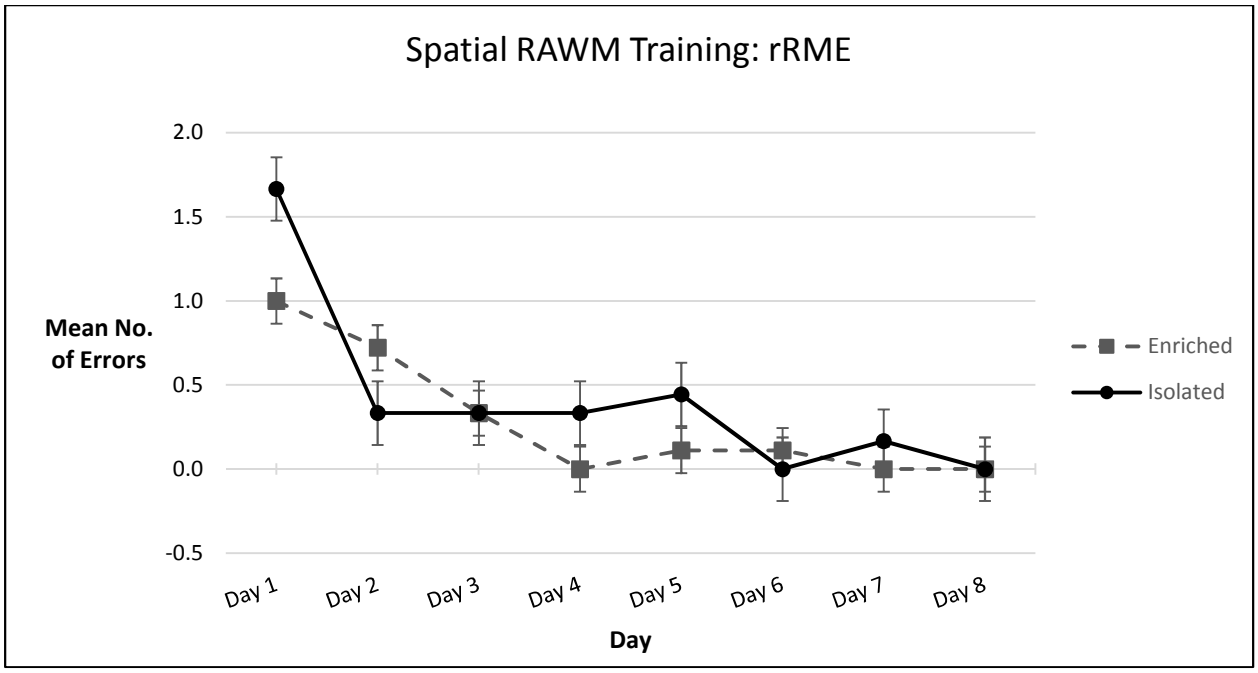

Figure 10. Repeated reference memory error across 8 days of spatial RAWM training. Two blocks of training were provided on days 2, 3, and 7; data points represent the average error rate per day.

These results for both RME and rRME suggest that the rats did, in fact, learn which arms contained the escape platforms.

Baseline RAWM testing. Testing in the spatial RAWM (i.e., previously visited arms with escape platforms were no longer blocked as in training) lasted for 10 days. Separate 2x10 [environment (between-subjects factor) x days (within-subjects factor)] mixed-design repeated measures ANOVAs were used to analyze each error (RME, rRME, WME). Spatial RAWM testing RME are illustrated in Figure 11. The overall number of errors during testing was at or below the average number of errors committed towards the final days of training (see Figure 9). Thus, despite the previous alleys no longer blocked during testing, the rats still performed fairly well in terms of not visiting arms that never contained an escape platform. The mixed-design ANOVA results confirm a main effect of days [$F(9, 144)=1.94$, $p=.05$, $\eta^2_{partial}=.11$], suggesting

that both groups of rats significantly decreased the average amount of errors over days. There was no statistically significant main effect of environment [$F(1,16)=.20$, $p=.66$, $\eta^2_{partial}=.01$], nor a days x environment interaction [$F(9, 144)=.31$, $p=.94$, $\eta^2_{partial}=.02$]. These results indicate that performance in terms of reference memory errors was near asymptote and somewhat improved with additional testing.

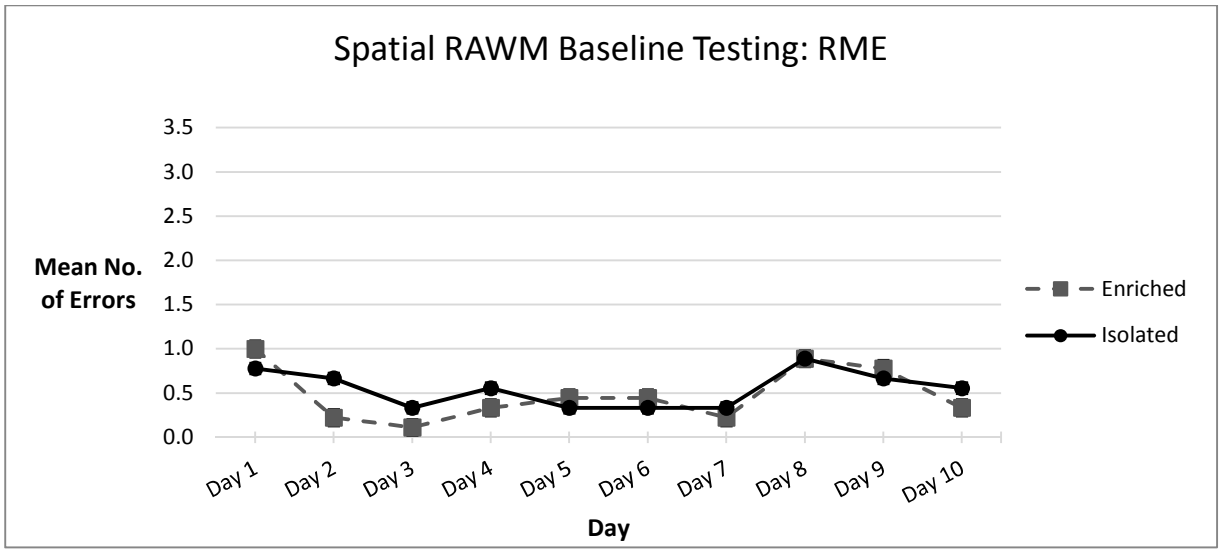

Figure 11. Average reference memory errors for each environmental group across 10 days of testing in the spatial RAWM.

Examining the average rRME during testing in Figure 12, both groups demonstrated good performance, as their error rates, on average, remained relatively low. Analysis using the mixed-design ANOVA revealed no statistically significant main effect of days [$F(9, 144)=.65$, $p=.76$, $\eta^2_{partial}=.04$] or environment [$F(1, 16)=2.57$, $p=.13$, $\eta^2_{partial}=.14$]. There was also no significant days x environment interaction [$F(9, 144)=.84$, $p=.58$, $\eta^2_{partial}=.05$]. As with RME, error rates remained at or below the average amount of rRME during the final days of training. This suggests that performance was at asymptote. When rats entered an arm that never contained a platform, they were unlikely to return to that arm again.

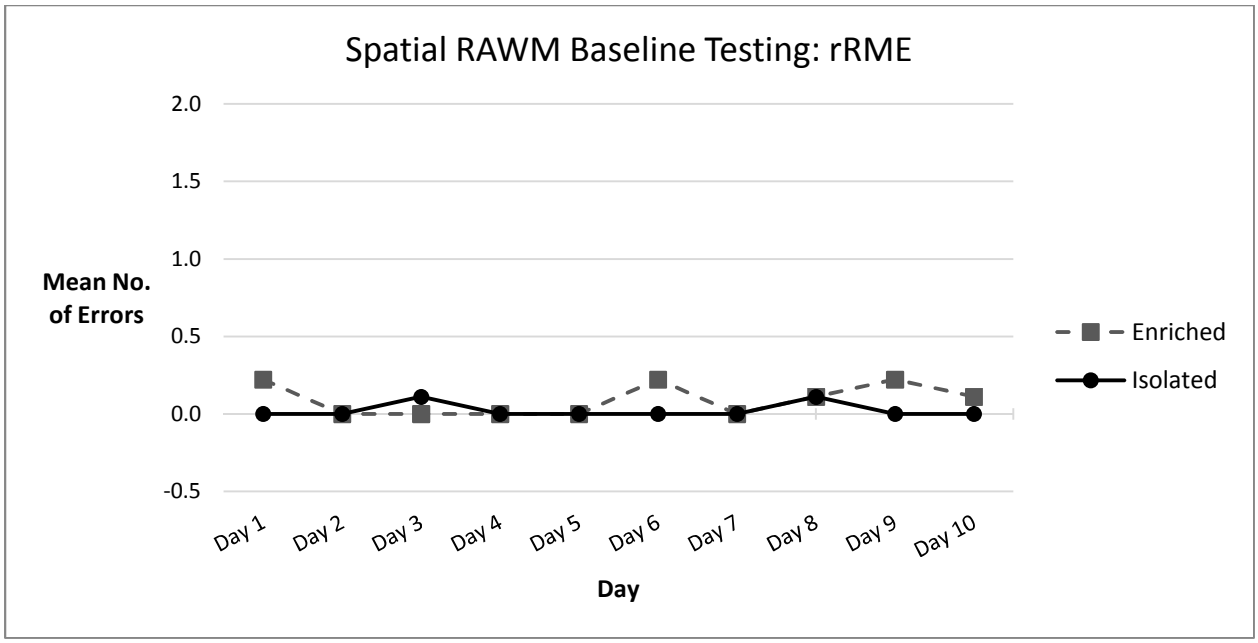

Figure 12. Average repeated reference memory errors for enriched and isolated rats across 10 days of testing in the spatial RAWM.

Figure 13 displays the average WME during testing for both groups. Patterns of performance appear to remain relatively stable across day, remaining consistently below 2 errors. It appears as though enriched rats performed worse than isolated rats, reflected in higher error rates. However, the 2x10 mixed-design ANOVA revealed no statistically significant main effect of days [$F(9, 144)=.30$, $p=.97$, $\eta^2_{partial}=.02$] or environment [$F(1, 16)=2.52$, $p=.13$, $\eta^2_{partial}=.14$], as well as no days x environment interaction [$F(9, 144)=.37$, $p=.95$, $\eta^2_{partial}=.02$]. These results suggest that, although the rats appear to have learned where the escape platforms are during training, during testing they were unable to improve in their ability to avoid entering arms that had previously contained platforms.

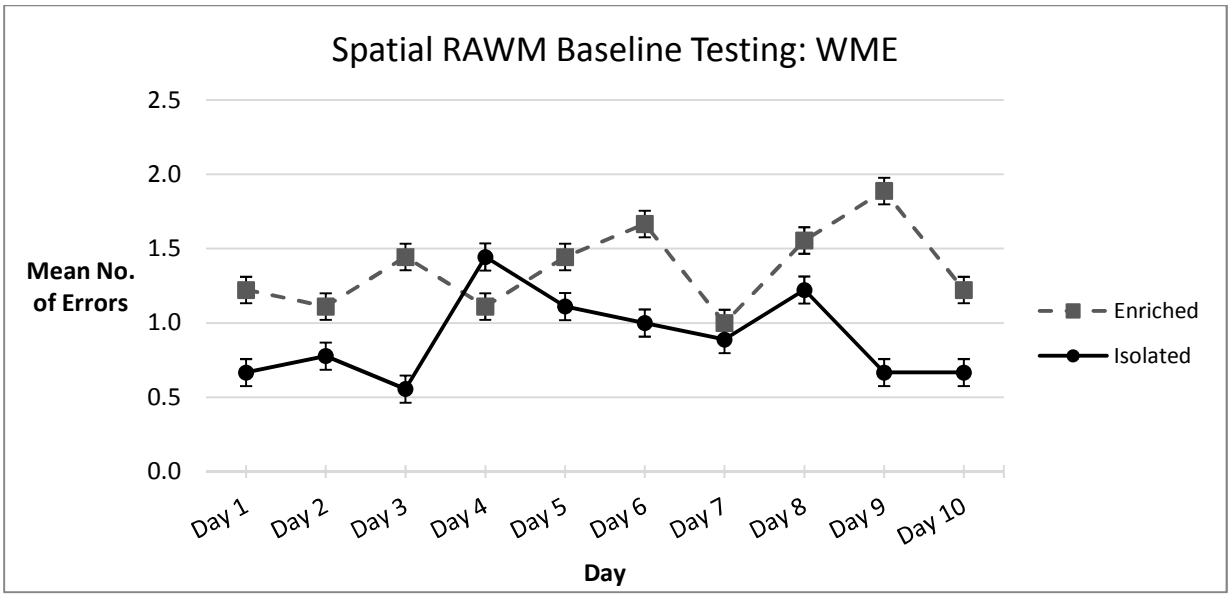

Figure 13. Average short-term memory errors across 10 days of testing in the spatial RAWM.

10% ethanol-Polycose gel consumption training. Following baseline, RAWM testing was suspended so rats could be trained to steadily consume the 10% Polycose gel. Figure 14 illustrates the average gel consumption across the 12 days of training for each group. It is evident that consumption increased over days. This was confirmed to be a significant increase through a 2x12 [environment (between-subjects factor) x days (within-subjects factor)] mixed-design ANOVA which revealed a main effect of days [$F(11, 176)=44.22$, $p<.001$, $\eta^2_{partial}=.73$]. Pairwise comparisons were performed using a Bonferroni correction to further analyze the data. Specifically, consumption increased rapidly over the first 6 days [$M_{D1}=.39$, $SD_{D1}=.73$, $M_{D6}=12.44$, $SD_{D6}=3.62$, $p<.001$] and remained stable for the final 6 days. The graph also suggests that there was little difference in consumption between treatment groups. Again, this was confirmed in the ANOVA, as no statistically significant main effect of treatment was found [$F(1, 16)=30.38$, $p=.44$, $\eta^2_{partial}=.04$], nor was there a significant days x environment interaction [$F(11, 176)=5.26$, $p=.79$, $\eta^2_{partial}=.04$].

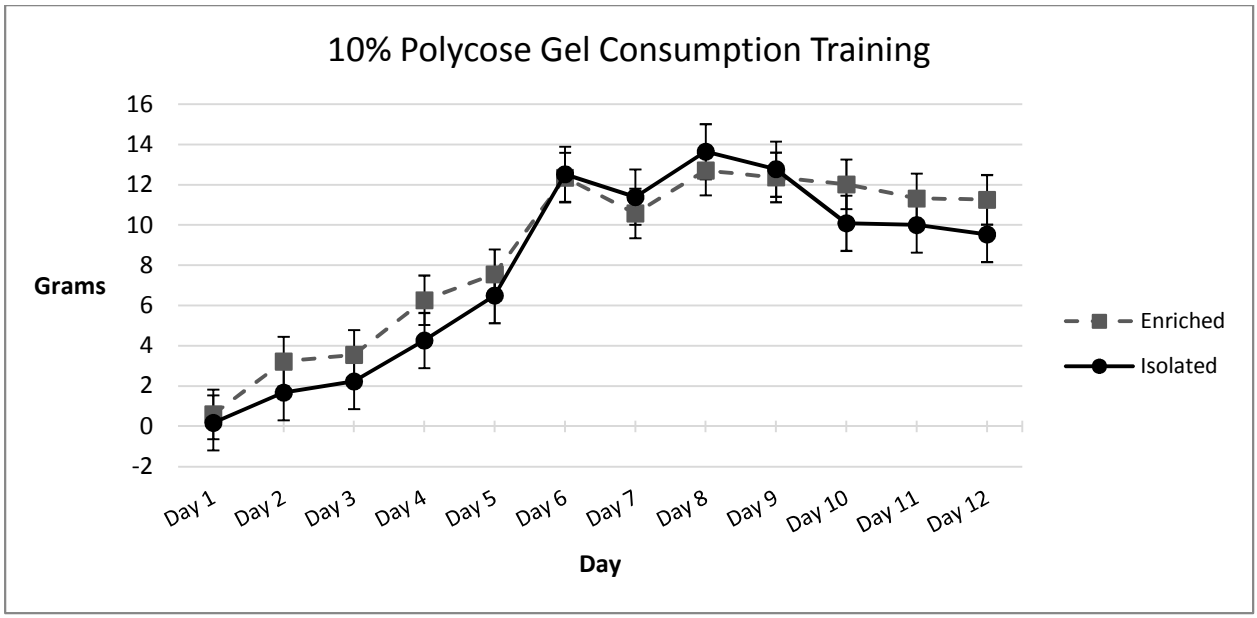

Figure 14. Average Polycose gel consumption for each group during 12 days of gel consumption training.

Figure 15 displays the average consumption of 10% ethanol-Polycose gel for each group across 10 days of training. Recall that rats were placed on food deprivation beginning on day 6 due to the decrease in gel consumption. Across the 10 days of consumption training, enriched rats consumed an average of 9.04 g of gel per day, while isolated rats consumed an average of 9.55 g of gel per day. However, both groups similarly display a large variation in gel intake across training days.

Paired samples t-tests were conducted to confirm changes in consumption for each group across days (see table 1). The isolated group produced a significantly sharp decrease in consumption between days 1-4, then increased the amount of gel ingested until day 8. Finally, on the last day, intake decreases.

Table 1

10% ethanol-Polycose gel consumption training-Isolated

Day	Avg.	*SD*	**Day**	Avg.	*SD*	*df*	*t*	*p*	*d*
1	11.33	2.35	**4**	5.31	1.40	8	5.38	<.01**	3.11
4	5.31	1.40	**8**	12.86	3.21	8	6.51	<.01**	-3.05
8	12.86	3.21	**10**	9.80	2.52	8	3.94	<.01**	1.06

Table 1. Significant changes in consumption by isolated rats during 10% ethanol-Polycose gel consumption training analyzed using paired-samples *t*-tests. **$p<.01$.

The enriched rats' consumption pattern is more varied than rats exposed to isolation (see table 2). They produced a significant decrease in gel ingestion from days 1-5. Consumption then begins to significantly increase until day 7. The rats varied between high and low consumption for the final 3 days. The enriched rats also display a decrease in consumption between days 9-10, however, this only trended towards significance.

Table 2

10% ethanol-Polycose gel consumption training-Enriched

Day	Avg.	*SD*	**Day**	Avg.	*SD*	*df*	*t*	*p*	*d*
1	12.67	4.33	**5**	6.41	1.97	8	4.60	<.01**	1.86
5	6.41	1.97	**7**	9.97	2.95	8	-3.69	<.01**	-1.42
9	10.81	5.70	**10**	8.69	3.49	8	2.27	.05	.45

Table 2. Significant changes in consumption by enriched rats during 10% ethanol-Polycose gel consumption training analyzed using paired-samples *t*-tests. **$p<.01$.

Despite this fluctuation in ethanol-gel intake, the rats from both groups were consuming significant amounts of ethanol per day with little consistent difference in voluntary ethanol intake between rats exposed to enrichment or isolation.

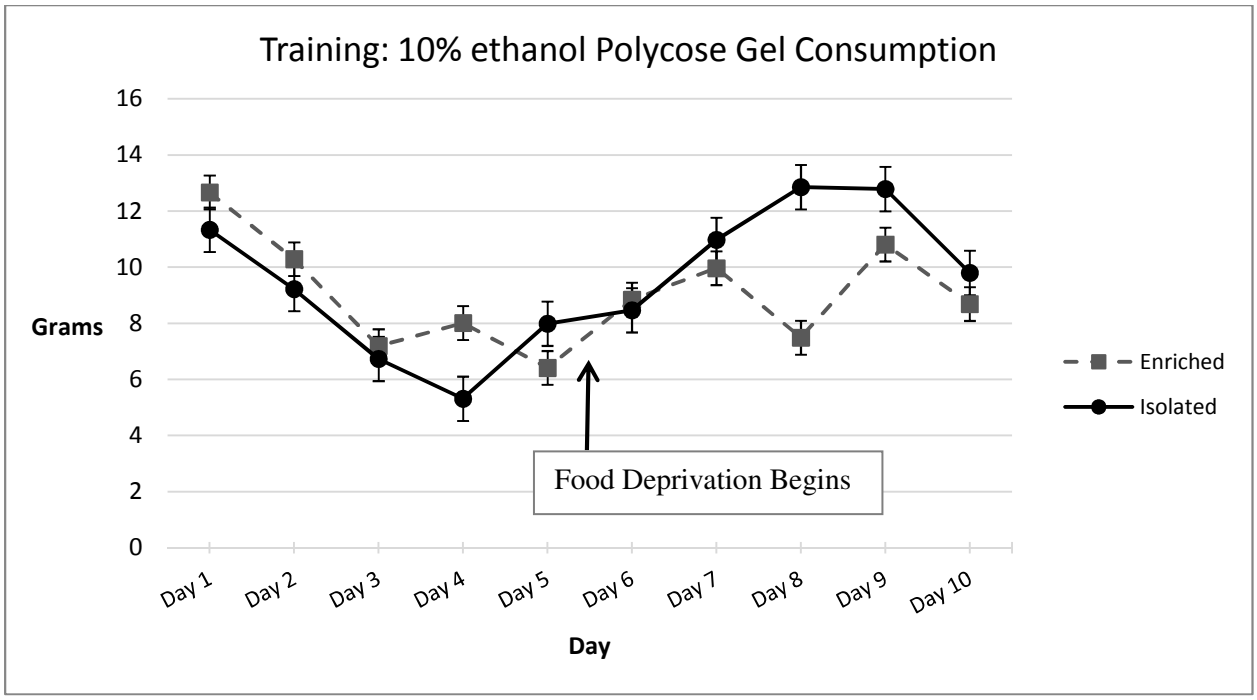

Figure 15. Average ethanol-Polycose gel consumption for each group across 10 days of training. Food deprivation was initiated after day 5.

Effect of ethanol-Polycose consumption of RAMW performance. Radial arm water maze testing was resumed after training in gel consumption was complete. The rats were given 4 days of 'reminder' blocks (4 blocks) in the maze (data not shown) and then tested after gel consumption. The average ethanol-Polycose gel consumption for each group during this phase is displayed in Figure 16. The graph shows that consumption remained fairly stable across days. Isolated rats produced consistently higher consumption rates than enriched rats [M_{ISO}=15.10 g, SD_{ISO}=5.49 g, M_{EE}=13.92 g, SD_{EE}=4.93 g], however, there was no statistically significant main effect of environment [$F(1, 16)=2.23$, $p=.16$, $\eta^2_{partial}= .12$] found when a 2 x 5 [environment (between-groups factor) x days (within-groups factor)] mixed-design ANOVA was used to analyze the data. There was also no significant main effect of days [$F(4, 64)=1.34$, $p=.26$, $\eta^2_{partial}=.08$], suggesting no differences in consumption patterns between groups or across days.

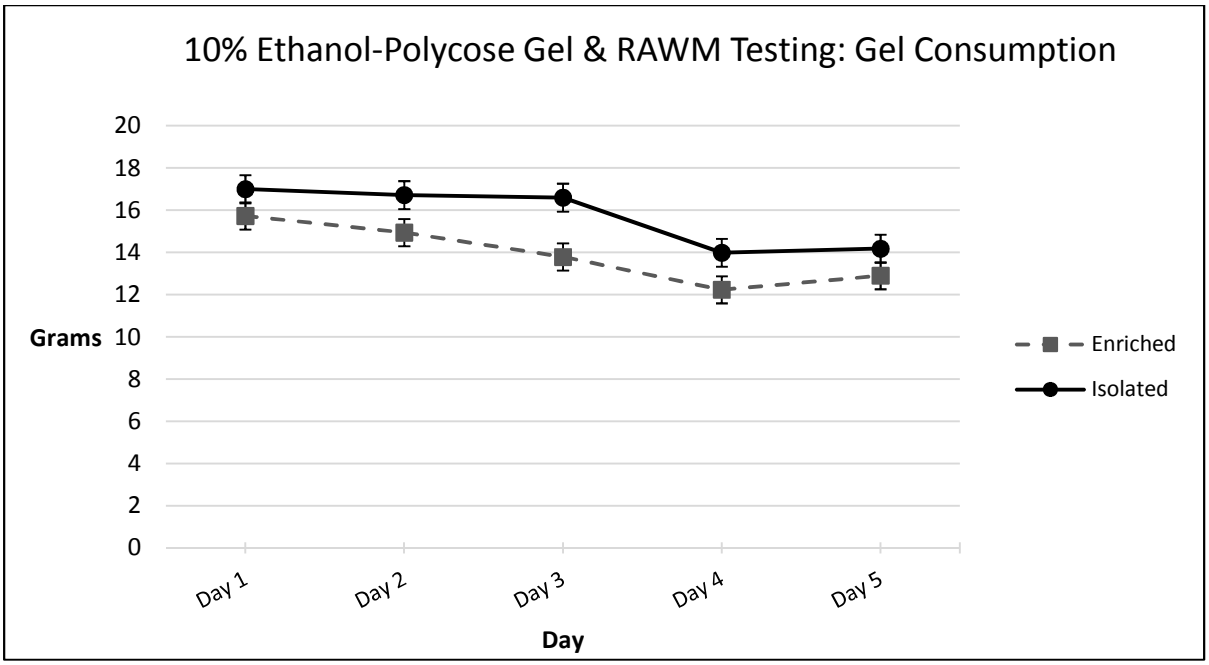

Figure 16. Average ethanol-Polycose gel consumption for each group during RAWM testing

Reference memory errors (RME). Figure 17 shows the average reference memory errors for each group across 5 days of testing after gel consumption. A 2 x 5 [environment (between-groups factor) x days (within-groups factor)] mixed-design ANOVA analyzing RME reveals no statistically significant main effects of environment [$F(1, 16)=1.10$, $p=.31$, $\eta^2_{partial}=.06$] or days [$F(4, 64)=1.55$, $p=.20$, $\eta^2_{partial}=.09$]. Additionally, the days x environment interaction failed to be significant [$F(4, 64)=.65$, $p=.63$, $\eta^2_{partial}=.04$]. On average, error rates appear to be slightly higher than performance during baseline (Figure 11), suggesting that ethanol-Polycose gel consumption may have adversely affected reference memory performance.

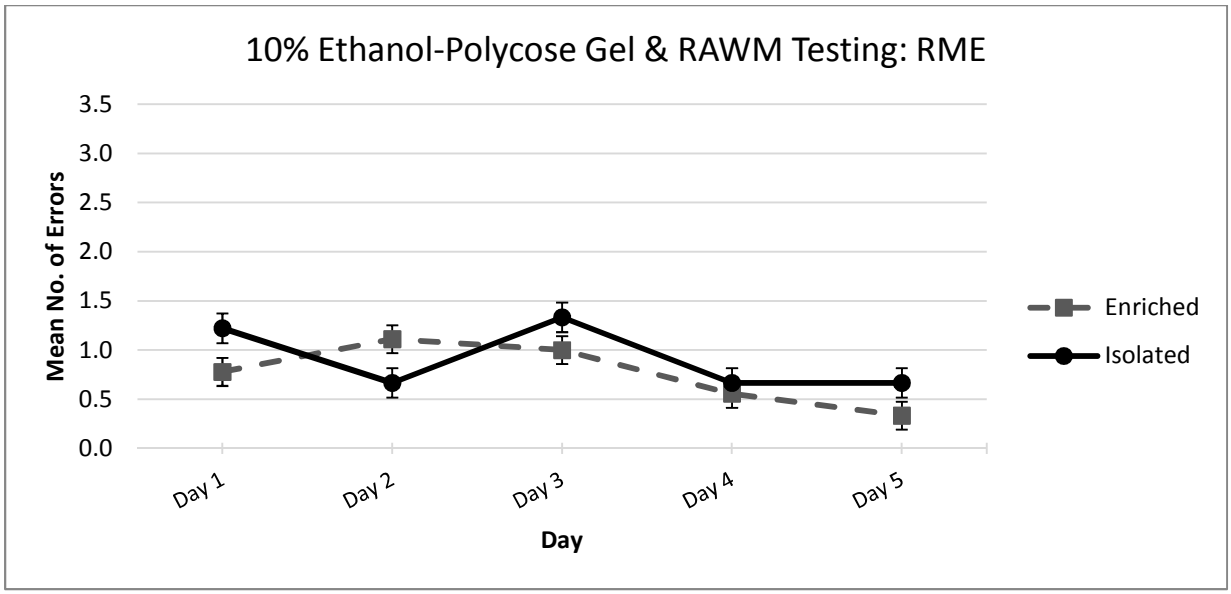

Figure 17. Average reference memory errors across 5 days of RAWM testing subsequent to 10% ethanol-Polycose gel consumption.

A 2 x 5 x 2 [environment (between-subjects factor) x days (within-subjects factor) x ethanol consumption (within-subjects factor)] mixed-design ANOVA for each type of error (RME, rRME, and WME) was used to compare baseline error rates, when there was no ethanol-Polycose gel consumption, to error rates after 30 minutes of gel consumption. Figure 18 illustrates the pattern of average RME rates for each group. There was a significant main effect of ethanol in the analysis [$F(1, 16)=12.59$, $p=.003$, $\eta^2_{partial}=.44$] indicating that, on average, the rats maintained higher error rates after consuming the ethanol gel than during baseline assessment. This is true for both treatment groups, particularly in days 1-3. There was no main effect of days [$F(4, 64)=1.23$, $p=.31$, $\eta^2_{partial}=.07$] or environment [$F(1, 16)=3.59$, $p=.08$, $\eta^2_{partial}=.05$], as well as no interactions.

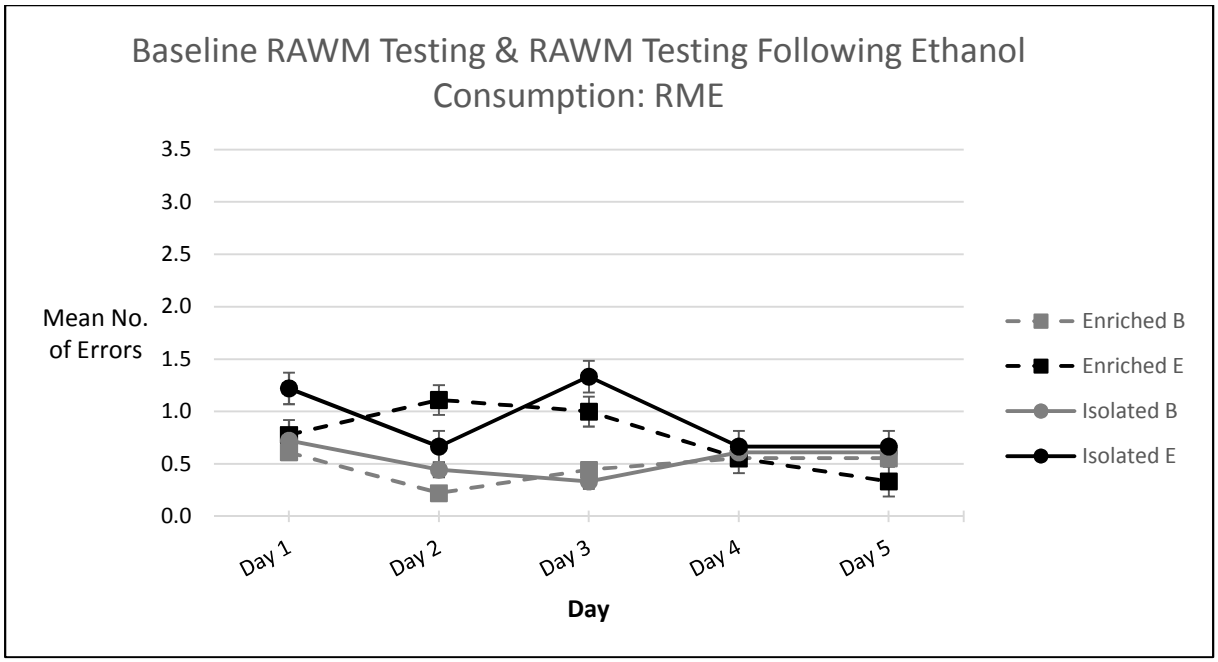

Figure 18. Average reference memory errors in the RAWM for enriched and isolated rats during baseline testing (10 days, collapsed every 2 days) and following 10% ethanol-Polycose gel consumption. B= baseline testing (grey); E=ethanol gel consumption testing (black).

Repeated reference memory errors (rRME). Repeated reference memory errors (rRME) occur when a rat returns to an arm of the RAWM that never contained an escape platform. Average rRME rates following ethanol consumption are plotted for each group in Figure 19. Error rates remain low, although they appear slightly higher than during baseline testing (Figure 12). A 2 x 5 [environment (between-groups factor) x days (within-groups factor)] mixed-design ANOVA did not reveal a significant main effect of environment [$F(1, 16)=2.75$, p=.13, $\eta^2_{partial}=.14$] or days [$F(4, 64)=0.63$, $p=.64$, $\eta^2_{partial}= .04$], as well as no days x environment interaction [$F(4, 64)=.38$, $p=.82$, $\eta^2_{partial}=.02$].

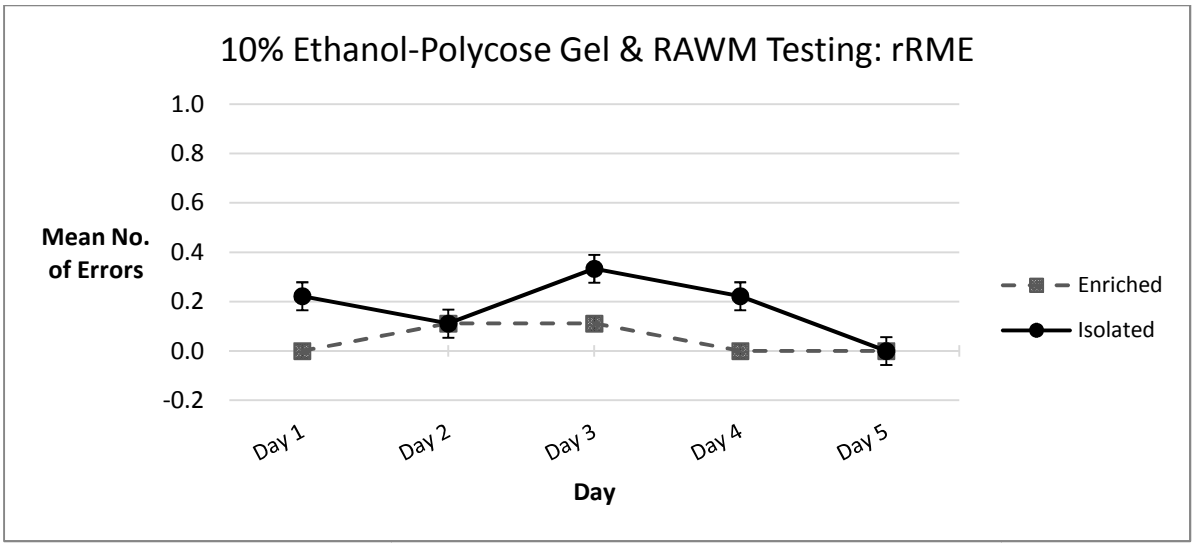

Figure 19. Average repeated reference memory errors across 5 days of testing subsequent to 10% ethanol-Polycose gel consumption.

Figure 20 illustrates the average rRME rates for each group during baseline testing and after ethanol-Polycose gel consumption. The 2 x 5 x 2 [environment (between-subjects factor) x days (within-subjects factor) x ethanol consumption (within-subjects factor)] mixed-design ANOVA revealed an ethanol x treatment interaction [$F(1, 64)=6.48$, $p=.02$, $\eta^2_{partial}=.29$]. The graph suggests that this interaction is driven by the effect of ethanol consumption on the isolated rats' performance but not the enriched rats. The isolated rats' error rates during those testing days remain higher, on average, than either group in baseline testing or enriched rats' performance following ethanol consumption.

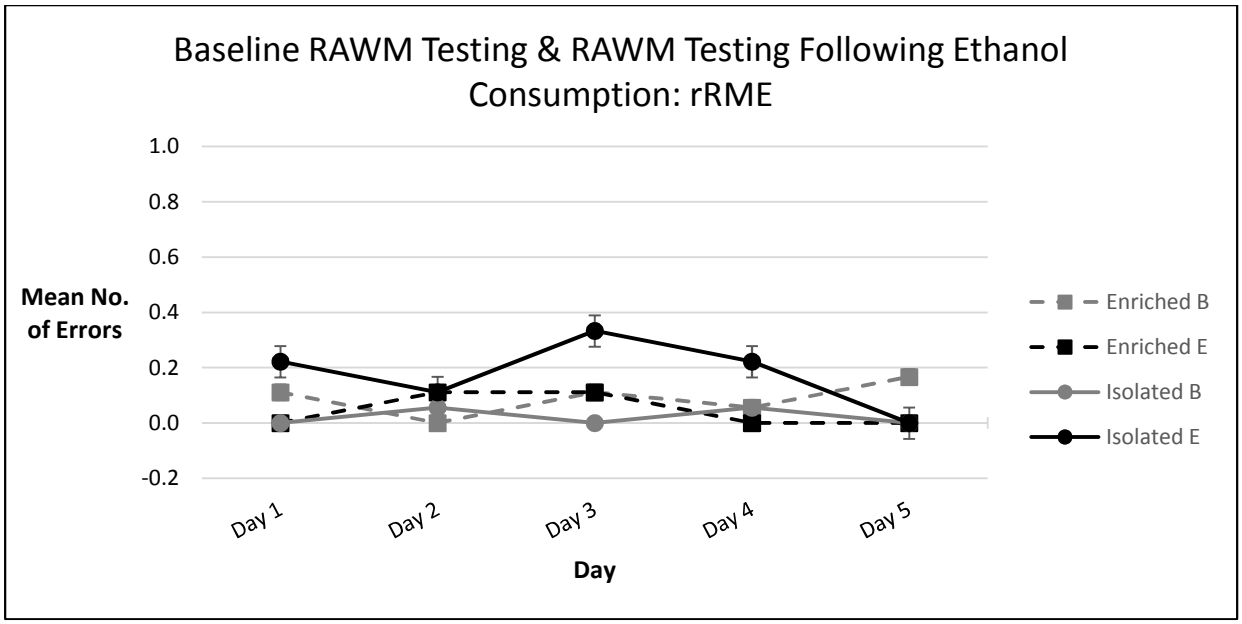

Figure 20. Average repeated reference memory errors in the RAWM for enriched and isolated rats during baseline testing (10 days, collapsed every 2 days) and following 10% ethanol-Polycose gel consumption. B= baseline testing (grey); E=ethanol-gel consumption testing (black).

Short-term memory errors (WME). Figure 21 illustrates the average short-term memory errors for each group across the 5 days of testing. Recall that a short-term memory error occurs when a rat returns to an arm of the RAWM that previously contained an escape platform. There is similar variability in error rates for both enriched and isolated rats. A 2 x 5 [environment (between-groups factor) x days (within-groups factor)] mixed-design ANOVA revealed no statistically significant main effect of environment [$F(1, 16)=1.11$, $p=.33$, $\eta^2_{partial}=.06$] or days [$F(4, 64)=1.00$, $p=.41$, $\eta^2_{partial}=.06$] as well as no significant days x environment interaction [$F(4, 64)=1.32$, $p=.27$, $\eta^2_{partial}=.08$]. There were no significant changes in error rates across individual days in either group. Compared to performance during baseline testing (Figure 22) consumption of ethanol-Polycose gels did not appear to further increase short-term memory errors.

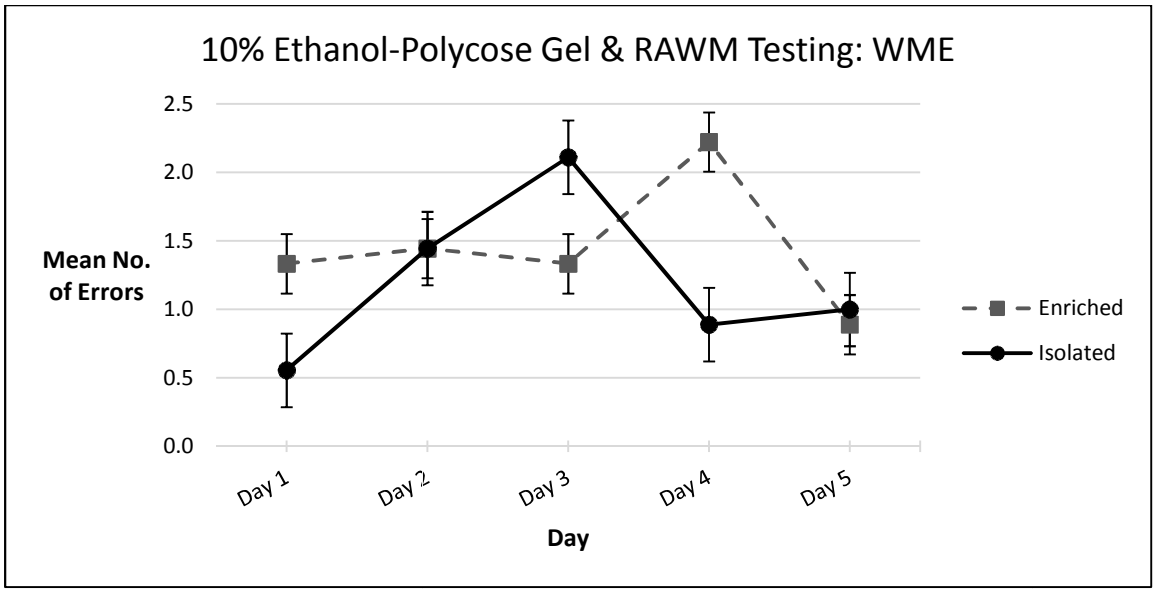

Figure 21. Average short-term memory errors in the RAWM for each group across 5 days of 10% ethanol-Polycose gel consumption and subsequent RAWM testing.

Average short-term memory errors for both groups during baseline and ethanol consumption testing are displayed in Figure 22. A 2 x 5 x 2 [environment (between-subjects factor) x days (within-subjects factor) x ethanol consumption (within-subjects factor)] mixed-design ANOVA did not reveal any significant main effects or interactions [days: $F(4, 64=1.23$, $p=.31$, $\eta^2_{partial}=.07$; environment: $F(1, 16)=3.59$, $p=.076$, $\eta^2_{partial}=.18$; ethanol: $F(1, 16)=.96$, $p=.34$, $\eta^2_{partial}=.06$]. The trend toward a main effect of environment appears to be driven by the isolated rats' performance, as the enriched rats' performance, on average, did not significantly differ following ethanol consumption. The isolated rats increased error rates following ethanol consumption on days 2 and 3, though paired sampled *t*-tests comparing performance on those days pre- and post-ethanol consumption were not significant ($p>.05$). These results suggest that 10% ethanol-Polycose gel consumption exerted little effect on short-term memory errors in the RAWM.

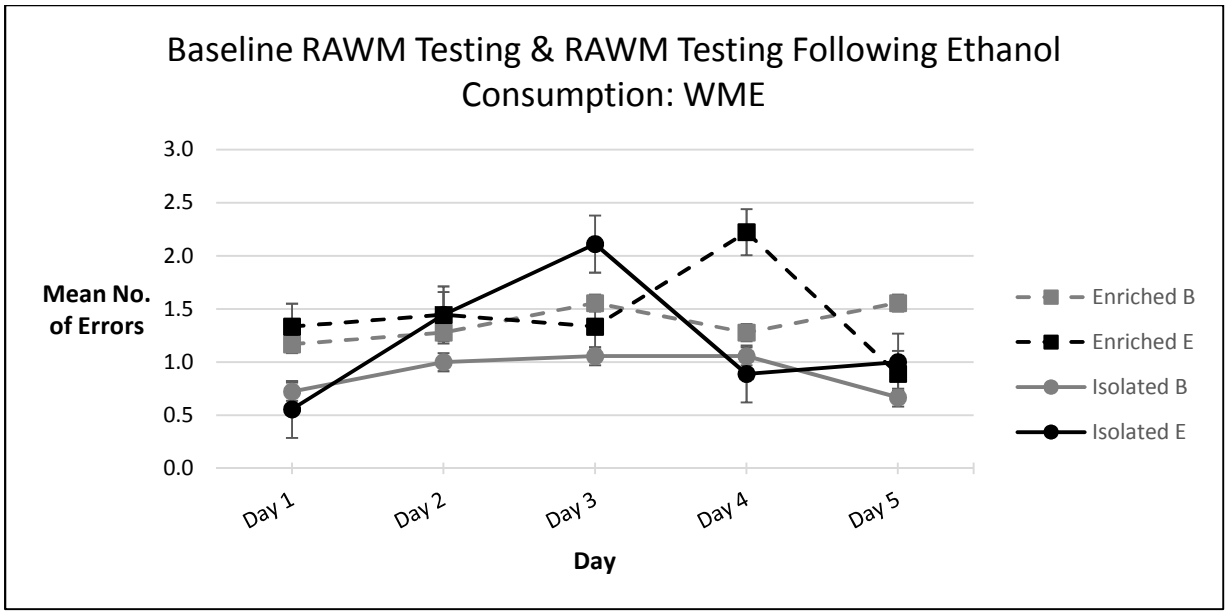

Figure 22. Average short-term memory errors in the RAWM for enriched and isolated rats during baseline testing (10 days, collapsed every 2 days) and following 10% ethanol-Polycose gel consumption. B= baseline testing (grey); E=ethanol gel consumption testing (black).

Over the course of training, performance in both RME and rRME provides evidence that the rats learned which arms contained escape platforms. This is apparent in their decreasing error rates across days. Rats were able to carry over their knowledge of platform locations to the testing phase, but improvement in RME and rRME was limited due to the already asymptotic nature of their performance.

Although they were able to learn where the platforms were located, the rats were not able to inhibit re-entering arms that previously contained escape platforms, akin to errors reported by Wahlsten, Cooper, and Crabbe (2005). This was reflected in high rates of WME, and is consistent with previous studies concluding that rats prefer to approach the place in a maze where they have previously been rewarded (e.g., Hebb, 1938; Matthews et al., 1999). In training, the rats were unable to commit WME due to the blockage of these arms; however in testing, these arms were accessible though they no longer contained an escape platform. Since the rats

had previously been rewarded for entering these arms (i.e., escape from the water), they returned to these arms.

This phenomena can also be explained as a form of sign tracking. Sign tracking is a classical conditioning preparation. In the most basic Pavlovian classical conditioning scenario, a conditioned stimulus (CS) is presented, followed by the response-independent presentation of an unconditioned stimulus (US, a reward), eliciting a conditioned response (CR). With repeated pairings, presentation of the CS elicits the CR without presentation of the US. Sign tracking evokes a sequence of behaviors directed at the CS, including preorganized tendencies of approaching or manipulating the CS (Brown & Jenkins, 1968). Though sign tracking can be harmless, maladaptive behaviors can be elicited by reward-related cues. One such example is when repeated presentations of the CS, followed by presentation of the US (reward) as described by Brown and Jenkins (1968). Pigeons were trained to sign track a key light CS that predicted the presentation of food on the opposite end of a long chamber. Pecking at the key light forced the pigeons to spend time walking across the box, which ultimately interfered with time to eat the presented food. The pecking behavior was maladaptive to obtaining the ultimate reward (food) since the birds were unable to prevent the performance of this preorganized behavior.

In the case of the current experiment, cues guiding the rats to escape (reward) were approached, even though traveling down an unbaited arm delayed or prevented delivery of the reward (escape). Which cues elicited this type of behavior is unclear, as both intra- and extra-maze cues were made available to the rats. Previous literature supports the notion that spatial cues and strategies are preferred over non-spatial when both types are available to guide behavior (Harrison et al., 2006; Olton & Samuelson, 1976; Restle, 1957). Therefore, it may have been the spatial cues that evoked the maladaptive behavior. However, it has been previously discussed

that the use of tactile and olfactory cues may have elicited maladaptive use of preorganized foraging behavior in the rats that interfered with maze performance. This theory supports sign tracking principles; thus; it may be possible that non-spatial intra-maze cues, not spatial extra-maze cues may elicited maladaptive sign tracking behavior.

Wishaw and Pasztor (2000) contend that in the case of escape behavior, rats are inclined to return to previous locations in which they have found the escape platform. This, too, can explain why the rats displayed relatively high rates of WME relative to other types of errors. It may be possible that instead of developing a win-shift strategy to successfully navigate the RAWM, a win-stay strategy was utilized, interfering with the ability to effectively choose an arm with a remaining escape platform.

Ethanol adversely affected RAWM performance when animals were tested after 30 minutes of 10% ethanol-Polycose gel consumption. Both groups produced significantly higher RME following consumption as compared to baseline RAWM performance. However, only isolated rats were negatively affected when analyzing rRME subsequent to ethanol consumption. Spatial short-term and reference memory has been shown to be impaired by acute ethanol administration (Matthews et al., 1995; White et al., 2000). This may be due to ethanol's effect on the hippocampus, disturbing interactions between hippocampal neurons and afferent structures, thereby negatively affecting memory performance (Matthews & Silvers, 2004).

The difference between treatment groups in rRME following ethanol consumption may be attributed to two factors. First, isolated rats consumed more 10% ethanol-Polycose gel than enriched rats. Though the literature is contradictory, our results are consistent with previous literature that found that rats exposed to isolated conditions consume higher amount of ethanol than rats exposed to enriched environments (Deatherage, 1972; Deehan et al., 2007; Deehan et

al., 2011). The difference in ethanol consumption between treatment groups in the current study was not significant; however isolated rats, on average, consumed more grams of ethanol than enriched rats. Since ethanol has been shown to impair memory in a dose dependent nature (Cacace et al., 2011; Matthews et al., 1995; Novier et al., 2012; Silvers et al., 2003), it may be that the higher consumption rate of isolated rats more negatively affected both their long- and short-term memory performance. Short-term memory appears to have been spared in enriched rats.

Second, it is possible that short-term environmental enrichment protected those rats' short-term memory against impairment due to ethanol. Ethanol and environmental enrichment influence neurotransmitter systems in opposing way. Ethanol is known to suppress short-term memory performance by inhibiting LTP induction. Glutamate level and hippocampal activity is also suppressed. However, ethanol does not affect the expression of LTP which was initiated prior to ethanol exposure. (Matthews et al., 1995; Matthews et al., 1999; White et al., 2000). There is evidence that environmental enrichment, on the other hand, enhances memory and LTP by increasing AMPA and NMDA receptor binding and promoting hippocampal activity (Rampon et al., 2000; van Praag et al., 2000). The degree to which environmental enrichment has already enhanced cognitive activity may be greater than degree to which ethanol can impair it. Thus, short-term memory was not negatively affected in enriched rats.

Experiment 3

Though the rats were able to learn the spatial RAWM, WME still remained high. Therefore, in experiment 3, we employed the use of the Morris water maze, one of the most frequently used paradigms to assess spatial reference and short-term memory due to its simplicity and easily varied protocols. This allows for easy application in studying search strategies employed by animals within the maze.

Method

Subjects. The same rats as experiments 1 and 2 were used for this experiment. Rats were approximately 19 weeks old at the start of this experiment. Animals were maintained on the restricted food diet as in experiment 2.

Apparatus. Rats were maintained on the same environmental exposure as in experiments 1 and 2. The same tub and one platform from experiments 1 and 2 was used to administer the Morris water maze (MWM). Shower curtains were completely removed from around the maze and all spatial cues were utilized. Stripes were affixed to the northern wall and circles were placed on the eastern wall. The experimenter stood at the southern point. A white shower curtain in front of the rack of metal holding cages marked the western side of the room. Objects in the room (e.g, sink, table, fume hood) provided additional extra-maze spatial cues. A schematic of the room can be seen in Figure 23.

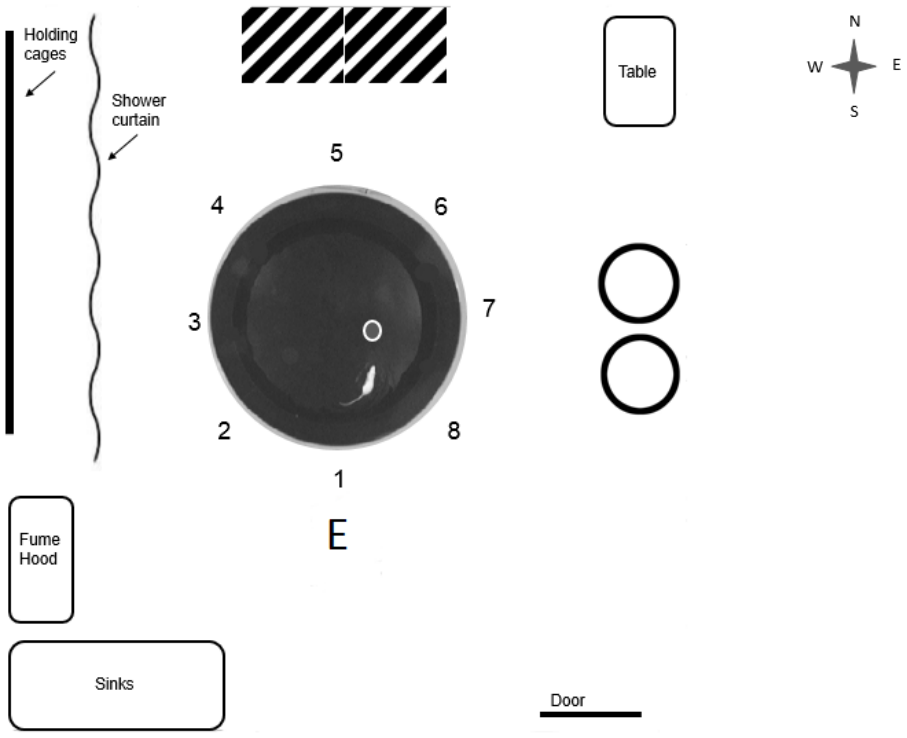

Figure 23. Schematic of the Morris water maze testing room. "E" represents where the experimenter stood.

Procedure. ***Acquisition.*** Platform was located in the northeast quadrant of the tub approximately 20 cm from the edge and remained fixed throughout acquisition. The top of the escape platform was 2 cm below the water surface. The surface of the water was covered with packing peanuts to hide the location of the platform. After each trial, the packing peanuts were evenly redistributed across the surface of the water. A randomized start point (a number 1-8 generated by www.random.org) was allocated for each trial of each rat. Animals were released facing the wall of the tub; latency to reach the platform was recorded. Once the animal found the platform it remained there for 10 s and was returned to its holding cage for 50 s, allowing for a 1 min total ITI. There were 4 trials/day; animals were run sequentially by trial in squads of six. Training lasted for 5 days.

Short-term memory testing-Baseline. Following acquisition, animals were introduced to a short term memory procedure. In this paradigm, the platform varied quadrants across days, but remained constant between trials. There were 2 trials per day, with a 5 min ITI. Animals were given access to an empty clay flower pot (those previously used to administer gel) for 30 min prior to the start of the first trial. Once 30 min had passed, the pots were removed and maze testing began. Testing occurred sequentially by trial in squads of six. Animals were released from a randomized start location (start position was consistent across rats) facing the outside wall of the tub. In the first trial, animals were required to locate the platform in its new location for that day. The second trial acted as a short term memory test to see how well the animals remembered the platform location. Latency to the platform was recorded. Short-term memory testing lasted 5 days.

Ethanol consumption and short-term memory testing. Ethanol and maze testing proceeded in the same manner as baseline short term memory testing except that the clay flower pots were filled with 10% ethanol-Polycose gel. Ethanol and maze testing lasted 5 days.

Results and Discussion

It was hypothesized that enriched rats would learn an effective swim strategy to successfully navigate the MWM quicker than isolated rats. This would be reflected as significantly faster acquisition over days and lower latency to reach the escape platform in both short term memory tests. We also hypothesized that enriched rats would consume significantly less ethanol gel as compared to isolated rats.

Acquisition. Figures 24 depict the average latency by trial to reach the escape platform for each group during training in the Morris water maze. Enriched rats display lower latencies than isolated rats on most trials. A 2x5x4 [environment (between-subjects factor) x days (within-

subjects factor) x trials (within-subjects factor)] mixed-design ANOVA was performed to confirm our hypotheses. This revealed a days x trials interaction [$F(12, 192)=3.30$, $p<.001$, $\eta^2_{partial}=.17$] suggesting that performance on the task improved over trials and across days. Figure 15 shows this decrease in latency, particularly across the first few days of acquisition. It also appears as though the enriched rats learned the maze faster than isolated rats, as their average latency to the escape platform appears lower than isolated rats, although the mixed-design ANOVA did not reveal any interactions with treatment. A main effect of treatment [$F(1,16)=5.90$, $p=0.03$, $\eta^2_{partial}=.27$) indicates that, on average, the enriched rats had significantly lower latencies to the escape platform than isolated rats.

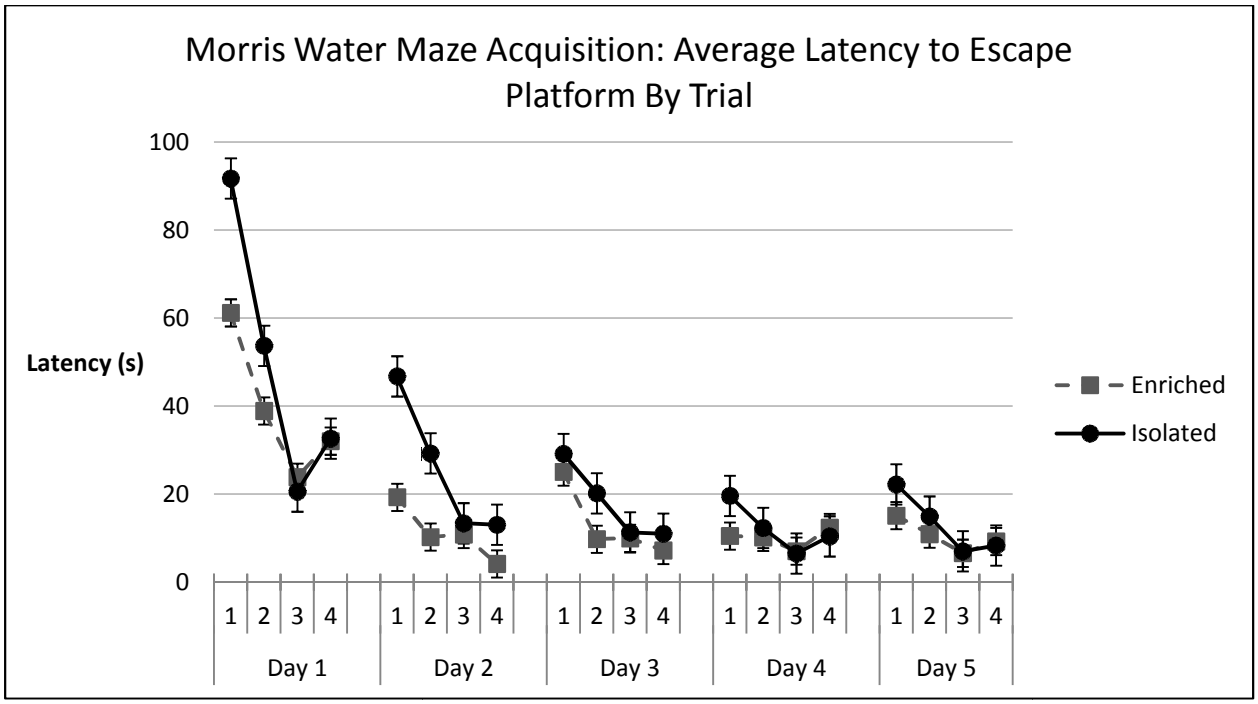

Figure 24. Average latency to reach the platform for each trial of MWM acquisition.

Long-term memory performance during acquisition. An index of long term memory (reference memory) was calculated by comparing latency to the escape platform on trial 1 of each day. Lower latencies on trial 1 across days would suggest consolidation of reference memory and indicate that the rats learned an effective search strategy. We hypothesized that rats

exposed to enrichment would display significantly lower latencies than rats exposed to isolation across days. Figure 25 displays the average performance of each group on trial 1 of each day of MWM acquisition. A 2x5 mixed-design ANOVA [environment (between-subjects factor) x days (within-subjects factor)] was performed to analyze group differences. There was a significant main effect of both days [$F(4, 64)=20.02$, $p<.001$, $\eta^2_{partial}=.56$] and treatment [$F(1, 16)=4.93$, $p=.04$, $\eta^2_{partial}=.24$]. The main effect of days suggests that both groups, on average, consolidated the memory of the platform location and improved performance across days. The main effect of treatment confirms the data displayed in the graph. Enriched rats were, on average, better able to remember the platform location than isolated rats. This was particularly evident on days 1 and 2, where an independent samples t-test reveals a tendency for lower latencies among enriched (see table 3).

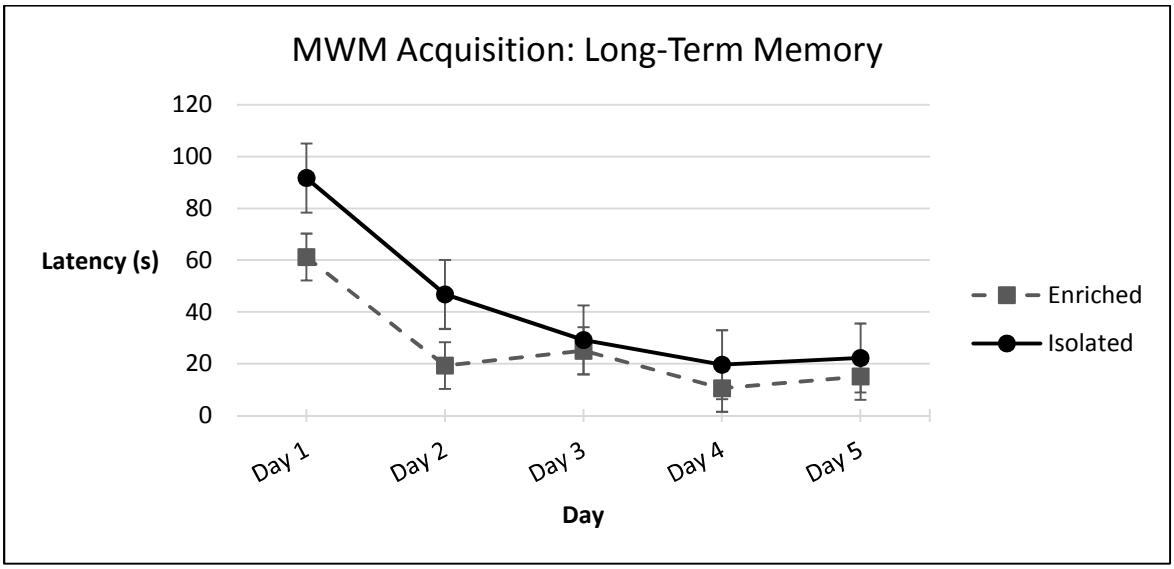

Figure 25. Average latency to the escape platform for each group on trial 1 of each day of MWM acquisition.

Table 3 *Between groups differences: MWM Acquisition-Reference memory*							
		Avg.	*SD*	*df*	*t*	*p*	*d*
Day 1	Enriched	61.33	44.50	16	-1.75	.10	-.83
	Isolated	91.78	27.11				
Day 2	Enriched	19.33	11.14	16	-2.08	.05*	-.98
	Isolated	46.67	37.85				

Table 3. Average between-group differences for days 1 and 2 of MWM acquisition-Long-term memory analysis. *p=.05.

Short-term memory performance during acquisition. Short-term memory performance was derived by subtracting trial 2 latencies from trial 1 latencies of each day. Difference scores below zero indicates poorer performance on trial 2 and hence poor short-term memory, whereas difference scores greater than zero indicates improved performance on the second trial as a result of utilizing information from short-term memory. Because the escape platform was always in the same location during this training phase it is not possible to infer the use of short-term memory in later trials when performance is at asymptote. It is sufficient for the rats to rely on their long-term memory to locate the platform because its position never changes; therefore, there is no need to rely on a short-term memory of the platform location. However, early in training when a long-term reference memory is not yet consolidated, the rats can utilize their short-term memory to find the escape platform on the trials after the first. Figure 26 suggests that this is precisely what happened during training. During the first days of training the higher difference scores suggest that both groups were relying on short-term memory to find the escape platform on subsequent trials, whereas during the final days the difference scores were close to zero suggesting that long-term memory was sufficient to find the escape platform. The absence

of difference scores below zero indicates that there was no deterioration in short-term memory, which of course, was not anticipated in this training phase of the experiment. It appears as though enriched rats displayed lower difference scores early in training, which would suggest that a reduced reliance on short term memory in the enriched rats as a result of better reference memory for the escape platform as discussed above. However, a 2 x 5 [environment (between-subjects factor) x days (within-subjects factor)] mixed-design ANOVA did not reveal any statistically significant differences between treatment groups [$F(1, 16)=.51$, $p=.49$, $\eta^2_{partial}=.03$].

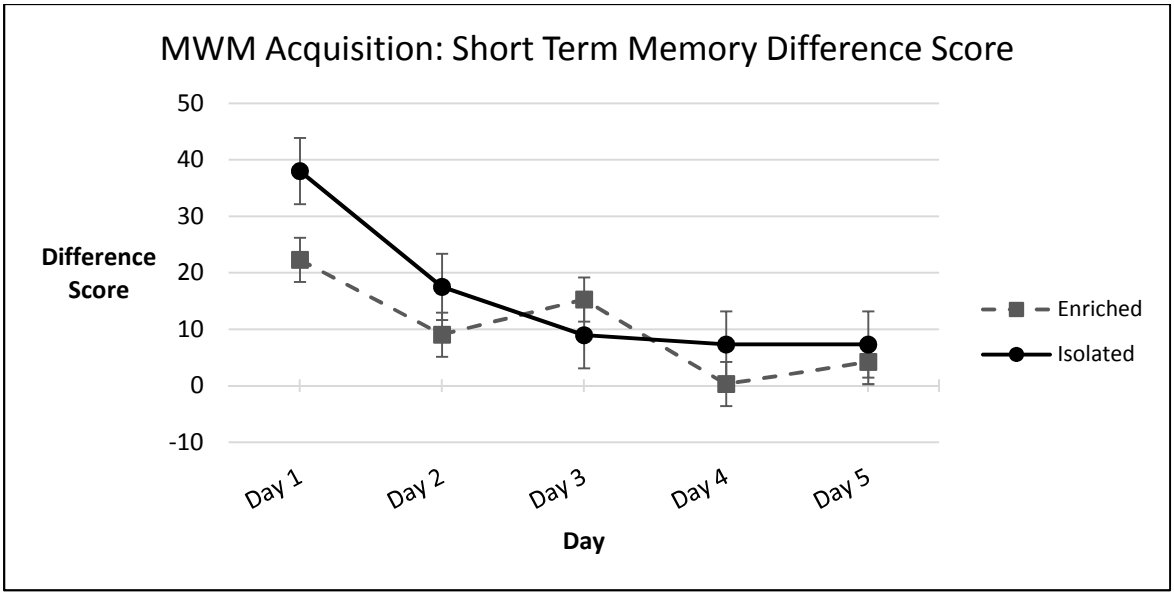

Figure 26. Average difference scores for each group, calculated by subtracting the latency to the escape platform on trial 2 from that of trial 1 for each day. A higher difference score indicates improvement in short term memory for the location of the escape platform during MWM acquisition.

Looking at MWM acquisition as a whole, enriched rats were able to learn the task at a quicker rate than isolated rats, suggesting a superior ability to consolidate a memory of the fixed location of the escape platform. Group differences in short-term memory performance are less clear because a water-maze task with a fixed platform location is not sufficient to isolate short-

term memory performance from long-term memory performance. To better examine short-term memory performance the procedure was changed in the next phase by changing the location of the platform daily so that the rats could not rely on their long-term memory of the platform location.

Short-term memory testing-Baseline. Following acquisition in the Morris water maze, rats' short-term memory (STM) was tested in a two-trial per day procedure. Platform location remained stable for both trials within a day, but was varied between days. Prior to looking at the short-term memory data we examined the latency to locate the platform over days. Figures 27 and 28 illustrate the average latency to find the escape platform for both groups. In Figure 28, each day has been collapsed over both trials. Isolated rats' performance over the 5 days of testing reveal a variable and inconsistent pattern as was expected since the platform was varied across days. Surprisingly, however, the enriched rats display a much more uniform decrease in latencies over days. Thus, the enriched rats developed a swim strategy that allowed them to improve their ability to locate the moving platform over days. Though the 2 x 5 x 2 [environment (between-subjects factor) x days (within-subjects factor) x trials (within-subjects factor)] mixed-design ANOVA used to analyze the data did not reveal any significant results.

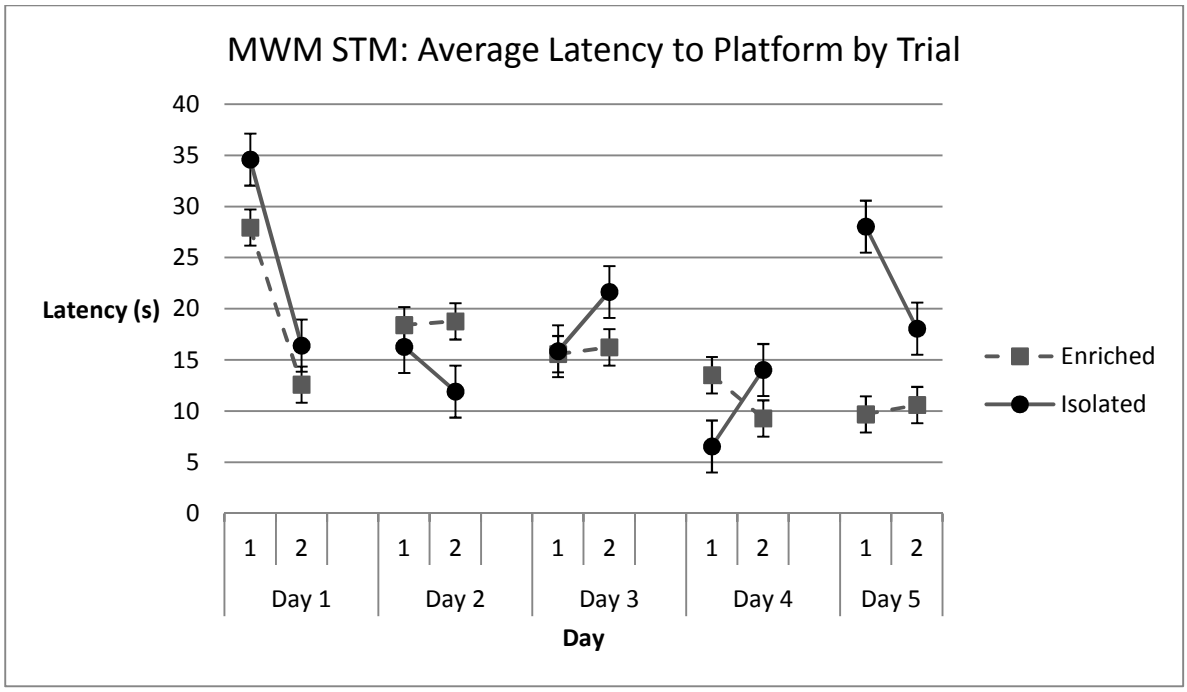

Figure 27. Average latency to the platform by trial for each group during short-term memory testing in the MWM.

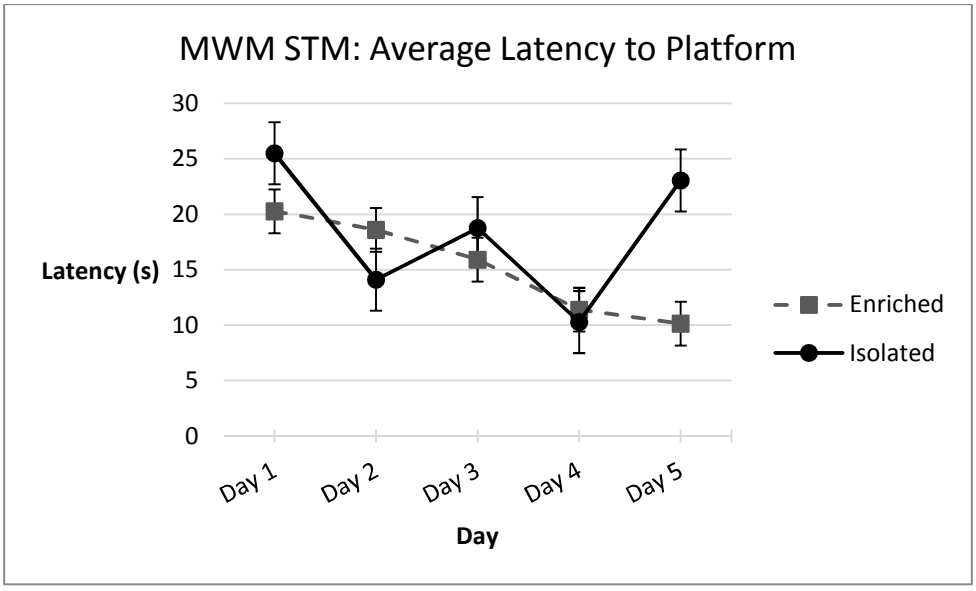

Figure 28. Average latency to the platform for each group, collapsed across trials for each day of short-term memory testing in the MWM.

Short-term memory was assessed by calculating the difference in latency between trial 1 and trial 2 is displayed in Figure 29. Positive difference scores represent lower latencies on trial 2, suggesting that the rats relied on their short-term memory to relocate the escape platform for that day. Negative difference scores represent higher latencies on trial 2, suggesting a lack of STM performance. Both groups of rats showed similar reliance on STM on the first day. Unfortunately, performance in the enriched rats was at zero for the remaining test days indicating that they were relying on a swim strategy that did not depend on short-term memory. Some reliance on short-term memory was more evident in the isolated rats, with slightly poor performance on days 3 and 4 and improved performance on day 5. However, this group difference in performance was not supported by a 2 x 5 [environment (between-subjects factor) x days (within-subjects factor)] mixed-design ANOVA. The analysis revealed no significant main effect of days [$F(4, 64)=1.57$, $p=.19$, $\eta^2_{partial}=.09$] or environment [$F(1, 16)=.04$, $p=.85$, $\eta^2_{partial}=0$], as well as no significant interaction [$F(1, 16)=.01$, $p=.92$, $\eta^2_{partial}=.02$].

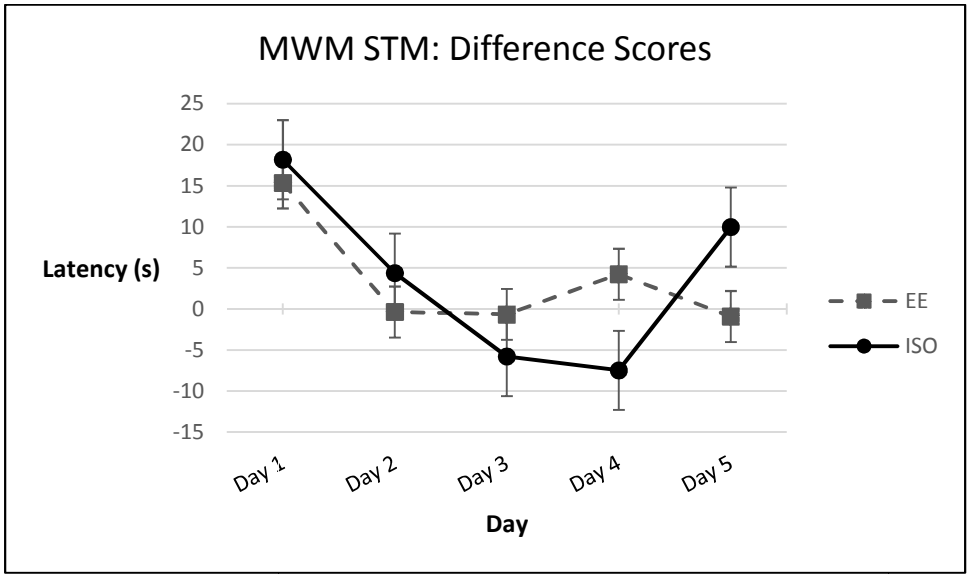

Figure 29. Average difference score for each treatment group during MWM short-term memory testing. Difference scores were calculated by subtracting the latency of trial 2 from those of trial

1. Positive sores indicate the successful use of STM to locate the escape platform on trial 2. Negative scores indicate higher latency on trial 2, suggesting a lack of STM performance. Scores close to zero indicate that the platform was located without the utilization of STM.

Overall, this task failed as a reliable measure of short-term memory. The enriched rats appear to have formed a swim strategy to quickly find the escape platform despite its variable location.

Ethanol consumption & STM testing. Despite the problems with the task just noted, short-term memory was assessed following ethanol (EOTH) consumption in the final phase. Recall that in the baseline STM testing, rats were given access to an empty clay flower pot for 30 minutes prior to testing. This paradigm follows the same procedure, except this time the flower pots were filled with 10% ethanol-Polycose gel.

The average gel eaten by each group is displayed in Figure 30. A 2x5 [environment (between-subjects factor) x days (within-subjects factor)] mixed-design ANOVA revealed a main effect of days [$F(4, 64)=11.79$, $p<.001$, $\eta^2_{partial}=.42$], as well as a trend toward a days x environment interaction [$F(4, 64)=2.42$, $p=.06$, $\eta^2_{partial}=.13$]. These two statistics, and large effect size, together indicate that gel consumption significantly varied as a function of days. Both groups show a similar pattern of gel consumption, volleying in the same manner between high and low intake. The significant interaction appears to driven by the isolated rats' sharp decrease in consumption on day 5, whereas enriched rats consumption slightly increase. Enriched rats display slightly lower consumption rates than isolated rats as seen in the previous experiment. On average, enriched rats consumed 0.83 g of alcohol each day (2.1 g/kg), while isolated rats consumed an average of 0.97 g of alcohol per day (2.48 g/kg).

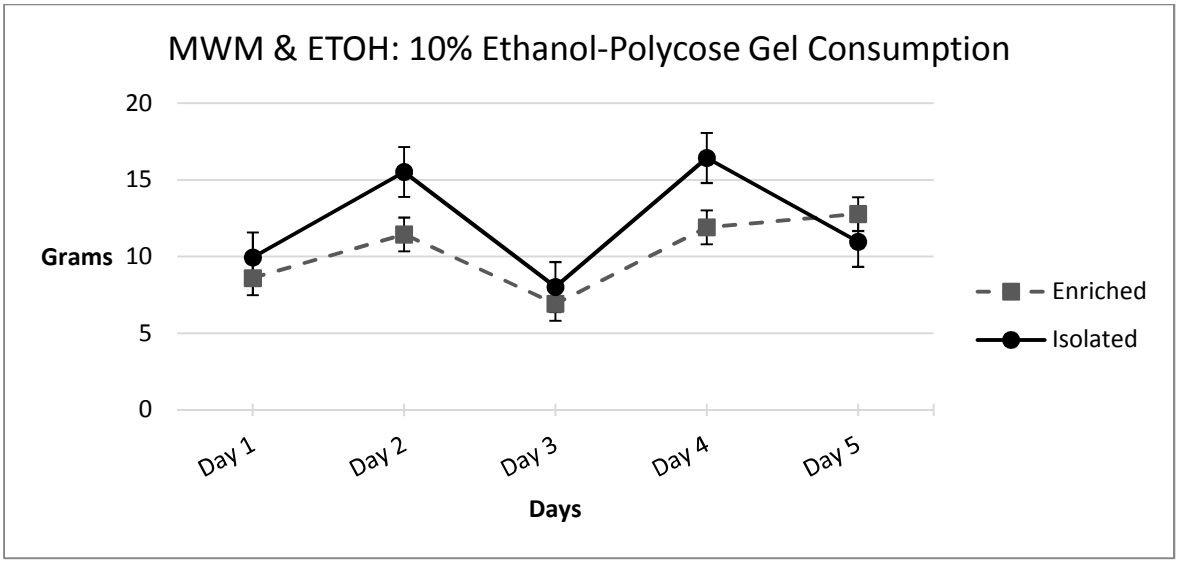

Figure 30. Average 10% ethanol-Polycose gel consumption for each group prior to STM testing in the MWM.

Difference scores were calculated as an index of STM following ethanol consumption as before. This data, displayed in Figure 31, shows some unanticipated findings. With the exception of Day 1, the enriched rats showed consistently worse performance on the second trial as indicated by the negative difference score. This result suggests that ethanol consumption disrupted performance specifically on the second trial. One possible reason for this behavior is that after finding the platform successfully on the first trial using the same swim strategy as in the previous phase, the enriched rats switched to a different strategy on the second trial. The isolated rats, however, did not show a similar pattern of change across trials as indicated by difference near zero or above. A 2 x 5 [environment (between-subjects actor) x days (within-subjects factor)] mixed-design ANOVA did not reveal any main effects or interactions [days: $F(4, 64)=.690$, $p=.602$; environment: $F(1, 16)=.4$, $p=.536$; days x environment: $F(1, 16)=1.898$, $p=.187$]

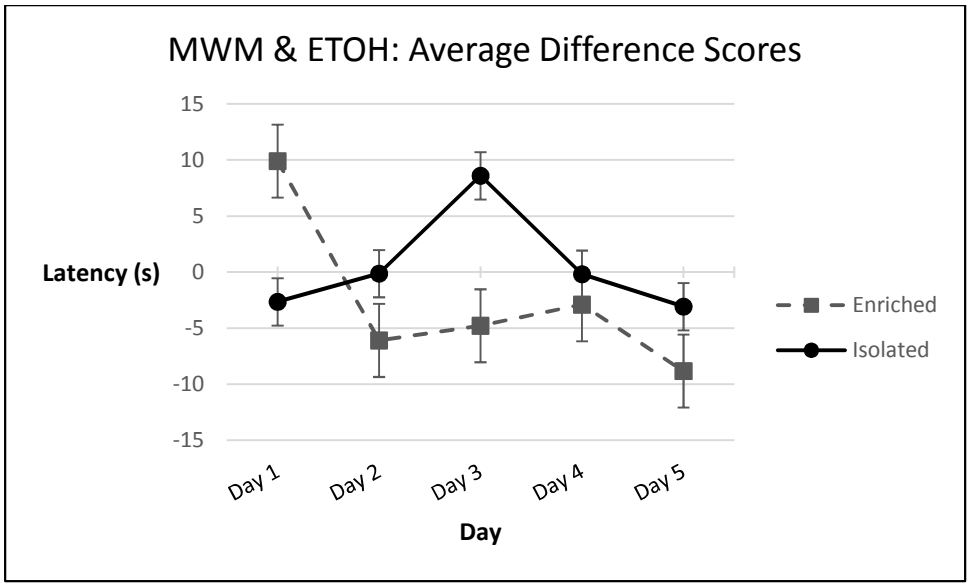

Figure 31. Average difference scores for each treatment group over 5 days of MWM short-term memory testing following 10% ethanol-Polycose gel consumption.

To examine this effect more closely, the average latency to the platform was analyzed with trials as a factor as shown in Figure 32. It is clearly seen that the latencies on trial 2 in the enriched rats are higher than those on trial 1 for days 2-5, whereas this pattern is not evident in the isolated rats. The enriched rats' performance was analyzed individually in order to more closely examine the general increase in latencies for trial 2. A 5 x 2 (days x trials) repeated measures ANOVA reveals a trend toward a days x trials interaction [$F(4, 32)=2.27$, $p=.08$, $\eta^2_{partial}=.22$]. This indicates that the rats tended to display higher latencies on trial 2 on the last 4 days after successfully finding the escape platform on the first trial despite the change in location across days. It is evident that performance was strikingly different for the enriched rats on Day 1 when they had difficulty finding the platform on the first trial 1, but were able to successfully locate the escape platform on the second trial. When day 1 was taken out of the analysis, a 4x2 (days x trials) repeated measures ANOVA revealed a main effect of trials [$F(1, 8)=10.11$, $p=.01$, $\eta^2_{partial}=.56$] confirming that enriched rats did perform worse on trial 2 as compared to trial 1

with a rather robust effect size. It appears as if they were unable to recall the platform location for that day. There was no main effect of days [$F(3, 24)=1.22$, $p=.33$, $\eta^2_{partial}=.13$] and no interaction between the two variables [$F(3, 24)=.22$, $p=.88$, $\eta^2_{partial}=.03$]. The lack of interaction when day 1 is removed from the analysis suggests that those data points were the driving force behind the statistics.

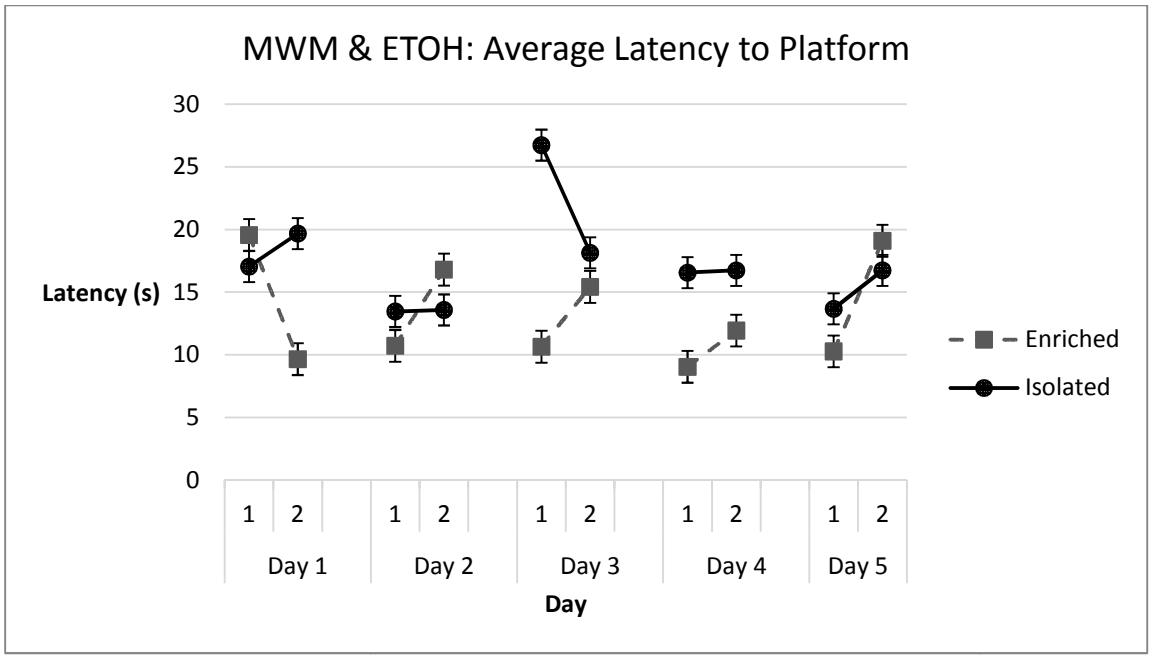

Figure 32. Average latency to the platform by trial for both groups in the MWM STM test following ethanol consumption.

Data from experiment 3 provide evidence that enrichment can facilitate spatial learning and memory. During acquisition of the spatial reference memory version of the MWM, enriched rats reliably displayed significantly lower latencies to reach the escape platform than isolated rats. This finding is consistent with multiple studies confirming enhanced spatial learning and memory processes, as defined by decreased latency to the platform in the MWM, following enrichment (Frick & Fernandez, 2003; Harburger et al., 2007; Harris et al., 2009; Simpson & Kelly, 2011; Speisman et al., 2013; van Praag et al., 2000).

Analysis of long- and short-term memory revealed that enriched rats tended to rely on long-term memory to find the escape platform, whereas isolated rats relied more on short-term memory, especially early in acquisition. One possible explanation of this effect is the enhanced transference of information from short-term memory to long-term memory seen in enriched rats (Schrijver et al., 2002). The enhanced speed with which enriched rats acquire, store, and retrieve spatial information into long-term memory may be explained neurologically. It has been previously described that environmental enrichment promotes neurogenesis, cell proliferation, augmented synaptic and dentritic length and plasticity, and increased AMPA and NMDA receptor binding facilitating LTP in the hippocampal formation. The dentate gyrus is a structure that particularly important for efficient exploratory behavior and formation of new memories (Bindu et al., 2005; Bindu et al., 2007; Bruel-Jungerman et al., 2005; Rampon et al., 2000; Will et al., 1977). The combined effect of these mechanisms likely contributes to superior memory and effective use of spatially precise, allocentric search strategies seen in the current experiment (Garthe et al., 2009; Rubio et al., 2012).

In order to more closely examine short-term memory, a modified MWM paradigm was established. In this paradigm, the escape platform varied locations between days. Ultimately this task failed as a measure of short-term memory because enriched rats appeared to develop a swim strategy to successfully locate the platform each day. This was apparent in a uniform decrease in latency across days, while isolated rats displayed more variable latencies and likely didn't rely on short-term memory strategies to find the platform. Due to the fixed distance of the platform from the edge of the pool, it is possible that enriched groups of rats developed a type of chaining strategy to locate the platform despite its variable location (Garthe et al., 2009). Swimming

around the pool a certain distance from the wall, rats would be able to locate the platform without utilizing the available spatial cues.

When tested in the same paradigm subsequent to ethanol gel consumption, performance on trial 2 was negatively affected in enriched rats. On average, it does not appear as though ethanol negatively affect isolated rats' performance in the maze. This may be because they did not have a particular strategy in place during STM testing without ethanol. Studies have shown that ethanol can selectively impair short-term memory (Gibson, 1985; Givens, 1995), suppress behavioral diversity (Devenport & Merriman, 1983), and can impair the use of memory strategies (Cain et al., 2002). These effects together can cause a less effective use of previously established strategies, and impair the ability of rats to obtain information and demonstrate knowledge about the location of the platform. By suppressing behavioral diversity and flexibility, ethanol consumption can put rats at a disadvantage when variability is rewarded in the task (Devenport & Merriman, 1983).

Though a chaining strategy may have been effectively used in locating the escape platform in the short-term memory paradigm and on the first trial of the short-term memory paradigm following ethanol consumption, the enriched rats were not able to utilize this strategy to locate the platform on the second trial, as evidenced by the increase in latency. This is consistent with previous literature which found that ethanol consumption can alter the type of information used in a memory task (Matthews et al., 1999), specifically causing a switch from using extra-maze (spatial) cues to utilizing intra-maze (non-spatial) cues. It is known that rodents prefer to use and process spatial information, even when both types of cues are available. They are able to use this spatial information to create cognitive maps of their environments (Matthews et al., 1995; Tolman, 1948). By impairing the ability to effectively use spatial information, and a

lack of non-spatial cues within the maze, it is possible that the rats employed a random search, scanning, or thigmotaxic strategy on the second trial of short-term memory paradigm following ethanol consumption. This led to increased latencies and an inability to successfully demonstrate their knowledge of the platform location on trial 2 of the short-term memory paradigm.

The hippocampus acts as a major structure in forming cognitive maps to guide behavior (O'Keefe & Nadel, 1978). Previous discussion of the effects of ethanol on hippocampal activity suggests that ethanol may have disrupted neuronal activity in the hippocampus and dentate gyrus, impairing the use of cognitive maps, allocentric search strategies, and lack of behavioral flexibility by the rats (Garthe et al., 2009; Matthews et al., 2002). Additionally, ethanol has been shown to disrupt activity of place-cells within the hippocampus (Matthews et al., 1996; White et al., 2000). By antagonizing NMDA receptors, establishment of new place-fields, as well as those fields that were previously established, is compromised. If place-cells in the hippocampus were unable to generate an accurate place-field, or any at all, rats could become disoriented within the maze (Knierim, Kudrimoti, & McNauhton, 1995). Without a clear representation of the platform location, rats likely engaged an inefficient search strategy to guide behavior.

General Discussion

Our hypothesis that short-term periods of daily environmental enrichment would facilitate short-term and reference memory was confirmed in experiment 3. Specifically, rats exposed to environmental enrichment, as opposed to social isolation, displayed significantly decreased latency to the platform location during MWM acquisition. This effect was mostly seen in long-term, reference memory, as superior performance occurred mostly early in the training. Unexpectedly, enriched rats were also able to successfully execute an effective search strategy, likely unreliant on spatial strategies or short-term memory (e.g., use of a chaining strategy, which does not rely on spatial configuration or memory), to locate the platform during the MWM short-term memory paradigm. Though this rendered the task inadequate to accurately measure short-term memory, it provides further evidence of enhanced learning and memory following environmental enrichment.

Ethanol produced slight deficits in memory, as suggested by slightly poorer performance compared to baseline in the final two experiments. Interestingly, isolated rats were more strongly affected in experiment 2, significantly increasing both RME and rRME in the RAWM following ethanol consumption; however, enriched rats were more adversely affected when tested in the short-term memory paradigm of the MWM. This may be due to the inherent failure of the paradigm itself. Due to the fact that enriched rats effectively reduced latency to the platform across days despite the variable platform location, the paradigm may not be effective in accurately quantifying short-term memory in enriched rats. As discussed previously, poor performance of enriched rats, particularly on the second trial may be due to the tendency for ethanol to disrupt effective use of strategies in maze performance (Cain et al., 2002). Since the rats had already established a strategy, there was a greater chance that ethanol would diminish its

use. The isolated rats did not display use of an effective swim strategy, suggested by variable latencies to the escape platform on both trials; therefore, there was little room for ethanol to disrupt performance.

In general, both groups of rats were readily able to learn and perform spatial versions of the RAWM and MWM. Learning was not apparent in experiment 1, a non-spatial version of the RAWM. One presumably apparent explanation could be the lack of training allowed in the maze. Rats were given 4 days (6 blocks) of initial training in the maze, followed by an unexpected break in maze running due to experimenter illness. Acquisition was assessed one week after initial training ended. Though this was not an ideal assessment of learning, this is not the primary reason for a lack of performance. Rats were unable to successfully solve the maze, suggested by consistently high error rates, during initial training, acquisition, and a secondary training period (3 days, 4 blocks) that was added following acquisition (data not shown). Statistical analyses of the entire experimental period did not reveal significant differences in performance from day 1 of initial training to the last day of secondary training. It is clear that subjecting rats to greater number of training blocks during non-spatial RAWM training and acquisition (15 blocks total, compared to 11 blocks of training in the spatial RAWM), did not improve performance in the maze. This supports previous literature supporting an inherent spatial bias, in that spatial tasks tend to be learned faster than non-spatial tasks (Harrison et al., 2006; Olton & Samuelson, 1976).

If the criterion of performance is the same on the non-spatial and spatial versions of the same maze, what factors can contribute to the apparent difference in learning ability? One possible element is the type of information being utilized by the rats to effectively solve the maze.

Not all maze cues are created equal, and the type of cue used can affect successful navigation of a maze depending on its relevance to the goal (Restle, 1957). It is generally accepted that animals prefer the use of spatial cues and are able to learn a spatial task quicker than a non-spatial task (Harrison et al., 2006; Olton & Samuelson, 1976).

Additionally, the salience of these cues is important in determination of location discrimination. Use of ambiguous stimuli can impede performance on learning and memory tasks (White, 2004). It is possible that in experiment 1, olfactory cues may have become mixed or diminished within the maze due to the rat's swimming activity. Additionally, because tactile cues were identical in each of the cued arms, the cue may not have been unique enough for the rats to discriminate between arms it had previously visited.

When spatial cues were introduced in experiment 2, rats were able to ignore the seemingly irrelevant intra-maze cues for preferential use of spatial cues. Restle's (1957) formula to determine an assumed rate of learning can be applied to both experiments when considering the ambiguity of cues. Restle (1957) contends that the rate of learning depends on the direct proportion of relevant cues to the total number of cues. In experiment 1, 6 total cues were available (2 cues, 1 olfactory and 1 tactile, in each arm). If we apply the assumption that none of the cues was relevant due to the ambiguity of the cues, we are left with a learning rate of 0 (0 relevant cues/6 total cues). In experiment 2, unambiguous spatial cues, circles and stripes affixed to the curtains, were available for the rats to use. Because it is unclear exactly how many total spatial cues were available, we will assume that the stripes and circles were the only 2 relevant spatial cues introduced. This would equate to 8 total cues and 2 relevant spatial cues (2 relevant cues/8 total cues = ¼). In this calculation, experiment 1 has a learning rate of 0, whereas

experiment 2 has a learning rate of ¼. Since ¼ > 0, it can be assumed that experiment 2 would have a faster average rate of learning, which is exactly the case in the current study.

Another possible factor may be the strategy required to successfully solve the maze. Tolman (1946) contends that spatial strategies such as place learning, utilizing the spatial configuration of distal cues to guide behavior to the goal, are more natural and easily learned than non-spatial strategies such as response learning, utilizing a learned sequence of movements in specific directions to guide oneself to the goal. The claim that place learning dominates response learning is controversial (Restle, 1957) due to the relative number of mazes and strategies that have been successfully employed.

Additionally, reinforcement of strategies that were not reliant on memory of the platform locations may have retarded learning in experiment 1. Non-spatial strategies, such as serial searching and chaining to adjacent arms, can be inefficient when solving mazes (Harrison et al., 2006; Janus, 2004). However, use of these strategies will eventually lead to the goal location and will therefore be reinforced. Using these types of strategies, animals are not required to form a map of the environment or associate certain cues with the rewarded behavior when the task is inherently difficult. To this end, weaker memory representations of the task are stored and not able to be used productively in the next test session. This can support high rates of errors in the non-spatial tasks. In spatial tasks, such as experiment 2, rats are readily able to for maps of the environment, strengthening memory representations of the task, improving performance across days.

One effect that has not yet been considered is the amount of environmental exposure the rats had received at the time of experiment 3 compared to experiment 1. By the time experiment 3 had begun, the rats had received over 16 weeks of environmental exposure. When experiment

1 started, the rats had received approximately 4 weeks of environmental exposure. It is possible that the length of exposure affected the subsequent results of the experiment. Should this be the case, environment would have been more of a contributing factor in experiment 3 than in experiment 1. This appears to have happened, with environmental exposure significantly contributing to differences seen, particularly seen in MWM acquisition and STM baseline testing. Additionally, large effect sizes, according to Cohen's (1992) conventions, suggest that these effects are rather robust. Rats were unable to learn the task in experiment 1, when exposure to environments had been at its least. One way to assess whether the length of environmental exposure had an effect on the two different tasks would be to perform experiment 1 again. Due to time constraints, this was unable to happen, though future studies should take this factor into account.

The findings from the current experiments support an enrichment effect of daily EE, in the form of enhanced performance in spatial learning and memory, though this appears to be task dependent. Additionally, it was shown that ethanol can adverse effects spatial memory, though daily enrichment protected against some of the detrimental effects on short-term memory. One possible reason for a smaller enrichment effect than hypothesized may reflect the fact that environmental enrichment may be dose dependent. Following this assumption, increasing the amount of time animals are exposed to an enriched environment, as well as increasing the level of stimulation, can produce a greater enrichment effects in terms of enhanced learning and memory. Future studies directly comparing enrichment paradigms in a dose dependent manner can contribute important information to influence the manner in which environmental enrichment is applied to other cognitive domains. The importance and relative contribution of various forms of intra- and extra-maze cues in non-spatial and spatial search strategies should

also be considered in future studies. Finally, it would be exciting to see whether the is an inherent threshold in the memory system at which environmental enrichment can no longer rescue deficits inflicted by acute ethanol administration, and what specific characteristics of enrichment affect this limit.

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