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RATS' (RATTUS NORVEGICUS) RETRIEVAL
OF INFORMATION FROM THEIR NON-
SPATIAL AND SPATIAL WORKING
MEMORY IN OBJECT RECOGNITION
TASKS

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RATS' (*RATTUS NORVEGICUS*) RETRIEVAL OF INFORMATION FROM THEIR NON-
SPATIAL AND SPATIAL WORKING MEMORY IN OBJECT RECOGNITION TASKS

by

Marium Arain

A Thesis
Submitted to the Faculty of Graduate Studies
through Biological Sciences
in Partial Fulfillment of the Requirements for
the Degree of Master of Science at the
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2011

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RATS' (*RATTUS NORVEGICUS*) RETRIEVAL OF INFORMATION FROM THEIR NON-
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August 30th, 2011

DECLARATION OF ORIGINALITY

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ABSTRACT

Spatial memory is important for animals to achieve successful foraging in structurally complex habitats. Animals use consistent patch location as a reference to locate food. To use objects as navigational landmarks, animals must encode the specific spatial location and non-spatial features of the location of hidden food within their working memory. How animals use both the spatial and non-spatial aspects of landmarks and beacons is a major theoretical question in the study of their cognitive processes. The two objectives of this thesis were to determine whether spatial encoding is absolute or relative and how much of the spatial and non-spatial information is encoded. Also, we expanded Brodbeck's (1994) method for investigating multi-dimensional cue preferences. Results showed the flexibility of rat's visuo-spatial working memory. The baseline results obtained from my study will be helpful in conducting comparative studies on working memory across species and to study various disease models affecting working memory.

DEDICATION

I dedicate my thesis to my family, especially my Mother whose love, care and support have enabled me to do so well in my academic and personal life.

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CHAPTER I
GENERAL INTRODUCTION

Working Memory in Foraging context

Animals require visuo-spatial working and reference memory to perform various functions in their daily activities. These include memory for remembering food locations and returning home, keeping track of dangerous areas and optimally selecting the most efficient route when foraging (Spetch and Kelly, 2006). Understanding the nature and capacities of these memory processes has implications in conducting comparative studies and medical pharmaceutical experiments. My MSc thesis focuses on working memory processes in foraging behaviour by Long Evans Rats (*Rattus Norvegicus*). Specifically, I investigated the flexibility of rats' visuo-spatial working memory encoding and retrieval capacity (Chapter 2) and the factors which influence their retrieving information required to forage successfully (Chapter 3).

Working and Reference Memory

Working memory refers to the process for temporarily storing information over short durations so that it can be retrieved later (Baddeley, 1992; Potter, 1993). For Baddeley (1992), the definition of working memory has evolved from more than simply as another term for short term retention. One criterion that distinguishes working from short term memory is the organism's ability to simultaneously store and process information in working memory whereas short-term memory refers simply to maintenance of information for only a brief period of time. Working memory has been considered to consist of three components: 1) Central executive 2) Visual spatial sketch pad and 3) Phonological loop (Baddeley, 1994). The central executive carries out executive functions such as determining retrieval strategies, selectively attending to various stimuli and coordination of the other two systems (Baddeley, 1996). Visual sketch pad

controls information retrieved from visual imagery (Baddeley & Hitch, 1974; Baddeley, 1994). Phonological loop is important when learning a language (Gathercole, 1999; Baddeley 1998).

There are different criteria when defining animal working memory; there is a fine line between working, reference and associative memory (Honig, 1978; Baddeley, 1974). ‘Reference memory’ is analogous to human long term memory. Moreover, it has two salient features. Firstly, it is mostly inactive until needed and it is activated by the representation of appropriate features and cues that help animals retrieve the information stored in their long term memory (Honig, 1978; Nairne, 2002). Secondly, information in reference memory should be stable to allow animals to accurately perform on many similar tasks. On the other hand, working memory is defined as temporary retention of information required for successful responding on a task at hand but not on subsequent (or previous) similar tasks (Honig, 1978). Another difference is that working memory has a limited capacity in the amount of information it can hold and retrieve and is transient in the duration that such information can remain active or held. Thus working memory holds newly supplied information or old, recently refreshed (activated) information from reference memory (Baddeley, 1992). In contrast, the duration and amount of information in reference memory is relatively greater. The ability of reference memory to hold information over longer periods of time without any loss or degradation may be responsible for its greater storage capacity. In working memory events are often associated with temporal or personal contexts while those in reference memory, events are processed independently of such contexts (Honig, 1978). Moreover, information in working memory is only processed serially (Garavan, 1998), whereas information in reference memory can also be processed in a parallel fashion for access (Logan & Stadler, 1991).

Working Memory Paradigms in Animals

The distinguishing features of working from reference memory have led to three different basic procedures to investigate it in animals. Results from these paradigms have contributed to further theoretical notions of working memory. The three basic tasks are as follows:

Delayed alternation: This task is conducted in a T-maze over successive trials in which rats typically spontaneously alternate in choosing side arms for rewards. Such alternating choice patterns, however, sometime decline as delays between trials increase even when choosing the same arm in succession is not rewarded. Such reductions are considered to reflect loss of visuo-spatial retention of the position of a previously visited arm (Honig, 1976). Capaldi and Stanley (1963) initially demonstrated working memory retention in three experiments (as described by Honig, 1976) by demonstrating above chance alternation for delays (retention intervals) of 20 minutes. In these experiments, rats also tended to run more slowly when they chose the same arm (an incorrect choice) than the opposite arm (a rewarded correct choice) over trials. The working memory displayed in these experiments declined as retention interval were increased but remained reliably above chance levels. A more recent study (Futter & Aggleton, 2006), however, found that rats reduced their alternation performance to chance when retention intervals were increased to only 10 seconds. It is believed that associative memory must be active in such experiments (Lett, 1975). This paradigm has also been used in other studies shown to effectively measure working memory (William, White & Messer, 2002; Wortwein, Mogensen & Divac, 1994; Sanchez, De Bruin, Heinsbroek, & Verwer, 1997; Markowska, Koliatsos, Breckler, Price & Olton, 1994).

Radial Maze Tests of spatial memory: The third procedure of measuring visuo-spatial working memory is very different from the two previously described paradigms and was first described and investigated by Olton and Samuelson (1976). The basic protocol consists of placing a rat in the center of an elevated open maze with 8 radially projected arms, each baited at the end with food. The rat is permitted to visit the arms in any order to obtain food from all the arms. Olton and Samuelson (1976) found that rats tend to seldom revisit previously baited arms before obtaining all the food, but on those occasions when they did revisit previously sampled arms they did so at the end of a trial. Furthermore, such revisits occurred to arms that were first visited at the beginning of the trials. Several of their experiments ruled out that such accurate foraging could not be attributed to rats making some type of fixed choice algorithms within or over trials by scent-marking randomly visited arms during a trial. Rather, results from their and other subsequent studies (e.g., Bond, Cook & Lamb, 1981; Cook, Brown, & Riley, 1985) suggest that rats were maintaining a working memory of the locations of arms visited (retrospective memory) or yet-to-be visited (prospective memory) in a trial. Moreover, patterns of arm visits remained at chance over successive trials and number of revisits did not systematically vary over trials. These effects suggested that working memory is reset to prevent rats from confusing the arm entries from the previous trials with having entered in current trial. There is no trace of decay in Olton's model of working memory; however, it is able to be reset if it is no longer in use. This has led researchers to conclude that working memory is stored with no time constraint, yet it can be reset or terminated depending upon its use in future (Sharma, Rakoczy & Brown, 2010). Our basic task consisted of aspects of these three types of paradigms that allowed us to investigate factors necessary for rats to retrieve information from their working memory.

Novel Object Recognition: A more recent and increasingly popular paradigm for assessing visuo-spatial working memory in animals is the Novel Object Preference (or Recognition) test first introduced by Ennaceur (Ennaceur & Delacour, 1988; Ennaceur, Cavoy, Costa & Delacour, 1989). The task takes advantage of the fact that when exposed to previously experienced (familiar) and a novel object, rodents will spend more time investigating the latter. Such preferences for the novel object were considered to reflect rats' working memory for the familiar object and hence this behaviour (NOP: novel object preference) is sometimes called object recognition memory. Unlike the other types of working memory tasks, NOP tasks can be observed within a single trial and hence need no training other than to habituate rats' fear of the area in which objects will be placed.

The “novelty preference model” of NOR proposed by Bahrck, Hernandez & Pickens (1997) maintains that memory for familiar objects must be highly accessible for a novelty preference effect to be observed. According to this model, as the retention time increases between the sample and choice phases, rats develop preferences for familiar over novel objects. This model suggests the existence of three memory phases: A recent memory phase that follows very short delays (e.g. 1 min) where novelty is highly preferred, a remote memory phase that occurs after very long delays (e.g. 7 weeks) that produces familiarity preferences, and an intermediate memory phase following delays between one day to two weeks where no preferences occur. Evidence supporting this model has come from studies with human infants (Bahrck & Pickens, 1995; Bahrck et al., 1997; Bahrck, Gogate & Ruiz, 2002; Spence 1996) and adults (Richmond, Colombo, & Hayne, 2007).

Sheldon (1969) found that rats showed a preference for a familiar object when exposed to a novel environment but switched to a consistent preference for novel objects after repeated

exposure to the environment. Mumby (2002) found that rats with lesions to the perirhinal cortex preferred familiar to novel objects after 15 min retention intervals while sham operated control rats continued to prefer the novel objects. Although lesions to rats' perirhinal cortex disrupt their bias to investigate novel objects, such novelty preference is necessary to determine object recognition as a function of working memory (Winters, Bartko, Saksida & Bussey, 2009). Lesions to the perirhinal cortex also disrupt accuracy in reinforced non-matching to sample tasks where a bias to prefer a novel item is less likely. Therefore insult to this area may also disrupt memory in general. Ennaceur (2010) suggested that novelty preference models and the innate novelty bias hypothesis do not distinguish between object preference and novelty preference. Rather rats may prefer an object to its physical properties and hedonic values associated with it. A representation of the idea of novelty needs to be established by the rat before its preference for such an object can be considered driven by working memory processes. Thus "novelty preference" is an ambiguous concept inappropriately used in place of novelty detection and exploration.

Ennaceur (2010) concludes that one trial object recognition tests are not valid measures of working memory because the animal does not yet realize that it will need to form a representation of the familiar objects to later compare with a novel object. That is because animals have no reason to expect a subsequent choice segment and therefore will not have received any explicit instructions to attend to particular stimuli. This problem suggests that more than single sample stimuli and test presentations are required to ensure a valid measurement of working memory.

Delayed matching to sample tasks (DMTS): The DMTS is considered a more valid measure of working memory than the NOP paradigm when the to- be-remembered stimulus is held in

memory only for the next test segment of the trial in a session of many trials over many sessions. Within this paradigm, assessment of working memory on non-spatial aspects of sample stimuli was possible with the introduction of object recognition tasks when animals are food deprived and tested over several similar types of trials. These tasks require an animal to encode a specific representation of a trial's sample stimulus to be retrieved only for its test segment and not for subsequent trials which may have different sample and comparison stimuli.

The DMTS task was first devised by Blough (1959) and is currently being used by many researchers. Blough (as described by Honig, 1976) used pigeons as subjects. The pigeons (*Columia Livia*) were presented with a sample stimulus of one lit key among an array of three keys. After the pigeon pecked the lit key, the key darkened and after a retention interval, two keys became illuminated with one of them being the original sample key. Reinforcement was contingent upon the pigeon pecking the sample key. The locations of the sample key and its non-reinforced alternate lit key randomly varied over trials. Accuracy for selecting the sample key during test segments was close to 90-95% with no delay in presentation of comparison stimuli but gradually decreased to 60-75% as delays increased to 5 sec. With expanded training, pigeons were able to maintain 60-70% accuracy over 60 sec retention intervals (Grant, 1975).

The delayed matching to sample tasks is a conditional discrimination task in which an animal is presented with a stimulus and later exposed to a delay without the stimulus followed by the same sample stimulus and at least another stimulus at different locations. The animal has to remember the previously exposed sample stimulus and is reinforced upon either choosing it (the delayed matching version) or selecting the other stimulus (the delayed non-matching version) selecting the to-be-remembered stimulus presented along with an alternative new stimuli.

The stimuli used for DM/NM sample tasks can either be trial- unique or repeated items over trials depending upon the type of memory the researchers want to assess. It has been shown that trial-unique stimuli are important in recognition memory and the use of small number of familiar objects is dependent upon recent memory (Mumby 2001). In one of their experiments, Mumby, Pinel, and Wood (1990) used trial unique objects and the rats were trained to obtain food from one of the sample object. After a delay of variable time, rats were exposed to sample as well as novel object at the opposite end of a runway and were rewarded for choosing the novel object. The delay in between the sample and test segments of each trial was increased from 4 s up to 600 s. A delay dependent decrease in choice accuracy is found but was still maintained above chance. Other studies with trial-unique stimuli have replicated these findings (Kesner, Bolland & Dakis, 1993; Mair, Burk & Porter, 1998).

A possible confounding variable that could account for above chance accuracy in this DNMS task was that rats might be using an odour cue to locate the new objects as the sample object is typically handled twice in a trial but the novel object is only handled once. To discount this possibility, Mumby (2001) tested rats with identical objects in both the sample and test segments with reinforcement being contingent on rats' choosing of most recently handled objects. While rats could learn and respond accurately on this task with 4-s delays, their accuracy declined to chance with 15-s delays. Furthermore, some studies have measured the rat's capacity to hold odour information in their working memory through the use of DNMS tasks. In one such study (Dudchenko, Wood & Eichenbaum, 2000), rats were exposed of a cup over scented sand which it had to dig into to uncover food in the sample segment and then were exposed to two cups each with different scented sand, one with the original scent and the other with a novel scent. Reinforcement during the test segment of a trial was contingent upon rats' digging in the

cup with the novel scented sand. After another delay they received three cups, two with the sand scented from the previous segment and a cup with novel scented sand and only baited with the hidden food. Rats received successive segments with added baited novel scented cups until they search a cup with a previously experienced scent. The rats were able to perform accurately until they received 24 different odour stimuli. Our experiments made use of delayed non-matching to objects sample task and we also designed our experiment to control for possible confounding differential odour cues as will further described.

Optimal Foraging Behaviour

Those working memory preparations that require animals to avoid previously exposed sample stimuli or visited maze arms in a trial correspond to animals' behavioural predispositions as they forage in their natural environment. That is, animals will tend to avoid previously sampled food sites in a patch and will tend to stop searching such patches after having exhausted most of their available resources. Barring the use of possible cues left by previous visits, e.g., physical disturbances or odour trails or markings, animals would have relied on their visuo-spatial working memory processes. Based on such reasoning, we decided to more formally assess rats' working memory processes in a laboratory environment that more closely approximates their natural environment. Given this goal, we will briefly discuss the basic tenets of optimal foraging theory (OFT). This theory was first identified and developed by MacArthur and Pianka (1966) to help explain the foraging behaviour in animals. Its major postulate is that organisms forage for necessary resources (e.g., food, shelter, mates), to maximize their net energy intake over their expenditure or unit time. In other words, their behaviour is optimally devised to consume the highest quality of food by utilizing the least amount of energy.

According to OFT, an individual's behaviour during foraging also determines its contribution (i.e. fitness) to the next generation by determining its duration of survival (Pyke, 1984).

Of relevance in the present topic is the ability of rats to learn foraging "rules" in order to optimally search among food-baited locations. In many previous experiments, rats were able to learn such rules. For example, Brown and Wintersteen (2004) allowed rats to forage among a 5 X 5 matrix of food-baited columns. There were no visual or olfactory cues to indicate which columns were baited, and the only cue available was the spatial location of the baited versus non-baited poles. The rats always received the same spatial configuration of baited poles relative to each other, although the distinct poles that were baited varied unpredictably. Thus, once the rat found its first baited pole, it was then able to find the other baited poles and avoid the non-baited poles by searching relative to the first baited location, based on the spatial configuration of baited poles experienced in previous trials. The rats were able to learn these spatial patterns to guide their foraging, albeit not perfectly. Rats were able to learn to efficiently forage based on checker board patterns, (Brown & Wintersteen, 2004), square patterns (Brown & Terrinoni, 1996), and linear patterns of baited poles (DiGello, Brown, & Affuso, 2002).

Summary and Thesis Objectives

The overall goal of my thesis was to assess the limits and capacities for working memory processes in rats' foraging behaviour. Chapter 2 investigated whether representations of object locations encoded into rats' visuo- spatial working memory are absolute or relative in nature and the effects of varying the relevance of the spatial component of each item on rats' performance. Chapter 3 investigated the possible dominance and overshadowing aspects of multiple, redundant spatial and non-spatial cues of objects in their retrieval from working memory. I modified a method originally used by Brodbeck (1994) that determined the dominance of visual and spatial

characteristics encoded from a target feeder in song birds. In the current research, we not only were able to assess the relative dominance of similar cues for rats but whether they are encoded as separate or combined representations within working memory. Together, these studies provide a more comprehensive view of the hierarchical system that rodents use in their foraging behaviour. As a more valid test for assessing working memory, this preparation will be useful for conducting comparative studies on such processes across different species and also may be more easily applied to medico-pharmaceutical investigations.

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CHAPTER 2

RELATIVE RULE LEARNING IN RAT (*RATTUS NORVEGICUS*)

Chapter Summary

Spatial memory is important for animals to achieve successful foraging in structurally complex habitats. Animals use consistent patch location as a reference to locate foods which are present at or some distance away from these patches. We investigated the effects of stability of an array of landmarks on a rat's working memory. We trained rats to obtain sunflower seeds from four object-cued baited food stations arranged in a square configuration in a large square arena. The array (patch) consisted of either four identical or different junk objects. In the study segment, rats had to select the three randomly experimenter-selected object-cued food sites while in the test segment they had to find the remaining object-cued food well, the "jackpot". In the first phase of the experiment, rats improved their accuracy for finding the jackpot in the second half of the 24 training trials. In the second phase of the study, rats' performed equally well when the location of the test segment was moved than when it stays the same as that of the study segment. In the third phase, increasing the distance between the four objects from the study segment reduced the rats' accuracy for finding the jackpot especially when the array was widened to the corners of the foraging arena and the jackpot location was moved from that of the study segment. These results indicate that the rats are able to learn a geometric relationship among objects thus demonstrating the flexibility of their memory processes. Moreover, these results show that rats flexibly use global and local location information of the jackpot site and memory of the missing specific object from the study segment during the test segment. As a pilot study, we suggested improvements to develop a better working memory task.

Introduction

In the wild, non-human animals from many phyla use their spatial and non-spatial memory to accomplish many tasks, for example locating food, water, or home territory. Mammals, for example, house mice (*Mus Musculus*) use landmarks to create routes in an experimental arena to retrieve pups (Aylan & Jander, 1994). In cephalopods (*Cephalopoda*), several species of octopuses use their visual-spatial memory for navigation when tactile stimuli are not available in both field and laboratory environments (Mather, 1991). In insects, for example ants of the species *Formica rufa* use visual-spatial memory to create stable routes based on the arrangement of beacons in an experimental arena, driven by the goal of finding food (Graham, Fauria, & Collet, 2003). These studies illustrate the importance of visual cues in visual-spatial memory in both natural and experimental settings.

A well-developed visual system is essential for navigation. Cognitive map navigation models state that as an animal explores a new territory, it assimilates visual information in the form of a spatial cognitive map used as a reference for exploration (Tolman, 1948). Animals do not form these cognitive maps instantaneously, but rather initially use general or their species-specific innate path-integration navigational systems, and as they become more familiar with the region, switch to a cue-controlled strategy (O'Keefe & Nadel, 1979). More recent hypotheses point towards a "snapshot" matching model of navigation, evident in many hymenoptera species, whereby animals store snapshots of their environment and attempt to match stored snapshots with their current field of view [Ants (*Cataglyphis bicolor*) (Wehner & R ber, 1979; Harris, Graham & Collet, 2007); bees (*Apis mellifera*) (Cartwright & Collett, 1983); wasps (*Sphecidae Cerci*), (Zeil, 1993a)]. Navigation in this model is accomplished when the animals reduce the discrepancy between stored and current snapshots (Cartwright & Collet, 1983; Cheng, 2008).

In contrast to spatial navigation through path-oriented strategies, using spatial and non-spatial landmarks is advantageous because it allows the animal to orient itself in any direction relative to a stable landmark (Aylan & Jander, 1994). Despite this advantage, when rats are given a short-range navigation task with a redundant landmark over many trials and then probed to determine whether their navigation strategy consisted of egocentric pathways or landmark cues, they used both equally well (Tamara & Timberlake, 2011). However, as rats became experienced with the task and then started navigating from a novel location, they first oriented themselves with a route-based strategy and then located food by a landmark-based strategy. Our study is designed to test various factors that are essential in the landmark based search strategy in rats.

Memory tasks can be designed to isolate or combine two memory processes critical for visual spatial memory. Animals use their long-term, or reference memory when performing a task involving landmarks that provide information on the location of food at a constant distance away from the landmark, and use their short term, or working memory to help complete a task involving beacons that indicate the location of food in the immediate vicinity (Spetch & Kelly, 2006). Rats have been shown to learn the relative relationship between a landmark and hidden food when they are geographically constant across trials, but not when the locations are relatively constant but geographically unstable (Biegler & Morris, 1993). These findings suggest that local cues within the area of foraging are distinct from global cues that are stable across the entire task. Studies have shown that one of the passerine species hierarchically code these cues in their environment (Brodbeck, 1994). By elaborately manipulating the variables in this study, Brodbeck found that black-capped chickadees (*Parus atricapillus*), a food storing species, hierarchically used foraging cues by first focusing on the target feeder's global location followed by its local (with array) location, and finally on its non-spatial visual patterned cues. Dark-eyed

Juncos (*Junco hyemalis*) a non-food storing species, on the other hand, used all three sources of information in a non-hierarchical search pattern. According to Brodbeck (1994), these findings highlight the importance a species' evolved ecological niche on its foraging strategy.

Depending upon the availability of landmarks or beacons, animals can use different cues to develop associations between them and food locations. They can rely upon objects' local or global locations, their orientations (i.e., where they are 'pointing'), and their non-spatial featural aspects (e.g., color, visual patterns, texture, odour). Spetch and Edwards (1988) demonstrated that pigeons encode multiple cues in a hierarchical manner to locate hidden food. In that study, pigeons learned to always collect hidden food from a middle canister of a set of three identical linearly adjacent canisters. The fixed location of the three cartons allowed the pigeons to find the correct middle carton by paying attention to location in the room (global cues) or its position in the array (local cue). In post-acquisition tests in which the array was shifted to place global and local position cues in opposition, pigeons first selected the canister in the 'correct' global position before selecting the canister in the 'correct' local middle position. When the array was shifted to a completely different location in the room, pigeons consistently selected the correct middle canister. This means that information from both cues were encoded. Research on ground squirrels (Vlasak, 2006) showed that the animals needed both local and global landmarks of the environment for successful navigation, and they could not use information of either to compensate for the removal of information from the other. But, they used local landmarks as secondary information for navigation and did not necessarily attend to them over a familiar route when global landmarks were present. In our study, we were interested in determining whether rats can remember the correct local food location when its global location is no longer relevant.

If so, then we may conclude that rats can use different redundant spatial information in their working memory in a flexible than in a rigid manner.

Another aspect of our study is to determine if the rats can learn the relational rule among different beacons to find the correct goal location when an array of 4 objects is expanded (widened) from that in which they were initially trained. A goal location in relation to a set of identical looking landmarks, also landmark arrays, has been extensively studied by the researchers in investigating the pattern of animal search behaviour. Collet (1986) trained gerbils (*Gerbillus amoenus*) to collect food that was present between two identical landmarks. The animals had to search in the middle and a calculated distance south of those landmarks which compelled the animals to pay attention to both landmarks. They were successful in determining the correct location between the landmarks and food. When the landmarks were spread apart, the gerbils maintained the correct location vector from individual landmarks showing their inability to learn the abstract relationship between the goal and landmark. Spetch, Cheng, and MacDonald (1996) performed the same experiment on humans and pigeons using the touch screen task. On the expansion tests, humans remained centered between the landmarks and tended to shift their searching downward. Pigeons responded much more like gerbils and showed no tendency to shift their searching downward or to respond in the middle of the landmarks. The similar results were found for monkeys showing their inability to use abstract relationship (Macdonald et al., 2004). Cartwright (1983) showed that honey bees are similar to humans when tested on this task. Bees learned to find food at a particular spot relative to a triangular array of three landmarks. When the distances between the landmarks were altered to expand or contract the array, the bees adjusted their distance of searching in such a way that they always found the correct location. Of relevance in the present topic is the ability of rats to learn the relational rule among the objects.

We want to see if rats can learn to relate the objects upon extending the array and if so to what extent.

In the present study, we sought to teach rats a rule-governed foraging strategy using object-cued food sites that were composed of identical or different objects as inanimate beacons. Our arena consisted of a 5X5 matrix of wells, which were covered with non-movable pucks except for four wells which contained foraging stations. I designed a study as part of my undergraduate thesis last year where we studied whether the same factors affecting rats' retention in their reference memory of food site locations based on navigational landmarks (Biegler & Morris, 1993, 1996, 1999) operate in the same way as their retention of beacon-cued food site locations in their working memory. In our previous study, we used random positions within a square array of different objects across trials. In the present study, we made the task easier by exposing each rat to a fixed configuration of objects in the array across all phases so that they could learn each object's local within array position. We expected this design to allow rats to better focus on objects' local positions to help answer our second research question to be described later.

In order to determine whether rats adopt the same search patterns to collect hidden food in a working memory task as they do in a reference memory task, we used the basic experimental procedure developed by Brown, Yang and Digian (2002) combined with a modification of segmented-trial 4-arm radial maze working memory task (Cohen and Bussey, 2003; Tremblay and Cohen, 2005) used in my honours thesis. In our present study task, rats had to approach three closely adjacent object-cued feeding stations located within a relatively large enclosed square foraging arena during a study segment and then, after a delay, had to find the fourth remaining baited cued (jackpot) station that completed a square configuration of the four objects.

This experiment consisted of 4 phases with each trial divided into a 3-object study segment and a 4-object test segment. In the study segment, the rats had to collect one seed from each of the three object-cued baited stations. After a 2-min delay, the rat had to find the fourth added object that completed the square array configuration and was baited with three seeds. The dependent variable for our experiment was the number of choices rats made to find the jackpot in the test segment. We were interested in determining the effects of nature of the object array (different vs. identical objects), the stability of the within-trial location of the array and the effect of expanding the local array of the four objects on rats' accuracy for finding the jackpot.

We asked three basic questions. First, do the rats find the missing object (jackpot) more easily among arrays with different than identical objects? In other words, does adding non-spatial featural information with the local and global spatial information help rats find the correct location? Secondly, is rats' accuracy for finding the jackpot better when the array location of the test segment stays the same than when it is moved within a trial? In other words, can rats use only the local positions of the objects within the array to locate the correct object when its global position is made irrelevant? Thirdly, will they be able to perform with the same level of accuracy when we expand the square array over a larger area? We hypothesized that rats will perform better with different than identical objects as they will be able to use additional featural information of each object with other spatial sources of information (local and global position). Such predictions are based on traditional principles of association learning theory (see Domjan, 2009). Also, we expected that moving the test configuration will reduce rats' accuracy for finding the jackpot. Widening the array also allowed us to determine whether rats' use of local position of the jackpot was primarily determined by relative position of objects or also by the distance between them. If the latter were true, then rats should switch from primarily using the

former to attempt to use the latter. Thus widening the array would have greater disruptive effects when the jackpot's global position was changed.

Method

Subjects:

Seven male Long-Evans hooded rats purchased from Charles River Breeding Farms, St. Constant, Quebec, served in this study. They were three months old and weighed over 300 g at the beginning of the study. They were fed 20-25 g of food (Rodent Chow) for 2 h in standard stainless steel individual holding cages after each experimental session before being returned to their large group holding cages (three rats per cage) in the colony room. Water was freely available in group and holding cages. This regimen maintained rats at approximately 90% of their free-feeding weights. The colony room was maintained on a 12:12 h dark/light cycle and experimental sessions began within three hours of the beginning of the dark cycle that commenced at 8:00 AM.

Apparatus and Materials:

The apparatus that we used is the same as that we used for my undergraduate honours thesis with some modifications. It consisted of a 1.2 m square aluminum foraging platform that stood 56 cm above the floor of the experimental room. It was enclosed by 46 cm high grey wood walls and was surrounded by a black curtain suspended from the room's ceiling. A rat could enter and exit this chamber when the observer raised a black plastic guillotine door that connected any one of four standard stainless steel individual holding chambers located midway along a wall of the arena. Each door was controlled by a string and pulley system from one location near the observer who viewed and recorded a rat's activities inside the arena from an externally located video monitor connected to an overhead camera.

The floor of the arena was fitted with 25 holes arranged in a 5 by 5 grid with 20.3 cm between each hole (Fig. 1). Each hole allowed the experimenter to either insert a foraging station or a metal cylinder that blocked off the hole. The foraging stations were made of a rectangular block of aluminum (16.5 cm by 7.6 cm by 2.5 cm) with a small horizontally sliding metal plate that the rat could push with its nose to expose a reward (Fig. 2). Plain sunflower seeds were used for rewards. The horizontal sliding plate could be unlocked to allow the rat to uncover the baited food well or locked so that rats could only partially uncover the baited food well without allowing them access to the seeds. This arrangement prevented food wells with accessible bait from having different food odors from those with inaccessible food. Because of the ease of labelling we call the former ‘baited’ and the later ‘unbaited’ food sites. A magnet was mounted onto the horizontally sliding plate that allowed the experimenter to attach a junk object to each foraging station. We used five different types of junk objects consisting of four white golf balls, four plastic angels, four bronze iron fleeces, and four green Lego® cube, metal springs. Figure 3 shows an example of each of these types of objects. The reason for using replicates of each type of object will be become clear in our procedure. Each object had a flat head metal screw in its base allowing it to be attached to the magnet on the food site station.

Procedure:

Phase 1- Object-cued disk training: Each rat received one trial per day for up to 10 minutes in the foraging arena over a two week period. Throughout the experiment, rats always entered and exited the foraging arena through the start chamber on the west wall. We placed 10 feeding stations randomly placed among the 25 disks in the arena. We filled the wells in the stations with the sunflower seeds and allowed those stations to remain uncovered over the first week. We used only four uncovered, object-cued food stations arranged in square array from the

second week on. We moved the square array of the object-cued food sites in a non-sequential pattern over trials so that locations of any objects from one trial never overlapped with those on the previous trial. The subjects were shaped to push open the small horizontal window on the foraging stations by first training them to eat sunflower seeds from the fully exposed well open, and then by gradually closing the cover in small increments. A rat reached criterion for successful training when pushed open the completely closed cover from each food well of the four foraging stations within five minutes. Once training was complete, the rats were moved onto the task. During shaping, we also reduced the number of seeds under each object from four seeds to one seed. As soon as a rat had sampled all baited object-cued wells or three minutes had elapsed, the experimenter lifted up the guillotine door of the start chamber to allow it to exit the foraging arena. Before advancing to the second phase of this experiment, a rat had to open each baited well. No rat required more than five sessions to achieve this criterion.

After having learned to slide the plate from each stations food well, we introduced each rats to its five objects. We randomly determined an array's object-cueing condition (identical or different objects) over trials, and the type of objects in the identical objects condition and of the different objects for each rat. The positions of each rat's set of different objects within the array remained fixed on each trial and over all trials in this experiment. This is something that we made differently from my undergrad thesis experiment. We note that this procedure departs from that in my honours thesis where rats received variable array configurations of different objects over trials. Thus each rat received a fixed array of objects at a different location on any one trial. Throughout all phases in this experiment, we misted the foraging area with a weak anti-bacterial detergent solution and wiped it dry between trials and, in subsequent phases, between each trial

segment. We also varied the order of rats to be run in each session to further prevent any build-up or maintenance of odour trails.

Phase 2 Segmented-trial Training: After a rat had learned to push aside each of the four different or identical object-covered feeding stations for sunflower seeds, the rats were moved onto the working memory version of the task. Each rat received two segmented trials per session over 24 daily sessions (total of 48 trials). Before receiving its second segmented trial of a session, a rat had to wait in its individual holding cage outside the experimental running room until all other rats had finished the first trial. This procedure generated approximately a 60-min inter-trial-interval for each rat. Within each trial, however, the rat experienced a study segment consisting of three experimenter-selected object-cued food stations, each baited with one seed, and a test segment consisting of all four object-cued sites with only the previously missing fourth object baited. We baited the remaining object-cued site with three seeds, equal to as many seeds as could be obtained in the study segment, to insure rats would continue searching for the jackpot in an otherwise less densely baited test array. The positions of the jackpot in the different object-cueing condition were determined so that a jackpot's position was never repeated on more than two successive trials. Moreover, the location of the study and test arrays were always the same within each trial but their locations continued to vary over trials. Also, we only used four different locations (one of each corner of the chamber) equally and distributed throughout this phase trials so that the same location across trials never occurred over more than two successive trials. After collecting its seeds from the three objects in a study segment and returning to the start chamber, a rat was removed from the apparatus and placed into a solid opaque polycarbonate holding cage located in the running room. It waited in that cage approximately

two minutes while the experimenter prepared the arena for its test segment. An example of a trial of this phase is shown in figure 4.

In this phase half the trials consisted of arrays with three and then four different objects and the other half of arrays with three and then four identical objects. These two object-cueing conditions were equally distributed over trials so that the same object-cueing condition never occurred over more than two successive trials. From this pool of five different types of objects, we randomly selected four types to occur on arrays of different object and one type of to occur on arrays with identical objects for each rat. Therefore, five rats had different sets of different and same object arrays but two rats had the same sets of different and same object arrays. However the configuration of the arrays of the different objects for these two rats was different. Moreover, after a study segment across each phase, we replaced the three study segment objects with their replicates in addition to adding the fourth object in the test array. From this phase on, the experimenter allowed the guillotine door of the start chamber to remain open until the rat exited the foraging arena in each segment.

Phase 3: Test array location manipulation: Rats were tested for the effects of the test array location variable under each object cueing condition over the next 48 trials. Rats were equally exposed to two test array location conditions (same and moved) under each object cueing condition. In the same location condition, the test array was not moved from the location of its study array. For the moved location condition, the test array was randomly moved from its study array (to a different corner of the arena). So basically study array can be moved to 3 different locations. For example if the study array is located on to the right upper corner of the square chamber, during the test segment it can be moved to left upper corner, right lower corner or left lower corner of the chamber. Each of these four test array location/object cueing conditions was

equally distributed over 48 trials in a different random sequence for each rat with the restriction that the same condition did not occur on the second trial within a session. Figure 5 shows an example of a trial of this phase.

Phase 4 Test array expansions: this phase was comprised of working memory testing condition where the new independent variable is the expansion as well as the jackpot location. The test segment of half of the total trials had an expanded array where the distance between the four objects is doubled while the in the other half trials, the distance between the four objects is quadrupled so that each object was shifted to one of the four corners of the chamber in the test segment. Moreover, the locations of the arrays in the study segment were in the middle of the chamber for the trials where its test segment arrays were expanded over the smaller area. The location of the study segments' arrays were always at a corner of the foraging chamber when their test segment arrays were expanded over the larger area. The jackpot's location in the test array was either in the same or different global location for half the trials within each expansion condition. However, the relative local position of the jackpot was not changed. Examples of these two expansion conditions are depicted in Figure 6.

Dependent measure: Accuracy for locating the jackpot: We noted the number of times a rat found the jackpot on its first choice over each block of 12 trials for each condition in phases 2, 3 and 4 and calculated the proportion of first choices rats found the jackpot. We statistically analyzed rats' test choice accuracy scores by various two-way within-Ss analyses of variance (ANOVA) and by selected paired-samples t-tests. Statistical effects were evaluated for $\alpha = .05$.

Results

Phase 2: We examined whether rats improved their test segment accuracy for finding the jackpot under each object-cuing condition in this phase. We calculated each rat's test

performance for each block of 24 trials (12 trials for each object cueing condition within each block) and analyzed these data by a 2 (object-cueing) x 2 (blocks) ANOVA. As seen in Figure 3 and confirmed by a significant effect for blocks, $F_{1,6} = 44.01$, $p = .001$, rats increased their relative accuracy for finding the jackpot on the second block of trials under each object-cueing condition. We note that rats in each block of this experiment and throughout this study always found the jackpot on their first choice significantly well above chance (.25). Although rats appeared to show slightly greater accuracy for finding the jackpot in test arrays of Identical than different objects, this difference was not significant, $F_{1,6} = 1.624$, $p = .250$. Figure 7 shows the interaction between objects and trials by their first choices where rats were performing better on second half of 12 trials in both object cueing conditions.

Phase 3: The effects of varying the location of test arrays under each object-cueing condition on test performance was assessed by a 2 (test array location) x 2 (object-cueing condition) ANOVA. As seen in Figure 8 and confirmed by non-significant effect for array location, $F_{1,6} = 0.790$, $p = .415$, moving the test array under either type of object-cueing condition did not affect rats' accuracy for finding the jackpot. The observed slight difference in performance between object-cueing conditions remained non-significant, $F_{1,6} = 0.729$, $p = 0.43$. The rats performed slightly better with different object cueing condition than with identical object cueing condition. However their performance with both cueing and location conditions were well above chance (90%). Figure 8 shows the proportion of trials rats found the jackpot on their first choice. The graph shows that the accuracy for finding the jackpot with both object cueing conditions did not vary as a function of test array location (same as vs moved from that of its study array).

Phase 4: The effect of varying the jackpot location and expanding the array under each object-cueing condition was assessed by a 2 (jackpot location) x 2 (object-cueing condition) x 2(expansion) ANOVA. This analysis uncovered a significant main effect for Test Array Expansion, $F_{1,6} = 56.00$, $p = 0.00$, and a significant interaction between this factor and Jackpot Location, $F_{1,6} = 7.00$, $p < .001$. We also uncovered a significant interaction between object-cueing and Jackpot Location, $F_{1,6} = 6.25$, $p = 0.047$. Figures 9B and A summarize the findings for the first and second interactions respectively. As seen in Figure 9B, rats generally responded more accurately in finding the jackpot within test array that were expanded over a smaller than larger area. However, they appeared to display better (perfect) accuracy in finding the jackpot that had been moved than not within the smaller expanded arrays but showed the opposite tendency in the larger expanded arrays. Paired comparisons t-tests, however, revealed that only the observed difference as a function of jackpot location was significant within the small expansion condition, $t_6 = 3.24$, $p = .018$ but not within larger expansion condition, $t_6 = 1.528$, $p = .177$. Figure 9 A summarize the data for the second double interaction. Although as seen in this figure, rats appeared to perform slightly better when a jackpot had not been moved in test arrays of identical objects but did so when it had been moved in test arrays of different objects, these effects were not significant, $t_6 \leq 1.698$, $p_s \geq .140$. Rather this interaction resulted from a slight but significantly greater accuracy by rats in finding the jackpot among identical than different objects when the jackpot had not been moved, $t_6 = 2.50$, $p = .047$.

Discussion

In our experiment, we investigated the effects of stability of an array of landmarks and their expansion on rats' working memory. We had hypothesized that rats should perform better when the test array location is the same as the location of the study array. We also expected that

their working memory would be better for arrays of different than identical objects. We also expected that expanding the array will decrease the rats' accuracy for finding the jackpot especially more so when it's global location was also moved. Our prediction for better performance with different objects and same test array location as that of study were not supported by the results. However, our data supported the prediction for an impaired performance upon expanding the test array location.

For phase 2, we found rats similarly improved their accuracy for finding the jackpot in the second half of training under each object-cueing condition. This indicates that rats learned both of the object cueing condition at the same rate. This also shows that training does have an effect on the rats' performance which is shown by their improved performance on the second half of the sessions. The improved learning in animals over trials has been shown in previous research as well where pigs were able to learn the spatial pattern by making fewer mistakes in subsequent trials (Johanna, Josef & Dinand, 2009). Moreover, we found similar results in our previous experiment (Arain, Paramesvaran & Cohen, 2010) in which the rats improved their performance over trials.

In phase 3, varying test array location did not affect rats' jackpot choice accuracy under either object-cueing condition. Thus these data do not support our hypothesis that rats would rely on or encode the global location of the jackpot within a trial along with its other characteristics. Consequently, rats relied upon the jackpot's local position within the array of identical objects and either that or its non-spatial features in arrays of different objects. These results differ from those found earlier (Arain, Paramesvaran & Cohen, 2010) where moving the test array disrupted rat's test performance slightly before enhancing it. Perhaps exposing rats to fixed configurations of different objects in this study restricted encoding to local spatial characteristics under either

object-cueing condition. Perhaps in arrays of identical objects rats were also remembering from reference memory where different objects always occurred in each position.

In phase 4, we found that rats performed better with a smaller scale expansion than with larger scale expansion. However, they still performed well above chance under either type of expansion suggesting that they used a relational rule among the feeding stations. Also, this shows that they were paying more attention to objects' local positions as well as to their features. We note that chicks trained to find food in the center of enclosed spaces of various geometric shapes divided their searches between areas corresponding to the absolute learned distance from the walls and an area in the center when tested in expanded enclosures (Tommasi & Vallortigara, 2000; Tommasi, Vallortigara, & Zanforlin, 1997). Similar results have been shown with pigeons trained to find food in the center of a square array of identical landmarks (Gray, Spetch, Kelly, & Nguyen, 2004; Spetch et al., 1997). However, the use of absolute metrics with landmark arrays may only reflect a preferred rather than the only search strategy. Other studies demonstrate that Clark's nutcrackers (*Nucifraga columbiana*) (Kamil & Jones, 1997; 2000) and pigeons (Jones, Antoniadis, Shettleworth, & Kamil, 2002; Spetch, Rust, Kamil, & Jones, 2003) show relational learning with landmark arrays that maintain shape but vary in their absolute metrics. Thus our results are consistent with the other research indicating a general preferred strategy to use absolute spatial information in most species. However, our rats did learn the relational rule as they were performing above chance with even with the completely expanded array.

Moreover, we also found that when the test array was expanded over a smaller scale and the jackpot location was moved, rats actually showed enhanced performance. This may be attributable to the fact that when the array is expanded over a smaller scale, the global jackpot location was only one degree moved away from its absolute original position in the study

segment. Moreover, the jackpot is still located on the same within array location during the test segment which helps the rat to remember it better as oppose to when the array is expanded on a larger scale where it's difficult to keep track of the relative distance between objects. Also, moving the test array starting from phase 3 might have helped the rats to not attend the global spatial position of the jackpot. The correct jackpot could be found by just retrieving the local positions of the objects within the array which always stays relatively the same or by just retrieving different objects. Their better performance when the jackpot location is moved during the small expanded trials maybe the result of improved retrieval of information when rats have to select from fewer sources of information. These results can also be explained on the basis of study segment location in the small versus the large expansion. During the small expansion trials, the study array was always in either of two middle areas. However, during the large expansion trials the study array was always located in one of the four corners. Hence the same jackpot location during the large expansion trials were easily remembered by the rats as it was still the same corner of the arena that he found his study segment stations in. But when the array is expanded and the jackpot location is moved, it actually moved to a different corner of the arena and that might have made the task a bit difficult for the rats to perform with the same level of accuracy.

To summarize, we found out that training improves the performance of rats in finding the jackpot over trials. Also, moving the test array has no effect on rat's working memory. Finally, rats were able to learn the relative relationship between the objects but they performed better when allowed to use absolute encoded representations. Therefore, we suggest that there are redundant spatial sources of information available to a rat while foraging and they can rely on any or all of them. The information of the study segment that a rat retains in their working

memory may include the global position of the array in relation to the chamber, local position of the objects within the array or featural information regarding the objects themselves. Rats may learn not to use the global location of the objects when the test array is moved as shown in phase 3. They might have attended to the local position within the array to find the jackpot or to the type of objects encountered or missed in the study segment. Also, rats might have learned to pay attention to just the objects or their relative local configuration when the array is overly expanded.

Our findings indicate that rats can flexibly use information from both objects' spatial global locations and their non-spatial object features. As soon as we make one cue irrelevant, rats can switch to other more reliable cues to maintain above chance search accuracy. Rats might be relying on global position of the objects too in the beginning of phase 2 but as moving the test array location in phase 3 and onwards was started, rats performed equally well showing their ability to use local and featural information. Also, expansion of phase 3 further supports their shift directly onto the object's features as all other cues were made irrelevant by extending the test array for every trial and introducing trials where jackpot is at a different absolute location in the test segment in comparison to that of the study segment.

Some of the results that we found in phase 2 and 3 were different than our previous experiment (Arain, Paramenvaras & Cohen, 2010). In our previous experiment, we were using all 25 food wells covered by movable pucks and the rats were to push open all of them. In our current study however, we made their foraging task easier by using only 4 feeding stations as compared to pucks all over the arena. This reduces their ability to make mistakes. Moreover, in our previous study, we were using random configuration of objects across trials for each rat while for this study, we used a fixed configuration of objects for each rat across each trial. This

might have helped them with different object cueing condition as they had a stable within array object configuration to choose the jackpot from. Moreover, we only used 4 locations in a chamber in both phase two and three which further reduces their memory load in the test segment.

Moreover, our experiment did show that rats are able to learn the relational rule while foraging which can help them in various ways while foraging. In their wild life, food locations with respect to the landmarks can be inconsistent depending upon their environment. Remembering different foraging rules and a flexible usage of them is essential for them to survive and reproduce better hence increases their overall fitness. Results of our experiments are helpful in conducting comparative studies across species. Our working memory task can also be used to study different disease models such as Schizophrenia, Dementia and Alzheimer's disease etc. We can also test various neuro-protective agents to see if these agents preserve efficient foraging search patterns of animals that might be otherwise at risk of neuro-degeneration. In future, one might further modify this study by disrupting the fixed array location and exposing rats to randomly varied array location to determine how easily they learn not to ignore such aspects of test arrays. Moreover, baseline results found in this study can be used to analyze gender differences. We only used male rats for our study and one future direction could be to use females on the same task and to see if the results are any different. In conclusion, the present experiment shows that rats can learn a relational rule while foraging among object-cued food-baited sites. Furthermore, we recommend some suggestions to make this task a more valid test for working and reference memory that can be applied in medico-pharmaceutical investigations.

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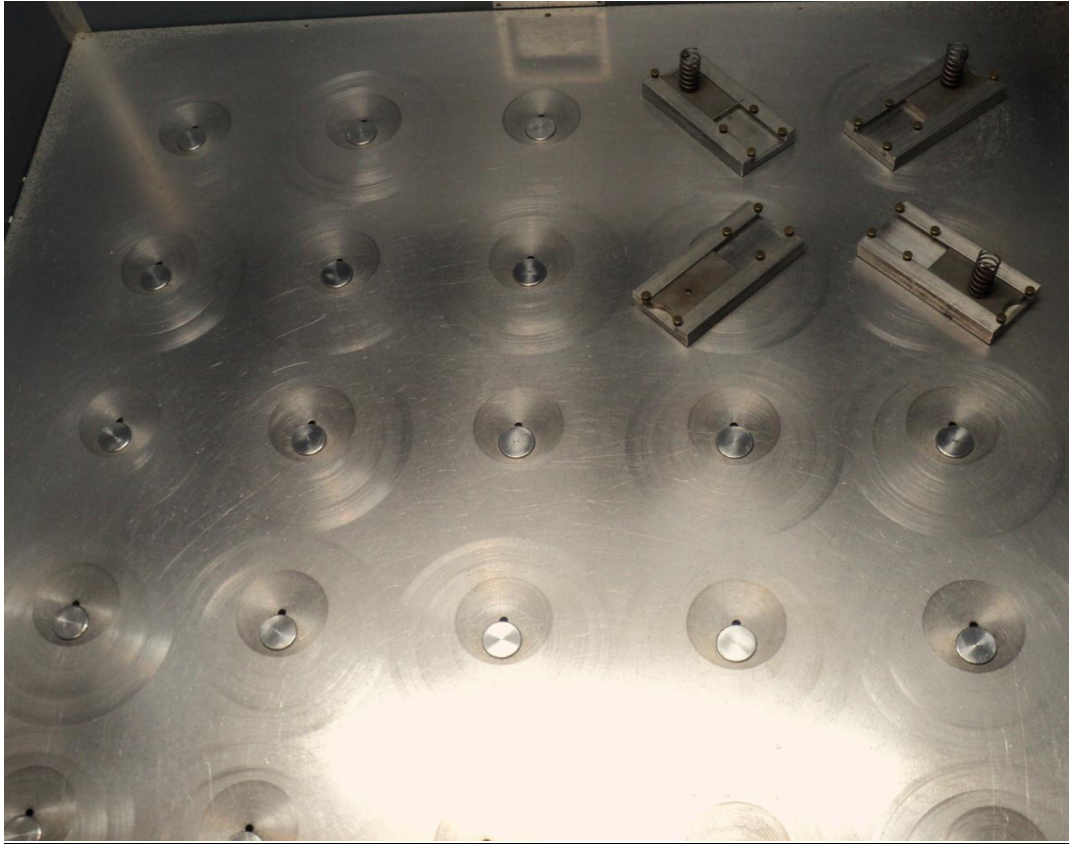
Figures

Figure 1: The foraging arena as it would appear with 4 foraging stations in the test segment. Camera angle is shown from the southern wall, the side from which the rat would enter.

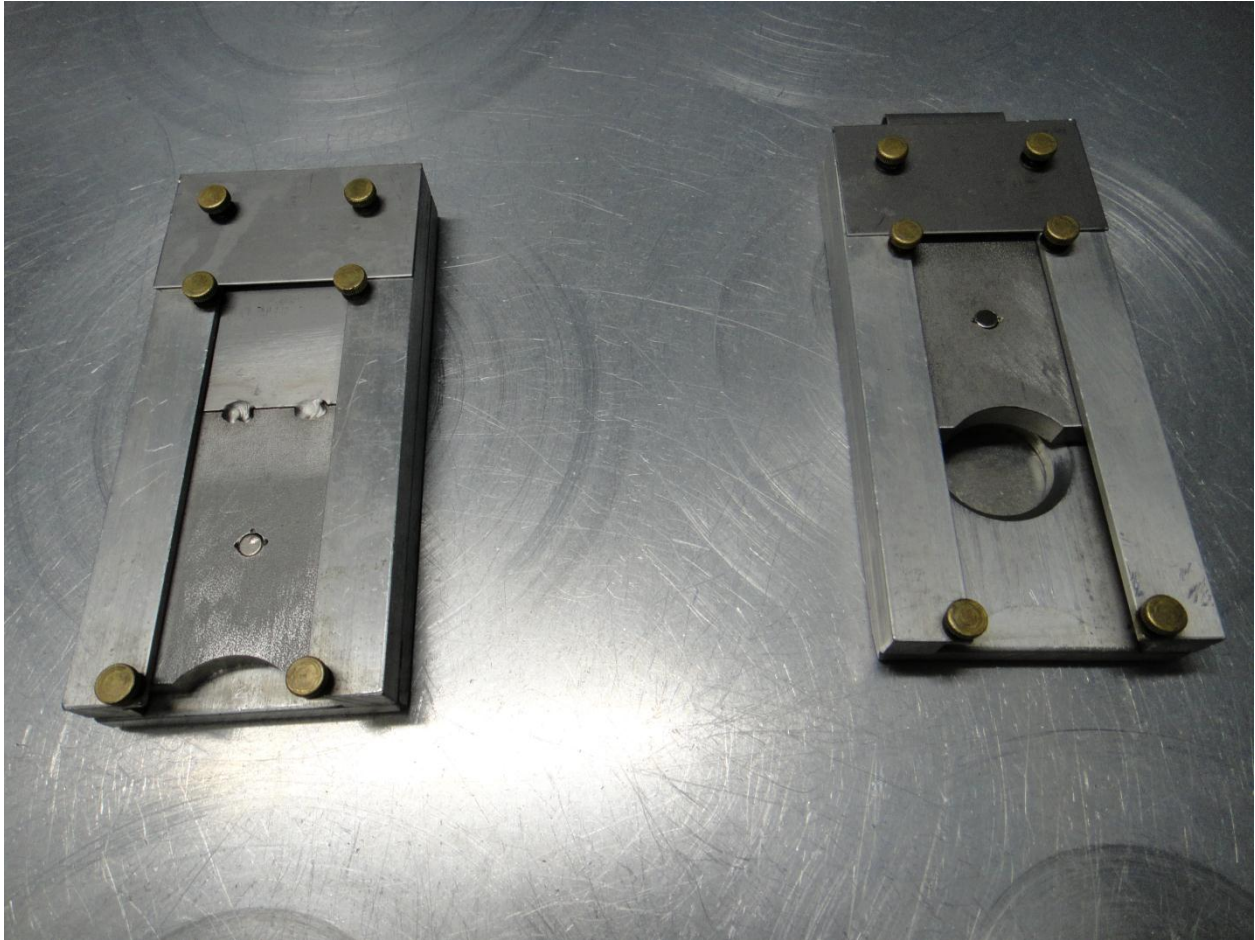


Figure 2: The foraging stations, as they would appear closed (left) and open (right).



Figure 3: Objects used in this experiment.

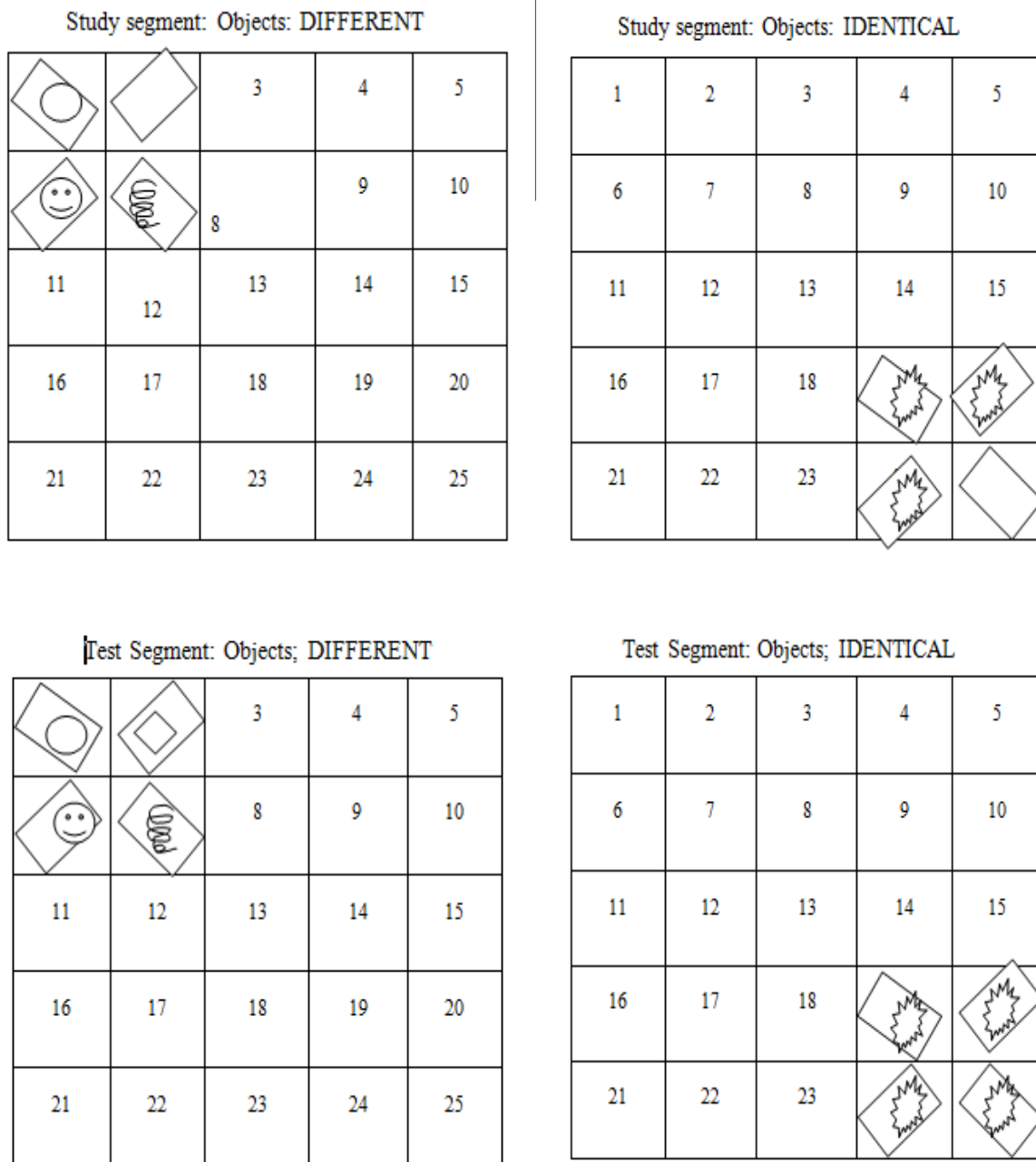


Fig 4: The spatial arrangement of foraging stations in phase 2, as they would appear in the study (top) and test (bottom) segment. The upper and lower right figures represent a trial where four or three identical objects were used. The upper and lower left figures represent a trial where four or three different objects were used.

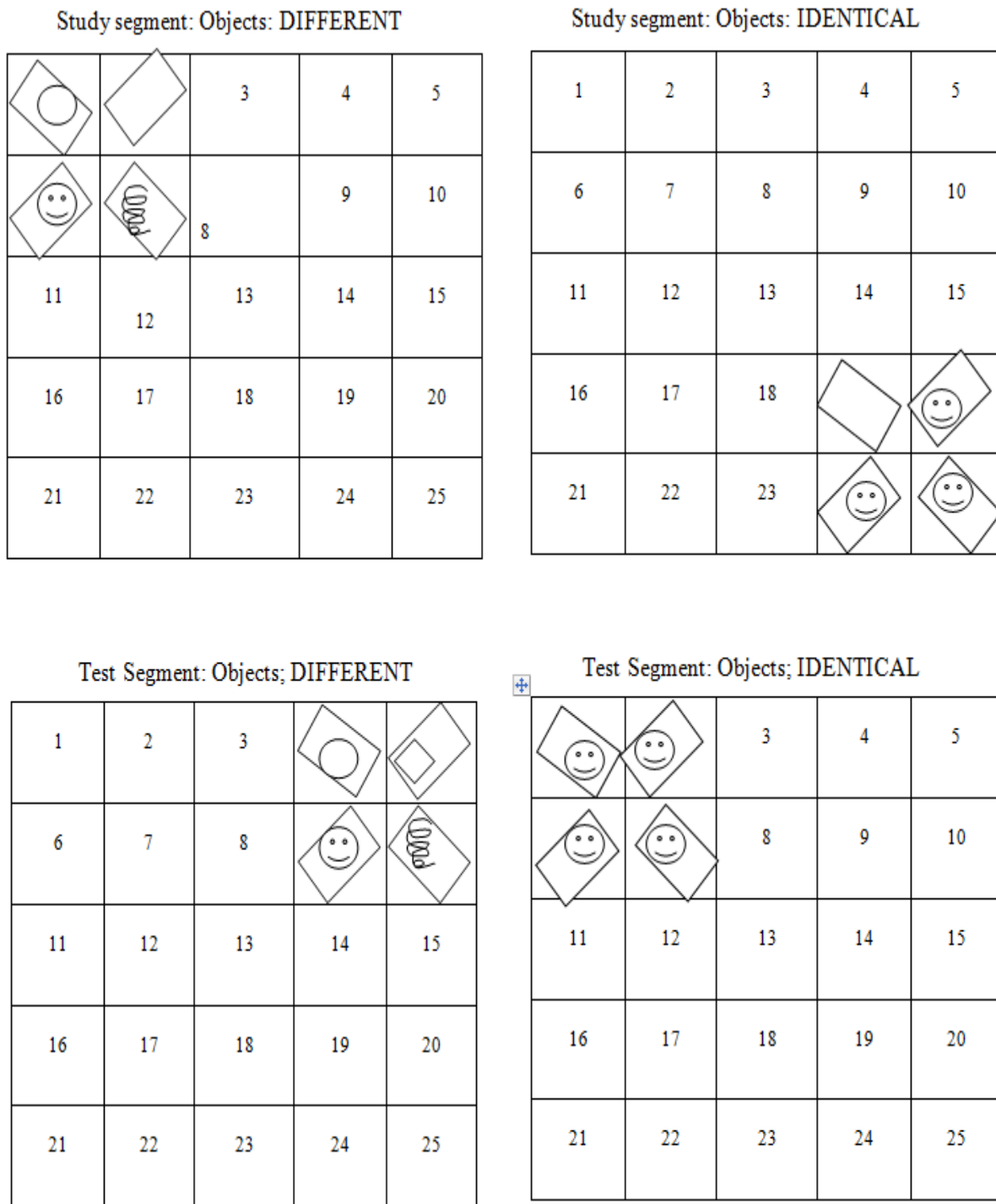


Figure 5: The spatial arrangement of foraging stations in phase 3, as they would appear in the study (top) and test (bottom) segment. The upper and lower right figures represent a trial where four or three different objects were used. The upper and lower left figures represent a trial where four or three identical objects were used.

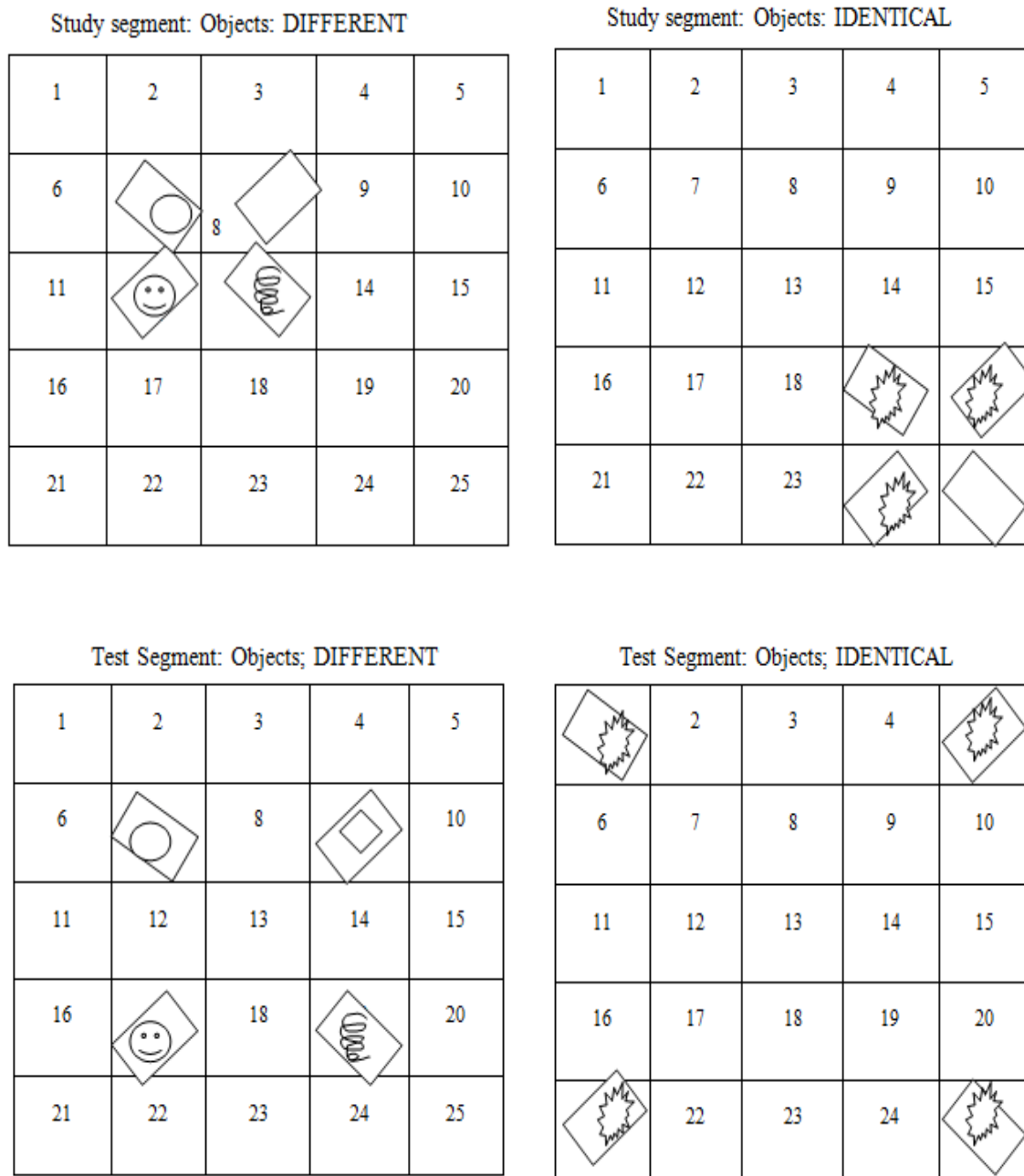


Figure 6: The spatial arrangement of foraging stations in phase 4, as they would appear in the study (top) and test (bottom) segment. The upper and lower right figures represent a trial where four or three different objects were used. The upper and lower left figures represent a trial where four or three identical objects were used.

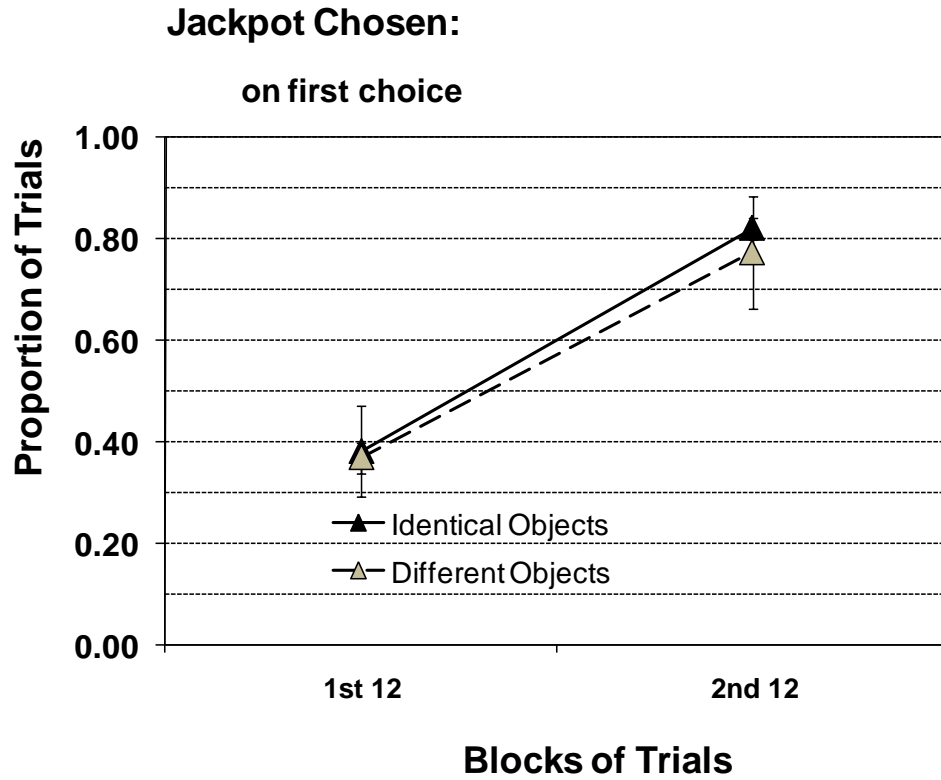


Figure 7: Proportion of trials rats find the jackpot on their first choice in the test segment as functions of the object-cueing and blocks of trials variables in phase 2 of the experiment. The graph depicts the mean proportions over all seven rats with vertical error bars denoting \pm SEMs. Chance accuracy was at 0.25 on first choice.

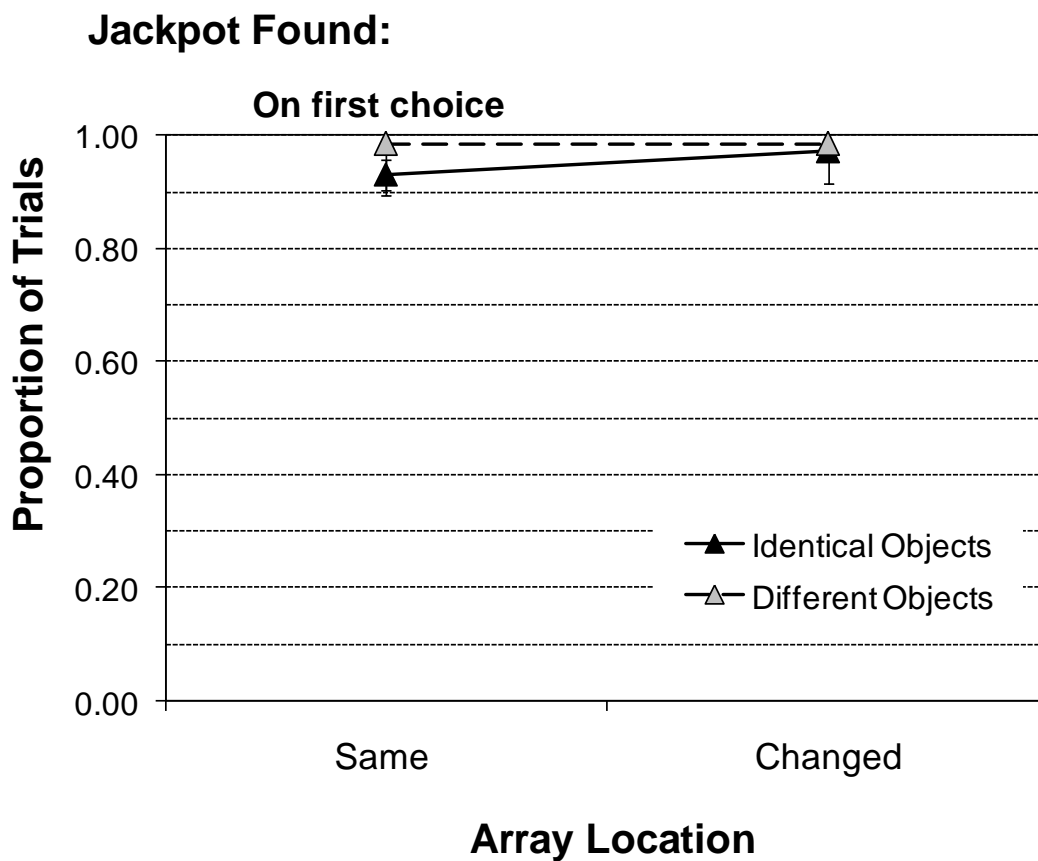


Figure 8: Proportion of trials rats find the jackpot on their first choice in the test segment as functions of object cueing and test array location in phase 3 of the experiment. The graph depicts the mean proportions of over all seven rats with vertical errors bars denoting \pm SEMs. Chance accuracy was at 0.25 on first choice.

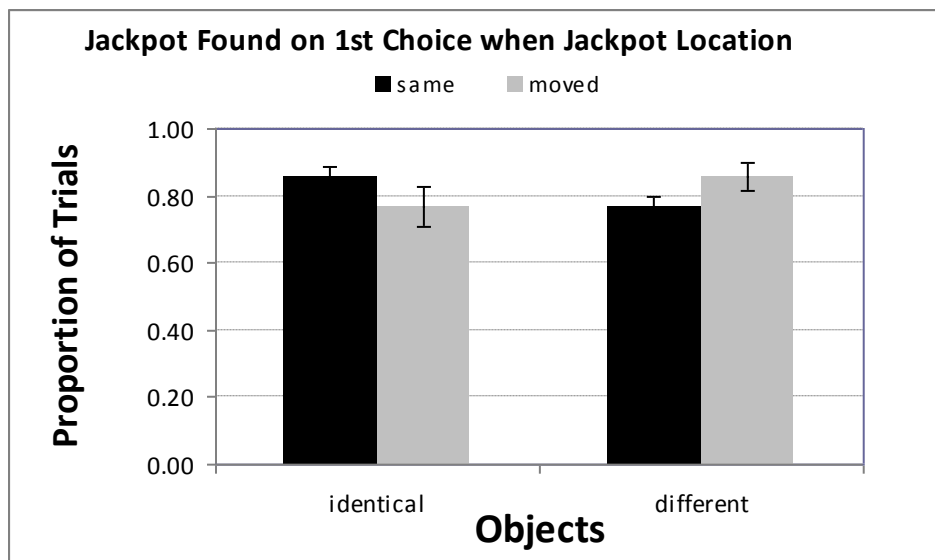


Fig 9 A: Proportion of trials rats find the jackpot on their first choice in the test segment as functions of objects and expansion in phase 4 of the experiment. The bars depict the mean proportions of over all seven rats with vertical errors bars denoting \pm SEMs. Chance accuracy was at 0.25 on first choice.

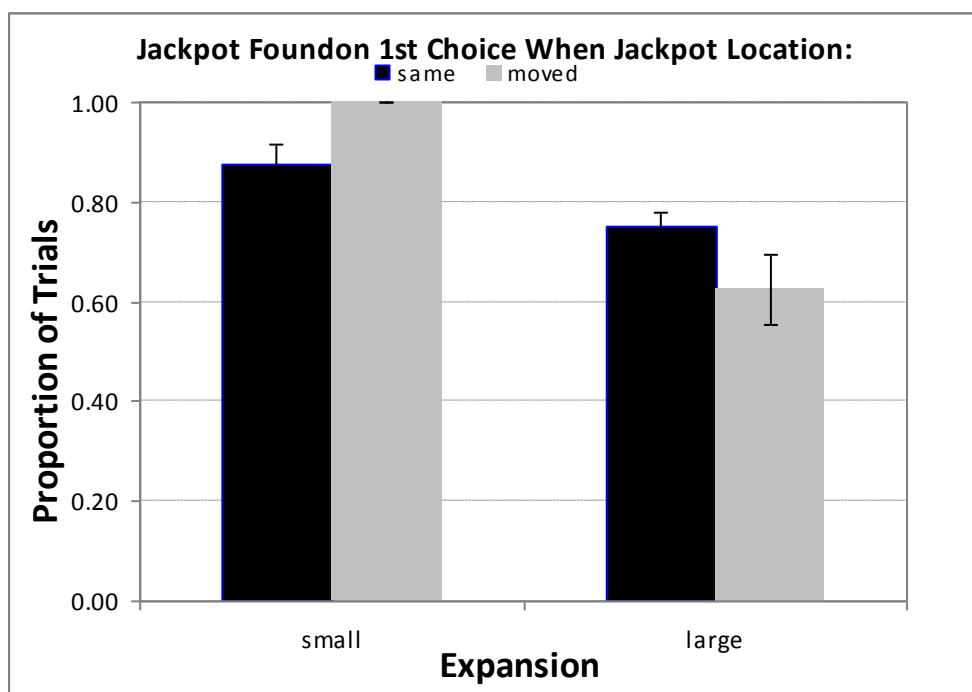


Fig 9 B: Proportion of trials rats find the jackpot on their first choice in the test segment as functions of object cueing and test jackpot location in phase 4 of the experiment. The bars depict the mean proportions of over all seven rats with vertical errors bars denoting \pm SEMs. Chance accuracy was at 0.25 on first choice.

CHAPTER 3
HIERARCHICAL USE OF INFORMATION IN RATS' (*RATTUS NORVIGICUS*) NON—
SPATIAL AND SPATIAL WORKING MEMROY IN AN OBJECT RECOGNITION
TASK

Chapter Summary

Animals use a variety of cues to navigate in their natural environment. They can rely upon objects' (landmarks') local position, global position, orientation, and surface patterns (e.g., texture, color, size, and odour). Over the last 20 years, considerable research has been conducted to examine differential use of such cues by different avian species. A less explored question is how rats use the different geometric or feature cues of an environment to locate hidden food places. We were interested in developing a working memory task that would allow us to determine if rats show any preferences for retrieving these cues and the extent to which degrees of dissociation among them would affect rats' use of each cue. To answer this question, we adopted the basic preparation used by Brodbeck (1994) with song birds. That is, we systematically placed all four cues in conflict with each other to observe the order, if any, that rats use to search for a remaining object. Probe tests placed local, global, feature and orientation cues in conflict. Results showed that feature cues exerted initial control over the rats' search preferences. The rats used local within- array location of jackpot site when the feature cue is unavailable or made irrelevant. The rats would attend to the correct orientation of the objects only after the correct local and pattern information was missing. The correct spatial global position was chosen only when this was the only cue available. They still chose the global position more often than can be accounted by chance. These results indicate the presence of a hierarchy in rats' retrieval of different information encoded within their visual spatial working memory. Our preparation could be used to provide behavioral data in studying various disease models affecting working memory. Moreover, baseline results found in this study may be helpful in comparative studies, analyzing gender differences or studying the effects of various neuro-protectants against particular ailments.

Introduction

Animals use multiple, redundant cues in their environment for different functions. It is critical for them to use that information for navigation, finding mates, searching for food, or finding safe places to reproduce (Gallistel, 1990). The use of such cues is dependent upon the environment in which animals navigate and the type of activity they want to perform. The differential exploitation of such cues varies across species. Animals either use internal cues such as proprioceptive or vestibular cues (Maaswinkel & Whishaw, 1999; Georgakopoulos & Etienne, 1994; Etienne, Maurer, & Seguinot, 1996) or they can also use external cues available in the environment such as colors, shape, smell or sound of different objects (Whishaw & Tomie, 1991; Brodbeck & Shettleworth, 1995). Our study focuses on the use of external cues by animals, specifically rats.

Animals adopt two basic strategies in using those cues. The first is a spatial strategy that makes use of geometric information such as spatial lay out of a place and distance or angular relation between two or more objects or landmarks. The second strategy is to use featural properties of different objects available in the surroundings. These properties include color, context, shape and patterns (for a review see Cheng & Newcombe, 2005). The spatial information of objects is further broadly divided into three main characteristics: their local position among other proximal objects, their global location determined by distal landmarks or compass 'readings', and orientation. In general, local cues represent situational or positional cues (goal's location within an array of objects) and the spatial cues that are provided by the beacons or landmarks located at or near the goal location. Global cues, on the other hand, provide information of the absolute goal's location within an arena (Spetch & Edwards, 1988), or geometrical relationship between objects near goals' location (Tommasi & Vallortigara, 2004).

Orientation cues, however, provide information regarding the direction or angle through which an animal must approach goals. In our study, we tried to investigate the preferential use of these four different types of cues in rats' visual spatial working memory.

Previous research has shown that animals can rely upon both spatial or feature properties in their environment but mostly prefer to use one type over the other. Some species, for example, goldfish (*Carassius auratus*), pigeons (*Columba livia*) and chicks (*Gallus domesticus*), place heavy emphasis on goals' featural properties (Vargas, Lopez, Salas & Thinus-Blanc, 2004; Kelly, Spetch & Heth, 1998; Vallortigara, Zanforlin & Pasti, 1990), while other species, for example, toads (*Bufo*), bees (*Apis mellifera*), bats (*Chiroptera*), pigeons (*Columba livia*), dogs (*Canis lupus familiaris*), lizards (*Cnemidophorus inornatus*) and human infants (Williams 1967; Gould & Marler 1987; Carter, Ratcliffe & Galef, 2010; Strasser & Bingman 1996; Dumas 1998; Day, Ismail & Wilczynski, 2003; Wang, Hermer & Spelke, 1999) rely more upon their spatial cues. The differential use of such cues is also dependent other factors such as sex (Vallortigara, 1996), species' ecological niche such as whether birds cache food as a primary foraging activity (Clayton & Krebs 1994; Brodbeck 1994; Brodbeck & Shettleworth 1995), the size of the spatial area available for animals' navigation (Healy & Hurly, 1998; Maes, Fontanari, & Regolin, 2009), and habitat stability (Odling & Braithwaite, 2003).

Although animals may prefer to use (retrieve) some non-spatial over spatial information or some types of spatial information over other types of spatial information, they still encode multiple kinds of spatial and non-spatial information that they can use in place of any preferred information that may be 'lost' or changed. Thus remembering more than one kind of information will aid animals in locating the goal's location better than only being able to use a single type of information. For example food-storing birds living in northern climates can still recover food-

caches that they stored in the fall even after the land is covered in snow (Sherry, 1992). Moreover, squirrels (*Spermophilus columbianus*) can still locate the hidden goal's location even after the removal of global landmarks (Vlasak, 2006). In many studies with different species where multiple goal cues have been placed in conflict with each other, animals (honey bees, dogs, humans and pigeons) have been shown to remember more than one type of spatial information (Collett & Kelber 1988; Fiset, Gagnon & Beaulieu, 2000; Goto, Wills & Lea 2004; Gray, Spetch, Kelly & Nguyen, 2004; Spetch & Edwards 1988). Some species of birds prefer to rely on a target's feeder global location over its color or pattern but can also use the latter when required (Brodbeck 1994; Brodbeck & Shettleworth 1995; Hurly & Healy 1996).

Although much research has been conducted with birds revealing differences in their hierarchical use of information across species, fewer studies of this kind have been carried out with rats. One pioneer study conducted by Cheng (1986) showed that when given transformational tests that provided conflicting information regarding featural and geometrical cues in a rectangular chamber, rats made more choices to the geometrically correct locations. This finding shows that they rely mostly on spatial, geometrical information. Another study conducted by Gibson, Wilks and Kelly (2007) showed that rats use geometry of goal's location relative to the arena in order to search accurately. Rats were able to search for the goal location even when the rectangular arrays of different objects were replaced with identical objects and when the array was expanded. However, they showed a strong preference to utilize feature cues when placed in conflict with geometric information. Similarly, Benhamou and Poucet (1998) conducted an experiment with rats where rats had to find the hidden platform under three objects differing in features but making an isosceles triangle. Rats were not able to learn the position of submerged platform even after 75 trials. Moreover, when in a different experiment, the local

versus the feature information was placed in conflict by switching two objects in an isosceles triangle, rats tended to go first to the location specified by geometry of the array and then to the location specified by the features. The goal of our study was to determine which cues are more significant for the rats when they have to rely upon their working memory to forage successfully in the environment.

In our experiment, we investigated which cues rats would use to locate a goal when given a task where all the different cues (global, local, feature, orientation) are in conflict with each other. To answer this question, we modified the basic task used by Brodbeck (1994). We replicated his experiment where he used a working memory version of a task in which chickadees and juncos were trained to find one of four widely spaced, distinctly different color patterned feeders they had previously found to contain food. Post-acquisition probe tests with displaced and varied test segment arrays revealed that chickadees initially select a feeder based on the baited feeder's global location on the wall before looking in the feeder that occupied the same local location within the array followed by choosing a feeder based on color pattern of the baited feeder. Thus these birds showed a hierarchical searching pattern in using the three different types of information about hidden food location. Juncos, a non food- storing species, were also equally capable to remembering which of the four feeders contained food in any trial, but during post-acquisition testing showed no hierarchy or preference for a particular cue. We were interested in developing a system where we can test whether rats have a hierarchical search pattern among redundant spatial and non-spatial cues in our specific working memory task.

This experiment consisted of five phases. We modified Brodbeck's (1994) task by using our segmented-trial preparation where rats had to find the missing object from the study segment during the test segment of a trial. We also extended Brodbeck's (1994) design by including a

third spatial “orientation” cue with local and global position cues along with non-spatial feature cues. Also we introduced trials of identical objects to remove the relevance of objects featural properties. Each trial of our experiment was divided into a study and test segment. In the former, the rats had to collect one seed from each of the three object cued baited station. After a 2-min delay, the rat had to find the fourth added object baited with three seeds that completed the array. Unlike the previous experiment, rats were exposed to arrays of adjacent objects that made up different geometrical configurations. The dependent variable for our experiment was the number of choices rats made to find the jackpot in the test segment. We were interested in determining which cues the rats would prefer when non spatial feature cues were in conflict with the rest of spatial cues (phase 2), when its global position is dissociated from the rest of the cues (phase 3), when global, local and feature cues were dissociated together (phase 4), and when all of these four types of information were in conflict with each other (phase 5). So each one of the four objects and its location represent one of the relevant cues to allow us to measure the proportion of times a rat chose each cue as their first, second, third or fourth choice. We further determined rats’ preferences for particular cues by analyzing which of them they would initially choose most of the time and whether such hierarchical preferences account for their working memory retrieval process.

Method

Subject:

Seven naïve male Long-Evans hooded rats the served in this experiment and received the same maintenance treatment as rats of previous experiment (Chapter 2).

Apparatus and Materials:

The same apparatus and objects from the previous experiment continued to be used.

Procedure:

Phase 1: Pre-training phase: The rats were given the same initial pre-training as that given to rats in Experiment 1 (chapter 2). They were initially trained to push open the small horizontal window on the foraging stations by gradually covering the windows in small increments. After acquisition of the basic task, rats were put directly onto the basic task.

Training Phase: The basic task was similar to the phase 1 of Experiment 1 (chapter 2) where the rats had to approach to three closely adjacent object-cued feeding stations located within a relatively large square arena during a study segment and then, after a delay, had to find the fourth remaining baited cued (jackpot) station that completes a square configuration of four objects. Each rat was run in this phase until it found the jackpot on its first choice on at least nine out of twelve consecutive trials that were equally divided into identical and different object-cueing conditions. The basic training trials consist of two types of trials; identical or different object cueing condition. From this phase on, we used pseudo-randomly determined an array's object-cueing condition (identical or different objects), the type of objects in the identical objects condition and the positions of different objects within the array for each animal. Thus each rat received an array of objects at a different location on any one trial. Moreover, we used 4 objects randomly selected out of the five objects to be given in different object cueing trials. The fifth object along with three other replicas was used during the identical object cueing trials. Thus each rat has one type of object for identical object trials and the other four objects for the different objects cuing trials. An object used for a different object trial for one rat might be the object for identical trials for a different rat. Moreover, we designed three types of geometrical arrays for this phase (figure 1) for both object cueing conditions. We rotated the array and changed the jackpot location of each of those three array designs to insure that each object and

location in each array equally served as a jackpot over trials. We insured that no more than two consecutive trials had the same array design, location or jackpot position. Throughout all phases in this experiment, we misted the foraging area with a weak lemon-scented anti-bacterial detergent solution and wiped it dry between trials and, in subsequent phases, between each trial segment. We also randomly varied the order of rats to be run in each session to further prevent any build-up or maintenance of odour trails. An example of a test configuration is shown in Figure 2.

Phase 2: Arrays of Different Objects - features versus spatial global, local and orientation cues. From this phase on, we introduced probe trials in addition to running the training trials of phase 1. From this phase on unless otherwise noted, we ran a total of 20 probe trials (10 different and 10 identical object cueing conditions) and 40 training trials (20 different and 20 identical object cueing conditions) in all the phases. Three trials were run on each day (2 training and 1 probe trial). The order of the trials was randomized throughout the experiment so that a probe test might occur in the first or in the second or in the last of the three trials in a session. Inter-trial-intervals during a session were maintained at 1 h.

Training Trials: In this and the following phases training trials were the same as in Phase 1. We recorded the number of choices the rat took to reach the jackpot. Therefore, for each of the following phases we only describe the probe trials.

Probe Trials: These probes were based on those in Brodbeck's (1994) first experiment. In order to find which of the two sets of cues (Spatial local/array or feature) the rats would prefer, we designed the test trials so a choice to one of the feeding stations would be governed by a correct local spatial cue while the choice of the other station was controlled by its correct featural and feeder orientation cues. Choices to the remaining two stations were incorrect and

thus did not represent any known control by encoded information. During this and all probe trials we waited until a rat opened all the four feeding stations or three minutes had elapsed, whichever occurred first, before removing it from the test arena.

For the different object cueing trials, we used the same basic trials as that of the training phase. The study segment is the same while the test segment had some changes. The fourth object that was missing in the study segment is switched with one of the different object already present. So the new object was placed at the location of a previous object and the previous object was placed in the jackpot location which had no object in the study segment. Moreover, the new object is randomly exchanged with one of the three objects present in the array. An example of a test configuration is shown in Figure 3.

Arrays of Identical Objects - Orientation, local and global position versus incorrect choices. For identical object cueing condition, we could not test for feature cue so we used the same trials as that of the training trials. The fourth object was added in the test segment in the correct location.

Another major difference in probe trials was that we did not bait any feeder in probe trials in this phase unlike the training phase. Since the rat did not know the order of trials, they were equally motivated to perform in the probe trial even though it was not rewarded. In order to keep their motivation level high, we gave them two training trials so that they would continue to respond. We recorded the time and measured the order of choices that a rat made to each of the four stations. If rats do not exercise any hierarchical search process it should chose each feeding stations in both object cueing conditions equally over its four choices. Hence chance performance at each choice for each feeding station should be $p = 0.25$.

Phase 3: Spatial global versus local position, orientation and feature cues for arrays for different objects / global position vs. local and orientation for arrays of identical objects: In order to find which of the two spatial cues (global or local position) the rats would prefer, we designed probe trials so that choice to one of the feeding stations would be governed by spatial local cues while the choice to the other would be controlled by spatial global information during test segments. These probes were based on those in Brodbeck's (1994) second experiment. The array of feeders was transformed so that a response to one feeder on each test trial showed control by its correct spatial global cue while visits to another feeder showed control by its correct local spatial or non-spatial features cues. The remaining 2 stations do not represent anything. To accomplish this in the probe trials under each cueing condition, we moved the test array over one location either to the left or right of its study array or up or down from it depending on the geometrical configuration of the array. The fourth missing object was always placed in the same local position within the test array and in the same orientation as the non-cued feeding station of the study array. In the test array in these probe trials, the missing object's 'correct' global position was now occupied by a different, previously presented object at its correct orientation. In probe trials with arrays of identical objects, only the non-cued feeding station's orientation occupied the same local position but had been moved to a new global position.

Thus a choice to the new object among arrays of different objects represents control by feature, orientation, or local position (or any combination); while a choice to the original jackpot location represents control by the global position. Among arrays with identical objects, a choice to the feeder at the correct location position represents control by either the local position or feeder orientation (or both) while choice to the feeder at the previously non cued location

represents control by the global position. Examples of probe trial configurations under each cueing condition are shown in Figure 4.

Phase 4: Spatial global versus local position and orientation cues versus feature cues for arrays of different objects. We ran a total of 10 probe trials and 20 training trials only with arrays of different objects. These probes were based on those in Brodbeck's (1994) third experiment. In order to find which of the three cues (Spatial global or local/array or feature) the rats would prefer, we designed the test segments of probe trials so that choice to one of the feeding stations would be governed by spatial local cues while choice to another station would be controlled by spatial global information and that to a third feeder would be controlled by its feature and orientation cues. Choice to a fourth feeder doesn't reflect control by any specific cue. To accomplish this we continued to move test arrays in probe trials in a similar manner as in phase 3. However, we switched the local positions of the missing object and that of a previously exposed object. Thus one of the previously exposed objects now occurred in the same local position at the same orientation as the non-cued feeding station from the study segment, another previously exposed object occurred on the global location of the study array's missing object, and the missing object occurred on a feeder in a different local position and orientation. An example of such a probe trial is seen in the left panel of Figure 5.

Local/global position vs. orientation for arrays of identical objects: We ran a total of 10 probe trials and 20 training trials with arrays of identical objects. Three-trial sessions containing these types of arrays were randomly interspersed among those with arrays of different objects. In the probe trials of these sessions, however, the location of a test array remained the same as that of its study array but the orientation of feeder with the missing object was switched with that of one of the previously object cued feeders. Thus a choice to the feeder at the same location of the

previous feeder without an object would indicate control by local or global position or both, while choice to the object at a feeder having the orientation of that previously without an object suggests control by orientation. We note that the introduction of testing for control only by object orientation was not tested by Brodbeck (1994). An example of such a probe trial with arrays of identical objects is shown in the right panel of Figure 5.

Phase 5: Spatial global versus local position versus orientation versus feature cues for arrays of different objects / Local versus global position vs. Orientation for arrays of identical objects: Probe trials in this phase followed that of Brodbeck's (1994) fourth and fifth experiments that tested separate control of birds' choice by the target's feeder global position, its local position, and its color/pattern features. In this phase we also tested for orientation as one of the cues. Thus we placed all four cues in conflict with each other for test arrays with different objects but could only place each of the three spatial cues in conflict with each other for test arrays with identical objects. The test array for each type of probe trial was moved one space as in phase 3. We switched the orientation of the last previously attended object in a trial's study segment with that of the non-object cued orientation. For arrays of different objects we also switched one of the previously exposed objects with the missing object in a location that was neither a previously local nor global position nor at a feeding station with the 'correct' orientation. We note that for arrays of identical objects, we could only test control of rats' choices by the three spatial cues. An example of a probe trial for each type of array is shown in Figure 6.

Data analysis. We adopted a similar approach as Brodbeck (1994) in analyzing the data. We examined the distributions of the number of choices to find jackpot (baseline data) in the training trials and the number of choices towards each station in the probe trials' test arrays. An

equal frequency of each number of looks would indicate chance performance. These distributions were analyzed using the G statistic (Sokol & Rohlf, 1981). This statistic is distributed as chi-squared, but differs from the chi-squared statistic in an important characteristic: results of groups of G tests can be summed and degrees of freedom can be partitioned as in an analysis of variance (ANOVA). This allows one to sum the distributions for each subject and look at an overall difference from a chance distribution. Variance in the distributions caused by differences between individual subjects can then be separated from the overall difference from chance and from any difference between conditions. This summing of distributions to get an overall picture would not be possible using chi-squared. A significance level of .05 was used for all statistical tests. Moreover, inter subject variation was calculated by Fisher Exact test which is based on exact probabilities from a specific distribution as opposed to Chi square tests which rely heavily on approximations.

Results

Phase 1: Six rats reached the 75% accuracy criterion within their first twelve trials for each object cueing condition trials. The jackpot was found by each rat on its first choice on nine out of the twelve trials (75% accuracy) containing identical object-cued configurations and on ten out the twelve trials (83% accuracy) containing different objects-cued configurations. Only one rat took fourteen trials to reach the 75% accuracy criteria.

Phase 2: Spatial features (global+local position +orientation) versus non-spatial features: During the training trials of this phase, the rats found the jackpot with 97% accuracy during all 20 trials of identical and with 95% accuracy with different object cueing condition. The baseline data for this phase is shown in Figure 7.

During the test trials of different objects condition, the rats overwhelmingly chose the correct object first and the correct Local/ spatial Global position on its second choice (Figure 8 A). The distribution of the number of choices taken to the correct object on test trials differed from chance [$G_{(3)} = 67.45$]. Moreover, inter subject variation calculated by a Fisher Exact test and was found to be non-significant ($p=0.114$). This shows the distribution of individual subjects did not significantly differ from the combined distributions. When the distribution of the number of choices to the feeder with the combined spatial features was examined, a significant difference from chance [$G_{(3)} = 25.51$] was obtained but no significant inter subject variation was found with a Fisher exact test ($p=0.103$). However, when the data from the correct object feature during the probe trials were compared with the baseline trials, a significant difference was found [$G_{(3)} = 6.92$]. Similar significant difference was found for the spatially correct feeder in probe trials with that of the baseline trials [$G_{(3)} = 127.0$]. That shows that the rats treated the probe trials feeding stations differently than that of the baseline trials.

During the test trials of identical object condition, the rat chose the correct jackpot (correct local and global position) at least more than 83% of the time (Figure 8 B). Since this phase was the same as training trial, we only counted the accuracy of each rat in finding the jackpot. So there could only be one correct location. When the distribution of the number of choices to the correct feeder with the combined spatial features was examined, a significant difference from chance [$G_{(3)} = 33.33$] was obtained but no significant inter subject variation was found with a Fisher exact test ($p=0.653$). We also looked at the distribution of choices for the probe trials and compared it with the baseline and found no difference [$G_{(3)} = 0.33$]. Hence the rats were treating the probe trials similar to that of the baseline trial.

Phase 3: Spatial global versus local, feature and orientation cue: During the training trials of this phase, the rats found the jackpot with 95-100% accuracy during all 40 trials of identical and different object cueing condition. The baseline data for this phase is shown in Figure 9.

During the probe test trials of different object condition, the rats overwhelmingly chose the correct object (having the correct orientation and local position) and then correct Global position on subsequent choices (Figure 10 A). The distribution of the number of choices made to the correct object at its correct local position and orientation on test trials differed from chance [$G_{(3)} = 91.23$]. Moreover, inter subject variation was calculated through fisher exact test and was found to be non-significant ($p=0.07$). This shows that each subject produced a similar pattern of choices. When the distribution of the number of choices to the correct global feeder was examined, a significant difference from chance was found [$G_{(3)} = 15.61$] and inter subject variation was found significant through fisher exact test ($p=0.00$). This shows that the subjects did produce dissimilar patterns for choosing the correct global position feeder. Moreover, the baseline and correct object, local and orientation cue distributions were compared and found to be significantly different [$G_{(3)} = 49.04$]. Similarly, similar picture emerges when baseline and global cue distributions across the probe trials are compared [$G_{(3)} = 44.84$]. The rats were treating the probe trials differently than how they treat the correct baseline feeder.

Spatial global versus and orientation cue: During the test trials of identical objects condition, rats overwhelmingly chose the correct within array Local position with correct orientation first and spatial Global position the second (Figure 10 B). The distribution of the number of choices taken to the correct Local array position on test trials differed from chance [$G_{(3)} = 78.97$]. Moreover, inter subject variation was calculated through fisher exact test and was

found to be non-significant ($p=0.24$). This shows that each subject has similar pattern of choices. When the distribution was examined of the number of choices to the correct global feeder, a significant difference from chance [$G_{(3)} = 40.82$] and inter subject variation was found through fisher exact test ($p=0.05$). This shows that the subjects did not have the similar patterns for choosing to the correct global position feeder. When the data from the correct spatial local location with correct orientation were compared with those of the baseline trials, a significant difference was found between the two distributions [$G_{(3)} = 28.51$]. Similar results were found with correct global position distribution [$G_{(3)} = 67.37$].

Phase 4: Spatial global versus local and orientation versus feature cue: During the training trials of this phase, the rats found the jackpot with 95-100% accuracy during all 40 trials of identical and different object cueing condition. The baseline data for this phase is shown in Figure 11.

During the test trials of different object condition, G test revealed similar results. The rats overwhelmingly chose the correct object first, correct local or within array position with correct orientation second and spatial Global position on its third choice (Figure 12 A). The distribution of the number of choices taken to the correct object on test trials differed from chance [$G_{(3)} = 71.26$]. Moreover, inter subject variation was calculated through fisher exact test and was found to be non-significant ($p=0.98$). When the distribution was examined of the number of choices to the correct Local position with correct orientation, a significant difference from chance was found [$G_{(3)} = 75.15$] with inter subject variation being not significant ($p=0.89$). The distribution of choices to the feeder that was in the correct spatial global position was also different significantly from chance [$G_{(3)} = 51.52$] and inter subject variation was not significant through fisher exact test ($p=0.16$). Also, by comparing all the three distributions in the probe trial with

that of the baseline, we found a significant difference in all three distributions; feature [$G_{(3)} = 32.18$], local position and orientation [$G_{(3)} = 128.16$] and global position [$G_{(3)} = 8.70$] which shows a differential strategy to reach towards the goal in probe trials in comparison to that of baseline trials.

Local/global position vs. Orientation for arrays of identical objects: During the test trials of identical object condition, the rats chose the correct within array Local and global position first and feeder with correct orientation the second (Figure 12 B). The distribution of the number of choices taken to the correct Local and global position on test trials differed from chance [$G_{(3)} = 92.17$] with non-significant inter subject variation ($p=0.14$). When the distribution was examined of the number of choices to the feeder with correct orientation, a significant difference from chance [$G_{(3)} = 46.25$] and no inter subject variation was found through fisher exact test ($p=0.20$). When the data from the correct orientation were compared with those of the baseline trials, a significant difference was found between the two distributions [$G_{(3)} = 51.11$]. Similar results were found with correct local and global position distribution [$G_{(3)} = 99.149$].

Phase 5: Spatial global versus local versus feature versus orientation cue: During the training trials of phase, the rats found the jackpot with 95-100% accuracy during all 40 trials of identical and different object cueing condition. The baseline data for this phase is shown in Figure 13.

During the test trials of different object condition, G test revealed the similar results. The rats overwhelmingly chose the correct object first, correct local or within array position second, correct orientation as a third choice and spatial Global position on its fourth choice (Figure 14 A). The distribution of the number of choices taken to the correct object on test trials differed from chance [$G_{(3)} = 72.39$]. Moreover, inter subject variation was calculated through fisher exact

test and was found to be non-significant ($p=0.92$). When the distribution was examined of the number of choices to the correct Local array feeder, a significant difference from chance [$G_{(3)} = 49.40$] and inter subject variation was not significant through fisher exact test ($p=0.85$). The distribution of choices to the feeder that was in the correct orientation position also different significantly from chance [$G_{(3)} = 82.11$] and inter subject variation was not significant through fisher exact test ($p=0.33$). Similarly, the distribution of choices to the feeder in globally correct location was also significantly different from chance [$G_{(3)} = 150.45$] and inter subject variation was not significant through fisher exact test ($p=0.82$). The ranking of cues by the rats was further evident when we compared these distributions with the baseline. All the four distributions in the probe trial were significantly different with that of the baseline distribution; feature [$G_{(3)} = 15.33$], local position [$G_{(3)} = 88.31$], orientation [$G_{(3)} = -46.34$] and global position [$G_{(3)} = -17.99$] which shows a differential strategy to reach the goal in probe trials in comparison to that of baseline trials.

Spatial global versus local versus orientation cue: During the test trials of identical object condition, the rats chose the correct within array local position first, feeder with correct orientation second and spatial Global position third (Figure 14 B). The distribution of the number of choices taken to the correct local array position on test trials differed from chance [$G_{(3)} = 65.41$] with a significant inter subject variation ($p=0.00$). This means the rat's pattern of choice was not consistent in choosing the local position as their first choice. When the distribution was examined of the number of choices to the feeder with correct orientation, a significant difference from chance [$G_{(3)} = 83.4$] and no inter subject variation was found through fisher exact test ($p=0.78$). Similarly, the distribution of choices to the feeder in globally correct location was also significantly different from chance [$G_{(3)} = 106.97$] and inter subject variation was not significant

through fisher exact test ($p=0.23$). All the three distributions in the probe trial were significantly different with that of the baseline distribution; local position [$G_{(3)} = 46.50$], orientation [$G_{(3)} = 117.63$] and global position [$G_{(3)} = -34.78$] which shows a differential strategy to reach towards the goal in probe trials in comparison to that of baseline trials.

Findings from this experiment show that featural cues or pattern information are heavily relied upon throughout all the phases. Only when we remove the informational values of non-spatial cues, did rats tend to choose local within array position most frequently followed by the correct orientation. Only when all four types of information are dissociated, rats did choose correct global position as their fourth choice. They still didn't adopt random search patterns which show their ability to develop a hierarchical system to be used during navigation.

Discussion

In this experiment, we investigated the preferential use of different cues available to rats in finding their hidden food location. We replicated Brodbeck's (1994) experiment but used rats and found results different from that of the earlier study. We found that the rats responded to the feature/pattern information first, followed by the local/within array information, orientation and spatial global information, when they had to choose among those cues to search for food.

Brodbeck found that a food storing bird species, chickadees, responded to the spatial information first followed by local position cues and pattern information when all the cues were dissociated.

We found opposite results with the rats. Moreover, he found that a non-food storing species, juncos, responded to all the cues equally well and they didn't make use of any hierarchical system in their search process. Thus we were able to extend a basic design of a hierarchical system that rats might use in order to search and navigate in their immediate environment.

When we compared our results of probe trials across all phases (2-5) with that of the training trials, we found significant differences. The rats tend to choose only the correct feeder (having the correct feature, local and spatial information) on their first choice and after retrieving the food would go back from the exit door without checking more stations. In test arrays of probe trials they tend to choose 3-4 feeding stations on average before exiting the chamber. This is partially because they weren't given food during the probe trials and they checked all the stations in the hope of getting food in one of them. They started from the station with the cue they considered significant. And that cue was found to be the pattern information of the objects, when available. Pattern information was heavily relied upon across all the phases of our study. And rats tend to choose local within array position as their first choice only after the pattern information is made irrelevant.

While the study by Cheng (1986) information in a working memory task and by Batty et al (2009) in a reference memory task had shown that rats prefer to use spatial or geometric cues, we found that they prefer to use feature cues in our spatial working memory task. This shows that rats are capable of using both kinds of cues but the strategy as to which cue to prefer seems to be shaped by a number of different factors. In Cheng's experiment, the feature information is provided by making the walls of the arena distinct and in Batty et al's experiment, an object is hanging from the wall while in our experiment we used different objects (in shape, color and texture) that served as beacons. The objects might have provided more reliable source of information to them and since they were adjacent to each other in the arrays allowing rats to touch and explore them more closely in time during their search. This might give them more information about their patterns. Similar results are found by Gibson et al (2007) in their reference memory task where the rats use feature information more preferably than the spatial

information and they also used salient objects for their testing. However, some previous research has shown that bonobos and rats switched to pattern information over the spatial information when the size of the arena is large (Kanngiesser & Call, 2010; Maes et al, 2009). In our experiment, the size always remained constant and the distance between objects was small enough to use spatial information as well.

Even though our study revealed that the rats use pattern information as the most preferred indication of a food location; we also found that they place heavy influence on the local/within array position of objects. This is because they always tend to choose local position as their first choice in all the identical object trials and as their second choice in different object trials. Similarly, they were preferring orientation cues more than spatial global cues. One of the reasons of their behaviour could have been is that rats cannot view an arena holistically or from top down view. The direction from which they opened a feeding station may appear to be more reliable compared to the global positioning of the object. Another contrast is that in our experiment during the study segment, the position of the feeding station without the object is referred to as the correct global position. During the test segment, that position was being filled by a new or a previously existing object which might encourage a rat to pay more attention towards the object in that position. Still, global location was chosen more than chance and more than the stations depicting none of the relevant spatial and non-spatial cues (probe trial phase 2, 3, 4). All the four types of information seem to have been remembered well and response to any of the cues was always done before choosing the feeding station without a cue.

Our results were comparable to Brodbeck's study (1994), in that our rats formed a hierarchical search pattern as did chickadees but in the opposite sequence. One more reason for our rats to choose pattern information before spatial cues might be due to our exposing each rat

to the same four different objects throughout this study. However, Brodbeck used more trial unique stimuli. The birds were given different random objects in each trial and there were ninety six objects used over all in the study. The birds might have been relying more on the other spatial information because they were exposed to new objects in each trial. Also, another difference is that they used the same wooden blocks (differing in size, shape, color and design) which have the same texture while our objects were differing in shape, color, size, design as well as texture. Therefore, future research with rats we should include trial unique stimuli to see if rats' search patterns approach those of song birds. That is, rats might also start to develop representations of spatial cues that they will retrieve before those of non-spatial pattern information during their search in working memory.

Our data indicate the rat's ability to flexibly use information from both the spatial global location as well as the non-spatial object features. As soon as we make one cue irrelevant, rats can switch on to other more reliable cues to increase their search accuracy. This information allows an animal to find places and take those routes that are much efficient and safer.

Results of our experiments are helpful in conducting comparative studies across species. We have made a systematic design through which we can test the essential cues that are important during foraging. Our working memory task can also be used to study different disease models such as Parkinson's, Alzheimer's disease etc. We can also test various neuro-protective agents to see if there is a difference in their behaviour by using the similar working memory tasks. Moreover, baseline results found in this study can be used to analyze gender differences. We only used male rats for our study and one future direction could be to use females on the same task and to see if the results are any different. Previous research on rodents shows that males are more likely to rely upon spatial cues while females tend to do better on tasks requiring

memory for patterns or position (Saucier, Shultz & Keller 2008). Similar results were found with human adults and prepubertal children (Chai & Jacobs, 2010; Newhouse, Newhouse & Astur, 2007). In conclusion, the present study shows that rats hierarchically use the information available to them during a working memory task and we recommend few suggestions for further improvements of this task, in hopes of developing a valid test for working and reference memory that can be applied in medico-pharmaceutical experiments.

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Figures



Figure 1: three array designs that we used in our study. Each one of them was further rotated from 1-4 degrees making 12 types of trials in total. The positions of the front (where the rat had to nose the slide to move the object) of each feeding stations was different and varied to further increase the number of different array configurations. The location of those arrays within the arena as well as the jackpot location was randomly chosen.

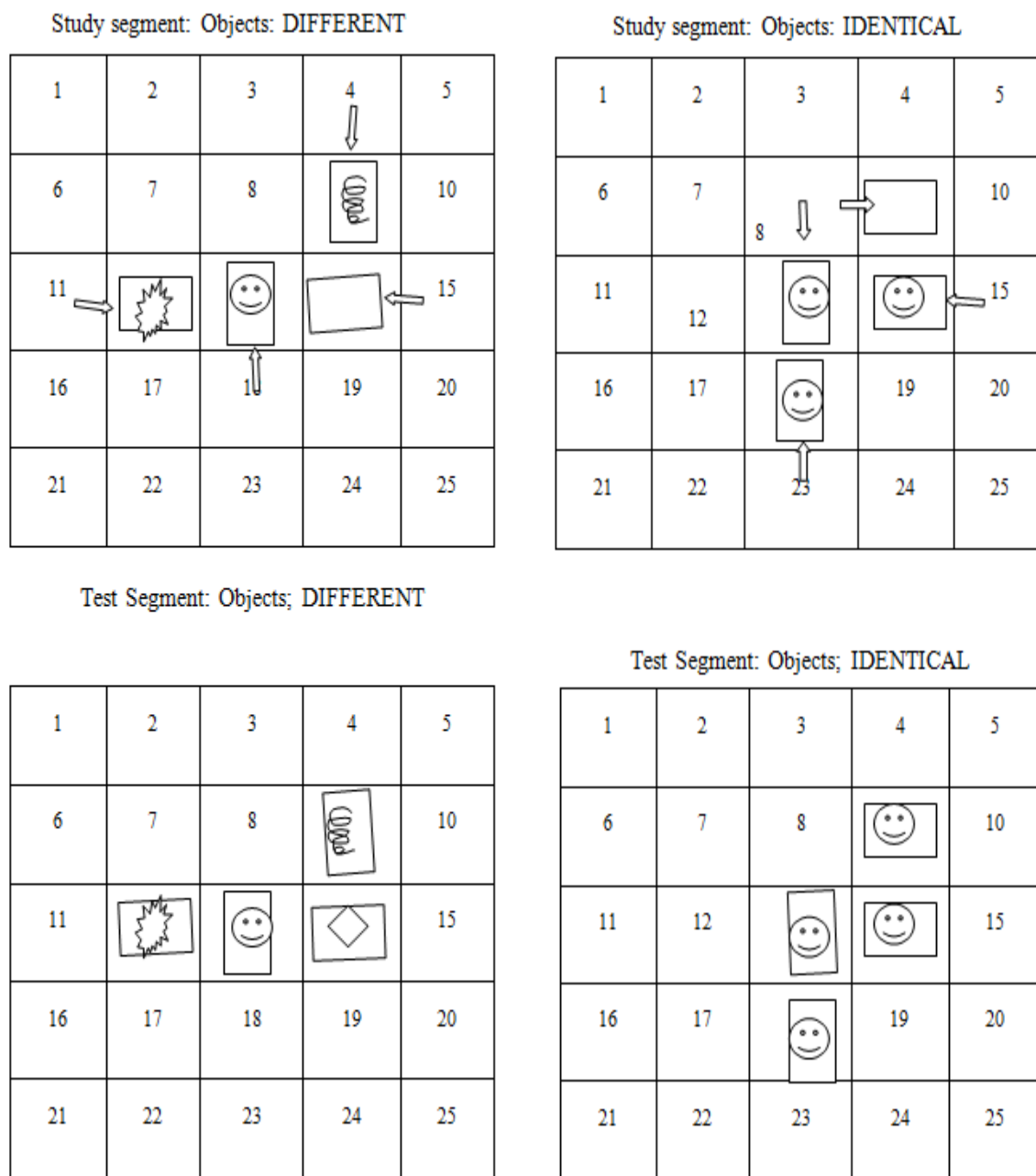


Figure 2: The spatial arrangement of foraging stations in training trials, as they would appear in the study (top) and test (bottom) segment. The upper and lower right figures represent a trial where four or three different objects were used. The upper and lower left figures represent a trial where four or three identical objects were used. The arrows represent the where the rat had to push to uncover the food well in the study segment which remained the same during the test segment on training trials.

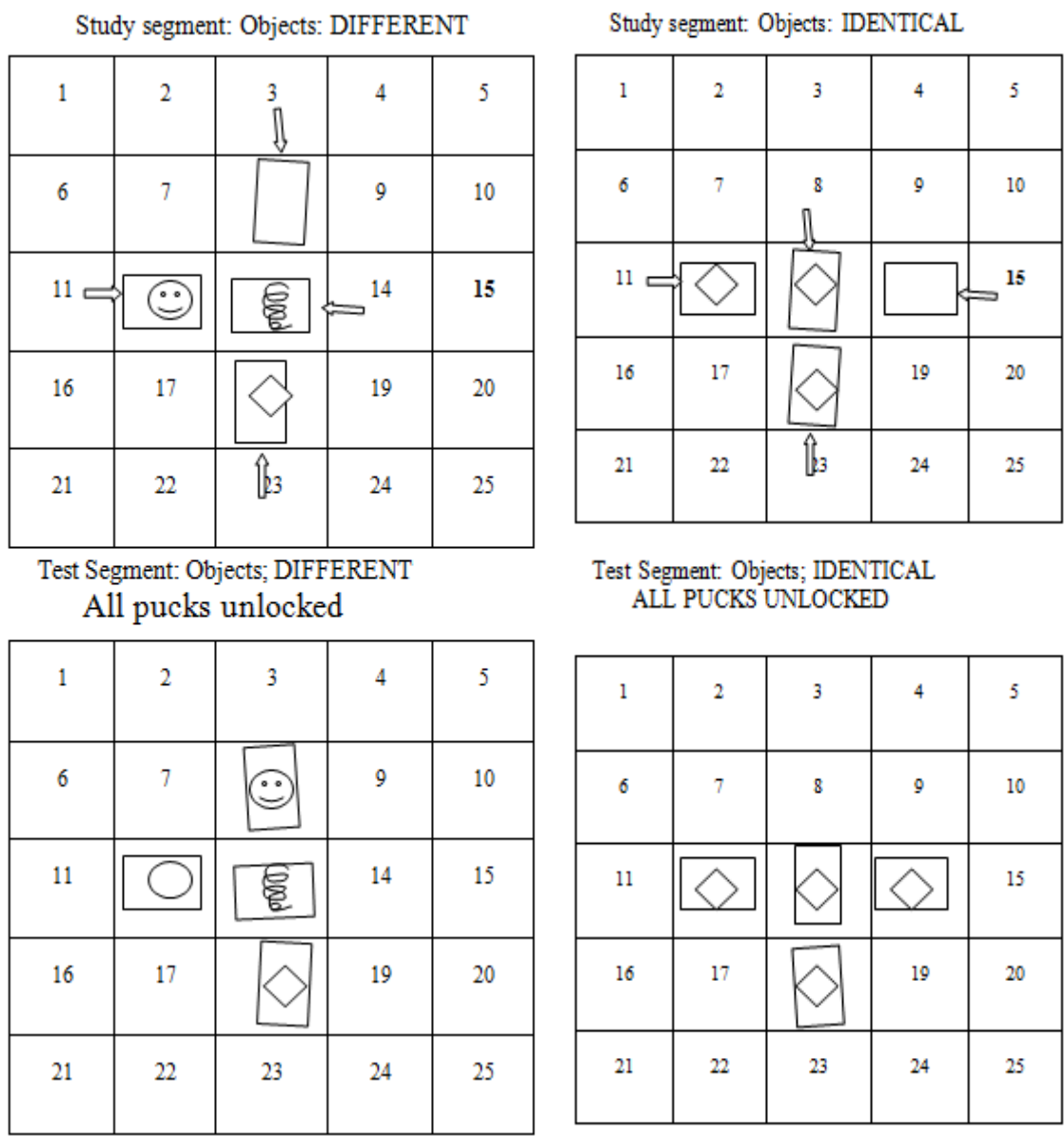


Figure 3: An example of a phase 2 probe trial. All the feeding stations were unlocked and unbaited during the test segment. The arrows represent the where the rat had to push to uncover the food well in the study segment which remained the same during the test segment on these probe trials.

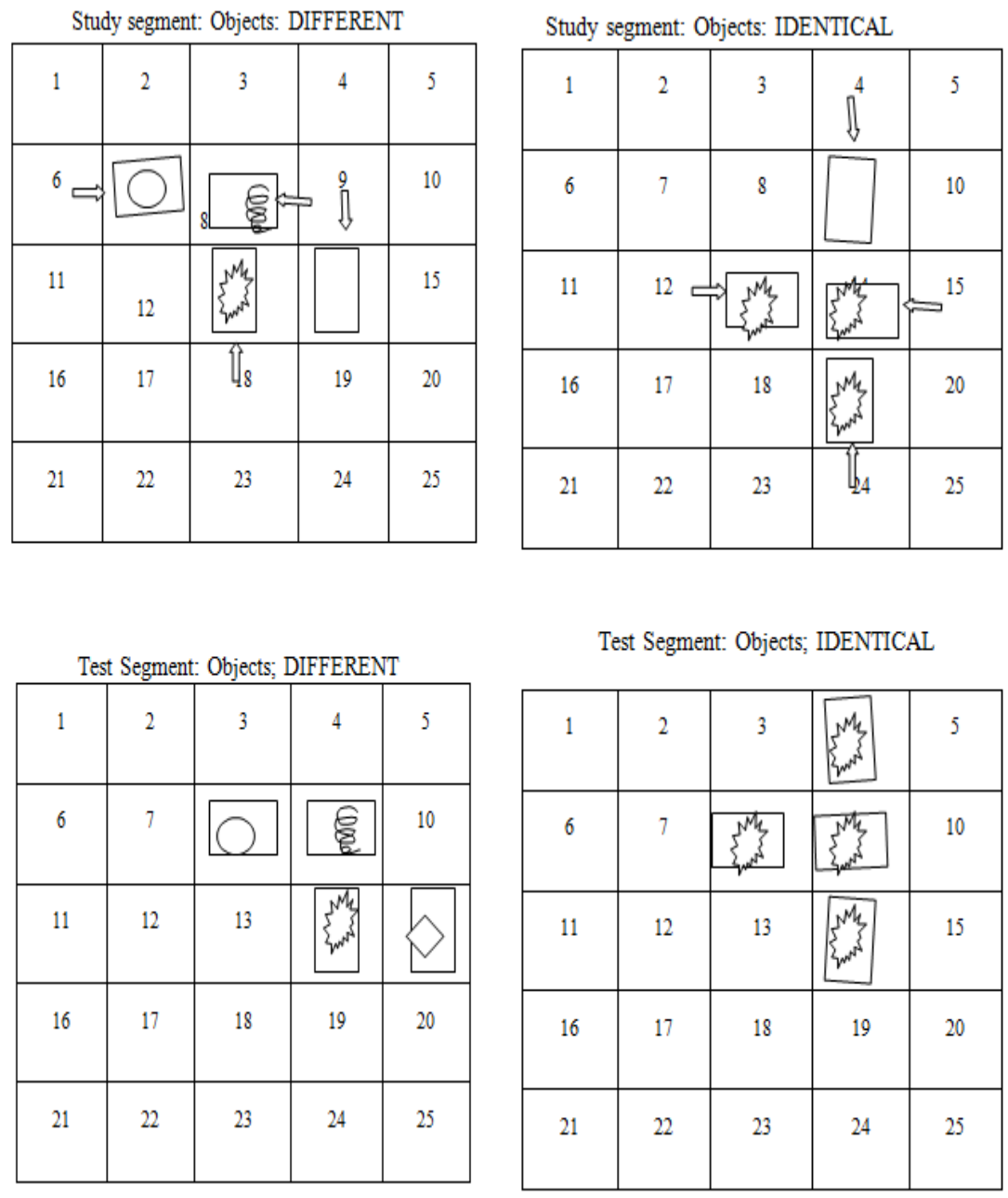


Figure 4: Examples of phase 3 probe trials. All the feeding stations were unlocked and un-baited. The arrows represent the where the rat had to push to uncover the food well in the study segment which remained the same during the test segment on these probe trials.

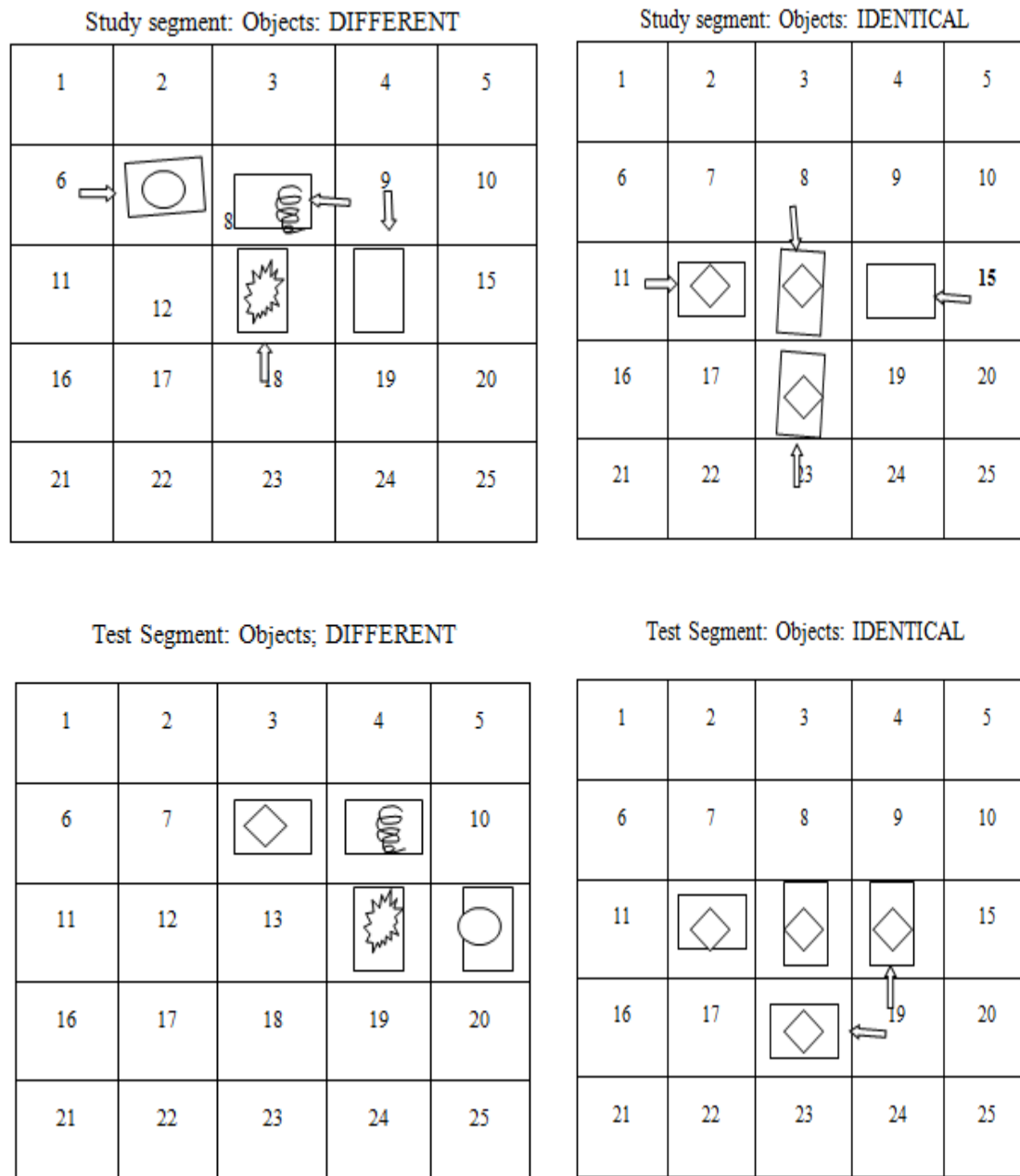


Figure 5: Example of phase 4 probe trials. All the feeding stations were unlocked and un-baited. The arrows represent the where the rat had to push to uncover the food well in the study segment which remained the same during the test segment on probe trials with different objects but were changed for probe trials with identical objects as illustrated in this example.

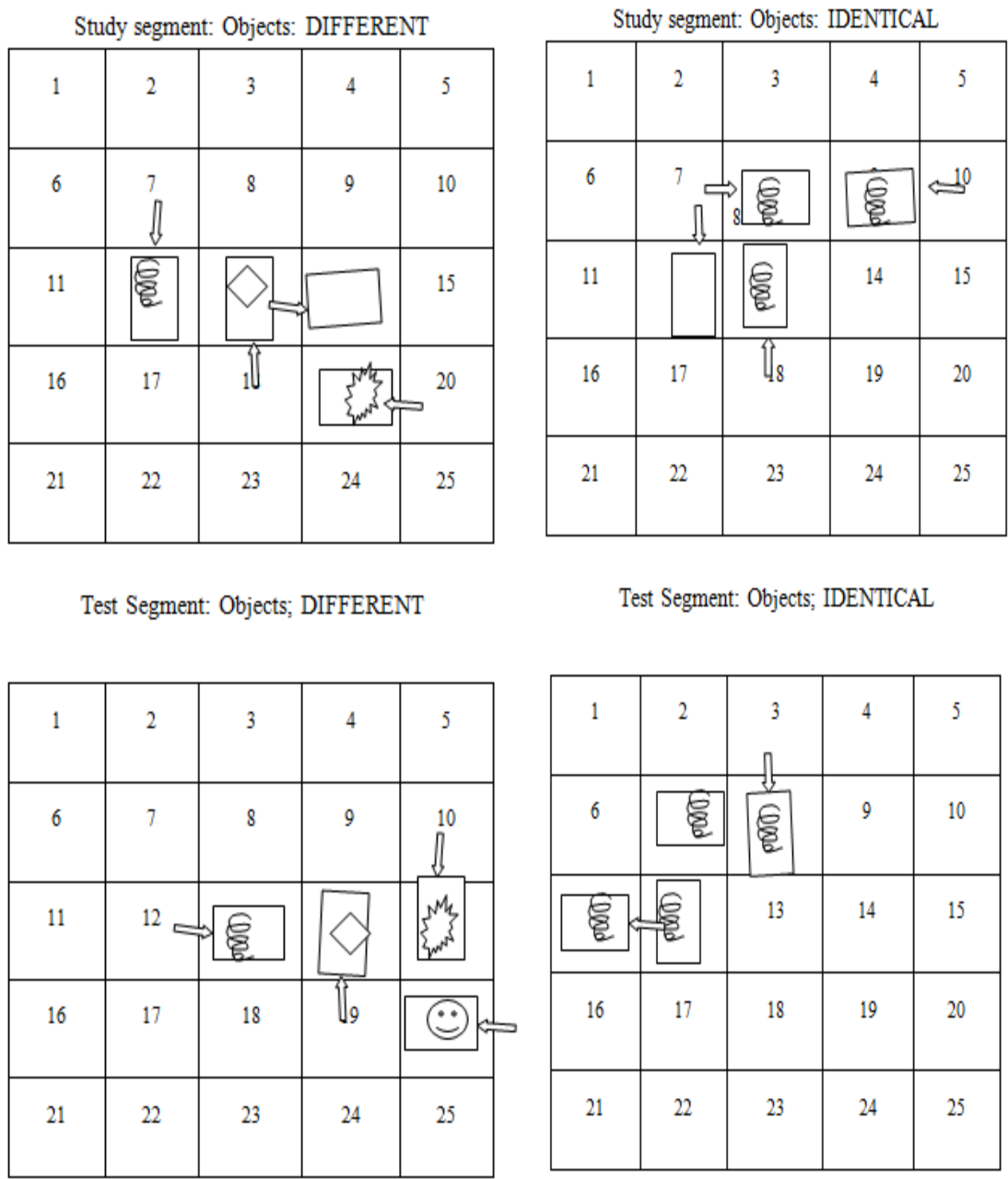


Figure 6: Examples of phase 5 probe trials. All the feeding stations were unlocked and un-baited. The arrows represent the where the rat had to push to uncover the food well in the study segment but were changed for probe trials with different and identical objects as illustrated in these examples.

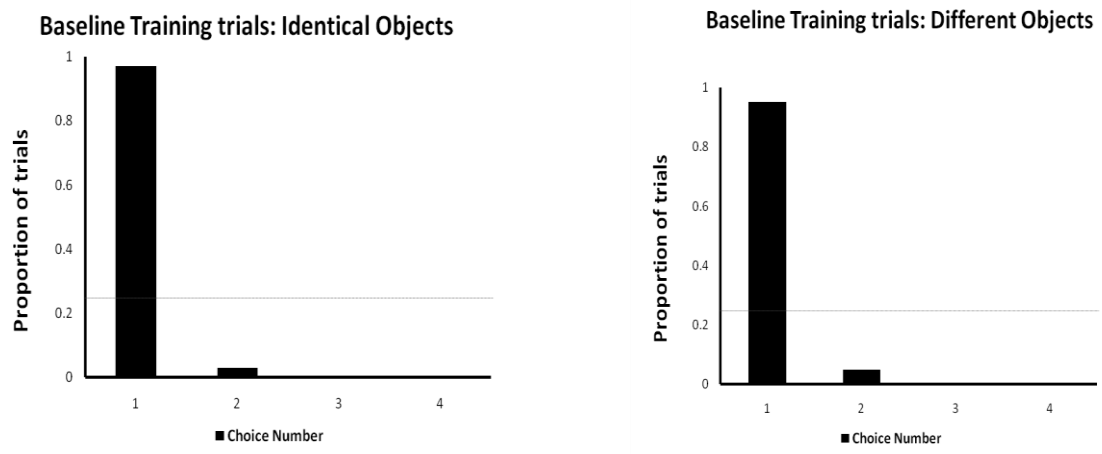


Figure 7: Distribution of number of choices to find the jackpot during the training trials with identical (left) and different (right) objects during the training trial of phase 2.

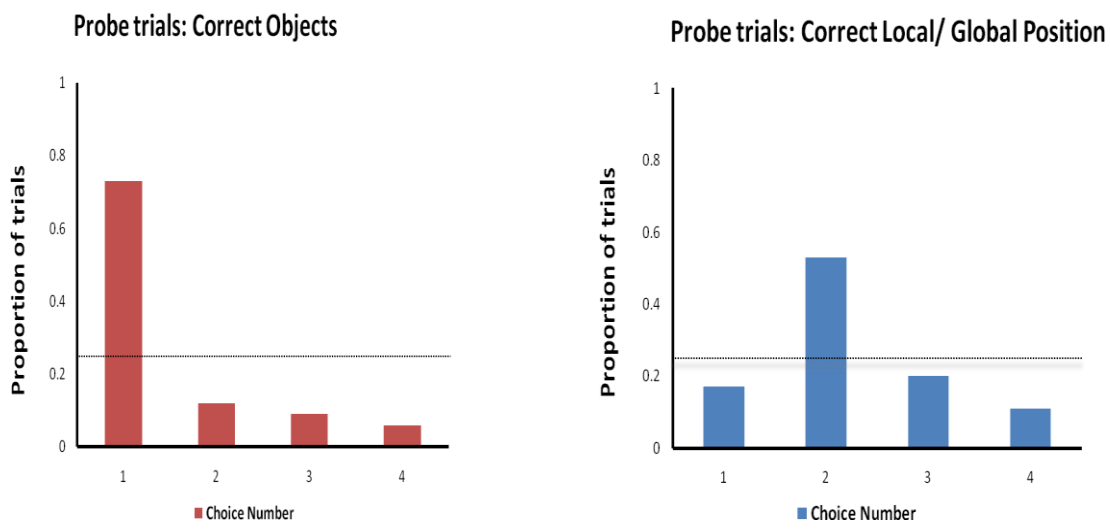


Figure 8 A: Distribution of number of choices to visit the correct object (pattern) (left) or correct local/global position (right) during the different objects probe trials of phase 2.

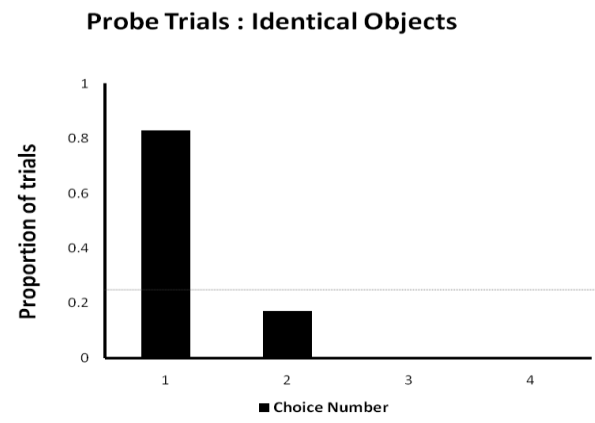


Figure 8 B: Distribution of number of choices to visit the correct object/ local/global position during the identical objects probe trials of phase 2.



Figure 9: Distribution of number of choices to find the jackpot during the training trials with identical (left) and different (right) objects during the training trial of phase 3.

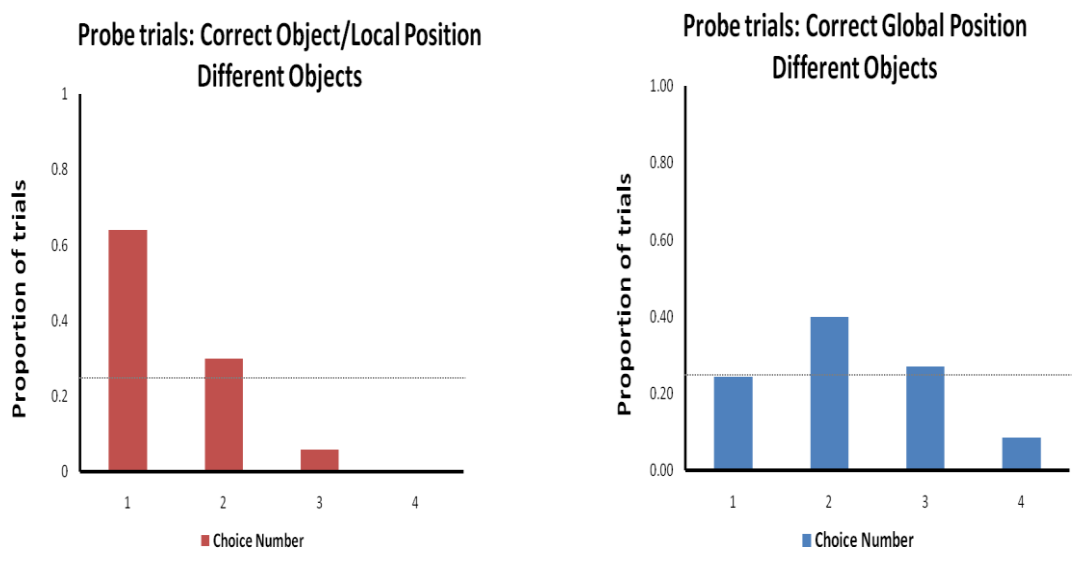


Figure 10 A: Distribution of number of choices to visit the correct object (pattern)/Local position (left) or correct global position (right) during the different objects probe trials of phase 3.

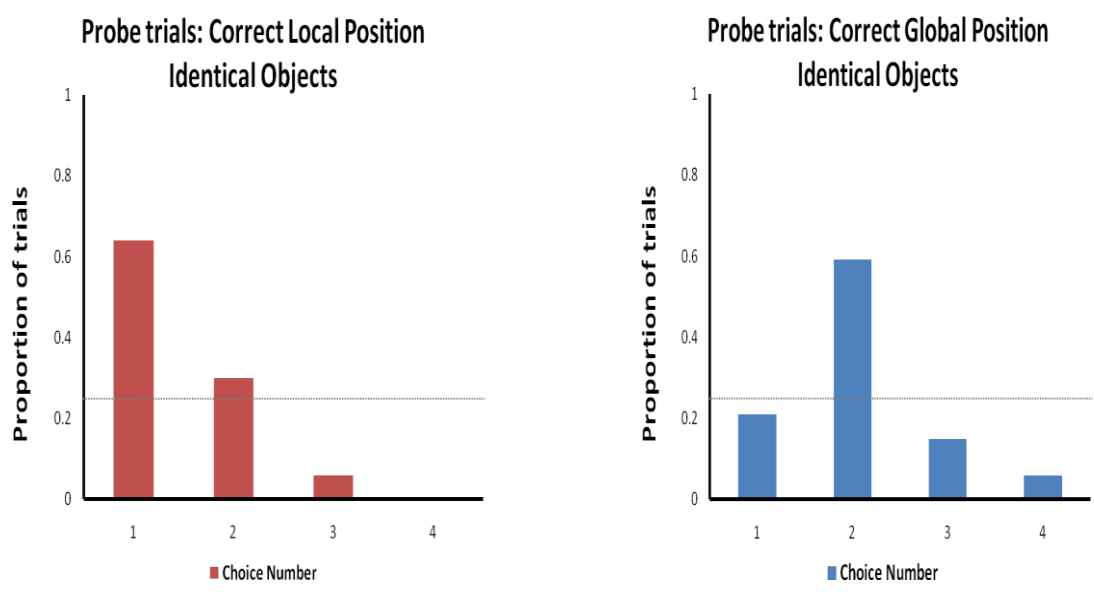


Figure 10 B: Distribution of number of choices to visit the correct Local position (left) or correct global position (right) during the identical objects probe trials of phase 3.

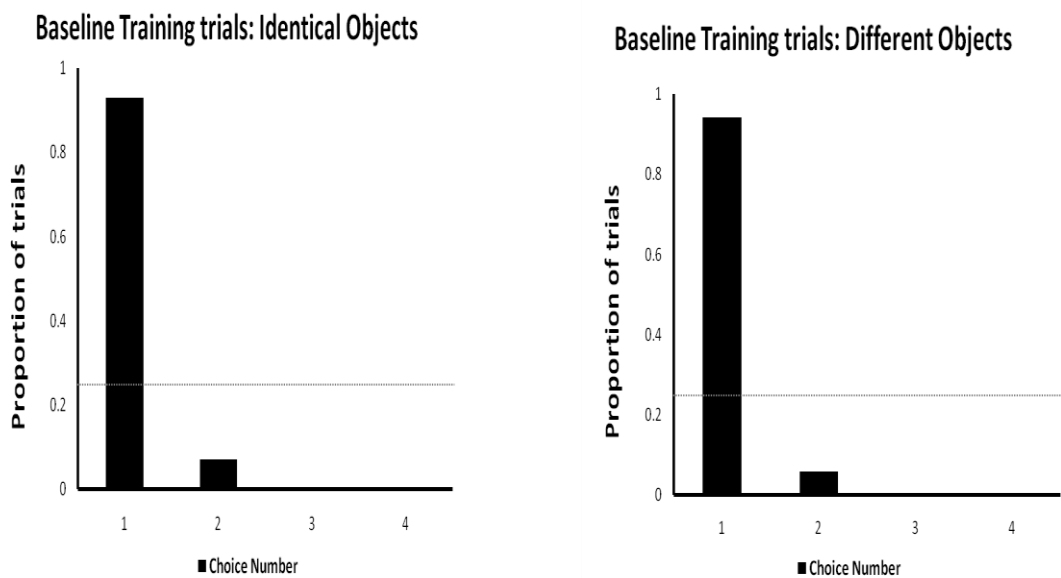


Figure 11: Distribution of number of choices to find the jackpot during the training trials with identical (left) and different (right) objects during the training trial of phase 4.

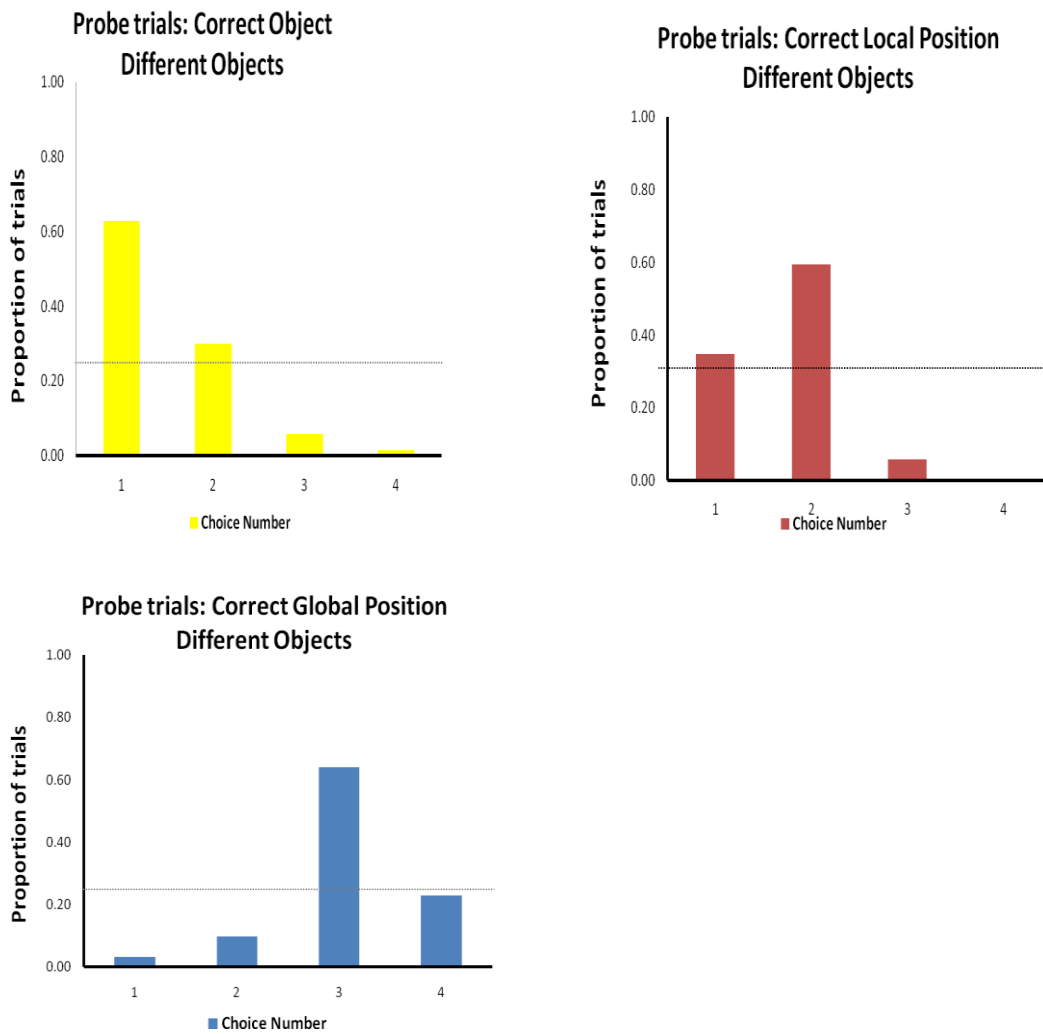
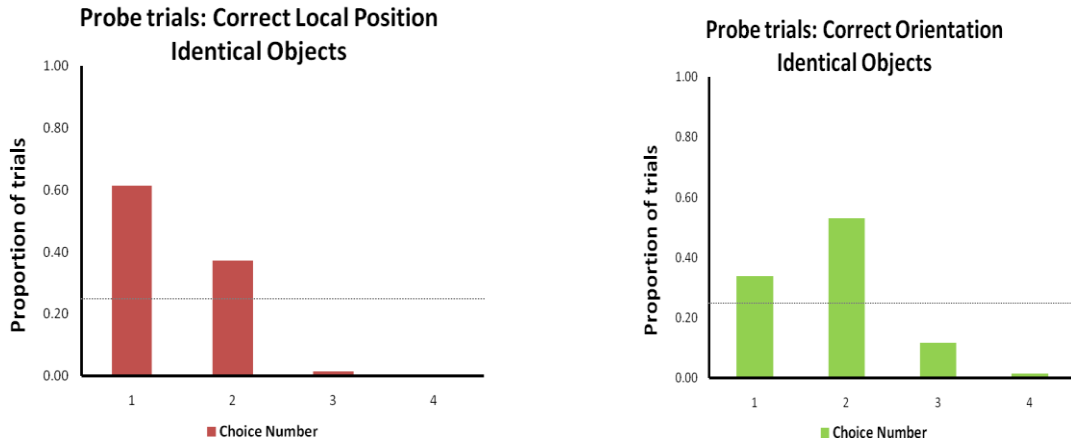


Figure 12 A: Distribution of number of choices to visit the correct object/pattern (top left) or correct local position (top right) or correct global position (bottom) during the different objects probe trials of phase 4.



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Figure 12 B: Distribution of number of choices to visit the correct Local position (left) or correct orientation (right) during the different identical probe trials of phase 4.

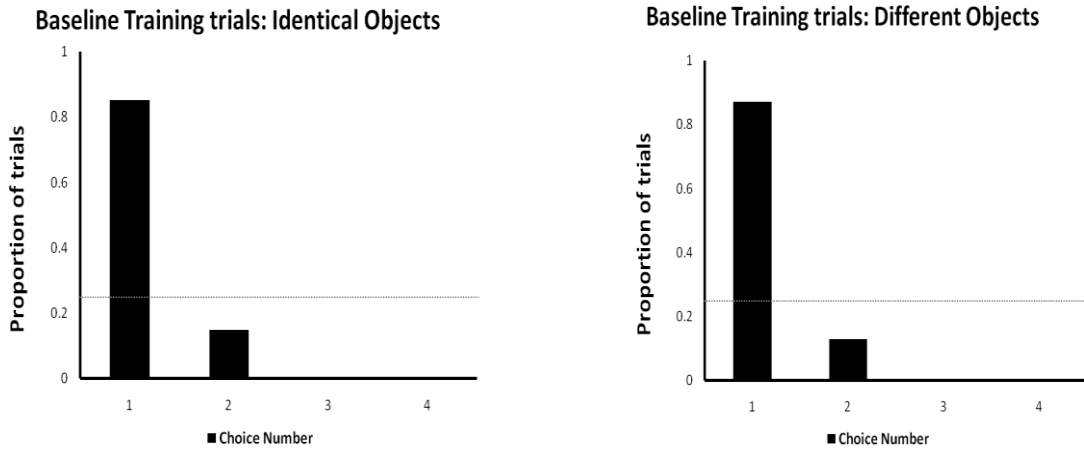


Figure 13: Distribution of number of choices to find the jackpot during the training trials with identical (left) and different (right) objects during the training trial of phase 5.

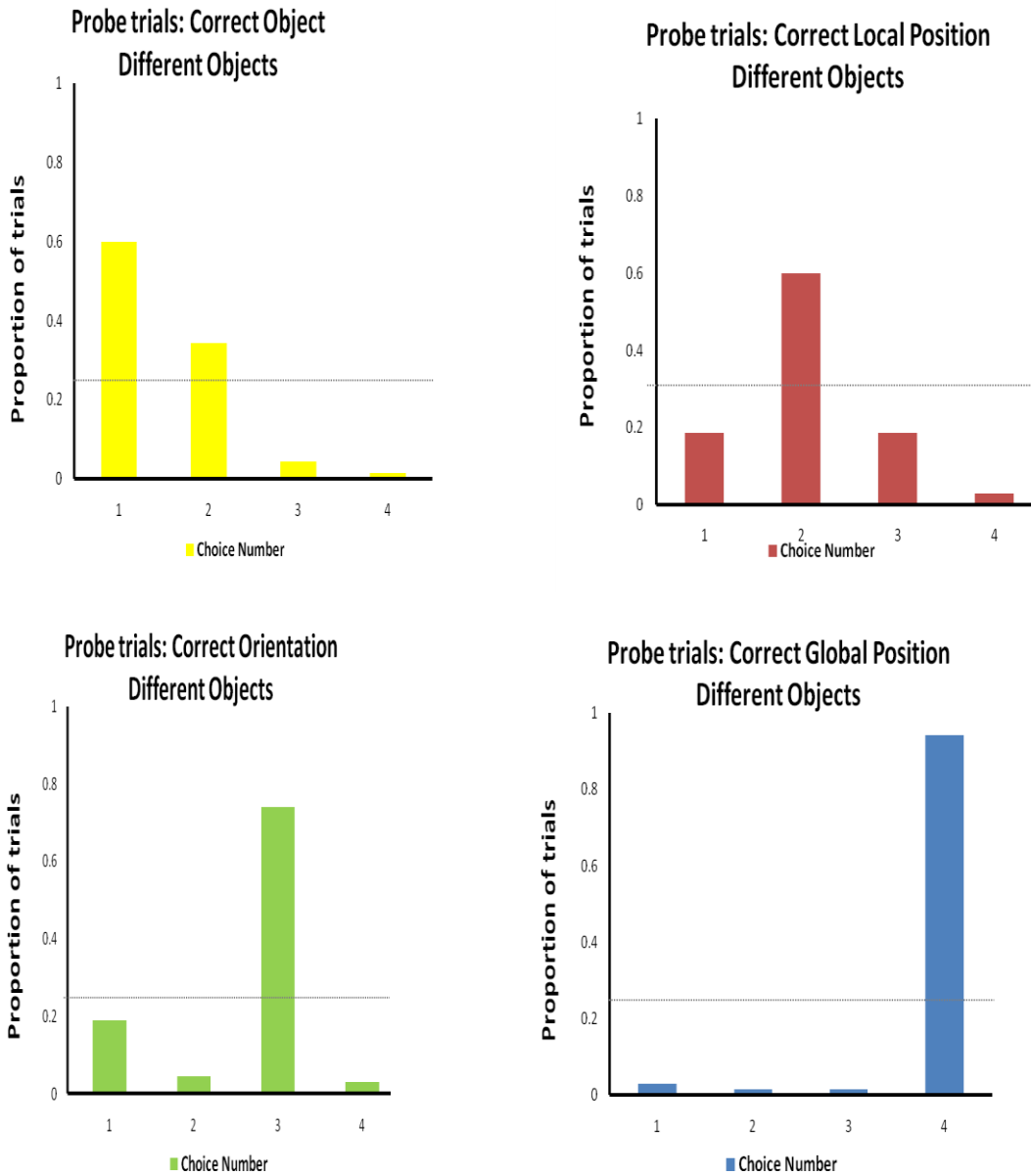


Figure 14 A: Distribution of number of choices to visit the correct object/pattern (top left) or correct local position (top right) or correct orientation (bottom left) or correct global position (bottom right) during the different objects probe trials of phase 5.

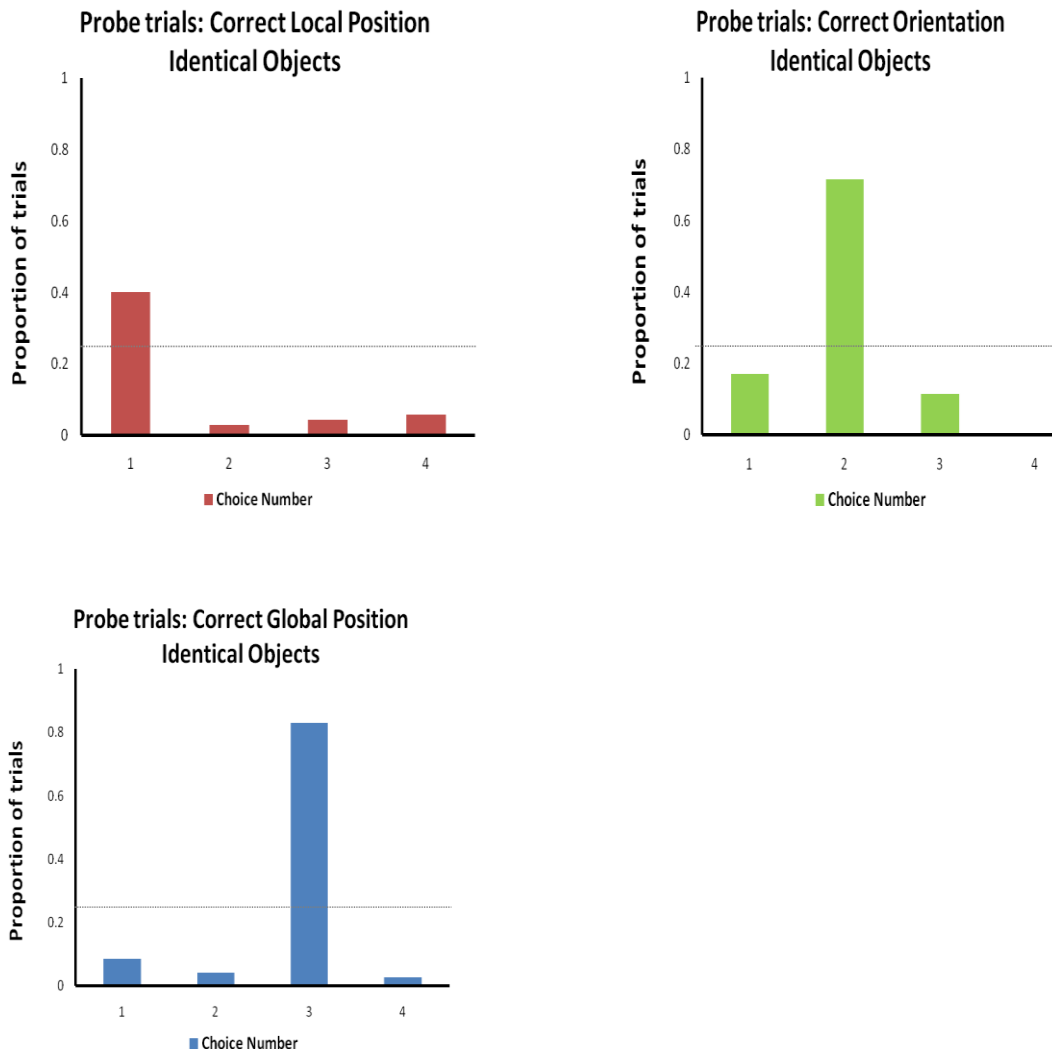


Figure 14 B: Distribution of number of choices to visit the correct Local position (top left) or correct orientation (top right) or correct global position (bottom) during the different identical probe trials of phase 5.

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