Title

Identifying Sex Differences in Spatial Cognition in Laboratory Mice

Author

Bettis, Tania

Publication Date

2011

Identifying Sex Differences in Spatial Cognition in the Laboratory Mouse

By

Tania Jean Bettis

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Psychology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Lucia Jacobs
Professor Frederic Theunissen
Professor Eileen Lacey

Spring 2011

Abstract

Identifying Sex Differences in Spatial Cognition in the Laboratory Mouse

by

Tania Jean Bettis

Doctor of Philosophy in Psychology

University of California, Berkeley

Professor Lucia Jacobs, Chair

Laboratory mice are the most commonly studied mammal today and they are frequently used as models of human disease. In the last decades, the mouse has become a popular model for understanding genes, brains and behavior. Therefore, it is of great importance that we develop a detailed understanding of the cognitive phenotype of the animal that we so frequently utilize in the laboratory. Sex differences are an important consideration in this phenotype. Sex differences in spatial behavior are seen in a number of species, suggesting a male advantage in all things spatial and a female advantage in object recognition memory. These data are consistent among humans and rats, but inconsistent in the mouse. The goals of this study were to discover whether mice would exhibit similar sex differences in a series of tasks designed to assess cue use at different spatial resolutions. The first experiment offers an assessment of cue use in a navigation task. Specifically looking at the use of geometry and large distal cues in the testing environment. The second experiment looks at the details of object recognition. The last experiment tests both object recognition and location memory, to look more closely at the nature of the sex difference with respect to proximal cues. We housed the mice in conditions that were more ethologically valid to allow a better comparison of our data to humans, as well as enriched laboratory animals and wild caught animals that must organize their behavior in much more complex environments than those provided in the lab. The navigation task revealed a male reliance on geometry and a female flexibility in the use of multiple sets of cues. The standard object recognition tasks all demonstrated a female advantage in discriminating the novel from the familiar object. Female mice distinguished novel from familiar objects when these objects had many similar features, while male mice only discriminated between them if the objects were unlike one another. Frequently, female mice also exhibited greater attention to the objects. The male mice demonstrated an advantage only when the change provided new directional information. The female mice displayed evidence of attention to the relationships between unique cues and the features encompassed within them. This is consistent with current literature on rats and humans and suggests that mice also exhibit sex difference in cue use strategies. This suggests

that, with attention to ethologically valid housing and testing, mice are an appropriate model of mammalian behavior and cognition.

This dissertation is dedicated to my family.

"One looks back with appreciation to the brilliant teachers, but with gratitude to those who touched our human feelings. The curriculum is so much necessary raw material, but warmth is the vital element for the growing plant and for the soul of the child."- Carl Jung

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to all of those who have supported me for so long in this endeavor. While this dissertation is an individual project, it would not have been possible without the support of many others. I am particularly grateful to my academic advisor, Lucia Jacobs for her guidance and constant support, which allowed me to conduct and complete this work. I am grateful for her guidance through some difficult setbacks and her consistent expression of faith in my ability to get through this work. Her enthusiasm and infectious zeal carried me through many challenging doldrums. I would also like to thank Eileen Lacey for her encouragement and inspiration as an instructor. She provided a calm and consistency that was always much appreciated while dealing with the ups and downs of graduate research. In addition, I would like to thank Frederic Theunissen for serving on my advisory committee as well as providing that extra push when I needed it. I also want to thank all of the members of our lab for active discussions of this research and for putting up with the repeated practice talks on the same topics time and again. Specifically, I want to thank Anna Waismeyer, Amy Cook, Jennifer Arter, Xiaoqian Chai, Mikel Delgado, Eric Abelson, Paul Elsen, Allison Gregor, and Molly Nichols for all of their input to the research and for their friendship.

Most importantly, none of this would have been possible without the love and patience of my family to whom this dissertation is dedicated. I am deeply grateful to my husband, Sam Evans, for the emotional support, sacrifice, and indulgence throughout this process. I never would have made it this far without his humor, love, and understanding. I would also like to thank my two daughters for their support and patience. It is done and now I can play outside. Last, but not least, I would like to thank my parents and in-laws for supporting all of us with many hours of babysitting and getting us, as a family, through many stressful weeks. For listening to all of the woes and for always helping me to make it over just one more hurdle- thank you.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION TO SEX DIFFERENCES IN SPATIAL BEHAVIOR AND LABORATORY MICE

> *"These creatures you call mice, you see, they are not quite as they appear. They are merely the protrusions into our dimension of vastly hyperintelligent pandimensional beings. The whole business with the cheese and the squeaking is just a front."*
>
> *The old man paused, and with a sympathetic frown continued. "They've been experimenting on you, I'm afraid"*
>
> *Arthur thought about this for a second, and then his face cleared.*
>
> *"Ah, no," he said, "I see the source of the misunderstanding now. No, look, you see what happened was that we used to do experiments on them. They were often used in behavioral research, Pavlov and all that sort of stuff. So what happened was that the mice would be set all sorts of tests, learning to ring bells, run round mazes and things so that the whole nature of the learning process could be examined. From our observations of their behavior we were able to learn all sorts of things about our own . . . ."*
>
> *Arthur's voice trailed off.*
>
> *"Such subtlety . . ." said Slartibartfast, "one has to admire it."*
>
> *- From The Hitchhiker's Guide to the Galaxy by Douglas Adams*
> *(Adams 1986)*

The above quote illustrates well the sense one gets both when reviewing the literature on spatial cognition in laboratory mice and when working with them as subjects in behavioral studies. Since the mapping of the mouse genome, mice have become the most commonly studied laboratory mammal in the world (Rosenthal and Brown 2007). Laboratory mice have many characteristics that make them ideal models of mammalian disease. They are relatively inexpensive and easy to maintain and they breed rapidly. In addition, mice share 99% of their genome with humans (Gunter and Dhand 2002). This results in very similar physiologies of internal systems and responses to disease treatments. In the last two decades, mice have also become popular as models of cognition, brain, and behavior. The formation of new transgenic models has given us a new insight into the genes and neural systems involved in learning and memory. For example, Rampon et al. (2000) produced mice that lacked an NMDA receptor only in the CA1 region of the hippocampus. These mice expressed a phenotype that was impaired in an object memory task relative to controls and this impairment was recovered with enrichment, thus demonstrating both the involvement of CA1 (and the NMDA receptor) in this kind of learning and its plasticity in response to environmental stimuli. The continued generation of conditional mutant models such as this will contribute much to our understanding of mammalian brain function and dysfunction. To more efficiently utilize these models we will need a clear understanding of the cognitive phenotypes of the background strains. As Medawar stated, "it is not informative to study variations of behavior unless we know beforehand the norm from which the variants depart" (Medawar 1967).

In laboratory studies of brains and behavior, often the norm is defined by observations made only from the male population. However, there is considerable evidence that suggests that this may lead to inappropriate generalizations (Cahill 2006). Sex differences are particularly important to consider when studying spatial cognition. Sex differences in spatial cognition and behavior have been found in a diverse range of species, including the molluscan cuttlefish (*Sepia officianalis*), blenniid fish (*Parablennius parvicorus* and *Lipophrys pholis*), ravens (*Corvus corax*), brown headed cowbirds (*Molothrus ater*), rats (*Rattus norvegicus*), kangaroo rats (*Dipodomys merriami*), meadow voles (*Microtus pennsylvanicus*), deer mice (*Peromyscus maniculatus*), chimpanzees (*Pan troglodytes*), rhesus monkeys (*Macaca mulatta*), and humans (*Homo sapiens*) (for a review see (Jones et al. 2003)). In addition, both anatomical and neurochemical sex differences have been observed in various brain structures including the hippocampus and amygdala (Cahill 2006).

The need for sex specific information is highlighted by the observed differences in incidence and/or nature of many psychological afflictions. For example, Alzheimer's disease and depression disproportionately affect women while attention deficit disorder and autism are more prevalent among male populations (Yue et al. 2005; Kessler 2003; Henningsson et al. 2009; Martel et al. 2008). Barnes et al. (2005) reports that the relationship between Alzheimer's disease pathology (presence of neurofibrillary tangles) and clinical diagnosis of the disease differs between men and women such that the pathology is more often expressed as dementia in women than men. Such differences suggest that the development of sex-specific treatments and/or preventative measures may be necessary for many central nervous system diseases. Therefore, it is critical to assess sex differences in laboratory mice as they are used as models of these diseases and as subjects to test the efficacy of various treatments. If we are to understand the evolution, development and mechanism of these learning and memory processes, then it is critical that sex differences are assessed in any model of mammalian cognition.

As Niko Tinbergen suggests, any understanding of a behavior should begin with a description followed by an answer to the four questions (i.e. causation, evolution, adaptive function and ontogeny) that he laid out in his paper " On aims and methods of Ethology" (Tinbergen 1963). Towards this end, the next sections will begin with a description of the sex differences seen in the field and in the laboratory. Following this review, I will discuss the goals and hypotheses that were the motivation for the research described in later chapters.

1.1 Sex differences in home range size

As mentioned above, sex differences in spatial behavior are seen in a diverse range of species. Outside of the laboratory, this difference is reflected in the relative home range sizes of the sexes within each of the species mentioned above. A home range is "the area traversed by an individual in its normal activities of food gathering, mating, and caring for young" as defined by Burt (1943). The size of a home range is determined by factors such as the dispersal of food, suitable shelter, and potential mates as well as the metabolic needs of the individuals under consideration (Harestad and Bunnell 1979). For example, in the polygamous wood mouse (*Apodemus sylvaticus*), male mice occupy home ranges that are 1.6 times larger than their female conspecifics outside of the breeding season (Attuquayefio et al. 1986). This difference in home range size outside of the breeding season was accounted for by the difference in mean body weight between the sexes. Male wood mice are larger than female wood mice, and therefore require a larger home range to acquire the necessary food resources. In addition, during the breeding season when potential mates are an important resource, the male wood mouse home range is expanded to 3.5 times that of the female wood mouse (Attuquayefio et al. 1986). As a result of the relationship between home range size and the dispersal of resources, Gaulin and FitzGerald (1988) postulated that relative home range sizes could be used as a predictor of mating systems. In polygamous species, the male typically occupies a larger home range than the female, especially during the breeding season when the distribution of potential mates becomes a significant factor in determining home range size. In monogamous species, the two sexes tend to occupy relatively similar spaces both in and out of the breeding season because food distribution is the critical determinant of home range size. For example, the polygamous male meadow vole (*Microtus pennsylvanicus*) occupies a home range that is, on average, 4.5 times larger than female con-specifics during the breeding season, while the monogamous prairie voles (*Microtus ochrogaster*) maintain home ranges that do not significantly differ between the sexes (Gaulin and FitzGerald 1988).

If mating systems are predictive of home range size, then determining the mating system employed by humans is a good place to begin to address sex differences in spatial cognition in humans. An assessment of the worldwide Y chromosome diversity in humans indicates a recent increase in the population size of breeding males. This suggests that humans have undergone a recent shift from an historically polygynous mating system, with a small effective population of males, to a more monogamous mating system and thus, a larger effective population of males and an increase in Y chromosome diversity (Dupanloup et al. 2003). Therefore, when one considers the polygynous mating system humans employed for much of our evolutionary history, one may also expect to see sex-specific patterns of space use that resemble those patterns seen in other polygamous mammals. In support of this, there is some evidence that men travel more widely (more km in a year) than women and participate in more "spatial activities" (Carlsson-Kanyama and Linden 1999; Newcombe et al. 1983). However, today human travel is determined more by cultural norms than a set of limiting resources, and the qualification of an activity as "spatial/ non-spatial" or "masculine/feminine" in the cited study was based on the judgments of participating undergraduate students and may also reflect cultural influences rather than an underlying sex difference in spatial cognition.

Laboratory mice are not subjected to this cultural influence and as a result, may offer a view of sex differences in spatial cognition that is unfettered by this confounding variable. However, it is unclear what the behavior of a laboratory mouse would look like in the field. Evidence from free-living, commensal and feral populations of mice (*Mus musculus*), from which the laboratory mouse is derived, suggests that home range size and territoriality vary greatly with both food availability and population density (Chambers et al. 2000; Latham 2004; Berry and Bronson 1992). In a study of two populations of feral house mice living in the wheatlands of Australia, Chambers et al. (2000; Attuquayefio et al. 1986) found that male mice tended to have larger home ranges than female mice (though home range sizes varied widely from 0.0002- 8.024 ha). This study also found that there was little to no overlap of female home ranges throughout the year indicating low tolerance of invaders, while male home ranges overlapped extensively towards the end of the breeding season and very little during the breeding season indicating a change in male territoriality across seasons. Other studies of feral mouse populations have found home ranges that range from 365 m^2 in open fields to 60,000 m^2 on the Russian steppes and 80,000 m^2 in the wheatlands of Australia (Berry and Bronson 1992; Latham 2004). In studies of commensal populations of mice, home ranges may vary from as little as a square meter to six square meters (Latham 2004). Commensal populations of mice display strong territorial defense with the territories of males overlapping those of females but never overlapping with other male territories (Bronson 1979). Thus, there is some evidence that supports the existence of a sex difference in space use patterns in wild populations of mice. This evidence also suggests that mice demonstrate great plasticity in response to changing environmental conditions, which indicates at least a need for close attention to housing practices in laboratory settings.

In summary, many polygamous mammals, including humans and mice, demonstrate a sex difference in home range size. Typically, males occupy a larger home range compared to female conspecifics and this sex difference in space use is either exaggerated or seen only during the breeding season. The next section will focus mainly on laboratory studies of spatial cognition. These studies provide a more detailed picture of the types of cues that are used by subjects to build a cognitive map and subsequently organize behavior in complex environments.

1.2 Cognitive maps: what information is encoded within a home range?

The term, “cognitive map” was first introduced by Tolman to denote a mental representation that can indicate a route or path and various relationships within an environment (Tolman et al. 1946). The idea that non-human animals might maintain any mental representation of the external world was extremely controversial and is still debated among scholars of animal behavior (Bennett 1996). One outcome of the discussion of spatial representations has been the proposal of a wide variety of definitions of cognitive maps. Gallistel (1989) provides one general definition- “A cognitive map is a record in the central nervous system of macroscopic geometric relations among surfaces in the environment used to plan movement through the environment.” Others define the cognitive map, operationally, as a representation that allows for novel routes between two locations in the absence of immediately perceptible features near a goal (Bennett 1996). Since the discovery of place cells in the hippocampus, frequently cognitive maps are implied if activation or normal functioning of the hippocampus is required to complete a task and navigate through an environment (O'Keefe and Dostrovsky 1971). For the purposes of this discussion, I will use the term, cognitive map, to mean an allocentric representation of an environment that supports the formation of novel routes.

The question regarding the various processes and types of information animals (both human and non-human) may use when organizing behavior in a complex environment has generated many studies. The results of which convey an appreciation for the various, functionally distinct operations that support complex spatial behavior. In addition to the cognitive map, animals may locate a goal via path integration (Alyan and Jander 1997; Etienne and Jeffery 2004), following a learned route (Galea and Kimura 1993), or by orienting towards a beacon (Gallistel 1989). Path integration (also referred to as dead reckoning) makes use of the internal cues of motion and direction to construct a vector that contains the distance and directional information necessary for an efficient return to a start location (Collett et al. 2003). This type of navigation is particularly well studied in hymenopteran insects (Schmidt et al. 1992). To follow a learned route, an animal may learn a particular sequence of landmarks without requiring any global positional information about the landmarks (Galea and Kimura 1993). In order to use a beacon to navigate, an animal must simply orient towards a landmark associated with a goal location and move towards it. Again, orienting towards a beacon is a process that does not require the positioning of the individual or the beacon in any larger spatial framework and therefore does not require the use of a cognitive map.

In addition to the many operations discussed above that do not require a cognitive map, there are many sources of information that can be utilized when building a cognitive map. Based on a large review of the literature regarding the hippocampus, Jacobs and Schenk theorized that the hippocampus may be responsible for the formation and maintenance of the cognitive map via the integration of two separate components or parallel maps, each relying on a different set of information or cue type (Jacobs and Schenk 2003). One of these components results in a coordinate system termed the bearing map, which is built on the integration of directional information provided in the environment. This directional information can come from gradients of the distribution of odor, light, or sound cues in the environment. Magnetic and sun compasses can also provide directional cues as well as large distal landmarks, such as mountain ranges, that do not change aspect ratio as an individual moves closer. Directional cues can also be provided in the geometry of an array of local landmarks. When combined, these cues can form a coordinate system that allows for the creation of novel routes. The second of the two parallel maps is called the sketch map and is based on the topographical arrangement of local positional cues. Positional cues consist of unique objects that are often near a goal and can provide distance information to that goal. Together these two components form the integrated cognitive map. In isolation, each of the two parallel maps supports different types of behavior. An animal using only the bearing map will be able to orient well directionally, but will possess little to no fine-grained detail regarding the distance relationships between goals and landmarks. An animal using only the sketch map will possess fine-grained detail of the relationships of local cues and can triangulate between these cues to find a goal, but will orient poorly in a global framework and thus have a poor sense of direction (Jacobs 2003).

This Parallel Map Theory provides an interesting framework in which to address the sex differences found in studies of spatial cognition. For example, as discussed in the previous section, Gaulin and FitzGerald (1986) found that polygamous meadow voles (*Microtus pennsylvanicus*) exhibit a sex difference in home range size. They also found a sex difference in performance on a sunburst maze. This task consists of training an animal to collect a reward at the end of a circuitous pathway leading off from a starting platform. After training, the circuitous path is blocked and a number of straight paths are then attached to the starting platform radiating outwards. Accuracy in choosing the arm with the most direct path to the previously rewarded location is used as a measure of spatial cognition. In regards to the Parallel Map Theory, this task measures bearing map function. The only available cues are directional and selecting the most direct path to the goal location does not require triangulation or distance estimates, only an estimate of direction. Male meadow voles (*Microtus pennsylvanicus*) performed this task with greater accuracy than female meadow voles (Gaulin and FitzGerald 1986). (A comparison of the performance of the monogamous male and female prairie voles (*Microtus ochrogaster*) did not reveal a significant sex difference.)

In addition to the sunburst maze, there are a few tasks that are more commonly used to assess spatial cognition in the laboratory. These are the water maze, the radial arm maze, mental rotation and object location memory tasks. Each of these tasks has been adapted for a number of species and consists of many slight variations on a theme, with the exception of mental rotation, which is only carried out with human subjects. In addition, each of these tasks can be used to assess biases in the reliance on different cue types (i.e. local landmarks, distal landmarks or gradients) and thus, cognitive map components.

The first task mentioned, the water maze, was originally developed to assess spatial memory in laboratory rats and has been adapted for a number of other species (Morris et al. 1982). More recently, the water maze has been developed as a virtual maze for human subjects (Astur et al. 1998). To successfully find the reward (either a hidden platform in the real version or a token in the virtual version of the maze), subjects must utilize the directional information provided by cues outside of the maze. The available cues can be controlled and can vary widely across labs, but generally include the geometry of the room, any furniture arranged around the room or posters on the wall. The use of randomly chosen starting points in the water maze also allows the experimenter to prevent the subject from utilizing internal cues to follow a learned route. In this configuration, like the sunburst maze, the water maze is another good indicator of the function of the bearing map.

Using the water maze task, Galea et al. (1994) found a seasonal sex difference in two populations of deer mice (*Peromyscus maniculatus*). In the non-breeding season, no sex differences were observed in either the latency to find a hidden platform or in the time spent searching the correct quadrant (were the platform had previously been). However, during the breeding season there was a significant sex difference. In the breeding season, male deer mice reduced their latency to the platform and increased the time spent in the correct quadrant while the observed latency to the platform for the female deer mice had increased and the time spent in the correct quadrant was reduced. The possible explanation given is that female spatial cognition is somewhat impaired during the breeding season, while male performance is enhanced and that this might allow for the longer distances males travel while courting females and it may cause the females to reduce their travels while breeding. Like the previous study of deer mice, rats and humans consistently demonstrate a male advantage in water maze tasks (for a review of the literature on laboratory rodents see (Jonasson 2005)) (Astur 2004; Dabbs et al. 1998).

Similar to the water maze, the radial arm maze was developed as a tool to assess spatial memory in laboratory rats and was later adopted as a method for humans in virtual mazes (Olton and Samuelson 1976). The radial arm maze consists of a number of corridors extending out from a central platform like the spokes of a bicycle wheel. During training, the subjects learn which of the arms are baited and can then use this reference memory to efficiently find and collect a reward at the end of all of the baited arms. Working memory can also be tested in this maze and is defined as a function of the number of errors (re-entering an arm within a trial). In the many versions of the radial arm maze, the available cues may vary from local cues, such as texture or color within the maze, to distal cues, such as the geometry of the room outside of the maze. In addition to testing both working and reference memory, the radial arm maze can be utilized to assess the types of cues that subjects rely on during navigation. For example, Williams and Meck (1990), showed that male rats rely on the geometry of a room to navigate the radial arm maze accurately, while female rats rely on both the geometry and the features of the local landmarks surrounding the maze. This indicates a male bias towards the use of the bearing map while female rats use both the bearing and sketch maps while navigating this maze.

Unlike the two tasks described above, the mental rotation task does not seem to have a correlate in the non-human animal literature. This task is a pen and paper task, which provides subjects with pairs of objects in different rotational positions. Subjects are then asked to identify whether or not the two objects within the pair are the same or different. Men are consistently more accurate when making these identity judgments and performance in this task correlates well with performance in the above two tasks when directional cues must be used for navigation (Astur 2004; Alexander and Evardone 2008). The relationship between the underlying operations of the mental rotation tasks and those of spatial navigation in a water maze and/or radial arm maze remain unclear. However, performance on this task correlates with navigational strategy, specifically with the use of the bearing map while navigating a virtual water maze (Saucier et al. 2002).

Like the mental rotation task, the object location memory task that is conducted with human subjects is a pen and paper task. This task provides subjects with an array of objects on a paper. After a brief interval subjects are given a similar array with minor changes in the location of some objects, the removal of others, and the addition of novel objects. Subjects are then asked to identify changes made to the array of objects. In terms of the Parallel Map Theory, this task would be a good measure of the function of the sketch map as it assesses the memory for objects and their relationships to one another. Women consistently demonstrate better memory for objects and their locations (for a review see (Voyer et al. 2007). In a similar task of object location memory in rodents, animals are provided with objects to explore in an arena. After an inter-trial interval subjects are returned to the arena where some of the objects have been moved to new locations. As in human subjects, laboratory rats demonstrate a female advantage in this task (Saucier et al. 2008).

In summary, the results from various tasks used to assess spatial cognition suggest a female advantage in the use of the sketch map and a male bias towards the use of the bearing map. The results regarding sex differences in spatial cognition in humans and non-humans alike have led to various hypotheses about the potential adaptive significance of such a sex difference. One such hypothesis, suggests that the human sex difference in spatial cognition reflects the disparate foraging roles of women and men (Eals and Silverman 1994).The suggestion is that this sex difference is a result of selection for hunting ability in men and gathering abilities in women (Postma et al. 1998); (New et al. 2007). This hypothesis has the advantage of explaining both the male advantage in tasks that require a bearing map and the female advantage seen in tasks that require the sketch map. However, this pattern of results is consistent with those seen in other mammalian species that have been tested and suggests that sex differences in spatial cognition have been conserved across the mammalian taxa. It is this similarity in spatial cognition among mammalian species that supports the validity of the use of laboratory rodents as models of human brains and behavior.

Others have suggested that these sex differences in spatial behavior and cognition maximize the reproductive success of both males and females by allowing males to travel further and track the status of potential mates and by minimizing the distance traveled by females and therefore reducing survival risks (Ecuyer-Dab and Robert 2004). This hypothesis about the potential adaptive significance of sex differences in spatial cognition is supported by the findings in deer mice of seasonally dependent sex differences in spatial ability as assessed in a water maze. It is also supported by the general findings in many species that the males occupy a larger home range and perform better on those tasks that measure the use of the bearing map. However, this hypothesis offers no explanation for those findings that suggest a female advantage in the use of the sketch map. In addition, this hypothesis focuses greatly on the theoretical increased metabolic needs of females during pregnancy and weaning. Since home range sizes frequently reflect metabolic requirements of the individuals occupying them, one might predict a larger home range size in lactating females than males or non-breeding females based on this hypothesis.

I would like to suggest an alternative hypothesis that the observed sex differences serve to improve the reproductive fitness of males and females by more appropriately organizing mate search and choice strategies in both males and females. In mice, the use and extension of the bearing map during the breeding season would allow for the inclusion and efficient tracking of many female territories. At the same time, a female increase in reliance on detailed information may allow for improved mate choice via the continual assessment of scent marks within a home range. In mice, there is evidence to suggest that female mate choice is based on chemical signals in the scent marks providing information about testosterone concentration, parasite load, and the identity of the major histocompatibility complex (a component of the immune system) detected in the urine of potential mates. There is also evidence to suggest that females prefer familiar males as detected via these scent marks and that males competitively mark the within the home ranges of potential mates (Arakawa et al. 2008; Hurst and Beynon 2004; Hurst et al. 2001; Zala et al. 2004). These differences in the mating strategies of males and females may offer selective pressures for divergent spatial behavior in polygamous species such as the mouse. This divergent spatial behavior in mice is hinted at in the sex differences seen in home range size in the field and should be apparent in a variety of tasks in the laboratory. The prediction from this hypothesis is that, as in other polygamous mammals, male mice will preferentially attend to and rely on the directional information provided by distal cues or gradients. It is this bias that provides an advantage in tasks such as the water maze and many versions of the radial arm maze as well as tasks that assess the memory of object locations (but here only when the transposition provides additional or changed directional information). In addition I predict that, as in other polygamous mammals, female mice will attend to and rely on the unique set of features of landmarks and their relationships to one another. This bias should provide an advantage in place-learning tasks that provide only local landmarks and should be apparent in tasks of object recognition and location memory, particularly when the spatial transposition of objects does not alter the geometry of the array or provide directional information via the polarization of the array.

1.3 The inconsistency of sex differences in laboratory mice

Evidence both from ecological and neuroanatomical studies provides some support for the existence of sex differences in spatial behavior in laboratory mice consistent with those seen in other mammalian species. As discussed above, commensal populations of mice display strong territorial defense with the territories of males overlapping those of females but never overlapping with other male territories (Bronson 1979) and, in feral populations of mice, male home ranges tend to overlap female ranges and tend to be larger than female home ranges (Chambers et al. 2000). Thus providing evidence for a functional sex difference in spatial behavior and cognition. In addition, there is some evidence for sex differences in the neuroanatomy underlying spatial behavior (Wimer and Wimer 1985; Tabibnia et al. 1999).

In studies of the spatial cognition of laboratory rats, males show a consistent advantage in the water maze and radial arm maze. Male rats find the hidden platform more quickly than females and spend more time in the appropriate quadrant during probe trials when the platform has been removed. In humans, the male advantages in mental rotation and in performance in virtual water mazes are robust to differences in methods and laboratories (Alexander and Evardone 2008; Driscoll et al. 2005; Maguire et al. 1999; Astur 2004). There are also consistent female advantages in tasks of object recognition and location in humans (for a review see, (Voyer et al. 2007)) as well as evidence for a similar advantage in rats (Saucier et al. 2008). Together, these lines of evidence support the hypothesis that males and females in these species rely on different sets of cues when operating in complex environments.

While sex differences in spatial behavior observed both in and out of the laboratory are consistent across the polygamous mammalian species that have been studied, they remain elusive and inconsistent in laboratory mice. One potential reason for this impression may be the relative scarcity of studies directly assessing sex differences in laboratory mice compared with the laboratory rat. This may seem contradictory to the opening paragraph, which suggests that mice are the most commonly studied lab mammals. However, relative to the laboratory rat, the laboratory mouse is still relatively new as a model of cognition. In addition, many of the studies carried out to gain insight into the genetic components of learning and memory tend to rely only on the male of the species as subjects, ostensibly to reduce variation. As a result of this scarcity, in a meta-analysis of sex differences in laboratory rodents focusing on results achieved using tests such as the water maze and radial arm maze, a total of five papers made up the literature for mice while rats were represented by 34 studies (Jonasson 2005).

A male advantage is generally observed in tests of spatial memory and place-finding abilities such as the water maze and radial arm maze. In mice the evidence for such a sex difference is still inconclusive. For example, Berger-Sweeney et al. (1995) report that male mice demonstrate a shorter path length and latency to the hidden platform than those observed in female mice only when the spatial version of this task (with a hidden platform) follows a cued version. When the order of testing is reversed, this advantage disappears. In another water maze task, female mice demonstrated a shorter latency to the platform, though only on the last day of acquisition (Lamberty and Gower 1988). While Frick et al. (2000a) finds no sex difference whatsoever in performance in a water maze. In a test of working memory only (all arms are baited) male ddY mice made fewer errors and learned the task more quickly than female mice (Mishima et al. 1986).

When the results of tests concerned with object recognition and location memory (where better female performance is predicted) are reviewed, the outcome is also mixed. One study finds that male mice differentially explore both a novel object and an object that has been moved to a new location, while female mice exhibit no such bias (Frick and Gresack 2003). Another study found no sex difference in object recognition (Benice et al. 2006). Yet another study of object recognition in males of four strains of laboratory mouse (i.e. C57BL, BALB/c, Swiss, and 129/Sv), subjects no longer distinguish between the novel and familiar objects in an arena after 24 hours (Sik et al. 2003). Here again, there are very few studies that include both sexes and most of these vary in methodology to some extent.

In addition to the scarcity of studies, and the variance in methodologies, some of the variance may be a result of the use of tasks such as the water maze, which were initially designed to assess learning in rats. Whishaw and Tomie (1996) show that rats perform better than mice in water tasks, but do not show this advantage on dry land mazes. This species difference is a result of adaptations to different ecological niches. Rats are semi-aquatic while the house mouse rarely swims. In addition, variation in training procedures, inter-trial intervals, or even in the cues available in the testing environment may influence the results in unintended ways (Chemero and Heyser 2005); (Ennaceur 2010b).

This variance, rather than a problem, provides an opportunity to investigate the potential factors influencing these outcomes and demonstrates the need for further studies. Specifically, studies that address both the ethological validity of housing and testing, as well as a more detailed analysis of the cues and features attended to by the sexes, will begin to provide a deeper understanding of sex differences in spatial behavior and cognition.

1.4 Goals, hypotheses and tests

The goal of these studies was to more precisely measure sex differences in a variety of tasks while making as many ethological considerations as possible in both housing and testing environments, thereby improving the ability to draw comparison between laboratory mice and other species that must organize their behavior in much more complex environments. The considerations taken when determining appropriate husbandry protocols included providing enrichment and extra shelter, as well as reducing the available food supply to a more appropriate level, and reversing the light cycle so that all testing was carried out during the active phase of the mice. Enriched housing conditions for mice have been shown to reduce the need for anxiolytic drugs as well as reducing stereotyped behaviors and conferring health advantages (Sherwin and Olsson 2004a; Benaroya-Milshtein et al. 2004a). In addition, there is evidence that enriched housing for laboratory mice does not increase the variation in behavior or reduce the reliability of behavioral tests similar to those included here (Wolfer et al. 2004). On the contrary, enrichment may actually improve the external validity of the results by normalizing the behavior of captive animals to some degree (Garner 2005).

I hypothesize that, when housed and tested in environments that are more ethologically valid and less stress inducing, laboratory mice will demonstrate sex differences consistent with the pattern observed in other mammalian species. To this end three experiments were conducted to assess attention to and memory for various cues at differing spatial resolutions. Specifically, Chapter 2 explores sex differences in the reliance on different cue types while navigating a place-finding task. This place-learning task is unique in that it does not rely on appetitive or aversive motivations that may create differences in motivation between the sexes. The predictions for this task are that, if the laboratory mouse follows the mammalian pattern of sex differences, males should rely on vector information gained from attention to distal cues and the geometry of the room while females should rely on local landmarks near the maze. Chapter 3 explores sex differences in relation to varying levels of similarity between novel and familiar objects in two consecutive object recognition tasks. For these experiments, the prediction is that sex differences will be more apparent when the novel and familiar objects are similar to one another and this sex difference will reveal a female advantage. Following this discussion, Chapter 4 describes sex differences found in an experiment designed to assess the attention and memory for object locations and thus, to relationships among local cues. The specific predictions for this task are that female mice will exhibit advantages in substitutions of familiar for novel object and in location switches between objects, reflecting a particular attention to objects and their spatial relationships. Male mice, on the other hand will exhibit advantages in the form of preferential exploration of displaced objects when that displacement causes a change in the geometry of the array of objects and as a result provides new/different directional information. Then the last chapter summarizes the conclusions drawn from this series of experiments and suggests some potential future directions for research in the field of sex differences in spatial cognition.

CHAPTER 2: SEX DIFFERENCES IN CUE USE IN A PLACE-FINDING TASK

Place-finding and navigation have garnered a great deal of interest from students of animal behavior over the years. With much of the controversy focusing on whether or not mental representations that may resemble topographical survey maps of an environment facilitate or are a necessary component of this behavior. Place-finding can involve the use of multiple sets of cues in order to orient towards a particular location in an environment. In addition, place-finding is usually defined operationally by what it is not. In place finding tasks, subjects are required to utilize allocentric cues. This would not be the case if a subject were allowed to perform the task via path integration or memorized routes. Therefore tasks that seek to measure place-finding ability must prevent these other potential mechanisms of orientation. Generally, this is done by forcing the subjects to begin from a number of randomly chosen starting points and/or disorienting the subject subsequent to the start of a trial. Once an experimenter can be sure that internal cues, memorized routes, or orienting to a beacon have been ruled out as potential means of orientation, the place-learning task can be utilized to assess reliance on various cue types. Thus, this type of task is an ideal place to begin to investigate potential sex differences in cue use strategies in laboratory mice.

As discussed in the previous chapter, one of the most commonly utilized tasks, in the quest for understanding this behavior, is a water maze. In this task, subjects must either locate a hidden platform (in the spatial version) or swim to a cued platform (in the non-spatial version). Generally the platform is a small area that allows the animal to rest out of the water, which is an aversive motivator. In order to complete this task quickly subjects must triangulate the location based on the available cues in the surrounding environment. Hippocampal lesions disrupt performance in this maze, leading to longer path lengths and latencies to arrival at the platform (Morris et al. 1982).

Another commonly used task is the radial arm maze, which was originally designed by Olton and Samuelson to test spatial memory (Olton and Samuelson 1976). In this task, subjects must enter corridors that branch off from a central platform like the spokes of a wheel to acquire a food reward or, in the water version, to find a platform. Choices are recorded when a subject enters an arm with working memory errors recorded when a subject re-enters an arm that was previously visited during the trial and reference memory errors recorded when a subject enters an unrewarded arm. Performance on this task is related to the size of the mossy fiber projection in the hippocampus in males of three inbred strains of mice (Crusio et al. 1987). In a classic study of the hormonal basis of the sex difference seen in spatial behavior in the laboratory rat by (Williams et al. 1990), female performance on a radial arm maze declined when the locations of extra-maze objects were randomized, while male performance was unaffected. When the geometry of the room was altered, performance dropped in males but not in females.

Sex differences in these types of place-finding tasks in laboratory mice have been less consistent than those seen in the laboratory rat (Jonasson 2005). Studies of laboratory rats have shown a consistent male advantage on both water and radial arm mazes while laboratory mice demonstrated inconsistent sex differences. Specifically, male rats rely more heavily on cues that provide directional information such as the geometry of a room, distributed cues or polarized arrays of objects (Benhamou and Poucet 1998; Williams et al. 1990). On the other hand, female rats have demonstrated sensitivity to positional information such as the relationships and unique features of discrete objects (Saucier et al. 2008; Sava and Markus 2005). While laboratory rats demonstrate this pattern of sex differences consistently in radial arm mazes and water mazes, laboratory mice have been tested less frequently and, in these few tests, do not display such a consistent pattern of sex differences. In one study of mice in water mazes, the sequence of testing was the critical variable when identifying sex differences, indicating that the sex differences observed were not a result of different cognitive abilities but a function of the testing protocol (Berger-Sweeney et al. 1995). Another potential explanation for this variance could be the use of a variety of protocols. In tests such as these, even the shape of the experimental room can determine the outcome. In addition, none of the described studies assessed cue use and availability as a determining factor in the performance of the subjects involved. These inconsistencies suggest the need for more extensive assessments of spatial cognition and cue use strategies in mice.

As with studies of spatial learning in complex mazes, studies of sex differences in laboratory mice on simpler spatial tasks show the same lack of consistency. One such task is the ability to recognize that a new object has appeared in a known location. In this task, two objects are presented for exploration. After a delay, a familiar object is replaced with a novel object. Evidence for object recognition is assayed by the bias in search time directed to the novel object (Ennaceur and Delacour 1988). This task is related to a paper-and-pencil task for humans where a pair of familiar objects are switched in location after a delay, a task that shows a female advantage (Silverman and Eals 1992). Similarly, in laboratory rats, there is a female advantage in the object recognition task (Sutcliffe et al. 2007b; Saucier et al. 2008). Yet, again, inconsistent with the pattern from other species, Frick and Gresack (2003) found a male, not a female, advantage in this task in C57BL/6J laboratory mice. In addition, other laboratories have found either no sex difference (Benice et al. 2006) or a female advantage (Podhorna and Brown 2002) in this type of task.

The lack of consistency between laboratory mice and laboratory rats could reflect a genuine species difference in cognition, or a physical limitation or disadvantage of laboratory mice being tested on tasks designed for laboratory rats. As Frick et al. (2000b) have written, “the mouse is not a little rat”. Differing by an order of magnitude in average body mass (300 g vs. 30 g), the laboratory rat is a domesticated strain of the semi-aquatic Norway rat while the laboratory mouse is derived from the house mouse, an adept climber. This is reflected in their tail morphology: the laboratory rat’s tail is stiff and can be used as a rudder while swimming, while the laboratory mouse’s tail is prehensile and is used as a fifth limb while climbing. Tasks that involve swimming should therefore give the laboratory rat an inherent advantage in addition to the better insulation a large body affords while swimming in cold water. In fact, in a direct comparison of different versions of the same maze (i.e., dry vs. water versions), Whishaw (1995) showed that laboratory rats outperformed laboratory mice only on the aquatic version of the task.

One of the goals of the current study is to develop a task that can provide a more appropriate comparison across species. As the species differences in performance in the above tasks indicates in increased level of stress for mice in aquatic mazes relative to other species, and as stress can have negative consequences for learning and memory, it is important to examine the phenotype of place-finding behaviors expressed in tasks that are designed more specifically for mice. In addition to providing a better means for crossspecies comparisons, the reduction of stress in testing can also reduce its confounding influence on the identification of sex differences. As an example of the differential effects of stress on spatial learning, one study demonstrated the modulation of sex differences in spatial learning by deer mice by the presence of environmental stressors such as predator odor and biting flies (Kavaliers et al. 1998; Perrot-Sinal et al. 2000). In our study we therefore used and/or developed tasks that did not depend on swimming or on food-restriction but instead shared the single, positive reinforcement of spatial exploration. In addition, mice were tested during their natural active phase and were housed in cages equipped with nest boxes and bedding. To reduce their anxiety in response to novel testing environments, mice were also allowed to explore complex mouse 'playgrounds' on a regular schedule. Because our goal was to measure sex differences in the adult, we also used mice that were fully mature (13 months old) and who had experienced similar tasks as young adults (3 months). We predicted that, under these conditions, C57BL/6J mice would show the pattern typical of the laboratory rat. Specifically, we predicted a female advantage on the object recognition task and sex-specific strategies in place learning, with males and females using different cues for orientation. We expected that males should preferentially orient using cues external to the apparatus, such as the shape of the room or objects on the walls. In contrast, females should preferentially orient to cues closer to the maze such as nearby objects. Such results would be consistent with results found in the laboratory rat (Williams et al. 1990) and in humans (Sandstrom et al. 2006). These sex differences in spatial strategies are also predicted by theories regarding the cognitive map as discussed in the previous chapter (Jacobs and Schenk 2003).

2.1 Subjects and husbandry

The subjects were C57BL/6J mice ($N = 22$, 11 female), which had been obtained at the age of two months and were tested at the age of three months on a series of object recognition tasks as part of a different study. They were tested again, in the current study, at the age of 13 months. The data for this study refer only to the test at 13 months. They were housed in same-sex groups, three per standard mouse cage (19 cm × 29 cm × 13 cm). Each cage was supplied with a disposable cardboard igloo and bedding material (Nestlets®). Mice were kept on a 12:12 light cycle (lights on 20:00, off at 08:00). Water was available ad lib. A ration of 8 g standard mouse chow was provided on alternate days to simulate natural cycles of food availability while maintaining a weight within 1-2g of ad lib weight. This amount of food meets the daily energetic requirements of the mice (Nutrition et al. 1995) and reduces the negative effects of ad lib feeding (Keenan et al. 1999). The mice in this study rarely finished the entire 48 hr ration, however, and were rarely without food for more than 4 h.

From the age of six months, mice were given access to larger environments with novel objects to explore for added enrichment. Enriched housing conditions for mice have been shown to reduce the need for anxiolytic drugs as well as reducing stereotyped behaviors and conferring health advantages (Olsson and Sherwin 2006; Sherwin and Olsson 2004b; Benaroya-Milshtein et al. 2004b)A cohort of three cage-mates was released for one hour per week into a covered, translucent plastic box (26 cm × 46 cm × 26 cm) with novel objects that could be chewed or climbed. The objects were changed weekly and included a running wheel, plastic tubes, wooden sticks, pine cones, nylon dog bones (Nylabones®), glass jars, pieces of Styrofoam®, tunnels made from wire mesh (i.e. chicken wire) and long upright barriers constructed from white acrylic plastic.

2.2 Apparatus and procedure for the object recognition task

This task consisted of three successive days of exposure to an environment with changing objects (Figure 2.1A below), for a period of 5 min per day. The arena was an opaque rodent cage (40 cm × 51 cm × 20 cm). Unique objects were constructed using Lego® blocks (Figure 2.1B). A video camera was mounted to the ceiling and was connected to a recorder, monitor, and computer in the adjacent room. The two rooms were connected through a partially open door. All experiments took place during the dark phase of the light cycle and were conducted in test rooms with low lighting. Extra-apparatus cues were not masked but were not prominent, as the sides of the arena were opaque.

On Day 1 (habituation phase), the mouse was released into the empty arena for 5 min of exploration and habituation. On Day 2 (sample phase), the mouse was again released into the arena, which now contained two identical objects built from Lego® blocks and centered in the arena. On Day 3 (choice phase), the arena now contained a duplicate of one object from the sample phase and a novel object (Figure 2.1). The location of the novel object was counterbalanced among mice to control for side biases. The object that was novel was also counterbalanced between the two object types shown in Figure 1B. The novel and familiar objects differed from one another in construction, but share some similarity of shape and pattern of contrasting colors. In addition the two objects are approximately the same height and occupy the same footprint. This level of similarity increases the difficulty of the discrimination and may contribute to the identification of a sex difference. The objects presented to the mice were both completely novel to the mouse, i.e., different objects were constructed for each replication of the task. In addition, ten months had elapsed between the first and second replication of this task, minimizing carryover effects between replications.

All behavior was recorded on videotape for subsequent analysis using Ethovision Pro (Noldus, Inc.). Two measures of behavior were made: the time spent within a zone of 4 cm distant from an object and the frequency of visits to this zone per object. The criterion for the mouse's presence within the 4 cm zone was the location of the body's high correlations between manual recording and automatic scoring in object recognition tasks in mice (Benice and Raber 2008; Roach et al. 2003). We also conducted a pilot study to examine this question and found significant agreement between manual scoring of mouse attention and automatic scoring of this behavior. Though possible, it was extremely rare that the Ethovision calculated center of gravity of the mouse crossed into the zone without the head of the animal being oriented towards the object. For this reason we felt confident relying on the tracking system rather than manually recorded behavior.

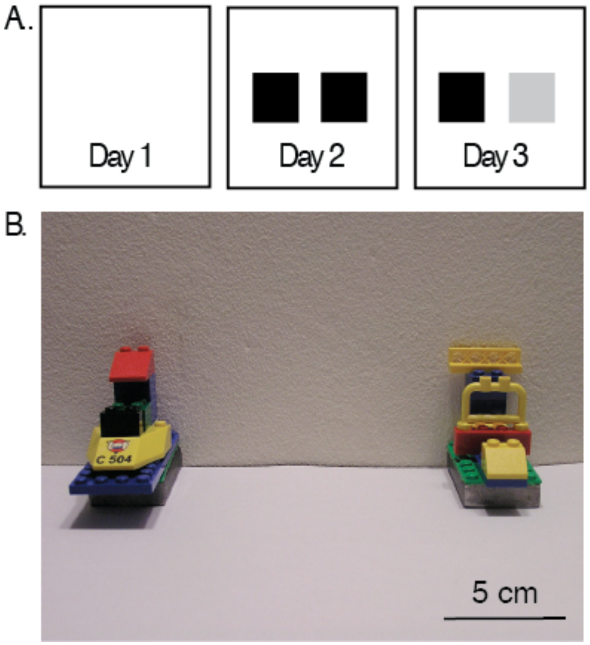


Figure 2.1: Experimental set-up and objects used in the object recognition task.

2.2 Results of the object recognition task

Two of the female mice were removed from the analysis because they did not attend to the objects during the sample phase, in the presence of two identical objects, and instead remained near a wall for the duration of the experiment. Thus bringing the number of female subjects in the data set to 9.

Using a 2 × 2 repeated measures ANOVA of time spent near object, with sex as the between-subjects factor and side as the repeated within-subject factor, we found a significant main effect of sex ($F_{1, 18} = 23.96$, $p < .01$). There was, however, no effect of side ($F_{1, 18} = 0.41$, $p = .53$) nor was there a Sex × Side interaction ($F_{1, 18} = 0.10$, $p = .76$). Further analyses using one-way ANOVA showed that females spent more time than males exploring both the left ($F_{1,18} = 12.12$, $p < .01$) and right ($F_{1,18} = 22.19$, $p < .01$) objects. Using the same analyses, the results for the frequency of visits showed a similar pattern. There was a significant main effect of sex ($F_{1,18} = 9.33$, $p < .01$) but no effect of side ($F_{1, 18} = 0.49$, $p = .49$) and no significant interaction between sex and side ($F_{1,18} = 0.83$, $p = .37$). Further analysis using one-way ANOVA showed that female mice made more visits than did male mice to both left ($F_{1,18} = 9.03$, $p < .01$) and right objects ($F_{1,18} = 7.17$, $p = .02$) than male mice.

In the choice phase on Day 3, a 2 × 2 repeated measures ANOVA with sex as the between-subjects factor and object type (novel vs. familiar) as the within-subjects repeated factor revealed significant main effects of both sex ($F_{1, 18} = 22.96$, $p < .01$) and object type ($F_{1, 18} = 8.33$, $p = .01$). This analysis also showed a significant Sex × Object type interaction ($F_{1, 18} = 17.80$, $p < .01$). Further one-way ANOVA revealed that females spent more time with the novel object ($F_{1, 18} = 50.45$, $p < .01$) than did males. The data on the frequency of visits reveal the same pattern of behavior. The 2 × 2 repeated measures

ANOVA shows significant effects of sex ($F_{1, 18}$ = 14.73, p < .01) and object type ($F_{1, 18}$= 4.69, p = .04) with a significant Sex × Object interaction ($F_{1, 18}$= 10.86, p < .01). Subsequent one-way ANOVA analyses showed that females visited the novel object significantly more than males did ($F_{1,18}$ = 47.10, p < .01) but they did not visit the familiar objects more than males did ($F_{1,18}$ = 3.16, p = .09). As revealed by paired-samples t-tests, females also spent more time exploring novel objects (t_8 = 8.9, p < .01) as well as making more visits to the novel object (t_8 = 3.1, p = .02) than to the familiar object. These differences were not significant for male mice (duration: t_{10}=-.79, p = .45, number of visits: t_{10}= -1.04, p = .32); see Figure 2.2. Finally, the discrimination indices calculated were significantly greater for the female mice than the male mice as determined with a one-way ANOVA ($F_{1,18}$= 7.36, p = 0.01). As revealed by one-sample t-tests, the discrimination index was significantly greater than zero for the female mice (p < 0.01) but not for the male mice (p = 0.63); see Figure 2.3.

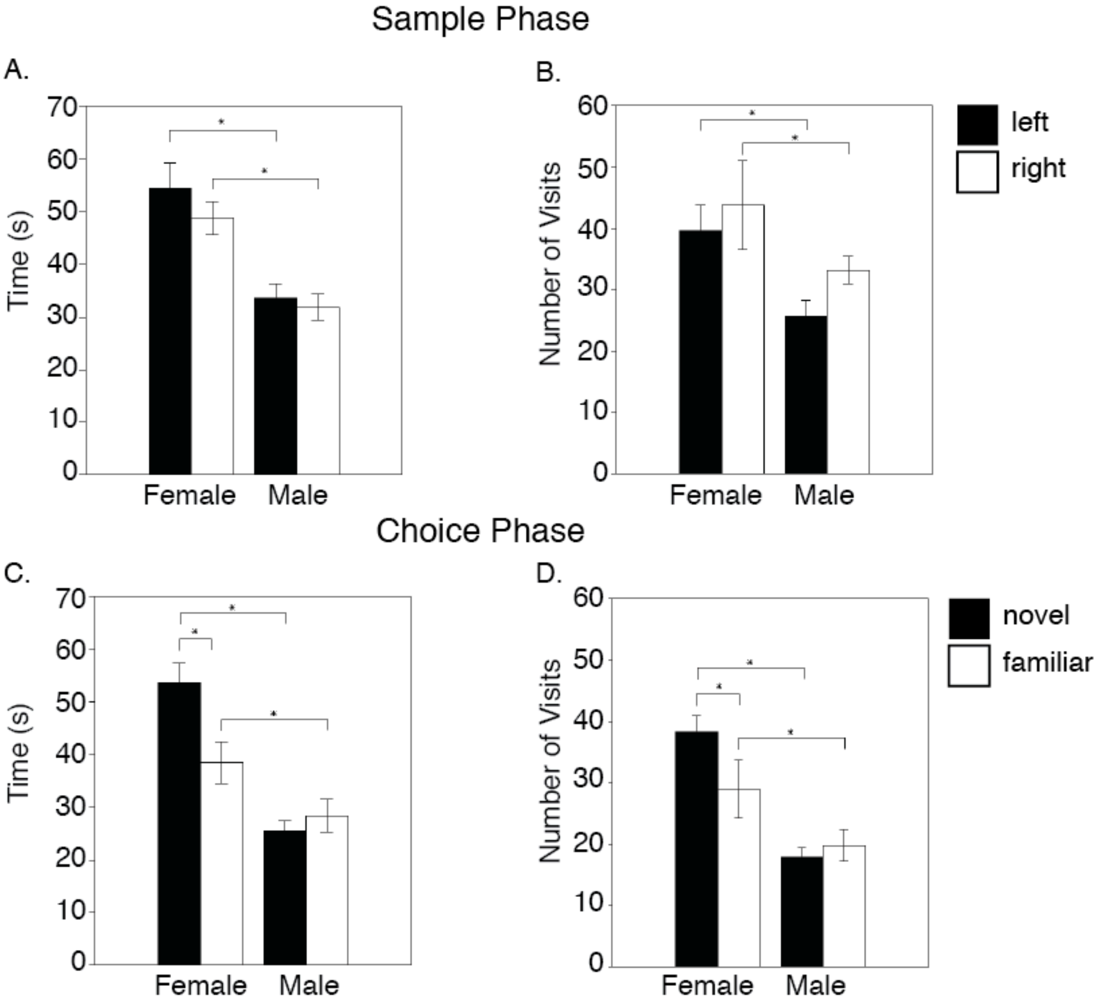


Figure 2.2: Results from object recognition task. A. Duration spent exploring objects during the sample phase. B. Number of visits to objects on sample phase. C. Duration

spent exploring objects during the choice phase. D. Number of visits to objects in the choice phase. * indicates p < .05.

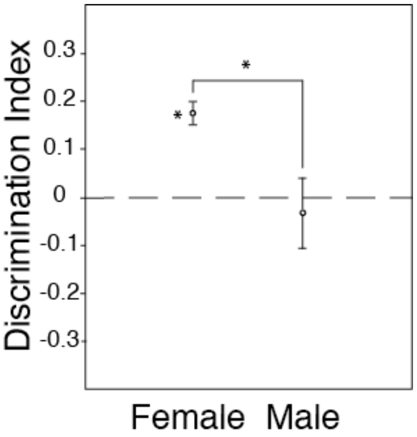


Figure 2.3: Discrimination indices for the novel object recognition task. Discrimination Index is calculated as the difference in exploration time between the novel and familiar objects, divided by the total time spent exploring both objects. * indicates p < 0.05.

2.3 Apparatus and procedure in the place learning task: an exploration-rewarded plus maze

The ladder-rewarded plus maze is a new mouse place-learning task that eliminates the need for food restriction or aversive conditioning. Versions of the elevated plus maze are commonly used to measure response to stress in unhabituated mice (Rodgers et al. 1999). In this ladder maze, we used standard plus-maze geometry to assess place learning but used a positive reinforcer, i.e., access to an unexplored area. Mice completed a trial by returning to the learned location of a hidden ladder that led to a novel space that contained tunnels to be explored. Thus, both tasks in our study - object recognition and place learning - exploited the natural tendency of mice to explore novel objects and novel environments.

The ladder maze is an elevated plus maze that is designed to reward performance with access to exploratory space that contains climbable objects (four tunnels). The maze was constructed from white sheet vinyl plastic. It was comprised of four perpendicular arms (45 cm × 4 cm). Each arm was also fitted with a ‘speed bump’. This was a U-shaped wire mesh structure (8 cm high) fixed to the maze arm (see Figure 2.4C). The function of the speed bump was to slow down the mice and allow for the attention to and utilization of allocentric cues when choosing to enter an arm. In pilot studies, without the speed bumps, the mice would run quickly across the maze and would not attend to their surroundings. Occasionally a mouse would run off the end of an arm, where they may have expected the escape ladder to be. In these pilot studies, the mice did not show evidence of learning the correct location. Once the speed bumps were added the mice slowed down and began to show evidence of learning.

The maze arms were elevated 31 cm above a white floor (122 cm × 122 cm × 5 cm) that contained four plastic tunnels that differed in color and texture (ca. 5 cm diameter × 14 cm length). The plastic ladder (4 cm × 34 cm) was constructed from commercially available white, latticed plastic (1 cm × 1 cm cubes). The layout of the test room is shown in Figure 2.4A. The maze and the exploration space were enclosed in a round barrier of transparent acrylic (70 cm high, 122 cm diameter) to prevent escape. Intra-maze cues were attached to this barrier at each of four directions (Northeast, Southeast, Southwest, Northwest), which were positions offset from the directions of the maze arms (North, South, East, West). The directions were absolute cardinal directions. The cues included artificial flowers, colored foam and hard plastic shapes (Figure 2.4B). Extra-maze cues in the room included two blue room doors, two spotlights on opposite corners of the test room, a rope of evenly distributed small white lights (decorator lights) that outlined the ceiling of the room and cast an even light, colored foam shapes and posters attached to the walls, a paper towel rack, a mop rack containing a mop and a metal bookshelf (for the room layout, see Figure 2.4A). For the extra-maze cue probe test, a white vinyl curtain was hung from a circular hoop (Hula Hoop®) that was suspended from the ceiling, encircling the maze. This curtain masked all visual cues outside of the maze.

Pre-training: Each mouse was given four pre-training trials in an adjacent room. This procedure habituated the mice to descending on ladders (Trials 1 and 2) and climbing the speed bump (Trials 3 and 4). The sample arm, speed bump and ladder led to the mouse's home cage; after each trial, the mouse spent a 20 sec inter-trial interval (ITI) in its cage.

Training: Each mouse was assigned either the East (E) or West (W) arm as its goal (ladder) location. The start arm of each trial was pseudo-randomly assigned from the three non-goal arms. The mouse was released on the top surface of the maze at the end of one arm. The mouse was rewarded with exploration when it chose the correct arm. A choice was defined as the mouse climbing to the top of the speed bump. Once the mouse made the correct choice, it descended the ladder and was allowed to explore the tunnels for 20 sec. Each mouse was trained across sessions of three trials per session. Thus each non-goal arm served as a start arm once per session. The ITI lasted 60 to 90 sec. During the ITI, the mouse was additionally rewarded with exploration of novel objects in a large arena with novel objects. Also, during the ITI, the maze arms were wiped and rotated to prevent mice from associating patterns of odor cues or imperfections in the symmetry of the maze with the goal arm location. All training and testing was conducted on a single day; testing lasted between 3 and 5 h. The criterion for learning the location was a first choice of correct arm for all trials in two consecutive sessions, ensuring the mouse oriented correctly twice from each start position.

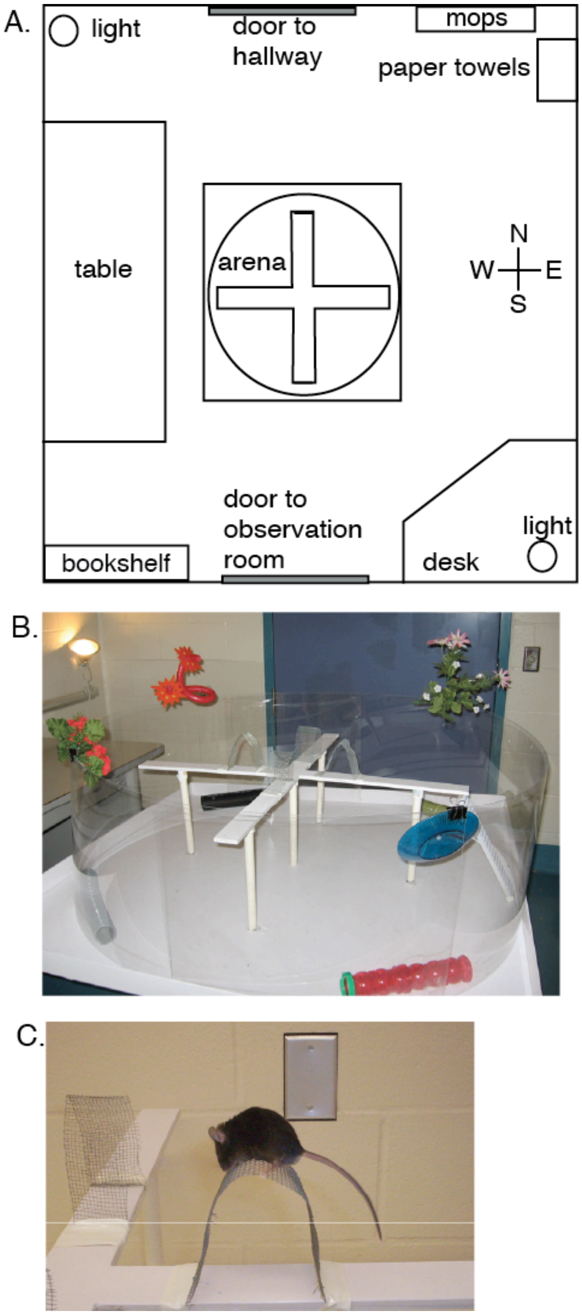


Figure 2.4: Experimental room layout (A) and the testing arena (B) with a picture of a mouse at the top point of a speed bump on one arm of the plus maze (C).

Probe tests: Probe sessions were carried out immediately after the training criterion was met. A probe session consisted of three training trials and one probe trial. This was done as a means of preventing extinction of the behavior and anticipation of the probe. The probe trial occurred either between training trial 1 and 2 or between training trial 2 and 3; the placement of the probe within the session was counterbalanced among subjects. No ladder was present on the probe trial. We recorded all choices made for 60 sec. However, the measure of performance was limited to the first choice. Three types of probe tests, in a fixed order, were used to assess cue use strategy: Probe 1, removal of intra-maze cues; Probe 2, masking of extra-maze cues; Probe 3, removal and masking of both cue sets simultaneously. In Probe 1, we removed all intra-maze cues, including the four colored tunnels and the cues that had been placed close to the maze. In Probe 2, we masked the extra-maze cues with the curtain. In Probe 3, both of the above manipulations were employed simultaneously. We counterbalanced the location of the goal arm across mice. We also counterbalanced the start arm across probe tests and subjects so that the subjects had a new start arm for each probe test. For example, half of the subjects with a West arm goal started from the North arm and the other half with the West arm goal were released onto the South arm in Probe 1. This was balanced so that subjects that started from the North arm in Probe 1 would start from the South arm in Probe 2. This design should have eliminated the effect of side-turning bias.

2.5 Results of the place learning task

Performance on a training trial was assessed by the number of errors made, i.e., the number of non-goal arms entered before the goal arm was entered. To complete training, mice were required to learn a rewarded location and escape from the maze on the first choice. Therefore, the first arm chosen is the best indictor of the mouse's memory for the correct location in probe tests. Beyond this choice, the behavior becomes increasingly difficult to interpret. As seen in Figure 2.5, females and males did not differ in their rate of acquisition of this task. The mean number (± SE) of trials to criterion was 35.4 ± 3.93 for females and 41.14 ± 4.69 for males, which was not significantly different as determined by a t-test (t_{15}= .94, p = .36). The first female mouse to reach criterion did so after 18 trials (6 sessions) and the last two female mice to reach criterion did so in 54 trials (18 sessions). The first male mice to reach criterion did so in 30 trials (10 sessions) and the last male mouse to reach criterion did so in 54 trials (18 sessions). Overall, after 20 sessions only one female failed to reach criterion while four males failed to reach criterion. These mice were removed from any further analysis thus the number of female subjects included in the data set was 10, and the number of male subjects included was reduced to 7. An analysis of errors per session using a 2 × 2 repeated measures ANOVA with sex as the between-subjects factor and session as the repeated within-subjects factor was carried out for the first six sessions with the data from the remaining 17 mice (10 female and 7 male). This revealed a main effect of session ($F_{5,11}$ = 9.37, $p < .01$), no effect of sex ($F_{1,15}$ = 3.04, p = .10), and no Sex × Session interaction ($F_{5,11}$ = 3.04, p = .06).

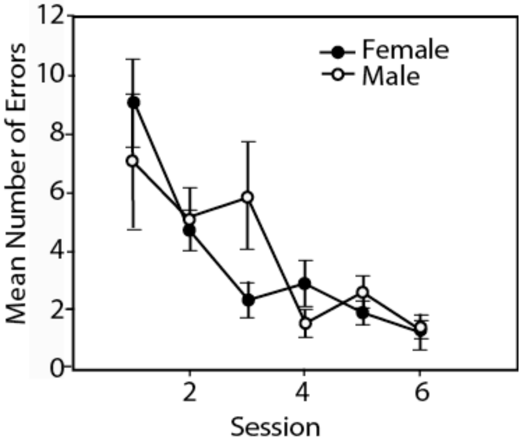


Figure 2.5: Task acquisition by female and male mice in the ladder maze assessing mean (±SE) number of errors per session (n=10 female and 7 male mice). Female mice began reaching criterion in the sixth session and male mice began reaching criterion in the tenth session. The last male and female mice to reach criterion did so in the eighteenth session. The average trials to criterion was 35.4 ± 3.93 for females and 41.14 ± 4.69 for males, which was not significantly different as determined by a t-test (t_{15}= .94, p = .36). Since mice began meeting criterion and were thus moved on to probe trials following the sixth session, only data from the first six sessions is included in this figure. *Note that the female and male mice show the same pattern of acquisition with nearly all of the mice remaining in training following the sixth session making one error per session.

The results from the probe tests are summarized in Table 2.1. A single male mouse refused to participate in Probes 2 and 3 by sitting in the start arm for five minutes, thus the sample size is reduced for these two probes. We used the binomial test to determine if more mice chose the correct location than expected by chance, within each group, on each probe. Mice could choose any of the four arms once they reached the center. Mice indeed on occasion (albeit rarely) reversed direction and reentered their start arm. For this reason, all four arms were potential choices and the probability of choosing one arm was set at 0.25. However, as the start arm was used less frequently, we repeated the binomial analysis more conservatively by calculating chance as one of three, non-start, arms, i.e., 0.33. When intra-maze cues were removed (Probe Test 1) both female and male mice were significantly above chance in choosing the correct arm (binomial test, $p < .01$). This was true both when the start arm was included as a choice possibility and when it was excluded (Table 2.1).

While, in the second probe, when extra-maze cues were masked (Probe Test 2), male choice behavior did not differ from chance (binomial test, p = 0.31) either when the start arm was included as a choice or not, females choose the correct arm significantly more than chance would predict (binomial test, p = .02). However, their choice was not significantly different from chance when the start arm was excluded as an available choice as it would be if we assume that mice should not return to an already traversed arm (binomial test, p = 0.05). It is important to note that one of the female mice did return to the start arm after entering the center of the maze and this was counted as the first choice. When all cues were removed or masked (Probe Test 3), the first arm choice of males was again not significantly different from chance and again females showed accurate performance, a greater proportion of females choosing the correct goal arm on the first choice than predicted by chance (binomial test, p < .01).

Table 2.1: Results for spatial probe tests on the ladder maze.

Type of Probe	Group	*Number correct*	*n*	*4 arms (p =.25)*	*3 arms (p =.33)*
Intra-maze cues deleted	Females	9	10	< .01 *	< .01 *
(Probe 1)	Males	6	7	< .01 *	< .01 *
Extra-maze cues masked	Females	6	10	.02*	.05
(Probe 2)	Males	2	7	.31	.31
All cues deleted/masked	Females	7	10	< .01 *	.01 *
(Probe 3)	Males	2	7	.31	.31

Note: This table only includes mice reaching training criterion (*N*=17). The binomial probabilities were calculated twice: first for chance levels with four maze arm choices (i.e., 3 choice arms + 1 start arm) and second with three maze arm choices, disqualifying the start arm as a possible choice (but see discussion in text). * p < .05.

2.6 Discussion of mice with objects and in elevated space

This study is the first to describe a clear sex difference in cue use strategies in laboratory mice in a place-finding task. The goal of this study was to determine if sex differences in cue use during spatial orientation in the C57BL/6J mouse strain are consistent with patterns observed in other species. The results of these tasks are largely concordant with those reported for other mammalian species. These results are also consistent with the hypothesis that attention to ethologically valid housing and testing measures as well as attempts at reducing stress may contribute to the expression and therefore identification of sex differences in object recognition and place learning tasks.

In the object recognition task, females were significantly more likely than males to discriminate the novel from the familiar object as evidenced by the differential exploration of this object in the choice phase. This result could not have been due to either side preference or odor cues. The side on which the novel object was placed was counterbalanced. In addition, during the test phase both objects were new to the mouse, as one was a duplicate of the object used in the training phase. There was also a sex difference in baseline exploration. It is possible that females collected more information during the sample phase than did males and, for this reason, this study cannot pinpoint the source of the female advantage which could potentially have arisen as a result of either increased attention and better encoding or more accurate recall of the familiar object. The goal of the study, however, was to determine not how but if male and female C57BL/J6 mice differ in their spatial encoding of a novel environment. The present results suggest that females and males pay attention differently to their surroundings, specifically that females pay more attention than males to novel objects and perhaps to discrete objects in general. While the female mice spent significantly more time with objects during the sample phase, the amount of time male mice spent exploring objects is representative of similar results in the field (Frick and Gresack 2003). Nonetheless, in the choice phase, they did not discriminate between novel and familiar objects.

The finding that male mice did not discriminate between novel and familiar objects may seem contrary to many other novel object recognition experiments in which male mice of this strain are capable of recognizing the novel object (Frick and Gresack 2003; Rampon et al. 2000; Tang et al. 1999). This may be due to the degree of similarity between the novel and familiar objects. The constructed objects in the present study, composed of Lego® blocks, were perhaps more similar to one another than in other studies, making the task more difficult. Further studies with standardized objects would clearly address this point. In addition, in other object recognition experiments we have found that increasing the difference between objects indeed results in an increase in males' ability to distinguish familiar from novel objects (see next chapter).

The results of the current study confirm the female advantage found in a related object recognition task in C57BL/6J mice (Podhorna and Brown 2002). This is significant because of several important methodological differences between the studies, most notably that the delay between the sample and choice phases in this study was only 15 min, as compared to 24 h in the current study. On the other hand, in both studies mice were tested during their natural active phase (dark phase of the cycle). The scheduling of testing may be critical. In a similar study that showed a male advantage in this task, after a delay of 24 h, C57BL/6J mice were tested during the light phase of the cycle, i.e. their natural period of inactivity (Frick and Gresack 2003). Although it is possible that the sexes vary in their sensitivity to activity phase, we can draw no real conclusions until all the factors (cycle, delay duration, object similarity) can be controlled. Such future research will determine the contribution of these factors to the size and magnitude of the sex differences in C57BL/6J mice on this task. Given the similarities in sex differences among other mammalian species, however, it is reasonable to expect that future research will confirm that laboratory mice show a similar pattern to that seen in laboratory rats and in humans, albeit with greater sensitivity to testing conditions.

The hypothesis that females attend more to small objects in their environment than do males was also supported by results from the place-learning task. Here males and females experienced the same duration of exploration and exposure to spatial cues and also showed the same pattern of task acquisition. Yet the sexes differed in their response to the removal or masking of cues. The masking of extra-maze cues impaired only male performance. Male performance was also impaired in the last probe test, when both extra-maze and intra-maze cues were masked or removed, but not in the probe test where only intra-maze cues were removed. This clearly points to a male reliance on using extra-maze cues for orientation, in agreement with studies of male vs. female humans (Sandstrom et al. 2006), desert kangaroo rats (Langley 1994), and laboratory rats (Williams et al. 1990).

Female performance on the place-learning task, however, was less affected by changes in visible cues, even the intra-maze cues. This is an unexpected result given their sensitivity to object features in the first task. In fact, female performance remained robust throughout all probe tests. This suggests that females had encoded not simply the visible landmarks but other information as well; some cue that was not controlled with the current experimental design. This interpretation would be consistent with a study of sex differences in laboratory rats, orienting to food rewards on a plus maze (Tropp and Markus 2001). In this study, females utilized both intra- and extra-maze cues initially and then after training, relied preferentially on extra-maze cues. Males, in contrast, rely preferentially on extra-maze cues from the beginning. In addition, some potential extra-maze cues that we could not control or mask were the auditory and olfactory cues surrounding the room, such as the noise from the observation room. It is possible that female C57Bl/6J mice show a similar pattern to the laboratory rats in this experiment as a result of the extended training that was necessary to confirm place learning from all release points in the current study.

In conclusion, future work must dissect the contributions of different parameters that influence sex differences in this species. Yet, the concordance of the present results with new models of spatial encoding and mapping (Jacobs and Schenk 2003), as well as with prior studies of sex differences in other mammalian species suggest that the pattern of cognitive sex differences in the C57BL/6J laboratory mouse will be similar to that found in other mammals. This opens the door for sophisticated work on the genetic and molecular basis of sex differences in cognition in mammals.

CHAPTER 3: SEX DIFFERENCES IN OBJECT RECOGNITION

As an animal moves through its environment it must respond appropriately to the objects surrounding it. This implies making a decision about an object's possible benefit, danger or insignificance and it requires the ability to discriminate between novel and familiar objects. By recognizing a familiar object, an animal can reduce the time spent exploring and can maximize the processing of non-redundant information (Mickley et al. 2000). Sokolov described the orienting-investigatory reflex of animals as "the series of reactions bringing the animal into contact with the object, and tuning the analysers of animal or man, so that perception of the stimulus takes place in the most favorable conditions" (Sokolov 1963). This direction of attention towards novelty facilitates long-term memory encoding (Tulving et al. 1996; Habib et al. 2003) and can be used as a tool to measure the strength of memories for familiar objects (Ennaceur 2010b).

As discussed in the previous chapters, sex differences in spatial behavior of mice have been found in both the field and in the laboratory. Yet, these sex differences can vary in size and direction from one lab to another (Frick and Gresack 2003; Podhorna and Brown 2002; Benice et al. 2006). In the previous chapter, a sex difference was reported for this task in which female mice differentially explored a novel object relative to a familiar object. Male mice, on the other hand, did not display this bias. This indicates that male mice did not distinguish between the two objects with respect to familiarity and this suggests a poor memory of the familiar object. These results are similar to those reported in experiments with rats (Saucier et al. 2008). The finding that male mice were unable to discriminate between novel and familiar objects may seem contrary to other novel object recognition experiments in which male mice of this strain respond differentially to the novel object (Frick and Gresack 2003; Rampon et al. 2000; Tang et al. 1999). These inconsistencies may result from differences in the amount of exposure to the objects during the sample phase, differences in the length of the inter-trial interval, the shape of the arena, or the types of objects used as stimuli in the study.

This experiment will look at the effects of the degree of similarity between the two objects on the sex differences observed in response to novel object recognition and will use a method originally developed by Ennaceur and Delacour (Ennaceur and Delacour 1988). As Ennaceur suggested in a recent discussion of the object recognition task, "The perception of differences in the orientation of stripes, however, does not necessarily imply that these objects are suitable for assessing memory of objects using variable retention intervals. Memory in rodents may not be able to afford such limited discriminative features between objects as these can be subject to high level of interferences than objects with redundant features. It may not be able also to afford large similarities between objects for the same reasons. Under these conditions, a deficit in object recognition can be observed as soon as a delay is interposed between the sample phase and the test phase" (Ennaceur 2010a). This review offers another caveat and recommends that one take careful precautions when choosing stimuli for this task to avoid objects with special affordances that might result in induced preferences. Thereby masking the detection and exploration of an object as a result of its novelty. Thus, one must choose objects that are both maximally discriminable and similar enough to not incite preferential exploration irrespective of novelty. This can be a difficult balance to attain particularly when so little is known about rodent object perception. Rather than being a variable to avoid, varying the similarity between the two objects may offer some insight into the degree of interference that is tolerable and provide valuable tool for assessing the quality of object recognition memory in mice.

As discussed in the introduction, sex differences in spatial behavior may reflect divergent selective pressures producing different mating strategies and reproductive behaviors. Accurate discrimination of objects in a home range may be particularly beneficial to female mice as they continuously assess the quality of potential mates via the scent marks left behind by competing males. Object recognition is one component of the sketch map (a mental representation of an environment built on the relationships and identities of local cues, which provide distance information relative to a goal or other local cues) (Jacobs and Schenk 2003). Since females tend to rely on this type of information, the hypothesis is that the biased exploration of the novel object will remain even when the objects to be distinguished are relatively similar. However, as a result of the male bias towards the use of a bearing map (directional information acquired from geometry and distal cues) to organize behavior, the hypothesis is that male performance will be impaired when the objects to be distinguished share many features.

3.1 Subjects and husbandry

The subjects were C57BL/6J mice ($N = 22$, 11 female, 11 male), which were 12 weeks old at the time of testing. In an effort to reduce as many stressors as possible and create a housing environment that was ethologically valid, the husbandry was modified. Group housed male rats have shown an increase in stress response and a decrement in object recognition relative to individually housed males while the opposite is seen in females (Beck and Luine 2002). In addition, male mice can become territorial and fight one another in the caged environment. Deacon (2006a) suggests that individual housing (though not isolation) of males is ethologically valid while housing female mice in groups is also ethologically valid. In addition, Harris et al. (2008b) find that isolation does not alter the results of a sex difference study in spatial cognition of rats. Thus females were housed in groups of three while male mice were individually housed in standard mouse cages (19 cm × 29 cm × 13 cm). Each cage was supplied with a disposable cardboard igloo and bedding material (Nestlets®). Mice were kept on a 12:12 light cycle (lights on 20:00, off at 08:00). Cages were all kept below the top tier of the racks in order to reduce the stress of being so near the light source. Water was available ad lib. A ration of 8 g standard mouse chow was provided on alternate days to simulate natural cycles of food availability while maintaining a weight within 1-2g of ad lib weight. This amount of food meets the daily energetic requirements of the mice (Nutrition et al. 1995) and reduces the negative effects of ad lib feeding (Keenan et al. 1999). The mice in this study rarely finished the entire 48 hr ration, however, and were rarely without food for more than 4 hr.

3.2 Apparatus and procedure for the object recognition task

This task was similar to the previously reported object recognition task (Bettis and Jacobs 2009) and consisted of three successive days of exposure to an arena and a set of objects in this environment (Figure 3.1A below), for a period of 5 min per day. The arena was an opaque rodent cage (40 cm × 51 cm × 20 cm). Unique objects were constructed using Lego® blocks (Figure 3.1B). A video camera was mounted to the ceiling and was connected to a recorder, monitor, and computer in the adjacent room. The two rooms were connected through a partially open door. All experiments took place during the dark phase of the light cycle and were conducted in test rooms with low lighting. Extra-apparatus cues were not masked but were not prominent, as the sides of the arena were opaque.

On Day 1 (habituation phase), the mouse was released into the empty arena for 5 min of exploration and habituation. On Day 2 (sample phase), the mouse was again released into the arena, which now contained two identical objects centered in the arena. On Day 3 (choice phase), the arena now contained a duplicate of the object from the sample phase and a novel object (Figure 3.1). The location of the novel object was counterbalanced among mice to control for side biases. The objects presented to the mice were both completely novel to the mouse, i.e., different objects were constructed for each replication of the task. In the first experiment two objects of relative similarity were used. Both objects were constructed with identical blocks on the bottom, thus the objects occupied identical footprints. In addition, these objects were constructed using the same color scheme and a similar pattern of alternating white/ dark blocks, which makes the contrast very striking and similar in the two objects. The most salient (to this human's eye) difference between the two objects is the difference in height. The second experiment was carried out one week later, and one of the objects was replaced with an object that was very different in terms of both shape and contrast. In this experiment the two objects differ in shape (one has only one tower and the other has two), height, and color. While color is probably not a feature that mice will attend to, contrast is (Jacobs 1993; Jacobs and Fenwick… 2001). And the contrast apparent in the pattern in the black, white and red object is greater than the contrast between colors in the yellow, blue, white, and red object. While these descriptions of the level of similarity between stimuli paired for these studies is entirely qualitative, they provide a starting point from which a more detailed understanding of the nature of object recognition in mice can be built.

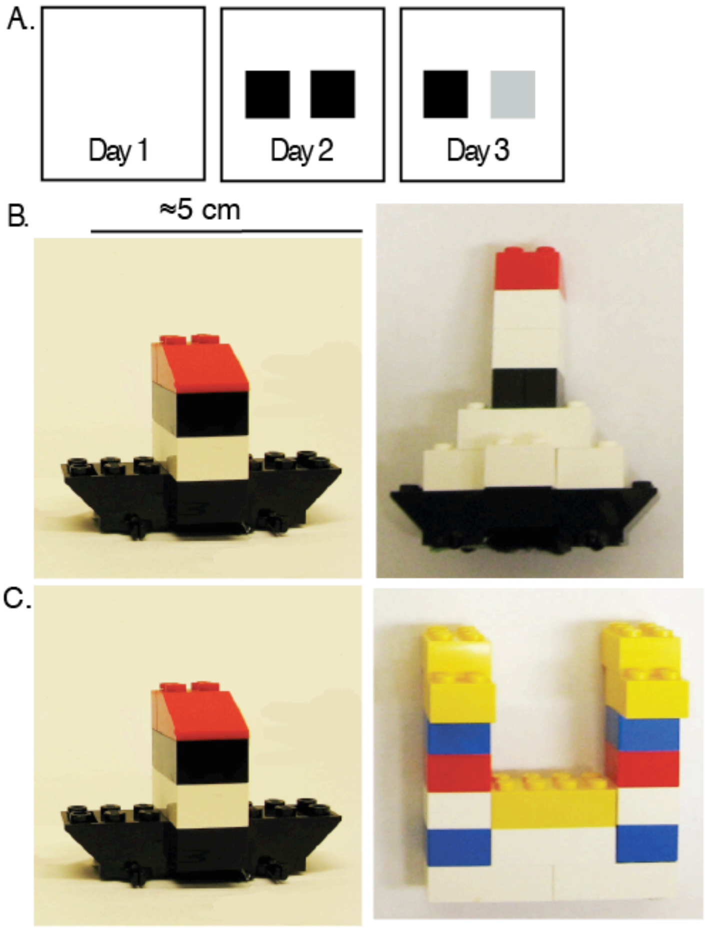


Figure 3.1: A: A representation of the experimental design. B. The two objects used in the first object recognition experiment in this series. C. The second set of objects used one week after the first experiment.

All behavior was recorded on videotape for subsequent analysis using Ethovision Pro (Noldus, Inc.). Two measures of behavior were made: the time spent within 4 cm of an object and the frequency of visits to this zone per object. The criterion for the mouse's presence within the 4 cm zone was the location of the body's center of gravity, as defined by Ethovision. The Ethovision tracking system results in high correlations between manual recording and automatic scoring in object recognition tasks in mice (Benice and Raber 2008; Roach et al. 2003) (See also Chapter 2).

3.3 Results of the first object recognition task with similar objects

During the sample phase, on Day 2 of the first experiment no significant differences in total duration were observed between objects for either female mice (paired samples t-test: $t_{10} = 0.54$, p = 0.14) or male mice (paired samples t-test: $t_{10} = 1.58$, p = 0.14). Nor were there any differences between the sexes in either measure of total duration (ANOVAs of right object: $F_{1,20} = 0.33$, p = 0.57, and left object: $F_{1,20} = 1.13$, p = 0.29) or percentage of time spent exploring the objects (ANOVA of right object: $F_{1,20} = 0.12$, p = 0.74, and left object: $F_{1,20} = 0.16$, p = 0.69) (see Figure 3.2A). In addition, none of the percentages differed significantly from 50% (one-sample t-tests for female mice: t_{10}= 0. 09, p = 0.93 and for male mice: t_{10}= 0. 5, p = 0.62).

On Day 3 of the first experiment, in which the novel and familiar objects were similar, significant differences were observed between the sexes (see Figure 3.2B). One-way ANOVA's reveal a significant difference between the sexes in total duration spent with both the novel ($F_{1,20}$ = 4.63, p = 0.04) and the familiar objects ($F_{1,20}$ = 5.88, p = 0.025). A two-tailed, matched-pairs t-test resulted in a significant difference in the total duration spent exploring the novel vs. the familiar objects by the female mice (t_{10} = 3.28, p = 0.01). The same test was not significant for the male mice (t_{10} = 1.74, p = 0.12). When percentage of exploratory time was analyzed, the female mice spent a significantly greater percentage of time exploring the novel object than the familiar object (one-tailed t-test: t_{10} = 1.95, p= 0.04). There was no significant difference in the percent of time devoted to each of the objects by the males (one-tailed, paired-samples t-test: t_{10} = 1.48, p = 0.06). In addition, one-sample t-tests revealed that the percentage of exploratory time spent with the novel object was significantly greater than 50% (t_{10} = 1.96, p = 0.04) for the female mice. The same test showed that the percentage of time spent with the novel object was not significantly greater than 50% for the male mice (t_{10} = 1.47, p = 0.08). Lastly, a discrimination index was calculated by subtracting the time spent with the familiar from the novel object and dividing by the total time spent exploring objects. An ANOVA of this discrimination index revealed no significant sex difference ($F_{1,20}$ = 0.0011, p = 0.97). However, one-sample t-tests of the distributions revealed that the discrimination index calculated for the female mice was significantly greater than zero (one-tailed t-test: t_{10} = 1.94, p = 0.04). The same test of the distribution from the male mice did not reveal a discrimination index that is significantly different from zero (t_{10} = 1.47, p = 0.09).

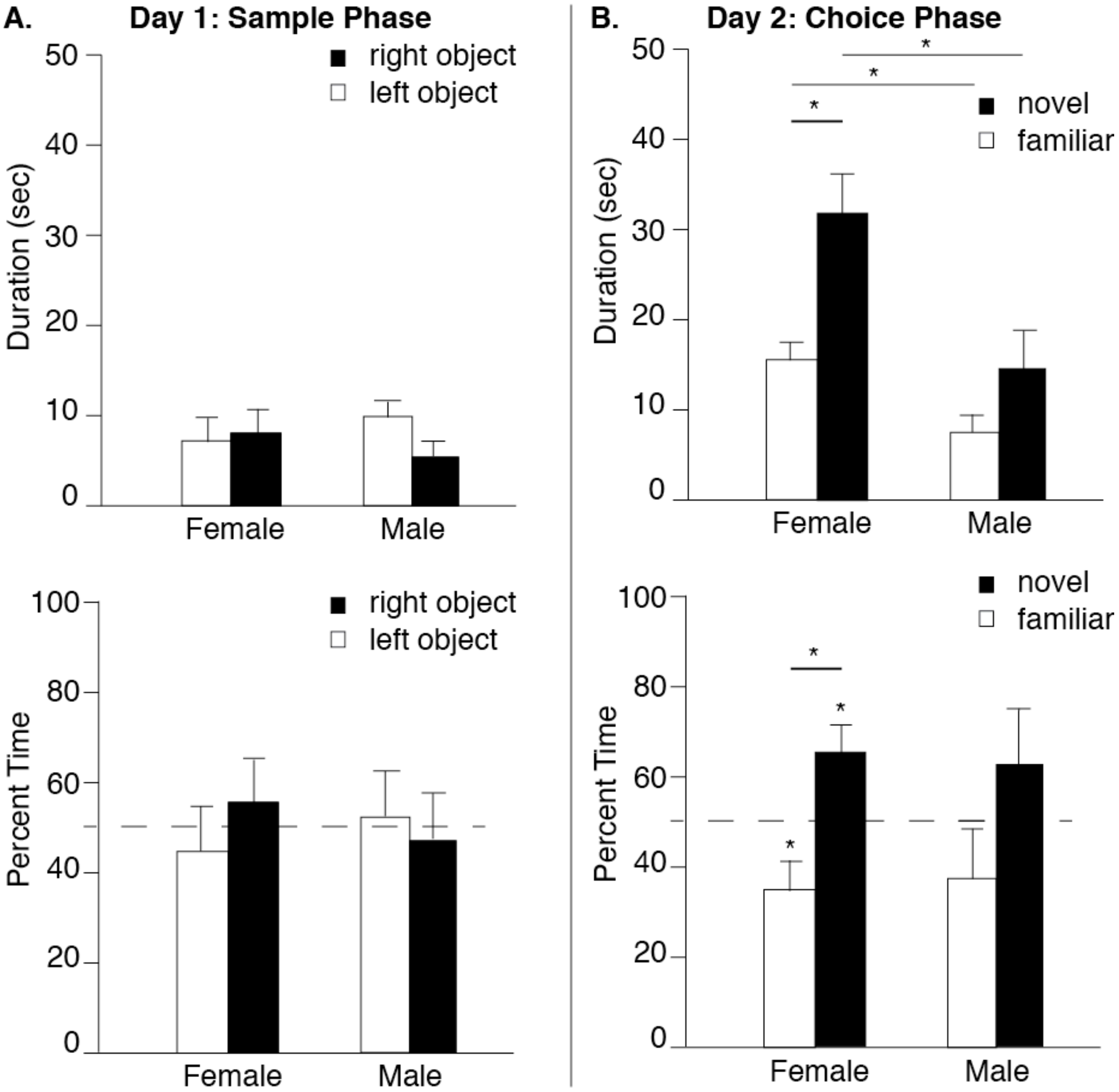


Figure 3.2: A. Duration and percent time spent exploring objects during the sample phase on Day 2 when the subjects are presented with identical objects. B. Duration and percent time spent exploring objects during the choice phase on Day 3 when one object is replaced with a novel object. * indicates $p < 0.05$.

3.4 Results of the second object recognition task with different objects

The sample size was reduced by one male mouse in the replication task, as this subject did not participate in the experiment due to health concerns. On Day 2, the sample phase of the second experiment, no significant differences were observed between sexes or objects and a repeated measures ANOVA with duration as the dependent measure reveals no significant effect of Sex ($F_{1,19} = 0.22$, $p = 0.65$) or Object ($F_{1,19} = 1.59$, $p = 0.22$) and no significant interaction ($F_{1,19} = 3.16$, $p = 0.09$). In addition, the percent of time devoted to each object was not significantly different than 50% for either the female ($t_{10} = 0.3117$, $p = 0.76$) or the male ($t_9 = 1.98$, $p = 0.08$) mice as analyzed with a one-sample t-test (see Figure 3.3A).

On Day 3, the choice phase, only a significant effect of Object type ($F_{1,19} = 80.66$, $p < 0.01$) was observed in a repeated measures ANOVA with duration as the dependent variable (see Figure 3.3B). There was no significant effect of Sex ($F_{1,19} = 0.70$, $p = 0.41$) and no significant Sex x Object interaction ($F_{1,19} = 0.0004$, $p = 0.99$). One-sample t-tests reveal that the female ($t_{10} = 10.4215$, $p < 0.01$) and male ($t_9 = 8.04$, $p < 0.01$) mice spent significantly more than 50% of their exploratory time with the novel object (and, by extension, significantly less than 50% of their time with the familiar object.) Matched pairs t-tests reveal that both the female ($t_{10} = 6.05$, $p < 0.01$) and male ($t_9 = 6.82$, $p < 0.01$) spent significantly more time with the novel than the familiar object. Lastly, one-way ANOVA of the discrimination index reveals no significant difference between the sexes ($F_{1,19} = 0.02$, $p = 0.88$). One-sample t-tests demonstrate that the discrimination index calculated for both the female ($t_{10} = 10.40$, $p < 0.01$) and the male mice ($t_9 = 8.06$, $p < 0.01$) were significantly different from zero (see Figure 3.4B).

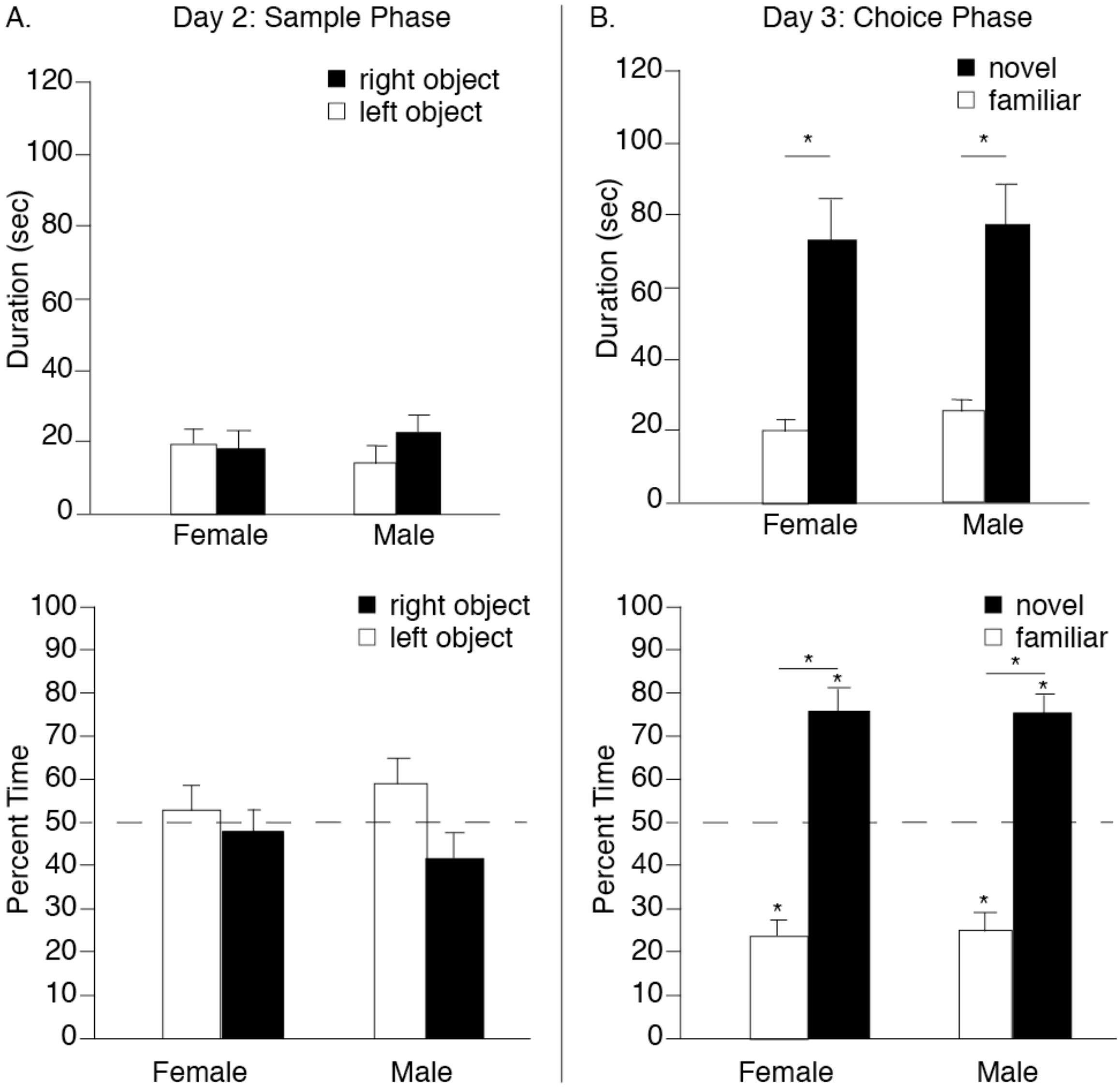


Figure 3.3: A. Duration and percent time spent exploring objects during the sample phase on Day 2 when the subjects are presented with identical objects. B. Duration and percent time spent exploring objects during the choice phase on Day 3 when one object is replaced with a novel object. * indicates $p < 0.05$.

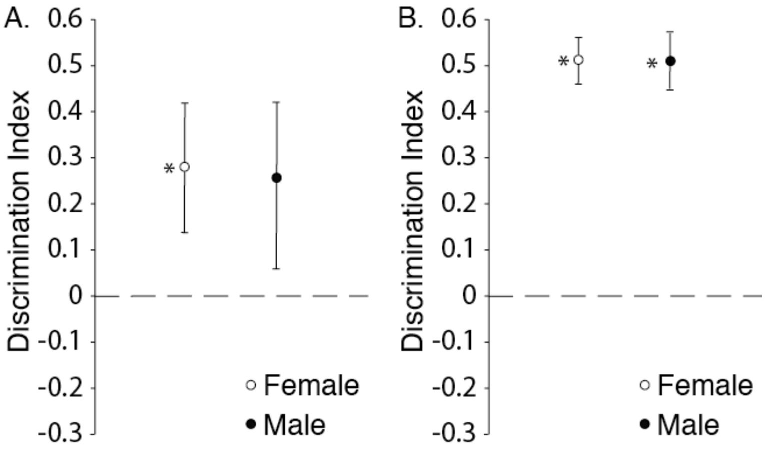


Figure 3.4: A. Discrimination indices for the first object recognition task with similar objects. (Discrimination index = duration with novel - duration with familiar/ total duration exploring objects) B. Discrimination indices for the second object recognition task with objects that differ. (* indicates $p < 0.05$)

3.5 Discussion of sex differences in object recognition

The results from the first of the two object recognition tasks replicated our earlier findings that female mice are better able to discriminate the identity of objects than male mice (Bettis and Jacobs 2009). This is consistent with the literature on sex differences in spatial cognition in other mammalian species. In this experiment, female and male mice both attended to the objects to the same degree during the sample phase, in the presence of identical objects. During the choice phase, female mice differentiated between the novel and familiar objects by exploring the novel object for a longer duration. Male mice trended towards this pattern of exploration, but the variance in responses between individuals made this an insignificant difference. While the first experiment demonstrated a female advantage, the second experiment, which used very distinct objects, demonstrated that male mice are capable of this task at inter-trial intervals of 24 hours. This is consistent with findings in labs that employ only male mice as subjects in tests of learning and memory (Tang et al. 1999).

The finding that there is a female advantage in novel object recognition in mice adds to the literature suggesting a sex difference in spatial navigation and cue use. It also supports the Parallel Map Theory, which suggests that females tend to rely more heavily on the local landmarks and the sketch map (Jacobs and Schenk 2003). In addition, this finding draws attention to yet another aspect to consider when choosing objects in this type of task. In these experiments the degree of difference between the objects was shown to affect the ability to differentiate between novel and familiar objects. Others have cited the need to attend to materials to control difference in odor absorption and texture, as well as the need to attend to affordances of objects (Chemero and Heyser 2005). The height of the objects may also cause an exploratory bias independent of the relative novelty (Alyan and Jander 1997). Future research will help determine which features of these objects influence the outcome of this kind of experimental manipulation (Ennaceur 2010a).

CHAPTER 4: SEX DIFFERENCES IN AN OBJECT LOCATION TASK

Tests of object location memory are typically cited as the only spatial task in which women consistently demonstrate an advantage (for a review see (Voyer et al. 2007)). In this task, subjects are exposed to an array of objects for a short period of time and then asked to identify various changes made to the array after an inter-trial interval. Women are consistently more accurate than men in identifying those changes.

In a similar task developed for rats, Saucier et al. (2007) reported a female advantage in Long- Evans rats. These tests rely on the animal's natural tendency to explore novel objects and arrangements of objects in their environment. The test usually involves habituation to a set of objects followed by a test of exploration after an inter-trial interval during which objects are rearranged or substituted (Ennaceur and Delacour 1988; Save et al. 1992). Female rats outperformed their male counterparts on an object location memory task by exhibiting a biased exploration of the displaced object over the non-displaced object. The results from these object location tasks taken together with those from the previous studies suggest that male and female in these species encode spatial locations using different strategies. In support of this, Williams, Barnett and Meck demonstrated that male laboratory rats rely on the geometry of the room while females tend to rely more on the landmarks for navigation in a radial arm maze (Williams et al. 1990).

While the evidence clearly supports the existence of a sex difference in spatial cognition, particularly in laboratory rats and humans, the evidence is much more sparse and not as clear for laboratory mice. While laboratory rats demonstrate a female advantage over long retention intervals and under a number of housing conditions (Sutcliffe et al. 2007b; Saucier et al. 2008), laboratory mice seem to be inconsistent in these types of tasks. For example, one study found no sex difference (Benice and Raber 2008), and another found a male advantage in recognizing both a shift in location and a novel object (Frick and Gresack 2003).

One potential contributor to the inconsistency in identifying sex differences in laboratory mice on this task may arise from the differences in the spatial arrangements of the testing arena and of the objects involved in these dishabituation tasks. Many of these studies of spatial cognition juxtapose a change that is "spatial" (either a switch in locations or a move to a new location) to one that is either non-spatial within the same trial. In these studies, all changes that involve a move to a new location or a switch of locations are categorized together as spatial while replacements of objects are considered non-spatial (Thinus-Blanc et al. 1996; Dere et al. 2007). However, not all spatial changes provide or alter the same kinds of information. Some changes in the arrangement of objects may provide new directional information by polarizing an array. Some changes may not alter the geometry of the array at all and therefore provide less directional information.

Therefore, the current study was carried out to assess both the existence of a sex difference in spatial strategy in mice and to study in more detail how the sexes might be attending to cue relationships in the environment. Given the current understanding of sex differences in spatial cognition and cue use, the prediction is that male mice will attend to alterations in the environment that change the available vector information by modifying the geometry of the array while female mice will attend to alterations in the relationships between the features of the objects and to substitutions. A study carried out with rats suggests that male rats in a water maze rely on the geometry of landmarks within the maze to the exclusion of the information provided by unique features (Benhamou and Poucet 1998).

The goal of our study was to determine, whether C57BL/6J mice would show the same pattern of sex differences as lab rats and other mammalian species. We first conducted a basic object recognition task similar to that designed by Ennaceur and Delacour (1988). The mice were habituated to the testing room in an empty open arena on Day 1. On Day 2, during the sample phase, they were exposed to two identical objects and, on Day 3, they were given a choice phase during which they were presented with a familiar object from the previous day and a novel object in same position as one of the old objects (see Fig. 4.1). The second experiment allowed us to test differences in response to various spatial changes, substitutions and additions (see Figure 4.2). We wanted to assess responses to changes in various types of relationships between the cues. Thus, we adopted an experiment similar to that used by Save et al. (1992). The hypotheses were that female mice should show an advantage in the object recognition task and in rearrangements that involve switching locations between objects and novel object substitutions, whereas male mice should show an advantage when the rearrangement involved a move to a new position or provided some additional directional information. In this task, a move to a new location changes the arrangement of the array of objects and provides directional information as a result of the polarization of the objects. It is this type of information that males rely on when constructing a bearing map and therefore should elicit a response. A switch in locations between objects offers no such information, but does change the relationship of the objects and the features contained within them. Based on the Parallel Map Theory described in Chapter 1, and on the hypothesis that the female advantage in object recognition and location memory observed in other species functions as a means for improving mate choice and discrimination the specific prediction is that a switch in location between two familiar objects should reveal a female advantage.

4.1 Subjects and husbandry

C57BL/6J mice (N = 30, 15 female) were obtained at the age of two months and were tested at the age of 3 months for the object recognition task. A subset of nine female and ten male mice were tested the following month in the serial dishabituation task. The females were housed in same-sex groups, three per standard mouse cage (19 cm x 29 cm x 13 cm). The males were housed individually in standard mouse cages. Each cage was supplied with a disposable cardboard igloo and bedding material (Nestlets™). If external validity is important, as it is when trying to assess drug effects and model mammalian behavior, then it is critical that we attend to the ethological relevance of the housing that we adopt for our animals (Wurbel 2002). In order to study sex differences in a more natural model, we chose to provide enriched housing for all of the animals by supplying nesting material and cardboard igloos for cover along with a toy to chew on. Standardized lab conditions are impoverished and can cause a suite of stereotyped behaviors and brain changes, which can be alleviated by providing the animals with relatively enriched living conditions (Mohammed et al. 2002; van Praag et al. 2000). It has also been shown that female mice benefit from an enriched cage without causing changes in variance across strains or labs on standard tests of cognitive performance; therefore demonstrating that enrichment does not hinder reliability of the results and may improve the life and cognitive function of the mouse (Wolfer et al. 2004).

In addition to the enrichment, the male mice were housed individually (though not entirely isolated) while female mice were housed in groups to replicate more natural circumstances. Wild female mice may live in groups, but male mice tend to be territorial and, when provided with enrichment or expanded space in a laboratory, setting may become aggressive (Deacon 2006b). Palanza et al. (2001) have shown that individually housed female mice have a higher anxiety profile and are less exploratory in an open field while male mice show the opposite pattern. The male mice housed individually spent more time in the open field while male mice that were group housed demonstrated high anxiety profiles. This type of chronic stress in group housed males may effect cognition and therefore can be considered a potential confound when determining sex differences in learning and memory. Individual housing still allows for olfactory and auditory communication between nearby cages containing male or female mice thus eliminating the stress of complete isolation while potentially causing changes in behavior and cognition that would be indicative of male territoriality (Brain 1975).

Mice were kept on a 14:10 light cycle (lights on 22:00, off at 08:00). All testing was conducted during the dark phase of the light cycle. Water was available ad lib. A daily ration of 4 g standard mouse chow was fed on alternate days, to simulate natural cycles of food availability while maintaining the ad lib weight. Mice rarely finished the entire 48 hr ration, however, and were never without food for more than 12 hrs.

4.2 Apparatus and procedure for the simple object recognition task

This object recognition task was adapted from Ennaceur and Delacour (1988). The task consisted of three successive days of exposure to an arena, in which various objects were placed, for a period of 15 min per day. The arena was an opaque guinea pig cage (40.2 cm x 50.8 cm x 20.32 cm). The objects were unique constructions from Lego® blocks. A video camera was mounted to the ceiling and was connected to a recorder, monitor, and computer in an adjacent room. External cues were available but were not prominent because the walls of the arena were opaque.

On Day 1, the mouse was released into the empty arena for 15 minutes of exploration and habituation. On Day 2, the mouse was again released into the arena for the sample phase, which contained two identical objects, placed near the center. On Day 3, the mouse was again released into the arena for the choice phase, which now contained a duplicate of an object from the sample phase and a novel object (see Fig. 1). The location of the novel object was counterbalanced among mice to control for side biases.

All behavior was recorded on videotape for subsequent analysis by Ethovision Pro (Noldus, Inc.). Two measures of exploratory behavior were made: the time spent within a zone of 4 cm distant from an object and the frequency of visits to this zone per object. The mouse's presence within the 4 cm zone was defined as its body's center of gravity, as defined by Ethovision. Sex differences were calculated using ANOVA tests.

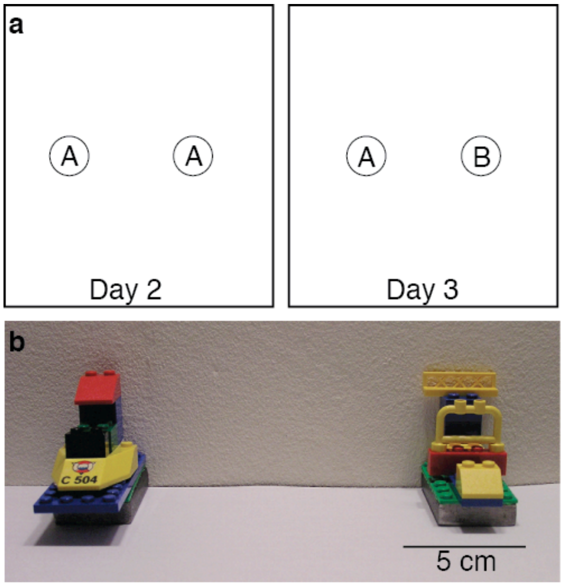


Figure 4.1: a. A representation of the experimental design and spatial layout. Note that the side of the arena in which the novel object was presented was counterbalanced across subjects. b. Lego™ objects used in the object recognition task

4.3 Results of the simple object recognition task

During the sample phase on Day 2, the female mice made more visits to both of the objects than the male mice did (left: ($F_{1, 28} = 11.27$, $p = 0.02$), right: ($F_{1, 28} = 14.19$, $p < 0.01$)). They also spent more time exploring the objects than the male mice did (left: ($F_{1, 28} = 12.60$, $p = 0.01$), right: ($F_{1, 28} = 23.20$, $p < 0.01$)) (see Fig. 4.2). Both male and female mice divided their exploration evenly between the two objects in the sample phase. In the choice phase on Day 3, female mice made more visits to the novel object than the male mice did ($F_{1, 28} = 17.36$, $p < 0.01$), but they did not make more visits to the familiar object than male mice ($F_{1, 28} = 3.54$, $p = 0.07$) (see Fig. 4.2). The female mice also spent more time with both the novel ($F_{1, 28} = 38.86$, $p < 0.01$) and the familiar objects ($F_{1, 28} = 14.13$, $p < 0.01$) than the male mice (see Fig. 3d). Paired samples t-tests reveal that females made more visits ($t_{14} = 2.35$, $p = 0.03$) and spent more time ($t_{14} = 2.84$, $p = 0.01$) with the novel objects than the familiar objects. The males did not make more visits ($t_{14} = 0.27$, $p = 0.80$) or spend more time ($t_{14} = 0.09$, $p = 0.93$) with the novel than the familiar object.

In summary, male mice spent the same amount of time and made approximately the same number of visits to all of the objects on both Day 2 and Day 3. The female mice spent more time with the objects overall and, on Day 3, selectively explored the novel object more than the familiar one.

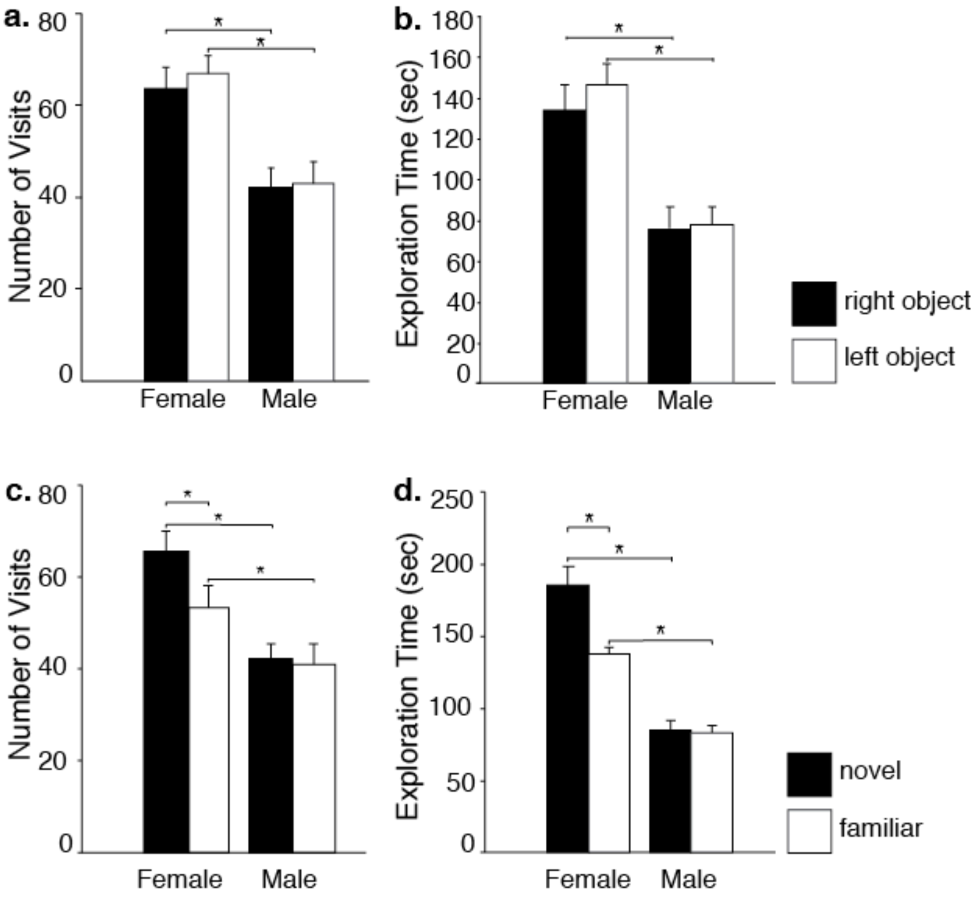


Figure 4. 2: a. Number of visits made by male and female mice to the objects during the sample phase. b. Duration of time spent exploring the objects during the sample phase on Day 2. Note: for a and b, closed bars indicate the object on the right and open bars indicate the object on the left. c. Number of visits to the objects during the choice phase on Day 3. d. Duration of time spent with the objects in the choice phase. (* indicates a significant difference, $p < 0.05$) Note: for c and d, closed bars indicate the novel object while open bars indicate the familiar object.

4.4 Apparatus and procedure for the seven-object recognition and location memory task

All trials took place on an elevated circular platform (90 cm diameter) constructed from white sheet vinyl plastic. This platform was elevated 31 cm above a white floor (122 cm x 122 cm x 5 cm). This elevation and lack of walls has been shown to increase object exploration (Clark et al. 2006). Trials were carried out during the dark phase of the light cycle under low light conditions. Extra-maze cues in the room included two blue room doors, two spotlights on opposite corners of the test room, a rope of evenly distributed small white lights that outlined the ceiling of the room and cast an even light; colored foam shapes and posters attached to the walls, paper towel rack, a mop rack containing a mop, and a metal bookshelf.

All of the objects used in this experiment were made of ceramic material, which was easily cleaned with ethyl alcohol between trials. All objects were of a similar height approximately 8- 10 cm (most of them were salt or pepper shakers) (see Fig. 4.3).

The design of this experiment was based on similar experiments carried out by Save et al., 1992. Each mouse was given 11 consecutive trials that lasted six minutes each with three-minute inter-trial intervals during which the mouse was placed in a holding cage. During the first trial, the mouse was released onto the empty platform for habituation to the environment. In trials two-four, the arena contained four objects in a square configuration with a fifth object in the center of the arena (see Fig. 4.3b). In trials five and six, the arena contained the same objects, however, the center object was moved to a position outside of the square. In trials seven and eight, two of the objects had switched positions. In trials nine and ten, a new object was substituted for an old object. In the last trial a new object was added to the array.

All behavior was recorded on videotape for subsequent analysis by Ethovision Pro (Noldus, Inc.). During trial 1, both distance traveled (cm) and velocity (cm/sec) were recorded in order to assess differences in baseline activity levels. This was done to assess whether or sex differences might be a result of general activity level rather than one of cue use. In all other trials, two measures of behavior were made: the time spent within a zone of 4 cm distant from an object and the frequency of visits to this zone per object. The mouse’s presence within the 4 cm zone was defined as its body’s center of gravity, as defined by Ethovision. An ANOVA was performed to assess any sex differences in initial activity level (average velocity and distance traveled). Habituation to the objects in trials two through four was analyzed using a repeated measures MANOVA with trial as the within subjects variable and sex as the between subjects variables. In order to assess sex differences in response to changes in the arrangement of the objects, MANOVA's were carried out on the difference in time spent with each object category (e.g. displaced vs. non-displaced) before and after the change. This number will be positive if the mice demonstrated an increase in attention to the object category and negative if the mouse habituated further to the object category. In order to assess a significant change in behavior towards the object categories, a one-sample t-test was done to test whether or not the distributions of the discrimination indices differed from zero. During the last trial, a new object was added and this does not allow for the same kind of analysis. In order to analyze this probe, we compared duration and number of visits to each object category using MANOVA tests.

Figure 4.3: a. Stimuli used for the serial dishabituation task. b. A representation of the experimental design and spatial layout.

Table 4.1: Predictions and outcomes of the seven-object task

	Probe 1	**Probe 2**	**Probe 3**	**Probe 4**
Predictions	Male +	Female +	Female +	No difference
Results	Male +	Female +	Female +	Female +

4.5 Results of the seven-object recognition and location memory task

Female and male mice behaved very similarly in the habituation trials. During the first trial, in an empty arena, female mice traveled an average of 26.06 ± 1.14 meters and male mice traveled an average of 25.21 ± 1.08 meters (mean ± standard error). The average velocity of the female mice was 8.93 ± 0.43 cm/sec and the average velocity for the male mice was 8.38 ± 0.42 cm/sec. Neither of these measures of activity were significantly different between the sexes (distance: ($F_{1,\,17} = 0.81$, $p = 0.38$), velocity: ($F_{1,\,17} = 0.30$, $p = 0.59$). These results indicate similar activity levels in the open arena. The duration spent in a zone in the center of the arena was also analyzed for sex differences. This center circle was 33.3 cm in diameter. Males spent 15.35 ± 2.67 sec in this zone. Female mice spent 7.39 ± 2.81 sec in the center zone of the open arena. This difference was not significant, though it was close ($F_{1,\,17} = 4.22$, $p = 0.056$). In summary, no sex differences were observed in measures of baseline behavior.

In trials two through four, when exposed to the five objects for habituation, both female and male mice habituated to the same degree. A repeated measures MANOVA, with number of visits to objects in each trial as the dependent measure, revealed a significant effect of trial ($F_{2, 16} = 56.62$, $p < 0.01$), but no effect of sex ($F_{1, 17} = 0.85$, $p = 0.37$) and no significant interaction ($F_{2, 16} = 3.27$, $p = 0.06$) (see Fig. 4.4a). The same pattern of results was seen using the time spent with objects as the dependent measure (significant effect of trial ($F_{2, 16} = 18.64$, $p < 0.01$), no effect of sex ($F_{1, 17} = 3.31$, $p = 0.09$), and no interaction ($F_{2, 16} = 0.24$, $p = 0.78$) (see Fig. 4.4b). It is interesting to note that the female mice spent more time with the objects in each trial compared to the male mice and that this data trended towards a sex difference.

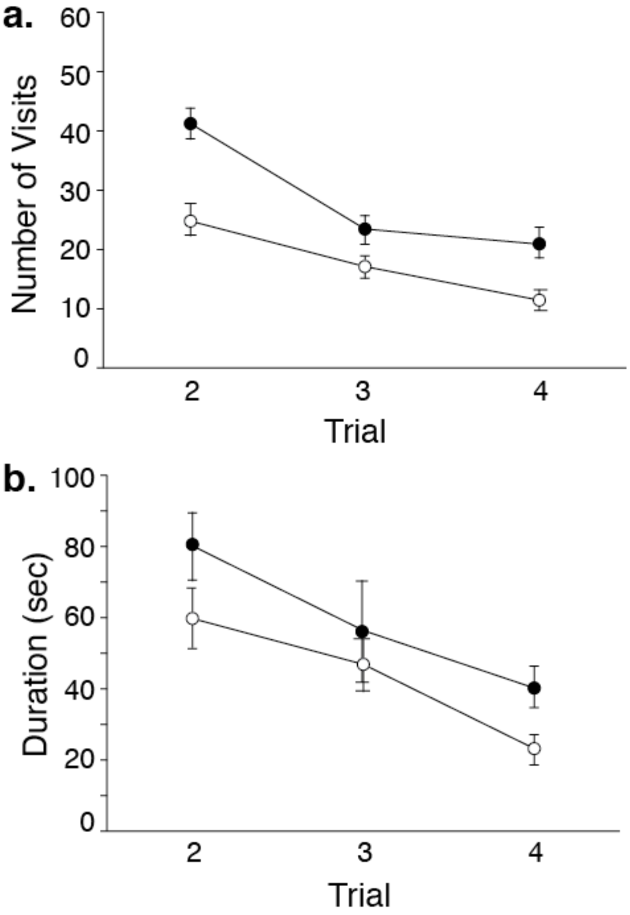
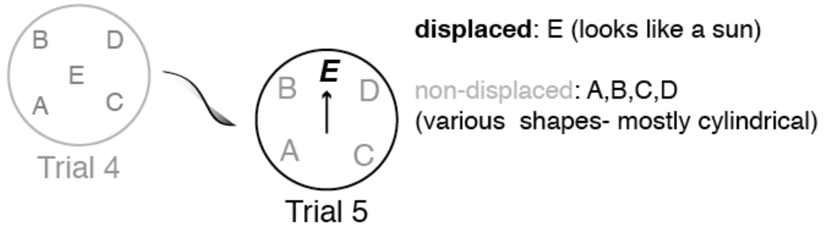


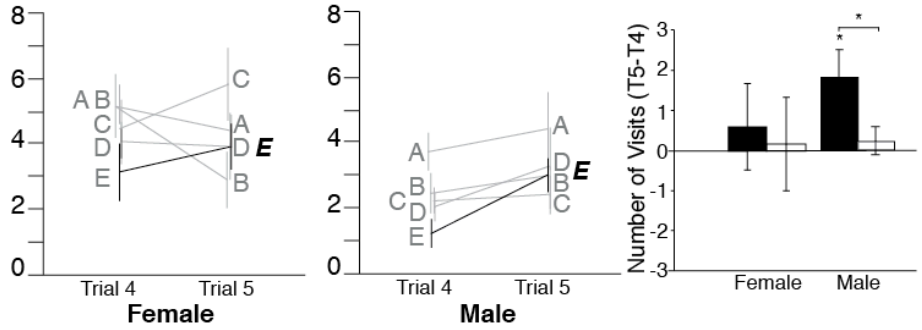
Figure 4.4: a. The mean number of visits to objects in each trial. Note that number of visits declines across trials for both male and female mice. b. The average duration spent exploring objects in each trial. No significant effect of sex was seen in either measure of habituation. There was a significant effect of trial, as is expected in habituation. Closed circles= females, Open circles= males

After the habituations trials, the central object was relocated to an outside position for the remainder of the trials. Male mice responded more strongly than female mice to this displacement of a familiar object to a novel location (see Fig. 4.5). There were no significant sex differences in the change in behavior after the displacement. The lack of sex differences applies to both to number of visits and duration of visits to both of the object categories (displaced and fixed). However, the male mice showed a significant increase (above a zero change level) in number of visits to ($t_9 = 2.52$, $p = 0.03$) and time spent exploring ($t_9 = 2.54$, $p=0.03$) the displaced object. The female mice did not show the same level of response. The change in number of visits ($t_8 = -0.35$, $p = 0.74$) and time spent exploring ($t_8 = 1.04$, $p = 0.33$) the displaced object did not differ from zero for the female mice. This suggests that the male mice increased their exploration of this object after it was displaced, while the female mice did not.

a.



b. Number of Visits



c. Duration (sec)

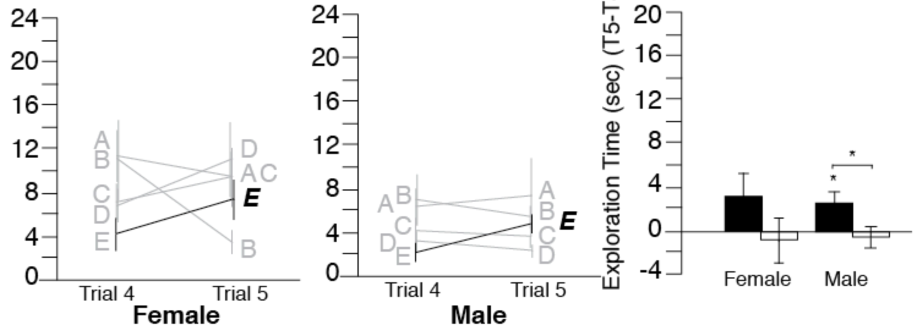


Figure 4.5: Discrimination results of the first displacement. a. The first displacement was of a familiar object to a new location. Note that the new location changes the geometry of the array. b. Change in mean number of visits between trials four and five for displaced and fixed object categories. c. Change in mean exploration time spent with objects between trials four and five for displaced and fixed object categories. (* indicates a significant difference from 0 in a t-test, $p < 0.05$)

Following the next shift, which was a switch in location between two of the objects, the females decreased their exploration of the fixed objects while they maintained the same level of exploration of the displaced objects (see Fig. 4.6). The change in the number of visits to the fixed objects was significantly less than zero (t_8 = -2.68, p = 0.03) indicating further habituation. While the change in the number of visits to the displaced objects across trials six and seven was not significantly different from zero (t_8 = 0.11, p = 0.92) for the female mice. The male mice displayed the opposite pattern of behavior; further habituating to the displaced objects and not the fixed objects. The change in time spent exploring the displaced objects differs significantly from zero (t_9 = -3.04, p = 0.01) indicating increased habituation. However, when we analyze the change in number of visits to each category the male mice did not differ from zero for either the displaced (t_9 = -2.02, p = 0.07) (though this is close) or the fixed objects (t_9 = -1.61, p = 0.14). Overall, this indicates that the female mice habituated further to those objects that did not move and the male mice did not change their behavior across trials unless it was to habituate further to the objects that were switched. In all of this, there were no sex differences in changes of behavior towards either object category as assessed by ANOVA's.

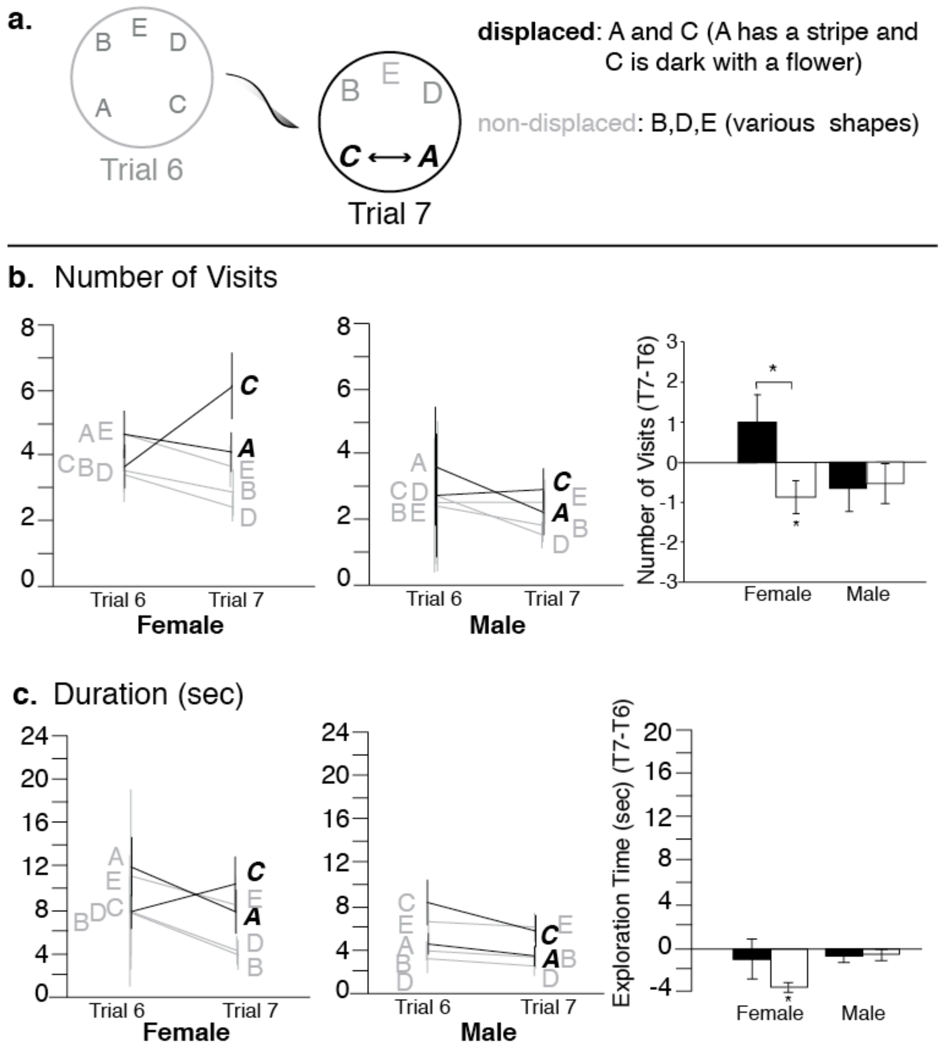


Figure 4.6: Discrimination results of the second spatial change. a. This change involved a switch of location between two familiar objects. b. Change in mean number of visits between trials six and seven for displaced and fixed object categories. c. Change in mean exploration time of objects between trials six and seven for displaced and fixed object categories. (* indicates a significant difference from 0 in a t-test, $p < 0.05$)

The next change in the object array involved a substitution of one of the objects for a novel object (see Fig. 4.7). While it looks as though the female mice habituated to the familiar objects and increased their exploration of the substituted object, none of these distributions differed from zero (number of visits to substituted object (T9-T8) $t_8 = 0.16$, $p = 0.87$; average number of visits to the familiar objects (T9-T8) $t_8 = -1.42$, $p = 0.19$; time spent with the substituted object (T9-T8) $t_8 = 1.15$, $p = 0.28$; average time spent with the familiar objects (T9-T8) $t_8 = -1.50$, $p = 0.17$). This indicates that the female mice did not change their behavior towards the objects between trials eight and nine. The male mice appeared to increase their exploration of both of the object categories but, again, these distributions did not differ significantly from zero (number of visits to substituted object (T9-T8) $t_9 = 1.10$, $p = 0.30$; average number of visits to the familiar objects (T9-

T8) $t_9 = 1.37$, $p = 0.21$; time spent with the substituted object (T9-T8) $t_9 = 1.46$, $p = 0.18$; average time spent with the familiar objects (T9-T8) $t_9 = 1.14$, $p = 0.29$). For this substitution, there are no sex differences and no significant response to the change.

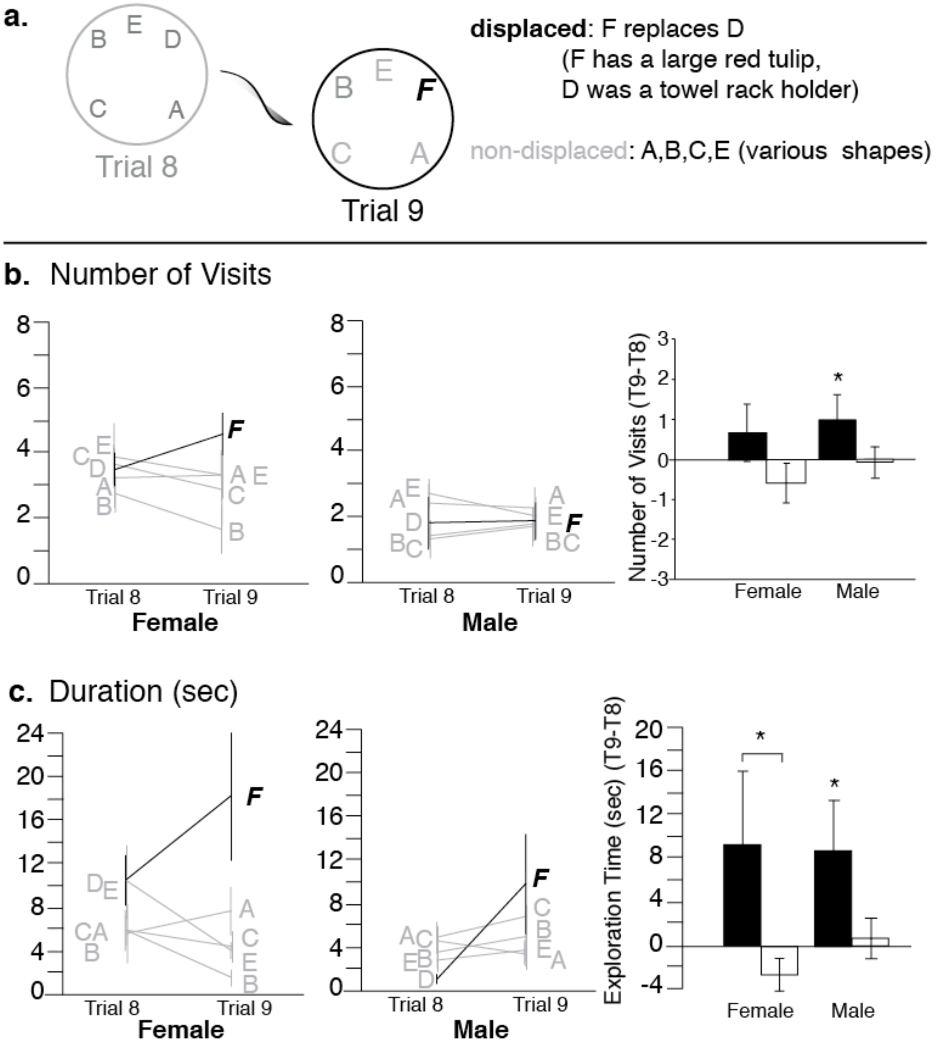


Figure 4.7: Discrimination results of the third change. a. This change was a substitution of a familiar object with a novel object in a familiar location. b. Change in mean number of visits between trials eight and nine for substituted and familiar object categories. c. Change in mean exploration time of objects between trials eight and nine for substituted and familiar object categories. Note that none of the measures differed significantly from zero.

The last change made to the array was an addition of an object to a new location. This addition changed the geometry of the array once again (see Fig. 4.8). The female mice made more visits to the familiar objects than the male mice did ($F_{1,17} = 4.86$, p =

0.04). The female mice made more visits to the novel object than to familiar objects (t_8 = 2.43, p = 0.04) and spent more time with the novel object than with the familiar object (t_8 = 2.53, p = 0.04) in trial 11. The male mice did not visit the novel object more than the familiar objects (t_9 = 1.00, p = 0.34) and they did not spend more time with the novel object than the familiar object (t_9 = 1.02, p = 0.33). This indicates that female mice attended to this change in the array but male mice did not.

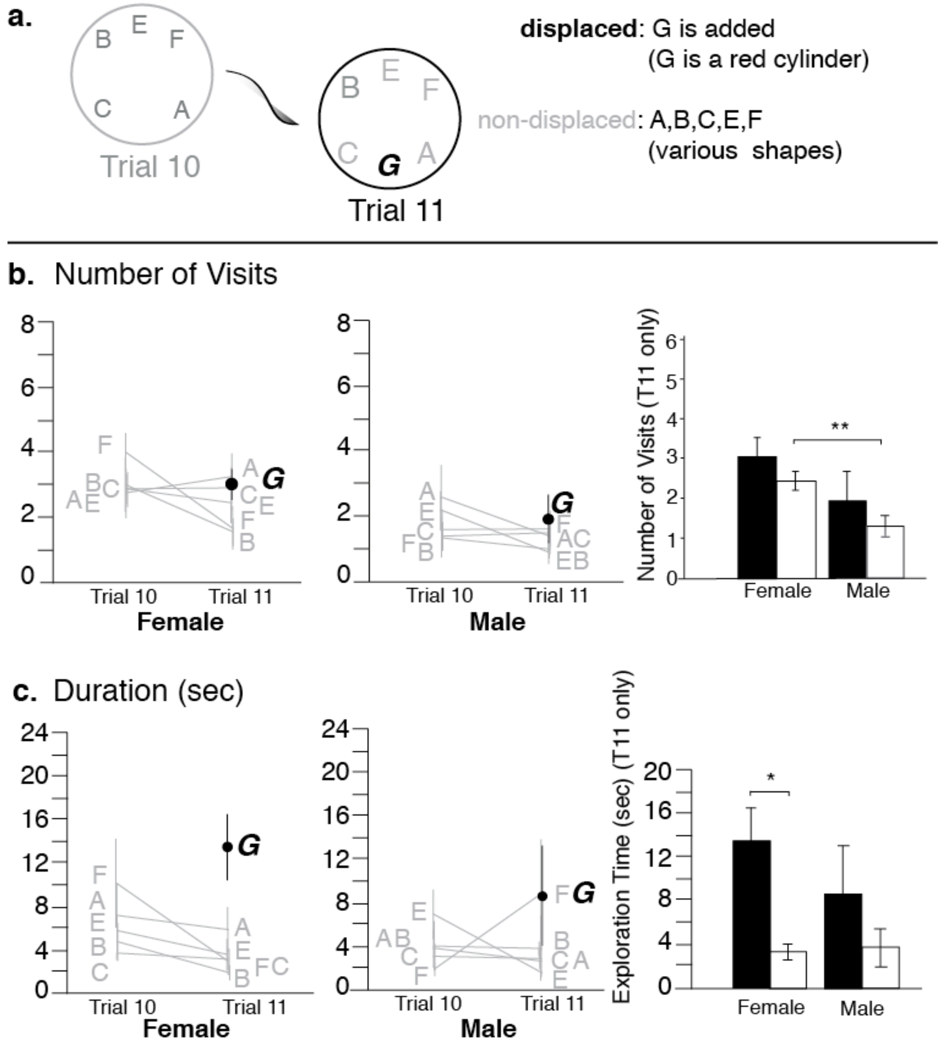


Figure 4.8: Dishabituation results after the last change. a. This change was an addition of a novel object to a novel location. b. Number of visits to the novel object and mean number of visits to the familiar objects. c. Exploration time spent with the novel object and mean exploration time of the familiar objects. (* indicates significant difference in a paired samples t-test, $p < 0.05$) (** indicates a significant difference using an ANOVA, $p < 0.05$)

4.6 Discussion of sex differences and objects in space

Overall our results are consistent with previous studies indicating sex differences in cue use in mammals. With attention to minimizing the chronic stresses of lab housing and the acute stressors of behavioral testing, the mice behaved as was predicted with some interesting results in the last trial. Female mice exhibit greater attention to local cue identity, while male mice show greater dishabituation to changes in the environment that alter geometry or provide larger directional information as in the first probe.

Female, but not male, mice discriminated between the novel and familiar objects in a standard object recognition task. This is consistent with studies in lab rats that demonstrate a female advantage in object recognition (Saucier et al. 2008; Sutcliffe et al. 2007a). Sutcliffe et al. demonstrated that male rats only discriminated the new object from the old up to an inter-trial interval of an hour, while female rats were able to recognize the novel object for longer inter-trial intervals. While this result is consistent with those seen in rats- it is inconsistent with a previous report in the same strain of mouse. Frick et al. have shown, in a very similar task, that males display an advantage in both object recognition and location (Frick and Gresack 2003). There are potentially many reasons for this discrepancy. Anything, from the housing of the animals to the shape of the arena could have resulted in different outcomes. One consideration is that the amount of attention to the objects during habituation was not controlled for, instead we controlled for the amount of exposure overall to the arena. Thus, the female mice attended more strongly to the objects during the sample phase than the male mice did. This increased attention may have resulted in improved memory for these objects and given them an advantage in recognition. Though we cannot say whether the sex difference that was observed is a result of better recall or increased attention in the females, this result still supports the hypothesis that the two sexes rely on different cue types to organize their behavior. This suggests that female mice, as in other species, rely more heavily on intra-maze cues than male mice and therefore respond more strongly than male mice to this type of environmental change.

In the seven-object task, when a familiar object was moved to a location that altered the geometry of the array, male mice increased their exploration of that object and demonstrate an advantage in the first probe. These results are consistent with the findings in rats that suggest a male reliance on geometry and those that endorse the importance of geometrical information as a guide for navigation (Benhamou and Poucet 1998; Williams et al. 1990; Cheng 1986). This is also consistent with studies in both mice and rats that find a male advantage in object location memory specifically (Frick and Gresack 2003; Sutcliffe et al. 2007a). In both of these studies the male animals responded more strongly than a female to an object that was moved to a new location. In the study on mice, the shift in location changed the geometry of the array as it does in this experiment. Also consistent with the literature on sex differences in cognition, female mice exhibit greater attention to changes in the relationships of featural cues and object identity. This was seen in the second probe when two objects switch locations and in the third probe when a familiar object is substituted with a novel one in an old location. These results are consistent with studies in both rats and humans. James and Kimura (1997) show, in a pen and paper task, that female participants perform better than male participants when the location of pairs of objects were exchanged but not when the location was shifted. Female rats also exhibit this same kind of advantage (Saucier et al. 2008). This spatial rearrangement offers no change in geometry but changes the spatial relationship of the objects with each other with particular respect to the unique features of the objects. This result suggests that females may attend more to the unique features of objects and their spatial relationships within the array while male mice attend to the geometry of the array and the positions of objects relative to extra-array cues without attending to the unique features of those objects. The final probe is interesting in that the addition of an object in a new location that creates symmetry in the geometry of the array thus changing the vector information available. It is interesting to note that, in this probe, the male mice did somewhat preferentially explore the novel object, but they also increased exploration of the last object that was added (which happens to be opposite the novel object in the array. The prediction was that male and female mice would demonstrate greater exploration of the additional object and that no sex difference would be seen. The female advantage observed after this change in array may be related to the loss of directional information provided by the local cues.

The effects of husbandry practices can have large effects on cognition, brains, and behavior in general, and on the outcome of studies such as these (For a review see (Rosenzweig and Bennett 1996)). In the early 1960's a team of scientists at UC Berkeley reported increased cortical thickness, as well as an increase in the number of dendritic spines and dendritic branches in response to enriched environments. Clearly, enrichment can help ameliorate some of the damaging effects seen in the impoverished conditions of laboratory housing. The question to address then becomes about what effects different types of enrichment might have on the outcome of studies of cognition.

In a recent study, rats performed equally well on tasks of object recognition regardless of housing situation- either paired or singly housed which suggests that housing should not have a large impact (Harris et al. 2008a). Though in a similar study in mice- individually housed males performed worse than group housed males on object recognition (Voikar et al. 2005). The question then remains what effect housing has on mice. If individual housing is stressful then it can cause negative effects on cognitive performance in mice, but studies have shown that group housing rather than individual housing causes an increased stress response in these mice. In the study by Voikar et al., male mice housed in groups demonstrated a higher stress response on an elevated plus maze. Therefore, the decreased performance in object recognition that is seen in individually housed mice must not be a result of increased stress, but may be a change in behavioral strategies to one that more closely mimics a natural adult male response and therefore a reliance on a different set of information in the environment.

Our results demonstrate that female mice exhibit better performance on tasks of object recognition and location when the location change involves a switch between two familiar objects and positions. These results also demonstrate that male mice perform better than their female counterparts in object location memory tasks when the change involves a switch to a new location. Thus, male mice do not exhibit advantages in all tests of spatial cognition. We suggest that these results were obtained due to differences in cue use and/or frame of reference. The female mice seem to attend more strongly to the unique features of objects and the spatial relationships of the objects within the arena. The male mice seem to utilize a different strategy, attending to the configuration of the objects without the identifying features of the objects information and the relationship of those objects to extra-array cues. These results are consistent with the human, rat, deer mouse and kangaroo rat literatures that suggest a male preference for the use of directional information and a female advantage in object recognition and location memory. Though it adds an important caveat- the assessment of object location memory is dependent on what kind of spatial change has been made and may be dependent on the housing conditions of the animals tested.

Future work will need to be done to assess the contributions of housing methods and reduced stress to the performance on these tasks. In addition, a better understanding of the integration of cues at various frames of reference will offer important information about the ways the information provided from these cues may be functionally combined to form the integrated cognitive map.

CHAPTER 5: CONCLUSIONS ABOUT SEX DIFFERENCES IN SPATIAL COGNITION IN LABORATORY MICE

To begin, the tasks and the housing described here offer a more ethologically valid test of mouse cognition than those currently employed and would be appropriate tools for such an endeavor. These tasks rely on the exploratory drive of the animal without the use of appetitive or aversive reward and without the need for food deprivation. In addition, they are more appropriate for the mouse than the water maze tasks that are commonly used to measure spatial learning in rats.

The results of these experiments support the hypotheses outlined in the introduction. Specifically, they demonstrate that laboratory mice, like laboratory rodents, wood mice, deer mice, meadow voles and humans, exhibit sex differences in spatial behavior. Together, these results support the validity of utilizing the laboratory mouse as a model of mammalian spatial cognition.

There were three tasks included in this series. In Chapter 2, the sex differences observed in the place finding task were consistent with a male bias towards the use of directional information, primarily gained from attention to the geometry of the experimental room or available distal cues and more flexible use of a variety of cue types in females. This is a particularly important result as it is the first study of sex difference in cue use in mice navigating to a goal.

The object recognition experiments, in Chapter 3 illustrate a greater attention to and discrimination of objects by female mice than male mice when the objects to be discriminated are relatively similar. This female bias towards local object cues and the unique relationship of features is again demonstrated in the object location task described in Chapter 4. The results from the seven-object recognition and location memory task suggest that not all spatial changes are equal. Therefore, careful consideration of the arrangement and alterations of environments should be made. These experiments also demonstrate the need for more precision in characterizing such variables as the stimuli, and cues available in testing environments as well as a more careful assessment of the ethological validity of standardized husbandry and testing procedures employed. These considerations may help to explain the variation across labs and may provide a more precise measure of the various spatial behaviors. In addition, they are more appropriate for the mouse than the water maze tasks that are commonly used to measure spatial learning in rats.

Together these results begin to develop a more detailed description of the nature of sex differences in spatial navigation in mice, which will help to more precisely guide future studies. As mentioned in the first chapter, it was Tinbergen’s opinion that to understand all of the reasons for a particular behavior to exist one must address questions of mechanism, development, phylogeny and adaptive significance. These studies only begin to address some of the immediate environmental stimuli that influence the expression of sex differences in cognition.

Other studies of the involvement of various brain structures will continue to broaden our understanding of the proximate mechanisms of these sex differences. The discovery of place cells in the hippocampus and the impaired performance in water maze tasks of subjects with hippocampal lesions indicate the importance of this brain region organizing behaviors in complex environments (O'Keefe and Dostrovsky 1971; Nadel

1991; Morris et al. 1982). And within species comparisons of total hippocampal volume (or the homologous region in birds and fish) between the sexes have discovered larger hippocampi in the sex that has the larger home range: i.e. the males in Merriam's kangaroo rat (*Dipodpmys merriami*) (Jacobs and Spencer 1994), and the females in brown-headed cowbirds (Clayton et al. 1997) and blenniid fish (Costa et al. 2011). When subregions of the hippocampal formation are analyzed interesting sex differences in neuroanatomy become apparent. For example, Wimer and Wimer (Wimer and Wimer 1985) reports that male mice have more granule cell neurons in the dentate gyrus of the hippocampus than female mice. This is consistent with hypotheses generated from the Parallel Map Theory that propose a male reliance on the bearing map for which there is evidence suggesting that this map is mediated by the dentate gyrus. Further studies of the quantitative differences in neuron structure and density of the various regions of the hippocampus in male and female mice will be critical in assessing the validity of the Parallel Map Theory as a model for this species (Jacobs and Schenk 2003).

Studies of the organizational and activational effects of hormones as well as genetic factors have provided a wealth of information regarding the development and modulation of sex differences in spatial cognition across the lifespan (Luine 2003; MacLusky et al. 2006; McEwen 2010; Galea et al. 1995; Gresack and Frick 2006). But many questions regarding the mechanisms of hormonal influence in the adult hippocampus remain.

The sex differences observed in these studies are consistent with the hypothesis that spatial behavior is related to home range size in the field. Specifically, performance in tasks that require the use of geometry or distal cues correlates with the occupation of large home ranges or territories and, generally, males demonstrate an advantage in these tasks. While tasks that require attention to the identities of objects and their relationships with one another tend to be related to the ways in which females organize behavior in complex environments and is not correlated with larger home range size. This leads to the question of the adaptive function of such specific attention to detail. Many have suggested that this attention to detail is a result of the greater demand for food resources females of the species in order to raise offspring or that, in humans, it is a result of the different foraging strategies of men and women (Healy et al. 2009; Ecuyer-Dab and Robert 2004; Silverman and Eals 1992; McBurney et al. 1997). Neither of these foraging hypotheses has been directly tested nor do they address the phylogenetic distribution of sex differences in spatial cognition. If the sex difference in spatial cognition was due to the greater need for food and/or a greater sensitivity to risk, one might predict that the sex differences seen in object recognition and location memory would still be present in monogamous species as this need is probably similar across species irrespective of mating system. Therefore a test of object recognition and location memory that demonstrates a relative female advantage in a monogamous species would be consistent with the hypothesis that increased metabolic need during pregnancy and lactation provides selective pressure for this spatial strategy.

Alternatively, it seems more parsimonious to first assume that these sex differences arise as a result of divergent selective pressures for differing mate choice strategies as proposed in the introduction. Generally in rodents, and specifically in mice this selection may lead to a preferential attention devoted to different levels of spatial resolution. Future experiments comparing the mating system, space use, and reproductive fitness of individuals in various populations will be needed to assess these hypotheses.

BIBLIOGRAPHY

Adams D (1986) The Ultimate Hitchhiker's Guide. Portland House, New York, New York

Alexander G, Evardone M (2008) Blocks and bodies: sex differences in a novel version of the mental rotations test. Hormones and Behavior 53:177-184

Alyan S, Jander R (1997) Exploration is sufficient but not necessary for navigation with landmarks in the house mouse (*Mus musculus*). Learning and Motivation 28:558-576

Arakawa H, Arakawa K, Blanchard DC, Blanchard RJ (2008) A new test paradigm for social recognition evidenced by urinary scent marking behavior in C57BL/6J mice. Behav Brain Res 190 (1):97-104. doi:10.1016/j.bbr.2008.02.009

Astur R (2004) Sex differences and correlations in a virtual Morris water task, a virtual radial arm maze, and mental rotation. Behav Brain Res 151 (1-2):103-115. doi:10.1016/j.bbr.2003.08.024

Astur R, Ortiz M, Sutherland R (1998) A characterization of performance by men and women in a virtual Morris water task: A large and reliable sex difference. Behav Brain Res 93 (1-2):185-190

Attuquayefio D, Gorman M, Wolton R (1986) Home range sizes in the wood mouse Apodemus sylvaticus: habitat, sex and seasonal differences. Journal of Zoology 210 (1):45-53

Barnes L, Wilson R, Bienias J, Schneider J, Evans D, Bennett D (2005) Sex differences in the clinical manifestations of Alzheimer disease pathology. Arch Gen Psychiatry 62:685-691

Beck KD, Luine VN (2002) Sex differences in behavioral and neurochemical profiles after chronic stress: Role of housing conditions. Physiology & Behavior 75 (5):661-673

Benaroya-Milshtein N, Hollander N, Apter A, Kukulansky T, Raz N, Wilf A, Yaniv I, Pick C (2004a) Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. Eur J Neurosci 20 (5):1341-1347. doi:10.1111/j.1460-9568.2004.03587.x

Benhamou S, Poucet B (1998) Landmark use by navigating rats (*Rattus norvegicus*): Contrasting geometric and featural information. J Comp Psychol 112 (3):317-322

Benice T, Rizk A, Kohama S, Pfankuch T, Raber J (2006) Sex-differences in age-related cognitive decline in C57BL/6J mice associated with increased brain microtubule-associated protein 2 and synaptophysin immunoreactivity. Neuroscience 137 (2):413-423. doi:10.1016/j.neuroscience.2005.08.029

Benice TS, Raber J (2008) Object recognition analysis in mice using nose-point digital video tracking. Journal of Neuroscience Methods 168 (2):422-430. doi:10.1016/j.jneumeth.2007.11.002

Bennett A (1996) Do animals have cognitive maps? J Exp Biol 199 (1):219-224

Berger-Sweeney J, Arnold A, Gabeau D, Mills J (1995) Sex differences in learning and memory in mice: effects of sequence of testing and cholinergic blockade. Behav Neurosci 109:859-873

Berry R, Bronson F (1992) Life history and bioeconomy of the house mouse. Biol Rev 67:519-550

Bettis TJ, Jacobs LF (2009) Sex-specific strategies in spatial orientation in C57BL/6J mice. Behav Process 82 (3):249-255. doi:10.1016/j.beproc.2009.07.004

Brain P (1975) What does individual housing mean to a mouse. Life Sciences 16 (2):187-200

Bronson F (1979) The reproductive ecology of the house mouse. Quarterly Review of Biology 54 (3):265-299

Burt W (1943) Territoriality and home range concepts as applied to mammals. J Mammal

Cahill L (2006) Why sex matters for neuroscience. Nat Rev Neurosci 7:477-484. doi:doi:10.1038/nm1909

Carlsson-Kanyama A, Linden A (1999) Travel patterns and environmental effects now and in the future: implications of differences in energy consumption among socio-economic groups. Ecological Economics 30 (3):405-415

Chambers L, Singleton G, Krebs C (2000) Movements and social organization of wild house mice (Mus domesticus) in the wheatlands of northwestern Victoria, Australia. J Mammal 81 (1):59-69

Chemero A, Heyser C (2005) Object exploration and a problem with reductionism. Synthese 147 (3):403-423. doi:10.1007/s11229-005-8363-7

Cheng K (1986) A purely geometric module in the rat’s spatial representation. Cognition 23:149-178

Clark B, Hamilton D, Whishaw I (2006) Motor activity (exploration) and formation of home bases in mice (C57BL/6) influenced by visual and tactile cues: Modification of movement distribution, distance, location, and speed. Physiol Behav 87:805-816. doi:10.1016/j.physbeh.2006.01.026

Clayton N, Reboreda J, Kacelnik A (1997) Seasonal changes of hippocampus volume in parasitic cowbirds. Behav Process 41:237-243

Collett T, Graham P, Durier V (2003) Route learning by insects. Curr Opin Neurobiol 13 (6):718-725. doi:10.1016/j.conb.2003.10.004

Costa SS, Andrade R, Carneiro LA, Goncalves EJ, Kotrschal K, Oliveira RF (2011) Sex differences in the dorsolateral telencephalon correlate with home range size in blenniid fish. Brain Behav Evolut 77 (1):55-64. doi:10.1159/000323668

Crusio W, Schwegler H, Lipp H (1987) Radial-maze performance and structural variation of the hippocampus in mice: a correlation with mossy fibre distribution. Brain Research 425 (1):182-185. doi:doi:10.1016/0006-8993(87)90498-7

Dabbs J, Chang E, Strong R, Milun R (1998) Spatial ability, navigation strategy, and geographic knowledge among men and women. Evolution and Human Behavior 19:89-98

Deacon RMJ (2006) Housing, husbandry and handling of rodents for behavioral experiments. Nat Protoc 1 (2):936-946. doi:10.1038/nprot.2006.120

Dere E, Huston JP, De Souza Silva MA (2007) The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav R 31 (5):673-704. doi:10.1016/j.neubiorev.2007.01.005

Driscoll I, Hamilton D, Yeo R, Brooks W, Sutherland R (2005) Virtual navigation in humans: the impact of age, sex, and hormones on place learning. Hormones and Behavior 47 (3):326-335. doi:10.1016/j.yhbeh.2004.11.013

Dupanloup I, Pereira L, Bertorelle G, Calafell F, Prata M, Amorim A, Barbujani G (2003) A recent shift from polygyny to monogamy in humans is suggested by the analysis of worldwide Y-chromosome diversity. J Mol Evol 57:85-97

Eals M, Silverman I (1994) The hunter-gatherer theory of spatial sex differences: Proximate factors mediating the female advantage in recall of object arrays. Ethology and Sociobiology 15 (2):95-105

Ecuyer-Dab I, Robert M (2004) Have sex differences in spatial ability evolved from male competition for mating and female concern for survival. Cognition 91:221-257

Ennaceur A (2010) One-trial object recognition in rats and mice: Methodological and theoretical issues. Behav Brain Res 215:244-254

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1.Behavioral-data. Behav Brain Res 31 (1):47-59

Etienne AS, Jeffery KJ (2004) Path integration in mammals. Hippocampus 14 (2):180-192. doi:10.1002/(ISSN)1098-1063

Frick K, Burlingame L, Arters J, Berger-Sweeney J (2000a) Reference memory, anxiety and estrous cyclicity in C57BL/6NIA mice are affected by age and sex. Neuroscience 95 (1):293-307

Frick K, Stillner E, Berger-Sweeney J (2000b) Mice are not little rats: species differences in a one-day water maze task. Neuroreport 11 (16):3461- 3465

Frick KM, Gresack JE (2003) Sex Differences in the Behavioral Response to Spatial and Object Novelty in Adult C57BL/6 Mice. Behav Neurosci 117 (6):1283-1291. doi:10.1037/0735-7044.117.6.1283

Galea L, Kavaliers M, Ossenkopp K, Hampson E (1995) Gonadal hormone levels and spatial-learning performance in the Morris water maze in male and female meadow voles, Microtus pennsylvanicus. Hormones and Behavior 29 (1):106-125

Galea L, Kavaliers M, Ossenkopp K, Innes D, Hargreaves E (1994) Sexually dimorphic spatial-learning varies seasonally in two populations of deer mice Brain Research 635 (1-2):18-26

Galea L, Kimura D (1993) Sex differences in route learning. Person Individ Diff 14 (1):53-65

Gallistel CR (1989) Animal cognition: the representation of space, time and number. Annual review of psychology 40:155-189

Garner J (2005) Stereotypies and other abnormal repetitive behaviors: Potential impact on validity, reliability, and replicability of scientific outcomes. Ilar J 46 (2):106-117

Gaulin S, FitzGerald R (1986) Sex differences in spatial ability: An evolutionary hypothesis and test. American Naturalist 127 (1):74-88

Gaulin S, FitzGerald R (1988) Home-range size as a predictor of mating systems in Microtus. J Mammal 69 (2):311-319

Gresack J, Frick K (2006) Post-training estrogen enhances spatial and object memory consolidation in female mice. Pharmacology Biochemistry and Behavior 84 (1):112-119. doi:10.1016/j.pbb.2006.04.013

Gunter C, Dhand R (2002) Human biology by proxy. Nature 420 (6915):509-509. doi:10.1038/420509a

Habib R, McIntosh A, Wheeler M, Tulving E (2003) Memory encoding and hippocampally-based novelty/familiarity discrimination networks. Neuropsychologia 41 (3):271-279

Harestad AS, Bunnell FL (1979) Home range and body weight--a reevaluation. Ecology 60 (2):389-402

Harris A, D'Eath R, Healy S (2008) Sex differences in spatial cognition are not caused by isolation housing. Behav 145:757-778

Healy SD, Bacon IE, Haggis O, Harris AP, Kelley LA (2009) Explanations for variation in cognitive ability: Behavioural ecology meets comparative cognition. Behav Process 80 (3):288-294. doi:10.1016/j.beproc.2008.10.002

Henningsson S, Jonsson L, Ljunggren E, Westberg L, Gillberg C, Rastam M, Anckarsater H, Nygren G, Landen M, Thuresson K, Betancur C, Leboyer M, Gillberg C, Eriksson E, Melke J (2009) Possible association between the androgen receptor gene and autism spectrum disorder. Psychoneuroendocrino 34 (5):752-761. doi:10.1016/j.psyneuen.2008.12.007

Hurst J, Beynon R (2004) Scent wars: the chemobiology of competitive signalling in mice. Bioessays 26:1288-1298

Hurst J, Payne C, Nevison C, Marie A, Humphries R, Robertson D, Cavaggioni A, Beynon R (2001) Individual recognition in mice mediated by major urinary proteins. Nature 414:631-634

Jacobs G (1993) The distribution and nature of colour vision among the mammals. Biological Reviews

Jacobs L (2003) The evolution of the cognitive map. Brain Behav Evolut 62:128-139. doi:10.1159/000072443

Jacobs L, Spencer W (1994) Natural space-use patterns and hippocampal size in kangaroo rats. Brain Behav Evol 44:125-132

Jacobs LF, Schenk F (2003) Unpacking the cognitive map: The parallel map theory of hippocampal function. Psychol Rev 110 (2):285-315. doi:10.1037/0033-295X.110.2.285

James T, Kimura D (1997) Sex differences in remembering the locations of objects in an array: Location-shifts versus location-exchanges. Evolution and Human Behavior 18 (3):155-163

Jonasson Z (2005) Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioral and biological data. Neurosci Biobehav Reviews 28:811-825

Jones C, Braithwaite V, Healy S (2003) The evolution of sex differences in spatial ability. Behav Neurosci

Kavaliers M, Colwell D, Choleris E (1998) Sex differences in opioid and N-methyl--aspartate mediated non-opioid biting fly exposure induced analgesia in deer mice. Pain 77 (2):163-171. doi:doi:10.1016/S0304-3959(98)00092-X

Keenan KP, Ballam GC, Soper KA, Laroque P, Coleman JB, Dixit R (1999) Diet, caloric restriction, and the rodent bioassay. Toxicological Sciences 52 (2):24-34

Kessler R (2003) Epidemiology of women and depression. J Affect Disorders 74 (1):5-13. doi:10.1016/S0165-0327(02)00426-3

Lamberty Y, Gower A (1988) Investigation into sex-related differences in locomotor activity, place learning and passive …. Physiol Behav 44:787-790

Langley CM (1994) Spatial memory in the desert kangaroo rat (*Dipodomys deserti*). Journal of Comparative Psychology 108 (1):3-14

Latham N (2004) From house mouse to mouse house: the behavioural biology of free-living *Mus musculus* and its implications in the laboratory. Applied Animal Behaviour Science 86:261-289. doi:10.1016/j.applanim.2004.02.006

Luine VN (2003) Rapid enhancement of visual and place memory by estrogens in rats. Endocrinology 144 (7):2836-2844. doi:10.1210/en.2003-0004

MacLusky N, Hajszan T, Prange-Kiel J, Leranth C (2006) Androgen modulation of hippocampal synaptic plasticity. Neuroscience 138 (3):957-965. doi:10.1016/j.neuroscience.2005.12.054

Maguire E, Burgess N, O'Keefe J (1999) Human spatial navigation: cognitive maps, sexual dimorphism, and neural substrates. Curr Opin Neurobiol 9:171-177

Martel MM, Gobrogge KL, Breedlove SM, Nigg JT (2008) Masculinized finger-length ratios of boys, but not girls, are associated with attention-deficit/hyperactivity disorder. Behav Neurosci 122 (2):273-281. doi:10.1037/0735-7044.122.2.273

McBurney D, Gaulin S, Devineni T, Adams C (1997) Superior spatial memory of women: Stronger evidence for the gathering hypothesis. Evolution and Human Behavior 18 (3):165-174

McEwen BS (2010) Stress, sex, and neural adaptation to a changing environment: mechanisms of neuronal remodeling. Ann Ny Acad Sci 1204 (S1):E38-E59. doi:10.1111/j.1749-6632.2010.05568.x

Medawar PB (1967) Art of the Soluble: Creativity and Originality in Science. Heinemann Young Books, London

Mickley G, Remmers-Roeber D, Crouse C, Walker C, Dengler C (2000) Detection of novelty by perinatal rats. Physiol Behav 70 (3-4):217-225

Mishima N, Higashitani F, Teraoka K, Yoshioka R (1986) Sex differences in appetitive learning of mice. Physiol Behav 37:263-268

Mohammed A, Zhu S, Darmopil S, Hjerling-Leffler J, Ernfors P, Winblad B, Diamond MC, Eriksson PS, Bogdanovic N (2002) Environmental enrichment and the brain. In: Hofman MA, Boer GJ, Holtmaat AJGD, Van Someren EJW, Verhaagen J, Swaab DF (eds) Progress in Brain Research, vol 138. Elsevier Science, pp 109-133

Morris R, Garrud P, Rawlins J, O'Keefe J (1982) Place navigation impaired in rats with hippocampal-lesions. Nature 297 (5868):681-683

Nadel L (1991) The hippocampus and space revisited. Hippocampus 1 (3):221-229

New J, Krasnow MM, Truxaw D, Gaulin SJC (2007) Spatial adaptations for plant foraging: women excel and calories count. P R Soc B 274 (1626):2679-2684. doi:10.1098/rspb.2007.0826

Newcombe N, Bandura M, Taylor D (1983) Sex differences in spatial ability and spatial activities. Sex Roles 9 (3):377-386

Nutrition SoLA, Nutrition CoA, Agriculture Bo, Council NR (1995) Nutrient requirements of laboratory animals. Fourth revised edition. Nutrient requirements of laboratory animals. , Fourth edn. National Academy Press, Washington D.C.

O'Keefe J, Dostrovsky J (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res 34:171-175

Olsson IAS, Sherwin CM (2006) Behaviour of laboratory mice in different housing conditions when allowed to self-administer an anxiolytic. Laboratory Animals 40 (4):392-399

Olton DS, Samuelson RJ (1976) Remembrance of places passed: Spatial memory in rats. Journal of Experimental Psychology: Animal Behavior Processes 2:97-116

Palanza P, Gioiosa L, Parmigiani S (2001) Social stress in mice: Gender differences and effects of estrous cycle and social dominance. Physiol Behav 73 (3):411-420

Perrot-Sinal T, Ossenkopp K, Kavaliers M (2000) Influence of a natural stressor (predator odor) on locomotor activity in the meadow vole (Microtus pennsylvanicus): modulation by sex, reproductive condition and …. Psychoneuroendocrino 25 (3):259-276

Podhorna J, Brown R (2002) Strain differences in activity and emotionality do not account for differences in learning and memory performance between C57BL/6 and DBA/2 mice. Genes, Brains, and Behavior 1 (2):96-110

Postma A, Izendoorn R, De Haan E (1998) Sex differences in object location memory. Brain and Cognition 36 (3):334-345

Rampon C, Tang Y, Goodhouse J, Shimizu E, Kyin M, Tsien J (2000) Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. Nat Neurosci 3 (3):238-244

Roach JT, Volmar C, Dwivedi S, Hammad O, Crawford FC, Mullan MJ (2003) Automated scoring of the mouse object recognition test. Society for Neuroscience Abstract Viewer and Itinerary Planner 2003:Abstract No. 718.718

Rodgers RJ, Haller J, Holmes A, Halasz J, Walton TJ, Brain PF (1999) Corticosterone response to the plus-maze: High correlation with risk assessment in rats and mice. Physiology & Behavior 68 (1-2):47-53

Rosenthal N, Brown S (2007) The mouse ascending: perspectives for human-disease models. Nature Cell Biology 9 (9):993-999

Rosenzweig M, Bennett E (1996) Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res

Sandstrom N, Kim J, Wasserman M (2006) Testosterone modulates performance on a spatial working memory task in male rats. Hormones and Behavior 50 (1):18-26. doi:10.1016/j.yhbeh.2005.09.008

Saucier D, Lisoway A, Green S, Elias L (2007) Female advantage for object location memory in peripersonal but not extrapersonal space. J Int Neuropsych Soc 13 (4):683-686. doi:10.1017/S1355617707070865

Saucier DM, Green SM, Leason J, Macfadden A, Bell S, Elias LJ (2002) Are sex differences in navigation caused by sexually dimorphic strategies or by differences in the ability to use the strategies? Behav Neurosci 116 (3):403-410. doi:10.1037//0735-7044.116.3.403

Saucier DM, Shultz SR, Keller AJ, Cook CM, Binsted G (2008) Sex differences in object location memory and spatial navigation in Long-Evans rats. Anim Cogn 11 (1):129-137. doi:10.1007/s10071-007-0096-1

Sava S, Markus E (2005) Intramaze cue utilization in the water maze: Effects of sex and estrous cycle in rats. Hormones and Behavior 48 (1):23-33. doi:10.1016/j.yhbeh.2005.01.011

Save E, Poucet B, Foreman N, Buhot M (1992) Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal-formation. Behav Neurosci 106 (3):447-456

Schmidt I, Collett T, Dillier F, Wehner R (1992) How desert ants cope with enforced detours on their way home. J Comp Physiol A 171 (3):285-288

Sherwin C, Olsson I (2004a) Housing conditions affect self-administration of anxiolytic by laboratory mice. Anim Welfare 13 (1):33-38

Sherwin CM, Olsson IAS (2004b) Housing conditions affect self-administration of anxiolytic by laboratory mice. Animal Welfare 13 (1):33-38

Sik A, van Nieuwehuyzen P, Prickaerts J, Blokland A (2003) Performance of different mouse strains in an object recognition task. Behav Brain Res 147 (1-2):49-54

Silverman I, Eals M (1992) Sex differences in spatial abilities: Evolutionary theory and data. In: Barkow J, Cosmides L, Tooby J (eds) The adapted mind: Evolutionary psychology and the generation of culture. Oxford University Press, New York, NY, pp 533-549

Sokolov E (1963) Higher nervous functions: The orienting reflex. Ann Rev Physio 25:545-580

Sutcliffe J, Marshall K, Neill J (2007) Influence of gender on working and spatial memory in the novel object recognition task in the rat. Behav Brain Res 177 (1):117-125. doi:10.1016/j.bbr.2006.10.029

Tabibnia G, Cooke B, Breedlove S (1999) Sex difference and laterality in the volume of mouse dentate gyrus granule cell layer. Brain Research 827 (1-2):41-45

Tang Y, Shimizu E, Dube G, Rampon C, Kerchner G, Zhuo M, Liu G, Tsien J (1999) Genetic enhancement of learning and memory in mice. Nature 401 (6748):63-69

Thinus-Blanc C, Save E, Rossi-Arnaud C, Tozzi A, Ammassari-Teule M (1996) The differences shown by C57BL/6 and DBA/2 inbred mice in detecting spatial novelty are subserved by a different hippocampal and parietal cortex interplay. Behav Brain Res 80 (1-2):33-40

Tinbergen N (1963) On aims and methods of ethology. Zeitschrift für Tierpsychologie

Tolman E, Ritchie B, Kalish D (1946) Studies in spatial learning. 1. Orientation and the short-cut. J Exp Psychol 36 (1):13-24

Tropp J, Markus E (2001) Sex differences in the dynamics of cue utilization and exploratory behavior. Behav Brain Res 119 (2):143-154

Tulving E, Markowitsch H, Craik F, Habib R, Houle S (1996) Novelty and familiarity activations in PET studies of memory encoding and retrieval. Cerebral Cortex 6:71-79

van Praag H, Kempermann G, Gage F (2000) Neural consequences of environmental enrichment. Nat Rev Neurosci 1:191-198

Voikar V, Polus A, Vasar E, Rauvala H (2005) Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences. Genes Brain Behav 4 (4):240-252. doi:10.1111/j.1601-183X.2004.00103.x

Voyer D, Postma A, Brake B, Imperato-McGinley J (2007) Gender differences in object location memory: A meta-analysis. Psychonomic Bulletin & Review 14 (1):23-38

Whishaw I, Tomie J (1996) Of mice and mazes: Similarities between mice and rats on dry land but not water mazes. Physiol Behav 60:1191-1197

Whishaw IQ (1995) A comparison of rats and mice in a swimming pool place task and matching to place task: some surprising differences. Physiol Behav 58 (4):687-693

Williams C, Barnett A, Meck W (1990) Organizational effects of early gonadal secretions on sexual-differentiation in spatial memory. Behav Neurosci 104 (1):84-97

Wimer R, Wimer C (1985) Three sex dimorphisms in the granule cell layer of the hippocampus in house mice. Brain Research 328 (1):105-109

Wolfer D, Litvin O, Morf S, Nitsch R, Lipp H, Wurbel H (2004) Cage enrichment and-mouse behaviour - Test responses by laboratory mice are unperturbed by more entertaining housing. Nature 432 (7019):821-822. doi:10.1038/432821a

Wurbel H (2002) Behavioral phenotyping enhanced - beyond (environmental) standardization. Genes Brain Behav 1 (1):3-8

Yue X, Lu M, Lancaster T, Cao P, Honda S, Staufenbiel M, Harada N, Zhong Z, Shen Y, Li R (2005) Brain estrogen deficiency accelerates A beta plaque formation in an Alzheimer's disease animal model. P Natl Acad Sci Usa 102 (52):19198-19203. doi:10.1073/pnas.0505203102

Zala S, Potts W, Penn D (2004) Scent-marking displays provide honest signals of health and infection. Behav Ecol 15 (2):338-344