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The Interaction Between Spatial Working and Reference Memory in Rats on a Radial Maze

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The Interaction Between Spatial Working and Reference Memory in a Radial Arm Maze with Rats: A Model for Human Memory Impairments?

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Abstract

The present study investigated the interaction between working and reference spatial memory in an effort to develop an animal model of this interaction. Twelve male Long-Evans rats were tested on an eight-arm radial maze in a two-phase procedure. In the study phase, a rat was allowed to enter four randomly selected arms for a food reward placed at the end of each arm. The test phase allowed the rat access to all eight arms, but only the previously unentered arms contained food. Two of the correct test arms were defined as reference memory arms because they were always correct. The other two correct test arms were defined as working memory arms because they varied randomly among trials. The percentage of correct working memory and reference memory arm entries made in the first 4 choices in the test phase were recorded to find out if rats showed better working memory or reference memory and in what order they chose to visit working and reference memory arms under a variety of conditions. Further research will use this model to analyze what interventions can reduce the type of confusion in working and reference memory seen in human memory impairments.

The Interaction Between Spatial Working and Reference Memory in a Radial Arm Maze with Rats: A Model for Human Memory Impairments?

Spatial memory is an important topic in animal cognition because it is a critical ability necessary for the survival of individuals and the evolution of their species. An animal must remember the locations of food, water and shelter in order to survive. Similarly, the locations of dangers in the environment (predators/ toxins) must be remembered. Spatial memories for the locations of objects are essential to evolutionary fitness and foraging optimization. Despite the variety of animal habitats, all mammals are able to learn about and remember locations within their environments.

William James originally hypothesized that there were two distinct mechanisms of memory; "primary" and "secondary" memory. They were later referred to as "short-term" and "long-term" memory (Atkinson & Shiffrin, 1968, p. 93), under the assumption that some short-term memories are converted to a long-term memory system, and others are available only for a short time. Today we denote the two memory systems as "working" and "reference" memory where working memories are those which last for short time periods and concern information regarding the immediate past, and reference memories are those that endure for long time periods and concern mental representations and associations (Baddeley & Hitch, 1974, p. 53). In animal cognition working memories are based on events from a specific trial, and reference memories are formed over repeated trials from the unchanging circumstances of a task (Honig, 1978).

Several theoretical distinctions can be made between working and reference memory. One is that working memories are useful for only one-trial in an experiment and lack useful long-term information; thus the animal is better off discarding them. Defining memory by "long-term" and "short-term" was misleading since one-trial working memories have been shown to last for

more than 24 hrs in rats (Beatty & Shavalia, 1980). Working memories are those that fade when they are no longer useful and reference memories are those that consolidate. Consolidation theory is the proposal that after an event, a period of rehearsal is required so that a memory can later be retrieved (Hebb, 1949, p. 112; Davies, Krebs & West, 2012, p. 42).

Working and Reference Memory Brain Structures

We study animal memory in order to gain an understanding of the neural processes responsible for the acquisition and storage of knowledge in humans and animals. Recent research out of Emory University has demonstrated that one-trial memory and habit are active simultaneously and independently in Rhesus Monkeys (Tu, Hampton & Murray, 2011). In this research one-trial memory is equivalent to working memory and habit is equivalent to reference memory. In one task, delay intervals selectively decreased one-trial memory scores, but did not affect habit scores. Biased reinforcement decreased habit scores but not one-trial memory scores (Tu & Hampton, 2013). This research supports the idea that working and reference memories are controlled by difference brain systems that contribute independently to performance on memory tests. The perirhinal cortex is implicated in memory function and is a crucial component of successful memory test completion (Baxter, 2009; Suzuki, 2010; Meunier et al., 1993; Buffalo et al., 1999). Tu et al. (2011) found that perirhinal cortex removal inhibited one-trial memory performance while leaving habits highly functional. Interestingly, habits were only relied on when one-trial memory failed. Therefore, working memory and reference memory could be defined as independent systems both cognitively and physically.

Episodic Memory

Tulving (1983) defined episodic memory as “be[ing] consciously aware of an earlier experience in a certain situation at a certain time” (p. 67). He also argued that episodic memory

has properties of autoneotic consciousness and self-awareness, both of which were previously considered uniquely human attributes. Autoneotic consciousness is the ability to mentally "time travel" into the past or future. Episodic memory involves memory for the what, when and where of events. Observations of animals such as Clark's nutcrackers, a species of bird in the corvid family who can store 33,000 seeds in the autumn and successfully recover them throughout the winter, have raised questions about the possibility of episodic memory in animals (Hitchcock & Sherry, 1990). Scrub Jays, another corvid species, have been shown to remember where they store food, what type of food they store, and how long ago they stored it (Clayton & Dickinson, 1999). Scrub Jays stored worms and peanuts on trays in a laboratory and were able to later consume preferred worms before peanuts at a delay when the worms were still fresh, but to consume the peanuts first at a longer retention interval when the worms had decayed. Clayton and Dickinson argued that jays could only have done this if they remembered what they stored (a worm or a peanut), where they stored it (which tray) and when worms and nuts were cached (long enough for the worms to be rotten or not). Clayton and Dickinson called this ability "episodic-like memory."

Rats have been shown to have impressive working memory capabilities on both a 17-arm radial maze (Olton, Collison, & Werz, 1977) and a 32-arm hierarchical radial maze (Roberts, 1979) where rewarded arm entries must be limited to previously unvisited arms. Episodic-like memory has been demonstrated in rats based on how long ago cues were encountered, suggesting that rats might have limited "mental time travel" which is an ability commonly experienced by humans (Roberts, 2006; Roberts et al., 2008). In a two phase test, Roberts et al. (2008) showed that rats were insensitive to when during the day they discovered a coveted cheese reward on a radial maze, but that they were able to keep track of how long ago a cheese

reward was encountered in order to infer whether or not that cheese would be replenished in a test phase. The researchers suggested that animals may be recalling how long ago events occurred by keeping track of time through circadian timers and their own behaviours, or the strength of their decaying memory traces (Roberts at al., 2008). Circadian timers are endogenous biological timers that are adjusted to the local environment and command a sense of time. In another experiment, rats were able to keep track of whether or not a food reward would be replenished on a radial maze depending on the time interval between the study and test phase (Naqshbandi, Feeney, McKenzie & Roberts, 2006; Babb & Crystal, 2005). The food reward was replenished after either a short or long interval, and rats learned whether or not to return to the initially rewarding arm based on the time interval they experienced on each trial. Therefore, rats show episodic-like memory on a radial arm maze.

The Radial Arm Maze

The radial arm maze was developed by Olton and Samuelson (1976) and has become an essential tool for testing memory in rats. Since its' invention, animals' performance on the radial arm maze has been shown to be a true measure of spatial memory. The radial arm maze features a number of open and elevated arms attached to a central platform. Each arm has a guillotine door such that an experimenter can impede entry into any particular arm. Each arm hosts a cup that can contain a food reward. Perfect maze performance results when a rat can enter each baited arm without repeating visits (Olton & Samuelson, 1976). Performance on the maze is not based on response algorithms such as an "always turn right" strategy (Roberts & Dale, 1981), or odor cues resulting from the placement of a rats scent to "mark its territory" as a sign that it has been there (Zoladec & Roberts, 1978; Olton & Samuelson, 1976; Olton & Papas, 1979). Roberts and Dale (1981) forced entry into four randomized arms of the maze before allowing rats to

search all of the arms for food rewards. The rats could not use an algorithmic strategy because the available arms were chosen for them and the experimenters found that performance on the maze was unaffected by forced entry. Zoladec and Roberts (1978) made rats temporarily anosmic and rats' performance on the maze was again, unaffected. Therefore, the radial maze is a true memory paradigm. Memory performance in the rat is a consequence of a categorical cue list in which an animal mentally "checks off" arms of a maze by using landmarks (Olton & Schlosberg, 1978; Healy, 1998, p. 18; Pearce, 2008) on a cognitive map (Healy, 1998, p. 119). Landmarks are the features within a testing environment; a chair, a desk, the experimenter, posters and the door. A cognitive map is the mental representation of these landmark cues and the maze itself. A match between an arm cue and an entered arm in a rats' cognitive map would lead to avoidance of an arm, and a match between an arm and an unchecked cue would trigger a decision to enter the arm (Olton, 1978; Healy, 1998, p. 18).

There are two types of cues used in the formation of a cognitive map. Allocentric cues are those that occur based on the relation of different landmarks to each other, such as the door and the chair in a testing room. Egocentric cues are those that occur based on the relation of a landmark and the animal itself, such as the door and the rat in the maze. Tolman (1948) displayed rats phenomenal spatial problem solving skills and was the first to hypothesize that rats formed a cognitive map. Tolman suggested that place learning, which associates an event with a location, occurs when landmarks are represented in relation to each other in an allocentric fashion. Tolman's work with latent learning, learning which excludes reinforcements, indicated that rats were able to take novel shortcuts to reach a reward with the aid of allocentric cues (Tolman, 1948). In contrast, Clark Hull argued that dead reckoning egocentric spatial localization in which landmarks are remembered relative to the organism's physical position by

means of response learning was responsible for cognitive mapping (Healy, 1998, p. 119; Shettleworth, 2010, p. 270). Response learning involves learning to perform particular behaviours based on rewards.

Working and Reference Memory in Rats

Beatty and Shavalia (1980) have demonstrated that working memory performance on the radial maze remains above 90% after a 4 hr retention interval and exceeds chance even after 24 hrs. Roberts and Dale (1981) showed that remembrance of places lasts in a demonstration of how rats do not "reset" their memory after each trial but instead switch to an algorithmic strategy when given massed trials that create proactive interference. Massed trials are trials that occur directly after each other such that the previous trials events become confused with the current trials events in what is referred to as a recency effect. Massed trials create proactive interference that confuses working memory capabilities via recency effects (Olton & Samuelson, 1976). The fact that confusion results because rats do not reset their memory indicates that they have a working memory of where they have previously been in an environment. Beatty and Shavalia (1980) tested memory in a two phase memory task: after forced visits to four randomly chosen arms in phase one, rats were required to visit the four previously unvisited arms in phase two for a food reward. Performance systematically declined after a 4 hr retention interval. To investigate pharmalogical interventions, the researchers found that exposure to barbiturate anesthetics during the delay interval did not disrupt memory. Therefore, this paradigm is a useful tool for studying the effects of pharmacological treatments on memory. The present study has the potential to lay the foundation for detailed research on the effects of various interventions on the interaction between working and reference memory. To test reference memory abilities in the radial maze, Olton and Papas (1979) trained rats to restrict their arm choices to those that were consistently

baited in order to create reference memories. The present study aims to fill a gap in the existing memory literature by examining the interaction between working and reference memories in a radial arm maze with rats.

This study used a radial arm maze to investigate how efficient rats' working and reference memories were under different conditions. After training trials that established certain arms on the maze as working or reference memory arms, rats were tested for each type of memory after different retention intervals. In a final experiment, the two types of memory were put in opposition to one another to examine their interaction at different retention intervals. This research could assist in the development of a rat model of the interaction between these two kinds of memory. This is useful because it would allow us to investigate the effects of various interventions (drugs or behavioural cognitive therapies) that could improve working and referential memory. Patients with a memory impairment such as; Alzheimer's Disease, or Dementia, often confuse memories of recent and past events. If this kind of confusion could be produced in a rat, it would be interesting to investigate what interventions could reduce that confusion.

Rats were exposed to a radial arm maze on which they searched for food rewards in the eight arms of the maze. In phase 1 (the study phase), only four arms were open, but those four arms were all baited with a food reward, and in phase 2 (the testing phase), only the opposite four arms were baited with food rewards even though all eight arms were open. The rat had to enter the four test arms to be correct and obtain rewards. Two of the test arms were reference memory arms because they were always baited for a particular rat. The other two test arms were working memory arms and varied randomly from trial to trial among the six remaining arms. Once rats learned to enter the test arms accurately, the retention intervals between the study and

test phase were varied. There are three independent variables in these experiments; trial, retention interval (0/1/24 hrs) and arms (reference/ working memory arms). Two dependent variables were recorded and analyzed, the percentage of correct working and reference memory arm entries made within the first four arm entries in phase 2, and the mean rank order of entry into each type of arm.

Hypothesis

I hypothesized that rats would prefer to enter working memory arms before reference memory arms. This behaviour should occur if rats have an accurate perception of the vulnerability of their working memories in comparison to their reference memories, and because of rat preference for win-shift tasks (Olton & Schlosberg, 1978). On the other hand rats might enter reference memory arms before working memory arms as an optimal foraging strategy. By foraging in locations that they are certain will contain food rewards, rats are insured of getting at least those rewards. I also acknowledge the possibility that in this task there may be no advantage to having a memory type preference under a short baseline training retention interval between study and test phases. When the test phase follows immediately after the study phase, rats' memory may be very good for both working and reference memory arms. When retention intervals between the study and test phase are increased, however, I predict that working memory performance will decline as demonstrated by Roberts and Dale (1981), Olton and Samuelson (1976), and Beatty and Shavalia (1980). Under longer retention intervals I hypothesize that reliance on reference memories to obtain food rewards will increase. I also hypothesize that when working memory and reference memory are put in opposition to one another, reliance on reference memories will become apparent at longer retention intervals as reference memory errors.

Method

Subjects

Twelve male Long-Evans rats that were approximately 100 days old at the beginning of the study were used. The rats were kept at 85% of their free-feeding weight (approximately 350 g) prior to testing. Rats were housed in standard polypropylene cages with water *ad libitum* on a 12:12 h light-dark cycle, with light onset at 7 a.m. and offset at 7 p.m. Rats were fed Pro Lab Rat Chow daily after testing in concordance with their target weights.

Materials and Apparatus

An eight-arm radial maze was designed of 2.5 cm plywood and consisted of a central platform with a diameter of 35.5 cm. Eight arms extended from the central platform, each 79 cm long and 9 cm wide, with equal angular distance between adjacent arms. A 30 cm tall wooden frame was connected to the central platform such that a guillotine door could impede entrance into each arm. The door was suspended on the frame by fishing line attached to a control board on the wall. The end of each arm held a blue PVC food cup that was 6 cm in diameter and 3 cm deep. The central platform was painted white, and the arms were painted black. The maze was elevated 61 cm off the floor and supported by nine pieces of wooden dowling (one under each arm and the central platform). A 45-mg Noyes Precision Pellet (PJAI-0045, Research Diets Inc., New Brunswick, NJ) was used as a food reward on the maze and was placed in the blue food cup. A single 60-W bulb was used in a desk lamp for dim lighting in the corner of the 3.5 m x 3.5 m testing room. The room contained a double-layered table, one chair, one stool, one metal transport cart, one plastic transport cart, and one unused curtain that could wrap around the periphery of the maze. A white noise generator on the desk emitted white noise at 60 dB to mask extraneous sounds.

Procedure

Arm assignment.

Each rat was assigned two arms that would always be correct (rewarding) and thus served as reference memory arms. Reference memory arms were chosen such that each arm on the maze was used equally often as a reference memory arm across rats. Therefore, each arm was used three times in the assignment of two reference arms for each rat. The distances between the two reference arms for each rat also occurred equally often. Therefore, distances of 0, 1, 2 or 3 arms between the two reference arms occurred equally often (see Table 1). Reference memory arms were always the same for each rat throughout all trials of all experiments. On each trial, a different set of two working memory arms was chosen randomly from among the remaining six arms. Working memory arms were selected by using a random number table generated with only numbers 1-8.

Experiment 1: Acquisition trials.

One month of preliminary training on the maze was required before the experiment could begin. In this training, rats were transported from their home cages to the testing room with dim lighting and white noise. Rats were allowed to explore the maze. The maze contained multiple rewards placed in each food cup and along the arms of the maze and on the central platform. Rats were typically exposed to the maze for 5 -10 min for five days/week during this month. Once rats were successfully travelling on the maze and eating from the food cups, their pattern of entry was recorded until they were consistently above 5.25 unrepeated arm entries (chance level) within the first eight arm entries.

After the initial training, rats were transported out of their housing room on a metal transport cart that held six cages. Therefore, rats were brought into the testing room in two

Table 1

Reference Memory Arm Assignments

Rat #	Reference Arms Assigned	Distance Between Arms
1	2, 4	1
2	3, 7	3
3	2, 8	1
4	1, 5	3
5	4, 8	3
6	6, 7	0
7	5, 8	2
8	1, 6	2
9	5, 6	0
10	3, 4	0
11	2, 7	2
12	1, 3	1

Note. The distance between arms is the number of arms between the two reference arms. Each arm is used as equally often (three times) and the distance between the two reference arms in a pairing occurs equally often (three times).

groups of six. In the testing room, a single desk lamp was lit to provide dim lighting, and white noise played throughout testing. All eight arms were baited with a 45-mg precision reward pellet. The reference and working memory arms remained closed while the other four arms were opened for the study phase (phase 1; see Figure 1). The rat was placed on the central platform of the maze, facing in a direction that changed randomly among trials. The rat was allowed to enter the four open arms and to consume the reward pellet in the food cup. An arm entry was defined as all four legs leaving the central platform, but in every experimental trial each rat travelled to the end of a chosen arm and ate the food reward. The order of entry was recorded. After all four food rewards were consumed, the rat was removed from the maze and temporarily placed on a nearby plastic transport cart. The four closed arms (the two reference and two working memory arms) were opened so that all eight arms were open for the test phase (Phase 2; see Figure 2). The rat was placed on the central platform for the second time and allowed access to all eight arms. The order of entry into successive arms was recorded, and the rat was removed from the maze once the four previously unvisited arms had been visited and their reward pellets consumed. The number of working memory and reference memory arm entries within the first four visits was recorded and used to calculate the percentage of correct working and reference memory arm choices. Perfect performance resulted when the rat entered the four previously un-entered arms within the first four arm entries of the testing phase. The rat was then placed back inside its cage and returned to the transport cart. When all 12 rats had completed experienced a trial, they were returned to their housing room and fed.

Experiment 2: Retention intervals (5 s, 1 hr, 24 hr).

The same procedure from Experiment 1 was used for Experiment 2, except that there was a time delay between the study and test phase. The three retention intervals used were 5 s, 1 hr,

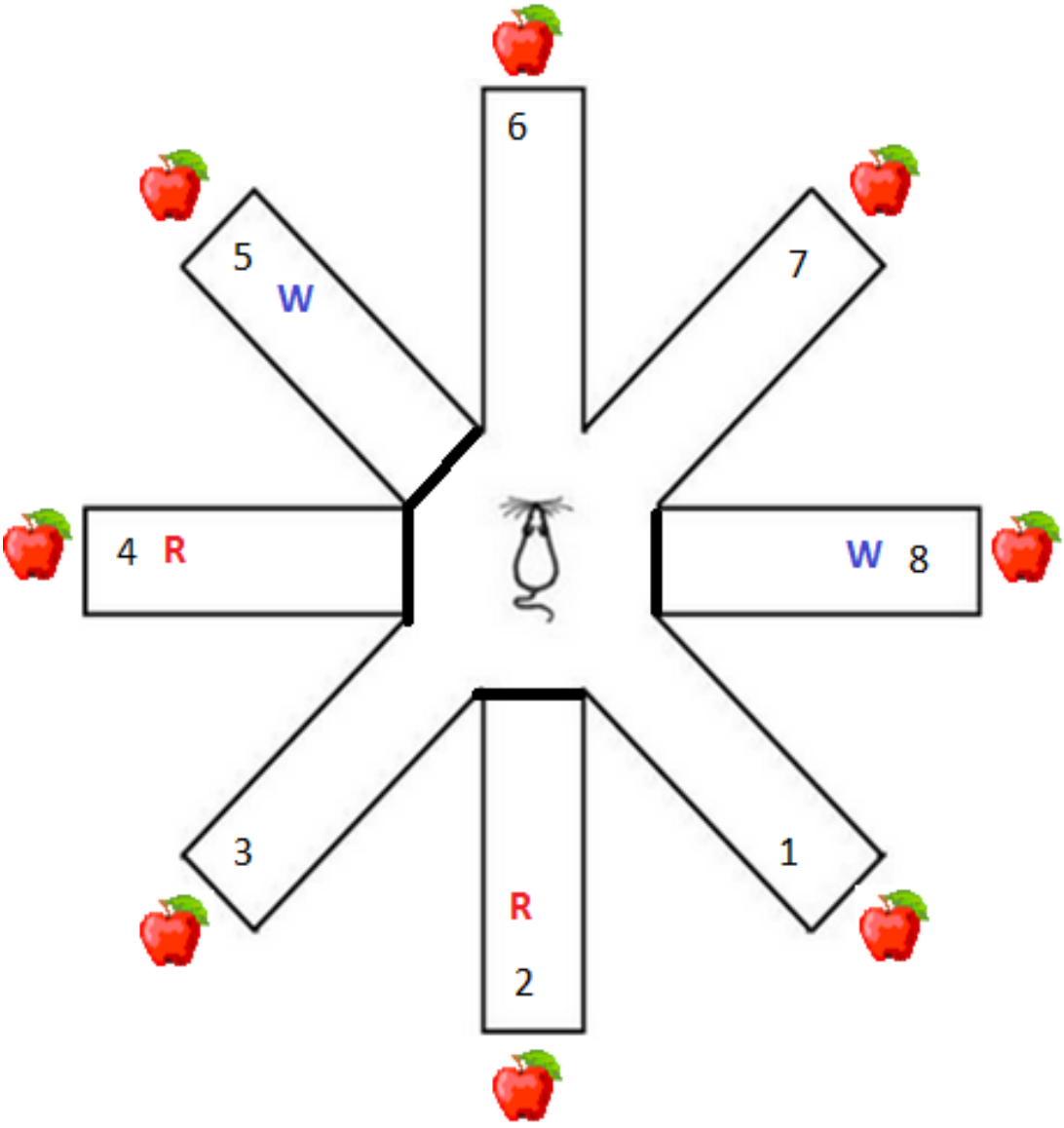

Figure 1. Study Phase (Phase 1): All eight arms are baited with a food reward. The two reference memory arms (2 & 4) and the two working memory arms (5 & 8) are blocked so that the rat (center) can only enter the other four arms (1, 3, 6 & 7). Apples represent that an arm is baited with a food reward.

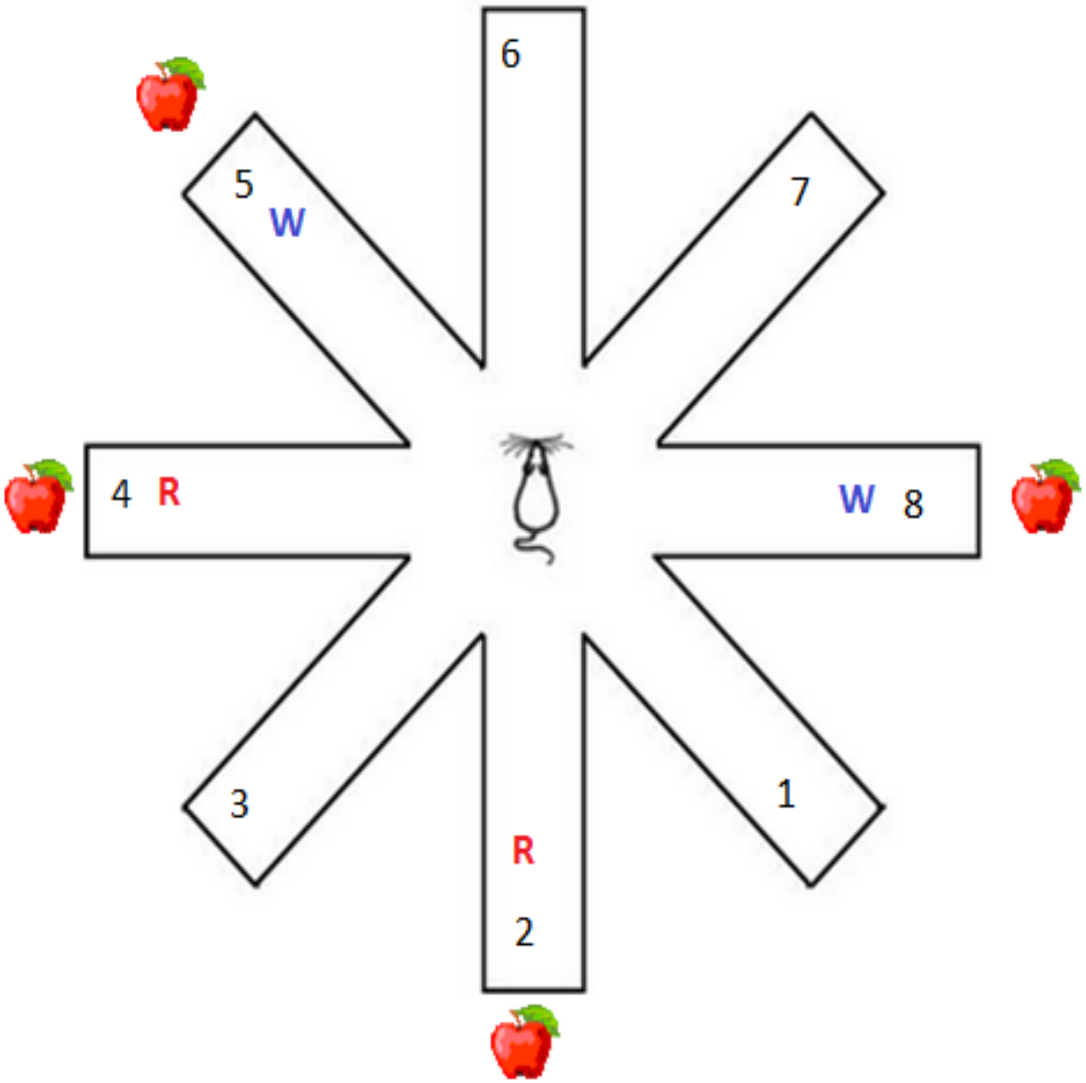

Figure 2. Test Phase (Phase 2): Only the two reference memory arms (2 & 4) and the two working memory arms (5 & 8) remain baited with a food reward. All eight arms are open to the rat. Apples represent that an arm is baited with a food reward.

and 24 hr. The retention intervals were each tested three times in three successive blocks of tests, with the order of the intervals counterbalanced over blocks in a Latin square design. As in Experiment 1, the order of arm entries on each trial was recorded, and the percentage of correct working and reference memory arm entries in the first four choices was calculated.

Experiment 3: Making reference memory arms working memory arms.

The same procedure from Experiment 2 was used for Experiment 3, except that rats were forced to enter the two reference memory arms in phase 1 along with two other randomly chosen arms. The three retention intervals used between the study and testing phases; were 5 s, 1 hr, and 24 hr. The retention intervals were each tested three times in three successive blocks of tests, with order of the intervals counterbalanced over blocks in a Latin square design. As in Experiment 1 and 2, the order of arm entries on each trial was recorded, and the percentage of correct working and reference memory arm entries in the first four choices was calculated.

Data Analysis

A 2 x 3 completely within subjects analysis of variance (ANOVA) was used to analyze percentage of correct arm entries and mean rank order of arm entry for both reference and working memory on the acquisition trials (Experiment 1). The within subjects factors were Memory Type (reference/ working memory), and Trial (grouped from 1-10, 11-20, 21-30). A second 2 x 3 completely within subjects ANOVA was used to analyze the percentage of correct arm entries and mean rank order of arm entry for reference and working memory at the three retention intervals (Experiment 2). The within subjects factors were Memory Types (reference/ working memory) and Retention Interval (0, 1 hr, 24 hr). A significant interaction was investigated with two one-way ANOVA's and three paired samples t-tests. A third 2 x 3 completely within subjects ANOVA was used for Experiment 3. The percentage of correct arm

entries and the percentage of both reference and working memory errors were recorded. The within subjects factors were Types of Memory Errors (reference memory errors/ working memory errors) and Retention Interval (0, 1 hr, 24 hr). A significance criteria of $\alpha = 0.05$ was used for all tests.

Results

The results indicated that rats showed no initial preference for using working or reference memories in the retrieval of a food reward at immediate intervals between the study and test phase. At long retention intervals rats preferred to rely on reference memories. When reference memory arms were made into working memory arms, rats had a nonsignificant tendency to make more reference memory errors at longer retention intervals (24 hrs) than at immediate and 1 hr intervals.

Experiment 1: Acquisition Trials

There were no significant differences between working and reference memory performance in this task, although, in all cases performance was significantly above chance. Performance increased over trials for both types of memory. A main effect was not found for memory type, $F(1.00, 11.00) = .108$, $p > .05$, therefore the percentage of correct arm entries did not differ as a function of memory type. A main effect was found for trial block, $F(1.65, 18.11) = 21.01$, $p < .001$, showing that the percentage of correct arm entries increased significantly across trials. An interaction of Memory Type X Trial Block was not seen, $F(1.97, 21.68) = 2.52$, $p > .05$, and therefore the memory types showed consistent patterns that did not differ significantly over trials (see Figure 3).

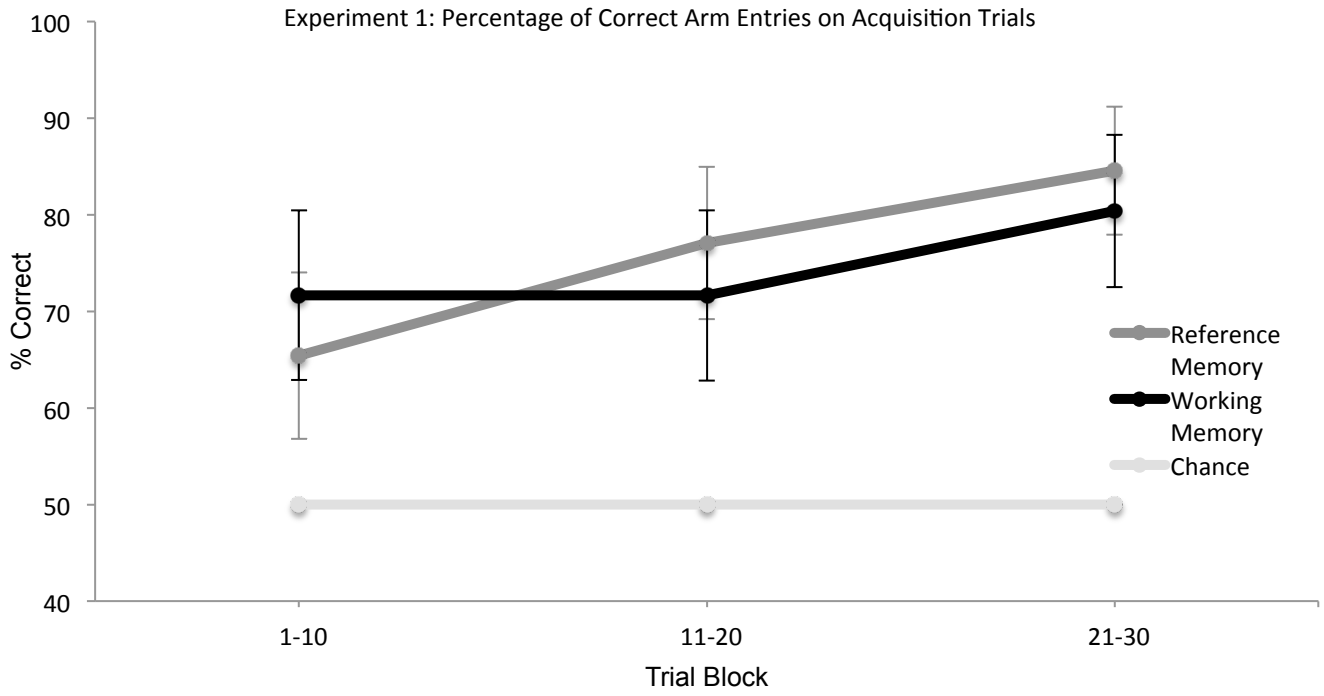

Figure 3. Experiment 1 (Acquisition Trials): The x-axis shows the trial groupings from trial 1-30, and the y-axis shows the percentage of correct arm entries made within the first four arm entries. Error bars represent standard error of the mean.

The reference memory acquisition percentage correct in each block of trials (Block 1 M = 65.42, SE = .1728; Block 2 M = 77.08, SE = .1576; Block 3 M = 84.58, SE = .1329) was significantly higher than chance (M = 50), $t(11) > 4.397$, $p < .01$. Therefore, rats rapidly formed reference memories of the locations of food rewards in the task and their performance for accurately visiting reference memory arms in the first four choices increased over trials.

The working memory acquisition percentage correct in each block also significantly exceeded the chance value of 50% (Block 1 M = 71.67, SE = .1754; Block 2 M = 71.67, SE = .1763, Block 3 M = 80.42, SE = .1579), $t(11) > 7.571$, $p < .001$. Therefore, rats also learned rapidly to respond accurately on test trials based on working memories and showed improvement over sessions in working memory accuracy.

Experiment 1: Acquisition Trials Mean Rank Order of Entry

Rats entered the reference memory arms before the working memory arms. The main effect for memory type was not significant, $F(1.00, 11.00) = 1.97$, $p > .05$, and the overall mean rank order of entry did not differ as a function of memory type. A main effect was found for trial block, $F(1.81, 18.89) = 9.943$, $p < .01$, showing that the mean rank order of entry into working and reference memory arms decreased as a function of trial block. An interaction of Memory Type X Trial Block was not seen, $F(1.80, 19.81) = 2.57$, $p > .05$, and therefore memory types showed consistent patterns of arm entry that did not differ significantly from one another over trials (see Figure 4).

Experiment 2: Retention Intervals (5 s, 1 hr, 24 hr)

At the immediate retention interval there was no significant difference between reference and working memory, but as the retention intervals increased, rats developed a preference for using reference memories to retrieve food rewards. A significant main effect was found for

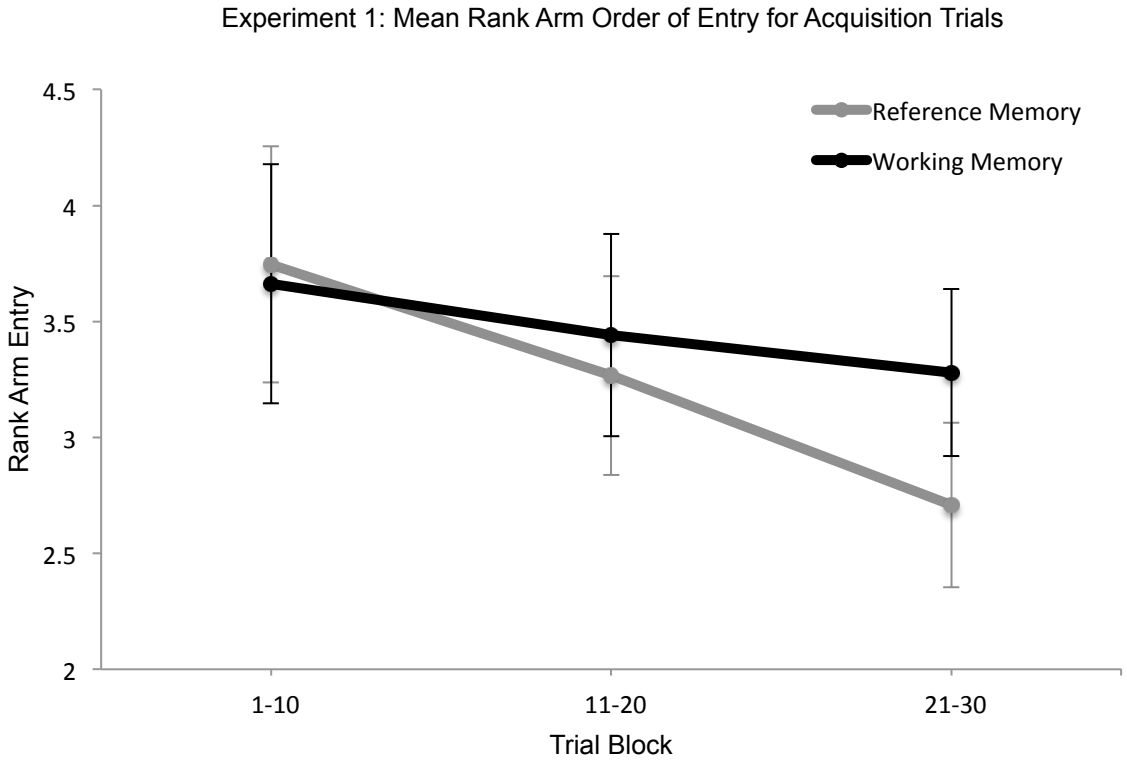

Figure 4. Experiment 1 (Acquisition Trials Mean Rank Order of Entry): The x-axis shows the trial groupings from trial 1-30, and the y-axis shows the mean rank of arm entries where values closer to 2 mean that rats are entering those arms earlier. Error bars represent standard error of the mean.

memory type, $F(1.00, 11.00) = 23.75$, $p < .001$, therefore the overall percentage of correct arm entries differed as a function of memory type across trials, such that the percentage of correct reference memory choices was significantly higher than the percentage of correct working memory choices. A significant main effect was found for retention interval, $F(1.39, 15.24) = 21.354$, $p < .001$, indicating that the percentage of correct arm entries decreased as a function of retention interval in the sense that performance suffered as a result of longer time intervals. There was also a significant interaction of memory type with retention interval, $F(1.40, 15.45) = 4.407$, $p < .05$, caused by the finding that the percentage of correct working memory arm choices decreased more as the retention interval increased, than did the percentage of correct reference memory arm choices (see Figure 5).

The reference memory percentage correct at 5 s ($M = 87.50$, $SE = 6.417$) was not significantly higher then the working memory percentage correct at 5 s ($M = 86.11$, $SE = 6.721$), $t(11) = .203$, $p > .05$, therefore, at an immediate retention interval there was no significant differences in performance due to memory type. The reference memory percentage correct at 1 hr ($M = 79.17$, $SE = 7.131$) was significantly higher than the working memory percentage correct at 1 hr ($M = 50.00$, $SE = 9.194$), $t(11) = 7.024$, $p < .001$, and the reference memory percentage correct at 24 hr ($M = 70.833$, $SE = 8.124$) was significantly higher than the working memory percentage correct at 24 hr ($M = 51.34$, $SE = 9.141$), $t(11) = 2.379$, $p < .05$. Therefore, performance was significantly higher for reference memory than it was for working memory at both 1 hr and 24 hr.

The percentage of correct reference memory arm entries made varied across the three retention intervals, $F(2.00, 105.00) = 17.487$, $p < .001$, Tukey's post hoc procedure indicated that reference memory at 5 s ($M = 87.50$, $SE = 6.417$) differed significantly from reference memory

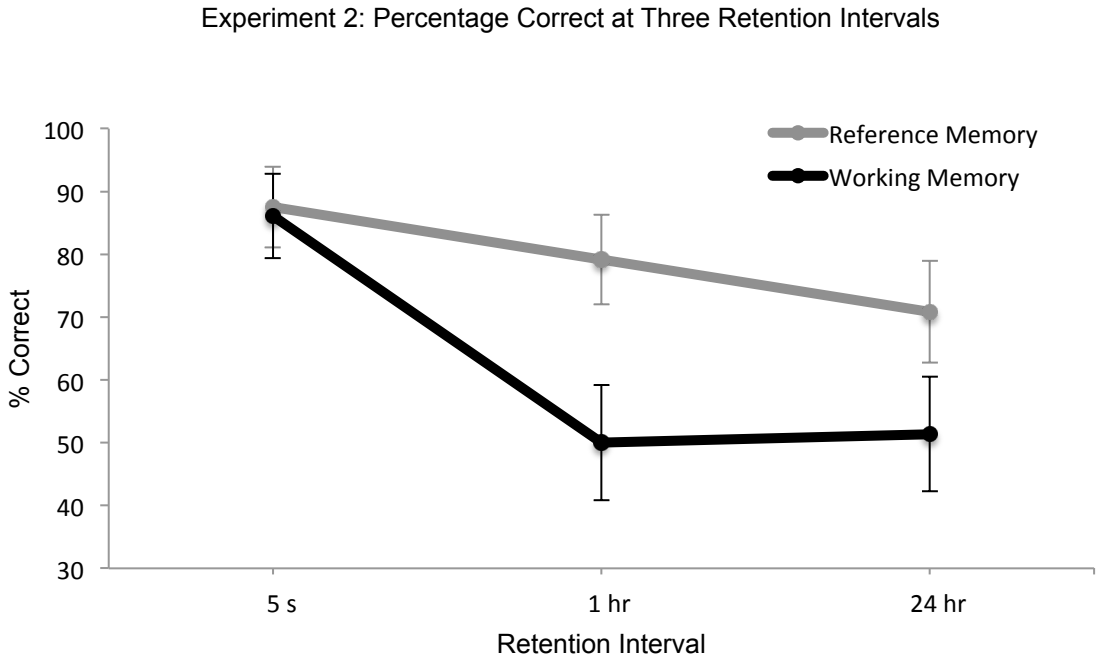

Figure 5. Experiment 2 (Retention Intervals): The x-axis shows the three retention intervals (5 s, 1 hr, 24 hr), and the y-axis shows the percentage of correct arm entries within the first four arm entries. Error bars represent standard error of the mean.

at 24 hr (M = 70.833, SE = 8.124), but that reference memory at 5 s and 1 hr (M = 79.17, SE = 7.131), and 1 hr and 24 hr, did not differ significantly. Therefore, reference memory lead to consistently high performance after a 1 hr retention interval.

The percentage of correct working memory arm entries made varied across the three retention intervals, $F(2.00, 105.00) = 17.487$, $p < .001$, Tukey's post hoc procedure indicated that working memory at 5 s (M = 86.11, SE = 6.721) differed significantly from working memory at 1 hr (M = 50.00, SE = 9.194), and 24 hr (M = 51.34, SE = 9.141), but that working memory at 1 hr and 24 hr did not differ significantly. Therefore, working memories lead to a decreased performance after a 1 hr retention interval.

Experiment 2: Mean Rank Order of Entry at Three Retention Intervals (5 s, 1 hr, 24 hr)

Rats entered reference memory arms before working memory arms across all retention intervals. The overall mean rank order of entry into reference and working memory arms differed as a function of memory type, indicating that reference memory arms were entered sooner then working memory arms, $F(1.00, 11.00) = 18.68$, $p < .01$. A significant main effect was found for interval, $F(1.48, 16.25) = 21.64$, $p < .001$, indicating that correct arms were entered earlier at shorter retention intervals when compared to longer retention intervals. Both reference and working memory arms had similar patterns of entry across retention intervals as shown by a nonsignificant Memory Type X Retention Interval interaction $F(1.22, 13.37) = 1.76$, $p > .05$ (see Figure 6).

Experiment 3: Making Reference Memory Arms Working Memory Arms

The percentage of correct arm entries decreased as the retention intervals increased. Both working and reference memory errors increased across retention intervals. There were no significant differences between working memory errors and reference memory errors in this task,

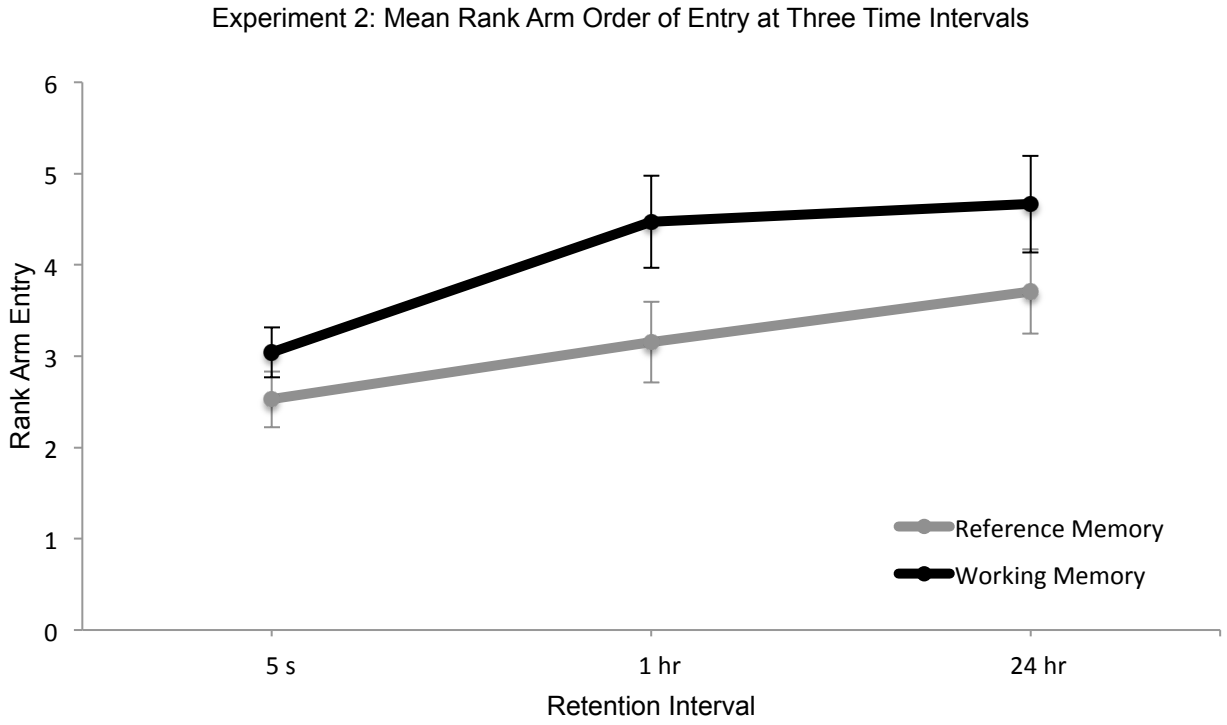

Figure 6. Experiment 2 (Retention Intervals): the x-axis shows the three Retention Intervals (5 s, 1 hr, 24 hr), and the y-axis shows the mean rank of arm entries where values closer to 2 mean that rats are entering those arms sooner. Error bars represent standard error of the mean.

although, the data suggest a tendency to enter reference memory arms before working memory arms even at immediate retention intervals. Performance decreased over trials for both types of memory. A main effect was not found for memory type, $F(1.00, 11.00) = 1.26$, $p > .05$, therefore the percentage of memory errors did not differ between reference and working memory. A main effect was found for retention interval, $F(1.96, 21.56) = 35.34$, $p < .001$, showing that the percentage of memory errors increased significantly at larger retention intervals. An interaction of Memory Type X Retention Interval was not seen, $F(1.42, 15.61) = 1.78$, $p > .05$, and therefore the memory types showed consistent patterns of error that did not differ significantly across retention intervals (see Figure 7).

The percentage of correct arm entries made varied across the three retention intervals $F(2, 35) = 40.73$, $p < .001$, Tukey's post hoc procedure indicated that the percentage of correct arm entries at 5 s ($M = 85.42$, $SE = 8.61$) differed significantly from the percentage of correct arm entries at 1 hr ($M = 59.72$, $SE = 10.27$) and 24 hr ($M = 54.17$, $SE = 9.21$) but that the percentage of correct arm entries at 1 hr and 24 hr did not differ significantly. Therefore, in congruence with results from Experiment 2, performance declined at longer retention intervals.

Discussion

The purpose of this investigation was to develop a model of the interaction between reference and working memory under conditions that varied: retention interval, and whether types of memory were or were not put in opposition to one another. This model could be useful for representing the confusion between recent and past events in human memory impairment. Unlike previous investigations, the present study was unique in its combination of working and reference memory on the radial arm maze at the same time. Therefore, we were able to manipulate the task to discover what conditions cause confusion in memory. The present

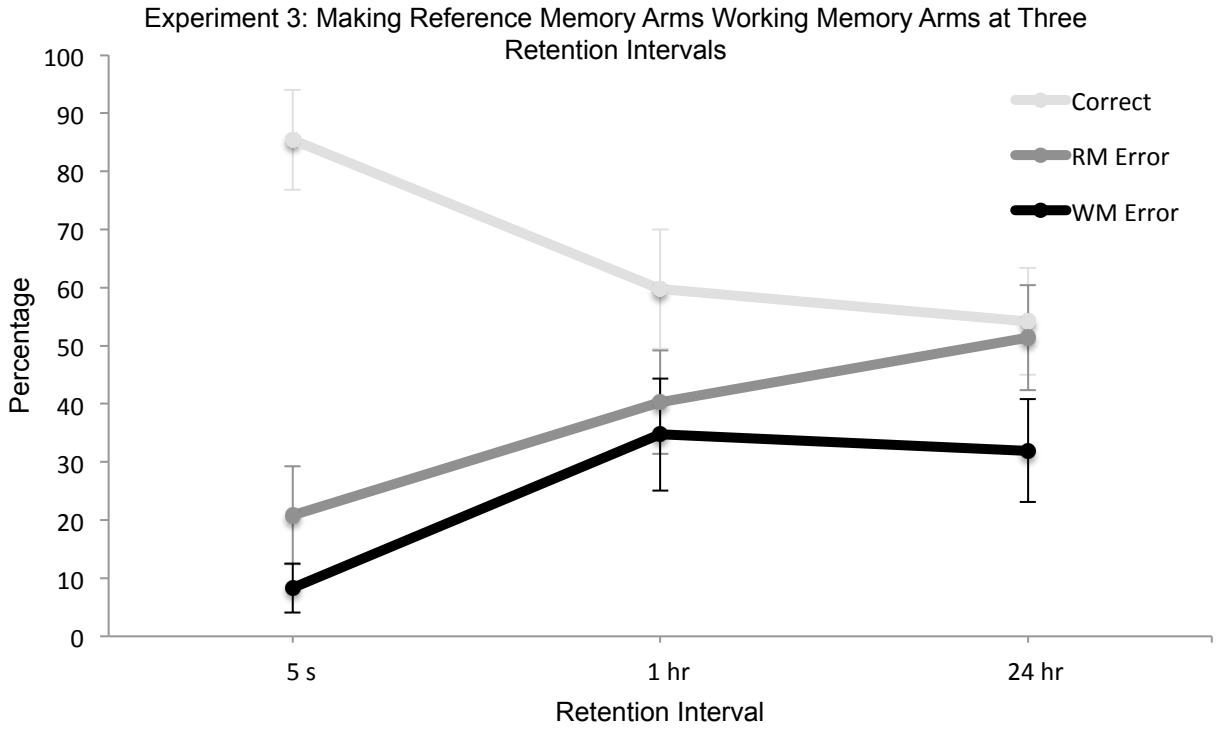

Figure 7. Experiment 3 (Retention Intervals): the x-axis shows the three Retention Intervals (5 s, 1 hr, 24 hr), and the y-axis shows the percentage of arm entries for correct arm entries, and working and reference memory errors as indicated by the legend. Error bars represent standard error of the mean.

investigation is important because it contributes new information to the field and allows future research to manipulate this model with various behavioural and drug interventions that could eventually apply to human memory impairments.

I hypothesized that rats would prefer to enter working memory arms before reference memory arms because of their preference for win-shift tasks and because of the vulnerability of working memories when compared to reference memories. In contrast, the results indicated that rats preferred to enter reference memory arms before working memory arms, which is indicative of an optimal foraging strategy. I also hypothesized that at longer retention intervals rats would rely on reference memories and that when reference memories become working memories rats would make more incorrect reference arm entries. The next step in future research is to firstly discover what conditions can bring working memory accuracy to the level of reference memory accuracy at longer retention intervals, and secondly, what manipulations can reduce the suggested confusion between incorrect reference memories and correct working memories when rats are forced to enter reference memory arms in a study phase.

To begin with Experiment 2 and the retention intervals, working memory performance was significantly worse than reference memory performance at 1 hr and 24 hr intervals, but not at the immediate retention intervals. At the same time, reference memory performance remained consistently high. Future research can investigate what kinds of interventions can bring working memory back to awareness, and thus increase performance at longer retention intervals. In order to do this a few strategies could be tested. Using a preferred food reward in the working memory arms may act as a trigger for reminding the rat which arms are to be remembered alongside the consolidated reference memories. Re-exposure to the central platform between retention intervals may act as a retrieval cue for working memories. The use of female rats, who use

different strategies for memory tasks during their estrous cycles, may provide a new strategy that differs from that used by the males in these experiments and might be a natural intervention that increases working memory performance.

When reference memory arms become working memory arms, as in Experiment 3, preferred food rewards could also be used in the working memory arms to reduce the tendency to return to previously visited reference memory arms. Similar to the future studies with Experiment 2, rats could also be re-exposed to the maze with the goal of the center platform acting as a consolidation cue. Also, female rats could be used to observe the different strategies for reward retrieval. Unique to Experiment 3, cue associations could be implemented. Brightness associations for example could be used to indicate to a rat that their reference memories are no longer reliable in a particular circumstance so that rats could avoid entering reference memory arms when they contain no reward.

Drug interventions that are already established as memory impairment aids should also be investigated to ensure the confusion created between recent and past events in our model mirrors the confusion in human memory impairment. Memantine and neramexane are two pharmaceuticals with promising effects for the treatment of Alzheimer's disease, particularly supported by an improvement in long-term spatial memory in both humans and rodents (Zoladz et al., 2006). Memantine is currently used for the treatment of moderate to sever Alzheimer's Disease (Roman, 2009). Therefore it should be tested in our model. In nonhuman primates, nicotine has been found to improve spatial working memory for 1 month after acute nicotinic treatment. Therefore experimenters have found that low doses of a nicotinic agonist can improve working memory and may play a role in the neural circuitry of working memory (Castner, 2011). Pharmaceuticals, which inhibit cyclic AMP specific phosphodiesterases, enhance memory in

rodents (Zhang, 2005). Some researchers have also found that MEM1018, and MEM1091 enhance working and reference memory in the radial arm maze (Zhang, 2005). As a final suggestion, recent research has indicated that a certain inhibitor (ROCK) improves spatial learning and working memory in rodent models (Huentelman et al., 2009). Therefore, these pharmaceutical interventions could be explored in our model to observe if they can improve the remembrance of working memories during retention intervals and reduce the confusion between reference and working memory arms when reference memory arms are made into working memory arms.

Before this model could be applied to human interventions it is crucial to investigate various interventions in the animal model in order to understand what types of interventions are effective. This would allow the human counterparts of these interventions to be used for reducing the confusion between working and reference memory that is seen in human memory impairments.

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