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Spatial memory for isolated arm locations on the radial maze

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Abstract

The ability to encode and retrieve information describing spatial locations allows organisms to solve a variety of natural problems. When multiple spatial locations vary with respect to foraging, predatory or environmental factors, successfully discriminating between these locations may result in increased fitness. Although, how do the spatial relations of multiple locations affect the encoding or retrieval of spatial information? Across three radial maze experiments, I addressed how spatial configuration affects working memory for multiple locations. Specifically, I asked 1) how spatial patterns affect memory for multiple locations, 2) how spatial isolation between to-be-visited locations may affect spatial memory, and 3) whether memory for spatially isolated locations is modulated by motivational factors. The findings support the hypothesis that spatial memory is sensitive to spatial configuration and are compared to the von Restorff effect. The absolute spatial distinctiveness of locations is proposed to be the underlying factor for the observed effects.

Keywords

Spatial Memory, Radial Arm Maze, Working Memory, Spatial Pattern, von Restorff, Isolation Effect, Prospective Memory, Retrospective Memory

Co-Authorship Statement

The experiments in this thesis were completed under the supervision of Dr. William A. Roberts and Dr. David F. Sherry. Although I conducted all of the experimental work, my supervisors worked closely with me to hone the focus of this thesis. Publications based on the work in this thesis will be co-authored by Hayden MacDonald and Dr. William A. Roberts. This research was also influenced by the input and feedback from the Roberts Lab group and the Sherry Lab group. Each experiment in this thesis benefitted from their suggestions about research questions, experimental design, statistical analyses and data visualization.

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Table of Contents

List of Figures

List of Appendices

List of Abbreviations and Symbols

°	Degrees
°C	Degrees Celsius
%	Percent
ANOVA	Analysis of Variance
POI	Point of Interpolation
RI	Retention Interval
a.m.	Ante meridiem; in the morning
cm	Centimeter
g	Grams
hr / hrs	Hour / Hours
m	Meter
min	Minutes
mg	Milligrams
p.m.	Post meridiem; in the afternoon

Chapter 1

1 General Introduction

1.1 Spatial Memory

Spatial memory is the cognitive ability to encode, maintain, and retrieve information which describes locations in extrapersonal space (Luciana, Collins, & Depue, 1998). Spatial memory is primarily used to navigate and manipulate environments. For example, humans use mental representations of space in order to navigate countries, cities, neighborhoods and buildings. Non-human animals (hereafter referred to as “animals”) can navigate equally large and dynamic environments. Remembering information pertaining to those environments can guide behaviour in ways that promotes increased fitness for individuals. In particular, spatial memory can facilitate the acquisition of additional or higher quality resources, additional or higher quality sexual mates, as well as the minimization of energy expenditure and exposure to risk (Olton, 1987; Shettleworth, 1983; Spritzer, Solomon, & Meikle, 2005; Spritzer, Meikle, & Solomon, 2005). Thus, spatial memory is an asset to many species for whom spatial cues are relevant.

Spatial memory can be used to achieve several different behavioural outcomes. For example, an animal that inhabits a central home base is given a choice between several patches that contain various amounts of food. Upon the first choice, the animal depletes food from the patch and returns to the home base. When the animal goes out again to retrieve more food, its spatial memory for the previously visited patch will inform the avoidance of that patch. This allows the animal to avoid wasting energy navigating to a patch that has already been depleted of its resources. Spatial memory would also help the animal avoid patches that never have food or have a higher risk of predation associated with them. Conversely, spatial memory for patches that always have food or access to mates would be remembered and visited more often. This is generally how spatial memory simultaneously promotes increased fitness while preventing decreases in fitness.

The interplay of different types of spatial information is facilitated by multiple memory systems. Spatial memory has been demonstrably divided into two separate systems: working memory and reference memory (Atkinson & Shiffrin, 1968; Baddeley, Bressi, Della Sala, Logie, & Spinnler, 1991; Olton & Papas, 1979; Packard, Hirsh, & White, 1989; Waugh & Norman, 1965). In human cognition, working memory is conceptualized as a system that processes and manipulates immediately available information through the perception of the environment or reference memory (Roberts & Santi, 2017). Typically, working memory in humans is inferred through conscious verbal recollection. In studies of animal cognition, working memory is inferred from nonverbal animals through behaviour. Working memory in animals is more generally defined as a system that flexibly handles information that is conditionally relevant over short or intermediate time scales (Roberts, Guitar, Marsh, & MacDonald, 2016). Conversely, reference memory handles robustly relevant information from repeated associations (e.g. Pavlovian conditioning) or discriminations over long time scales (Honig, 1978; Skinner, 1950). Drawing from the previous example, working memory would be responsible for the avoidance of the most recently visited patches, since it is conditional on the fact that the food was recently depleted. Reference memory would be responsible for avoidance of riskier or barren patches, since their status and position are fixed.

1.2 The Radial Arm Maze

So how do we test spatial memory in animals? A classic apparatus used to study spatial memory is the radial arm maze (see Figure 1; Olton & Samuelson, 1976). The apparatus consists of a number of arms that radiate from a central hub, much like the shape of an asterisk. The actual number of arms in the maze varies widely across studies, but is usually eight. At the end of each maze arm is a food cup where food rewards are placed. Typically, the maze is elevated from the ground in order to prevent animals from wandering off the maze while also allowing them to encode distal spatial cues. The simplest testing paradigm in the radial maze is a one-phase working memory test. At the beginning of this test, all arms in the maze are baited with food rewards and the animal is placed in the center of the maze. Assuming that the animal has been habituated to the apparatus and testing environment, it will search the maze arms, one at a time, until all

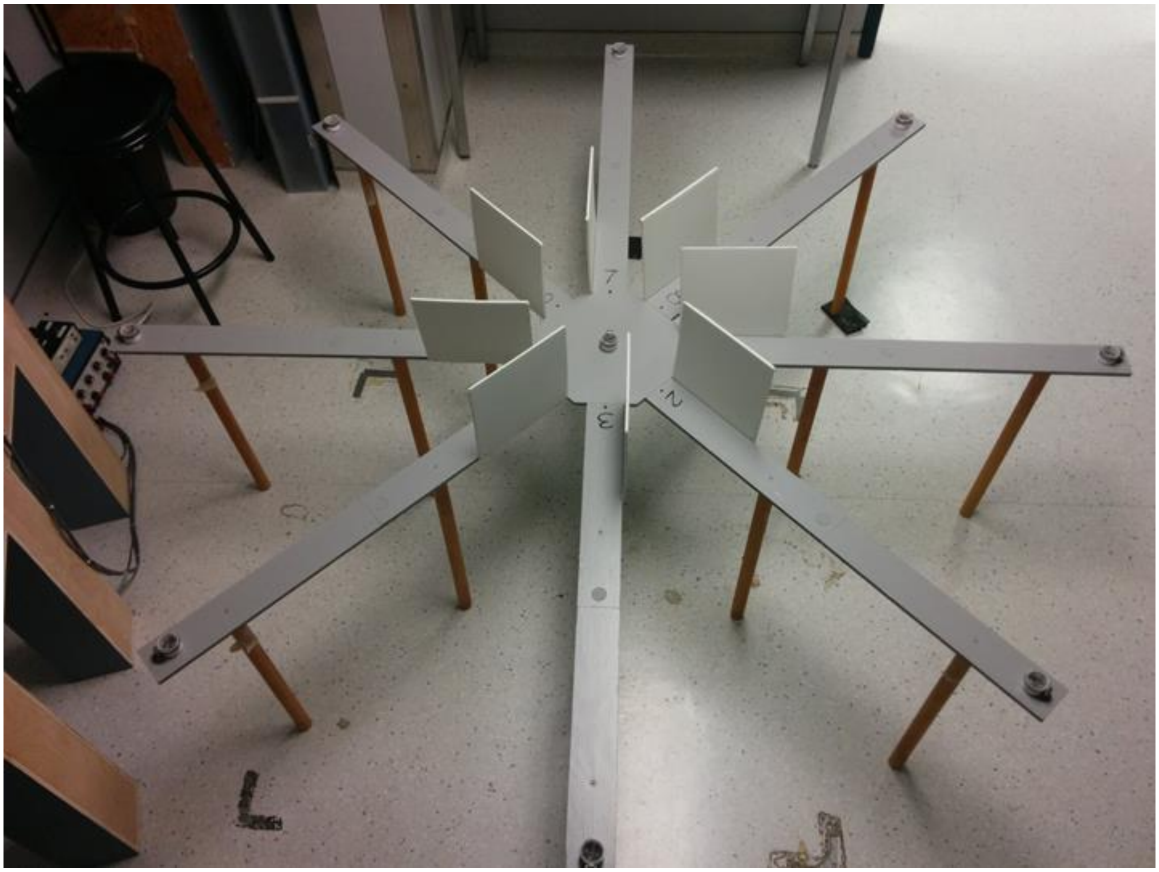

Figure 1. Overhead view of the 8-arm radial maze used in Experiment 1. The central food cup pictured was not present in this experiment. The barriers used in this experiment were inserted into the holes just outside the central platform. Both barrier types occluded the numbers written on the surface of each arm so that they could not be used to guide choice behaviour.

food rewards are depleted. Measurements of working memory in this task could be quantified as the number or errors (i.e., re-entries into previously visited arms), the total number of arm visits prior to depleting all food rewards, or the percentage of correct choices within the first *n* choices, where *n* equals the total number of arms. These test scores are proxy measurements of working memory for previously visited arms. In this procedure, high working memory accuracy could be quantified as achieving a minimal number of errors or the lowest possible number of total arm entries before finding all of the food rewards in the maze. Alternatively, achieving the highest possible percent correct score is representative of accurate performance. In Olton and Samuelson, rats performed with high accuracy in the above procedure within five days of testing. The strong findings from Olton's lab ushered in a wave of studies that used the radial maze to test spatial memory (Beatty & Shavalia, 1980; Einon, 1980; Maki, Brokofsky, & Berg, 1979; Olton, Collison, & Werz, 1977; Olton & Schlosberg, 1978; Olton & Werz, 1989; Suzuki, Augerinos, & Black, 1980; Zoladek & Roberts, 1978). Although the maze was originally designed for rats, many other species have been tested in the maze with varying degrees of success (Hilton & Krebs, 1990; Hughes & Blight, 1999; Mizumori, Rosenzweig, & Kermisch, 1982; Picq, 1993; Roberts & Van Veldhuizen, 1985). A number of testing paradigms have since been used on the maze to differentiate the types of spatial memory. The simplest test of reference memory in the maze consists of a single phase where the positions of rewarded arms remain fixed across sessions (Olton & Papas, 1979). Typically, half of the arms in this procedure are rewarded, with the remaining arms kept as non-rewarded throughout testing. Over time, rats learn to avoid non-rewarded arms and only visit the fixed rewarded arms. Measurements of reference memory in this type of procedure also include the number of errors or the total number of arm entries prior to finding all of the food rewards. Alternatively, percent accuracy is defined as the percentage of correct choices within the first *n* choices, where *n* equals the total number of rewarded arms.

Another popular test is the two-phase working memory test (Beatty & Shavalia, 1980; Maki et al., 1979; Olton & Schlosberg, 1978; Zoladek & Roberts, 1978), which was developed as an alternative working memory paradigm to the original one-phase working memory test in Olton and Samuelson (1976). In the two-phase working memory test, a predetermined delay or retention interval (RI) is typically inserted after the rat has entered half of the arms on the maze. This interruption forms two phases in the test, an initial study phase and a following test phase. Which arms are entered during the study phase may be determined by the rat (i.e., free choice) or by the experimenter (i.e., forced choice), chosen either randomly or arbitrarily. Following the RI, the rat is placed back on to the center of the maze for a test phase, in which the rat searches for food rewards in the remaining unvisited arms. Measurements of working memory accuracy are similar to measurements of reference memory accuracy, including the number of errors / re-entries and the total number of arm entries prior to finding all of the food rewards. Conversely, accuracy is calculated as the percentage of correct choices within the first $\frac{n}{2}$ choices in the test phase, where *n* equals the total number of arms. Generally, rats take much longer to achieve high accuracy in these reference memory paradigms than in working memory paradigms due to their natural tendency to seek out novelty and explore previously unvisited locations (Olton & Samuelson, 1976; Olton & Schlosberg, 1978). Rats can achieve high accuracy in working memory tasks in very few sessions, whereas reference memory tasks take upwards of 20 sessions to reach the same level of accuracy (Guitar & Roberts, 2015; Roberts et al., 2016). There are a variety of other procedures in the radial maze that address more complex learning and memory concepts such as episodic-like memory (Babb & Crystal, 2005, 2006), source memory (Crystal, Alford, Zhou, & Hohnmann, 2013), retroactive interference (Maki et al., 1979) and proactive interference (Hoffman & Maki, 1986; Roberts, MacDonald, Brown, & Macpherson, 2017). The radial maze has also seen use in studies on the behavioural effects of pharmacological substances (Burešová & Bureš, 1982; Levin, 1988; Olton, 1987). In the interest of this thesis, I will review literature that examines what factors influence the cognitive performance of rats in the radial maze.

Suzuki et al. (1980) elegantly demonstrated which cues rats were primarily attuned to in the radial maze. In a series of experiments, explicit extramaze cues (i.e., cues that lie beyond the boundaries of the maze arms) were assigned to each arm in an eight-arm radial maze. Suzuki et al. (1980) were firstly interested in whether rats used extramaze cues at all, and if they did, whether they encoded extramaze cues separately or as a part of a larger configuration. Their first experiment demonstrated that rats performed better when these specific extramaze cues were present as opposed to when they were absent. Secondly, performance in two-phase working memory task was highly disrupted when extramaze cues were randomly transposed in between the study and test phase. This performance decrement was observed only after extramaze cues were transposed, not rotated 180°. If rats independently associated extramaze cues to single maze arms, then random transposition of cues should not have affected performance. Instead, performance was disrupted in this condition and only minimally disrupted when cues were rotated 180° together. This implies that, although extramaze cues were still displaced from their original position, rats were still able to use their relative configuration to guide choices in the test phase. Furthermore, Suzuki et al. suggested that it is unlikely that extramaze cues are processed like items in a list. Rather, they may be processed like components of a cognitive spatial map.

How these components might be coded into memory was explored in a series of experiments by Cook, Brown and Riley (1985). When the experimenter introduces a delay in the middle of a radial maze test, the task imposes a cognitive memory load on to the animal. This memory load contains information pertaining to the spatial locations in the maze, but which locations are retained and how are they represented in memory? Cook et al. (1985) hypothesized that rats code into working memory the spatial information associated with either previously visited arms, to-be-visited arms or both during the two-phase working memory task. In the case of the first hypothesis, rats use retrospective memory of past experiences to avoid previously visited locations. Rats encode visited locations during the study phase, which accumulate to form a higher memory load as the rat visits more unique locations in the maze. The second hypothesis posits that rats use prospective memory for anticipated events to guide their choices to previously unvisited locations. Here, rats encode to-be-visited locations during the study phase, which dissipate and relieve the rat from a high memory load as it visits more unique locations in the maze. Lastly, the third hypothesis states that rats flexibly use both types of memory coding to find food rewards on the maze. Under this hypothesis, rats switch between using retrospectively and prospectively coded representations as the test progresses. In order to actually test these hypotheses, rats were subjected to a two-phase working memory task with the RI inserted after different numbers of study phase choices were made. That is, the point at which the RI was inserted varied across conditions. On a 12-arm maze, rats were allowed to make 2, 4, 6, 8, 10 choices before being removed for a 15 min RI and then allowed to complete the remaining choices. On control trials, testing proceeded as a one-phase working memory task, where rats were allowed to search for all 12 food rewards without an interpolated RI. Performance in their task was measured as the differential probability of errors from control trials (see Cook et al. 1985; Experiment 1 for calculations). As one would expect, rats made significantly more errors in trials with RIs than in control trials. The differential probability of errors from controls was calculated for each level of the point of interpolation (POI; i.e., the number of choices that were made before the RI was inserted) manipulation. If rats were using retrospective coding exclusively, errors would peak as the memory load maximizes at later POIs. If rats were using prospective coding exclusively, errors would peak at earlier POIs and wane at later POIs, where memory load would be maximized and minimized, respectively. The data instead revealed that the POI had a significant quadratic trend on errors relative to control trials. Specifically, rats showed an inverted-U trend in errors that peaked at the POI = 6 condition. This observation suggests that rats take on a minimized memory load throughout the task by using retrospective memory earlier in the test and switching to prospective memory later in the test. At the midpoint of the test (i.e., POI = 6), the differential probability of errors is maximized because both retrospective coding and prospective coding carry equally high memory loads. These data support the dual-code hypothesis of memory coding, which posits that rats flexibly switch between retrospectively and prospectively coded representations of locations in order to minimize memory load and achieve high accuracy in the radial maze.

1.3 Rat Behaviour in the Radial Arm Maze

Despite the fact that the radial maze has been used to address questions about memory, there was (and still is) interest in how animals behave in the maze. Initially, Olton and Samuelson (1976) were interested in whether their apparatus was truly a test of memory. It is possible that rats could use odour cues, instead of memory, to guide their choices in the maze. Researchers have used various odour controls in radial maze testing and have all found that odour cues are not a primary cue for rats in this task (Burešová & Bureš, 1981; Einon, 1980; Maki et al., 1979; Olton & Collison, 1979; Olton & Samuelson, 1976; Zoladek & Roberts, 1978). That being said, researchers routinely implement odour controls in their experiments in order to prevent odour cue usage (Einon, 1980).

Another concern when using the radial arm maze is the use of response patterning or response strategies. It is possible that instead of using spatial memory, rats opt to choose arms according to a pattern of responses or a simple rule of responding. For example, in the original one-phase working memory test, a rat could enter the first arm at random and, upon exiting that arm, turn the next arm to the right (Olton and Samuelson, 1976). Then by continually choosing consecutive arms in a counterclockwise fashion, the rat could achieve perfect accuracy in the task without explicitly using memory to retrieve food rewards (Eckerman, Gordon, Edwards, MacPhail, & Gage, 1980; Watts et al., 1981). This potential confound has been addressed across a number of studies, with most indicating that intramaze cues and response strategies are not primary strategies for rats (Babb & Crystal, 2003; Olton & Collison, 1979; Olton & Samuelson, 1976; Olton & Schlosberg, 1978). Alternatively, other studies have suggested that juvenile rats are more likely to adopt response strategies than adults (Einon, 1980; Hall & Berman, 1995). It has been suggested that juvenile rats may opt to use these strategies because their hippocampus and visual system are not fully developed, whereas they are in adults (Hall & Berman, 1995). Additionally, Einon (1980) found that rats were more likely to adopt the adjacent response strategy if male and socially reared. These findings belong to a body of literature containing inconsistent findings about response strategies in the radial maze (Brown, 1990; Dale & Roberts, 1986; Dallal & Meck, 1990; Foreman, 1985; Roberts & Dale, 1981; Yoerg & Kamil, 1982). Generally, response strategies are regarded as non-essential for high accuracy in forced-choice radial maze tasks (Maki, Beatty, Hoffman, Bierley, & Clouse, 1984; Olton & Werz, 1978). In forced-choice tasks, rats are typically given randomly chosen sets of arms between the study and test phase. In these randomly-generated sets, the adoption of a response strategy would not be effective considering that only half of the arms would contain food rewards. If, however, arms were arranged in specific spatial patterns, response strategies may be advantageous.

1.4 Spatial Patterns and Configurations

Given that performance in the radial maze has been shown to be reliant on the cognitive mapping of spatial locations by extramaze cues (Mazmanian & Roberts, 1983; Suzuki et al., 1980), one might expect that being able to represent multiple locations together as a configured “chunk” would yield certain benefits. Chunking of cognitive map components may relieve higher memory loads and lead to more accurate performance in the maze (Dallal & Meck, 1990; Macuda & Roberts, 1995; Miller, 1956). Grouping together locations by pattern would rely on the spatial relations between arms as a cue to guide choices in the maze. Spatial patterns or spatial configurations have been shown to control choice behaviour in a variety of spatial tasks (Brown & Terrinoni, 1996; Brown, DiGello, Milewski, Wilson, & Kozak, 2000; Brown & Giumetti, 2006; Brown, Zeiler, & John, 2001; Dallal & Meck, 1990; Roberts et al., 2017), but it is unclear if these effects are robust (Hoffman & Maki, 1986; Olthof, Sutton, Slumskie, D’Addetta, & Roberts, 1999; Roberts et al., 2017). Previous work has also demonstrated spatial configurations of intramaze cues controlling choice behaviour in various radial mazes (Cohen & Bussey, 2003; Tremblay & Cohen, 2005; Vollmer-Conna & Lemon, 1998). Regrouping spatial locations in working memory into larger units would be a compelling observation, but only in the absence of response strategies.

Dallal and Meck (1990; Experiment 3) tested rats in a 12-arm maze task where the same four arms were never baited and the remaining eight were always baited. This task requires the rat to maintain a reference memory of the never-baited arms in order to avoid them across sessions. Conversely, working memory is also required in order to encode and retrieve representations of visited and unvisited rewarded locations within sessions. Two control groups were formed. One control group experienced random configurations of baited and unbaited arms across sessions and the other control group experienced a standard one-phase working memory test with all arms baited. Relative to the control groups, rats in the chunking group, where the configurations of baited and unbaited arms were consistent across sessions, were significantly less likely to exhibit response strategies than rats in the control groups. As one might expect, rats in the chunking group were significantly more likely to enter all of the baited arms prior to entering the unbaited arms. They were also significantly less likely to alternate between baited and unbaited arm choices than those in control groups. These results imply that rats maintained a reference memory for unbaited arms and that those arms were avoided during searches for food rewards. This observation alone does not convey whether rats were chunking multiple locations into larger units. The compelling observation was that rats in the chunking group, upon incorrectly entering an unbaited arm, were significantly more likely to enter additional unbaited arms within the next three choices. This finding implies that rats were forming chunks of baited and unbaited arms and tended to enter sequences of arms based on these chunks. Additionally, when tested in a brand new testing environment, the chunking group that did not receive a reversal in the configuration of baited and unbaited arms did not experience a significant performance decrement. That is, even though they were tested in a substantially different context, their performance on their assigned configurations was preserved. Taken together, these results suggest that rats were able to successfully use a chunking strategy on the radial maze and in the absence of response strategies.

As a direct counter to Dallal & Meck (1990), a series of experiments by Olthof et al. (1999) found no evidence for the use of abstract patterns or configurations on the radial maze. In a task similar to Dallal and Meck (Experiment 3), each rat was assigned a configuration of arms, where the same six arms were always baited and the opposite six arms were never baited. After sufficient training on these configurations, rats experienced a reversal where their respective configurations were rotated or randomly reassigned. If rats were able to form a representation of the chunked pattern of arms, then rats that had their configurations rotated should transfer to the new task with greater accuracy than if their configurations were randomly reassigned. Regardless of the degree of rotation, rats were unable to show differential rates of transfer between the two tasks (Olthof et al., 1999; Experiment 1; Experiment 2). This indicated that rats had not learned to chunk multiple locations together when those locations were in a pattern. Over the course of another three experiments, Olthof et al. continually attempted to replicate Dallal and Meck's findings but with no success. Despite their attempts to replicate the exact experiments in Dallal and Meck (1990), they found no evidence that rats were able to learn patterns in the maze.

A finding from Dallal and Meck (1990; Experiment 2) highlighted how rats trained in a chunking condition were generally more resistant to proactive interference than control groups. Generally, proactive interference is the tendency of earlier memories to interfere with the formation and retrieval of more recent or current memories. Roberts and Dale (1981) demonstrated notable proactive interference effects after repeated daily sessions in the maze, but Hoffman and Maki (1986) took particular interest in attempting to protect working memory from proactive interference. One of their notable manipulations is the use of spatial patterns (Experiment 1) during radial maze testing. Hoffman and Maki integrated spatial patterns into two-phase control trials as well as experimental proactive interference trials, which used a three-phase procedure. These patterns on the 8-arm maze were either a “cross” pattern or a “side” pattern. The cross pattern included four arms that were separated 90° from one another (e.g. 1 3 5 7 or 2 4 6 8), whereas the side pattern included four arms that were all next to one another (e.g. 4 5 6 7 or 1 2 3 8). Spatial patterns in this experiment were unsuccessful in protecting working memory from proactive interference and also failed to produce substantial differences in two-phase control trials. In a very similar experiment, Roberts et al. (2017) used the same spatial patterns in an attempt to protect working memory from proactive interference. The “Alternate” and “Adjacent” patterns in Roberts et al. are equivalent to Hoffman and Maki’s cross and side patterns, respectively. The only major difference between this study and Hoffman and Maki was the use of a 5 min RI instead of a 2 hr RI. Although, the spatial patterns also failed to protect working memory from proactive interference in this study, surprising differences between spatial patterns appeared in the two-phase control trials. Performance accuracy was highest in the Control Alternate condition (98.96%), followed by the Control Random condition (89.58%) and the Control Adjacent condition (75%). Even with a short RI, spatial patterns were shown to produce large differences in working memory accuracy.

Brown and Giumetti (2006) employed a forced-choice two-phase working memory test that also involved a spatial pattern manipulation of radial maze arms. The experiment involved a between-subjects design where groups of rats experienced a no-pattern (i.e., random) or alternation pattern on an eight-arm radial maze. This alternation pattern was identical to the cross pattern in Hoffman and Maki (1986) and the Alternate pattern in

Roberts et al. (2017). Rats received 20 training sessions with either pattern but in a free-choice one-phase working memory test. Rats were lowered into an open maze with four arms baited and four arms unbaited according to the pattern they were assigned. After training, rats received 15 sessions of forced-choice tests, where rats were forced into two of the baited arms while all other arms were blocked. After retrieving food rewards from those arms, they were removed for a 1 min RI. After the RI elapsed, the rat was returned to the maze to find the remaining two baited arms or until 4 min had elapsed. Rats in the alternation pattern group achieved significantly lower mean serial position scores for baited arms compared to unbaited arms. That is, rats were more likely to visit baited arms earlier in the test phase than they were to visit unbaited arms. Additionally, rats chose baited arms as their first choice significantly more often than expected by chance and significantly more often than choosing an unbaited arm as their first choice. Although Brown and Giumetti did not present explicit accuracy data for the two unvisited baited arms, they concluded that choice behaviour in the alternation group was controlled by pattern.

There have been other instances in which spatial patterns have been noted to control choice behaviour in rats. In the pole box task, spatial configuration has been shown to control choices (Brown & Terrinoni, 1996). In this task, rats are trained to stand upright in order to investigate the contents of a reward well located at the top of a pole. These poles are arranged in a matrix, in which only a subset of poles are baited. In Brown and Terrinoni’s experiments, the positions of baited poles were configured in a square or in a line (row or column). These configurations were randomly positioned about the pole box so that their position on any given trial was unpredictable. This, in addition to the fact that the pole box was randomly rotated to prevent use of extra-apparatus cues, meant that it would be very difficult for the rats to develop a robust strategy to find the rewards. Brown and Terrinoni also took precautions to prevent the rats from finding the food rewards through visual or olfactory cues. Firstly, the heights of the poles were calibrated so that rats could not see into the reward well and would only be able to choose poles by retrieving the reward with their tongues. Secondly, the procedure also included a series of probe trials where some of the possibly baited locations within the square configuration were not actually baited during the test. Their analysis of responses in these probe trials revealed that rats did not respond differently when approaching possibly baited poles that were, in fact, unbaited. Under these conditions, rats were able to reliably choose poles in the square and line patterns significantly more often than was predicted by chance. The replication of results using the line pattern in addition to the square pattern revealed that rats were not attuned to using the spatial averages of previously rewarded locations to guide subsequent choices (Cheng, 1989). Taken together, these results suggest that rats learned to respond to pole choices on the basis of geometric pattern. This conclusion was corroborated by findings from Brown et al. (2000). In this pole box experiment, one group of rats was trained to retrieve food rewards in a square configuration when the reward was sucrose pellets and in a line configuration when the reward was sunflower seeds. The other group received the same treatments but the pairings of reward and configuration were reversed. Rats in this experiment replicated the findings of Brown and Terrinoni by showing control of choice by pattern, but with the additional finding that control by pattern was conditionally determined by reward type.

1.5 Objectives

The three experiments presented in this thesis expand upon previous research by addressing the role of spatial configurations in working memory for multiple locations. In Experiment 1, I asked whether working memory for spatial locations in the radial maze would be enhanced by spatial configuration effects. The experiment specifically investigated the effect of spatial patterns on working memory over various RIs. I hypothesized that rats would show differential performance across spatial patterns and that this differential performance would vary across levels of RI. In Experiment 2, I asked whether the performance differences across spatial patterns observed in Experiment 1 were due to the inherent number of spatially isolated arms within each pattern. Experiment 2 expanded upon the work in Experiment 1 by controlling for the number and position of spatially isolated arms across subjects and sessions. I hypothesized that spatial working memory accuracy would vary with the number of spatially isolated arms in the patterns and that performance would vary differentially across different levels of RI. Experiment 3 addressed whether motivational factors were responsible for the enhanced working memory for spatially isolated locations. Were rats more accurate on spatially isolated arm locations simply because they were always found in between two previously rewarded locations? I hypothesized that accuracy for isolated locations was modulated by motivational factors. In these three experiments, I addressed how multiple locations are encoded, maintained and retrieved in spatial working memory.

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Chapter 2

2 Experiment 1

2.1 Introduction

The current experiment investigated whether rat spatial working memory for multiple locations is enhanced when locations are arranged in a pattern. Similar to Hoffman and Maki (1986) and Roberts et al. (2017), rats were trained and tested in a radial maze where sets of arms were positioned into specific patterns. The "cross" and "side" patterns from Hoffman and Maki and "Alternate" and "Adjacent" patterns from Roberts et al. (which were equivalent, respectively) were used in this experiment. This first experiment formed the basis of the experiments to follow, as I expected to observe differences in how rats accessed memories for multiple locations. If rats were not using response strategies or algorithmic responding, spatial working memory was expected to vary with arm pattern and also vary differentially across increasing levels of RI. I predicted rats to perform more accurately on the Alternating pattern than the Random or Adjacent pattern, as previously observed with 5 min (Roberts et al., 2017) and 2 hour RIs (Hoffman & Maki, 1986).

2.2 Methods

2.2.1 Subjects

Eight adult male Long-Evans rats (*Rattus norvegicus*) were used. These subjects had previous experience in a win-shift radial maze task and were highly habituated to the maze at the beginning of the experiment. Rats were allowed to eat Pro Lab Rat Chow *ad libitum* for 2 weeks before being reduced to 85% of their respective free-feeding weights (*M* = 335g). They were pair-housed in polypropylene cages, given water *ad libitum*, and kept in a home room maintained at 22°C with a 12:12 h light-dark cycle (7 am onset, 7 pm offset). All testing occurred between 9 a.m. and 5 p.m. five days per week. Rats were fed in accordance with their individual weight reduction and were always fed after testing sessions were completed.

2.2.2 Materials

The eight-arm radial maze (see Figure 1) was made of 2.5 cm plywood and was painted grey. The central platform of the maze was an octagon with a diameter of 30.5 cm. Each of the eight arms extending from the central platform measured 91.5 cm in length and 7.5 cm wide, and arms were equidistant from one another. The maze was elevated 61 cm from the floor by wooden legs. White plastic food cups that were 3.5 cm in diameter and 1.5 cm deep were attached to the ends of each arm by small squares of Velcro. A piece of white foam board (approximately 23 cm by 21.5 cm) was stapled to the right side of each arm close to the central platform. This barrier prevented rats from jumping between arms and forced rats to fully exit one arm before entering another. Two types of wooden barriers were used in this experiment. Large grey wooden barriers (15 cm high, 7.5 cm wide) with threaded bolts on the bottom could be inserted into holes drilled at the start of each arm to prevent entry into arms during the study phase. Small grey barriers (7.5 cm high, 7.5 cm wide) with wooden dowels attached to the bottom could be inserted in the same holes during either phase. These barriers were placed at the start of open arms and could be climbed over by rats. These small barriers were used in order to reduce the frequency of ballistic choices (i.e., impulsive arm choices made at the beginning of either phase) by making arm choices more energetically costly to the rat. All food rewards in the experiment were 45 mg Noyes Precision sucrose pellets (PJAI-0045, Research Diets Inc., New Brunswick, NJ, USA). The radial maze was centered in a 3.8 m × 3.8 m testing room. A single 60W incandescent light bulb was used to create a dimly lit testing environment and a white noise generator was used to prevent extraneous noise from affecting performance. Although rats have been shown to not use odour cues in the radial maze (Olton & Collison, 1979), I attempted to control for odour cues potentially produced by the sucrose pellet rewards. Plastic bags with 8 sucrose pellets each were taped to the wooden legs under the ends of the arms. Holes were poked into the bags so that odour from the sucrose pellets would diffuse throughout the testing room. The room contained several extramaze cues including a table, a stool, an upright double T-maze, a stack of plywood from an old radial maze, two transport carts, and the experimenter’s chair.

2.2.3 Procedure

Habituation. Rats were habituated to testing conditions over a period of 4 weeks through daily 15 minute sessions of handling and exploratory time on the maze. Sucrose pellets were placed in each of the food cups and were spread across the floor of the maze to encourage rats to travel down the arms. After rats were comfortably navigating the maze and no longer showing neophobia to the testing environment or food rewards, they were transferred to a set of training trials.

Training. The rats were initially trained on a two-phase working memory tasks with randomized arm positions and a 5 min RI (see Figure 2). At the beginning of each trial, every arm in the maze was baited with a single sucrose pellet. In the study phase, four randomly chosen arms were blocked by the large barriers while the remaining four arms had the small barriers at the start of the arm. The rat was allowed to navigate the maze to find the food rewards in the open arms or until 5 min had elapsed. The rat was then removed for a 5 min RI, which was spent in their home cage. During the RI, all arms were opened by replacing large barriers with small barriers. After the RI elapsed, the rat was placed back onto the central platform of the maze to search for the remaining food rewards in the originally blocked arms. Spatial working memory accuracy in this task was measured as the percentage of correct arm entries made in the first four choices. What arms were visited, the order they were visited in and the total number of arm visits were also recorded. After two trials of training, rats were sufficiently experienced with the two-phase procedure to move on to testing.

Testing. After completing the training trials, experimental testing commenced. The procedure for the experimental testing was also a two-phase working memory task. Three arm patterns were tested: Random, Adjacent and Alternating (see Figure 3). In the Random patterns, the spatial positions of the four study phase arms were determined by a random number generator (Urbaniak & Plous, 2013). Due to the nature of the Alternating pattern, there were only two possible configurations available for testing in my eight-arm radial maze (1 3 5 7 and 2 4 6 8). In order to compensate for the small number of

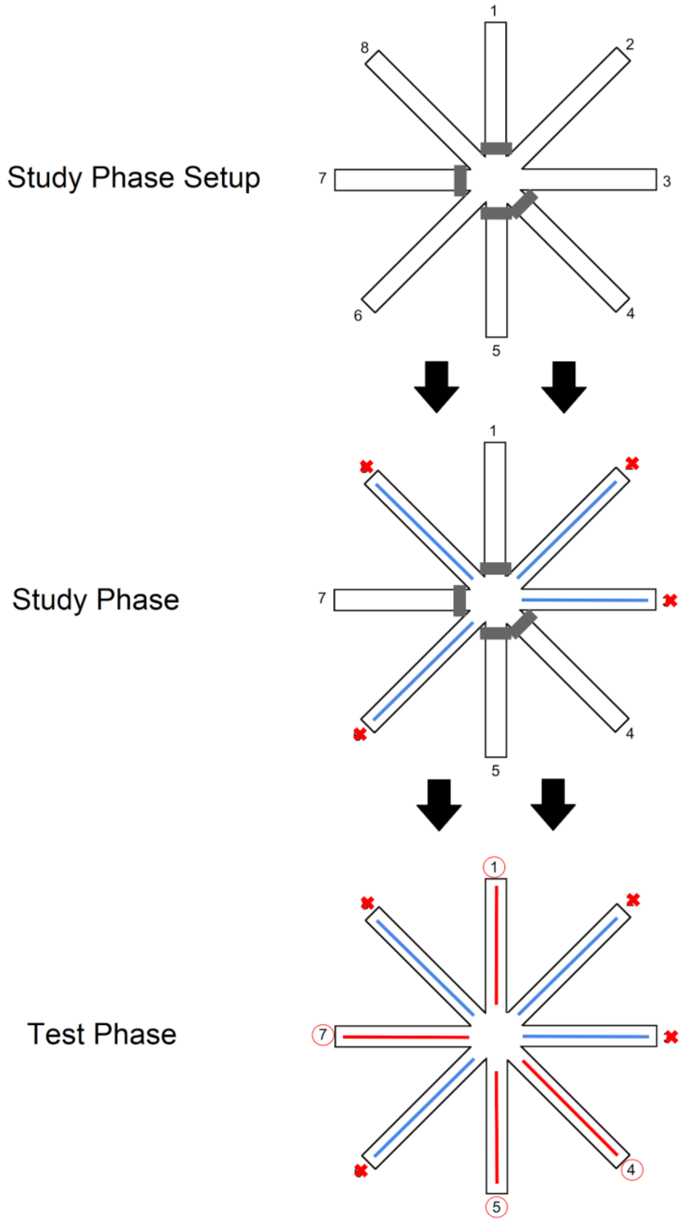

Figure 2. Two-phase spatial working memory test with a random pattern. In the study phase, half of the arms in the maze were blocked. Rats were forced into entering open arms (marked with blue lines) in order to retrieve food rewards. After entering all of the open arms, the rat was removed from the maze for a RI. After the RI elapsed, the rat was lowered back into the maze with all arms open for the test phase, in order to find the remaining food rewards (marked with red circles) in unvisited test phase arms (marked with red lines).

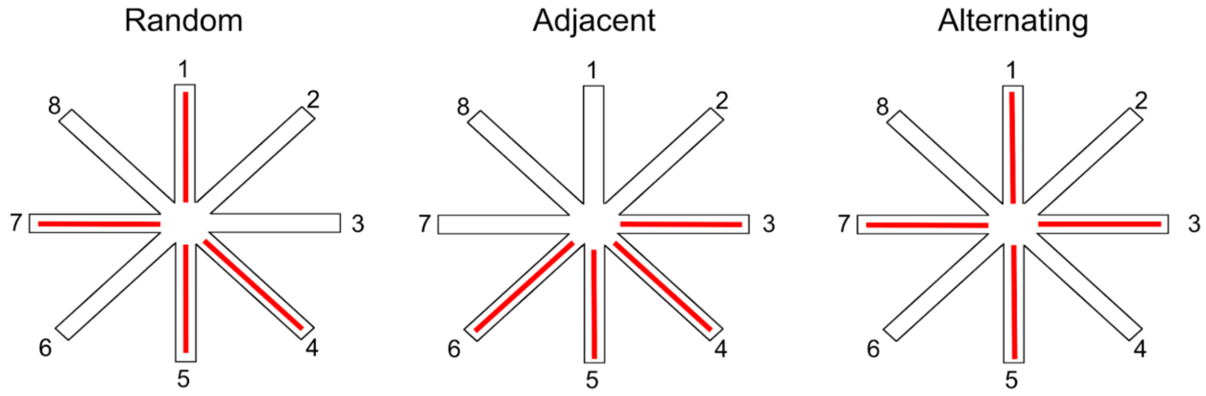

Figure 3. Schematics for example arm patterns in Experiment 1. Positions of test phase arms (marked in red) in the Random pattern were generated by a random number generator. Adjacent patterns featured four study phase arms and four test phase arms that were all next to one another. Alternating patterns featured study and test phase arms that were arranged so that every other arm was another study or test phase arm, resulting in a "plus" or cross shape.

configurations in the Alternating pattern, I arbitrarily chose two specific adjacent patterns from all the possible configurations (3 4 5 6 and 1 2 7 8). This precaution was taken in order to counteract the argument that rats might perform better on the Alternating pattern simply because they only experience two possible configurations. The RI manipulation had four levels: 5 min, 1 hr, 4 hrs, and 24 hrs. Each testing condition was a combination of the three arm patterns and four RIs. The experiment used a within-subjects design so that each rat experienced each of the 12 unique testing conditions twice across the 24 sessions completed. All rats experienced the same conditions in the same order, and one testing session was conducted per day. First, rats were tested in two consecutive sessions for each level of arm pattern, in the order of Random, Adjacent, and Alternating, at the 5 min RI. This same testing order was completed at a 1 hr RI, 4 hr RI, and then finally at a 24 hr RI. The same performance measurements taken in the training phase were also collected during the testing phase of this experiment.

2.3 Results

2.3.1 General Accuracy

Figure 4 illustrates the general accuracy data for each arm pattern across RIs. In the 5 min RI, rats seemed to perform equally well across the three arm patterns (*M* = 98.96%, *SD* = 1.93). They maintained over 90% accuracy in 1 hr RI (*M* = 92.71%, *SD* = 2.95), with the Alternating pattern remaining at 96.88%. As RI increased, the performance differences across patterns maximized at the 4 hr RI. At this RI, performance in the Alternating pattern remained at an average of 95.31%, followed by the Random pattern at 82.81% and the Adjacent pattern at 75% accuracy. Performance differences were still apparent in the 24 hr RI, but were less pronounced. Across all conditions, rats performed well above chance performance (41%; for calculations see Beatty & Shavalia, 1980). Analysis of general accuracy on the task was conducted through a two-way (3 × 4) repeated measures analysis of variance (ANOVA) of arm pattern and RI on spatial work memory accuracy (percentage of correct choices out of the first four choices in the test phase). The analysis revealed a main effect of RI, $F(3, 21) = 69.45$, $p < .001$, $\eta_{\rho}^{2} = .71$, a main effect of arm pattern, $F(2, 14) = 17.43$, $p < .001$, $\eta\rho 2 = .91$, and a significant interaction of arm pattern

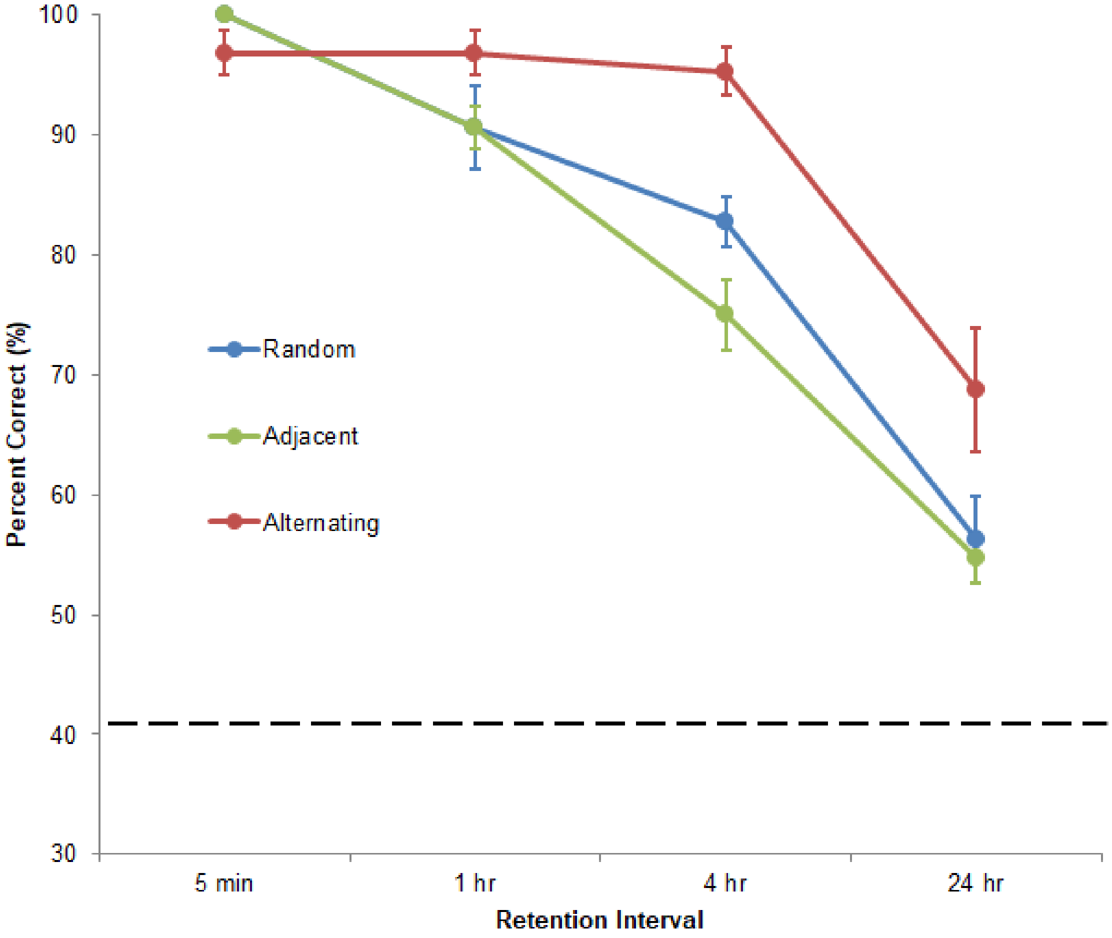

Figure 4. Spatial working memory accuracy in each arm pattern across increasing levels of RI. Spatial working memory was measured as a percentage of correct choices within the first four choices of the test phase. Errors bars represent standard error. Chance accuracy (41%) is denoted by the dotted line.

and RI, $F(2, 17) = 3.48$, $p < .05$, $\eta_\rho^2 = .33$. An analysis of the simple main effects revealed an significant effect of pattern at the 4 hr RI, $F(2, 6) = 17.00$, $p < .01$, $\eta_\rho^2 = .85$, but not at the 24 hr RI, $F(2, 6) = 2.81$, $p = .14$, $\eta_\rho^2 = .48$.

2.3.2 Response Patterning

One concern about rat behaviour in the radial maze was the use of response strategies. Response strategies could undermine the measurement of working memory in a variety of radial maze tasks because they allow high accuracy to be achieved in the task without the use of memory. For example, it is possible for rats in a one-phase working memory test (with all arms baited and open) to visit consecutive arms in a clockwise or counterclockwise fashion in order to retrieve all food rewards in the maze with no errors. Although overt response strategies in the radial maze were historically not a concern (Olton and Samuelson, 1976), it is possible that rats used response strategies in either the Adjacent or Alternating pattern to solve the task with high accuracy.

In the Adjacent pattern, rats could have remembered one of the two outer arms in the pattern and made sharp, consecutive clockwise (e.g. 3→4→5→6) or counterclockwise (e.g. 6→5→4→3) turns in order to find all four test phase arms. Conversely, rats given the Alternating pattern could have remembered one of the four test phase arms and then made 90° clockwise (e.g. 1→3→5→7) or counterclockwise turns (e.g. 7→5→3→1) after exiting each arm. In order to address this concern, the probability of using a response strategy was calculated for each pattern. Assuming no errors, there were 4! (= 24) different orders in which rats could choose the correct test phase arms in either Adjacent or Alternating patterns. If arms were arranged in an Adjacent pattern, they could be retrieved in the order of 6 5 4 3 or 3 4 5 6. These two orders were classed as the adjacent response strategies described earlier. According to chance, these choice orders were expected to be observed in 8.33% of trials (two out of twenty-four possible orders). In the current experiment, the use of either response order was only observed on two separate instances across two rats. A one sample t-test comparing the percent of trials in which these response strategies were observed ($M = 3.13\%$, SD = 5.79) to the chance value of 8.33% resulted in a significant difference, $t(7) = -2.54$, $p < .05$ (two-tailed hypothesis).

Conversely, if the arms were arranged in the Alternating pattern, rats could enter one of the four correct test phase arms during their first choice (one of 1 3 5 7 or 2 4 6 8), followed by a 90° turn clockwise or counterclockwise turn, followed by two additional 90° turns in the same direction. For either alternating configuration, there were two possible orders for each possible first choice test phase arm, meaning that there were a total of eight response orders among the 24 possible orders that were classed as response strategy orders. Therefore, there was a 33.33% probability of observing a rat using one of these response orders by chance alone. In the current experiment, the response orders in question were observed in 16 separate instances across six subjects. A one sample t-test comparing the percent of trials in which these response strategies were observed (*M* = 25.00%, SD = 21.13) to the chance value of 33.33% resulted in no significant difference, *t*(7) = -1.12, *p* = .30 (two-tailed hypothesis).

2.4 Discussion

The analysis of response orders in the Adjacent pattern revealed a significant difference between the observed and chance usage of response orders classed as response strategies. The rats showed significantly less use of response strategy orders than was predicted by chance. Considering that my results were drawn from a sample of 64 trials in total, there could be random variance or sampling errors affecting my observations. Regardless, this significant difference suggests that rats were not using response strategies as a primary strategy to solve the Adjacent pattern. Comparatively, response strategy usage in the Alternating pattern did not differ significantly from chance. Therefore, it is unlikely that rats were using a 90° turning strategy to solve the Alternating pattern. Importantly, these results suggest that the rats were not relying on response strategies to guide their responses in the maze. It can be confidently suggested that rats were in fact using memory to navigate the maze in order to retrieve food rewards.

My analyses of the working memory task revealed that arm pattern significantly affected the variance in general accuracy across conditions. These results counter that of Hoffman and Maki (1986), who found no significant differences in performance across the same patterns at a 2 hr RI. Although rats did not show any performance differences at the 5 min

RI, the current experiment’s results show that rats performed better on the Alternating pattern, followed by the Random and then Adjacent patterns, as in Roberts et al. (2017). An interaction between pattern and RI was also observed, such that the variance produced by arm pattern differed across different levels of RI. The decreased differences between patterns at the 24 hr RI may be attributed to general/overnight forgetting that occurred between testing days in that condition. These results were in line with my hypothesis and predictions of a main effect of arm pattern as well as an interaction of arm pattern and RI on spatial working memory accuracy.

These results did not support the hypothesis that rats “chunk” multiple locations in memory using general spatial cues. If rats were using a chunking strategy, one would expect them to perform better on the Adjacent pattern than on the Alternating pattern. This clearly was not the case in this experiment, as rats performed the worst on the Adjacent pattern at the 4 hr and 24 hr RIs. The rats’ performance on the Alternating pattern suggested that rats encoded the specific spatial cues unique to each study and test phase arm. Although this memory strategy may incur a higher memory load, it may have facilitated the high performance in the Alternating pattern. If rats were in fact encoding spatial cues unique to each arm location, how specific and mutable were their memories for these arm locations? The spatial cues present in the testing room were fairly large objects that were not centered or aligned in reference to any particular arm. The radial maze itself was elevated and had no walls with the exception of the barriers and white boarding. Although rats are not a particularly visual species, the conditions of the testing room allowed for a large scope or range of spatial cues to be encoded from the ends of each arm. It is possible that full or partial spatial cues overlapped between arm locations. If memory for these spatial cues was not specific, then arms that were adjacent to one another may have caused mutual spatial interference with one another due to the overlap in their associated spatial cues. This hypothesis would explain the rats’ high performance on the Alternating pattern, where all of the arms in the configuration were maximally separated in absolute space, and their low performance in the Adjacent pattern, where arms were minimally separated. The rats may have been experiencing high spatial interference in the Adjacent pattern, since the spatial cues of the study and test phase arms were highly overlapping. Conversely, the low amount of overlap between spatial cues in the Alternating pattern would have facilitated low spatial interference and thus better retention of arm locations. Alternatively, these results could be viewed as a spatial analogue to the von Restorff effect (Hunt, 1995; von Restorff, 1933). The von Restorff effect was originally found in human recall tasks, where subjects were asked to memorize items in a list. If one item within the list was incongruous or particularly distinct from the other items, memory for that item would be enhanced. In the context of the current experiment, arms that appear spatially distinct from other arms may be retrieved with higher accuracy. The analogous von Restorff effect would then explain the high accuracy achieved in the Alternating pattern, whose arms were all spatially distinct from one another, and the low accuracy in the Adjacent pattern, whose arms were all spatially proximate to one another. If this were true, then one would also expect that rats perform differentially across individual test phase arms that were separated from or flanked by other test phase arms.

In order to quantify a performance measurement that addressed the differential performance on test phase arms, a post-hoc analysis was devised. Specifically, choice accuracy data from within test phase trials were analyzed. Within any given test phase, rats could encounter isolated or non-isolated test phase arms (see Figure 5). Isolated test phase arms were correct test phase arms that had no other correct test phase arms directly adjacent to them. Non-isolated test phase arms were correct test phase arms that had at least one other correct test phase arm directly adjacent to them. I was interested in whether rats would perform better on isolated test phase arms than on non-isolated test phase arms when both arm types were being held in working memory. Since the Adjacent and Alternating patterns only contained non-isolated and isolated test phase arms, respectively, the data in this post-hoc analysis were drawn exclusively from the Random pattern data. Accuracy for isolated arms was calculated by dividing the total number of correct isolated arm choices within the first four choices of a test phase trial by the number of opportunities for isolated arm choices in that test phase trial. Accuracy for non-isolated arm choices was calculated similarly. These accuracy data are illustrated in Figure 6. At the 5 min RI, rats showed equal accuracy on isolated and non-isolated test

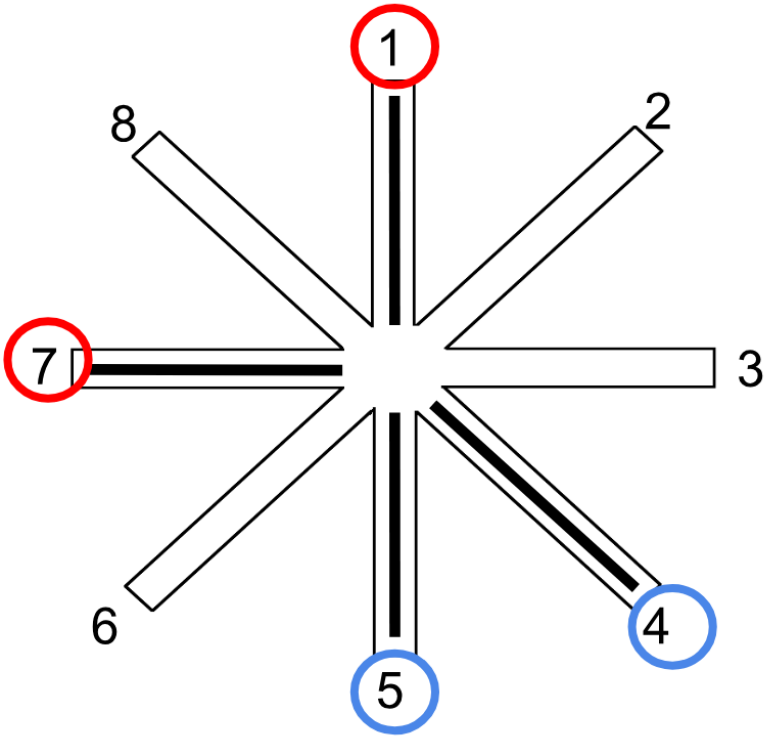

Figure 5. Example schematic of differentially isolated test phase arms. Arms 1 and 7 are defined as “isolated” (i.e., both arms have no other correct test phase arm directly adjacent to them) and arms 4 and 5 are defined as “non-isolated” (i.e., both arms have at least one other correct test phase arm directly adjacent to them).

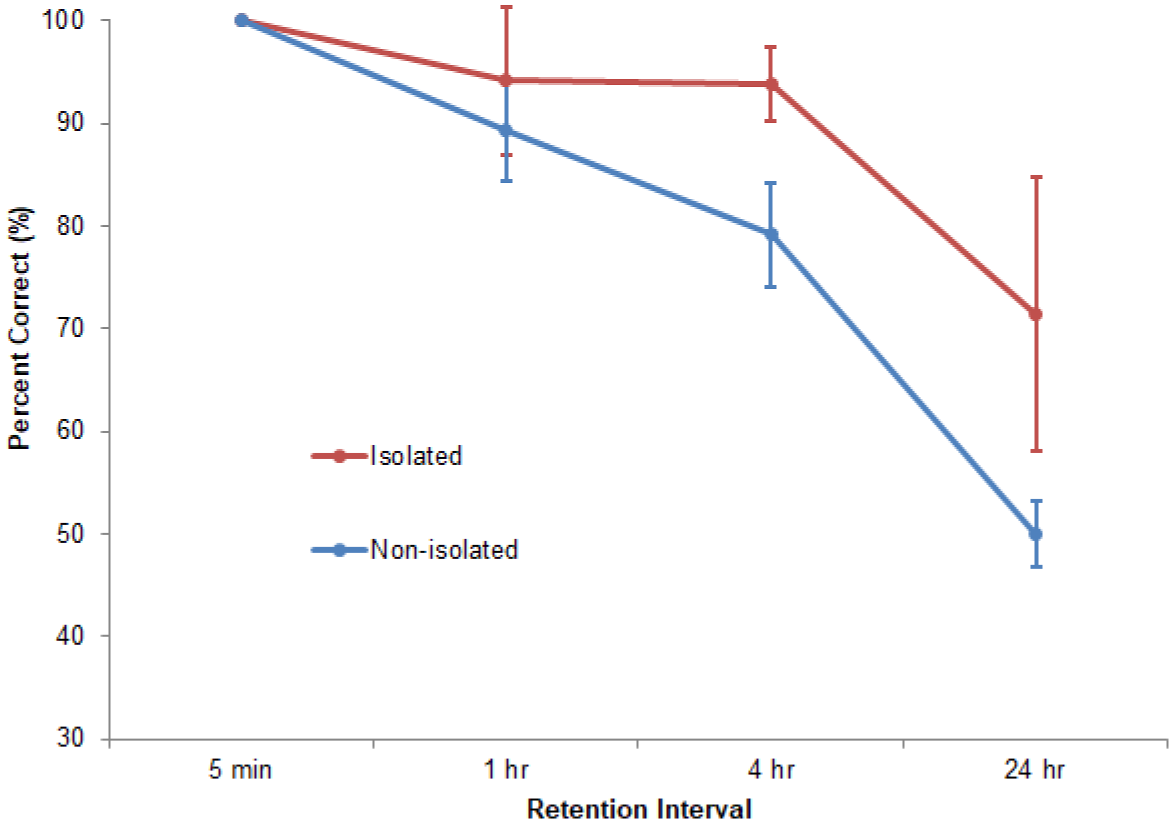

Figure 6. Within-trial spatial working memory for isolated and non-isolated test phase arms across increasing levels of RI. Percent accuracy was calculated as the number of correct choices for isolated/non-isolated arms within the first four choices of the test phase over the number of opportunities for isolated/non-isolated arm choices in the test phase. Error bars represent standard error.

phase arms. Differences in accuracy began to show at the 1 hr RI but were maximized as the RI is increased to 24 hrs, with isolated arm choices remaining at 71.43% accuracy and non-isolated arm choices declining to 50% accuracy. Generally, the data suggests that rats performed better on isolated arms than non-isolated arms in the test phase. That being said, no statistical analyses were run on these data because the number and positions of spatially isolated arms in the Random pattern varied across rats, RIs and sessions. The number of opportunities to visit isolated test phase arms varied between zero and four across rats and different levels of RI. The number of opportunities to visit non-isolated test phase arms varied between four and eight opportunities across rats and different levels of RI. This variation was due to the random generation of study and test phase arms before a main effect of arm pattern and interaction of arm pattern and RI were observed. In order to conduct an appropriate statistical analysis of these data, the number and position of isolated and non-isolated arms should be controlled.

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Chapter 3

3 Experiment 2

3.1 Introduction

In Experiment 2, I attempted to control for the number and position of isolated test phase arms in order to analyze the potential isolation effect noted in Experiment 1. A similar testing procedure, that included a new set of pseudo-random patterns, was used. These pseudo-random patterns were designed to vary the degree of spatial isolation of arms on the maze. The results of this experiment were suitable for statistical analyses, unlike the data in Experiment 1 (see Figure 6). I assessed the hypothesis that spatial working memory for multiple locations varies with the spatial isolation of the locations in reference to one another. I predicted that rats would perform with higher accuracy on isolated test phase arms than non-isolated test phase arms in any given test phase trial. It was also expected that the effect of RI on spatial working memory accuracy would be replicated and that an interaction of arm isolation and RI on spatial working memory accuracy would be observed.

Were the performance differences across pattern in Experiment 1 due to the number of isolated arms within the patterns? In order to address this question, the number of isolated test phase arms was incrementally increased across the pseudo-random patterns in this experiment. If rats remember isolated test phase arms better than non-isolated test phase arms, then general spatial working memory accuracy will vary with the number of isolated arms in a pattern. Increasing the number of isolated arms in a pseudo-random pattern was expected to result in increased general accuracy in this task. I also predicted that the effect of RI on spatial working memory would be observed in the general accuracy data.

3.2 Methods

3.2.1 Subjects

A new set of ten male Long-Evans rats were used in this experiment. Because rats were still juvenile (approximately 100 days old) upon their arrival in the lab, they were given 6 weeks to gain weight and reach maturity before testing began. Rats were allowed to eat Pro Lab Rat Chow *ad libitum* for 2 weeks before being reduced to 85% of their respective free-feeding weights (M = 377.8g). Housing and feeding conditions were identical to those in Experiment 1. All testing occurred between 9 a.m. and 5 p.m. five days per week.

3.2.2 Materials

The experiment was conducted under the same conditions as Experiment 1, except that a 12-arm radial maze was used instead of an eight-arm radial maze (see Figure 7). This new maze was made of 2.5 cm plywood and was painted grey. The central platform was now in the shape of an equilateral dodecagon. Each of the twelve arms extending from the central platform measured 91.5 cm in length, 7.5 cm wide and were equidistant from one another. The maze was elevated 61 cm from the floor by wooden legs. The white plastic food cups, white foam boards, scent bags, and both types of wooden barriers were reused on this new maze. The same sucrose pellets from Experiment 1 were used as food rewards.

3.2.3 Procedure

Habituation. Four weeks after arriving in the lab, rats were habituated to the maze through daily 15 min sessions on the maze. Sucrose pellets were placed in each of the 12 food cups and were spread across the floor of the maze to encourage exploration. These sessions were completed in full lighting and with no active noise generator. During this time, rats were also handled for 15 min before or after their time on the maze in order to habituate them to the experimenter and reduce handling stress. After completing 16 sessions, rats were navigating the maze at a sufficient pace and depleting food rewards from all 12 arms on the maze. Rats were immediately transferred to the training procedure.

Training. In seven training sessions, rats were tested in a two-phase working memory task with the same procedure as used in Experiment 1. Arm positions were randomized through a random number generator (Urbaniak & Plous, 2013) and a 5 min

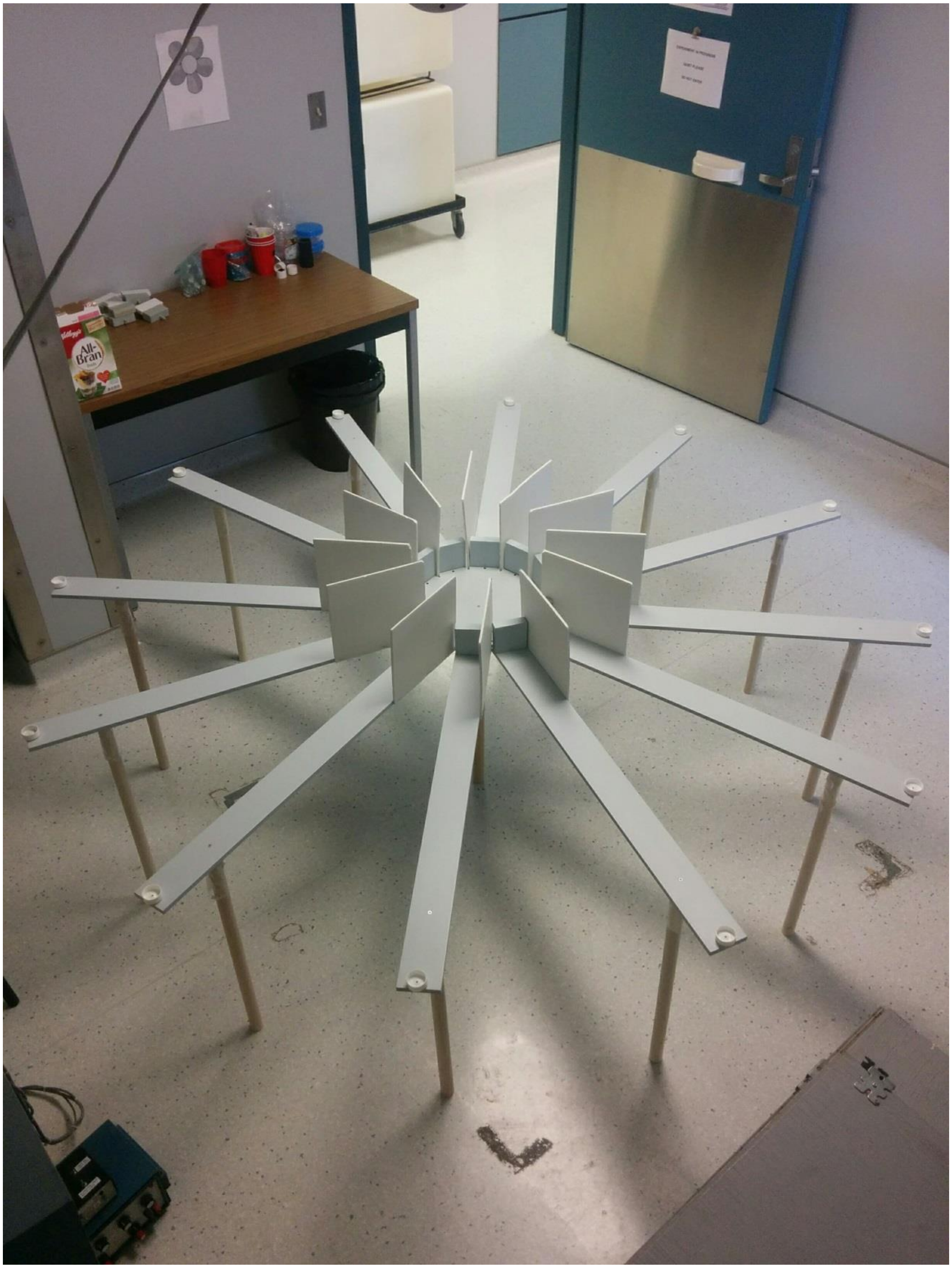

Figure 7. The 12-arm maze used in Experiment 2 and Experiment 3. Short barriers are pictured at the entrance of each arm.

RI was used between the study and test phase. At this time, the noise generator and dim lighting were introduced. Spatial working memory accuracy was measured as the percentage of correct choices made within the first six choices in the test phase. Additional data including the order of arm entries and the total number of arm entries were recorded. In the last training session, rats achieved an average of 85% accuracy and required 7.1 mean arm entries to collect all six rewards. At this point, rats had clearly habituated to and became proficient at the two-phase working memory task. Experimental testing proceeded immediately after.

Testing. During experimental testing, RI and the position of test phase arms were manipulated in a two-phase working memory task. The RI manipulation included two levels: 5 min and 4 hrs. These RIs were chosen because, in the previous experiment, rats showed minimum and maximum differences in performance accuracy at 5 min and 4 hr RIs, respectively. The positions of test phase arms were manipulated through pseudo-random patterns. This manipulation included six unique patterns in which the number of isolated arms varied (see Figure 8). The 0-isolated and 6-isolated arm patterns were analogous to the Adjacent and Alternating patterns from Experiment 1. Since the 6-isolated arm pattern only had two possible configurations, only two configurations were used for the 0-isolated pattern. The other patterns contained 1, 2, 3 and 4 isolated arms (note: it is impossible to create a 5-isolated arm pattern with six arms in this maze). Each of these pseudo-random patterns was designed so that isolated arms were positioned opposite to non-isolated arms. Additionally, 1-isolated, 2-isolated, 3-isolated, and 4-isolated patterns were allowed to randomly rotate across sessions in order to control for position and side preferences in the maze. By combining the two levels of the RI manipulation and the six pseudo-random patterns, 12 unique testing conditions were created. Experimental testing involved a within-subjects design, where each rat experienced each of the 12 unique testing conditions. A Latin square randomization was used in order to counterbalance the testing order of the pseudo-random patterns, so that any order effects across pseudo-random patterns were controlled. Rats were assigned randomly to one of six Latin square orders. Each rat completed their assigned Latin square order at the 5 min RI and then again at the 4 hr RI. In order to control for order effects, a second round of testing was completed in the reverse order. The assigned Latin

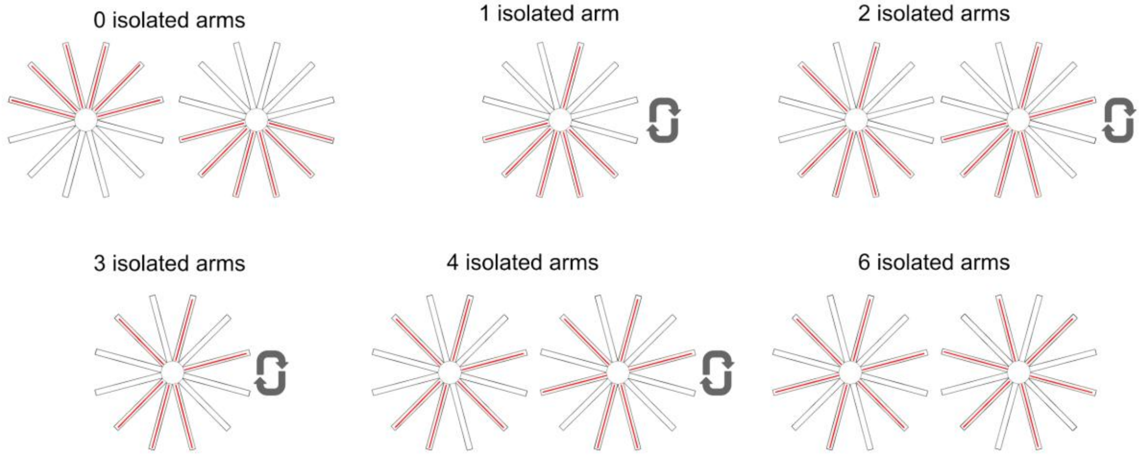

Figure 8. Examples of pseudo-random arm patterns in Experiment 2. In order to maximize the isolation of the isolated test phase arms, isolated arms were placed opposite to grouped non-isolated test phase arms. Additionally, the number of isolated test phase arms was incrementally increased from zero to six across patterns. The rotation symbols denote the patterns that were randomly rotated to prevent side or position preferences.

square orders were reversed and completed at the 4 hr RI, followed by the 5 min RI. Therefore, each rat experienced each combination of pseudo-random pattern and RI twice over a total of 24 experimental testing sessions. Spatial working memory accuracy was measured as the percentage of correct choices within the first six arm choices. Order of arm entries and the total number of arm entries were also recorded.

3.3 Results

3.3.1 Response Patterning

Just as in Experiment 1, it was possible to solve some of the pseudo-random patterns through response strategies. The 0-isolated pattern could be solved through retrieving food from the outer arms in the pattern before making consecutive clockwise (e.g. 10→11→12→1→2→3) or counter-clockwise (e.g. 3→2→1→12→11→10) choices. There were two orders that were classed as response strategies out of the 720 (6!) possible orders in which rats could choose the correct test phase arms. Therefore, these response strategy orders should be observed in 0.56% of trials by chance alone. These response strategies were not observed in any of the sessions across the 10 rats. Therefore, I was confident that rats were using memory to solve the 0-isolated pattern.

In the 6-isolated pattern, rats could achieve high accuracy by choosing any one of the six correct test phase arms and then choosing arms based on an every-other arm rule or through consecutive 60° turns in a clockwise (e.g. 2→4→6→8→10→12) or counter-clockwise (e.g. 2→12→10→8→6→4) fashion. There were 12 response orders were classed as such response strategies out of the 720 (6!) possible orders to choose among the correct test phase arms. Therefore, one should expect to observe these response strategies by chance in 1.67% of trials. In this experiment, none of the 10 rats exhibited these response strategies. Rats clearly did not employ a response strategy to solve the 6-isolated pseudo-random pattern.

It was unlikely that rats developed response strategies for all other patterns due to their irregular layouts. Likewise, using either of the two response strategies described earlier in the other patterns would likely result in mediocre accuracy compared to relying on memory. For these reasons, other patterns were not analyzed for response strategy usage. Given the results of these response patterning analyses, I was confident that this second set of rats also used memory to solve the task.

3.3.2 Within-trial Responses

A primary interest of this experiment was to observe how rats would perform on isolated and non-isolated test phase arms when they in were in competition. Therefore, performance accuracy data for within test phase trials were taken from 1-isolated, 2-isolated, 3-isolated and 4-isolated patterns only and were aggregated for analysis. Accuracy for isolated arms was calculated by dividing the total number of correct isolated arm choices within the first six choices of a test phase trial by the number of opportunities for isolated arm choices in that test phase trial. Accuracy for non-isolated arm choices was calculated similarly. Figure 9 illustrates the compiled data from the four patterns. At both RIs, rats achieved higher accuracy on isolated test phase arms than non-isolated test phase arms. Due to the fact that the number and position of isolated arms were directly controlled in this experiment, I found it appropriate to conduct statistical analyses on these data. A two-way (2 × 2) repeated measures ANOVA was conducted to investigate the effects of arm isolation and RI on spatial working memory accuracy. The ANOVA revealed a main effect of arm isolation, $F(1, 9) = 7.20$, $p < .05$, $\eta_\rho^2 = .44$, a main effect of RI, $F(1, 9) = 22.83$, $p < .001$,$\eta_\rho^2 = .72$, but no interaction of arm isolation and RI on accuracy, $F(1, 9) = 0.45$, $p = .52$, $\eta_\rho^2 = .05$.

3.3.3 General Accuracy

General performance data are shown in Figure 10. Chance performance was recalculated using the same method described in Beatty & Shavalia (1980). A list containing each possible permutation of correct and incorrect choices in the first six choices of the test phase was generated. The probability of each permutation occurring by chance was calculated and multiplied by the number of correct choices in that permutation. This produced the expected number of correct choices for each permutation. These values were summed across all permutations to produce the total number of correct choices expected by chance across the first six choices of the test phase, 2.405. This value divided

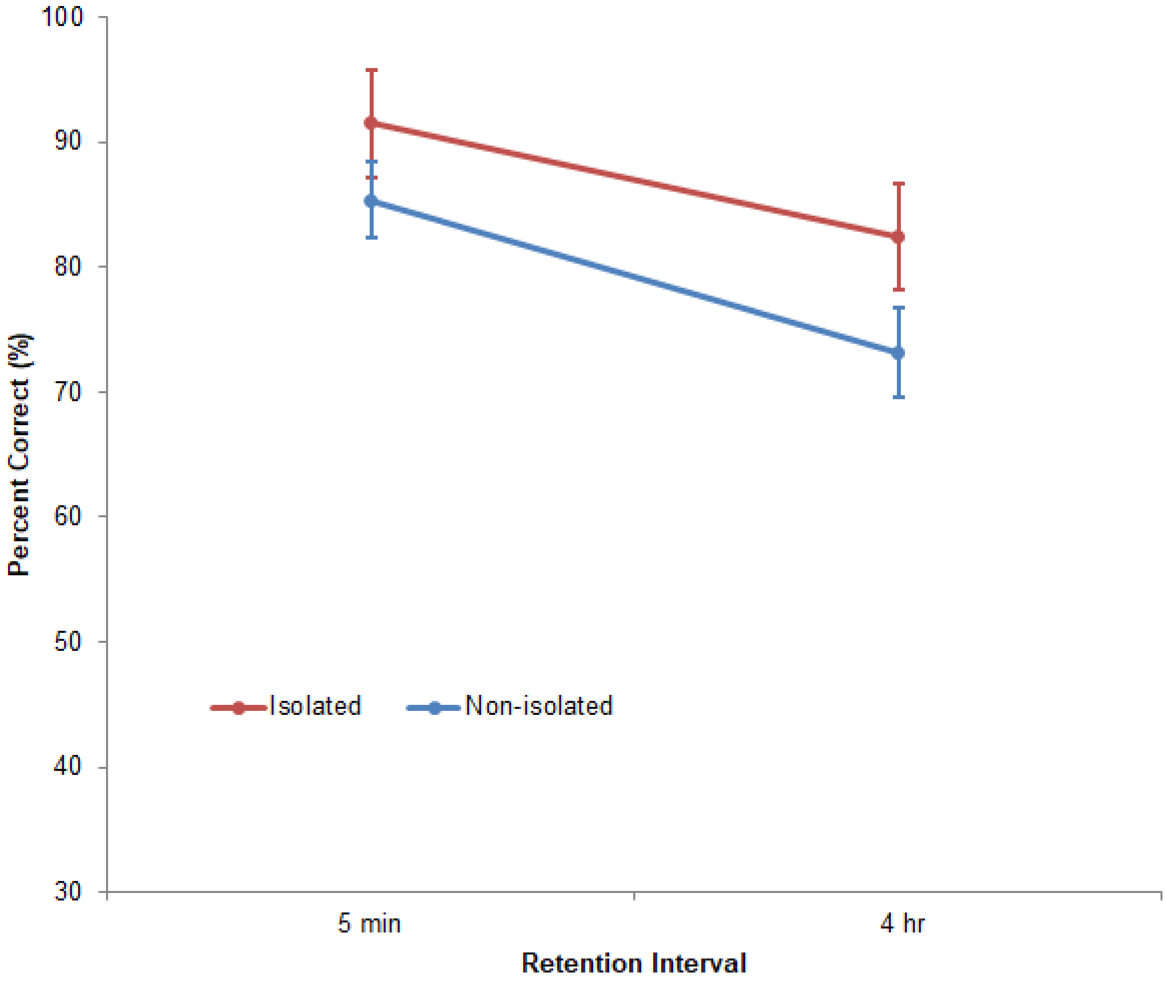

Figure 9. Spatial working memory accuracy for isolated and non-isolated test phase arms at 5 min and 4 hr RIs. Error bars represent standard error.

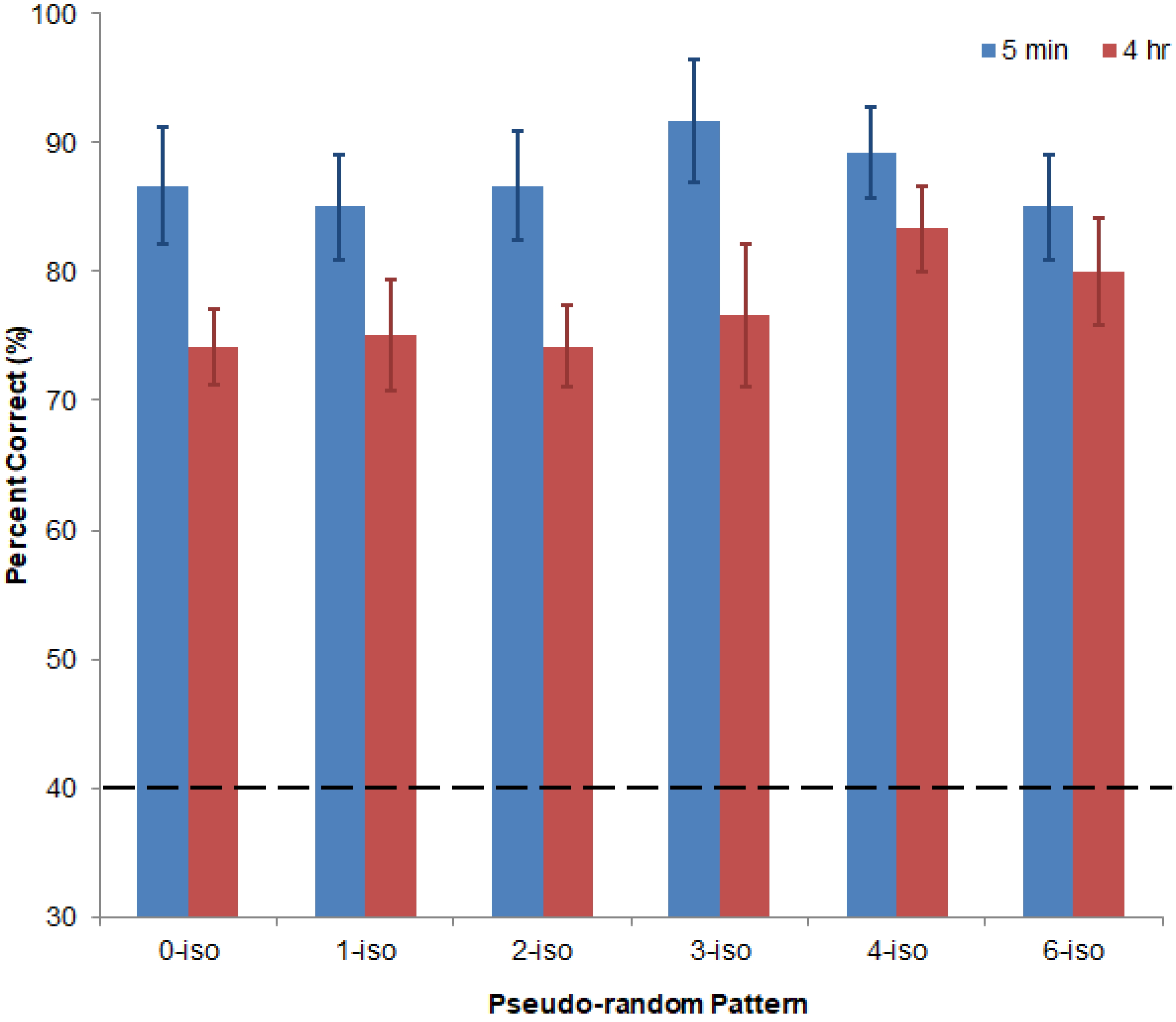

Figure 10. General spatial working memory accuracy at 5 min and 4 hr RIs across the six pseudo-random arm patterns. Error bars represent standard error. Chance accuracy (40.08%) is represented by the dashed line.

by six equals a chance performance of 40.08%. Despite the fact that the 12-arm maze reduced general accuracy in the task compared to Experiment 1's results, performance across pseudo-random patterns remained well above chance even in the 4 hr RI.

Analysis of general accuracy in the task was conducted through a two-way (6 × 2) repeated measures ANOVA of number of isolated arms and RI on spatial work memory accuracy (percentage of correct choices out of the first six choices in the test phase).The main effect of RI on spatial working memory accuracy was replicated in this experiment, $F(1, 9) = 41.31$, $p < .001$, $\eta_\rho^2 = .82$. Across the pseudo-random patterns, very little variation was observed in spatial working memory accuracy. No main effect of number of isolated arms on spatial working memory accuracy was observed, $F(5, 45) = 1.69$, $p = .157$, $\eta_\rho^2 = .16$. No interaction of number of isolated arms and RI on spatial working memory was observed, $F(5, 45) = 0.91$, $p = .482$, $\eta_\rho^2 = .09$.

3.4 Discussion

In Experiment 2, a 12-arm radial maze was used instead of an 8-arm radial maze. The use of this new maze had two purposes: to increase the difficulty of the two-phase working memory task and to allow for more pseudo-random patterns to be used. Given the high accuracy scores observed in Experiment 1, I was concerned that performance in this maze task could be subjected to ceiling effects. If high accuracy was too easy to achieve in the task, the experimental manipulations may not reveal observable effects. By making the task more difficult across all conditions, differences caused by the experimental manipulations could be observed more clearly. The 12-arm maze, under the same conditions as the 8-arm maze, imposes a higher memory load on rats in each trial, since rats have to remember six to-be-visited locations instead of four. Additionally, the extra maze cues beyond the boundaries of the maze become less informative with each additional arm added to the maze. That is, each extramaze cue could have been associated with even more maze locations than before, making each cue less informative about the spatial locations in the maze. At a 5 min RI, rats performed better in the 8-arm maze (M = 98.96%, SD = 3.68) than in the 12-arm maze (M = 87.36%, SD = 12.97). This was also true at the 4 hr RI, with rats performing better in the 8-arm maze (M = 84.38%, SD =

11.21) than the 12-arm maze (*M* = 77.22%, *SD* = 12.55). Since these data were drawn from different rats in different experiments, we did not conduct statistical analyses but, generally, performance in the 12-arm maze was lower than in the 8-arm maze. The second purpose of the 12-arm maze was to increase the number of possible pseudo-random patterns. In an 8-arm maze, only four pseudo-random patterns are possible (i.e. 0-isolated, 1-isolated, 2-isolated and 4-isolated). In a 12-arm maze, six pseudo random patterns are possible (see Figure 8). In order to properly assess whether the number of isolated arms in a pattern affects general accuracy, I opted to expand the maze in order to use as many pseudo-random patterns as possible.

My analysis of response strategy usage in the 0-isolated and 6-isolated patterns indicated that rats were not using a response strategy in either pattern to solve the task. Therefore, I assumed that rats were using memory to retrieve food rewards from unvisited arms.

The design of this experiment allowed the problem of unequal and limited observations across subjects and RIs to be addressed. In Experiment 1, performance on isolated test phase arms was based on an average of 17.75 observations per RI. Conversely, performance on non-isolated test phase arms was based on an average of 46 observations per RI. The randomized positions of study and test phase arms in Experiment 1 meant that the number of accuracy observations for isolated and non-isolated test phase arms were also unequal across subjects. The current experiment’s design (including the use of pseudo-random patterns and two extra rats) allowed for an increased and equal number of observations across subjects and RIs. Performance accuracy for isolated test phase arms was now based on 200 observations across both RIs, while performance accuracy for non-isolated test phase arms was now based on 280 observations in both RIs. This ultimately allowed performance accuracy to be approximated with greater resolution than in Experiment 1.

Despite this, a remaining issue with the experimental design was that the total number of observations across arm isolation conditions was not equal. Across RIs and subjects, the number of isolated and non-isolated arms was equal, but the patterns selected for these analyses inherently have an unequal number of isolated and non-isolated arms (with the exception of the 3-isolated pattern). For example, the 1-isolated arm pattern has one isolated arm and five non-isolated arms. For every 1-isolated pattern trial, five observations were taken for non-isolated arm accuracy but only one observation was taken for isolated arm accuracy. This discrepancy skews the analysis since it is more likely for random or sampling errors to occur in isolated arm choices than non-isolated arm choices. The discrepancies across the pseudo-random patterns resulted in 200 total observations for isolated arm choices and 280 total observations for non-isolated arm choices. In the context of this experiment, the discrepancy in observations across arm isolation conditions should not be of great concern. In an experiment with fewer observations per condition (e.g. less than 20), random errors and sampling errors could have a greater effect on performance measurements. Given the large number of observations in both arm isolation conditions, the effect of random or sampling errors should be fairly small.

Controlling for the number and position of test phase arms made the data suitable for analysis. In this follow-up experiment, rats demonstrated enhanced working memory for isolated test phase arms in the radial maze. The results suggest rats are more accurate on isolated test phase arms than non-isolated test phase arms. Additionally, spatial working memory accuracy varied significantly with RI. These results fell in line with the within-trial response data from Experiment 1 (see Figure 6), which implied that isolated arms were being retained better than non-isolated arms. Interestingly, the data from Experiment 1 also hint at an interaction between arm isolation and RI. In this experiment, there was no interaction of arm isolation and RI on spatial working memory accuracy, suggesting that these two factors independently affected the variance in accuracy. In addition to the apparatus differences between the two experiments, I also reduced the number of RIs at which rats were tested. Perhaps an interaction could have been observed if the 24 hr RI was included in Experiment 2’s design. However, no difference between isolated and non-isolated arms appeared at the 5 min RI in Experiment 1, but a clear difference appeared at 5 min in Experiment 2.

Lastly, the analysis of general accuracy demonstrated that rats did not show performance differences across the pseudo-random patterns. Regardless of how many isolated arms were in these patterns, there were no significant variations in general accuracy. These findings are interesting, as they suggest that rats in Experiment 1 did not perform better on the Alternating pattern because of the number of isolated arms. This suggestion, with the added context that rats were not using response strategies to solve the Alternating pattern, is peculiar. It may be possible that the increased number of test phase arms (isolated or non-isolated) being held in working memory inhibited the spatial isolation effect. That is, enhanced working memory for isolated arms may only have pronounced effects on general accuracy when memory load is low. This could possibly explain the larger variation in general accuracy in Experiment 1, where only four arm locations were held in working memory, compared to the smaller variation in Experiment 2, where six locations were held in working memory. It would be interesting to see if enhanced memory for isolated arms varied with memory load by testing rats on radial mazes with different numbers of arms. The main effect of RI was reproduced in this experiment, but there was no interaction between the number of isolated arms and RI on spatial working memory. Further exploration of the spatial isolation effect and its mechanisms was pursued in Experiment 3.

3.5 References

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Chapter 4

4 Experiment 3

4.1 Introduction

In Experiment 2, rats demonstrated enhanced working memory for isolated locations on the radial maze. The next research question of interest was: what factors or cognitive mechanisms are responsible for the spatial isolation effect? I had two hypotheses concerning which factors were the primary drivers for this effect. The first hypothesis was that the spatial isolation effect is driven by prospective memory use. According to this hypothesis, rats enter the maze and search for previously unvisited arms (i.e., “Where do I need to go?”) as opposed to avoiding previously visited arms (i.e., “Where have I been?”), in order to seek out test phase arms (Cook et al., 1985). Using prospective memory, rats maintain representations of isolated test phase arms better due to their distinct absolute spatial location in reference to other test phase arms. As discussed in Experiment 1, isolated test phase arms benefit from a spatial analogue of the von Restorff effect (Hunt, 1995; von Restorff, 1933). In reference to other test phase arms, isolated test phase arms’ absolute spatial distinctiveness results in enhanced within-trial accuracy for those locations. The second hypothesis proposes that rats are more accurate on isolated test phase arms because motivational factors cause rats to prioritize isolated arm choices. By definition, isolated arms always sit in between two previously visited (and therefore, rewarded) study phase arms. These consecutive arm locations (i.e., two outer study phase arms and an inner isolated test phase arm) may be analogous to a food patch. According to this hypothesis, rats remember that they were previously rewarded in a general area on the maze and may be rewarded again if they returned to that general area via the unvisited isolated test phase arm. In this sense, rats may be using their memory for multiple locations on the maze to make judgements about reward probability. If the rat is sufficiently motivated to retrieve food rewards, then it may be prioritizing isolated arms in memory due to a perceived higher reward probability. Judgements of reward probability would also dissuade rats from entering arms in an area of the maze where they had not been rewarded earlier in the session (i.e., non-isolated test phase arms).

In order to measure the prioritization of isolated arms, the mean rank orders of the isolated arms could be analyzed. If rats were prioritizing isolated arm choices (i.e., choosing isolated arms earlier in the test phase) based on motivational factors, the mean rank order of isolated arms should be lower than the mean rank order of non-isolated arms. Unfortunately, mean rank order of isolated and non-isolated arm choices is confounded by accuracy. If rats are more accurate on isolated test phase arms, they are choosing these arms more often within the first six choices of the test phase. Conversely, lower accuracy on non-isolated test phase arms means that rats are less likely to choose non-isolated arms within the first six choices of the test phase. Knowing this, a different way of testing whether motivational factors modulate the spatial isolation effect was required.

In Experiment 3, I developed a simple manipulation to modulate the potential motivational factors that could be driving the spatial isolation effect. I reasoned that if motivational factors are driving the spatial isolation effect, removing the motivational factors should cause the spatial isolation effect to disappear. That is, if retrieving sucrose pellets in adjacent locations to an isolated arm increases choice accuracy for that isolated arm, then removing the sucrose pellets from the adjacent locations should reduce the choice accuracy for the isolated arm. Across four levels of a sucrose depletion manipulation, sucrose pellets were removed from specific arm locations that varied in distance from an isolated arm. I hypothesized that enhanced working memory for isolated test phase arms is modulated by motivational factors. Additionally, I predicted that depleting sucrose pellets from study phase arms adjacent to isolated test phase arms would decrease working memory accuracy for isolated test phase arms.

4.2 Methods

4.2.1 Subjects

The same set of ten male Long-Evans rats was used in this experiment. This experiment began 9 weeks after Experiment 2 had finished. Over these 9 weeks, rats were allowed to eat Pro Lab Rat Chow *ad libitum* until they reached their free-feeding weights. Prior to the training and testing periods, their weights were reduced to 90% of their respective free-feeding weights (*M* = 414g). Otherwise, housing and feeding conditions were identical to those in Experiment 1. All testing occurred between 9 a.m. and 5 p.m. five days per week.

4.2.2 Materials

Experiment 3 used the same 12-arm radial maze, testing room and sucrose pellets as in Experiment 2. All experimental testing conditions were identical to those in Experiment 2.

4.2.3 Procedure

Training. In two training sessions, rats were tested in a one-phase working memory task. The purpose of these trials was to ensure that rats were still comfortable navigating the maze after the nine week hiatus from testing. In this task, all arm locations were open and baited with sucrose pellets. Rats were allowed to navigate the maze for food rewards until all were depleted or 5 min had elapsed. Arm entries were recorded in the order they were entered. At the end of the second session, it was clear that rats were still accurately navigating the maze under the same testing conditions they had experienced before. Experimental testing proceeded immediately after these two sessions were completed.

Testing. A two-phase working memory task was used to assess spatial working memory accuracy for isolated arm locations under sucrose depletion and RI manipulations. The RI manipulation, again, included the 5 min and 4 hr levels. In this experiment, the 1-isolated pseudo-random pattern from Experiment 2 was used exclusively (see Figure 8). This pattern was selected on the basis that it contained one arm that was maximally isolated from the non-isolated arms in the pattern. This pattern was allowed to randomly rotate across sessions in order to control for position and side preferences in the maze. Within this pattern, the sucrose depletion manipulation was implemented (see Figure 11). In the control condition, arm locations were baited as normal, with 12 sucrose pellets distributed across the 12 arm locations. In the Isolated-Depleted condition, two sucrose pellets were depleted from the two study phase arms on

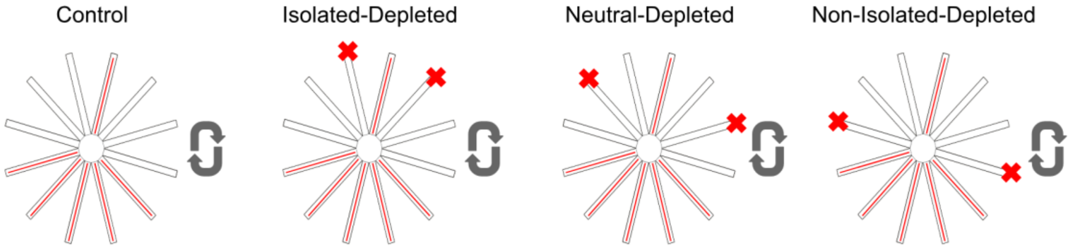

Figure 11. Schematics of the sucrose depletion manipulations on the 1-isolated pseudo-random pattern. Test phase arms are marked in red and sucrose depleted locations are marked with red Xs. Rotation symbols denote that pattern could be rotated randomly in order to control for position and side preferences.

either side of the isolated test phase arm prior to the beginning of the study phase trial. In the Neutral-Depleted condition, the two study phase arm locations halfway between the isolated and non-isolated arms had their sucrose pellets removed before the study phase trial began. Lastly, the Non-Isolated-Depleted condition entailed removing sucrose pellets from the two study phase arms adjacent to the non-isolated arms before the study phase began. The levels of RI and sucrose depletion were combined to create eight unique testing conditions. Again, testing was completed using a within-subjects design, in which each rat experienced each of the eight unique testing conditions. A Latin square randomization was used in order to counterbalance the testing order of the sucrose depletion condition, so that any order effects across the sucrose depletion manipulation were controlled. Each rat was assigned randomly to one of four Latin square orders. Each rat completed their assigned Latin square order at the 5 min RI and then again at the 4 hr RI. In order to control for order effects, a second round of testing was completed in the reverse order. The assigned Latin square orders were reversed and completed at the 4 hr RI, followed by the 5 min RI. Therefore, each rat experienced each combination of sucrose depletion and RI twice over a total of 16 experimental testing sessions. Spatial working memory accuracy was measured as the percentage of correct choices within the first six arm choices. Order of arm entries and the total number of arm entries were also recorded.

4.3 Results

Although rat i5 successfully completed the experiment, the data from this rat was excluded from the following analyses due to its erratic and erroneous choice behaviour. Although this particular rat had performed well in Experiment 2 and in pre-training, its performance and general behaviour during testing were quite different from the other rats. Anecdotally, it seems that this rat was choosing arms based on a win-stay strategy instead of the win-shift strategy that it was supposed to learn across Experiments 2 and 3. Even at the 5 min RI, rat i5’s accuracy scores were as bad as 33.33% while other rats consistently achieved 83.33% or 100%. Rat i5’s averaged accuracy of 57.88% was substantially lower than that of the other nine rats that achieved 91.28%. At the 4 hr RI, i5 achieved an average accuracy of 39.38% while the others performed at 83.01%

accuracy. The decision to exclude rat i5's data was made about halfway through the testing procedure, but testing continued until he had completed the whole procedure.

4.3.1 Response Patterning

As in Experiment 2, it was unlikely that rats developed response strategies for the 1-isolated pattern due to its irregular layout and randomized rotation about the maze. It was possible that rats developed a response strategy to find the five non-isolated test phase arms in the pattern. For example, the majority of the food rewards could be retrieved with high accuracy if rats entered an outer non-isolated test phase arm before making consecutive clockwise (e.g. 4→5→6→7→8) or counter-clockwise (e.g. 8→7→6→5→4) choices. These patterns would result in a minimum accuracy score of 83.33% and would entail a lower memory load than remembering all six test phase arms independently. For the sake of consistency, I analyzed order-of-entry data in the 1-isolated pattern in order to be sure that the rats' prior experience on the maze did not incentivize response strategy usage. There were only two response orders (described above) out of the 120 (5!) possible response orders that could be classed as response strategies. Therefore, one should expect to observe these response strategies by chance in 1.67% of trials. Across all nine rats and 16 testing sessions, the described response strategies were not observed even once. Given these results, I was confident that the rats continued to use memory to solve this newly modified task.

4.3.2 General Accuracy

General accuracy across the four levels of sucrose depletion is shown in Figure 12. In general, the data do not seem to vary distinctly across the different levels of sucrose depletion and their respective standard error bars frequently overlap. As one might expect, there is a general decline in performance across the increasing RIs, but the data do not seem to diverge at the 4 hr RI. Chance performance in this task remained at 40.08%. A two-way (4 × 2) repeated measures ANOVA was conducted to analyze the effects of sucrose depletion (control, Isolated-Depleted, Neutral-Depleted and Non-Isolated-Depleted) and RI (5 min, 4 hr) on spatial working memory accuracy (measured

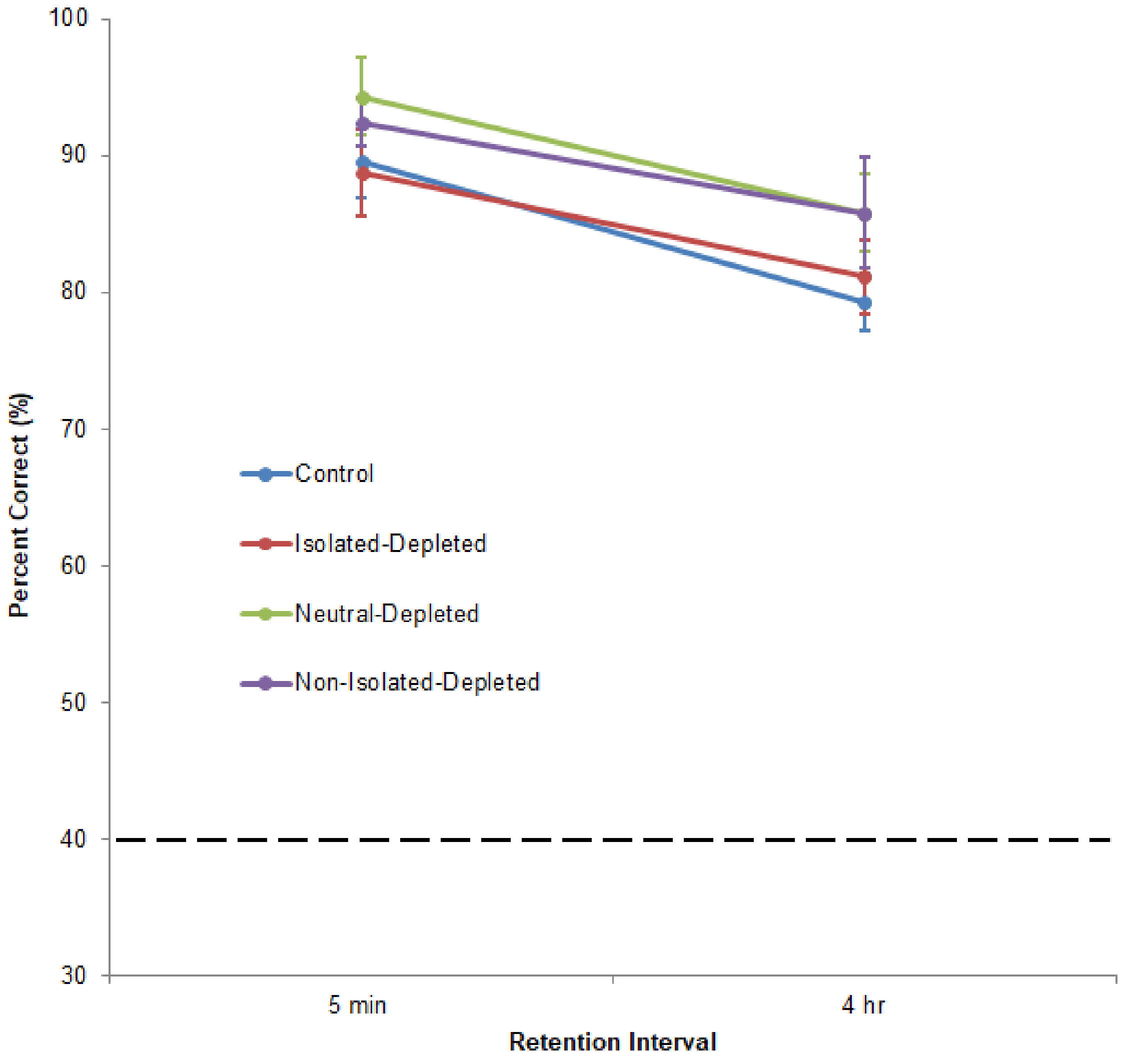

Figure 12. General spatial working memory accuracy (measured as percent correct choices out of the first six choices in the test phase) in the four sucrose depletion conditions across RI. Chance accuracy (40.08%) is denoted by the dashed line.

as the percentage of correct choices within the first six choices of the test phase). The ANOVA revealed a main effect of RI, *F*(1, 8) = 16.96, $p < .01$, $\eta_\rho^2 = .68$, but no main effect of sucrose depletion, *F*(3, 24) = 2.60, $p = .08$, $\eta_\rho^2 = .24$, on general working memory accuracy. No interaction of RI and sucrose depletion on general working memory accuracy was found, *F*(3, 24) = 0.38, $p = .77$, $\eta_\rho^2 = .05$.

4.3.3 Within-trial Responses

Spatial working memory accuracy for isolated arm choices was calculated by dividing the total number of correct isolated arm choices within the first six choices of a test phase trial by the number of opportunities for isolated arm choices in that test phase trial. Accuracy for non-isolated arm choices was calculated similarly. The data are illustrated in Figure 13. In the control condition, performance accuracy across RI mirrored the results from Experiment 2 (see Figure 9), but with smaller differences observed at the 4 hr RI. In the Isolated-Depleted condition, the data show a reversal of accuracy at the 4 hr RI, as rats were more accurate on non-isolated arms than isolated arms in this condition. In order to address the main hypothesis, accuracy data specifically from the control and Isolated-Depleted condition were run through a three-way (2 × 2 × 2) repeated measures ANOVA. Just as in Experiment 2, arm isolation was a factor containing the two levels of isolated and non-isolated arm choices. RI had the two levels of 5 min and 4 hrs, while sucrose depletion levels selected for this analysis were the control and Isolated-Depleted conditions. The ANOVA revealed a main effect of RI, *F*(1, 8) = 10.09, $p < .05$, $\eta_\rho^2 = .56$, no main effect of arm isolation, *F*(1, 8) = 0.41, $p = .54$, $\eta_\rho^2 = .05$, and no main effect of sucrose depletion on spatial working memory accuracy, *F*(1,8) = 0.05, $p = .82$, $\eta_\rho^2 = .01$. Additionally, no significant two-way or three-way interactions were revealed by the ANOVA.

4.4 Discussion

Although general accuracy data were not particularly important in addressing the hypothesis of this experiment, the data were analyzed to see if the manipulations produced any effects on general accuracy in the task. The analysis revealed that the

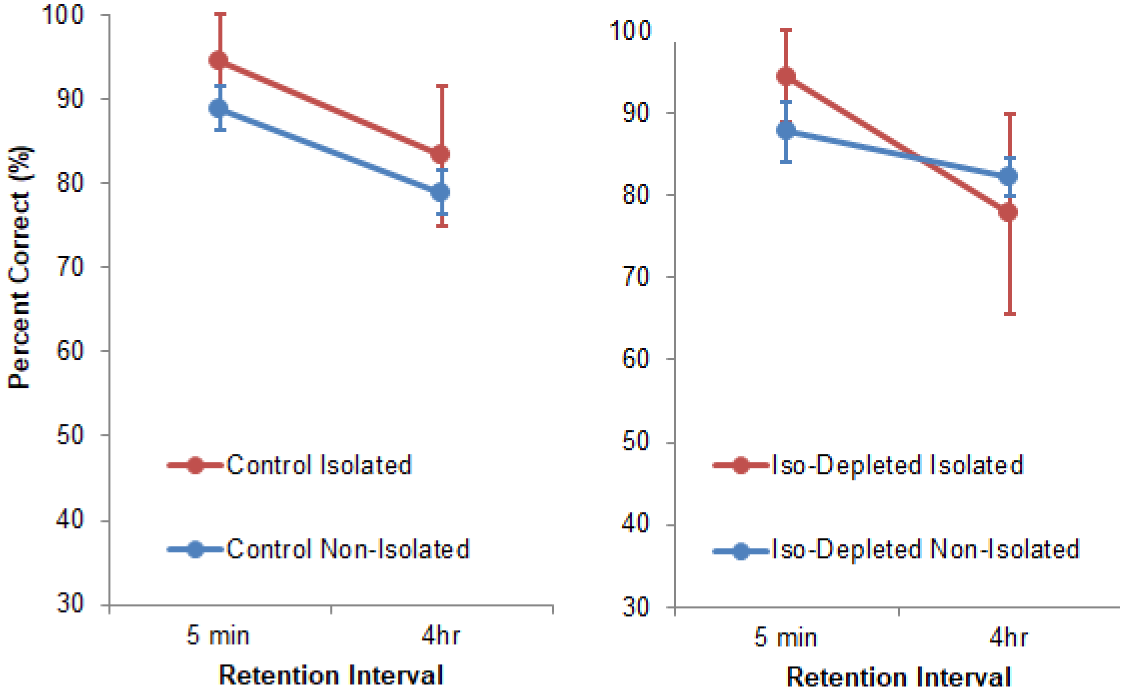

Figure 13. Spatial working memory accuracy data across RI, subsetted by sucrose depletion. Error bars represent standard error.

sucrose depletion manipulation did not affect performance in the task, although the effect did approach statistical significance. Importantly, the general accuracy data did vary significantly with RI, as in the previous experiments. The most pertinent data in reference to the hypothesis were the within-trial response data. The results suggest that motivational factors did not affect the accuracy on isolated test phase arms. In order to confidently support the hypothesis that motivational factors modulate the spatial isolation effect, a two-way interaction of arm isolation and sucrose depletion on spatial working memory accuracy would be required. A three-way interaction of RI, arm isolation and sucrose depletion would also be sufficient, but no interactions were revealed by the ANOVA. Therefore, I cannot suggest that motivational factors modulate the spatial isolation effect.

Importantly, the spatial isolation effect was not replicated in this experiment. Although differences between accuracy for isolated and non-isolated arms were comparable between the two experiments, reduced differences between isolated and non-isolated arm accuracies were observed at the 4 hr RI in Experiment 3. In order to compare these accuracy data, additional context is required. In Experiment 2, the within-trial accuracy data were aggregated from performance on the 1-isolated, 2-isolated, 3-isolated and 4-isolated pseudo-random patterns. Combining the data from across these pseudo-random patterns increased the number of observations of choices for isolated and non-isolated arms to 200 and 280, respectively. In Experiment 3, all testing conditions were performed on the 1-isolated pattern since it allowed the sucrose depletion manipulation to be easily implemented. Unfortunately, using the 1-isolated pattern in this experiment reduced the number of observations for isolated arms relative to non-isolated arms. This, in addition to running fewer sessions with fewer rats, resulted in a skewed and reduced total number of observations for isolated and non-isolated arm choices throughout the experiment. Accuracy for isolated arm choices was based on 18 total observations in both of the control and Isolated-Depleted conditions, whereas accuracy for the non-isolated arm choices was based on 90 observations in both control and Isolated-Depleted conditions. The reduced number of observations may have made observations of choice accuracy more susceptible to random error in either the isolated or non-isolated conditions. Random error would have been more detrimental to performance measurements in the isolated condition than in the non-isolated condition, but both conditions in Experiment 3 would have been affected more than the accuracy data in Experiment 2. The reduced and unequal number of observations between the isolated and non-isolated conditions could account for the reduced difference in accuracy (4.45% in Experiment 3 compared to 9.29% in Experiment 2) at the 4 hr RI.

A further limitation to this study was the lack of control for reward probability. Part of the motivational factors hypothesis relied on the proposal that rats used retrospective memory to make reward probability judgements on the isolated arms. Throughout this experiment, the study phase arms that were adjacent to the isolated arm were rewarded in 75% of trials, which did not differ across rats. Perhaps this decrement in reward probability was not sufficient enough to affect the rats' judgement of reward probability. In a separate experiment, manipulating the reward probability across control and Isolated-Depleted conditions could yield effects not observed in the current experiment. Reducing the reward probability of the study phase arms to 0% or 50% could reveal a main effect of sucrose depletion on accuracy compared to control levels at 100% reward probability. In a within-subjects design, rats could experience changes in reward probability, from 0% to 50% to 100% and vice versa, in order to see if accuracy for isolated test phase arms changed dynamically according to changes in reward probability. This manipulation, in addition to a greater number of sessions (i.e., number of observations for accuracy), would allow for a further and more controlled analysis of potential motivational factors on the spatial isolation effect.

Ultimately, the current experiment's results did not replicate the spatial isolation effect and failed to support the hypothesis that motivational factors modulate the spatial isolation effect. The effect of RI was replicated, but with a reduced effect size compared to Experiment 2. Further experimentation, like the proposed design above, would be required to make further inferences on the relation, if any, between motivational factors and the spatial isolation effect. If that experiment failed to produce sufficient evidence to support that hypothesis, then the hypothesis that prospective memory use modulates the spatial isolation effect should be considered.

4.5 References

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Chapter 5

5 General Discussion

5.1 Overview of Thesis

The current set of experiments investigated how rats behave in the radial maze. In Experiment 1, rats demonstrated differential performance across three arm patterns. Although rats performed equally well across arm patterns with a 5 min RI, differences became clearer at longer RIs (i.e., 4 hrs). Rats were most accurate in the Alternating pattern, in which study and test phase arms were placed 90° or 180° from one another. Rats were less accurate on Random patterns and even less accurate in Adjacent patterns, in which study and test phase arms were all placed next to one another. Performance in all three arm patterns remained well above chance. At the 24 hr RI, performance in all arm patterns seemed to suffer from general or overnight forgetting, but rats were still more accurate on Alternating patterns than Random or Adjacent patterns. Analysis of the order of arms visited revealed that rats were not using response strategies to solve arm patterns, which implies that they were using memory. These results support the hypothesis that rat spatial working memory for multiple locations is sensitive to their relative configuration. An analysis of within-trial responses suggested that rats on the random pattern condition were more accurate on test phase arms if they were spatially isolated from the other test phase arms in a trial. It is possible that rats show greater accuracy for unvisited arms that are isolated compared to non-isolated arms. This hypothesis would explain the effect observed across pattern in Experiment 1.

Experiment 2 followed up the results from Experiment 1 by standardizing the number and positions of isolated and non-isolated test phase arms. General accuracy in this task remained high across all conditions, well above the 40.08% predicted by chance. The effect of RI on spatial working memory was observed in both general accuracy and within-trial accuracy. Although the number of isolated arms in the test phase did not significantly affect general accuracy, rats were significantly more accurate on isolated than non-isolated test phase arm choices. The within-trial data agreed with the data from Experiment 1, but the general accuracy data from Experiment 2 did not support the notion that rats were performing better on the Alternating pattern in Experiment 1 due to the number of isolated arms in that pattern.

Experiment 3 addressed one of the possible mechanisms behind the spatial isolation effect; that rats were incentivized to remember isolated arms over non-isolated arms because they always occurred between two previously rewarded study phase arms. Having been rewarded in a general area of the maze, rats made judgements about reward probability based on retrospective memory. These reward probability judgements incentivized returning to areas in the maze that were adjacent to previously rewarded arms as opposed to novel areas in the maze. This scenario in the maze is analogous to foraging among patches, where rats prefer to return to a patch where they were previously rewarded. I hypothesized that the spatial isolation effect was modulated by motivational factors and predicted that removing sucrose pellets from study phase arms that were adjacent to isolated test phase arms would decrease working memory accuracy for isolated test phase arms. The data did not support this hypothesis or prediction, with only the effect of RI reaching statistical significance. The spatial isolation effect was not replicated in this experiment and the sugar depletion manipulation had no effect on general or within-trial accuracy.

Taken together, the three experiments presented in this thesis support previous evidence that working memory for multiple locations is sensitive to spatial configuration (Brown et al., 2000; Brown & Giumetti, 2006; Brown & Terrinoni, 1996; Brown, Zeiler, & John, 2001; Dallal & Meck, 1990). This phenomenon was first demonstrated when rats showed differential performance across Random, Adjacent and Alternating patterns in Experiment 1. The arrangement of arms seemed to have a strong effect on their working memory for unvisited test phase arms. The effect of configuration was more specifically demonstrated in Experiment 2, where rats were found to be more accurate on isolated than non-isolated test phase arms. The rodent model presented in this thesis suggested that spatial memory for individual unvisited locations can be affected by their spatial separation from other unvisited locations.

All three of these experiments were vulnerable to the possibility of rats using response strategies to partially or fully solve the tasks. In particular, there were patterns across all three experiments that had a series of consecutive correct test phase arms on one side of the maze. Additionally, every other arm was an unvisited test phase arm in the Alternating and 6-isolated patterns from Experiment 1 and 2, respectively. These arm configurations could be solved via response strategies instead of working memory. Previous studies have shown large variation in response strategy usage across individuals, apparatus and experimental design (Einon, 1980; Foreman, 1985; Olton, et al., 1977; Roberts & Dale, 1981; Watts et al., 1981). I did not find evidence that rats employed response strategies to solve any of the patterns used in these three experiments. Response strategies (or the response orders that could be classified as response strategies) were either observed less often than predicted by chance or in zero percent of the testing trials in each experiment. These findings were consistent despite switching between an eight-arm and 12-arm radial maze and two sets of rats. It should be noted that in all experiments, rats were at least 142 days old by the time testing commenced. Einon (1980) suggested that there might be a relationship between response strategy usage and age. Specifically, rats at 45-70 days old were observed using response strategies in just less than 50% of testing trials and 150 day old rats did not use response strategies at all. Even though the incentives for using response strategies do not change, there may be other behavioural factors that modulate choice behaviour as rats age (Geinisman, Toledo-Morrell, Morrell, 1986; Rapp & Amaral, 1992). Regardless, the conclusion that rats were not using response strategies to solve the task in each experiment was confirmed through the analysis of their response orders.

Another legitimate concern with this task was the use of odour cues. Although, the use of odour cues was originally found to be a secondary or an otherwise unimportant cue for radial maze navigation (Burešová & Bureš, 1981; Einon, 1980; Maki et al., 1979; Olton & Collison, 1979; Olton & Samuelson, 1976; Zoladek & Roberts, 1978), researchers should not disregard odour entirely, as it is a salient cue to rodents (April, Bruce, & Galizio, 2013; Branch, Galizio, & Bruce, 2014; Devore, Lee, & Linster, 2013; Eichenbaum, 1998; Kulvicius, Tamosiunaite, Ainge, Dudchenko, & Wörgötter, 2008; Panoz-Brown et al., 2016). For the three present experiments, I attempted to control odour cues from food rewards by using hidden sucrose pellets in perforated plastic bags. This control followed the same logic as the control implemented in Einon (1980). Theoretically, these bags would disperse the same odour as the food reward, but from underneath the surface of the maze. With a bag taped under the distal end of each arm, the odour of the food reward should be evenly dispersed across the testing room. It is possible that the distribution of odours changed over the course of a session, both when sucrose pellets were eaten or depleted. Although not likely, it is possible that rats were guided by the asymmetries in odour salience in the testing room. However, this strategy would require a profound acuity in olfactory perception. Additionally, this specific control did not account for odour trails left in previously visited arms. In order to prevent odour trails from providing cues, the maze was routinely cleaned with a 10% acetic acid solution once every five testing days and only after a session was completed. The maze was not cleaned in between trials as it would introduce an additional odour cue in the middle of the session more than it would be removing an odour (Einon, 1980; Olton & Samuelson, 1976). When the maze was cleaned, the solution was allowed to evaporate and disperse overnight and was assumed to leave a neutral odour on the barriers and maze floor after 24 hrs. Under this control, odour trails should have been controlled with some degree of certainty, especially when the maze was cleaned immediately if rats urinated or defecated on the maze, although those situations became increasingly rare as rats habituated and matured. Based on previous findings about odour cue use in the radial maze (Burešová & Bureš, 1981; Means, Hardy, Gabriel, Uphold, 1971; Olton & Collison, 1979) and how appropriate controls were implemented, I was confident that odour cues did not control choice behaviour. Between the evidence against response strategy usage and the sufficient controls for odour, I am confident that the set of experiments presented here were tests of working memory. Therefore, I will contrast the current work with previous literature.

5.2 Comparisons with Previous Literature

Hoffman and Maki (1986) used a spatial pattern manipulation in a series of experiments investigating proactive interference on the radial maze. The patterns used in Hoffman and Maki (Experiment 1) and my first experiment were identical. Interestingly, Hoffman and

Maki failed to find any significant main effect of spatial pattern. The differences between the “cross” and “side” (i.e., Alternating and Adjacent) patterns, although in the right order, were small and did not suggest that spatial pattern affected working memory or protected working memory from proactive interference. Notably, Hoffman and Maki did not include a random clustering pattern as a control condition.

The experiments presented in Hoffman and Maki (1986) influenced a series of experiments presented in Roberts et al. (2017). In particular, Experiment 3 of Roberts et al. also used spatial pattern manipulations in an attempt to protect working memory from proactive interference. Just as in Hoffman and Maki (1986), spatial pattern failed to protect working memory from proactive interference but Roberts et al.’s observed substantial differences between spatial pattern conditions in control trials, where no proactive interference manipulation was implemented. In particular, rats were most accurate in the Alternate arm pattern (98.96%), followed by Random (89.58%), and then Adjacent (75%) patterns. In Experiment 1, my results replicated the findings of Roberts et al., as rats were most accurate on the Alternating, Random, and then the Adjacent pattern. A notable procedure difference in Roberts et al. was that results were observed at a 5 min RI. In Experiment 1 of this thesis, similar differences across patterns were observed at a 4 hr RI. Roberts et al. did not analyze response data, but confirming that response strategies were not employed would make these two studies agree more conclusively about the control of working memory by spatial pattern.

Superficially, my results agree with the findings of Brown and Giumetti (2006), where rat spatial working memory for multiple locations was enhanced when those locations were defined by an abstract pattern. Furthermore, the results synergistically suggest that the alternation / Alternating pattern controls choice behaviour and enhances working memory for unvisited locations compared to no-pattern / Random conditions. That being said, there are substantial differences between the experimental designs of Brown and Giumetti and Experiment 1 in this thesis. Differences found in Brown and Giumetti were observed at the 1 min RI, whereas the performance differences between patterns in Experiment 1 were observed at the longer RIs. Brown and Giumetti opted to train rats on a one-phase working memory test in their assigned patterns, whereas Experiment 1’s training procedure employed a two-phase working memory task with a random pattern. In addition to training differences, Brown and Giumetti used a between-subjects design in which groups were only exposed to a single pattern. In Experiment 1, performance differences across patterns were compared within each subject. Sampling errors in group assignments, individual differences, random error and experience effects were less of a concern under this within-subjects design. The main effect of arm pattern in Experiment 1 was observed in rat performance relative to their own control, which makes the results more compelling.

Additionally, Brown and Giumetti's (2006) task involved an asymmetric memory load across the two phases (Cook et al., 1985). In the study phase, rats were given two forced choices which were encoded in retrospective memory. Upon entering the test phase, the rat maintained a retrospective memory load of two locations and a prospective memory load of six unvisited locations (only two of which were baited). In the current Experiment 1, all rats had equal retrospective and prospective memory loads of four locations in each phase, regardless of pattern manipulations. This was the case because rat performance in the study phase was always terminated after they had visited the four forced choice arms. Given the evidence found by Cook et al., it should be expected that rats would have made fewer errors on the first two choices in Brown and Giumetti's test phase than in the first two choices in Experiment 1's test phase. If rats are in fact using retrospective memory prior to switching to prospective memory, then removing rats from the maze after fewer choices would make the task less demanding in terms of cognitive resources (Cook et al., 1985; Riley, Cook, & Lamb, 1981). Therefore, the task described by Brown and Giumetti may be artificially less difficult than the task described in Experiment 1. I also controlled for a potential alternative explanation noted by Brown and Giumetti, which was that rats performed more accurately in an alternation / Alternating pattern because it had fewer possible configurations in an eight-arm maze. It could be argued that rats learned to categorize configurations into pattern categories and that fewer possible configurations in a category led to higher accuracy. I addressed this concern by only using two configurations for the Adjacent pattern throughout Experiment 1. This counterbalance meant that rats could not perform better on the Alternating pattern on the basis of learning fewer configurations that belonged to the same pattern category (Vaughan, 1988). Overall, Brown and Giumetti's experiment shares interesting parallels with Experiment 1 of this thesis, and differences in experimental design make the combination of conclusions interesting. Both experiments demonstrated control of working memory by spatial pattern, but without observing explicit response strategies. It would be interesting to see if rats in Brown and Giumetti's no-pattern control condition also exhibited the spatial isolation effect, which was suggested in the within-trial data of my first experiment.

5.3 Limitations

The most consistent limitation among the three experiments was the skewed number of observations between conditions. Most notably, in the first experiment's analysis of within-trial responses, there were no controls in place to account for variation in the number or positions of isolated or non-isolated arms. Additionally, the number of observations was generally low between conditions. Statistical analysis of these data was avoided until the variation in number and positions of isolated and non-isolated arms was properly (or at least partially) controlled. This limitation was less of a concern in Experiment 2, where isolated arms were almost always opposite to non-isolated arms, and the number of observations was high between conditions. Due to the number of observations across the 1-isolated, 2-isolated, 3-isolated and 4-isolated patterns, the data were less vulnerable to random error and provided greater resolution on isolated and non-isolated arm performance. Unfortunately, this limitation returned in Experiment 3 due to the exclusive use of the 1-isolated pattern. This pattern only provides one observation for isolated arm accuracy and five observations for non-isolated arm accuracy per session. Since rats only experienced each condition twice, rats were only able to achieve 0% (0/2 correct isolated arm choices), 50% (1/2 correct isolated arm choices) or 100% (2/2 correct isolated arm choices) as their final performance measurement in each condition. Aggregated across the nine rats, each isolated arm condition was based on a total of 18 observations. This was far below the 200 observations taken in Experiment 2. Future experiments addressing arm isolation should use designs that control the number of observations across conditions, particularly for isolated and non-isolated arm choices. If possible, aggregating data across patterns that counterbalance the number of opportunities for isolated and non-isolated choices (e.g. 2-isolated, 3-isolated and 4-isolated together) should be considered. This approach would prevent unequal observations across conditions while also exposing the rat to a variety of patterns that feature different proportions of isolated and non-isolated arms.

5.4 Future Directions

In addition to addressing the limitations outlined above, there are several lines of experiments that could extend upon the current work. Replicating the spatial isolation effect in a variety of experimental designs and testing environments would bolster the conclusions drawn here. It is possible that increasing the number of observations across conditions could have led to a replication the effect in Experiment 3, but future experiments are required to confirm this. Building on Experiment 3, future experiments might address the same hypothesis of motivational factors modulating the spatial isolation effect, but perhaps with different methodology. The major idea behind Experiment 3's hypothesis was that retrospective memory for previously visited study phase arms informed reward probability judgements about unvisited test phase arms. This retrospective memory use was hypothesized to be responsible for the spatial isolation effect and that motivational factors (i.e., motivation to return to previously rewarded spatial area) modulated their judgment about visits to isolated or non-isolated test phase arms. If motivational factors are shown to not modulate the spatial isolation effect, then the spatial isolation effect may not be the result of retrospective memory use. From there, future research should address the alternative hypothesis presented in Experiment 3. This alternative hypothesis suggests that the absolute spatial distinctiveness of isolated test phase arms, in reference to other test phase arms, enhances working memory for those locations. This hypothesis is derived from the von Restorff effect (Hunt, 1995; von Restorff, 1933) and relies on the assumption that rats use representations of anticipated events in working memory (i.e., prospective memory) to guide choice behaviour in the test phase. Cook et al. (1985) demonstrated flexible use of retrospective and prospective memory in a two-phase working memory task on the radial maze. Rats should use prospective memory in the test phase because it is cognitively efficient compared to continually using retrospective memory (Cook et al., 1985; Riley et al., 1981). To test if rats are behaving this way, an experiment could be designed to observe the use of prospective memory and quantifiably manipulate the degree of spatial distinctiveness of isolated and non-isolated test phase arms. If absolute spatial distinctiveness is varied, then its potential modulation of the spatial isolation effect could be observed. Further experiments are required to support this hypothesis as well as discount motivational factors more confidently.

There is the additional concern of extramaze cues and how they may affect the spatial isolation effect. In all three experiments, the extramaze cues were large objects that were placed at least 30 cm from the ends of maze arms (see Experiment 1 Methods). These cues were not aligned with any particular arm and generally could be viewed from several arm locations. It has been well demonstrated that rats are attentive to visual extramaze cues in radial maze tasks (Babb & Crystal, 2003; Olton & Collison, 1979; Suzuki et al., 1980). Most notably, Suzuki et al. (1980) disrupted working memory performance on the radial maze by randomly redistributing extramaze cues during the RI of a two-phase task. It would be interesting to see if the specificity of extramaze cues affects the spatial isolation effect. Perhaps the effect may not be observed when each arm location is given a unique extramaze cue. Evidence supporting that suggestion would imply that the spatial isolation effect may only occur when spatial cues are general enough to be associated with multiple locations. Simple manipulations of spatial cue size and position could be useful in an experiment and could reveal further findings about the dynamics or conditions in which this spatial isolation effect occurs.

5.5 Concluding Statement

In conclusion, I conducted three experiments that examined how rats maintain and differentially retrieve information about multiple locations in spatial working memory. In Experiment 1, rats were found to perform differently across spatial arm patterns without the use of response strategies. Analysis of within-trial responses suggested that rats were performing better on isolated test phase arms than non-isolated test phase arms. In Experiment 2, I investigated whether the performance differences across pattern were due to the number of isolated arms found in each pattern. I found that the number of isolated test phase arms in pseudo-random patterns did not affect general accuracy, but confirmed that within-trial accuracy was greater for isolated arms than non-isolated arms. I then investigated a possible underlying mechanism for the spatial isolation effect. I asked if rats were more motivated to visit isolated locations because they were between previously rewarded study phase arms. Depleting sucrose pellets in study phase arms that were adjacent to isolated test phase arms did not affect within-trial accuracy for isolated arms. Instead of motivational factors modulating the spatial isolation effect, the absolute spatial distinctiveness may explain performance differences across isolated and non-isolated test phase arms. Further experimentation is required to uncover the specific conditions and mechanisms of this spatial isolation effect.

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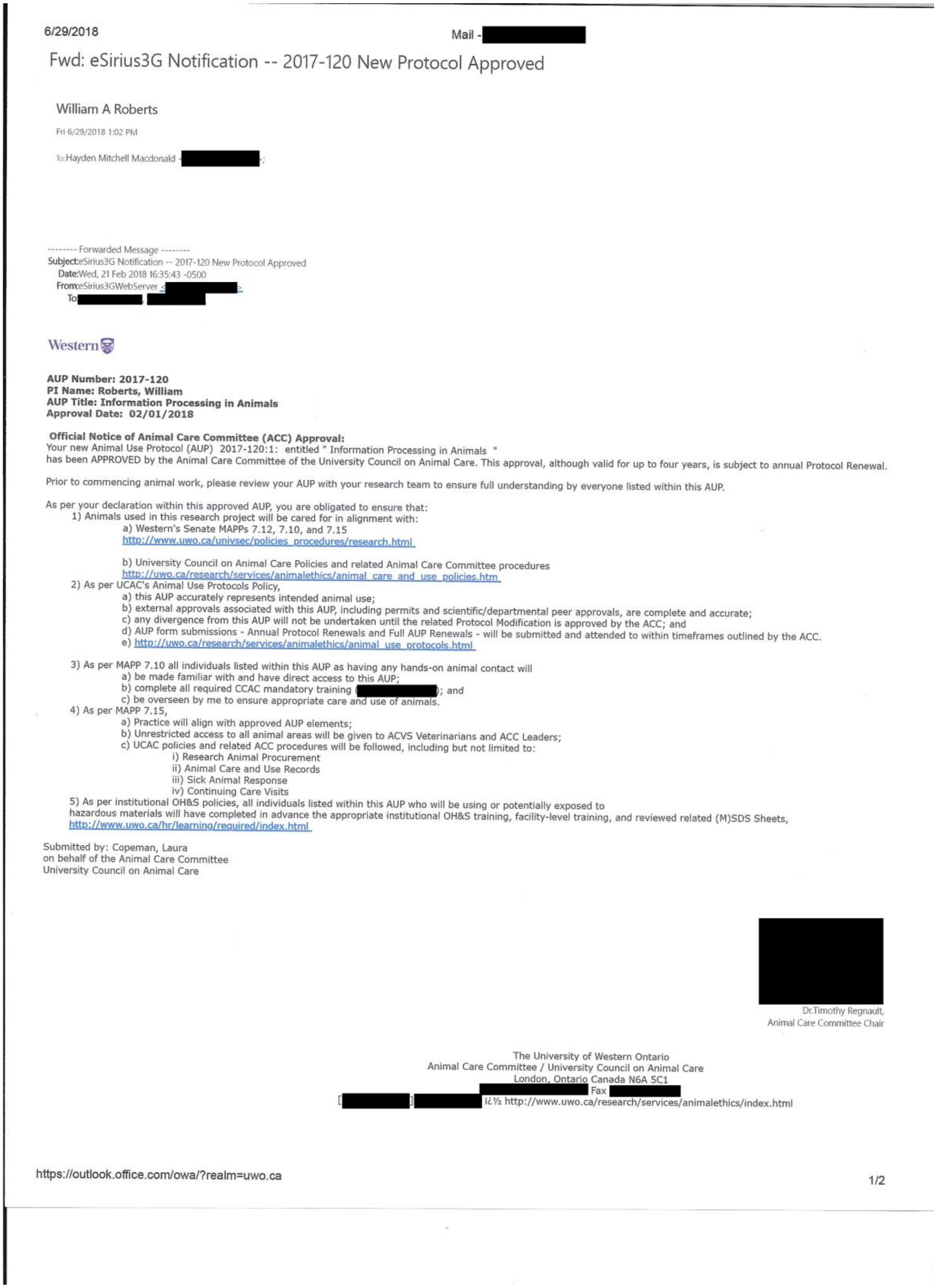
Appendix 1



6/29/2018 Mail - [redacted]

Fwd: eSirius3G Notification -- 2017-120 New Protocol Approved

William A Roberts

Fri 6/29/2018 1:02 PM

To:Hayden Mitchell Macdonald <[redacted]>;

-------- Forwarded Message --------
Subject:eSirius3G Notification -- 2017-120 New Protocol Approved
Date:Wed, 21 Feb 2018 16:35:43 -0500
From:eSirius3GWebServer <[redacted]>
To[redacted], [redacted]

Western

AUP Number: 2017-120
PI Name: Roberts, William
AUP Title: Information Processing in Animals
Approval Date: 02/01/2018

Official Notice of Animal Care Committee (ACC) Approval:
Your new Animal Use Protocol (AUP) 2017-120:1: entitled " Information Processing in Animals "
has been APPROVED by the Animal Care Committee of the University Council on Animal Care. This approval, although valid for up to four years, is subject to annual Protocol Renewal.

Prior to commencing animal work, please review your AUP with your research team to ensure full understanding by everyone listed within this AUP.

As per your declaration within this approved AUP, you are obligated to ensure that:
1) Animals used in this research project will be cared for in alignment with:
    a) Western's Senate MAPPs 7.12, 7.10, and 7.15
    http://www.uwo.ca/univsec/policies_procedures/research.html

    b) University Council on Animal Care Policies and related Animal Care Committee procedures
    http://uwo.ca/research/services/animalethics/animal_care_and_use_policies.htm
2) As per UCAC's Animal Use Protocols Policy,
    a) this AUP accurately represents intended animal use;
    b) external approvals associated with this AUP, including permits and scientific/departmental peer approvals, are complete and accurate;
    c) any divergence from this AUP will not be undertaken until the related Protocol Modification is approved by the ACC; and
    d) AUP form submissions - Annual Protocol Renewals and Full AUP Renewals - will be submitted and attended to within timeframes outlined by the ACC.
    e) http://uwo.ca/research/services/animalethics/animal_use_protocols.html

3) As per MAPP 7.10 all individuals listed within this AUP as having any hands-on animal contact will
    a) be made familiar with and have direct access to this AUP;
    b) complete all required CCAC mandatory training ([redacted]); and
    c) be overseen by me to ensure appropriate care and use of animals.
4) As per MAPP 7.15,
    a) Practice will align with approved AUP elements;
    b) Unrestricted access to all animal areas will be given to ACVS Veterinarians and ACC Leaders;
    c) UCAC policies and related ACC procedures will be followed, including but not limited to:
        i) Research Animal Procurement
        ii) Animal Care and Use Records
        iii) Sick Animal Response
        iv) Continuing Care Visits
5) As per institutional OH&S policies, all individuals listed within this AUP who will be using or potentially exposed to hazardous materials will have completed in advance the appropriate institutional OH&S training, facility-level training, and reviewed related (M)SDS Sheets,
http://www.uwo.ca/hr/learning/required/index.html

Submitted by: Copeman, Laura
on behalf of the Animal Care Committee
University Council on Animal Care

[redacted]
Dr.Timothy Regnault,
Animal Care Committee Chair

The University of Western Ontario
Animal Care Committee / University Council on Animal Care
London, Ontario Canada N6A 5C1
[redacted] Fax [redacted]
[[redacted]] [redacted] ï¿½ http://www.uwo.ca/research/services/animalethics/index.html

https://outlook.office.com/owa/?realm=uwo.ca 1/2

Curriculum Vitae

Name:	Hayden MacDonald
Post-secondary Education and Degrees:	The University of Western Ontario London, Ontario, Canada 2012-2016 B.Sc.
	The University of Western Ontario London, Ontario, Canada Expected Graduation 2018 M.Sc.
Honours and Awards:	NSERC Canada Graduate Scholarship - Masters 2017-2018
	Dean's Honor List 2014-2015, 2015-2016
Related Work Experience	Teaching Assistant The University of Western Ontario Fall 2016, Winter 2017, Winter 2018
	Research Assistant Roberts Comparative Cognition Lab The University of Western Ontario Summer 2015, Summer 2016

Publications:

Roberts, W. A., **MacDonald, H.** & Lo, K. H. (2018). Pigeons play the percentages: Computation of probability in a bird. *Anim Cogn*, *21*(4), 575-581. doi: 10.1007/s10071-018-1192-0

MacDonald, H., & Roberts, W.A. (2018). Cognitive flexibility and dual processing in pigeons: Temporal and contextual control of midsession reversal. *J Exp Psychol Anim Learn Cogn*, *44*(2), 149-161. doi:10.1037/xan0000161

Roberts, W.A., **MacDonald, H.**, Brown, L., & Macpherson, K. (2017). Release from proactive interference in rat spatial working memory. *Learn Behav*, *45*(3), 263-275. doi:10.3758/s13420-017-0263-4

Roberts, W.A., Guitar, N.A., Marsh, H.L., & **MacDonald**, **H**. (2016). Memory systems in the rat: effects of reward probability, context, and congruency between working and reference memory. *Anim Cogn*, *19*(3), 593-604. doi:10.1007/s10071-016-0964-7

Conferences

MacDonald, H., & Roberts, W. A. (2018, April). *Spatial memory for isolated arm locations on the radial maze*. Five minute talk presented at the International Conference On Comparative Cognition, Melbourne, Florida.

MacDonald, H., & Roberts, W. A. (2017, April). *Time versus context in a visual mid-session reversal task*. Poster presented at the International Conference On Comparative Cognition, Melbourne, Florida.