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Spatial Memory in Adult Male Rhesus Macaques:
Relationship between Season and the Influence of Testosterone

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Master of Arts

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Spatial Memory in Adult Male Rhesus Macaques: Relationship between Season and the Influence of Testosterone

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B. A., Franklin & Marshall College, 2003

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An abstract of
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Abstract

Spatial Memory in Adult Male Rhesus Macaques:

Relationship between Season and the Influence of Testosterone

By Shannon Brooke Zoe Stephens

Spatial memory research in humans and rodents generally find a sex difference favoring male performance on such tasks. One hypothesis for the observed male advantage is the influence of testosterone on spatial memory. Research investigating the activational and organizational effects of testosterone in rodents and humans has shown that testosterone can improve or enhance spatial abilities. Unlike the results in humans and rodents, results from a previous study in rhesus macaques suggest there is a female advantage on certain spatial tasks and testosterone had no impact on performance (Herman, 2006). The previous study in rhesus macaques tested males during the nonbreeding season, when testosterone levels were low. Using the methodology of Herman (2006), the current study examined male performance during the breeding season, when testosterone is elevated in order to further investigate the effects of testosterone on spatial task performance. Adult male rhesus macaques had been prenatally exposed to a control vehicle or flutamide, an androgen receptor blocker, either early or late in gestation. Results of the current study found a male advantage when only local cues were available and a female advantage on initial performance when only reliable spatial information was present. A positive correlation between testosterone and performance existed for control males when multiple cues were available, while a negative correlation was found when global cues were present in the absence of local cues. These correlations were not found in subjects receiving prenatal flutamide. In addition, control males' performance improved during the breeding season on a task requiring the use of local landmarks, while subjects receiving prenatal flutamide showed no improvement between seasons. Thus, the activational effects of testosterone are dependent on the strategies required to complete the task and these effects differ based on prenatal androgen exposure.

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Table of Contents

List of Tables

List of Figures

Food-caching in birds and mammals allows an animal to store food and retrieve it at a later time. Seasonal migratory patterns, such as those seen in birds, whales and fish, show animals will often return to the same locations each year. These behaviors require the use of spatial memory. Spatial memory refers to the ability to process spatial information about a complex environment, such as direction, distance, orientation, and landmarks, and is typically divided into two components: global and local spatial memory. Global spatial memory incorporates more distant environmental features or landscapes and identifies the general location of an item. Local spatial memory uses landmarks specific to the area to identify the exact location of an item (Tomasello & Call, 1997). Spatial navigation refers to the ability to move around the environment efficiently from one location to another without wasting energy getting lost. Food-caching animals use spatial navigation and global memory to return to the general area of the food, such as a particular patch of trees and then use local spatial memory to recall the exact location of the food, such as the specific tree under which the food is stored. Migratory animals use spatial navigation to reach their general destination and then may use local spatial memory to identify the precise location, such as a particular patch of woods or an area of the river. Evidence of spatial memory has been found in many species such as goldfish (Lopez, Broglio, Rodriguez, Thinus-Blanc, & Salas, 1999), squirrels (Vlasak, 2006), turtles (Lopez, Vargas, Gomez, & Salas, 2003), pigs (Held, Baumgartner, KilBride, Byrne, & Mendl, 2005), birds (Kamil, Balda, & Olson, 1994: Hurly, 1996; Bednekoff & Balda, 2006), chicks (Tommasi & Vallortigara, 2004), rodents (Morris, 1981; Werboff & Laverty, 1970; Jones, McGhee, & Wilkie, 1990) and nonhuman primates (Garber, 1989; Menzel, 1973; Garber & Paciulli, 1997). Spatial memory and navigation is important for

the survival of many species, allowing animals to recall the location of food or to travel far from home to find food or potential mates and returning home when the task is completed. Thus it seems highly likely that spatial memory and the processes which subserve it have been the subject of significant selective pressure.

Current research in spatial memory and navigation is shifting from an evolutionary focus, concentrating on interspecific variation, to a proximate focus, investigating the role of genetics, development and physiological processes on intraspecific variation in spatial memory. However, understanding the evolution and importance of spatial navigation and spatial memory and its underlying physiological processes in primates will provide necessary insight in understanding intraspecific variation, primarily sex differences, in nonhuman primates and ultimately be relevant to understating spatial cognition in humans.

Spatial Memory in Nonhuman Primates

Few studies of nonhuman primates have been conducted in the wild investigating spatial memory and spatial navigation. Studies of natural foraging patterns of moustached and saddle-back tamarins reveal evidence that such foraging employs spatial memory and spatial navigation. While feeding, tamarins minimized the distance traveled between feeding sites by selecting the closest feeding tree and rarely backtracked or crossed previous routes to reach a new feeding site (Garber, 1989). Similar efficient foraging patterns have been observed in free-ranging brown capuchins (*Cebus apella*), suggesting the use of a cognitive map while foraging (Janson, 1998). An experimental field study designed to test the spatial memory of white-faced capuchins (*Cebus capucinus*) used real and plastic bananas on feeding platforms and observed the ability to

recall the sites of real bananas (Garber & Paciulli, 1997). Capuchins learned which platforms contained real bananas when the feedings sites remained consistent. The second phase of the experiment varied the location of the rewarding platforms and presented a yellow block on the rewarding platforms. The capuchins performance decreased in this phase, suggesting capuchins use global spatial information more than local visual landmarks when locating food (Garber & Paciulli, 1997).

Since field experiments are relatively difficult to conduct, spatial memory has been largely studied in a laboratory. Several studies have used a naturalistic approach and looked at navigational success and strategies in an open environment in which the animal is required to travel between food sites. One study tested a macaque's ability to locate food in an open area, more similar to a foraging environment than a small cage (Hampton, Hampstead, & Murray, 2004). Without prior training, the macaques' performance was no different than chance in finding the hidden food. However, after training the macaques on the task by reducing the delay between trials, altering the array of objects each trial to prevent confusion and showing the food during the trial, the macaques' performance improved significantly. Additional tests were conducted to determine if the macaques were using a cognitive map. If the macaque's point of release into the room was altered and the available cues remained in the same location, the macaque's performance declined but was still above chance. The results not only support the use of a cognitive map by macaques, but also indicate the formulation of a cognitive map is not determined by the animal's original position (Hampton et al., 2004).

Squirrel monkeys have also shown evidence of spatial memory when tested in large, open settings (Ludvig, Tang, Eichenbaum, & Gohil, 2003). Having no previous

training on the task, subjects were allowed to explore the test area to search eight stationary containers, four of which contained hidden food. The same four boxes were baited for all trials on the five days of testing. Evidence supporting short-term memory in squirrel monkeys was apparent on the first day, which consisted of thirty trials, as the average performance on the first day was significantly better than chance. At the end of the second day, performance had significantly improved from the first day and the number of incorrect visits had significantly declined. Performance continued to improve during the five days of testing, suggesting the use of reference spatial memory by squirrel monkeys to complete the task (Ludvig et al., 2003).

Research in apes has also indicated the use of spatial memory in foraging experiments (Menzel, 1973). A young chimpanzee was carried around a field enclosure and watched an experimenter hide pieces of fruit in different sections of the compound. The informed animal than returned to the capture area with the rest of the group and the entire group was released two minutes later. Of the six chimpanzees, four of the chimpanzees served as the informed animal for two trials each, while the other animals served as controls for the trial. The chimpanzee given the opportunity to watch the food being hidden retrieved significantly more food than did the other chimpanzees and in most trials used a strategy traveling the least distance between sites, which differed from the route taken by the experimenter to hide the food. The second part of the experiment used similar methods except fruit, a highly desired item, and vegetables, a less desirable item, were hidden in separate places. The informed chimpanzee again minimized travel between sites, but selectively visited the sites containing fruit rather than vegetables. Chimpanzees were able to remember the location of the food, but also what type of food

was hidden at each location and made few working memory errors, if any, during the trials. The superior performance by the informed chimpanzee and the efficient travel routes used lend support to the presence of a cognitive map in chimpanzees (Menzel, 1973).

In addition to the naturalistic spatial tasks, computerized kiosks are being used in the laboratory to test spatial memory. Macaques were able to navigate a virtual maze using a joystick to locate a target and were capable of locating the target even when it was presented in a novel alley of the maze, suggesting rhesus macaques use spatial navigation and cognitive spatial maps to locate targets without physically interacting with the environment (Washburn & Astur, 2003).

The results from field and laboratory experiments demonstrate several primate species use spatial memory and spatial navigation to move around in the environment, at least while searching for food. Few studies have examined which spatial strategies are used by primates, such as global cues in capuchin monkeys and cognitive maps in rhesus macaques (Garber & Paciulli, 1997; Hampton et al., 2004). Although spatial memory is present in several primate species, the mechanisms or strategies used to complete spatial tasks are less understood.

Sex differences in Spatial Memory in Nonhuman Primates

Few studies investigating spatial memory in nonhuman primates have been able to examine sex differences due to small sample sizes. An aged-matched comparison of spatial ability using a delayed response spatial task found females performed significantly better than males. However, the males were more distracted during the task than the females suggesting differences in concentration or attention may account for the results

(McDowell, Brown, & McTee, 1960). Another experiment using a delayed recognition span test tested sex differences by using a 3x6 matrix of wells and using discs to hide food in selected wells (Lacreuse, Kim, Rosene, Killiany, Moss, Moore, Chennareddi, & Herndon, 2005). The number of discs added to the array increased as correct responses accumulated and only the displacement of the new disc would result in a reward. In order to examine working memory, the location of the initial discs presented changed each trial so the information available to identify the new disc was trial specific. In the reference memory task, the location of the initial discs remained the same throughout the trials so subjects were able to memorize the initial locations of the discs and use this information repeatedly when identifying the new disc. In this experiment, subjects were aged-matched and the results showed males performed better than females at locating new discs on the array and finding the hidden food. In addition, a male advantage was only apparent for working memory and not reference memory suggesting memorization of the locations of the discs was important in female performance (Lacreuse et al., 2005). The sex differences in spatial performance only appeared in young rhesus monkeys, 4-7 years of age, and were not apparent in older monkeys, 20-27 years of age, suggesting age is important when examining sex differences in spatial ability (Lacreuse, Herndon, Killiany, Rosene, & Moss, 1999).

The most recent study investigating sex differences in spatial memory and navigation utilized an open environment in which adult rhesus macaques were required to find hidden food in identical goal boxes (Herman & Wallen, 2007). Five of the twelve goal boxes contained rewards. In the initial phase of the experiment, both landmark and spatial cues were present. Blue discs identified the locations containing food and the

food locations remained constant throughout this phase. Subjects were then exposed to two tasks requiring the use of either global or local cues and testing order was counterbalanced so an equal number of subjects of each sex received the Spatial task or Marker task first. In the Spatial task, the blue discs were removed from the goal boxes and the locations of the food remained the same as the initial phase. In the Marker task, the blue discs were indicative of the goal boxes containing food, but the location of the goal boxes containing food varied for each trial (Herman & Wallen, 2007).

There was no sex difference in working memory errors or the number of trials required to reach criterion, although female performance scores were higher than males in the last four trials of the initial phase (Herman & Wallen, 2007). In the Spatial task, female performance was significantly better than male performance. Males exhibited a significant decrease in performance in the first trial of the Spatial task and showed little improvement throughout the task, while females showed a slight, but insignificant decrease in the first trial and continued improvement throughout the Spatial task. The results of the Spatial task suggest females were using global cues more than the local landmark, while males were more dependent on the blue disc to find the food, or on the presence of redundant cues. In the Marker task, both males and females showed a dramatic and significant decrease in performance on the first trial. However, female performance in the Marker task was above chance and improved throughout the task, while male performance never differed from chance across all trials. The results of the Marker task, in conjunction with the results of the Spatial task, suggest females integrate and use both global and local cues to find food. Male performance on the Spatial and Marker task suggest both global and local cues are necessary for finding food, or male

subjects were more sensitive to the change in task demands. As in the initial phase, there were no sex differences in the number of working memory errors in the Spatial task or Marker task (Herman & Wallen, 2007). Despite the sex differences found in task performance, route efficiency to the goal boxes did not vary by sex for any of the tasks (Herman, 2006).

The few studies investigating sex differences in rhesus macaques have used different methods to test spatial memory and navigation, resulting in contradictory findings. While studies have found attention, age, and type of task are important factors when investigating spatial memory, the inconsistent methodologies employed makes it difficult to compare results across studies (McDowell et al., 1960; Lacreuse et al., 2005; Lacreuse et al., 1999; Herman, 2006; Herman & Wallen, 2007). Therefore, it is unclear whether sex differences in cognitive spatial abilities exist in nonhuman primates and more research is needed in nonhuman primates to identify sex differences and the factors contributing to these differences.

Sex differences in Spatial Memory in Rodents

Sex differences in spatial memory and navigation have also been studied in rodents. Spatial navigation tasks require the use of both reference and working memory in order to travel efficiently in the environment. A water version of the radial-arm maze was used to test working and reference memory in male and female mice and rats (Bimonte, Hyde, Hoplight, & Denenberg, 2000). At the beginning of each test day, four of the eight arms contained an escape platform. The locations of the escape platforms at the beginning of each test session remained the same in order to investigate reference memory. Once the escape platform was found and used by the subject, it was removed,

decreasing the number of escape platforms available to the subject for the rest of the testing session. The results indicated female mice and rats exhibited superior working memory and were better able to recall the arms of the maze visited that day. However, male mice and rats had superior reference memory and were better able to recall the original positions of the escape platforms (Bimonte et al., 2000). A similar experiment in mice resulted in slightly different findings. Males made fewer working and reference memory errors than females in the experiment. During the acquisition phase, males made fewer working memory errors than females. As the number of arms in the maze visited increased, females made more reference working memory errors than males. The data suggest there is a sex difference in both working and reference spatial memory on navigation tasks in mice, favoring a male advantage (Gresack & Frick, 2003). The results of these two studies stress the importance of investigating both working and reference memory in spatial tasks, especially with regard to sex differences.

Another study using a Morris water maze task found that males take a more direct route with shorter distances traveled than do females when the starting position is altered (Roof & Stein, 1999). Additional tests revealed no sex difference in path length when the starting positions were altered, but landmark cues were provided, suggesting male and female rodents may use different strategies in spatial navigation tasks (Roof & Stein, 1999). In contrast, local and global cues provided during a radial-arm maze resulted in significantly higher male performance than female performance in rats and the number of cues presented did not alter performance or the sex difference observed (Seymoure, Dou, & Juraska, 1996).

Several studies in rodents have also examined environmental factors such as rearing condition to determine its effect on sex differences in spatial cognition. In an 8-arm radial maze, males rats raised socially had significantly higher performance than male rats reared alone in cages. However, there were no differences in performance when comparing socially reared and isolated female rats (Einon, 1980). In contrast to these results, another study found male performance in a radial-arm maze to exceed female performance regardless of the rearing condition. Socially reared rats performed better than rats raised in isolation; however a similar effect was seen in both sexes (Seymoure et al., 1996).

Although more spatial cognition research has been conducted in rodents than in nonhuman primates, the findings are still conflicting. While Bimonte and colleagues (2000) found a female advantage on a working memory task, most research in rodents favors a male advantage in spatial cognition tasks, which is not evident in nonhuman primates. Comparing the methodology, most of the spatial research in nonhuman primates has centered on the search for food, similar to the radial maze used in rodents. However, the Morris water maze used in rodents requires animals to find an escape platform while submerged in water, which may result in different physiological responses or motivation to complete the task. Thus, the differences in these two maze tasks must be considered when comparing the findings of sex differences in spatial memory.

Sex Differences in Spatial Memory in Humans

In human research, there is growing evidence suggesting sex differences exist in spatial cognition. Male performance in mental rotation tasks has exceeded female performance in numerous studies and has been considered the most reliable human

cognitive sex difference (Saucier, McCreary, & Saxberg, 2002a; Geary, Gilger, & Elliott-Miller, 1992; Rahman, Abrahams, & Jussab, 2005). One study investigating spatial memory in humans focused on mental rotation of letters and the angle of rotation. The results indicated males were better able to identify rotated images and the angle of rotation (Geiger & Litwiller, 2005). A test of undergraduate males and females revealed a sex difference favoring male performance of the Vandenberg three-dimensional mental rotation (Astur, Tropp, Sava, Constable, & Markus, 2004). The results of these studies suggest a true sex difference in the ability to perform mental rotation tasks.

In other aspects of spatial memory, results suggest females are better at object location tasks than males. Tottenham and colleagues (2003) tested object location memory in males and females. Subjects played a computerized version of the game Memory, where objects were shown for several seconds and the subjects were required to match pairs of the hidden objects based on the location. The results indicated females were better at the task than males (Tottenham, Saucier, Elias, & Gutwin, 2003). Females were also better at locating and identifying objects in a complex array (Neave, Hamilton, Hutton, Tildesley, & Pickering, 2005). Another study found female performance in a radial arm maze had significantly fewer errors than male performance. However, further analysis revealed the result was primarily due to an increased recognition of objects rather than location in the radial arm maze (Rahman et al., 2005). The results support the previous findings suggesting females are better at object location tasks, but also suggest female performance may result from other factors separate from spatial memory such as object recognition. In contrast to these results, a three-dimensional spatial task revealed no sex difference in the object location task, although males were better at identifying the

distance between objects (Iachini, Sergi, Ruggiero, & Gnisci, 2005). Another experiment using a computerized array of objects found male performance to be significantly higher than female performance in recalling object locations (Postma, Jager, Kessels, Koppeschaar, & van Honk, 2004). In a virtual Morris water task, males were significantly better at locating a hidden platform, while no sex difference was apparent when the platform was visible (Astur et al., 2004). Studies focusing on the recall of object location have been inconclusive at identifying sex differences in spatial memory and additional aspects, such as object recognition, may interfere with the spatial memory task.

Several studies have focused on navigation as a means to study spatial memory. When required to learn a route to a location using a novel map, males required fewer trials than females to reach the location (Galea & Kimura, 1993). Males used more Euclidean properties such as distance and direction to find the location, whereas females paid close attention to landmarks and street names along the route. Tests were also completed to determine if there was a difference in landmark recognition and the results indicated no significant sex differences for the landmarks passed along the route. This analysis suggests both males and females observed landmarks, but females chose to use them significantly more than males when searching for a location (Galea & Kimura, 1993). In one experiment, subjects were required to find their way back to an original location using a reverse route. Subjects were guided from the original destination to a novel destination, but were shown an indirect route by taking the subjects in circles or backtracking on parts of the route. Males were significantly faster at finding the original location than females (Postma et al., 2004).

One study investigated the ability of men and women to use either Euclidean directions, providing distance and direction, or landmark directions in a navigation task around a university (Saucier, Green, Leason, MacFadden, Bell, & Elias, 2002b). There were no significant differences in the number of errors or time required to reach the destination for males given Euclidean or landmark directions or females given landmark directions. However, females given Euclidean instructions made significantly more errors and took significantly longer to reach the destination. In contrast to other studies, males were also able to recall significantly more landmarks than the females (Saucier et al., 2002b). The experiment highlights the differences in abilities and strategies used by men and women in navigation tasks. The results indicate males are capable of using either Euclidean or landmark directions similarly, suggesting previous research identifying the use of cardinal or distance factors by males observed a preference in strategy rather than a difference in ability. Female performance on the task suggests a difference in ability, not strategy, as females were significantly impaired when given Euclidean directions.

Expanding on these results, an experiment using symbols on a grid focused on the differences between landmark and Euclidean directions and the role of interference when given these directions (Saucier, Bowman, & Elias, 2003). Subjects were presented with ten different pictures of objects and given either Euclidean directions, such as move north five spaces, or landmark directions, such as move five spaces up from the house. In addition, subjects were asked to name days of the week or to tap a specified symbol while performing the task. Both males and females were significantly better at the task when given instructions relating to symbols on the grid rather than Euclidean instructions.

However, when given cardinal directions, male performance was better than female performance. The two types of interference did not affect male performance for either task, while females were affected by verbal interference in both tasks suggesting women rely more on verbal instruction regardless of the information, Euclidean or landmark, provided in the instruction. The test also revealed women recalled more of the symbols used in the grid suggesting the linguistic strategy used by women is responsible for the recollection and use of landmarks (Saucier et al., 2003).

Sex differences in spatial cognition have been studied more in humans than in nonhuman primates or rodents, with most research revealing a male advantage in spatial cognition tasks consistent with most rodent spatial memory research. The spatial navigation research in humans indicates males may prefer or use different strategies depending on the task, while females are more reliant on landmark cues and verbal instruction (Galea & Kimura, 1993; Saucier et al., 2002b; Saucier et al., 2003). Comparisons of human spatial memory and navigation research is difficult since factors such as object recognition and language may confound any comparisons between studies (Tottenham et al., 2003; Rahman et al., 2005; Saucier et al., 2003). Thus, investigating spatial memory and navigation in nonhuman primates may provide a better understanding of the factors affecting these cognitive processes without the interference of language.

Possible explanations for Sex Differences in Spatial Memory

Much of the previous research has focused on the appearance of sex differences rather than discussing the causal mechanisms driving sex differences in spatial cognition. In order to accurately study cognition in humans and animals, it is imperative to understand the biological factors affecting performance. Several theories have been

proposed to explain the sex differences observed in spatial cognition in humans and nonhuman primates.

One possible explanation for sex differences in spatial cognition observed in humans is gender role socialization. However, a study looking at the radial arm maze in humans found gender role socialization has little influence on the sex differences found in mental rotation tasks. The only trait correlated with the differences is engaging in stereotypic masculine behaviors and the correlation is weak (Saucier et al., 2002a). In addition, gender role socialization is only adequate to explain human sex differences in spatial cognition and would not account for any sex differences observed in nonhuman animals.

Socio-economic status has been found to influence sex differences observed in humans (Levine, Vasilyeva, Lourenco, Newcombe & Huttenlocher, 2005). Children in second and third grade from low, middle and high socio-economic backgrounds were given an aerial map task, mental rotation task, and verbal task. A sex difference favoring male performance on the aerial map and mental rotation tasks was found in the middle and high socio-economic students. However, there were no differences between males and females on these tasks in the low socio-economic subjects. No sex differences were found on the verbal task suggesting differences in overall intelligence cannot explain the observed sex differences. Childhood activities were discussed as a possible mechanism to explain the observed sex differences. Children in lower socio-economic groups may not have access to the same toys and tools for spatial learning, which would explain the absence of a sex difference in this economic class. The results of this study suggest environmental conditions present during development affect observed sex differences in

spatial memory in children (Levine et al., 2005). Although socioeconomic status may affect sex differences observed in human spatial memory, it cannot account for the sex differences observed in animals.

While childhood experiences may affect spatial abilities in humans, the presence of sex differences in spatial abilities in animals suggests there must be an underlying biological factor affecting spatial memory, in addition to any experiential factors. In contrast to the previous study, one study investigating spatial memory and another study investigating spatial navigation in pre-pubertal children found no sex differences in performance, errors or task completion time, suggesting sex differences in spatial cognition emerge in adulthood (Barnfield, 1999; Leplow, Lehnung, Pohl, Herzog, Ferstl, & Mehdorn, 2003). Therefore, another widely studied explanation for observed sex differences in cognition is an effect of gonadal hormones. In particular, current research has focused on the role of testosterone in spatial cognition, since spatial cognition research usually results in higher male performance. Unlike the other hypotheses which are only applicable to humans, the major advantage of the testosterone hypothesis is the ability to explain the sex differences observed in nonhuman animals.

Activational Effects of Testosterone on Spatial Cognition in Nonhuman Primates

Only one study has investigated the role of testosterone on spatial navigation in male and female rhesus macaque monkeys. Subjects completed navigation tasks in which spatial information and landmarks were provided as well as tasks where spatial information or landmarks were absent. Testosterone measures did not correlate to performance for any of the tasks in males or females (Herman, 2006). However, female monkeys were tested during the breeding season in the fall while males were tested

during the spring and summer, when testosterone levels are extremely low (Robinson, Scheffler, Eisele, & Goy, 1975; Gordon, Rose, & Bernstein, 1976). Therefore, seasonal differences in hormone production may influence the role of testosterone on spatial navigation.

Activational Effects of Testosterone on Spatial Cognition in Rodents

Research investigating the role of endogenous testosterone in spatial memory has also been conducted using rodent models. One group investigating seasonal differences in cognition in deer mice found male performance on a Morris water maze task was significantly greater than female performance during the breeding season when testosterone levels are high. However, no sex differences were observed during the non-breeding season, suggesting testosterone mediates spatial performance (Galea, Kavaliers, Ossenkopp, Innes, & Hargreaves, 1994). A similar study was conducted in meadow voles, but the males and females were divided into two groups based on high or low testosterone or estradiol, respectively. The results indicated no significant differences in performance between the two groups of males, suggesting testosterone levels were not correlated to performance (Galea, Kavaliers, Ossenkopp, & Hampson, 1995).

A comparison of adult castrated rats to intact rats on two water maze tasks revealed no significant differences in performance (Sandstrom, Kim, & Wasserman, 2006). However, when delays of sixty minutes were implemented between two trials, castrated males had a significant decline in performance in comparison to intact males. Delays of ten minutes produced no significant differences between treatment groups. In addition, testosterone replacement in the castrated males improved performance after a sixty-minute delay, resulting in no differences between the castrated and intact males.

The results suggest testosterone affects reference memory, but not spatial working memory and the use of testosterone replacement therapy can eliminate spatial deficits in castrated males (Sandstrom et al., 2006).

The limited findings regarding the effects of testosterone on spatial cognition in rodents are inconclusive. Testosterone improves performance during the breeding season in deer mice (Galea et al., 1994); however, castrated rats only show spatial impairments after delays of sixty minutes (Sandstrom et al., 2006). Thus, the presence or absence of testosterone seems to affect spatial memory in some capacity, while differences in high and low circulating testosterone levels has less affect on spatial abilities (Galea et al., 1995).

Activational Effects of Testosterone on Spatial Cognition in Humans

Many studies have utilized endogenous testosterone measures in humans when investigating a correlation to spatial cognition. In a study investigating sex and age differences in spatial cognition using a virtual Morris water maze task, male performance was assessed with relation to circulating testosterone levels by focusing on subjects of three age-classes: age 20-39, age 40-59, and over 60. The results indicated males performed better than females in all three age classes. Interestingly, testosterone levels were negatively correlated with performance measures. Men with lower testosterone levels performed better than men with higher testosterone levels, as seen by the difference in performance between age groups after age as a factor was controlled (Driscoll, Hamilton, Yeo, Brooks & Sutherland, 2005).

A positive correlation was also found between testosterone and spatial ability in pubertal males (Davison & Susman, 2001). Males between the ages of 10-14 were tested

on mental rotation tasks and block design tasks from the Wechsler Adult Intelligence Scale-Revised (WAIS) once every six months for a total of three test sessions. The block task in this experiment required subjects to build a 3-D model using colored blocks and a 2-D picture of the target design. Testosterone and spatial ability increased linearly as the number of test sessions progressed supporting the positive role of testosterone in spatial ability (Davison & Susman, 2001). Similar results were found in a population-based study, using over 1100 male participants. Higher free testosterone levels were positively correlated with performance on a block design task (Thilers, MacDonald & Herlitz, 2006).

On the contrary, results of one study indicated testosterone was not correlated with cognitive performance in men on a mental rotation task. The men, mean age 28, were all tested during the same season and at the same time of day in order to control for any fluctuations in endogenous testosterone. Therefore, the results from this study suggest testosterone does not influence some spatial tasks, including mental rotation tasks (Halari, Hines, Kumari, Mehrotra, Wheeler, Ng, & Sharma, 2005). In support of this study, another study investigating endogenous testosterone and spatial ability in older men, mean age 69 years, found no correlation between testosterone and spatial ability on a mental rotation task or a spatial memory task (Wolf & Kirschbaum, 2002).

Several studies have also directly evaluated the role of exogenous testosterone on spatial ability in men and women. Older men, age 50-80 years, given a single injection of testosterone were tested three and six weeks after the injection on navigation and block design tasks (Cherrier, Asthana, Plymate, Baker, Matsumoto, Peskind, Raskind, Brodkin, Bremner, Petrova, LaTendresse & Craft, 2001). Hormonal analysis revealed testosterone

treated individuals still exhibited significantly higher levels of testosterone after six weeks in comparison to control males. The testosterone treated individuals performed significantly better on the navigation task after three weeks. Although their performance remained at the same level after six weeks, the difference was not significant due to an improvement in performance by control males at six weeks. In addition, testosterone treated males had significantly higher performance on a block design task, but only at the six week interval. The results of this study suggest testosterone can enhance performance on spatial navigation and memory tasks; however, the latency to improvement on these tasks varies according to the task (Cherrier et al., 2001). A similar methodology was used to test older men, age 63-85 years, suffering from Alzheimer disease or displaying mild cognitive impairment (Cherrier, Matsumoto, Amory, Asthana, Bremner, Peskind, Raskind & Craft, 2005). Testosterone treated individuals showed improved performance on both the navigation and block design tasks in comparison to control males and continued to have elevated testosterone levels at six weeks. However, the difference was only significant at six weeks after the testosterone injection. In addition, all subjects were tested again during a washout phase, twelve weeks after the testosterone or placebo injection. The performance on the navigation and block design tasks of the men that received a testosterone injection decreased to levels comparable to control subjects, suggesting testosterone had a direct effect on performance and learning or repeated exposure to the task cannot explain the improved performance (Cherrier et al., 2005).

In contrast to these results, another study found a single testosterone injection did not improve male performance on spatial tasks (Wolf, Preut, Hellhammer, Kudielka, Schurmeyer & Kirschbaum, 2000). Older men, age 65-70 years, received either a

testosterone injection or placebo injection and were tested on several cognitive tasks five days after the injection. Individuals were shown a city map with a route drawn on the map and were exposed to periods of short delays, 2 minutes or long delays, 10 minutes, and asked to redraw the route on a new map. In addition, subjects were also administered a mental rotation task. The results indicated no significant differences in performance on any of the three tasks between individuals treated with testosterone and controls (Wolf et al., 2000). Although the results of this study conflict with previous research in older men, there were several methodological differences that could contribute to the differing results. In this experiment, men were tested five days after the injection whereas in other experiments testing occurred weeks later. In addition, the men in this experiment received three times the amount of testosterone than men in the previous studies, suggesting testosterone dosage may affect results. Due to these methodological differences, it is difficult to compare the results of the different studies.

In addition to the studies conducted with elderly men, few studies have begun to investigate the effects of testosterone on spatial ability in women. An object-location memory task was conducted with young women, age 18-35 years, five hours after receiving either a placebo or testosterone injection (Postma, Meyer, Tuiten, van Honk, Kessels & Thijssen, 2000). Using a computer, subjects were required to recall the position of objects and the precise location of each object. The task was completed immediately after removal of objects on the screen as well as after a three-minute delay. Women receiving testosterone showed enhanced performance in comparison to controls after the three-minute delay. However, there were no significant differences between treatment groups when the task was completed immediately. The results suggest

testosterone enhances certain aspects of spatial ability, in particular, aiding in the retention of specific information, while working memory did not appear to be directly affected by testosterone (Postma et al., 2000). A positive effect of testosterone administration was also found in young women completing a mental rotation task. Like the previous study, young women were tested on a mental rotation task five hours after receiving either a placebo or testosterone treatment. Testosterone increased performance on the mental rotation task in comparison to control subjects (Aleman, Bronk, Kessels, Koppeschaar & van Honk, 2004). These two studies support previous research indicating testosterone enhances spatial ability. However, more studies are required in order to better understand the effects of testosterone on spatial ability in women and to determine whether testosterone affects spatial ability similarly in men and women.

In summary, the activational effects of testosterone on spatial ability in males are inconclusive. Endogenous testosterone was positively related to performance on spatial navigation tasks in men, which contrasts the results found in nonhuman primates (Driscoll et al., 2005; Herman, 2006). The effect of endogenous testosterone on spatial memory and rotation tasks was less conclusive, which is surprising since mental rotation tasks generally show strong sex differences with a male advantage. However, the age of men used in these studies varied, which may explain the differences in the results. In contrast to endogenous testosterone, exogenous testosterone given to older men and young women improved performance on spatial memory and navigation tasks in most studies. In addition, the dosage of testosterone and the timing between testosterone administration and testing are important factors which should be considered when comparing the results of these studies.

Organizational Effects of Testosterone on Spatial Cognition in Nonhuman Primates

Herman and Wallen (2007) investigated the organizational effects of testosterone on a spatial navigation task in male and female adult rhesus macaques. Subjects were treated either early or late in gestation with flutamide, an androgen receptor blocker, androgen, or DMSO to serve as a control. The females were tested during the breeding season (September-February) and the males were tested during the non-breeding season (March-August). In the testing area, there were 12 possible locations for the subject to find food, only five of which contained food at any one time. The experiment consisted of three tasks, which investigated the use of spatial orientation and local landmarks. In the first task, the baited locations remained constant and a local landmark, a blue disc, was provided at each location. The first task allowed the subject to use both the spatial location and the local landmark as cues for retrieving the food. In the Spatial task, the baited locations remained the same as in the first task; however the local landmarks were removed from the baited locations, forcing the subjects to rely solely on the spatial location of the food. The Marker task required subjects to rely only on the local landmark, since the food locations changed each trial but were always marked with the blue discs.

Subjects treated with flutamide, an androgen receptor blocker, and androgen treated animals did not differ from control animals in the number of acquisition trials to reach criterion or in working memory errors. There were no significant differences between control males and flutamide treated males on the Spatial task, when local landmarks were removed. However, females that received flutamide late in gestation had a significant decline in performance in the first trial. In addition, females that received

androgen late in gestation showed improvement across the four trials of the task, which was not present in any other female treatment groups. Thus, increased prenatal androgen exposure improved performance in females, while blocking prenatal androgens impaired initial performance. The Marker task required subjects to use local landmarks to identify baited locations that changed each trial. There were no significant differences in performance for any of the female groups. However, males treated with flutamide early in gestation performed significantly better than did control males (Herman & Wallen, 2007). On the Marker task, blocking prenatal androgens improved performance in males, while prenatal androgen exposure did not affect performance in females. The results of this study suggest prenatal androgen exposure can affect spatial memory in adult rhesus macaques. However, the effects of prenatal androgens may differ depending on the strategy required to complete the spatial memory tasks.

Organizational Effects of Testosterone on Spatial Cognition in Rodents

Several studies have been completed using rodent models to assess organizational effects of testosterone on spatial memory. Male and female gonadally-intact rats were given either a high or low dose of exogenous testosterone one week after birth, a period of sexual differentiation in rats, and tested on radial-arm maze and Morris water maze tasks as adults (Roof, 1993). Controls males performed better than control females on both tasks. In addition, control males performed better than both groups of testosterone treated males and the high dosage testosterone group had the worst performance of all males. In contrast, testosterone improved female performance with both testosterone groups performing better than control females and the high dosage testosterone group having the highest performance. A follow-up study tested 21-day old gonadally-intact

rats to determine whether the effects of neonatal testosterone on spatial memory were present prior to puberty. If neonatal testosterone has an activational effect on spatial memory, then the treatment differences in spatial memory performance should not be apparent until after puberty. However, if neonatal testosterone has an organizational effect on spatial memory, then the differences in performance due to treatment should be observable in prepubescent subjects. There were no differences in performance between control males, testosterone treated males and testosterone treated females, all of which performed significantly better than control females. Therefore, the effects of neonatal testosterone treatment could be produced in females at 21 days of age but were not present in males, suggesting neonatal testosterone may have an organizational effect on spatial memory in females and an activational effect on spatial memory in males. The results of these two studies suggest the presence of testosterone early in life during brain development can influence performance on spatial tasks in adulthood and in females, the effect can be seen prior to adulthood (Roof, 1993).

One experiment observed the effects on spatial cognition of treating gonadally-intact adult rats prenatally with flutamide, an androgen receptor blocker, or testosterone (Lund & Lephart, 2001). Subjects were then tested as adults in an eight-arm radial maze. Control males and testosterone treated males and females completed the acquisition phase of the task in fewer days than control females and flutamide treated males or females. With regards to task performance, control and testosterone treated subjects performed significantly better than did flutamide treated subjects and control males performed significantly better than control females. There were no significant sex differences in performance for testosterone or flutamide treated subjects. No direct

comparisons were made between control and testosterone treated individuals, therefore it is unclear whether testosterone treatment influenced performance in comparison to control animals. Although the results do not support a benefit of increased testosterone during the prenatal period, the impairment of flutamide treated individuals suggests the importance of androgen action during the prenatal period and its effect on adult spatial cognition (Lund & Lephart, 2001). Other experiments have found that performance on a Morris water maze task by females prenatally treated with testosterone exceeded performance by control females and the performance of testosterone treated females was comparable to control males (Roof & Havens, 1992: Isgor & Sengelaub, 1998).

In rodents, the organizational effects of testosterone are unclear. It appears the absence of testosterone during a period of gestation can impair spatial abilities as an adult since flutamide, an androgen receptor blocker, treatment impaired spatial performance. However, the result of increased testosterone during the prenatal period is less clear and may have more of an effect on females than males. In females, neonatal testosterone seems to have both an organizational and an activational effect since improvements in performance in comparison to control animals were observed both prior to puberty and as adults. However, neonatal testosterone produced no differences in performance in prepubescent testosterone treated males in comparison to control males and impaired performance as an adult. Thus, it appears neonatal testosterone only has an activational effect in males, while both an organizational and activational effect is present in females.

Organizational Effects of Testosterone on Spatial Cognition in Humans

Studies looking at the organizational effects of testosterone on cognitive ability in humans are difficult to conduct for ethical reasons. However, there are a few studies that

have assessed cognitive ability in relation to prenatal hormone levels using various methods. Measuring testosterone from umbilical cord blood at birth, one group was able to obtain perinatal testosterone levels for males and females. The children were tested at the age of six on a battery of tasks, including spatial tasks. In males, spatial ability did not correlate to perinatal testosterone levels. In females however, there was a significant negative correlation between perinatal testosterone and spatial ability (Jacklin, Wilcox & Maccoby, 1988). The results suggest testosterone may affect spatial ability in males and females differently and that perinatal testosterone levels may affect cognitive abilities later in life. Similar to the findings in rats, testosterone influenced female performance prior to puberty, but had no effect on performance in prepubescent males. However, in this study with children, a negative correlation between testosterone and performance was found in females, while a positive correlation exists in rats.

Congenital adrenal hyperplasia (CAH) is a disease in which individuals are exposed to high levels of prenatal and neonatal adrenal androgens as a result of lacking one of two enzymes necessary to produce glucocorticoids. This prevents normal negative feedback control of the adrenal cortex resulting in over production of adrenal androgens. Cognitive studies with CAH females found better spatial abilities in CAH females in comparison to control females (reviewed in Berenbaum, 1995). In addition, another study found CAH females had greater spatial abilities than their unaffected sisters even though general intelligence measures did not differ (reviewed in Berenbaum, 1995). In contrast, one study with CAH males found impairment in spatial ability, suggesting extremely high androgen levels are not optimal (Hampson, Rovet & Altmann, 1998). Another possible explanation is CAH males have reduced levels of testosterone because

adrenal testosterone may suppress the higher levels of testicular testosterone via negative feedback. Therefore, impaired spatial ability could also be a result of reduced testosterone levels, which would be more compatible with the findings in females.

As in rodents and nonhuman primates, the data for humans suggest prenatal androgens affects female spatial ability and these effects are present prior to puberty. Increased prenatal androgen exposure in females had a positive effect in humans, similar to the positive effects produced in rodents and nonhuman primates. In contrast to females, increased prenatal androgen exposure in human males impairs performance, but these deficits are not apparent until after puberty. The activational effects of prenatal testosterone exposure in humans were similar to the results found in rodents.

Current Study

The current study investigated the role of testosterone in a spatial navigation task using adult, intact, male rhesus macaques during the breeding season, when testosterone levels are higher than in the nonbreeding season (Robinson et al., 1975: Gordon et al., 1976). Three tasks were used to assess the use of spatial information and landmarks separately. The three spatial tasks selected can also provide information on the strategies used by individuals as well as the ability to shift strategies during the task. Testosterone measures were collected regularly to identify any correlations between endogenous testosterone levels and performance on the three spatial tasks. In addition, seven of the male subjects received flutamide, an androgen receptor blocker, prenatally as part of another study, allowing us to investigate the organizational effects of testosterone and its consequences on spatial memory. The results of this study are compared to results collected previously during the nonbreeding season to examine the effects of seasonality

and testosterone production on spatial cognition (see Herman, 2006 and Herman & Wallen, 2007 for nonbreeding season results). Finally, male and female performance on these tasks during the breeding season is compared to examine sex differences in spatial performance when circulating gonadal hormones are elevated in both sexes (see Herman & Wallen, 2007 for female performance results). The results of this research help clarify the role of testosterone in spatial cognition by directly evaluating cognitive performance, both within and between subjects, as it is influenced by seasonal changes in testosterone production, natural variance in endogenous testosterone levels, and the presence or absence of androgens during critical time periods in gestation.

When both consistent spatial information and local landmarks are available, it was expected control males and prenatal flutamide treated males would not differ in performance. However, it was hypothesized control males would have superior performance when only consistent spatial information was available. Altering the spatial information and requiring the subjects to use local landmarks should result in a significant decrease in performance in all males. However, it was expected that flutamide treated males would perform better than control males when only local landmarks were reliable cues. Performance during the breeding season was expected to exceed performance during the nonbreeding season on the Spatial task, while performance on the Marker task during the breeding season was expected to decline or remain constant. Testosterone was expected to have a positive relationship with performance measures on tasks when spatial location remained consistent and a negative relationship with performance measures when the use of local cues was required. Finally, male performance during the breeding season was predicted to be greater than

females when local landmarks are removed, while female performance should be greater than males when spatial information is not reliable and the use of local cues is required.

Method

Subjects

Subjects were 15 adult male rhesus macaques, age seven or eight years, housed in multi-male, multi-female, social groups or small bachelor groups at the Field Station of the Yerkes National Primate Research Center. Subjects were offspring of female rhesus macaques who received prenatal hormone treatments. The pregnant females received twice daily injections of flutamide (dissolved in dimethyl sulfoxide (DMSO) at a concentration of 500 mg/ml and administered at 30 mg/kg), an androgen receptor blocker, or DMSO as a vehicle control. The flutamide or DMSO was received during gestation (approximately 170 days) either early (starting on gestational day 40 for the eight-year old males and gestational day 35 for the seven-year old males) or late in gestation (starting on gestational day 115 for eight-year old males and gestational day 110 for seven-year old males) and twice daily treatments continued for 30 days (eight-year old males) or 35 days (seven-year old males) (Zehr et al., 2000). The fifteen males used in this project were previously exposed to this testing procedure (Herman, 2006). Data from six control female subjects was also obtained and used in comparisons to examine sex differences (R. Herman, personal communication, October, 2005). Subjects were removed from their social groups each day of testing using a separation procedure familiar to the subjects. The research was approved by the Institutional Animal Care and Use Committee and developed in accordance with the *NIH Guide for the Care and Use of Laboratory Animals*.

Testing Facility

The testing facility was a 4.9m x 4.9m x 2.4m area with chain link walls and ceiling and a concrete floor (Figure 1). On the north side of the testing area, there was a small enclosure from which the animals were released into the facility and where the researcher collected the behavioral data. On the northwest side of the facility, there was a small capture area with a sliding door, which the subjects were given access to and entered upon completion of the trial. A carport covered the testing facility, providing shade and protection from rain, and obstructed visual access to the west side of the testing facility where additional macaque groups were located. Visual access to macaque groups on the north and east sides of the testing facility were blocked by weaving 2.5cm vertical slats through wire fencing. The south side of the testing facility remained open since no macaque groups were housed in this area and visual access was limited to woods and initial stages of construction. Although the testing facility was located next to some macaque groups and vocalizations could still be heard, it was distant from all subjects' social groups.

Twelve goal boxes were used during testing and were attached to the wire fencing on the west, east and south sides of the facility in an irregular configuration. The goal boxes were approximately 13 cm tall and 5 cm in diameter and were made of PVC Tees and plugs. The "T" extension of the PVC Tee was removed, resulting in a 5cm opening in the middle of the pipe (Figure 2). The locations of the goal boxes remained consistent throughout training and all phases of testing. Blue discs (10cm in diameter) were attached to the bottom of the PVC pipe for the Dual Cue Acquisition and Marker task trials.

Training

Subjects had been previously habituated to the testing facility (Herman & Wallen, 2007). During training, each of the twelve goal boxes contained 3 M&Ms, 3 raisins and 2 peanuts. Subjects were released into the testing area and given the opportunity to explore the area and the goal boxes for five minutes or until all twelve goal boxes were visited, whichever occurred first. Training was completed when subjects visited at least 5 different goal boxes within five minutes for two consecutive days.

Testing Procedure

All subjects were tested during the breeding season (mid-November through mid-January) when circulating levels of testosterone are at the highest in males (Robinson et al., 1975: Gordon et al., 1976). Since subjects were previously exposed to the testing procedure and tasks, subjects were tested at least three months after the completion of the prior experiment (subjects used in Herman, 2006). Testing occurred at least five days a week with no more than two days in between testing sessions and only one test session a day. Subjects were briefly removed from their social group in the morning and returned to the group when finished testing. Trials were completed in the morning unless weather conditions delayed the start of testing. Subject testing order was randomized each day to eliminate the possibility of an order effect. The testing procedure used was derived from Herman (2006) and consisted of four phases for all subjects: the Dual Cue Acquisition phase, the Spatial task, the Repeated Dual Cue Acquisition phase, and the Marker task.

Dual Cue Acquisition.

In the Dual Cue Acquisition phase, subjects were given two cues, the location and a landmark, to find the baited goal boxes. The twelve goal boxes remained in the same

location as during training sessions, however only five of the twelve goal boxes contained food. The five goal boxes each contained 3 M&Ms, 3 raisins and 2 peanuts. The selection of which five goal boxes contained food was pseudorandom to ensure the baited goal boxes were not clustered in one area. In addition, each of the five baited goal boxes had a 10cm blue disc attached to the bottom of the goal box, serving as a landmark for the subjects. The location of the food in the five goal boxes and blue discs remained constant throughout the Dual Cue Acquisition phase.

A Dual Cue Acquisition trial was five minutes unless a subject visited all five baited goal boxes in less than five minutes, in which the trial ended as soon as the subject left the fifth baited goal box. The Dual Cue Acquisition phase was complete once a subject reached criterion or 24 trials were completed. A subject reached criterion if four of the first five goal boxes visited were correct for two consecutive days of testing. Subjects were given a maximum of 24 trials to reach criterion in the Dual Cue Acquisition phase before continuing to the next phase, either the Spatial or Marker task.

Spatial task.

In the Spatial task, the same baited goal boxes were used as in the Dual Cue Acquisition phase. However, the blue discs were removed from the baited goal boxes. Each subject had four trials of the Spatial task and each trial lasted a maximum of five minutes. Like the Dual Cue Acquisition phase, if a subject visited all five rewarding goal boxes in less than five minutes, the trial ended when the subject left the fifth baited goal box.

Marker task.

In the Marker task, different goal boxes were chosen pseudorandomly to contain food for each test trial. However, the physical locations of the goal boxes did not change. For each of the four trials, at least three of the five baited goal boxes did not contain food the previous trial. The blue discs were located at the bottom of each baited goal box for each Marker task trial. Like the Dual Cue Acquisition and Spatial tasks, the trial ended at five minutes or once the subject visited all five baited locations.

Upon completion of training, each subject advanced to the Dual Cue Acquisition task. Subjects were given 24 trials to reach criterion in the Dual Cue Acquisition task and then exposed to either the Spatial task or the Marker task for four trials. After four trials of the Spatial task or Marker task, the subject received four additional trials of the Dual Cue Acquisition task followed by four trials of the remaining task, either the Spatial task or Marker task. Half of the subjects received the Spatial task first, while half of the subjects received the Marker task first and all subjects completed both the Spatial task and Marker task only once. The four trials of the repeated Dual Cue Acquisition task between the Spatial and Marker tasks were added to bring the subjects back to the original task and to ensure performance on the upcoming task was not affected by the previous task.

In all of the trials for the Dual Cue Acquisition, Spatial and Marker tasks, the subjects were released into the testing area from the same location, the north side of the testing area, and each trial was a maximum of five minutes. Once a trial ended, the subject exited the testing area by entering the small capture area before entering a transfer

box to return to the group. If a subject did not visit any goal boxes during a trial, the trial was not counted and the same trial was completed the following test day.

Data Collection

The researcher was located in the Northern end of the facility in a separate section to collect all behavioral data. Each trial began once the subject was released into the testing facility. Data were collected using a PDA with an attached keyboard using the program that produced a time stamp for each data entry (handobs, Center for Behavioral Neuroscience, Atlanta, GA. A visit to a goal box was defined as the subject either reached or looked into the goal box. The researcher recorded each goal box visited as well as differentiated between investigations and reaches into the goal box. Each trial was also videotaped using Sony DCR-VX2000 miniDV digital cameras (Sony Corp, Tokyo) from approximately 5m outside the testing facility on the south and east sides. One researcher, who had been previously trained on the data collection procedures used in Herman &Wallen (2007), collected a majority of the data. In order to avoid breaks in testing of more than two days, two additional researchers were trained to collect the data. They each collected 141 trials (34.22%) and 81 trials (19.66%) out of the total of 412 trials in the study.

Hormonal Measures

Blood samples were collected from each subject for testosterone analysis. Subjects were separated from the group and entered a small caging unit where the subject presented his leg out a small hole in the caging. All of the subjects were familiar with the procedure and each blood collection required less than two minutes per subject. Samples were collected at least once a week between 11:30am-1:30pm to prevent differences

between subjects due to diurnal fluctuations in testosterone (Goodman, Hotchkiss, Karsch & Knobil, 1974). The blood sample was centrifuged and the serum was stored at -20^{o}C until analysis. Testosterone assays were conducted at the Endocrine Core Laboratory at the Yerkes National Primate Research Center Main Center using a commercially prepared kit by Diagnostic Systems Laboratories (Webster, TX). The sensitivity of the testosterone assay was 0.05 ng/ml and the intra-assay and inter-assay coefficients of variation were 4.3% and 8.2% respectively. At least one testosterone measure was collected during each of the four phases of the experiment: Dual Cue Acquisition task, Spatial task, Repeated Dual Cue Acquisition and Marker task.

Statistical Analysis

Performance was measured in two ways: a percent score, which is traditionally used in analysis and a performance score (Herman & Wallen, 2007).

Percent score.

The percent score is based only on the first five goal boxes visited and does not reveal any information regarding performance after the first five visits. The number of correct goal boxes visited out of the first five visits was divided by five and the quotient multiplied by 100 to calculate the percent score.

Performance score.

Although the percent score is traditionally used in statistical analysis, it is based on the assumption that a subject will visit at least five goal boxes each trial. If a subject visits less than five goal boxes during a trial, the percent score will be less than 100% even if all of the goal boxes visited were correct. However, the performance score is not based on an assumed number of visits but rather calculates probabilities based on the

number of correct and incorrect visits for each trial. Therefore, the performance score provides a more accurate measure of performance when analyzing trials with less than five total goal box visits.

The performance score is based on a combination of probabilities and is calculated by subtracting the chance score from the difference score (Herman, 2006). The difference score is defined as the number of correct visits minus the number of incorrect visits or errors. The chance score is the difference score that would result from chance performance and will be calculated for each number of possible goal box visits, 1-12 (Table 1).

If an individual only visits one goal box in the trial, the probability of visiting a correct goal box is 5/12, or 0.417, while the probability of visiting an incorrect goal box is 7/12, or 0.583. These probabilities are then multiplied by the corresponding difference scores and the sum of the products results in a chance score. If the only goal box visited was correct, the difference score equals 1 (1 correct visit- 0 incorrect visits= 1). However, a difference score of -1 is obtained if the goal box visited was incorrect (0 correct visits- 1 incorrect visit= -1). Therefore, the chance score for visiting one goal box is (1*0.417) + (-1*0.583) = -0.1667. There are three possible outcomes that would result if two goal boxes were visited: two correct visits, two incorrect visits, or one correct and one incorrect. If only two goal boxes are visited, the probability of visiting two correct goal boxes is (5/12)*(4/11) = 0.152. If both goal boxes are incorrect, the probability is (7/12)*(6/11) = 0.318. When the first goal box visited is correct and the second goal box visited is incorrect, the probability is (5/12)*(7/11) = 0.265. However, the first goal box visited may be incorrect and the second goal box visited may be correct, which would

result in a probability of (7/12)*(5/11) = 0.265. Therefore, the probability of visiting one correct goal box and one incorrect goal box accounts for both possible outcomes and is calculated by adding the two probabilities (0.265 + 0.265), producing a total probability (0.530) for one incorrect and one correct visit. The chance score for two goal boxes visited is thus (2*0.152) + (0*0.530) + (-2*0.318) = -0.333.

The percent score and the performance score was calculated for each trial and all statistical comparisons used both the percent score and the performance score. SPSS 15.0 was used for all statistical analyses. An alpha level of .05 was used for all tests and a trend in the data was discussed when probability values greater than .05 but less than .10. Correlations of performance and percent score were completed for each task to ensure the two measures were reliable. Comparisons in performance were made between control males and early flutamide males to assess treatment differences using paired t-tests. Since only two late flutamide males were used in testing, their results are discussed qualitatively in comparison to the other two treatment groups. Paired t-tests were used to compare performance measures across tasks for each prenatal treatment group, while independent t-tests were used to compare performance measures between control males and EFMs. Repeated measures ANOVAs were used to compare performance within the Spatial and Marker tasks. In addition, performance for the Marker task was compared to chance performance using paired t-tests. Correlations were calculated to identify relationships between testosterone level and performance and between percent score and performance score.

Independent t-tests were used to compare the data from this project to the data obtained from the same subjects during the non-breeding season to evaluate the possible

effects of testosterone and seasonality on spatial navigation. Independent t-tests were also used to compare the performance of control males and females during the breeding season to examine sex differences on the three tasks. Data from control females during the breeding season and males during the nonbreeding season were obtained from R. Herman for these comparisons (personal communication, October, 2005).

Results

Breeding Season

Trials with Zero Visits.

Trials in which a subject did not visit a goal box were rare and the occurrence of null trials did not differ between tasks. Seven of the 15 subjects had trials with zero visits with a mean frequency of 1.71 ± 0.42 SEM null trials per subject. There was no significant difference in the frequency of zero trial visits between control males (*M*= 0.56, *SD*= 0.73) and early flutamide males (EFMs) (*M*= 0.50, *SD*= 1.00), $t(11)= 0.11$, $p= .911$. Late flutamide males (LFMs) had a higher mean frequency of null trials (*M*= 2.50, *SD*= 2.12) than control and EFMs. However, one of the two LFMs had four null trials, which was the maximum number of null trials for one subject.

Frequency of Goal Box Visits.

During the Dual Cue Acquisition task, 14 of the 15 subjects had a mean frequency of five or more visits per trial. One control male visited fewer than five goal boxes per trial (*M*= 3.54) and was also the only subject that did not reach criterion in 24 trials of the Dual Cue Acquisition task. Although this subject's mean percent score on the last four trials of the Dual Cue Acquisition was lower than other subjects, the mean performance score on the last four trials of the Dual Cue Acquisition task and the mean performance

and percent scores on the Spatial task and Marker task were within the range of other subjects. These results suggest the subject learned the task but was more selective about the goal boxes visited and therefore, this subject's data were included in the analysis.

Order of Testing.

The testing order of the spatial and Marker tasks was counter-balanced and seven males completed the Spatial task first, while eight males completed the Marker task first. There were no significant differences in performance or percent scores for the spatial or Marker tasks based on testing order (Table 2) and data from these two groups were combined for analysis.

Dual Cue Acquisition.

Percent and performance scores were the two dependent variables used to measure performance on each trial and percent and performance scores were strongly correlated for DCA trials, $r(185) = .74$, $p < .001$. Subjects were given 24 trials to reach criterion (two consecutive days of a percent score of at least 0.8) on the DCA task. Fourteen of 15 subjects reached criterion in 24 trials or less ($M = 10.86$, $SD = 1.15$). Two control males reached criterion in five trials, one LFM in six trials and one control male in seven trials. There were no significant differences in trials to criterion between control males ($M = 11.56$, $SD = 6.31$) and EFMs ($M = 13.50$, $SD = 3.51$), $t(11) = 0.57$, $p = .581$. LFMs ($M = 9.00$, $SD = 4.24$) required fewer trials to reach criterion than both control males and EFMS.

The mean performance score (Table 3) on the first four DCA trials did not significantly differ between control males and EFMs, $t(11) = 0.77$, $p = .461$. LFMs had performance scores similar to EFMs. Similar to performance scores, control males and

EFMs had comparable mean percent scores (Table 3) on the first four DCA trials, $t(11)= .14$, $p= .893$. The mean percent score on the first four DCA trials of LFMs was lower than both control males and EFMs. On the last four DCA trials, performance scores, $t(11)= 0.97$, $p= .351$, and percent scores, $t(11)= .45$, $p= .662$, did not significantly differ between control males and EFMs (Table 3). Performance scores of LFMs on the last four DCA trials were more similar to control males, while percent scores were similar to both control males and EFMs.

Working memory errors during the DCA task were rare and eight of fifteen subjects never made a working memory error. Mean working memory errors per trial (0.11 ± 0.05 SEM) and the number of goal box visits that were a working memory error in DCA trials ($M= 0.01 \pm 0.005$ SEM) did not significantly differ based on prenatal treatment, $U= 9.00$, $p= .144$.

Spatial task.

Performance and percent scores on all spatial trials were strongly correlated, $r(60)= .87$, $p< .001$. On the Spatial task, mean performance scores of control males ($M= 2.91$, $SD= 1.26$) did not significantly differ from EFMs ($M= 2.86$, $SD= 1.37$), $t(11)= 0.05$, $p= .959$. Mean percent scores on the Spatial task for control males ($M= .58$, $SD= .15$) and EFMs ($M= .59$, $SD= .19$) were also comparable, $t(11)= 0.10$, $p= .921$. Performance scores ($M= 2.59$, $SD= 0.30$) and percent scores ($M= .58$, $SD= .11$) for LFMs were similar to both control males and EFMs. A repeated measures ANOVA (Spatial task trial as repeated measure and prenatal treatment as between group factor) of performance score for control males and EFMs showed no main effect of trial, $F(3, 33)= 0.68$, $p= .572$; no main effect of prenatal treatment, $F(1,11)= 0.003$, $p= .959$, and no interaction of prenatal

treatment and trial, $F(3, 33)= 0.64$, $p= .597$. A repeated measures ANOVA of percent score on the Spatial task for control males and EFMs showed no main effect of trial, $F(3, 33)= 1.19$, $p= .331$; no main effect of prenatal treatment, $F(1,11)= 0.01$, $p= .921$; and no interaction of trial and prenatal treatment, $F(3, 33)= 0.45$, $p= .718$.

A comparison of the four trials prior to the Spatial task and the four Spatial task trials (Figure 3) showed no significant difference in mean performance scores for control males, $t(8)= 0.25$, $p=.811$, or EFMs, $t(3)= 0.80$, $p=.480$. There was also no significant change in mean percent scores between the four trials prior to the Spatial task and the Spatial task for control males, $t(8)= 1.42$, $p=.194$, and EFMs, $t(3)= .00$, $p= 1.0$. LFMs had mean performance and percent scores similar to both control males and EFMs.

Performance scores on the first spatial trial in comparison to the previous trial showed no decline in performance for control males, $t(8)= 1.56$, $p=.157$, or EFMs, $t(3)= 0.51$, $p=.643$ (Figure 4a). There was a trend for a significant decline in percent score in the first spatial trial for control males, $t(8)= 1.89$, $p=.095$, $d= 0.69$, but there was no significant change in percent score for EFMs (Figure 4b) (means were the same, t not computed). Changes in performance and percent scores for LFMs were more similar to control males. There were no significant differences in performance scores of control males ($M= 3.86$, $SD= 1.27$) and EFMs ($M= 2.64$, $SD= 1.68$) on the trial prior to the Spatial task, $t(11)= 1.46$, $p= .174$. Percent scores on the trial prior to the Spatial task did not differ between control males ($M= .76$, $SD= .17$) and EFMs ($M= .55$, $SD= .25$), $t(11)= 1.77$, $p= .105$. Therefore, the significant decline of percent score in control males' performance on the first Spatial task trial is not due to a greater percent score on the previous trial. A repeated measures ANOVA (previous trial vs. first spatial trial and

prenatal treatment as between groups factor) of performance score for control males and EFMs showed no main effect of trial, $F(1,11)= 1.44$, $p= .255$; no main effect of prenatal treatment, $F(1, 11)= 1.46$, $p= .253$, and no interaction of trial and prenatal treatment, $F(1,11)= 0.57$, $p= .467$. A repeated measures ANOVA of percent score showed no main effect of trial, $F(1,11)= 1.51$, $p= .245$; no main effect of prenatal treatment between control males and EFMs, $F(1, 11)= 1.96$, $p= .189$; and no significant interaction of trial and prenatal treatment, $F(1,11)= 1.51$, $p= .245$.

There were only three working memory errors made by a total of two subjects (1 EFM and 1 LFM) during the four Spatial task trials and all three working memory errors were made in the first trial.

Marker task.

Performance and percent scores on all marker trials were highly correlated, $r(60)= .49$, $p< .001$. In comparison to the previous four trials, control males showed a significant decline of performance score, $t(8)= -5.01$, $p= .001$, and percent score, $t(8)= -9.17$, $p< .001$, on the Marker task (Figure 5). EFMs showed a similar decline in performance score, $t(3)= -4.21$, $p= .024$, and a trend for a decline in percent score, $t(3)= -2.90$, $p= .063$. Similar to control males and EFMs, LFMs showed a similar decline in performance and percent score during the Marker task. Control males ($M= 1.48$, $SD= 0.86$) performed significantly greater than chance (performance score of 0) across the four Marker task trials, $t(8)= 5.17$, $p=.001$. However, EFMs ($M= 0.55$, $SD= 0.74$) performed at chance levels over the four Marker task trials, $t(3)= 1.47$, $p= .237$. Control males showed a trend for a significantly higher mean performance score on the Marker task than EFMs, $t(11)= 1.88$, $p=.088$. There was no significant difference in mean

percent scores between control males (*M*= .46, *SD*= .09) and EFMs (*M*= .48, *SD*= .10), *t*(11)= 0.36, *p*=.727. LFMs had mean performance and percent scores similar to the EFMs on the Marker task (performance score: *M*= 0.69, *SD*= 1.46; percent score: *M*= .45, *SD*= .14).

In comparison to the previous trial, there was a significant decline in performance score on the first marker trial in control males, *t*(8)= -3.90, *p*= .005, and a trend existed for EFMs, *t*(3)= -3.18, *p*= .050. The decline in performance score on the first marker trial was also evident in LFMs (Figure 6a). A repeated measures ANOVA for control males and EFMs on the first marker trial and the previous trial showed a significant decline for all males on the first marker trial, *F*(1, 11)= 20.93, *p*= .001; no main effect of prenatal treatment, *F*(1, 11)= 0.09, *p*= .776; and no interaction of trial and treatment, *F*(1, 11)= 0.003, *p*= .954. Similar to performance scores, there was a significant decline in percent score on the first marker trial for control males, *t*(8)= -6.11, *p*< .001. There was a trend for lower percent scores on the first marker trial for EFMs, *t*(3)= -2.78, *p*= .069. Like control males and EFMs, LFMs showed a similar decline in percent score on the first marker trial (Figure 6b). Comparing the percent scores on the first marker trial and the previous trial for control males and EFMs, a repeated measures ANOVA revealed a significant decline in percent score on the first trial for all males, *F*(1, 11)= 34.66, *p*< .001; no main effect of prenatal treatment, *F*(1, 11)= 0.05, *p*= .826; and no interaction of trial and treatment, *F*(1, 11)= 0.46, *p*= .514.

On the first Marker task trial, performance scores were not significantly above chance for control males (*M*= 0.63, *SD*= 2.32), *t*(8)= 0.82, *p*= .436, or EFMs (*M*= 0.36, *SD*= 1.79), *t*(3)= 0.40, *p*= .716. Performance score on the first Marker task trial (*M*=

-0.80, *SD*= 3.72) of LFMs was lower than both control males and EFMs. Analysis of the first marker trial showed similar performance scores and percent scores in both control and EFMs. Performance scores on the first Marker task trial did not differ for control males and EFMs, $t(11)= 0.21$, $p= .838$. There was also no significant difference in percent score on the first marker trial between control males (*M*= .38, *SD*= .19) and EFMs (*M*= .45, *SD*= .25), $t(11)= 0.58$, $p= 0.571$. LFMs had percent scores (*M*= .40, *SD*= .28) comparable to control and EF males. Since subjects could not anticipate the change in task, lower performance on the first marker trial was not surprising. Thus, mean performance score on the second, third, and fourth marker trials was compared to chance for control males and EFMs. Control males (*M*= 1.56, *SD*= 1.14) performed significantly better than chance on the remaining three marker trials, $t(8)= 4.11$, $p= .003$. However, mean performance on the remaining marker trials (*M*= 0.61, *SD*= 0.52) remained equal to chance for EFMs, $t(3)= 2.33$, $p= .102$. LFMs had performance scores (*M*= 1.18, *SD*= 0.70) greater than EFMs, but less than control males on these three marker trials.

A repeated measures ANOVA of performance score on the Marker task showed no main effect of trial, $F(3, 33)= 1.58$, $p= .212$; there was a trend for an effect of prenatal treatment, $F(1, 11)= 3.52$, $p= .088$, with control males having higher performance scores than EFMs, and no significant interaction of trial and prenatal treatment, $F(3,33)= 0.32$, $p= .808$. Within each prenatal treatment group, there was no main effect of trial for control males, $F(3, 24)= 2.16$, $p= .120$, or EFMs, $F(3, 9)= 0.44$, $p= .730$, and LFMs showed a similar trend in performance (Figure 7a). A repeated measures ANOVA of percent score (Figure 7b) for control males and EFMs on the Marker task showed a trend for a main effect of trial, $F(3, 33)= 2.36$, $p= .09$; no main effect of prenatal treatment,

$F(1, 11)= 0.13$, $p= .727$, and no significant interaction of trial and prenatal treatment, $F(3, 33)= 1.15$, $p= .343$. The main effect of trial resulted from a significant decline in percent score between the third and fourth trial, $F(1, 11)= 4.96$, $p= .048$. For control males, there was a significant main effect of trial on the Marker task, $F(3, 24)= 6.14$, $p= .003$. A repeated contrast showed a trend for a significant increase between the first and second trial, $F(1, 8)= 5.14$, $p= .053$; no difference between the second and third trial, $F(1, 8)= 0.00$, $p= 1.0$; and a significant decline between the third and fourth trial, $F(1, 8)= 8.24$, $p= .021$. Unlike control males, percent scores for EFMs did not change over trials, $F(3, 9)= 0.18$, $p= .905$, and LFMs had comparable percent scores.

Since the Marker task was the only task in which food locations changed each trial, perseveration behavior is one explanation for the decline in male performance and the chance performance of EFMs. Performance and percent scores on the four Marker task trials were rescored as if the trials were DCA trials to determine if males continued to visit previous food locations. If the males exhibit perseveration behavior and continue to visit previously baited locations rather than the new locations marked by the blue disc, then perseveration scores should remain consistent across trials. Perseveration performance scores did not decline across the four Marker task trials in control males, $F(3, 24)= 2.17$, $p= .118$, or EFMs, $F(3, 9)= 0.64$, $p= .611$ (Figure 8a). LFMs also showed no decline in perseveration performance scores. A comparison of perseveration performance scores between control males and EFMs showed no main effect of trial, $F(3, 33)= 1.77$, $p= .171$; no main effect of treatment, $F(1, 11)= 1.86$, $p= .20$ and no interaction of trial and treatment, $F(3, 33)= 0.45$, $p= .717$. Like performance scores, perseveration percent scores did not decline over trials for control males, $F(3, 24)= 1.24$, $p= .319$, or

EFMs, $F(3, 9)= 0.55$, $p= .663$ (Figure 8b). A repeated measures ANOVA comparing control males and EFMs resulted in no main effect of trial for the perseveration percent scores on the Marker task, $F(3, 33)= 1.34$, $p= .277$; no main effect of prenatal treatment, $F(1, 11)= 0.03$, $p= .862$; and no significant interaction of trial and prenatal treatment, $F(3, 33)= 0.29$, $p= .831$. On the first and third marker trial, perseveration percent scores of LFMs were similar to both control males and EFMs. However, LFMs had lower perseveration percent scores on the second and fourth trials.

In control males, the mean Marker task performance score (M=1.48, SD= 0.86) was significantly higher than the perseveration performance score (M= 0.59, SD= 1.39), $t(8)= 2.40$, $p= .043$. These results suggest control males greater than chance performance was due to the use of the markers and perseveration behavior was not a strategy used by control males. The mean percent score for the control males (M= .46, SD= .09) did not differ from perseveration percent score (M= .46, SD= .12), $t(8)= -0.12$, $p= .907$, which suggests control males may have initially exhibited some perseveration behavior by visiting correct DCA locations. However, this initial perseveration behavior seen each trial did not continue throughout the trial since the actual performance scores on the task were greater than the perseveration performance scores. Perseveration behavior could explain the performance of the EFMs and LFMs, since their performance did not significantly differ from chance and there was no significant change in perseveration scores across trials. The mean Marker task performance score for EFMs (M= 0.55, SD= 0.74) did not significantly differ from the mean perseveration performance score (M= 0.05, SD= 0.71), $t(3)= 0.93$, $p= .423$. LFMs had means similar to the EFMs. Although the perseveration performance score remained consistent across trials for EFMs, these

results do not support a perseveration strategy. If EFMs were using a perseveration strategy, than the perseveration scores should be significantly greater than chance performance. However, the perseveration performance scores for the EFMs were not significantly greater than chance, $t(3)= 0.13$, $p= .904$. Thus, the poor performance scores of the EFMs and LFMs are not consistent with the perseveration behavior strategy. Like the control males, the mean actual percent score for the EFMs (M=.48, SD=.10) (and LFMs) did not significantly differ from the perseveration percent score (M= .45, SD= .04), $t(3)= 0.48$, $p= .664$. Since the perseveration percent scores did not decline across trials for the EFMs, perseveration behavior may be evident in the first five visits of each trial. However, the performance scores of the EFMs do not suggest perseveration behavior was maintained throughout the trial, indicating another search strategy was used in addition to perseveration behavior.

Working memory errors were more common in the Marker task. The mean frequency of working memory errors per Marker task trial (0.33 ± 0.12 SEM) and the mean proportion of visits that were working memory errors (0.35 ± 0.01 SEM) did not differ between control males and EFMs, $U= 8.00$, $p= .104$. Seven subjects (47%) never made a working memory error during the Marker task. Of the total 20 working memory errors made in the Marker task trials, 10 of them were made in the first Marker task trial and all were to previously correct goal boxes. Four subjects (27%) made working memory errors after the first marker trial.

Relationship Between Testosterone and Performance.

There was a significant positive correlation for both performance and percent scores to testosterone levels in control males on the DCA task (Table 4). In contrast,

there was a strong negative correlation for control males between testosterone and performance and percent scores during the Spatial task. However, there was no significant correlation for control males between testosterone and performance or percent scores on the Marker task. There were also no significant correlations between testosterone and perseveration performance score, $r(8) = .37$, $p = .370$, and testosterone and perseveration percent score, $r(8) = .15$, $p = .732$, for control males on the Marker task. Testosterone was not related to performance on the DCA task or Spatial task in EFMs. Conversely, in EFMs, there was a trend for a positive correlation between testosterone and performance score on the Marker task. On the DCA task, LFMs had no significant correlations with testosterone and either performance measure. Additional correlations were not possible for LFMs due to the small number of data points.

Testosterone levels ranged from 1.41- 24.62 ng/ml. There were significant differences in mean testosterone levels during the breeding season between EFMs, LFMs, and control males, $F(2, 75) = 3.69$, $p = .03$. Tukey tests showed LFMs had significantly higher mean testosterone levels ($M = 15.10$ ng/ml ± 1.59 SEM) than control males ($M = 10.47$ ng/ml ± 0.85 SEM, $p = .041$, and EFMs ($M = 9.71$ ng/ml ± 1.40 SEM), $p = .036$. However, there was no significant difference in testosterone levels between control males and EFMs, $p = .882$. Mean testosterone levels were significantly higher during the breeding season ($M = 10.55$ ng/ml ± 1.33 SEM) than during the nonbreeding season ($M = 3.14$ ng/ml ± 0.62 SEM), $t(14) = 5.53$, $p < .001$.

Summary.

There were no significant prenatal treatment differences on the Dual Cue Acquisition task. Performance on the Spatial task did not differ between control males

and EFMs. However, control males showed a trend for a significant decline in percent score on the first spatial trial, which was not due to a higher percent score on the previous trial. There was no significant decline in percent score on the first Spatial task trial in EFMs. Although all males showed a significant decline in performance on the Marker task, control males performed significantly above chance on the Marker task, while EFMs performed at chance levels. There was a trend for higher performance scores in control males across the Marker task, while percent scores did not differ between control males and EFMs. The decline in performance scores on the Marker task are not the result of perseveration behavior for control males or EFMs, though perseveration behavior could explain the decline in percent scores. In control males, testosterone was positively correlated to performance when consistent spatial information and landmarks were available and negatively correlated to performance when local landmarks were removed. Performance was not related to testosterone levels on the DCA or Spatial tasks in EFMs, but there was a trend for a positive relationship between testosterone and percent score on the Marker task.

Seasonal Differences in Performance

Dual Cue Acquisition.

The number of trials to reach criterion for control males was greater during the nonbreeding season (M= 16.67, SD= 4.74) than the breeding season (M= 11.56, SD= 6.31), t(8)= 4.05, p=.004. In contrast to control males, EFMs required a comparable number of trials to reach criterion for the nonbreeding season (M= 21.50, SD= 5.00) and the breeding season (M= 13.50, SD= 3.51), t(3)= 2.12, p= .118. LFMs showed a decline

similar to control males from the nonbreeding season (*M*= 18.50, *SD*= 7.78) to the breeding season (*M*= 9.00, *SD*= 4.24).

The average number of visits did not significantly differ between the two seasons for control males (Nonbreeding: *M*= 6.39, *SD*= 1.56; Breeding Season: *M*= 6.27, *SD*= 1.63), *t*(8)= 0.23, *p*= .821. Similar to control males, EFMs had an equal number of visits per DCA trial for the nonbreeding season (*M*= 6.42, *SD*= 2.25) and the breeding season (*M*= 7.48, *SD*= 0.62), *t*(3)= -0.74, *p*= .512. The mean number of visits per DCA trial by LFMs for the nonbreeding season (*M*= 5.06, *SD*= 3.08) and breeding season (*M*= 6.46, *SD*= 0.30) were similar to both control males and EFMs.

The best performance score on the DCA task did not differ between the nonbreeding season (*M*= 4.67, *SD*= 0.76) and the breeding season (*M*= 4.41, *SD*= 0.70) for control males, *t*(8)= 0.75, *p*= .477. EFMs also showed no difference in best DCA performance score between the nonbreeding season (*M*= 4.55, *SD*= 0.72) and the breeding season (*M*= 4.60, *SD*= 0.47), *t*(3)= -0.10, *p*= .929. The best DCA performance score in the nonbreeding season (*M*= 3.67, *SD*= 1.90) and the breeding season (*M*= 3.42, *SD*= 0.59) of LFMs was lower, but similar to both control males and EFMs.

Performance scores on the first four DCA trials were significantly higher during the breeding season than the nonbreeding season for both control males, *t*(8)= 3.51, *p*= .008, and EFMs, *t*(3)= 3.87, *p*= .03. A similar difference in performance score on the first four DCA trials was seen in LFMs (Table 5). Percent scores on the first four trials of control males were significantly greater during the breeding season than the nonbreeding season, *t*(8)= 3.52, *p*= .008. A similar trend with percent scores being greater during the

breeding season was found for EFMs, $t(3)= 2.42$, $p= .094$, with LFMs having comparable percent scores (Table 5).

Performance scores on the last four DCA trials did not differ between seasons for control males, $t(8)= 0.60$, $p= .566$, or EFMs, $t(3)= -0.06$, $p= .960$. LFMs had a larger difference between seasons than control males and EFMs, with higher performance scores during the breeding season (Table 6). Like performance scores, percent scores on the last four DCA trials were comparable between the breeding and nonbreeding seasons for control males, $t(8)= 1.51$, $p= .169$, and EFMs, $t(3)= 1.77$, $p= .174$. LFMs also had similar percent scores between seasons (Table 6).

The frequency of working memory errors per DCA trial did not significantly differ between the two seasons for control males, $t(8)= .76$, $p= .467$, or EFMs, $t(3)= 0.47$, $p= .671$. The occurrence of working memory errors per DCA trial by LFMs was similar to both control males and EFMs (Table 7). In control males, there was no difference in the percent of DCA visits that were WMEs between the two seasons, $t(8)= 1.03$, $p= .332$. The percent of DCA visits that were WMEs also did not differ for EFMs between seasons, $t(3)= 0.85$, $p= .459$, and LFMs showed similar results (Table 7).

Spatial task.

Performance scores on the four trials prior to the Spatial task did not differ between the nonbreeding ($M= 2.72$, $SD= 0.51$) and the breeding season ($M= 2.81$, $SD= 0.82$) for control males, $t(8)= -0.27$, $p= .794$. In EFMs, there was no significant difference in performance score for the four trials before the Spatial task for the nonbreeding season ($M= 3.40$, $SD= 0.65$) and the breeding season ($M= 2.59$, $SD= 1.27$), $t(3)= .93$, $p= .420$. Performance scores of LFMs during the nonbreeding season ($M=$

2.25, *SD*= 0.87) and the breeding season (*M*= 2.72, *SD*= 0.41) on the four trials prior to the Spatial task resembled performance scores of control males. Unlike performance scores for control males, percent scores on the four trials prior to the Spatial task showed a trend of being greater during the nonbreeding season (*M*=.71, *SD*=.08) than the breeding season (*M*= .64, *SD*= .07), *t*(8)= 2.14, *p*= .064, *d*= 0.93. However, percent scores on the four trials prior to the Spatial task did not differ between the nonbreeding season (*M*= .72, *SD*= .05) than the breeding season (*M*= .59, *SD*= .13) for EFMs, *t*(3)= 1.75, *p*= .178, *d*= 1.29. Mean percent scores of LFMs between the nonbreeding season (*M*= .74, *SD*= .20) and breeding season (*M*= .63, *SD*= 0.04) were more comparable to the means of control males.

Mean performance score on the Spatial task did not differ between the nonbreeding season and breeding season for control males, *t*(8)= 0.61, *p*= .559, or EFMs, *t*(3)= -0.16, *p*= .884. LFMs also showed comparable performance scores on the Spatial task between seasons (Figure 9a). The mean percent score on the Spatial task of control males was greater during the nonbreeding season than the breeding season, *t*(8)= 3.72, *p*= .006, *d*= 1.16, and a similar trend existed for EFMs, *t*(3)= 2.99, *p*= .058, *d*= 1.12. There was a smaller difference in percent score between seasons for LFMs (Figure 9b).

On each trial of the Spatial task, performance scores did not differ between seasons within each treatment group except for the performance scores of control males on the third spatial trial. Control males had greater performance scores on the third trial during the nonbreeding season than the breeding season (Table 8). Percent scores on the first spatial trial did not differ between seasons for each prenatal treatment group. However, the percent scores during the nonbreeding season were greater for the second

and third spatial trial for control males and there was a trend for greater percent scores during the nonbreeding season on the fourth trial for EFMs (Table 9).

Marker task.

Performance scores of control males on the four trials prior to the Marker task did not differ between nonbreeding season (M= 3.70, SD= 0.51) and the breeding season (M= 3.52, SD= 1.12), $t(8)$= 0.39, p= .711. The same result was found for EFMs between the nonbreeding season (M= 3.26, SD= 1.39) and the breeding season (M= 3.44, SD= 1.06), $t(3)$= -0.15, p= .890. There was a greater difference in performance scores on the four trials prior to the Marker task between the nonbreeding season (M= 2.50, SD= 0.11) and the breeding season (M= 3.32, SD= 0.74) in LFMs. In contrast to performance scores, the percent scores of control males on the four trials prior to the Marker task were significantly higher in the nonbreeding season (M= .85, SD= .10) than the breeding season (M= .69, SD= .11), $t(8)$= 2.78, p= .024. However, like performance scores, percent scores of EFMs on the four trials prior to the Marker task did not differ between the nonbreeding season (M= .82, SD= .05) and the breeding season (M= .70, SD= .11), $t(3)$= 1.91, p= .152. There was less difference in percent scores on the four trials prior to the Marker task between the nonbreeding season (M= .69, SD=.19) and breeding season (M= .68, SD=.04) for LFMs, in comparison to control males and EFMs.

The mean performance score of control males on the Marker task was significantly higher during the breeding season (M= 1.48, SD= 0.86) than the nonbreeding season (M= -0.63, SD= 0.67), $t(8)$= -6.75, p< .001 (Figure 10a). In contrast to control males, there was no difference in performance score on the Marker task between the nonbreeding season (M= 0.15, SD= 0.58) and the breeding season (M= 0.55,

SD= 0.74) for EFMs, *t*(3)= -0.90, *p*= .433. The difference in performance scores on the Marker task between the nonbreeding season (*M*= -0.53, *SD*= 0.10) and the breeding season (*M*= 0.55, *SD*= 0.74) for LFMs was greater than EFMs, but less than control males.

As in performance scores, percent scores of control males on the Marker task were greater during the breeding season (*M*= .46, *SD*= .09) than the nonbreeding season (*M*= .36, *SD*= .07), *t*(8)= -2.48, *p*= .038 (Figure 10b). Also similar to performance scores, EFMs showed no difference in percent scores on the Marker task between the nonbreeding season (*M*= .41, *SD*= .06) and the breeding season (*M*= .48, *SD*= .10), *t*(3)= -1.13, *p*= .340. In LFMs, the change in percent scores between the nonbreeding season (*M*= .374, *SD*= .04) and the breeding season (*M*= .45, *SD*= .14) was similar to the change in percent scores of control males.

Looking at each trial individually within treatments, performance scores were greater on the first three trials of the Marker task during the breeding season and there was no difference between seasons on the fourth trial. Performance scores of EFMs did not differ on any trial except the second marker trial, which was higher during the breeding season (Table 10). Percent scores of control males were greater during the breeding season on the second and third trials of the Marker task, but scores were greater during the nonbreeding season on the fourth marker trial. In EFMs, the percent score on the second marker trial was greater during the breeding season, while no other marker trial differed between seasons (Table 11).

Summary.

Performance during the first four trials of the Dual Cue Acquisition task was higher during the breeding season for all males, but performance on the last four trials of the task did not differ between seasons for control males or EFMs. Performance scores on the Spatial task did not differ between seasons for control males or EFMs. However, percent scores on the Spatial task were greater during the nonbreeding season for control males and EFMs showed a similar trend. Performance on the Marker task were significantly higher during the breeding season for control males, while EFMs showed no seasonal differences in performance on the Marker task.

Sex Differences in Spatial Memory

Control males' performance measures during the breeding season were compared to the performance of control females during the breeding season to examine sex differences in performance on the three tasks (female data obtained from R. Herman, personal communication, October, 2005). On the last four DCA trials, when both consistent spatial information and local landmarks were present, performance scores of control males (M= 2.75, SD= 0.88) did not differ from control females (M= 3.37, SD= 1.19), $t(13)$= -1.18, p= .261. In contrast to performance scores on the last four trials of the DCA task, control females (M= .76, SD= .08) had higher percent scores than control males (M= .64, SD= .07), $t(13)$= -2.87, p= .013, d= 1.49. Performance scores on the Spatial task for control males (M= 2.90, SD= 1.26) and control females (M= 3.41, SD= 1.51) during the breeding season did not differ, $t(13)$= 0.72, p= .486. In addition, mean performance score on the four trials prior to the Spatial task did not differ between control males (M= 2.81, SD= 0.82) and control females (M= 2.89, SD= 1.07), $t(13)$=

0.16, p= .874. Control females (M= .75, SD= .12) had greater percent scores on the Spatial task than control males (M= .58, SD= .15), t(13)= 2.43, p= .03, d= 1.30. However, the percent scores on the four trials prior to the Spatial task did not differ between control males (M= .64, SD= .07) and control females (M= .70, SD= .13), t(13)= 1.20, p= .253. Unlike the DCA and Spatial tasks, control males (M= 1.48, SD= 0.86) showed a trend for greater performance scores on the Marker task in comparison to control females (M= 0.54, SD= 1.07), t(13)= 1.87, p= .084, d= 0.96. The trend for greater performance scores in males on the Marker task was not due to greater performance scores on the previous four trials since control males (M= 3.52, SD= 1.12) and control females (M= 3.55, SD= 1.46) had comparable performance scores on the four trials prior to the Marker task, t(13)= 0.05, p= .958. Similar to performance scores on the Marker task, control males (M= .46, SD= .09) showed a trend for greater percent scores on the Marker task during the breeding season than control females (M= .38, SD= .08), t(13)= 1.78, p= .098, d= 0.96. However, on the four trials prior to the Marker task females showed a trend for greater percent scores (M= .82, SD= .12) in comparison to males (M= .69, SD= .11), t(13)= 2.15, p= .051, d= 1.12. Thus, the trend for greater male percent scores was limited to the Marker task and was not present on the previous four trials.

Summary.

Performance scores on the Dual Cue Acquisition and Spatial tasks did not differ between males and females. However, females had higher percent scores on these two tasks and these effects were large. Males showed a trend for greater performance and percent scores on the Marker task than females and these effects were also large.

Discussion

Sex Differences in Spatial Memory

When spatial information remained constant and local landmarks also indicated the stocked locations, the performance score results were as expected and males and females did not differ on the last four Dual Cue Acquisition trials. Females, however, had greater percent scores and visited more correct goal boxes in the first five visits of each of the last four DCA trials than did males. Herman (2006) found no sex difference in performance or percent scores when global and local cues were provided. The difference in percent scores between males and females during the breeding season may be an effect of a repeated testing of the males. Since the males completing the task in the breeding season were previously exposed to the task and fewer trials were required to reach criterion, it is likely that control males had steeper learning curves during the breeding season. The criterion for the DCA task was two consecutive days of a 0.8 or higher percent score. Thus, a steeper learning curve during the DCA task would result in a lower percent score over the last four trials. A greater percent score on the last four trials would suggest more trials were required to reach criterion and a smaller sloped learning curve. However, the effect of steeper learning curves in male performance only affected initial goal box visits during the breeding season. Despite the superior initial performance by females on each trial, males and females were still visiting a comparable number of correct and incorrect goal boxes each trial since performance scores did not differ between sexes. It is possible a sex difference was only observed in percent score because the criterion was based on percent scores. If criterion incorporated a minimum performance level for both performance and percent scores, it is possible a sex difference

would have been observed in performance score as well as percent score. Therefore, it seems probable the observed female advantage on percent scores of the last four DCA trials found in this study is an effect of repeated exposure to the task by males and is not a true sex difference in performance. Although repeated exposure of males to the task is a concern, the effects of repeated testing of the males during the breeding season only seemingly affected initial performance and did not affect overall performance on each of the last four DCA trials.

Consistent with the present results, there was no sex difference in performance on a spatial memory task in humans when both global and local cues were provided (Leplow, Holl, Zeng, & Mehdorn, 2000). In contrast, male rats had greater performance measures than females on a radial-arm maze when both global and local cues were provided (Seymoure et al., 1996). Since both humans and nonhuman primates rely heavily on visual sensory information, it is not surprising that the lack of sex differences in performance were similar between species. However, rodents may use multiple sensory modalities to complete a task and the use of different sensory information may differ between sexes. Therefore, the male advantage observed in rodent spatial memory may result from focusing on visual information when providing other environmental cues. The conflicting sex differences observed between rodents and primates when multiple cues are present reflect unique sensory adaptations within species.

Contrary to the hypothesis that males would exceed female performance when unvarying spatial information was provided in the absence of local landmarks, the removal of local landmarks produced no difference in performance scores between males and females, though females had higher percent scores than males. As in the case of the

last four trials of the Dual Cue Acquisition task, females visited more correct goal boxes in the first five visits, but ultimately visited a comparable number of correct and incorrect goal boxes in comparison to males. The lack of a sex difference in performance scores on the Spatial task is consistent with previous results in rhesus macaques (Herman & Wallen, 2007). However, Herman and Wallen (2007) found no sex difference in percent scores when control females were tested during the breeding season and control males were tested during the nonbreeding season. Since neither performance nor percent scores on the four trials prior to the Spatial task differed between males and females, the lower percent scores in males cannot be attributed to a steeper learning curve or an effect of repeated testing. The lower percent scores on the Spatial task by control males in the breeding season may also be due to a decrease in motivation to complete the task. However, the lack of a sex difference in performance scores on the Spatial task shows males and females were visiting a comparable number of correct and incorrect goal boxes. Thus, a decrease in motivation in males during the breeding season does not appear to explain the lower percent scores by males on the Spatial task. The female advantage present in percent scores on the Spatial task was a large effect and suggests testosterone in males may have a negative effect on the initial performance of each trial when only spatial information is available.

The failure to find a sex difference in performance scores on the Spatial task is consistent with findings in rodents that show no sex differences in performance on a radial-arm maze when local cues are removed (Williams, Barnett, & Meck, 1990). However, the greater initial performance on each trial in females is not supported by the findings of Williams and colleagues. In contrast to the results of this study, male rats

performed better and made fewer working and reference memory errors during a radial arm maze when only distal cues were present (Gresack & Frick, 2003). Gresack & Frick (2003) also found males made fewer initial reference memory errors and initially visited more correct locations than females, which is also conflicting with the results of this study. The inconsistencies within the rodent data make it difficult to compare the results of the Spatial task in the current study, but again the species comparisons suggest the mechanisms underlying sex differences in spatial memory in nonhuman primates may differ from that of rodents.

The Spatial task results of the current study do not support previous findings in humans. Sex differences in humans show a male advantage when spatial information must be used and landmark cues are not provided (Saucier et al., 2002b; Saucier et al., 2003). Since factors such as socio-economic status and language have been shown to affect spatial memory performance producing a male advantage, the sex differences observed in humans may be complicated by multiple complex cognitive processes (Levine et al., 2005; Saucier et al, 2003). Thus, the discrepancies in results between the nonhuman and human data may be explained by more complex cognitive abilities mediating spatial memory in humans. The differences could also reflect that in human studies subjects relied upon either spatial or local cue behavior, whereas in our study the monkeys first learned the tasks with both cues and one type of cue was removed. Possibly compensating for a missing cue is different than relying on one cue or the other.

It was expected that females would exceed male performance when the food locations changed and local landmarks were the only reliable cue present. However, this hypothesis was not supported. Altering the location of the food and providing a

corresponding landmark resulted in a trend for higher performance and percent scores in control males. Although these results were not significant and only indicative of a trend, the effect sizes for both results were large. Therefore, it seems likely the failure to find significant sex differences in performance and percent scores on the Marker task was due to small sample sizes in each group. The results of this study do not support the previous finding that females had higher performance measures than males when required to use local cues (Herman & Wallen, 2007). The improvement in male performance during the breeding season reversed the original sex difference observed and suggests testosterone enhances performance on the Marker task. Since this was the second time that the males were tested on these tasks, we cannot rule out that this difference in performance reflects more experience with the task.

Research in humans has shown males are able to use either spatial information or landmarks to navigate their environment, while female dependence on local cues is coupled with an inability or a decrease in performance when only spatial information is provided (Saucier et al., 2002b). In support of the human findings, performance in female rodents was not impaired when landmarks were provided but the geometry of the room was modified. However, unlike humans, male rodents showed a decline in performance on this task suggesting males were more reliant on the spatial information while females were able to use either the spatial information or the landmark cues independently (Williams et al., 1990). The results of this study have shown a trend for a male advantage when spatial information is variable and local landmarks are the only reliable cue, which is not supported by previous research in humans and rodents. Considering the results of both the Spatial and Marker tasks, male macaques are able to

use both global and local spatial information independently, similar to the results in humans. However, in contrast to the human data, female nonhuman primates appear to use global spatial cues rather than local spatial cues. Therefore, it appears the sex differences in strategies used to complete spatial memory tasks differs between species and the presence of sex differences in strategies are dependent on the conditions of the task.

Activational Effects of Testosterone on Spatial Memory

In the Dual Cue Acquisition task, the performance and percent scores on the first four trials were greater during the breeding season for control males and EFMs, with LFMs showing a similar trend. In addition, control males required fewer trials to reach criterion during the breeding season. While EFMs had no significant difference in trials to criterion between the two seasons, the mean number of trials for the two seasons resembled the decline shown in control males. Thus, the lack of an effect for fewer trials to reach criterion in EFMs may be the result of the small sample size. LFMs also showed a comparable decline in trials to criterion from the nonbreeding season to the breeding season. Fewer trials to criterion in conjunction with the higher performance and percent scores on the first four DCA trials could indicate that males were showing a practice effect due to repeated testing. Although there was evidence for practice effects on the initial trials of the DCA task, there was no difference in the mean number of visits per trial or the best performance score on the task for both control males and EFMs, with LFMs showing similar results. In addition, there was no difference in performance or percent scores between seasons for control males and EFMs on the last four DCA trials. There was a larger seasonal difference in performance scores for the two LFMs with

greater performance scores during the breeding season. However, percent scores of LFMs on the last four DCA trials were comparable to both control males and EFMs. Thus, any practice effects apparent in the initial DCA trials did not result in fewer visits per trial or better performance in later DCA trials. These results are consistent with the findings in voles that show when both consistent spatial information and local cues were provided, males grouped by either high or low testosterone levels did not differ in performance (Galea et al., 1995).

Considering the males previous exposure to the task, it is difficult to determine whether testosterone enhanced the acquisition of the task during the breeding season. However, the degree of correlation between testosterone and performance measures should indicate whether testosterone had any effect on performance. While there were no significant correlations between testosterone and performance measures for EFMs or LFMs on the Dual Cue Acquisition task, there were significant positive correlations between testosterone and both performance measures in control males. Thus, higher testosterone levels were related to higher performance and percent scores, but only for animals that did not receive prenatal flutamide, an androgen receptor blocker. In contrast to these results, Herman (2006) found no correlations between performance measures and testosterone.

Contrary to the hypothesis, testosterone did not enhance performance on the Spatial task during the breeding season. Performance scores on the Spatial task did not differ between seasons for control males or EFMs and LFMs showed a similar result. Similarly, the performance scores on the four trials prior to the Spatial task did not differ between seasons within each prenatal treatment group. In addition, control males, EFMs,

and LFMs showed no difference in performance scores between the four trials prior to the Spatial task and the Spatial task during the breeding season. These results suggest there was no change in performance score on the Spatial task and increased levels of testosterone in the breeding season did not affect performance scores. However, there was a strong negative correlation between testosterone and performance score on the Spatial task in control males that was not present in EFMs. In contrast to these results, Herman (2006) found no relationship between testosterone and performance scores on the Spatial task. However, previous research in humans using a virtual Morris water maze also found a negative correlation between testosterone and performance when only reliable distal cues were available to locate a hidden platform (Driscoll et al., 2005). Though the results were not significant, the direction of the results for EFMs appeared to be positive, rather than negative as in control males. Although an overall increase in testosterone in the breeding season produced no differences in Spatial task performance scores, variance in circulating levels of testosterone interacted with prenatal androgen exposure and resulted in a negative relationship to performance score that was only observed in control males.

In contrast to performance scores, percent scores on the Spatial task were greater during the nonbreeding season than the breeding season in control males and EFMs showed a similar trend. There was a smaller, but similar seasonal difference in percent scores for LFMs. Since the effect sizes were large for both control males and EFMs, it is likely the difference in percent scores for EFMs did not reach significance due to the small sample size. The lower percent scores of males during the breeding season suggest previous exposure to the task during the nonbreeding season did not enhance

performance and may again suggest a decrease in motivation to complete the task during the breeding season. However, there was no difference in performance scores on the Spatial task, which means males visited a comparable number of incorrect and correct goal boxes during the breeding and nonbreeding seasons. Thus, it does not seem probable the lower percent scores on the Spatial task during the breeding season are due to a decrease in motivation to complete the task. Therefore, the results suggest testosterone has a negative effect on initial performance on the Spatial task, which does not extend to overall performance on each trial of the Spatial task.

Previous research has shown no relationship between testosterone and percent scores on the Spatial task (Herman, 2006). However, the percent scores on the four trials prior to the Spatial task were higher during the nonbreeding season for control males. While there was no statistical difference in percent scores on the four trials prior to the Spatial task between seasons for EFMs, the mean percent scores reveal a difference similar to control males and produced a large effect size suggesting the small sample size reduced the power to find significant results. In addition, there was no difference in percent scores between the four trials prior to the Spatial task and the Spatial task for both control males and EFMs within the breeding season. Therefore, the lower percent scores on the Spatial task during the breeding season did not result from a decline in performance, but rather possibly maintaining lower percent scores that were observed on the previous four trials. Thus, testosterone may have a negative effect on initial performance of each trial, but this effect does not appear to be limited to the Spatial task. Testosterone levels were also negatively correlated to percent scores in control males, which further supports the conclusion that testosterone has a negative effect on initial

performance on the spatial trial. The lack of a relationship between testosterone and percent scores in EFMs may be due to the small sample size or a difference in activational effects of testosterone based on prenatal hormone treatment.

Increased testosterone during the breeding season improved performance on the Marker task in control males, but not EFMs. Performance and percent scores were significantly higher on the Marker task during the breeding season in control males, while EFMs had comparable performance and percent scores on the Marker task between seasons. The seasonal difference observed in LFMs was greater than EFMs but less than control males and showed greater performance and percent scores during the breeding season. These results contradict previous work in rodents which found no difference in performance on a water maze task between intact and castrated males when spatial location of the platform varied, but local cues were provided (Sandstrom et al., 2006). There was no direct relationship between testosterone and either performance measure for control males on the Marker task, which supports previous findings in nonhuman primates (Herman, 2006). However, EFMs had a trend for a positive correlation, producing a large effect size, between testosterone and performance score on the Marker task. This correlation is surprising since there was no difference between the seasons in performance score for EFMs.

There were no seasonal differences in performance scores on the four trials prior to the Marker task, either the last four DCA trials or the repeated DCA trials, for both control males and EFMs. LFMs had a larger difference in performance scores in comparison to control males and EFMs with performance scores being higher during the breeding season. Thus, the improvement in performance score on the Marker task during

the breeding season by control males was not related to better performance on the previous trials. The percent scores on the four trials prior to the Marker task were significantly higher during the nonbreeding season for control males, while there was no seasonal difference in these scores for EFMs or LFMs. Thus, the greater percent scores on the Marker task during the breeding season for control males were also not the result of greater percent scores on the previous four trials. Since the males were previously exposed to the task during the nonbreeding season, it is possible the improvement in performance by control males is an effect of prior experience. However, if prior experience was related to performance, than we would expect all males to improve on the task and not just control males. Therefore, increased testosterone during the breeding season seems to improve performance but does not directly correspond to performance measures for control males. In contrast, increased testosterone in the breeding season produced no seasonal difference in EFMs, though performance scores were positively associated with circulating levels of testosterone during the breeding season. In comparison to control males and EFMs, LFMs performance for the two seasons was more comparable to EFMs, which suggests prenatal flutamide treatment inhibits the positive activational effects of testosterone on the Marker task.

It is difficult to compare the activational effects of testosterone in this study to human research since most research investigating the role of testosterone in humans has focused on spatial memory tasks such as mental rotation or block design tasks (Davison & Susman, 2001; Thilers et al., 2006; Wolf & Kirschbaum, 2002). Cherrier and colleagues (2001, 2005) found testosterone increased performance in elderly men on a navigational task when both consistent spatial information and local landmarks were

provided independently. However, the performance on the two types of tasks was combined and testosterone was shown to improve overall performance (Cherrier et al., 2001; Cherrier et al., 2005). Thus, the impact testosterone has on performance of each task type cannot be determined.

In summary, seasonal changes in testosterone do not appear to affect performance when both consistent spatial information and local landmarks are provided. However, circulating levels of testosterone were positively associated with performance for control males. Overall performance on a Spatial task when local landmarks are removed is not affected by an increase in testosterone during the breeding season, though circulating levels of testosterone do appear to impair initial performance on each trial in control males. The observed sex difference of higher percent scores in control females in comparison to control males supports the conclusion that testosterone impairs initial performance. When local landmarks are the only reliable cue, seasonal fluctuations in testosterone positively affect performance measures in control males but testosterone levels do not directly correspond to performance measures. In support of this finding, the observed sex differences showing higher performance and percent scores in control males also suggest testosterone enhances performance on the Marker task in control males. In contrast, variation in circulating testosterone levels is positively related to performance scores in EFMs, but seasonal changes in testosterone do not affect performance on the Marker task.

Organizational Effects of Testosterone on Spatial Memory

Organizational differences in testosterone were assessed by comparing performance of control males to males receiving flutamide, an androgen receptor blocker,

both early (EFMs) and late (LFMs) in gestation. Since there were only two LFMs, the results of their performance were discussed qualitatively in comparison to control males and EFMs. During the breeding season, there were no significant differences in the number of trials to reach criterion in the Dual Cue Acquisition task between control males and EFMs. In addition, there were no significant differences between control males and EFMs in performance or percent scores on the first four or last four DCA trials. LFMs required fewer trials to reach criterion than both control males and EFMs, though the required number of trials was closer to the mean of control males. LFMs had performance scores more similar to EFMs on the first four DCA trials and percent scores lower than both control males and EFMs. Performance scores of LFMs on the last four DCA trials resembled performance scores of control males, while percent scores were similar to both control males and EFMs. Working memory errors during the DCA task were rare, but mean working memory error per trial and the mean number of visits that were a working memory error did not differ between control males and EFMs. Thus, there were no differences in performance on the DCA task during the breeding season related to prenatal hormone treatment. The results of this study are consistent with previous findings in rhesus macaques which found no differences in performance when multiple cues were available based on prenatal androgen exposure (Herman, 2006).

Testosterone levels were significantly higher in LFMs in comparison to control males and EFMs, while there was no difference in testosterone levels between control males and EFMs. Despite the differences in testosterone levels, there were no performance differences on DCA task based on prenatal treatment. However, testosterone was positively correlated to performance and percent scores on the DCA task

for control males, while no correlation existed for EFMs or LFMs. Therefore, blocking androgen receptors either early or late in gestation results in organizational differences present in adulthood that affect the relationship between testosterone and performance measures on spatial memory tasks.

On the Spatial task, there were no significant prenatal treatment differences except for the change in percent score on the first Spatial task trial. Control males showed a trend for a decrease in percent scores on the first spatial trial which was a moderate effect, while EFMs showed no change in percent score between the previous trial and the first Spatial task trial. LFMs showed a decline in percent scores on the first Spatial task trial similar to control males. These results suggest the control males and LFMs were more reliant on the markers during the DCA. When the markers were removed during the Spatial task, these subjects were impaired for the first trial, but then were able to shift strategies and rely on the spatial location of the goal box rather than the identifying marker. The lack of impairment in the first trial for the EFMs, which is similar to that reported for females, suggests these subjects were less reliant on the marker and more reliant on the spatial location during the DCA and thus, a shift in strategies was unnecessary when the markers were removed. Prenatal flutamide exposure, at least early in gestation, appears to block the impairment on initial performance on the first trial when local landmarks are removed.

The failure to find significant differences in performance on the Spatial task between control males and EFMs is consistent with the previous study in rhesus macaques (Herman, 2006). Interestingly, the only prenatal treatment difference observed in males on the previous study was the initial drop in performance on the first Spatial task

trial. In contrast to the results of this study, all treatment groups showed a significant drop in performance scores. EFMs showed a significant decline in percent score on the first Spatial task trial, while control males exhibited a trend for a decline in percent scores. LFMs showed a decline in percent score similar to EFMs (Herman, 2006). While there was no difference in performance score on the first trial for control males or EFMs during the breeding season, the decline in percent scores was only observed for control males and LFMs. Therefore, the positive effects of early prenatal flutamide exposure apparent on the first Spatial task trial are only present during the breeding season, when circulating levels of testosterone are increased.

As in the DCA task, there were significant correlations between testosterone and performance and percent scores for control males, but not EFMs. However, there were negative correlations between testosterone and performance measures on the Spatial task for control males. Although most performance measures on the Spatial task did not differ between prenatal treatments, exposure to prenatal androgens resulted in a negative relationship between circulating testosterone levels and performance scores when local landmarks were not available. However, blocking prenatal androgen exposure, at least early in gestation, eliminates the relationship between circulating testosterone and performance on the Spatial task.

Performance on the Marker task resulted in the most striking difference between prenatal treatment groups. Control males performed significantly above chance, while EFMs performed at chance level and there was a trend for a treatment difference in performance scores across the Marker task. There was no prenatal difference in performance scores on the first trial, which was expected since males could not anticipate

the change in task. However, EFMs still performed at chance level when the first trial was excluded from analysis. LFMs had performance and percent scores comparable to those of EFMs. These results suggest the control males learned the significance of the marker during the DCA and were able to shift strategies to rely on the location of the marker rather than the spatial location when visiting goal boxes. Since the EFMs did not significantly differ from chance performance, it is possible the EFMs did not use the marker in their search strategy during DCA, and therefore were unable to perform well during the Marker task using the previous spatial location strategy. Therefore, exposure to prenatal androgens seems to enhance spatial memory when consistent spatial information is not available and the use of local landmarks is required. Blocking prenatal androgens in gestation appears to affect spatial strategies by impairing the ability to use local landmarks to complete a spatial memory task. Percent scores did not differ between control males and EFMs, which suggests initial performance on each Marker trial was not affected by prenatal hormone treatment.

The results from the nonbreeding season showed control males performed significantly worse than chance on the Marker task, while EFMs performed at chance level. In addition, EFMs had significantly higher performance scores and a trend for higher percent scores on the Marker task. Performance and percent scores of LFMs on the Marker task were similar to control males' performance measures (Herman, 2006). The improvement of control males' performance scores during the breeding season reversed the direction of the prenatal treatment differences found in the previous study by Herman (2006). Although performance scores of control males showed a trend for being higher than EFMs, there was no difference in percent scores during the breeding season.

However, percent scores on the Marker task improved during the breeding season for control males, while there was no improvement in the breeding season for EFMs. In addition, LFMs had performance and percent scores comparable to EFMs. Thus, increases in testosterone do not affect performance in subjects receiving prenatal flutamide, while increases in testosterone improve performance in subjects exposed to normal levels of prenatal androgens.

On the Marker task, testosterone was not significantly related to performance for control males. However, testosterone showed a trend for a positive correlation to performance scores for EFMs and the effect size of this result was large. The results suggest activational effects of testosterone affect control males and EFMs differently and the variation in circulating testosterone levels can affect performance on the Marker task for EFMs, but not control males.

Since EFMs performed at chance level and performance did not improve across trials, it is possible EFMs were exhibiting perseveration behavior and failed to stop visiting previously correct goal boxes. Perseveration performance and percent scores did not decline across trials for EFMs, which is expected if a perseveration strategy was used. In addition, if a perseveration strategy was used, perseveration performance scores should be greater than chance performance. However, the perseveration performance score of EFMs did not differ from chance and did not differ from actual performance scores on the Marker task. Therefore, perseveration behavior cannot account for the lower performance scores observed in EFMs and LFMs had comparable perseveration scores. Perseveration behavior was also not a likely strategy used by control males throughout the trial as the actual Marker task performance scores were significantly higher than

perseveration performance scores. Since perseveration percent scores did not decline and did not differ from actual percent scores for both control males and EFMs, it is possible perseveration behavior was used initially on each trial. However, this strategy was not maintained throughout the trial for either control males or EFMs since perseveration performance scores were not consistent with a perseveration strategy.

Research investigating organization differences in testosterone in rodents and humans is limited and primarily focuses on increased testosterone levels in females during development (Roof & Havens, 1992; Isgor & Sengelaub, 1998; Berenbaum, 1995). However, one study found intact adult male rats exposed to prenatal flutamide performed significantly worse than control males on a radial arm maze (Lund & Lephart, 2001). The results of the Marker task in the current study support this finding in rodents. However, exposure to prenatal flutamide only impaired performance on the Marker task and did not impair performance on other tasks. Therefore, prenatal androgen levels can affect adult spatial memory performance, but the effects are dependent on the parameters of the Spatial task.

In summary, the results suggest control males were able to use both spatial information and local landmarks independently to complete the tasks. In contrast, EFMs seemed to rely only on spatial information and were unable to use local landmarks when the spatial information was inconsistent. Conclusive results regarding the LFMs are difficult, but it appears LFMs are more sensitive to changes in the task and the strategies used resemble those of EFMs.

Conclusion

Sex differences in spatial memory in rhesus macaques are affected by the levels of circulating gonadal hormones. When spatial information was consistent and local landmarks were removed, there was a female advantage on initial performance, but only in comparison to male performance during the breeding season. If local landmarks were available, but spatial information was unreliable, there was a female advantage on the task in comparison to male performance during the nonbreeding season. However, there was a male advantage observed on the task when males were tested during the breeding season. Thus, the improvement of the males during the breeding season when local cues were the only reliable information reversed the direction of the sex difference. Activational effects of testosterone were present, but varied depending on prenatal androgen exposure. There was a positive correlation between testosterone and performance when both reliable spatial information and landmarks were provided, but this relationship was only present in control males. When consistent spatial information was provided without local cues, there was a negative relationship between testosterone and performance for control males, but not EFMs. Blocking androgen receptors early in gestation eliminated the impairment on the first Spatial trial that was observed in control males. However, exposure to normal levels of prenatal androgens was necessary for the improvement on the Marker task during the breeding season. The results of this study demonstrate the importance of activational and organizational effects of testosterone on spatial memory and these effects are dependent on the strategies required to complete the task.

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Table 1
Calculations of Chance Scores

		Difference Score													Score by Chance
		-7	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	
Total Visits	**1**							0.583		0.417					**-0.1667**
	2						0.318		0.530		0.152				**-0.3333**
	3					0.159		0.477		0.318		0.045			**-0.5000**
	4				0.071		0.354		0.424		0.141		0.010		**-0.6667**
	5			0.027		0.221		0.442		0.265		0.044		0.001	**-0.8333**
	6		0.008		0.114		0.379		0.379		0.114		0.006		**-1.0051**
	7	0.001		0.044		0.265		0.442		0.221		0.019			**-1.1894**
	8		0.010		0.141		0.424		0.354		0.044				**-1.3864**
	9			0.045		0.318		0.477		0.088					**-1.5707**
	10				0.152		0.530		0.159						**-1.6667**
	11					0.417		0.265							**-1.5152**
	12						0.417								**-0.8333**

(taken from Herman, 2006)

Table 2
Mean Performance and Percent Scores on the Spatial and Marker tasks Based on Testing Order during the Breeding Season

	Spatial Task			
	Spatial Task First	Marker Task First	*t*	*p*
Mean Performance Score	3.00	2.71	0.48	.641
Mean Percent Score	.62	.54	1.04	.318
	Marker Task			
	Spatial Task First	Marker Task First	*t*	*p*
Mean Performance Score	1.13	1.12	0.20	.984
Mean Percent Score	.45	.47	0.39	.700

Table 3
Mean Performance and Mean Percent Scores on the First and Last Four Dual Cue Acquisition Trials for Male Rhesus Monkeys Tested during the Breeding Season by Prenatal Treatment

	First Four Trials		Last Four Trials	
	Performance Score			
	Mean	Standard Deviation	Mean	Standard Deviation
Control Males	1.49	0.95	2.75	0.83
Early Flutamide Males	1.12	0.20	3.23	0.69
Late Flutamide Males	1.24	1.20	2.61	0.26
	Percent Score			
	Mean	Standard Deviation	Mean	Standard Deviation
Control Males	.52	.11	.64	.07
Early Flutamide Males	.53	.07	.66	.05
Late Flutamide Males	.45	.07	.65	.00

Table 4
Pearson Correlations (one-tailed) of Testosterone and Performance or Percent Scores on the Three Tasks by Male Rhesus Monkeys Tested during the Breeding Season

	Control Males	
	Pearson Correlation	*p*
Dual Cue Acquisition		
Percent score	.50*	.012
Performance score	.45*	.024
Spatial task		
Percent score	-.75*	.017
Performance score	-.79*	.011
Marker task		
Percent score	.04	.460
Performance score	-.03	.476

	Early Flutamide Males	
	Pearson Correlation	*p*
Dual Cue Acquisition		
Percent score	-.09	.401
Performance score	-.31	.196
Spatial task		
Percent score	.81	.198
Performance score	.58	.302
Marker task		
Percent score	.49	.337
Performance score	.97**	.082

	Late Flutamide Males	
	Pearson Correlation	*p*
Dual Cue Acquisition		
Percent score	.312	.274
Performance score	.251	.318

Table 5
Seasonal Differences in Mean Performance and Mean Percent Scores of Male Rhesus Monkeys on the First Four Dual Cue Acquisition Trials by Prenatal Treatment

	Performance Score			
	Nonbreeding Season	Breeding Season	*t*	*p*
Control Males	-0.02	1.49	3.51	.008
Early Flutamide Males	-0.05	1.12	3.87	.030
Late Flutamide Males	0.36	1.24		
	Percent Score			
	Nonbreeding Season	Breeding Season	*t*	*p*
Control Males	.35	.52	3.52	.008
Early Flutamide Males	.43	.53	2.42	.094
Late Flutamide Males	.43	.45		

Table 6
Seasonal Differences in Mean Performance and Mean Percent Scores of Male Rhesus Monkeys on the Last Four Dual Cue Acquisition Trials by Prenatal Treatment

	Performance Score			
	Nonbreeding Season	Breeding Season	*t*	*p*
Control Males	4.45	4.10	0.59	.566
Early Flutamide Males	3.19	3.23	0.06	.960
Late Flutamide Males	2.50	3.42		
	Percent Score			
	Nonbreeding Season	Breeding Season	*t*	*p*
Control Males	.87	.78	1.51	.169
Early Flutamide Males	.74	.66	1.77	.174
Late Flutamide Males	.90	.80		

Table 7
Seasonal Differences in Working Memory Errors of Male Rhesus Monkeys by Prenatal Treatment

	Control Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(8)	*p*
Working Memory Errors per DCA Trial	0.16	0.22	0.12	0.24	0.76	.467
Percent of DCA visits that were Working Memory Errors	0.20	0.03	0.01	0.03	1.03	.332

	Early Flutamide Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(3)	*p*
Working Memory Errors per DCA Trial	0.16	0.14	0.14	0.06	0.47	.671
Percent of DCA visits that were Working Memory Errors	0.03	0.03	0.02	0.01	0.85	.459

	Late Flutamide Males			
	Nonbreeding		Breeding	
	Mean	Standard Deviation	Mean	Standard Deviation
Working Memory Errors per DCA Trial	0.06	0.03	0.00	0.00
Percent of DCA visits that were Working Memory Errors	0.01	0.003	0.00	0.00

Table 8
Seasonal Differences in Mean Performance Score of Male Rhesus Monkeys on the Four Spatial Trials by Prenatal Treatment

	Control Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(8)	*p*
Trial 1	2.32	1.00	2.97	1.40	-1.12	.296
Trial 2	3.10	1.50	2.80	1.61	0.60	.562
Trial 3	3.88	1.51	2.94	1.78	2.34	.047
Trial 4	2.96	1.68	2.71	1.47	0.14	.894

	Early Flutamide Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(3)	*p*
Trial 1	2.35	1.38	2.44	1.59	-0.07	.952
Trial 2	2.96	0.48	2.81	2.10	0.12	.911
Trial 3	2.56	1.26	3.63	1.16	-0.97	.405
Trial 4	2.86	1.44	2.56	1.26	0.30	.781

	Late Flutamide Males			
	Nonbreeding		Breeding	
	Mean	Standard Deviation	Mean	Standard Deviation
Trial 1	1.01	0.69	2.34	0.95
Trial 2	3.46	0.08	2.64	0.00
Trial 3	1.45	1.58	3.08	1.06
Trial 4	2.85	1.90	2.25	1.06

Table 9
Seasonal Differences in Mean Percent Score of Male Rhesus Monkeys on the Four Spatial Trials by Prenatal Treatment

	Control Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(8)	*p*
Trial 1	.67	.13	.64	.17	0.38	.716
Trial 2	.77	.15	.51	.23	2.31	.049
Trial 3	.80	.22	.62	.21	3.41	.009
Trial 4	.66	.28	.53	.25	1.01	.343

	Early Flutamide Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(3)	*p*
Trial 1	.66	.14	.55	.25	0.60	.591
Trial 2	.85	.19	.55	.34	1.73	.182
Trial 3	.74	.19	.70	.12	0.68	.547
Trial 4	.80	.23	.55	.25	2.61	.080

	Late Flutamide Males			
	Nonbreeding		Breeding	
	Mean	Standard Deviation	Mean	Standard Deviation
Trial 1	.54	.19	.60	.00
Trial 2	.80	.28	.60	.00
Trial 3	.55	.07	.60	.28
Trial 4	.74	.09	.50	.14

Table 10
Seasonal Differences in Mean Performance Scoreof Male Rhesus Monkeys on the Four Marker Trials by Prenatal Treatment

	Control Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(8)	*p* value
Trial 1	-1.51	2.03	0.63	2.32	-3.14	.014
Trial 2	-0.39	1.69	2.22	1.43	-3.78	.005
Trial 3	-0.15	1.20	2.27	1.50	-4.24	.003
Trial 4	-0.46	0.89	0.80	1.75	-1.80	.110

	Early Flutamide Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(3)	*p*
Trial 1	-0.11	2.51	0.36	1.79	-0.25	.817
Trial 2	-0.27	1.32	1.21	1.45	-12.39	.001
Trial 3	-0.01	1.22	0.57	0.87	-0.57	.606
Trial 4	0.99	1.86	0.04	1.69	1.12	.343

	Late Flutamide Males			
	Nonbreeding		Breeding	
	Mean	Standard Deviation	Mean	Standard Deviation
Trial 1	-0.42	0.12	-0.80	3.72
Trial 2	-0.99	2.14	2.09	1.30
Trial 3	-0.75	0.59	0.79	1.98
Trial 4	0.04	2.07	0.67	1.18

Table 11
Seasonal Differences in Mean Percent Score of Male Rhesus Monkeys on the FourMarker Trials by Prenatal Treatment

	Control Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(8)	*p*
Trial 1	.25	.17	.38	.19	-1.51	.169
Trial 2	.33	.17	.58	.16	-2.82	.023
Trial 3	.38	.17	.58	.12	-3.49	.008
Trial 4	.46	.09	.29	.23	2.89	.020

	Early Flutamide Males					
	Nonbreeding		Breeding			
	Mean	Standard Deviation	Mean	Standard Deviation	*t*(3)	*p*
Trial 1	.45	.38	.45	.25	0.00	1.00
Trial 2	.35	.10	.45	.19	-1.00	.391
Trial 3	.39	.10	.55	.10	-2.88	.064
Trial 4	.47	.21	.45	.30	0.09	.934

	Late Flutamide Males			
	Nonbreeding		Breeding	
	Mean	Standard Deviation	Mean	Standard Deviation
Trial 1	.47	.19	.40	.28
Trial 2	.10	.14	.40	.28
Trial 3	.40	.00	.60	.00
Trial 4	.54	.19	.40	.00

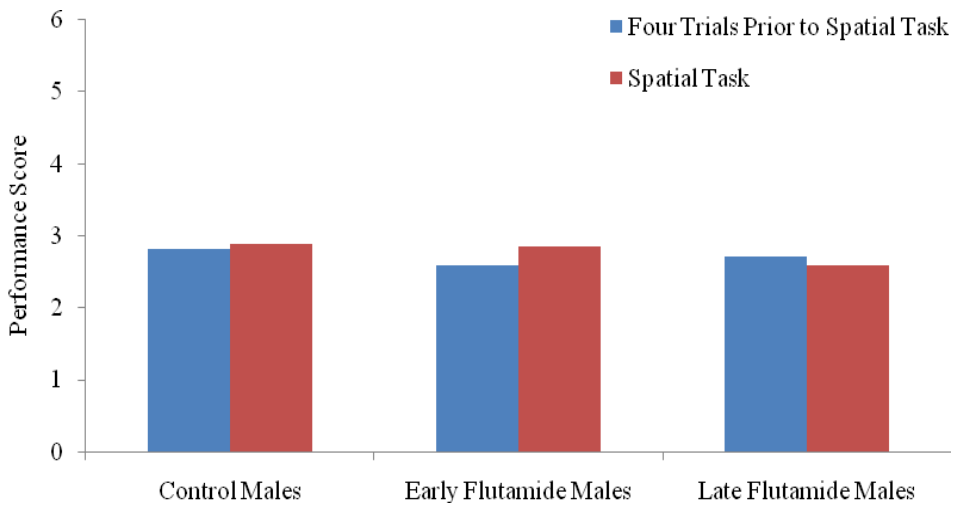
List of Figures

Figure 1. The testing facility (view from the east side of the facility).

Figure 2. A goal box used during the experiment.

Figure 3. Mean a) performance scores and b) percent scores during the breeding season on the four trials prior to the Spatial task and the Spatial task based on prenatal treatment.

a)



b)

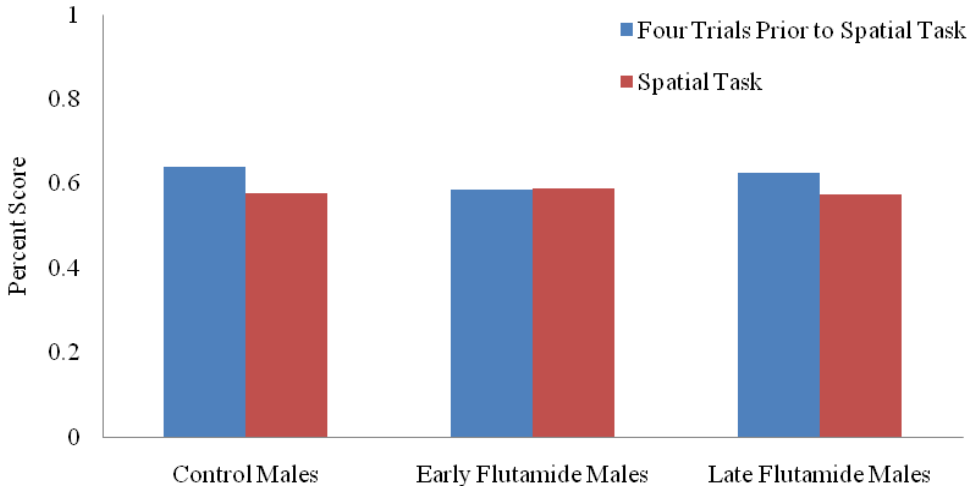
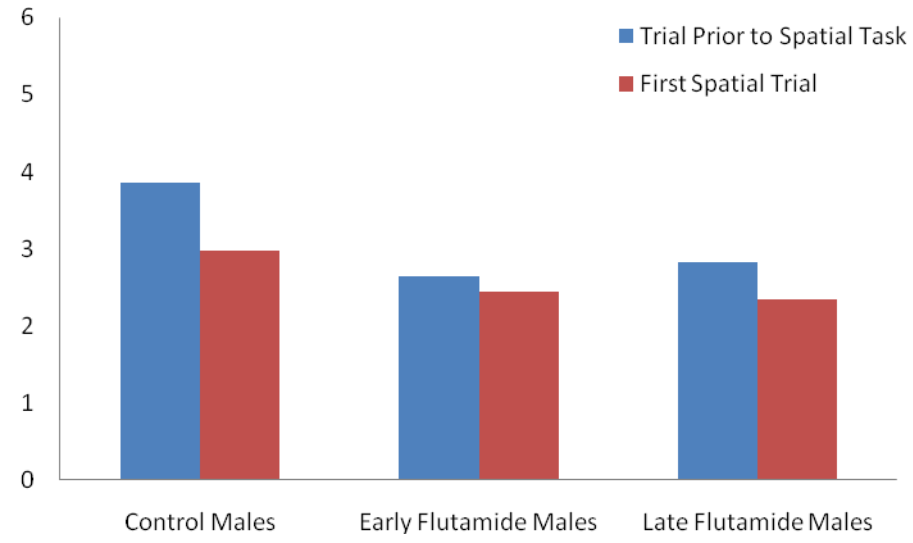


Figure 4. Mean a) performance scores and b) percent scores during the breeding season on the trial prior to the Spatial task and the Spatial task based on prenatal treatment.

a)



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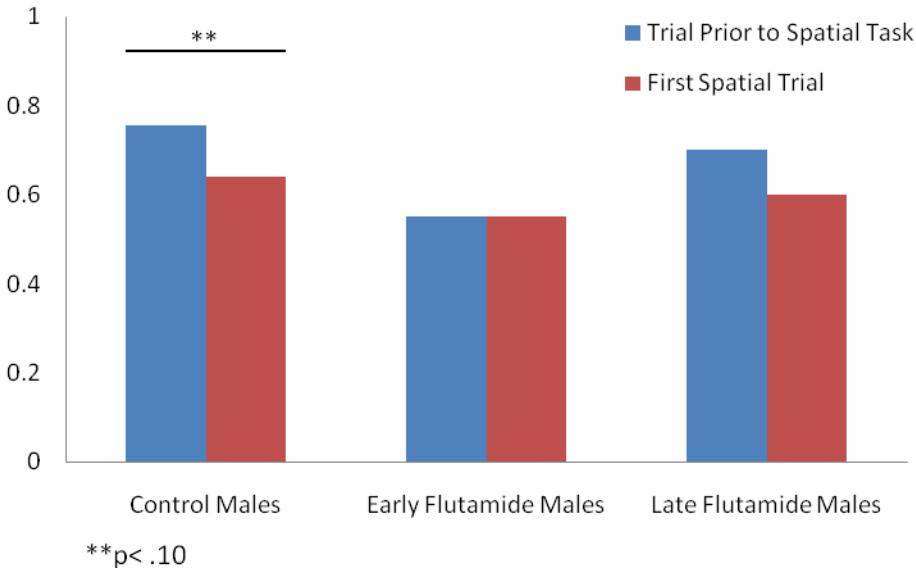
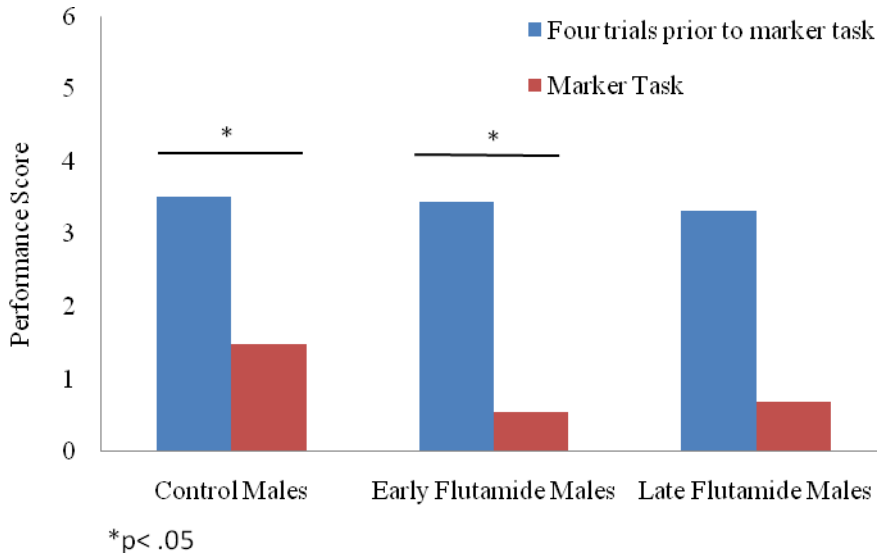


Figure 5. Mean a) performance scores and b) percent scores during the breeding season on the four trials prior to the Marker task and the Marker task based on prenatal treatment.

a)



b)

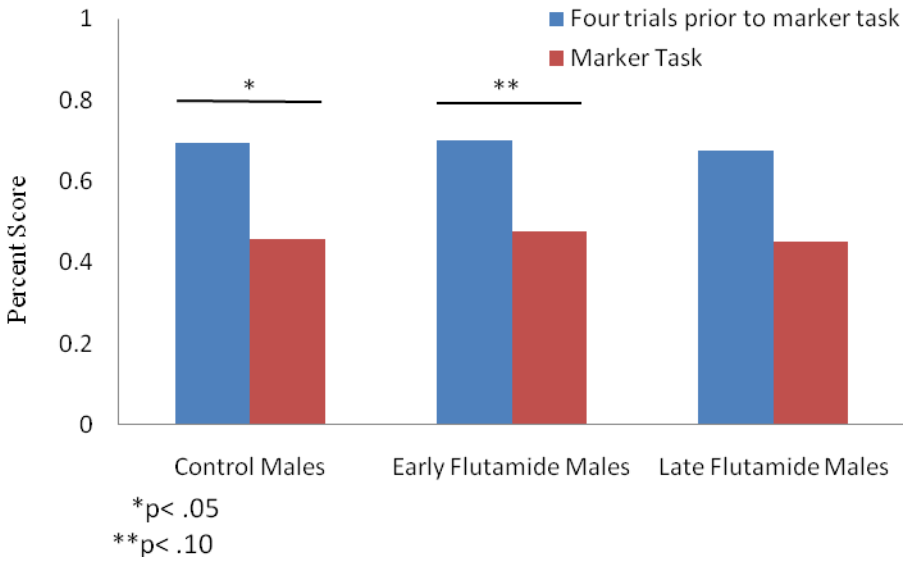
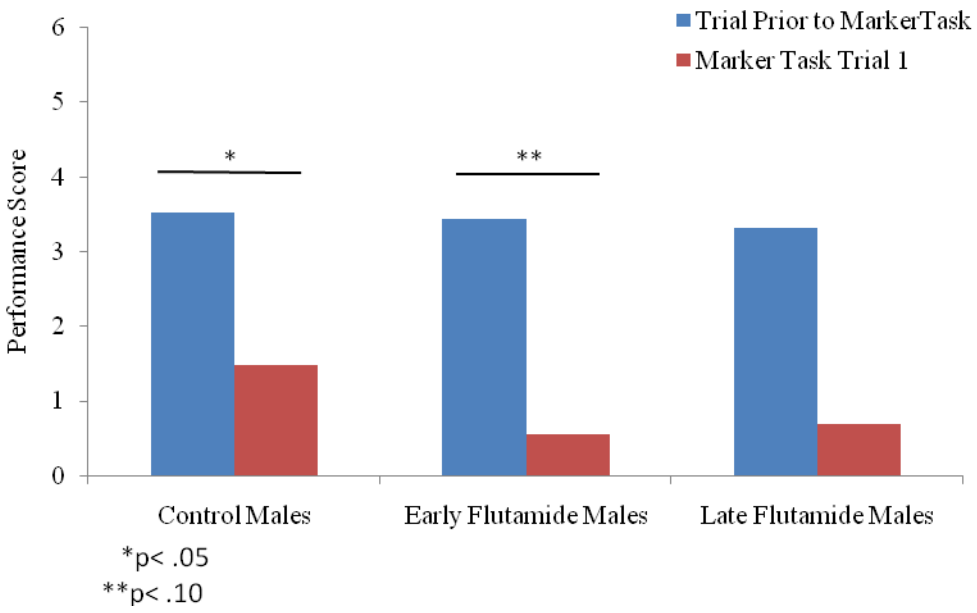


Figure 6. Mean a) performance and b) percent scores on the trial prior to the Marker task and the first Marker task trial based on prenatal treatment during the breeding season.

a)



b)

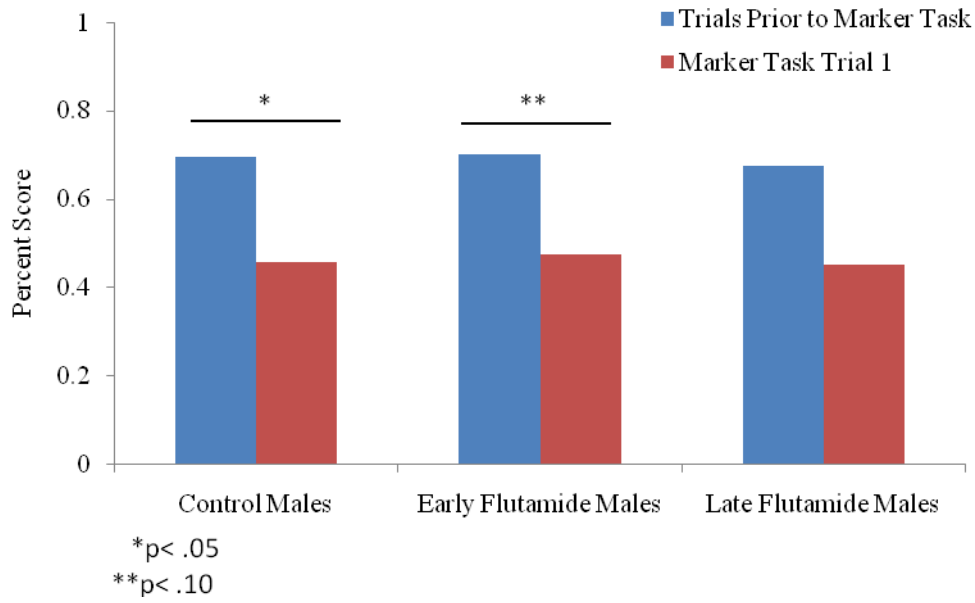
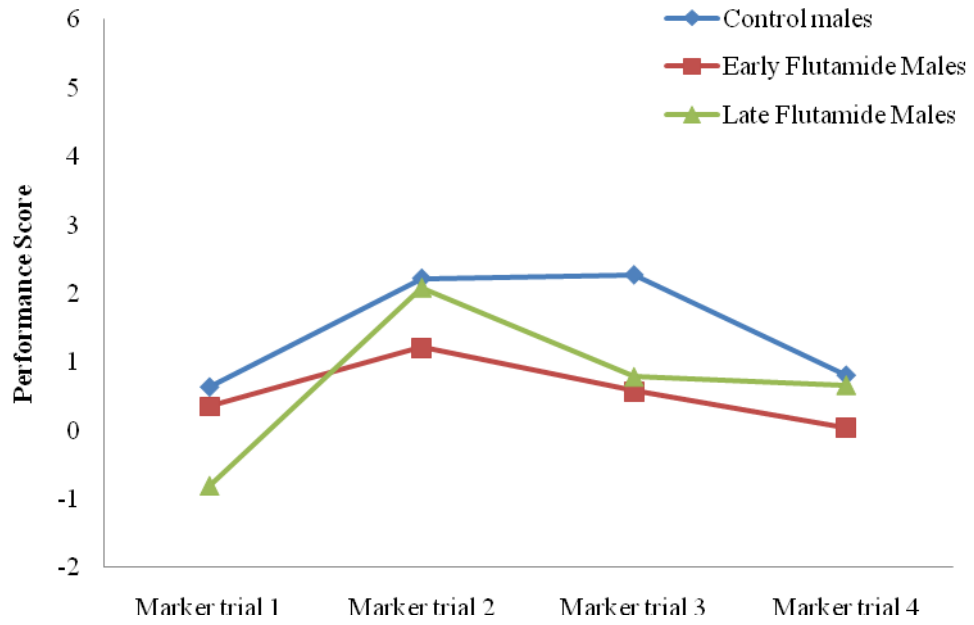


Figure 7. Change in a) performance and b) percent scores over the four Marker task trials for each prenatal treatment group during the breeding season.

a)



b)

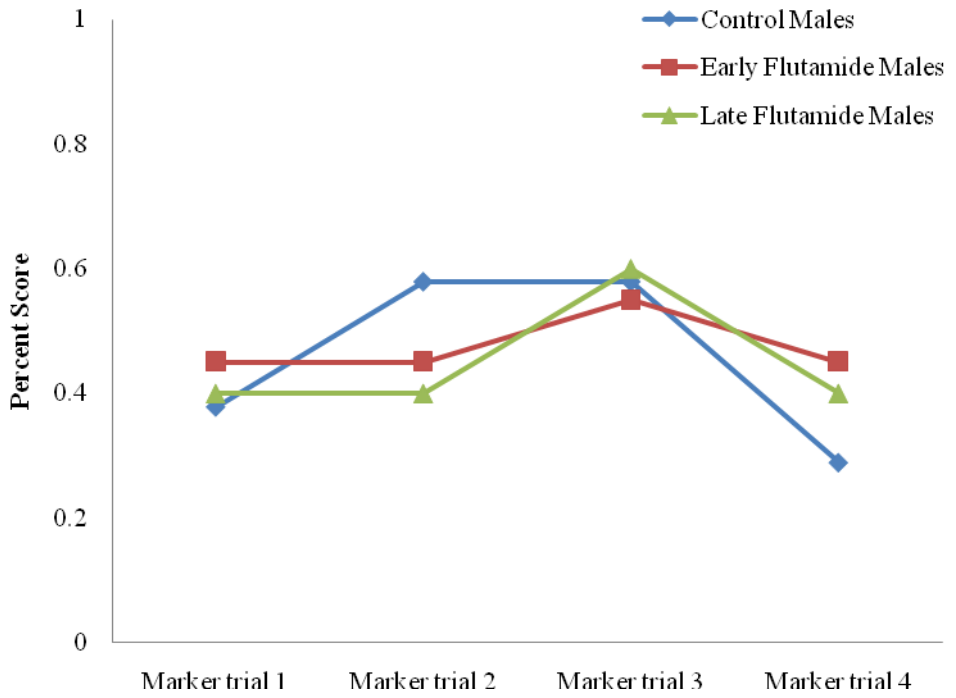
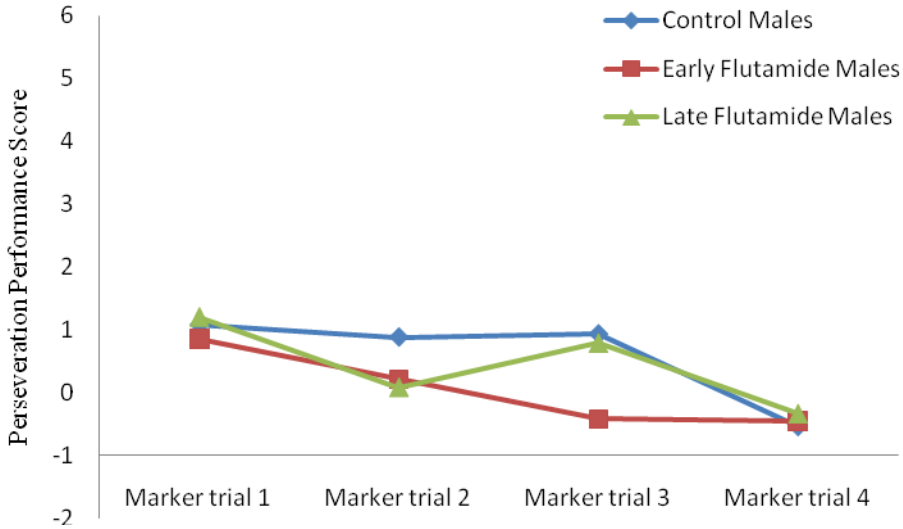


Figure 8. Perseveration a) performance and b) percent scores on the Marker task during the breeding season for each prenatal treatment.

a)



b)

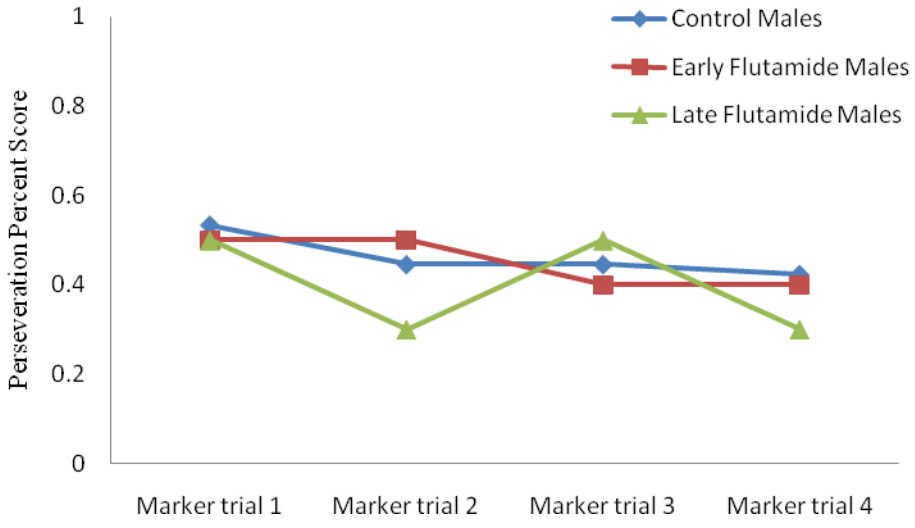
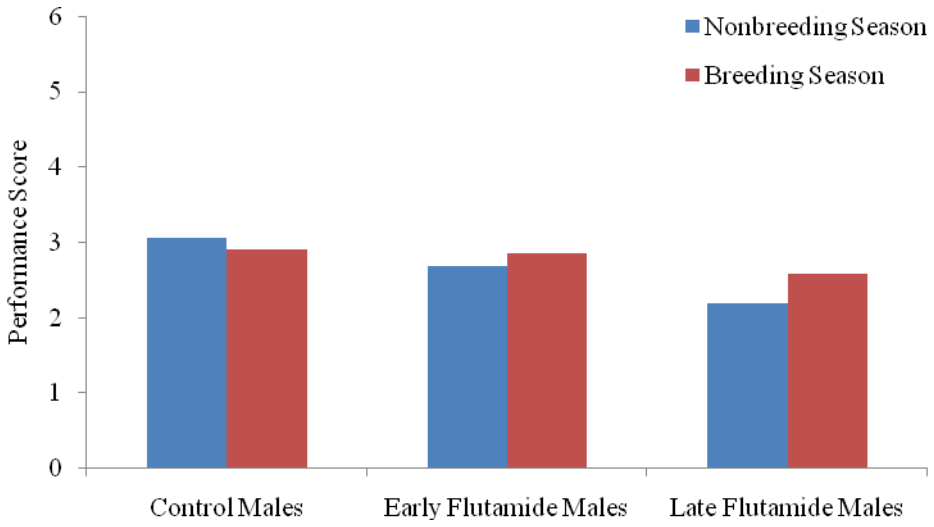


Figure 9. Mean a) performance and b) percent scores on the Spatial task during the breeding and nonbreeding seasons.

a)



b)

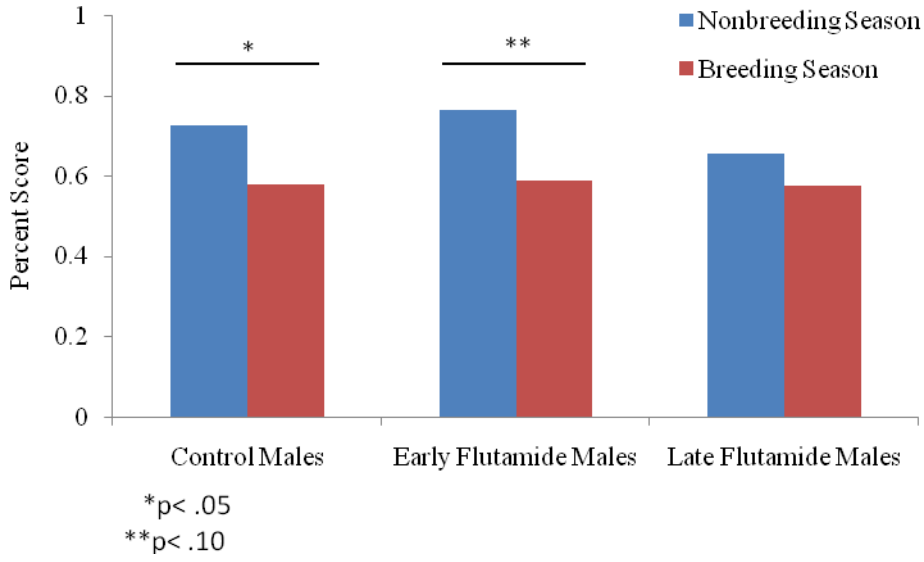
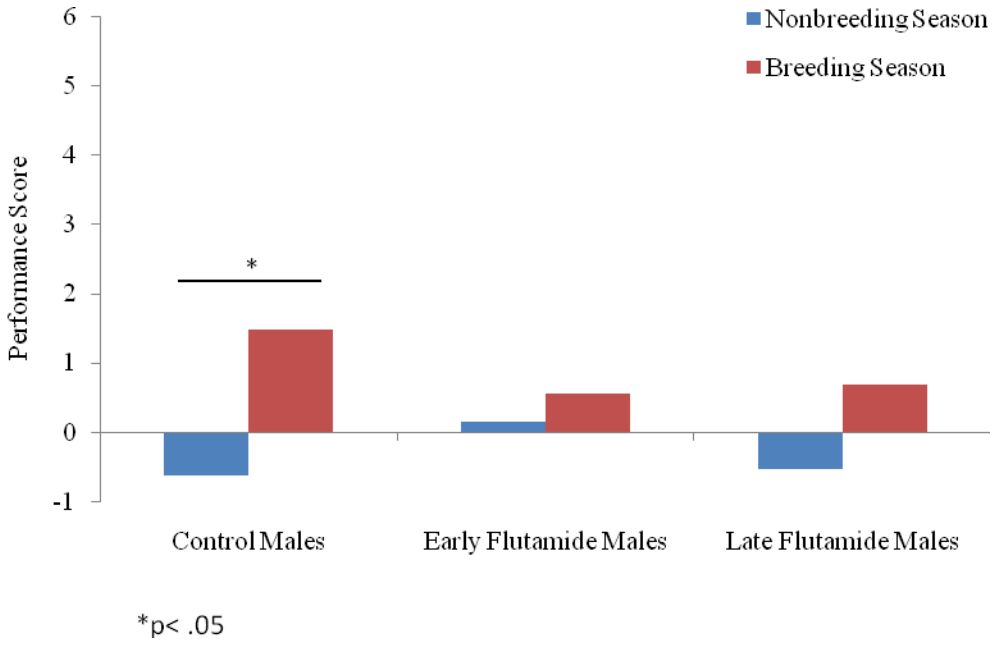


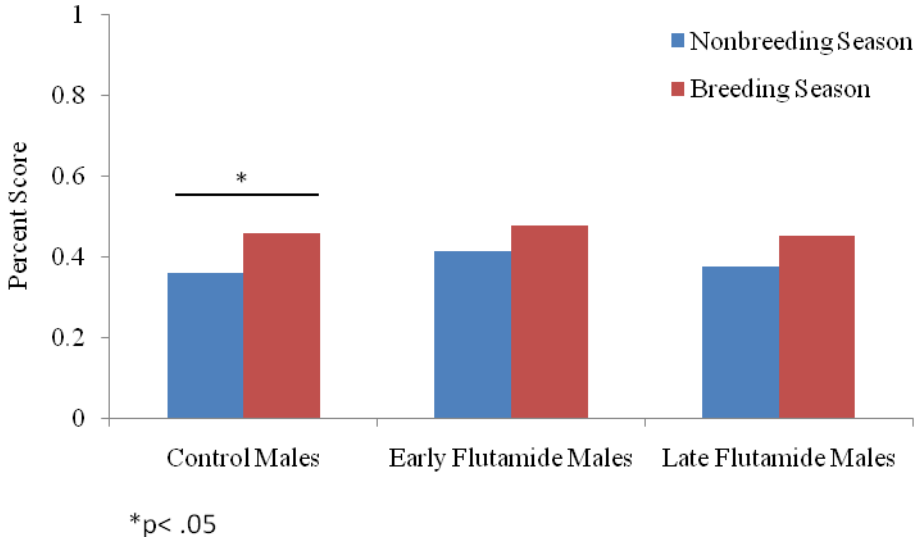
Figure 10. Mean a) performance and b) percent scores on the Marker task during the breeding and nonbreeding seasons.

a)



*p< .05

b)



*p< .05