

**SEX DIFFERENCES FOR OBJECT LOCATION MEMORY IN RATS:
THE CONTRIBUTION OF THE DENTATE GYRUS**

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Dedication

I would like to dedicate my thesis to my parents, Ken and Marianne Ehresman.

Abstract

Females exhibit superior object location memory (OLM) compared to males, but the reasons for this sex difference remains unknown. This thesis investigates the role of the dentate gyrus (DG) in an OLM task in normal rats (Experiment 1) and after bilateral adrenalectomy (ADX; Experiment 2). ADX is known to reduce volume of the DG and impair spatial learning. There was no sex difference for OLM in Experiment 1 but females exhibited superior OLM in Experiment 2. Experiment 2 found a significantly smaller DG due to ADX but this had no effect on behaviour. The male DG was significantly larger than the female DG in both experiments. Behaviour during the OLM task was not a predictor of DG volume, although a larger than average DG was related to poor OLM memory in females. Thus, the DG involvement for OLM appears to differ between the sexes.

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List of Abbreviations

ADX = bilateral adrenalectomy
BrdU = bromodeoxyuridine
CORT = corticosterone
DAPI = 4',6-diamidino-2-phenylindole
DG = dentate gyrus
INC-ADX = incomplete bilateral adrenalectomy
i.p. = intraperitoneal injection
MRI = magnetic resonance imaging
MRT = mental rotations task
MWT = Morris water task
OLM = object location memory
PBS = phosphate buffered saline
PC = parietal cortex
PFA = paraformaldehyde
RAM = radial arm maze

Chapter One

General Introduction

Sex differences are irrefutable when it comes to the physiology involving reproductive behaviours (Feder, 1984), while the biological basis for sex differences in cognition is still under investigation in the scientific community (McCarthy & Konkle, 2005). However, cognitive differences between the sexes are commonly observed, particularly during tasks of spatial ability (McCarthy & Konkle, 2005). For spatial ability, males tend to excel at tasks involving navigational skills (Astur et al., 1998; Saucier et al., 2002; Williams et al., 1990), whereas females outperform males on tasks that require remembering object location (Eals & Silverman, 1994; James & Kimura, 1997; McBurney et al., 1997; Tottenham et al., 2003). It is notable that sex differences in spatial ability are found in non-human animals, such as rats (Saucier et al., 2008; Williams & Meck, 1991), favoring a neurobiological rather than a cultural explanation for these behaviours. There are a number of possible explanations for sex differences in spatial abilities, but the affects of steroid hormones in the brain are a possible cause (Hausmann, et al., 2000; Jonasson, 2005; Williams & Meck, 1991).

The purpose of this thesis is to investigate sex differences in spatial memory and the contribution of the hippocampus, a structure that is impacted by sexually disparate steroid hormones. Navigational skill, a form of spatial ability that male humans and rodents excel at, depends critically on the integrity of the hippocampus (Astur et al., 2002; Sutherland et al., 1982), particularly a subregion of the hippocampus, the dentate gyrus (DG; Hunsaker et al., 2008). In contrast to the majority of existing research that focuses on the male's dominant navigational ability, the focus of this thesis is on superior

ability for object location memory (OLM) of the female. The OLM task measures the memory for object locations in an environment, as opposed to measuring spatial memory through performance on navigational tasks. Importantly, OLM has been commonly found to be superior in females in the human literature (Eals & Silverman, 1994; James & Kimura, 1997; McBurney, et al., 1997; Tottenham, et al., 2003), and in rats (Saucier et al., 2008). However, the neural basis of OLM is unknown. The experiments reported here investigated the contribution of the DG to memory for object locations.

Role of the hippocampus in learning and memory.

The notion that the hippocampus is a critical structure for learning and memory was notably published by Scoville and Milner (1957) after neuropsychological testing of human patients. Before this observation, it was generally thought that memory was distributed throughout the brain, with no particular structure implicated (Lashley, 1950). However, Milner discovered severe memory deficits in Scoville's patients after the bilateral removal of the medial portion of the temporal lobes (i.e., the hippocampus). These original reports stated that removal of the hippocampus caused both retrograde amnesia (the loss of remote memories) and anterograde amnesia (the inability to retain new memories; Scoville & Milner, 1957). Furthermore, it appears as though the amount of damage to the hippocampus is positively correlated with the severity of memory impairment (Scoville & Milner, 1957).

Research on the role of the hippocampus has revealed its importance for learning, memory and stress responses (McCarthy & Konkle, 2005). The hippocampus is anatomically organized into separate regions – the DG, CA1, CA2, CA3 and CA4 – all of which contribute to spatial learning and memory (Kesner et al., 2004). The DG primarily

receives projections from another structure important for memory, the entorhinal cortex (for a review see Amaral et al., 2007). The DG granular neurons in turn project to the CA3 pyramidal neurons via the mossy fibers. The neurons that comprise the CA3 connect to the CA1 region by the Schaffer collateral fibres. A number of theories regarding hippocampal processing exist, but importantly research provides evidence for its role in autobiographical memory, particularly spatial memory (Bird & Burgess, 2008). Accordingly, damage to the hippocampus in humans causes memory problems such as getting lost in a previously familiar environment and forgetting where objects have been placed (Abrahams et al., 1999; Astur et al., 2002).

In experimental research with rats, the most commonly used paradigm to investigate spatial learning and memory is the Morris Water Task (MWT; Morris, 1981). This task involves locating an escape platform that is submerged in water within a circular pool. Rats are typically placed in the pool at various starting locations and thus need to remember spatial cues from the surrounding environment in order to locate the platform. Importantly, performance of the MWT critically depends on the hippocampus, as rats with hippocampal damage are unable to utilize the spatial and contextual information that is necessary for remembering the location of the hidden platform and perform the task very poorly (Jarrod, 1993; Sutherland et al., 1982). Lesions to the hippocampus also impair visual recognition memory (Broadbent et al., 2004), which could affect the ability to use environmental features while navigating. Thus, the rat research reveals a dependence on the hippocampus for accurate spatial memory comparable to findings in humans.

The exact function of the hippocampus in relation to memory remains controversial, but it appears to include the processing of both spatial and nonspatial relational information (Sutherland & Rudy, 1989). Many memories and surroundings can contain a great degree of overlap and thus may interfere with correct memory recall (McClelland et al., 1995). It has been suggested that the hippocampus is responsible for creating representations of the environment and for highlighting differences between overlapping representations (Sutherland & Rudy, 1989). The hippocampus contains relatively few active neurons at a given time, suggesting a functional difference in comparison to the surrounding cortical areas (O'Reilly & McClelland, 1994). The lowest neuronal activity patterns occur in the DG at 0.4% of total neurons active in comparison to 2.5% in CA1 and CA3 (O'Reilly & McClelland, 1994). This sparseness of neuronal activity in the hippocampus may be responsible for reducing interference of overlapping information during memory retrieval and consolidation.

The specific subregions of the hippocampus - the DG, CA1 and CA3 - appear to make unique contributions to spatial memory and processing of spatial information (Kesner et al., 2004). For example, blocking the receptors of either the CA1 or CA3 regions in rats, using localized injections of a receptor antagonist, produces different spatial memory deficits (Lee & Kesner, 2002). Blocking the receptors of the CA3 region caused deficits in spatial working memory in a novel environment but not in a familiar environment. Thus, CA3 is considered responsible for the rapid acquisition of novel spatial information necessary for short-term memory applications (Lee & Kesner, 2002) and is suggested to be able to recall a complete memory from a fragment of input (Treves & Rolls, 1992). In contrast, blocking the receptors of CA1 caused no impairments in a novel location;

however these rats are unable to maintain or retrieve spatial memories after an intermediate delay. Accordingly, CA1 may be involved with the temporal ordering of spatial information and mediating the consolidation of memories to other brain regions via its projections back to the cortex (Kesner et al., 2004; Rolls, 2000). The DG has an additionally complex role that includes: encoding inputs from multiple sensory modalities, differentiating between overlapping spatial information, and encoding memories in conjunction with CA3 (Kesner, 2007).

Role of the dentate gyrus in learning and memory.

The DG is composed of three cellular layers: the molecular layer, the granular cell layer and the polymorphic cell layer (for a review see Amaral et al., 2007). The DG is particularly interesting because it is in a position to control the flow of information into the rest of the hippocampus (Amaral et al., 2007; Xavier et al., 1999). In addition, the DG is one of the rare brain regions that exhibits neurogenesis in adulthood (Altman & Das, 1965). Neurogenesis in the DG has been related to performance in the MWT (Ambrogioni et al., 2000; Dupret et al., 2008). Lesions to the DG result in impaired spatial abilities (Jeltsch et al., 2001; Spanswick et al., 2007; Xavier et al., 1999). These behavioural deficits are particularly evident if there is overlap or similarity between environmental cues required to learn the MWT (Kesner, et al., 2004). In addition, rats with lesions to the dorsal region of the DG appear impaired at detecting the changes in distance between stimuli and discriminating environmental changes, such as the geometric shape of a room (Hunsaker, et al., 2008). The amount of damage to granule cells within the DG appears to be linearly related to poor spatial memory; that is, more damage leads to a greater deficit in acquiring new spatial information (Kesner, 2007).

However, even a 40% reduction of granule cells can result in rats exhibiting deficits during spatial tasks (Spanswick et al., 2007). Thus, the granule cells of the DG provide an important contribution to accurate performance of tasks of spatial abilities, such as the MWT.

Successful extraction of both adrenal glands (i.e., adrenalectomy; ADX) is one procedure that damages the DG without direct intervention in the brain, and leads to a loss of granule cells within a few weeks (Sloviter et al., 1993). The adrenal glands produce glucocorticoids, the loss of which after ADX leads to neurodegeneration within the dorsal region of the DG, where glucocorticoid receptors are prevalent (Joels, 2007). Adrenalectomized rats that exhibit granule cell loss display spatial learning deficits during the MWT (Rooszendaal et al., 1998; Spanswick et al., 2007). These deficits are similar to those found after destruction of about 90% of granule cells (Xavier et al., 1999). Therefore, the ADX procedure causes impaired performance on spatial tasks similar to that of more invasive lesioning procedures (Spanswick et al., 2007).

It is apparent from the behavioural data that the DG is required for the performance of certain types of spatial information. The DG appears to work in conjunction with the CA3 via the mossy fibers for the encoding, but not retrieval, of spatial information (Kesner et al., 2004). Importantly, the DG receives multiple sensory inputs, such as olfactory, visual and auditory, and uses this information to maintain spatial representations (Kesner, 2007). In addition, the DG appears to be necessary for the ability to combine object identity with spatial memory (Hunsaker, et al., 2008; Xavier et al., 1999). Accordingly, it has been suggested that the DG may be critical for detecting subtle changes within an environment, such as adjustments in the distance between

objects (Hunsaker et al., 2008). Thus, it seems parsimonious that the DG is a likely candidate for contributions to performance of tasks of OLM.

Sex differences in neuroanatomy.

In humans, volumetric studies using magnetic resonance imaging (MRI) find that the male brain is significantly larger than the female brain, including larger frontal and temporal lobes (Allen et al., 2002). After controlling for brain volume, female brains exhibit more grey matter than males, but male brains have a larger percentage of white matter and cerebrospinal fluid than female brains (Gur et al., 1999; Gur et al., 1991).

At a more discrete level, the nucleus of the preoptic area is five to seven times larger in male rats than in females (McCarthy & Arnold, 2008). Conversely, the anteroventral periventricular nucleus of the preoptic area is three to five times larger in females (McCarthy & Arnold, 2008). These regions contribute to reproductively discrepant sexual behaviours and thus the disparity between the sexes is not surprising. However, structures that subserve higher cognitive functions, such as the hippocampus, also exhibit sex differences in overall volume and subregional connections.

The hippocampus of male rats has an overall greater volume compared to female rats (Nunez et al., 2000; Roof & Havens, 1992) and other polygynous mammals, such as meadow voles (Galea et al., 1999; Jacobs et al., 1990). In human research, the hippocampus is found to be similar in size between men and women (Cosgrove et al., 2007; Gur et al., 2002), but this has not been rigorously investigated.

Androgens and estrogens may contribute to the sex difference in hippocampal size (Zhang et al., 2008). The DG, CA1 and CA3 regions, and their neural connections within the hippocampus have been found to exhibit marked sex differences that are structurally

altered by steroid hormones during prenatal development (Isgor & Sengelaub, 1998). Following gonadectomy, male rats show a reduction of spine density in the CA1 region that can be reversed with testosterone treatment but not with estrogen treatment (Leranth et al., 2003). Similarly, the removal of estrogens via ovariectomy in female rats also decreases dendritic spine density in the CA1 region (Gould et al., 1990). This effect in females appears to be specific to the CA1 region, as the removal of estrogens did not alter dendritic spine density within the DG or CA3 (Gould et al., 1990). Natural fluctuations of hormones during the estrous cycle also alter dendritic spine density in the CA1 region, such that spine density values positively correlate to circulating levels of estradiol (Woolley et al., 1990). Furthermore, prenatal treatment with androgens can increase CA3 volume in females to levels approaching that of males (Isgor & Sengelaub, 1998). Conversely, males receiving prenatal antiandrogen treatments develop CA3 pyramidal cell volumes similar to those found in females and will be significantly smaller than control males. These data indicate that steroid hormones have significant effects on hippocampal structure, both during brain development and throughout adulthood.

In certain mouse and rat strains the DG contains more granule cells and is thicker in males than in females (Wimer & Wimer, 1989; Roof & Havens, 1992). Similar to the CA1 and CA3 regions, the DG is affected by prenatal exposure to steroid hormones, with prenatal exposure to testosterone in females altering the width of the DG to that equivalent to adult males (Hajszan et al., 2007; Joels, 2007; Jones & Watson, 2005; Roof & Havens, 1992). There are also sex differences in neurogenesis, with females actually producing more newly born neurons than males, particularly when circulating estrogen levels are high; females also exhibit more neural degeneration (Tanapat et al., 1999).

Therefore, a transient increase in granule cell production in females does not necessarily lead to a greater number of granule cells (Tanapat et al., 1999). However, it has been suggested that these newly born granule cells are making connections to the CA3 region, which would account for the greater number of mossy fiber synapses in the CA3 region in females compared to males (Madeira et al, 1991; Tanapat et al., 1999). Steroid hormones contribute to the organization and function of the DG (Hajszan et al., 2007), although this has not been substantially researched in comparison to other hippocampal subregions.

Sex differences in behaviour.

There are numerous sex differences in cognitive behaviours (for review see Kimura, 1999). Females typically possess superior verbal memory and verbal fluency skills (Chipman & Kimura, 1998; Kimura & Clarke, 2002) and are faster at relaying a speech sequence (Nicholson & Kimura, 1996). Males, however, are typically superior at visuospatial abilities, the most reliable being the Mental Rotations Task (MRT; Collins & Kimura, 1997; Vandenberg & Kuse, 1978; Voyer et al., 1995), a task in which individuals must match rotated items with each other. Although the female superiority at verbal skills has been limited to human research, the sex differences in spatial abilities have been found across species.

Experiments with rats find sex differences during the MWT, with female rats exhibiting longer latencies to solve the MWT compared to male rats (Beiko et al, 2004; Perrot-Sinal et al., 1996; Jonasson, 2005; McCarthy & Arnold, 2008). Female rats display more thigmotaxis, which is the tendency to swim alongside the walls of the pool, and is an index of anxiety (Perrot-Sinal et al., 1996). Thus, females may take longer to reach the

platform, even when it is visible, due to their thigmotaxic trajectories (Beiko, et al., 2004). The longer acquisition times seen in females thus may be due to the adoption of an inefficient strategy in order to reduce stress (Beiko et al., 2004). However, sex differences in strategy are not always reported alongside the male advantage during the MWT (Jones & Watson, 2005) and steroid hormones contributing to differences in brain morphology are an alternative explanation. Accordingly, perinatal testosterone treatment in females can facilitate performance on the MWT (Roof, 1993), and these females exhibited decreased latency to solve the task compared to their untreated counterparts, and were nearly the same as males (Roof, 1993). In contrast, females with high levels of circulating estradiol, whether naturally occurring or exogenously administered, perform more poorly on the MWT than females with low levels of estradiol (Frye, 1995). Taken together, these data suggest that sex hormones affect MWT performance.

Another spatial task that exhibits sex differences in rats is the radial arm maze (RAM; Olton & Samuelson, 1976). The RAM is an elevated platform connected to a number of equally spaced arms (four to seventeen), some of which will be baited with food. Similar to the MWT, RAM measures spatial working memory because the rats must use distal visual cues to remember which arms contain the reward and which arms have already been visited. Males make fewer errors during the RAM compared to females; that is, males are better able to remember which arms contain the food reward (Jonasson, 2005; Williams & Meck, 1991). Male performance can be negatively affected by performing neonatal castration, which reduces testosterone during the perinatal period and presumably negatively impacts the hippocampus (Williams & Meck, 1991). Furthermore, neonatal testosterone treatment in female rats produces improvements in

RAM performance (Roof, 1993). Hence, the RAM is another measure of spatial ability that exhibits sex differences and sensitivity to manipulated sex hormones.

Ultimate causes for sex differences in spatial ability.

The neural basis for sexually dimorphic spatial abilities is thought to have arisen from separate evolutionary pressures on the different sexes. Two theories in particular have been used to explain sex differences in spatial ability, one based on the mating system of polygynous species (Ecuyer-Dab & Robert, 2004; Gaulin & Fitzgerald, 1986) and the other on a division of labor between the sexes (Eals & Silverman, 1994). The former emphasizes spatial abilities that would be selected for in polygynous species, where males require navigational skills to maintain a large range size in order to search for potential mates (Gaulin & Fitzgerald, 1986). In contrast, females would likely maintain a semi-permanent, smaller range size in order to rear their offspring. Thus, females would likely attend to the position of objects within their home range either for future use or to detect invasion of their territory. As a result, sex differences in spatial ability would be due to the ancestral range size used for mating strategies. Accordingly, range size has been positively correlated to spatial skills and the size of the hippocampus (Jacobs et al., 1990).

In contrast, the division of labour hypothesis argues that sex differences in spatial abilities originate from the labour skills of our hominid ancestors, where males were predominantly hunters and females were predominantly gatherers (Eals & Silverman, 1994; Silverman & Eals, 1992). Males would travel long distances through unfamiliar territory in order to track and hunt game. Thus, the success of a male would depend on his navigational ability and capacity to maintain an accurate orientation over a large

spatial range. In contrast, females would be solving spatial tasks in a smaller range size that would include remembering the position of edible plants. Sex differences in spatial ability would then result from separate methods for acquiring provisions.

Proximate causes for sex differences in spatial ability.

Learning and experience.

Human research has contributed to our understanding of how males and females use different strategies to solve the same spatial task. For instance, navigational strategy varies, although strategy can be partitioned into topographic strategies, which include the use of landmarks and an egocentric point of view as a referent, and Euclidean or metric strategies, which include the estimate of distances and reference to cardinal directions, such as North. Females tend to prefer topographic strategies while navigating and are also more proficient at utilizing landmark information compared to males (Choi & Silverman, 2002; Galea & Kimura, 1993; Saucier et al., 2002b; McFadden et al., 2003). In contrast, males are more inclined to use Euclidean strategies and also exhibit superior ability at navigating with Euclidean-based instructions. Thus, males and females attend to different features of the same environment that they later utilize while navigating or giving directions.

Functional MRI suggests that even when men and women are performing the same spatial task, differences in activation patterns are observed (Hugdahl et al., 2006; Weiss et al., 2003). For example, although as expected, performance of both navigational and mental rotation tasks result in significant activation in hippocampal regions (Iaria et al., 2008; Maguire et al., 1998), women also exhibit a significant amount of frontal activation and this is not observed in men (Hugdahl et al., 2006; Weiss et al., 2003).

These different activation patterns suggest that men and women use different regions of the brain to solve spatial tasks, which may reflect strategy differences and subsequent behavioural differences (Gron et al., 2000).

It is tempting to attribute sex differences in cognitive behaviours to societal gender roles affecting life experience, but comparable sex differences are found in animals that remain unaffected by culture (Beiko et al., 2004; Jones & Watson, 2005; Saucier et al., 2008). It is difficult to attribute observed sex differences in the behavior of non-human animals to gender biased experience. Furthermore, sex differences in spatial behaviours have been found across different cultures (Lippa et al., 2009; Silverman et al., 2007) and in young age groups (Kerns & Berenbaum, 1991). In western culture, gender stereotypic behaviours such as sports, vocational interests and social interactions do not significantly predict the large sex difference seen for the MRT (Saucier et al., 2002b). These data support the idea that sex differences in spatial tasks reflect some underlying difference that is not easily attributable to cultural biases and gendered behaviours.

Hormones.

Organizational effects of hormones occur prenatally and can persist into adulthood but in adulthood endogenously circulating hormones can also influence brain functioning and behaviour (Williams & Meck, 1991). These are known as the activational effects of hormones. The natural occurrence of endogenous hormone levels has been found to influence spatial abilities in males and females. In males, there is an inverted U-shaped relationship between spatial ability and testosterone levels, where the lower normal range is related to enhancement of spatial abilities (Moffat & Hampson, 1996). However, female spatial performance is enhanced if their testosterone levels are in a

higher normal range (Moffat & Hampson, 1996; Burkitt et al., 2007). Behaviourally, female performance on some spatial tasks fluctuates in accordance with phase of the menstrual cycle; specifically, higher estrogen levels are correlated with poorer performance on certain tasks of spatial ability (Kimura & Hampson, 1994). Furthermore, studies have demonstrated that endogenous levels of steroid hormones affect cortical activation across the menstrual cycle (e.g., Schoning et al., 2007; Protopopescu et al., 2008). Further, manipulating steroid hormones exogenously can affect also spatial learning and memory in rats and humans (Isgor & Sengelaub, 1998; Postma et al., 2000).

As reviewed above, the hippocampus is sensitive to steroid hormones and has been implicated as a critical structure in sexually dimorphic spatial abilities (McCarthy & Konkle, 2005). For example, female rats treated with testosterone *in utero* showed a masculinized DG structure and escape latencies in the MWT equivalent to that of males (Roof & Havens, 1992; Roof, 1993). Similarly, Isgor and Sengelaub (1998; 2003) found that female rats treated with androgen during the last week of gestation reversed the sex differences in volume typically found in the CA1 and CA3. Importantly, androgen treated female rats also exhibited spatial abilities more similar to the male behaviour than to the untreated females. Thus, proximate causes for spatial abilities have been characterized by hormone exposure both *in utero* (i.e., organizational effects) and throughout the postnatal period (i.e., activational effects; Jones et al., 2003). The organizational and activational effects on the hippocampal subregions may be capable of providing insights into the cause for sex differences in spatial behaviours.

Object location memory (OLM).

Although males typically exhibit superior performance for navigational abilities, females tend to be superior at OLM. Several tasks have been developed in order to study this specific component of spatial ability within an experimental setting. The most commonly used task was developed by Eals and Silverman (1994; Silverman & Eals, 1992), where participants are given a piece of paper containing 2D drawings of a variety of common objects. After one minute of observing the array, participants are given a new array of the same objects but some objects have switched positions with each other. Participants indicate which items remain in the same location and which ones have moved to a new location. Performance on this simple paper and pencil task typically results in superior performance of women compared to males (Eals & Silverman, 1994, Silverman & Eals, 1992). Notably, this finding has been replicated across ethnicity and in at least 35 different countries (Silverman et al., 2007).

Research has expanded to include other tasks that find superior female performance for OLM tasks. For example, on both computerized and paper versions of the game Concentration, women outperform men (McBurnery, et al., 1997; Sykes Tottenham et al., 2003), both in terms of number of errors and in terms of efficiency. More recently, a virtual grocery store was designed to measure OLM for common items in a 3D environment (Spiers et al., 2008). Similar to the findings of 2D tasks, females were superior at remembering the location of items within the grocery store compared to males.

The data from human experiments have contributed to the understanding of sex differences in spatial abilities; however, comparative studies are important to provide

evidence for the ultimate causes for these behavioural differences. Saucier et al. (2008) made a necessary contribution to furthering OLM research by developing a rodent paradigm that is consistent with the OLM task developed by Eals and Silverman (1992). In the rat version, the OLM task consists of six sessions: one habituation trial (to familiarize the rat with the testing environment), four training trials, and one testing trial. During the training trials, rats are individually placed in an arena containing four common items that remain in constant locations. During the testing trial, however, two of the four objects exchange positions with one another, keeping the absolute positions consistent with the training sessions; thus, the paradigm is consistent with the human task in which half of the objects exchange positions with each other. Rats spend more time interacting with what is novel (Ennaceur & Delacour, 1988), thus OLM is operationally defined as the amount of exploration with the exchanged objects (the items that are in a novel position) during the testing trial. For the testing day, female rats explored the objects that were exchanged more than the objects that remained in the same location (Saucier et al., 2008). Furthermore, the amount of exploration with these objects significantly increased from the previous training session. Males, however, did not display significant changes in behaviour between training trials and the testing trial. Thus, comparable to the human research, Saucier et al. (2008) found that female rats exhibited superior OLM compared to males.

Summary.

The hippocampus not only plays an essential role in spatial memory, but is also a target for the organizational and activational effects of steroid hormones (Hajszan et al., 2007). The DG is of particular interest because of its role in the encoding of the spatial

location of objects as well as distal cues (Hunsaker et al., 2008). Furthermore, the DG contains estrogen and androgen receptors, and the DG is modulated by sex steroids (Hajszan et al., 2007). Hence, the DG may be the critical structure within the hippocampus for understanding sex differences in OLM. For that reason, this thesis presents two experiments to explore the role of the DG in OLM. The first experiment investigates adult male and adult female rat behaviour during the OLM task with no manipulation and assesses naturally occurring volumes of the DG. Based on previous research, I predict that females will display superior OLM in comparison to males. Furthermore, given the role of the DG in spatial memory, I expect that DG volume will be positively related to superior OLM.

The second experiment investigates adult male and adult female rat behaviour during the OLM task after ADX. Recall that ADX is a non-neurally invasive way of causing a selective volume reduction of the DG. Based on previous research, I predict that rats that undergo ADX will exhibit a significantly smaller DG compared to their sham surgery counterparts. I predict that this subsequent decrease in DG volume will negatively affect OLM and thus these rats will display inferior performance on the OLM task compared to the control group. Therefore, I hypothesize that DG volume will be positively correlated to OLM. Furthermore, I expect ADX to have a negative effect on OLM regardless of sex, but for female control rats to exhibit superior OLM in comparison to male control rats.

Chapter Two

Experiment One: Sex Differences in Object Location Memory and the Dentate

Gyrus

Males tend to outperform females on tasks of navigational ability and females outperform males on OLM tasks (Astur et al., 1998; Beiko et al., 2004; Saucier et al., 2008; Tottenham et al., 2003). The hippocampus has been implicated as the necessary structure to solve many spatial tasks; however the neural basis of the OLM task has yet to be investigated. This experiment will investigate the role of a subcomponent of the hippocampus, the DG. The DG could be the necessary component for combining object and spatial memory and be critical for detecting changes in the relationships between objects (Hunsaker et al., 2008; Kesner, 2007). The dorsal region of the DG in particular has been implicated as a necessary component for spatial memory (Hunsaker et al., 2008; Spanswick et al., 2007), whereas the ventral region of the DG has a role in anxiety-related behaviours (Bannerman et al., 2004). The DG is known to be sensitive to gonadal hormones and to the release of corticosterone, which also varies between the sexes and can affect performance of spatial tasks (Beiko et al., 2004). Taken together, the DG appears to be likely to play a role in OLM.

Furthermore, the DG is a site for adult neurogenesis, with the potential for new granule cell neurons to be integrated into the DG structure (Kempermann et al., 2004). In order to study neurogenesis in the DG, researchers commonly inject bromodeoxyuridine (BrdU; Taupin, 2007). BrdU is a thymidine analog that is incorporated into the DNA during the cell cycle (Dolbeare, 1995). Thus, cells that are replicating DNA at the time BrdU is available allows for immunohistochemical labeling. Steroid hormones and sex

can affect adult neurogenesis and thus BrdU has been used to detect sex differences in cell survival and proliferation. For example, it has been found that females have higher levels of cell proliferation in the dentate gyrus during periods of high estrogen levels compared to males (Tanapat et al., 1999). However, studies using BrdU need to be interpreted with caution because factors such as dose, number of injections, and duration between BrdU administration and perfusion can affect the number of BrdU labeled cells (Taupin, 2007).

I predict that memory for object locations will be reflected by interactions and duration of exploration, such that rats will explore the objects that have exchanged positions more than those objects that remain in the same location. Based on the results from Saucier et al. (2008), I predict that female rats will have significantly increased exploration with the objects that exchange position on the testing day compared to the objects that remained in a constant position. Furthermore, I expect that females will increase their exploration with the objects that have been exchanged compared to the previous training session (training session four). Although I expect males to exhibit similar behaviour, I predict it to be greater in females, indicating superior OLM. Post-mortem volume estimates of the dorsal DG and the entire DG will be correlated to behaviour during the OLM task. The dorsal region of the DG has been implicated as an important structure for spatial memory more so than the ventral region; thus, dorsal DG volume and its contribution to OLM will be assessed separately from the entire DG volume. I expect that dorsal DG volume will be positively related to superior OLM because of its important role in spatial memory. Accordingly, I predict that the overall DG volume will be positively related to behaviour during the OLM task.

Methods.

Subjects.

Subjects for this experiment were Long Evans rats (39 males, 38 females; Charles River). All female rats (postnatal day 62-80) and male rats (postnatal day 63-88) were handled daily for one week prior to the first day of the experiment. All rats were group housed (two to three rats per cage) in a 12-hour light/dark cycle and were only handled and tested during the rats' light cycle. Rats had *ad libitum* access to food and water throughout the experiment and were weighed on all testing days. All procedures were approved by and conducted according to The University of Lethbridge Animal Welfare Committee guidelines.

Rats received BrdU injections to investigate the relation between neurogenesis and behaviour, although this is not analyzed in this thesis. Thus, rats were randomly assigned to one of five groups. There were three control groups: 1. rats that received no injections (the no injection control group; seven males, six females); 2. rats that received an intraperitoneal (ip) injection of sterile phosphate buffered saline (PBS; 1 ml/kg) 20 minutes prior to placement in the arena for the training sessions and the testing session (days two, three, and four; the vehicle control group; eight males, eight females); and 3. rats that received an ip injection of BrdU (150mg / kg body weight dissolved in PBS) 20 minutes prior to placement in an empty arena for days two, three and four (the activity control group, seven males, seven females). There were also two experimental groups: 1. Rats that received an injection of BrdU 20 minutes prior to placement in the arena for all training trials (days two and three; eight males, eight females) and rats that received an injection of BrdU 20 minutes prior to placement in the arena only for the testing trial (day

four; nine males, nine females). For each of the five groups, there were different perfusion days; half were perfused 24 hours after testing and half 28 days after testing.

OLM task.

The OLM task (Saucier et al., 2008) is a task in which rats are tested individually in a circular arena with a diameter of 152.5 cm. All activity was recorded by an overhead camera and recorded onto digital videocassettes. The OLM task consisted of six sessions: one habituation trial, four training trials, and one testing trial. For the habituation trial rats were placed in an empty arena for five minutes (giving a baseline measure of activity and habituating the rat with the arena). 24 hours following habituation, the four training trials began (two trials per day for two days, spaced eight hours apart). For the training trials, the rat was placed in the arena and allowed to explore the arena, which contained four objects, for five minutes. To reduce possible bias for object locations, there were three possible configurations of the four objects (randomly assigned among rats). For an individual rat, the configuration of the objects was constant for all trials. The objects in the arena were common items: a brown empty bottle, a clear bottle filled with unscented pink liquid (water and food colouring) with a sealed cap, a plastic toy with no removable parts and a ceramic statue. The objects were chosen to avoid potentially confounding interactions, such as the rats lying on top of the object or chewing on its parts. The objects were cleaned with 50% ethanol between each rat.

The testing trial began 24 hours after the last training trial (training session four). In the testing trial, the rat was placed in the arena for five minutes and allowed to explore. Unlike the training trials, during which the positions of the objects remained constant, in the testing trial two of the four objects exchanged positions with one another (the objects

that were moved were counter-balanced among rats) while the other two objects remained in the same location. Thus, in the testing trial all of the absolute positions of objects were the same as the training sessions, but the relative positions of two of the objects differed (Figure 1). Two coders (one blind to the groups) coded the videotapes and recorded both the number of interactions with each object and the time spent with each object. Interactions were operationally defined as the rat contacting the object with either the paw or vibrissae or the vibrissae being at a distance of less than two cm from the object (Saucier et al., 2008). Each rat was scored for the total interactions with each object, and for the total time spent with each object for each training session and on the testing day. These variables were scored individually for each object and then were summed into either the Exchanged object category (i.e., the objects to be moved on the testing day) or Constant object category (i.e., the objects to remain in the same position on the testing day) for statistical analysis.

Euthanasia and brain fixation.

Rats were either euthanized 24 hours after the testing trial or 28 days after the testing trial (see Table 1 for group composition). Rats were euthanized with an injection of Euthanosol® and perfused transcardially with PBS (approximately 200 ml) followed by a transcardial injection of approximately 200 ml of 4% Paraformaldehyde (PFA). Brains were extracted, stored in brain bottles containing 4% PFA and refrigerated for 24 hours and then transferred to sucrose solution for at least three days.

Histology.

Coronal sections were cut with a microtome at a thickness of 40 μm and 12 series were taken and stored in 1 X PBS with a 1:1000 concentration of sodium azide. Two

series of tissue (i.e., every sixth section) were stained with 4',6-diamidino-2-phenylindole (DAPI) in PBS buffer. The tissue was mounted onto glass slides and covered with a glass slip using fluorescent mounting media. Stereo Investigator® (MicroBrightField Inc., 2008, Version 8) was used to assess volume of both the dorsal DG (every sixth section) and the total DG (every 12th section) by employing the cavalieri estimator counting technique (Slomianka & West, 2005). The dorsal region of the DG was officially defined as any DG existing above the choroid plexus (the mid-dorsal ventricle in a coronal section; see Figure 2). An 80 μ m grid was randomly placed on each section containing the DG and all crosses in which the upper right quadrant was overlaying the structure were counted in order to determine an unbiased volume estimate. The intervals were adjusted in order for the coefficient of error to remain within a reliable range (< 0.05) and to maintain systematic-random sampling.

Analyses.

All analyses compared the last training session (training session four), when the rats are most habituated to the configuration, to their behaviour on test day. The total number of interactions and the total time spent interacting with the objects were the dependent behavioural measures. These scores were then examined with a 2 x 2 x 2 (Object [Exchanged objects, Constant objects] x Day [training session four, testing day] x Sex [male, females]) mixed measures analysis of variance (ANOVA) using PASW Statistics 17.0. An alpha level of .05 was used for all statistical tests.

Results.

Histological analysis- dorsal DG.

A one-tailed Pearson Correlation revealed the volume of the dorsal DG to be significantly correlated to the volume of the entire DG, $R(75) = .696, p < .0001$. An ANOVA found no significant sex difference for the volume of the dorsal DG, $F(1, 75) = 1.396, p = .241$, nor was there an effect of BrdU injection that could be detected on volume, $F(1, 75) = .027, p = .870$.

Histological analysis- DG.

A one-way ANOVA detected a significant sex difference for the volume of the DG, $F(1, 75) = 5.068, p = .027$, with male volumes being larger than female volumes (see Figure 3). A one-way ANOVA found no significant impact of BrdU injection volume of the DG, $F(1, 75) = 0.421, p = .518$.

Relation between DG volume and behavior.

A linear regression was performed; dorsal DG volume was the criterion and the predictors were the total interactions and total time with objects (Constant and Exchanged) during training session four and the testing day. The model failed to reach significance, $R^2 = .122, F(8,54) = 0.938, p = .493$.

Separate linear regressions for males and females were run to investigate possible sex differences in the contribution of the DG for this task. DG volume was the criterion and the predictors were the total interactions and total time with objects (Constant and Exchanged) during training session four and the testing day. The model failed to reach significance for males, $R^2 = .300, F(4, 30) = 1.234, p = .324$, and only the amount of time spent with Exchanged objects on the testing day approached significance as a predictor, $\beta = .451, t(54) = 1.772, p = .090$, two-tailed. The same model also failed to reach significance for females, $R^2 = .319, F(4, 31) = 1.290, p = .299$.

To further investigate a possible effect of volume on behaviour, all rats were divided into either a below average size DG group (below 2.50 mm³ for females, below 2.73 mm³ for males) or an equal to and above average size DG group. The variable Volume was added as another between-subjects variable (High volume, Low volume) in the ANOVA. There were no significant effects of Volume on the Time variable, but there was a significant Sex x Volume interaction for the Interactions with objects, $F(1, 58) = 5.028, p = .029$. Fisher LSD post hoc analysis revealed that High volume females had significantly fewer interactions with the Exchanged objects on the testing day compared to High volume males, $p = .017$, Low volume males, $p = .034$ and approaching significance compared to Low volume females, $p = .070$ (see Figure 4). High volume females also had significantly fewer interactions with the Constant objects on the testing day, $M = 8.941, SD = 4.520$, compared to Low volume females, $M = 11.733, SD = 3.614, p = .027$. High volume females also had significantly fewer interactions with Exchanged objects during training session 4, $M = 8.823, SD = 2.766$, compared to Low volume females, $M = 11.866, SD = 5.767, p = .048$ and High volume males, $M = 12.368, SD = 3.684, p = .016$.

Behavioural analysis- interactions with objects.

A significant Object x Day interaction, $F(1, 59) = 30.592, p < .0001$, was revealed. Fisher LSD post hoc analysis revealed a significant increase in the number of interactions with the Exchanged objects on the testing day, compared to their interactions with Exchanged objects during training session four, $p < .0001$, and to Constant objects on the testing day, $p < .0001$ (see Figure 5). There was no significant main effect of Sex, $F(1, 59) = 2.545, p = .116$ or significant Sex interactions with other variables.

To ensure no confounding effects of BrdU injection on behaviour, the variable BrdU (BrdU, no-BrdU) was added as another between-subjects measure into the mixed measures ANOVA. BrdU was not found to be a significant factor for number of interactions with objects, $F(1, 59) = .114, p = .736$, and revealed no significant interactions.

Behavioural analysis- time with objects.

The ANOVA revealed a significant Object x Day interaction, $F(1, 59) = 19.258, p < .0001$. Fisher LSD post hoc analysis revealed a significant increase in time spent with the Exchanged objects on the testing day, $M = 51.987, SD = 24.243, p < .0001$, compared to the time spent with these objects during training session four, $M = 31.768, SD = 18.923$. The time spent with Exchanged objects on the testing day was significantly different from the time spent with Constant objects on the testing day, $M = 33.067, SD = 11.492, p < .0001$. There was also a significant main effect of Sex, $F(1, 59) = 4.184, p = .045$, with males spending significantly more time with all objects than females (see Figure 6). There were no significant Sex interactions.

A separate ANOVA including the BrdU variable revealed a significant BrdU x Sex effect on duration with objects, $F(1, 59) = 6.396, p = .014$ (see Figure 7). Fisher LSD post hoc tests revealed BrdU-males spent significantly more time with the objects than BrdU-females, $p = .005$. There was a tendency for BrdU-females to differ from no-BrdU-females, $p = .087$ and BrdU-males to differ from no-BrdU-males, $p = .140$.

Discussion.

The purpose of the present study was to investigate the relationship between the volume of the DG and performance on the OLM task. The behavioural results indicated

that all rats exhibited OLM; the rats spent more time and had more interactions with the objects that had exchanged positions on the testing day than with those that remained constant, and this had significantly increased from the previous training session. In contrast to previous findings by Saucier et al. (2008), this study did not find that females outperformed males on the OLM task. There was a main effect of sex, with males interacting with the objects for longer periods of time, regardless of whether they had exchanged positions or remained in the same location. This effect was not anticipated, although a similar, but not significant, sex difference in behaviour was previously reported (Saucier et al., 2008).

Contrary to predictions, neither the dorsal DG volume nor overall DG volume was significantly related to behaviour during the OLM task. There was no sex difference in the dorsal region of the DG, but males had a significantly larger DG overall compared to females. This is consistent with research on meadow voles (Galea et al., 1999), mice (Wimer & Wimer, 1989) Sprague-Dawley rats (Roof & Havens, 1992) and guinea pigs (Severi et al., 2005). Overall, the male advantage for DG volume was not predicted by behaviour during the OLM task. However, females with a higher than average volume of the DG had significantly fewer interactions with the Exchanged objects on the testing day compared to all males, and approached significance in comparison to females with a below average volume of the DG. This finding indicates that having a large DG is related to poor OLM in females, whereas DG volume was not a performance factor in males. However, previous research has found that females rely on the CA3 region of the hippocampus more than the DG to solve spatial tasks (Mendez-Lopez et al., 2009). Furthermore, it has been found that females increase synaptic contacts between CA3 cells

and the mossy fibers projecting from the DG, perhaps as a compensatory mechanism for fewer granule cells (Madeira et al., 1991). Recalling a spatial location likely involves the DG and CA3 (Kesner, 2007) and perhaps the sexes differentially depend on these structures. Therefore, a larger DG in females may be detrimental if this prevents the necessary connections to the CA3 that they rely on for the OLM task.

BrdU is a marker of DNA synthesis and is currently the most common label to study neurogenesis and thus its pitfalls are often discussed at a histological level (Taupin, 2007). However, the idea that injecting BrdU could alter animal behaviour is not at the forefront in the literature with the only reports primarily limited to the effects of prenatal or perinatal exposure. For instance, BrdU injections given to pregnant rats result in significant problems for the offspring including physical deficits, such as cleft palate (Taupin, 2007), abnormal incisor growth (Kolb et al., 1999), and neural abnormalities, including defects in proliferation and migration of cells in the cerebellum (Sekerikova et al., 2004). Behaviourally, prenatal injections of BrdU have been found to negatively alter performance during spatial tasks in adulthood (Kolb et al., 1999), but injections of BrdU in adulthood typically show no behavioural deficits (Taupin, 2007). Nevertheless, the behavioural differences between the BrdU injection groups in this study were striking and cannot be ignored. An injection of BrdU increased the exploration behaviour of males compared to their non-injected counterparts, indicated by the amount of time spent with objects. Conversely, females injected with BrdU decreased their exploration of objects compared to females who did not receive BrdU. This led the BrdU-females to spend significantly less time with the objects than the BrdU-males. As the sexes were differentially affected by the injection of BrdU, behavioural changes would be hard to

detect when investigating a single sex (typically males) and could account for the lack of behavioural findings in the literature. Furthermore, it appears that it was exploration behaviour that was affected and thus this behavioural change may not be detectable in other behavioural paradigms, such as the MWT.

Chapter Three

Experiment Two: The Object Location Memory Task after Adrenalectomy

The granule cells of the DG express both mineralocorticoid and glucocorticoid receptors and require some level of circulating corticosterone (CORT) to avoid apoptotic processes (Joels, 2007). Consequently, bilateral adrenalectomy (ADX) which results in CORT abolition causes the granule cells of the DG to degenerate, a progression that begins within days after surgery and continues for months (Sloviter et al., 1993). This degeneration is limited to the DG, leaving other regions of the hippocampus intact (Sloviter et al., 1993). However, to be effective both adrenal glands must be successfully removed; otherwise the surgery is considered incomplete and no behavioural differences are found (Armstrong et al., 1993). Even miniscule amounts of circulating glucocorticoids will leave the granule cell layer mostly intact (Sloviter et al., 1993).

Consistent with a role for the DG in spatial memory, adrenalectomized rats that exhibit granule cell loss also exhibit impaired performance on spatial tasks, such as the MWT (Roosendaal et al., 1998; Spanswick et al., 2007) and the RAM (Vaher et al., 1994). ADX does not alter activity or exploration behaviour, thus deficits likely reflect impaired spatial memory (McCormick et al., 1997). Further, as CORT replacement was given to all rats for one-week prior to (and throughout) testing in the above studies, it is not likely that these differences are due to the lack of CORT, which can contribute to sex differences during spatial tasks (Beiko et al., 2004). Rather, the deficits observed in the above studies likely reflect impairments in the DG, which does not exhibit significant recovery during the span of CORT replacement. Accordingly, it may be that

adrenalectomized rats may exhibit deficits in other spatial tasks, such as OLM. As of yet, no studies have tested the effect of ADX on an OLM paradigm.

This experiment investigates the effect of ADX and sex in the OLM task. I expect rats with a complete ADX to have a significantly smaller DG compared to those with either incomplete ADX (INC-ADX) surgeries or sham surgery rats. Given the results of Experiment, I expect males to have a significantly larger DG than females for both the sham surgery and INC-ADX groups. I predict that sham surgery females will have superior performance on the OLM task compared to sham surgery males, as previously shown by Saucier et al. (2008). Specifically, I predict that sham surgery female rats will have significantly increased exploration with the objects that exchange position on the testing day compared to the objects that remained in a constant position and that these females will have increased exploration with the objects that have been exchanged compared to the previous training session (training session four). Further, I hypothesize that ADX rats will exhibit inferior performance on the task compared to INC-ADX rats and sham surgery rats, regardless of sex, due to the decreased volume of the DG.

Methods.

Subjects.

Subjects for this experiment were Long-Evans rats (21 males, 22 females; Charles River). Housing and feeding conditions were the same as in Experiment 1. All experiments were approved and conducted according to The University of Lethbridge Animal Welfare Committee guidelines.

Adrenalectomy.

All rats received buprenorphine (0.05 mg/kg, i.p.) approximately ten minutes before surgery to reduce post-operative pain. The rats were anesthetized using a 4% concentration of isoflurane, and maintained at decreasing concentrations throughout the surgical procedure. Rats underwent either a bilateral adrenalectomy (ADX; 13 males and 14 females) or sham surgery (Controls; eight males and eight females). The average weight for males at the time of surgery was 320 g (postnatal day 57-62) and the average weight for females was 235 g (postnatal day 64-71). The ADX procedure consisted of a three cm incision through the skin on the left and right flank. The underlying muscle was separated in order to access the inner abdomen adjacent to each kidney. Both adrenal glands and a small amount of surrounding adipose tissue were removed using forceps and/or small scissors. The fascia overlying the abdominal muscles was sutured along with the flank incisions. The sham surgery consisted of a three cm incision through the skin on the left and right flank, followed by suturing the skin bilaterally. Polysporin® was applied to the incision area and the rats were placed individually into a home cage on a heating pad until they awoke from anesthetic. Rats were housed individually for 24 hours to observe recovery and to ensure that their sutures remained intact before being returned to their home cages. Immediately post-surgery, and for the rest of the duration of the study, ADX rats were given 0.9% saline as a replacement for drinking water to maintain their electrolyte balance. Rats were weighed once weekly for ten weeks, as attenuated weight gain can be used as an indicator of successful ADX (McCormick et al., 1997), and were also weighed daily throughout the behaviour paradigm. Rats were handled daily for

one week before the onset of testing. Male rats began behavioural testing on postnatal day 130-136 and female rats began on postnatal day 136-142.

Hormone treatment.

Replacement CORT was provided to all ADX rats for one week prior to behavioural testing and continued throughout the testing period. One mg of CORT was administered daily suspended in 2.5 ml of sesame oil and delivered orally through a 1 g cookie. Control rats were given 2.5 ml of sesame oil on a 1 g cookie.

Behavioural testing.

The OLM task was performed as in Experiment 1. To ensure granule cell death in ADX rats (McCormick et al., 1997; Roozendaal et al., 1998; Sloviter et al., 1993), rats began behavioural testing 70 days after surgery. All rats were euthanized 24 hours following completion of the experiment. Euthanasia and brain fixation followed the same procedures as Experiment 1.

Histology.

Frozen coronal sections were cut with a microtome at a thickness of 40 μm and 12 series were taken and stored in 1 X PBS with a 1:1000 concentration of sodium azide. One series of tissue (i.e., every twelfth section) were stained with DAPI using a PBS buffer. Sections were mounted onto glass slides and covered with a glass slip using fluorescent mounting media. DG volume was computed with Stereo Investigator® using the same methods as in Experiment 1.

Analyses.

All analyses compared the last training session (training session four), when the rats were the most familiar with the configuration of the objects, with their behaviour on the

testing day. The total number of interactions and the total time spent interacting with the objects were the dependent behavioural measures. For behavioural analyses, the Control group was combined with the INC-ADX group to compare the behaviour of rats with an intact DG (non-ADX group) with those who underwent successful ADX. Importantly, INC-ADX has previously been found to have no effect on behaviour (Armstrong et al., 1993). The behavioural measures were analyzed with a 2 x 2 x 2 x 2 (Object [Exchanged objects, Constant objects] x Day [training session four, testing day] x Sex [male, females] x Group [ADX, non-ADX) mixed measures ANOVA using PASW Statistics 17.0.

Results.

DG volume estimates.

Successful ADX was defined as any volume estimate of the DG that fell below three standard deviations from the mean volume of the same-sex Control group (i.e., below 2.239 mm³ for males and below 2.196 mm³ for females). Those ADX rats that have volumes larger than that were considered to have an incomplete adrenalectomy (INC-ADX; Figure 8). For males, there were seven successful ADX surgeries, $M = 1.443$ mm³, $SD = .288$ mm³, leaving six INC-ADX, $M = 2.716$ mm³, $SD = .126$ mm³ and eight controls, $M = 2.955$ mm³, $SD = .237$ mm³. For females, there were eight complete surgeries, $M = 1.366$ mm³, $SD = .621$ mm³, and six INC-ADX, $M = 2.629$ mm³, $SD = .239$ mm³ and eight controls, $M = 2.586$ mm³, $SD = .130$ mm³. A linear regression revealed that the weight of male rats on perfusion day, $M = 544.47$ g, $SD = 78.08$, was a significant predictor of the volume of the DG, $R^2 = .560$, $F(1, 20) = 24.17$, $p < .0001$, but was not a significant predictor for females, $M = 329.22$ g, $SD = 28.09$, $R^2 = .004$, $F(1, 20) = .078$, $p = .783$ (Figure 9).

For DG volume, a univariate ANOVA with sex (male, female) and group (sham surgery, INC-ADX, ADX) as the between subjects measures failed to reveal an effect of Sex nor a Sex x Group interaction for DG volume. There was a significant effect of Group, $F(2, 37) = 77.562, p < .0001$, with a Bonferroni post hoc analysis detecting no difference between the Controls, and INC-ADX groups; however, both of these groups were significantly larger than the ADX rats (Figure 10).

Rats that did not exhibit reductions in DG volume (i.e., sham surgery and INC-ADX rats; $n = 28$) were combined into one group (non-ADX) and DG volume was re-examined for sex differences in volume. Male non-ADX rats had significantly larger DG volumes than female non-ADX rats, $F(1, 26) = 10.338, p = .003$ (Figure 11). ADX rats, when examined by themselves, did not exhibit a sex difference in DG volume, $F(1, 13) = .090, p = .769$.

Behavioural analysis- interactions with objects.

The ANOVA revealed a significant main effect of Sex $F(1, 39) = 8.441, p = .006$ but no effect of Group. There were trends for an Object x Sex interaction $F(1, 39) = 3.495, p = .069$, an Object x Day interaction, $F(1, 39) = 3.282, p = .078$ and an Object x Day x Sex interaction $F = 3.161, p = .083$. Independent samples t-test revealed that females interacted more with Exchanged objects compared to males during training session 4, $t(41) = -2.357, p = .023$. There was a tendency for females to interact more with the Constant objects, $M = 13.909, SD = 3.803$, than males, $M = 11.666, SD = 3.705$ during training session four, $t(41) = -1.957, p = .057$. Females also spent significantly more time with Exchanged objects than males on the testing day, $t(41) = -3.751, p = .001$. A paired samples t-test revealed that female rats had significantly more interactions

with the Exchanged objects on the testing day compared to the interactions with Constant objects, $t(21) = 2.243$, $p = .036$ and compared to the number of interactions with the Exchanged objects during training session four, $t(21) = 2.752$, $p = .012$ (Figure 12). The same analyses failed to reveal significant differences in the male behaviour.

A linear regression was performed with DG volume as the criterion and the total interactions and total time with objects (Constant and Exchanged) during training session four and the testing day as predictors. The regression failed to reach significance, $R^2 = .042$, $F(3, 42) = 0.420$, $p = .793$.

Behavioural analysis- time with objects.

ANOVA failed to reveal a main effect of Sex or Group for the amount of time spent with the objects. There was a significant effect of Day, $F(1, 39) = 6.036$, $p = .019$, with the rats spending significantly more time with the objects on the testing day, $M = 44.663$, $SD = 2.051$, than during the previous training session, $M = 37.180$, $SD = 2.076$. There was a significant Object x Sex interaction, $F(1, 39) = 6.803$, $p = .013$, with female rats spending more time with the Exchanged objects, $M = 92.945$, $SD = 26.924$, $p = .002$, than males, $M = 68.524$, $SD = 22.043$; although this can mostly be attributed to the female behaviour during the testing day, where females spent significantly more time with the Exchanged objects, $M = 56.599$, $SD = 21.979$, $t(41) = -3.721$, $p = .001$, compared to males, $M = 36.560$, $SD = 11.468$. Accordingly, the Object x Sex x Day interaction, neared significance at $F(1, 39) = 1.932$, $p = .172$.

A linear regression was performed with DG volume as the criterion and the predictors were the total interactions and total time with objects (Constant and

Exchanged) during training session four and the testing day. The regression failed to reach significance, $R^2 = .074$, $F(3, 42) = 0.756$, $p = .560$.

Discussion.

Successful ADX led to a reduced volume of the granule cell layer of the DG. As expected, the DG in ADX rats was significantly smaller than that of sham surgery and INC-ADX rats, regardless of sex. These results are consistent with other reported effects of ADX on the DG (Sloviter et al., 1993; Spanswick et al., 2007). There was a significant sex difference in DG volume for rats that did not undergo successful ADX (i.e., sham surgery and INC-ADX rats). This finding is consistent with the volumetric sex difference found in Experiment 1 and suggests that this sex difference is consistent from at least postnatal day 60 until postnatal day 130 in Long Evans rats. Moreover, there was no sex difference in volume for ADX rats, suggesting that the sexes undergo the same degenerative processes after removal of the adrenal glands. Consistent with Experiment 1, behaviour during the OLM task did not predict DG volume.

Consistent with other studies (McNeil et al., 1991), weight was a significant predictor for complete ADX; however this was only found for male rats. Indeed, the sham surgery females weighed less on average than the ADX or INC-ADX females at the end of behavioural testing. It is possible that the individual differences in size outweigh the metabolic effects of ADX in females. These results suggest that weight gain is not a predictive element of ADX in female rats, although this has been repeatedly found with male rats (McCormick et al., 1997; McNeil et al., 1991). Future research should investigate CORT assays or saline consumption as corroborating evidence of successful

ADX in females, as these are also known indicators for successful ADX in males (McCormick et al., 1997; McNeil et al., 1991; Spanswick et al., 2007).

Female rats interacted more with the objects overall, which differs from the results of Experiment 1, but is consistent with other reports of females displaying more exploratory behaviour than males (Lynn & Brown, 2009; Russell, 1975). Females also displayed superior OLM compared to males. In other words, they significantly increased their exploration of the exchanged objects on the testing day compared to the constant objects, and this increase was significant when compared to the previous training session. In contrast, male behaviour remained relatively unchanged during the testing day compared to the fourth training session. Importantly, this sex difference in behaviour was observed regardless of surgery-type; there were no surgery effects or interactions detected for the OLM task. These results differ from previous studies that found that ADX resulted spatial memory impairment (Rooszendaal et al., 1998; Spanswick et al., 2007; Vaher et al., 1994); however, these studies focused on different spatial tasks (MWT, RAM) after ADX.

Unlike the tasks that showed performance deficits following ADX, the OLM task may be measuring topographical spatial memory as opposed to Euclidean or metric spatial information. Recall that topological spatial memory is characterized by the memory for relative relationships between objects, as opposed to precise distances and angles that are important for accurate metric spatial memory (Goodrich-Hunsaker et al., 2008). The parietal cortex (PC) is associated with processing topological information (Goodrich-Hunsaker et al., 2005), thus future studies should investigate the role of the PC in OLM.

CHAPTER FOUR

General Discussion

Experiment 1 investigated the relation between performance of OLM and the volume of the DG in male and female rats. I expected that memory for object locations would be reflected by number of interactions and duration of exploration, such that rats would explore the objects that exchanged positions more than the objects that remained in the same location. Based on the results from Saucier et al. (2008), I expected females to exhibit this behaviour more so than males, indicating superior OLM in females. As predicted, all rats spent significantly more time and had more interactions with the objects that were exchanged on the testing day compared to those that remained in a constant location. The exploration behaviour with the exchanged objects on the testing day also significantly increased compared to the previous training session. This is consistent with other research that finds rats will spend more time investigating a novel object or with objects that have exchanged location with each other (Dix & Aggleton, 1999; Ennaceur et al., 1997; Saucier et al., 2008). However, in contrast to the findings of Saucier et al. (2008), there was no significant sex difference for exploration of the exchanged objects on the testing day, indicating no sex difference in OLM.

Importantly, it appears that the behaviour in Experiment 1 was confounded by the BrdU injections that differently affected male and female behaviour. A significant interaction revealed that males that received BrdU increased their time spent interacting with the objects whereas females that received BrdU decreased the amount of time they spent interacting with the objects. Furthermore, males that received BrdU also spent more time interacting with the objects more than their non-injected counterparts, whereas

females injected with BrdU spent less time with objects compared to females that did not receive BrdU. This interaction may also explain the unexpected main effect of sex, where males spent significantly more time with all the objects across days compared to females. This finding was not anticipated as BrdU injections into adult rats are typically not found affect behaviour (Taupin, 2007). However, prenatal exposure to BrdU can cause both physical deficits and impaired spatial abilities in adulthood (Kolb et al., 1999). For instance, BrdU injections given to pregnant rats result in cleft palate (Taupin, 2007), abnormal incisor growth (Kolb et al., 1999), and neural abnormalities, including defects in proliferation and migration of cells in the cerebellum (Sekerikova et al., 2004). Rats receiving prenatal BrdU treatment also showed impaired memory during the MWT, although this may have been due to impaired motor and perceptual skills (Kolb et al., 1999). Thus, the possibility of BrdU injections causing differential effects on behaviour in males and females requires further investigation.

Although males had a significantly larger volume of the DG, behaviour during the OLM task was not a significant predictor for DG volume for either sex. However, it cannot be concluded that DG volume is entirely unrelated to OLM due to another unanticipated finding. It was discovered that females who had an above average DG volume (compared to the rest of their cohort) performed more poorly than all males on the OLM task. Females with a below average DG volume also exhibited superior performance to females with an above average DG volume, although this was not significant. Thus, a larger than average DG was related to poor spatial memory in females. In contrast, there was a tendency for the amount of time spent with exchanged objects on the testing day to be a predictor for DG volume in males.

The reason for a large DG volume to be related to poor OLM in females is not currently clear. It may be that the DG is not directly contributing to the OLM task, but perhaps a large DG in females interferes with other important connections to solve this task. Females have a higher volume of mossy fiber synapses in the CA3 area of the hippocampus compared to males, which may compensate for the fewer granule cells found in the DG (Madeira et al., 1991). Furthermore, it is possible that females rely on the CA3 region of the hippocampus more than the DG to solve spatial tasks (Mendez-Lopez et al., 2009). Both the DG and CA3 contribute to the memory of spatial locations (Kesner, 2007) and it may be that the sexes differently depend on these structures, given the diverse effects of steroid hormones on these regions (Hajszan, et al., 2007; Isgor & Sengelaub, 2003). Accordingly, there was a tendency for the amount of time spent with the exchanged objects on the testing day to be a predictor for DG volume in males, but not for females. In conclusion, the DG involvement for OLM was not made apparent from the results of this experiment, but it appears to differ between the sexes.

Experiment 2 investigated the effects of sex and ADX in the OLM task. I expected rats with complete ADX to have a significantly smaller DG compared to those with either INC-ADX surgeries or sham surgery rats. Given the results of Experiment, I expected males to have a significantly larger DG than females for both the sham surgery and INC-ADX groups. As predicted, rats with successful ADX possessed significantly smaller DG compared to sham surgery rats and rats with INC-ADX. Indeed, the ADX rats had an approximately 50% smaller DG compared to the other groups. Also as predicted, the volume of the DG was significantly larger in males compared to females for both sham surgery rats and INC-ADX rats.

I hypothesized that successfully adrenalectomized rats would exhibit inferior performance on the task compared to INC-ADX rats and sham surgery rats, regardless of sex, due to the decreased volume of the DG. Contrary to predictions, there was no significant effect of ADX on behaviour. Both males and females who received successful ADX performed in a manner similar to their sham surgery counterparts. Indeed, behaviour during the OLM task was not a predictor for DG volume for either sex. Thus, the findings from Experiment 2 suggest that an intact DG is not critical for OLM because ADX had no effect on behaviour. There are competing theories regarding the functioning of the dorsal region of the hippocampus, including the DG region damaged through ADX. A dissociation of function between the PC and the dorsal hippocampus has been proposed (Goodrich-Hunsaker et al., 2005) suggesting that the latter is responsible for metric information processing and the former is responsible for topological information processing. Recall that topographical information includes remembering where objects are located in relation to one another, whereas metric information includes measurable distances and cardinal directions (Dabs et al., 1998).

Investigations of how lesions to either the PC or the dorsal hippocampal region affected behaviour during topographical or metric spatial memory tasks (Goodrich-Hunsaker, et al., 2008), demonstrated that lesions to the PC caused significant memory impairment for the topological task, whereas the lesions to the dorsal hippocampus caused significant impairment for the metric task. It is important to note that the topographical task was similar to the OLM task used here, in that the rats were habituated to an array of objects, two of which exchanged locations on a subsequent trial. Therefore, if the OLM task used here is a measure of topographical memory, then these results are

consistent with the theory presented by Goodrich-Hunsaker and colleagues, which suggest that damage to the dorsal hippocampus would have no effect for this type of memory.

I predicted that sham surgery females would perform better on the OLM task compared to sham surgery males, as previously demonstrated by Saucier et al. (2008) and as predicted for Experiment 1. Surprisingly, all females in this experiment displayed superior OLM to males, regardless of surgery and despite the decreased DG volume in the ADX rats. Comparable to the findings by Saucier et al. (2008), female rats had significantly more interactions with the exchanged objects on the testing day, compared to the interactions with objects that remained in a constant position. These interactions significantly increased compared to the number of interactions with the exchanged objects during training session four. The males, however, showed no differences in behaviour from the previous training session and did not discriminate between objects on the testing day.

The females in this study exhibited superior OLM compared to the males, a finding that has previously been established in humans (Eals & Silverman, 1994; James & Kimura, 1997; McBurney et al., 1997; Tottenham et al., 2003) and in rats (Saucier et al., 2008). The female superiority for OLM found in Experiment 2 is also consistent with experiments that find females exhibit superior topographical memory compared to males (Saucier et al., 2002; Dabs et al., 1998). Accordingly, research that finds ADX causes impaired spatial ability (Spanswick et al., 2007; Roozendaal et al., 1998) has used behavioural measures that require metric information as opposed to topographical information. Therefore one alternative explanation is that the OLM task is selectively

engaging the PC and thus damage to the DG after ADX would not cause memory impairments. However, the contributions of the DG, CA3 and CA1 to spatial memory are currently debatable (Goodrich-Hunsaker, et al., 2008). Accordingly, the OLM task may rely on any combination of these structures rather than the PC.

Conclusions

A clear sex difference for the size of the DG is reported in this thesis, with males possessing a significantly larger volume than females. This is consistent with reports for mice (Wimer & Wimer, 1989), meadow voles (Galea et al., 1999), Sprague-Dawley rats (Roof & Havens, 1992) and guinea pigs (Severi, et al., 2005) but differs from some reports (Isgor & Sengelaub, 1998; Jones & Watson, 2005). The findings in this thesis suggest that a sex difference for DG volume persists from at least postnatal day 60 (Experiment 1) until postnatal day 130 (Experiment 2) in Long Evans rats. This is consistent with previous reports that male rats and other polygynous mammals possess a greater overall hippocampal volume compared to females (Jacobs, et al., 1990; Nunez et al., 2000; Williams et al., 1990). Larger hippocampal volume has been correlated to superior spatial behaviours (Biegler et al., 2001; Maguire et al., 2000), but a larger DG volume did not correlate with superior OLM. Indeed, a larger DG volume was related to poor OLM in females (Experiment 1) and females that displayed an OLM advantage over males (Experiment 2) also had a significantly smaller DG volume.

Contrary to predictions, behaviour during the OLM task did not predict the volume of the DG. This is consistent with the finding from Experiment 2, that a 50% decrease of granule cells in the DG had no impact on OLM. However, it does not appear that the DG is irrelevant, given that a larger than average DG volume was found to be

significantly related to poor OLM in females. Furthermore there was a tendency for behaviour during the testing day to be a predictor for DG volume in males, although this was not significant. There is evidence that the DG is important for detecting changes within an environment, including object placement (Hunsaker et al., 2008). Thus, it is likely that the DG is necessary for some element of OLM. The role of the DG in the OLM task may become apparent if assessed in conjunction with the mossy fiber synapses and the CA3. All three of these components of the hippocampal circuit work collectively to encode spatial information (Kesner et al., 2004). Males and females may utilize these structures differently while solving spatial tasks (Mendez-Lopez, 2009) because of structural differences between the sexes (Madeira et al., 1991; Isgor & Sengelaub, 1998) and the activational effects of hormones (Hajszan et al., 2007).

A female superiority for OLM was found in Experiment 2, but no difference was observed for Experiment 1. The protocols for the OLM task were precisely the same for both experiments but it is possible the BrdU injections administered in the first experiment masked a possible sex difference. The BrdU injections had a significant effect on behaviour that differentially affected each sex. Although there was no sex difference found for OLM between the rats that did not receive BrdU injections, this was likely due to the reduced sample size for analysis. The age difference between the rats in Experiment 1 and Experiment 2 was also considered for a possible confound because the first cohort was approximately 70 days younger than the second cohort. However, the rats that did not exhibit a sex difference for OLM (Experiment 1) were the same age and weight as the rats in the Saucier et al. (2008) experiment, where a female advantage was established. Furthermore, all rats had reached adulthood at the time of testing and thus

possible differences in post-pubertal hormone levels should not have affected performance between groups.

Although research typically finds that males are superior at spatial tasks (Kimura, 1999; Jonasson, 2005; McCarthy & Arnold, 2008), this thesis provides evidence that the sexes demonstrate differences in ability, depending on the task (Eals & Silverman, 1994; Ecuyer-Dab & Robert, 2004; Gaulin & Fitzgerald, 1986). This thesis contributes to the validity of the OLM task developed by Saucier et al. (2008) as a way to study OLM in rats that is comparable to the human literature. Females' superior ability for OLM has previously been attributed either to the differences in the range size of polygynous species (Ecuyer-Dab & Robert, 2004; Gaulin & Fitzgerald, 1986) or to the division of labor between the sexes (Eals & Silverman, 1994). The rats in the present experiments do not maintain a division of labour but they are a polygynous species. Hence, the data here support the polygynous-range size hypothesis for sex differences in spatial ability, which posits that females would have acquired superior OLM from maintaining a small range size and concentrating on nearby spatial cues.

Suggestions for improvement.

The experiments presented here would have benefited from volume estimates of the entire hippocampal structure. This would allow comparisons of the DG as a ratio to overall hippocampal volume. The volume of the DG may be more informative if quantified in comparison to the rest of the hippocampus. It has been proposed that the DG and CA3 work in combination to encode spatial locations (Kesner et al., 2004) and that females rely on the CA3 of the hippocampus more so than the DG for spatial memory (Mendez-Lopez et al., 2009). Thus, it may have been beneficial to analyze the volume of

the CA3 in conjunction with the DG analyses. Similarly, correlating the number of mossy fiber synapses with DG volume may provide insight to the finding that a large DG was related to poor OLM in females. Furthermore, testing rats on the MWT would have revealed if a large DG in females was related to poor performance for other types of spatial memory. The next objective for this project will be to analyze neurogenesis that was occurring throughout the OLM paradigm, made possible by the BrdU injections. Both the extent of neurogenesis and the number of surviving neurons has been correlated to spatial learning and memory (Ambrogini et al., 2000; Dupret et al., 2008). Therefore, this data may provide greater insights into the DG contribution for OLM than DG volume alone.

Future directions for research.

The results presented in this thesis give rise to a plethora of unresolved issues. ADX had no effect on OLM, but a sex difference for the role of the DG was implicated. Further investigations are required to discover what types of manipulations would affect OLM. There appears to be a linear relationship between the amount of DG damage and spatial memory deficits (Kesner, 2007), hence it is possible that more widespread damage would cause OLM impairment. In addition, Goodrich-Hunsaker et al. (2008) propose that the PC would be critical for the OLM task and thus it would be informative to compare behaviour after PC lesions to behaviour after a severe DG lesion. However, the sex difference revealed for OLM (Experiment 2; Saucier et al., 2008) suggests sexually dimorphic hormones and brain structures are involved. Subsequent research should begin by investigating if female's OLM varies in accordance with hormone fluctuations during the menstrual/estrous cycle, which has been found for other spatial tasks (Frye, 1995;

Hausmann et al., 2000; Kimura & Hampson, 1994). It will be important for future studies to investigate the biological substrate underlying the female OLM advantage and also the contributions of organizational and activational effects.

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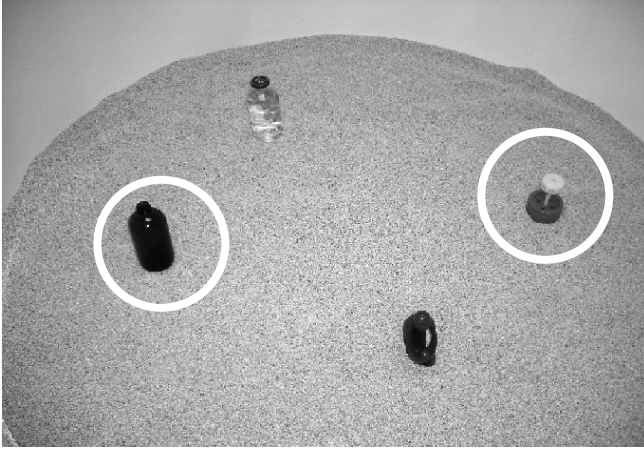
Table 1

The assignment of individual rats to groups

<u>Injection Group</u>	<u>Perfusion Day</u>	
	<u>24 hrs after the testing day</u>	<u>28 days the testing day</u>
No injection controls	(3 males, 2 females)	(4 males, 4 females)
Injection controls (PBS)	(5 males, 5 females)	(3 males, 3 females)
Activity controls (BrdU)	(3 males, 3 females)	(4 males, 4 females)
Learning group ^a	(4 males, 4 females)	(4 males, 4 females)
Memory group ^b	(4 males, 4 females)	(5 males, 5 females)

Note. a = received BrdU injections during the training trials. b = received BrdU injection during the testing trial

A. Training trials



B. Testing day

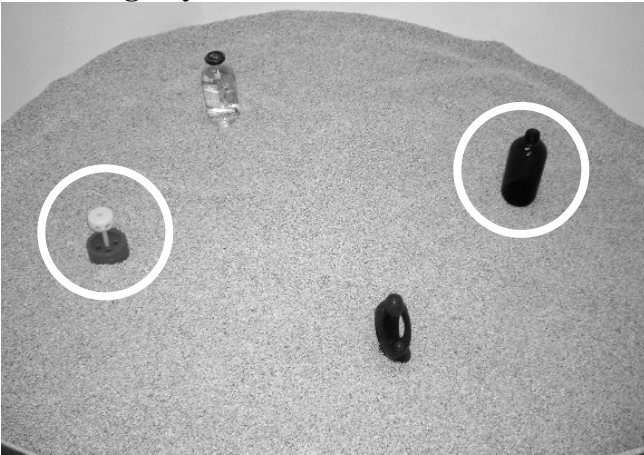
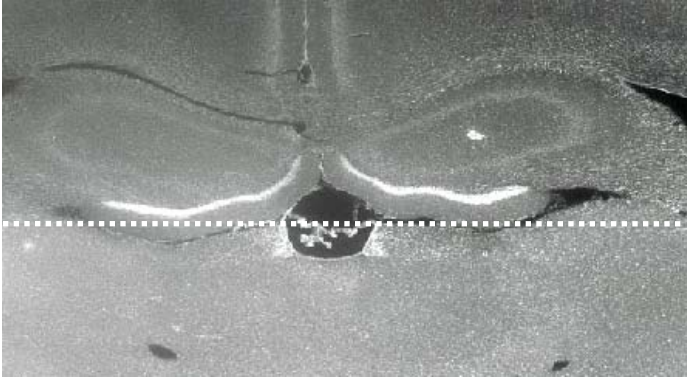
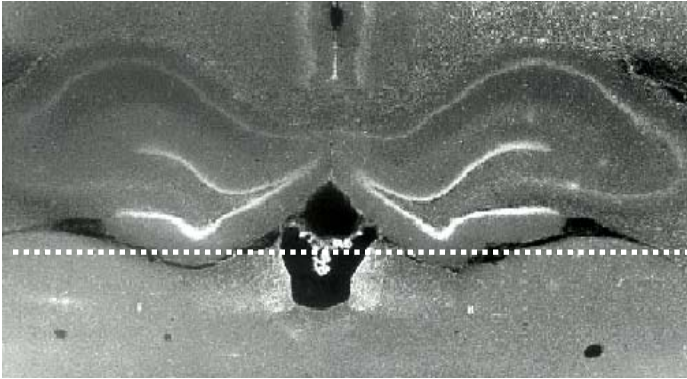


Figure 1. Sample session of the object location memory task during training trials (A) and the testing day (B). Note the switching of the positions of the circled objects.

A.



B.



C.

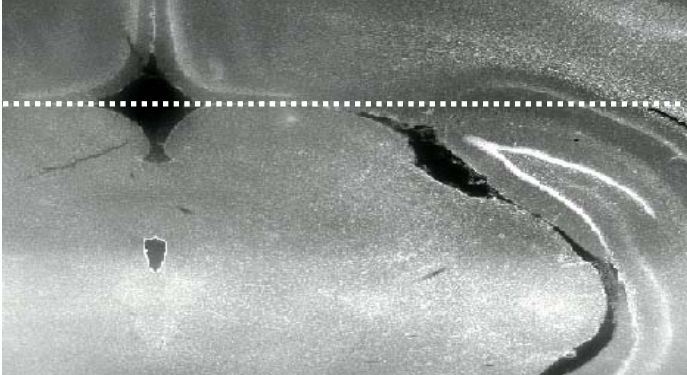


Figure 2. The dorsal region of the dentate gyrus (DG) was defined as any DG appearing above the choroid plexus (**A** and **B**). The DG beginning below the choroid plexus (indicated by the dotted line) was not included in the volume estimate for the dorsal DG, but was included in the volume estimate for the entire DG.

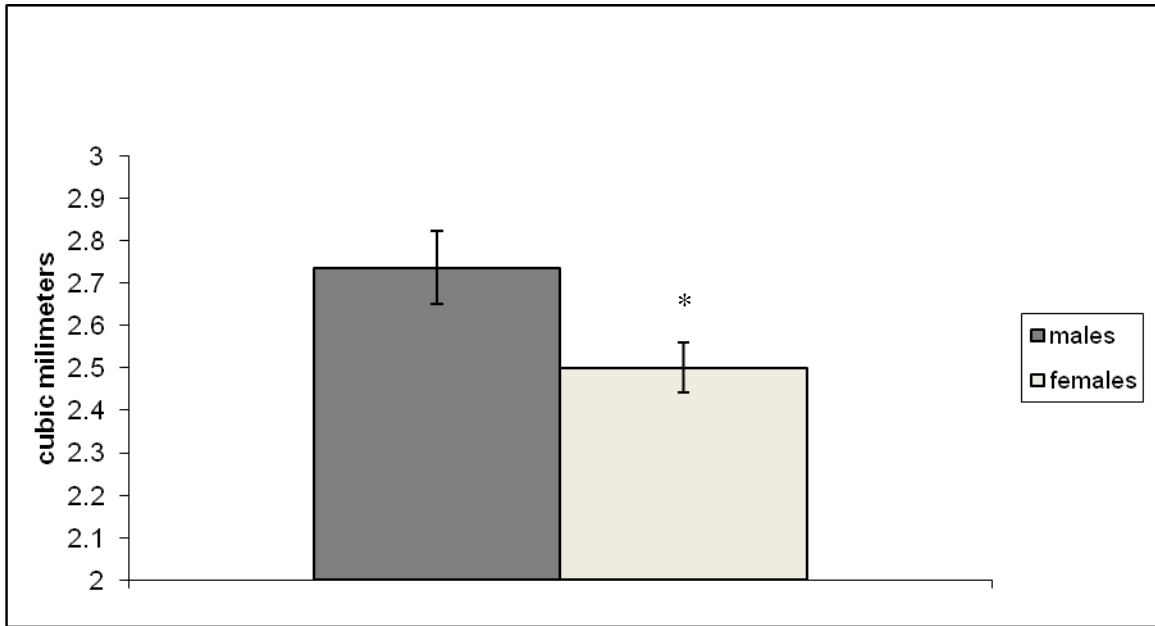


Figure 3. Volume (mm³) of the entire dentate gyrus (DG). Male rats have a significantly larger DG volumes compared to females. Error bars represent the standard error of the mean.

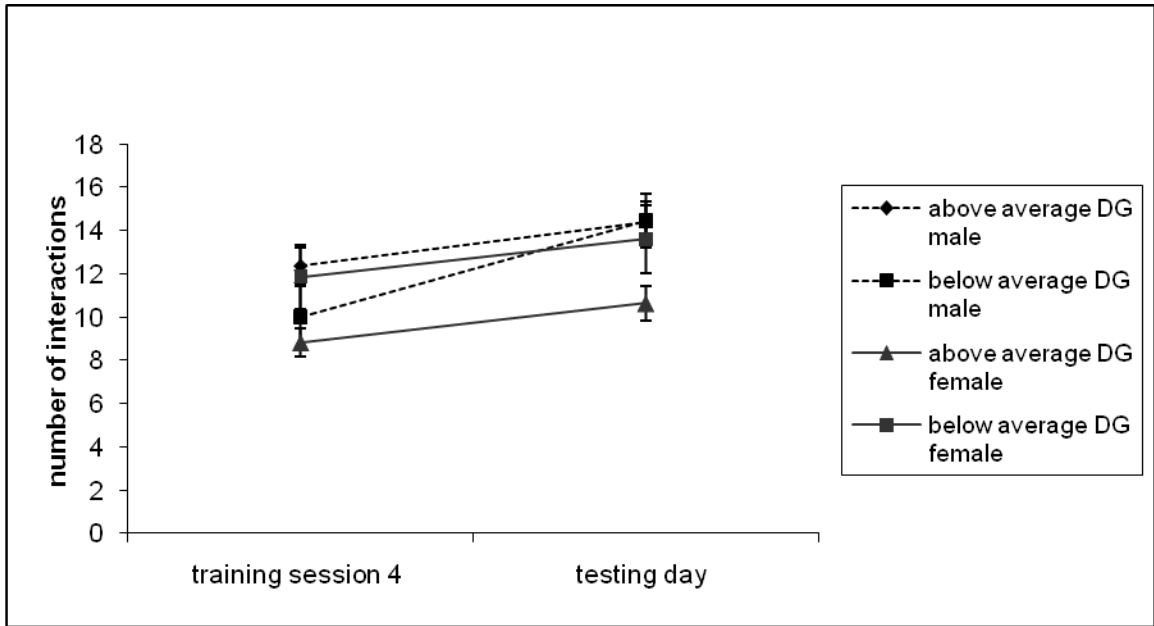


Figure 4: Sum of the total interactions for only the exchanged objects. Females with dentate gyrus (DG) volumes larger than average (above average DG females) spent significantly less time with the exchanged objects on the testing day compared to all other groups. Error bars represent the standard error of the mean.

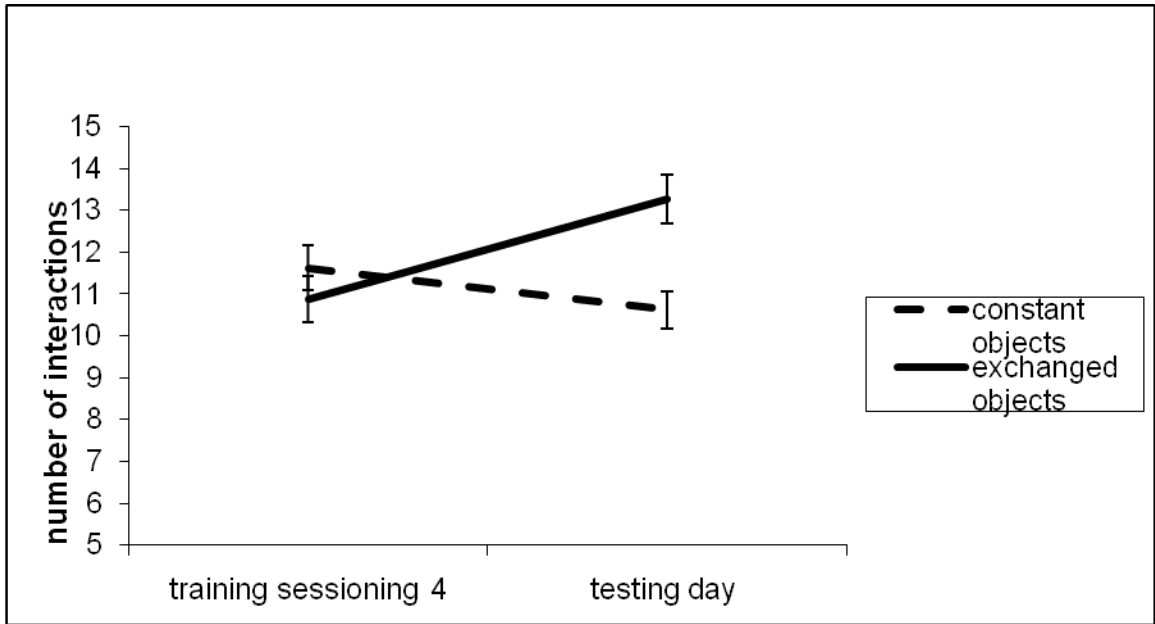


Figure 5: Sum of the interactions with the objects (constant, exchanged). The interactions with the exchanged objects significantly increased from training session four to the testing day. The interactions with the exchanged objects significantly differed from the number of interactions with constant objects on the testing day, but not on the training day. Error bars represent the standard error of the mean.

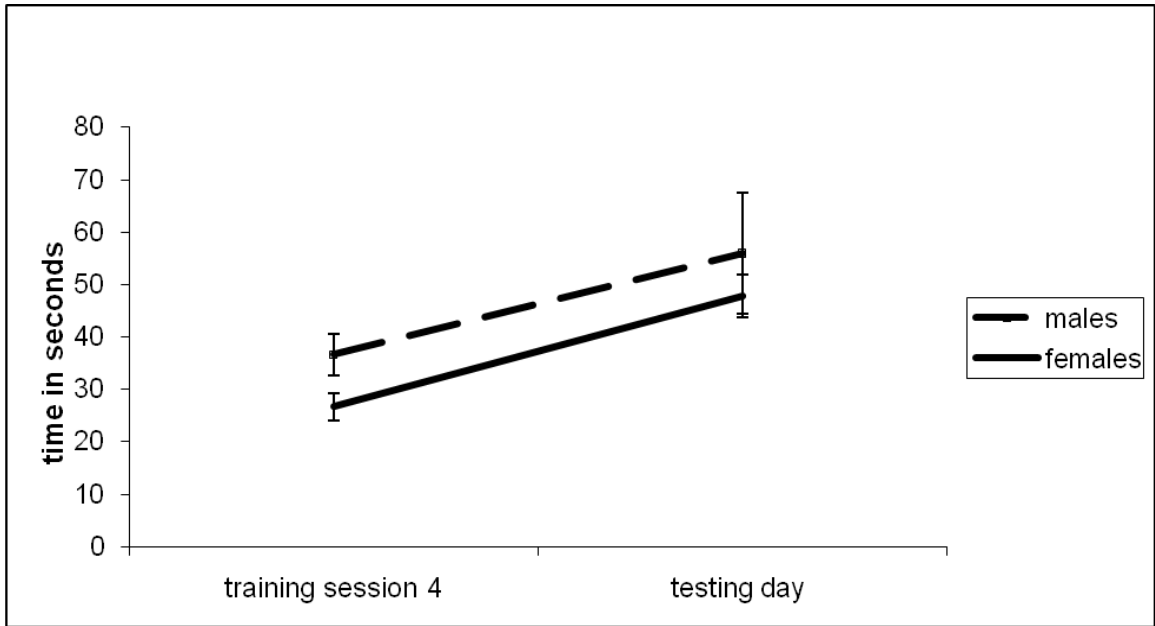


Figure 6. The sum of total time spent interacting with the objects. Only the main effect of sex was significant, with males spending significantly more time with the objects than females. Error bars represent the standard error of the mean.

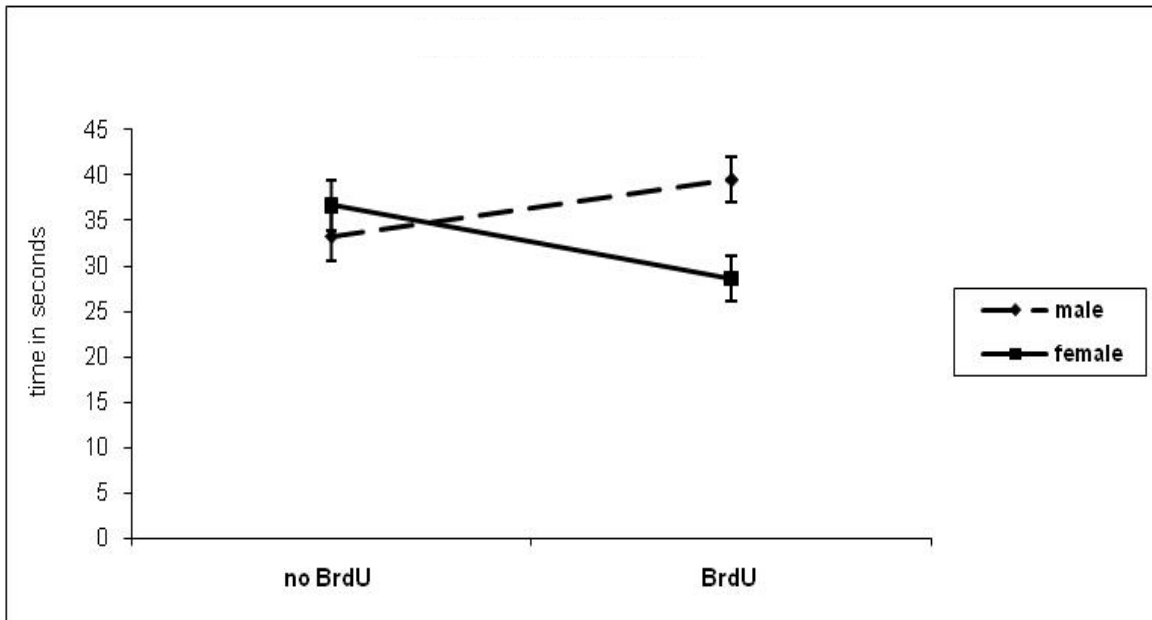
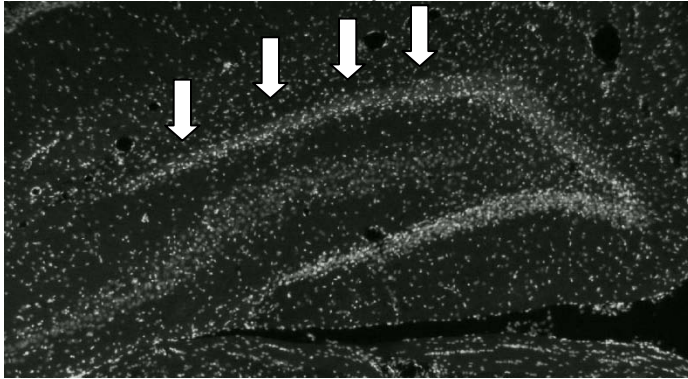
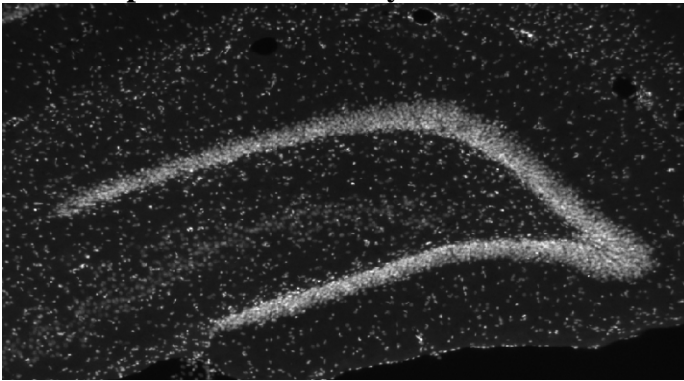


Figure 7. Total time spent interacting with the objects, regardless of day. Males that were injected with BrdU spent significantly more time interacting with objects than females that were injected with BrdU. Error bars represent the standard error of the mean.

A. Successful adrenalectomy



B. Incomplete adrenalectomy



C. Sham surgery

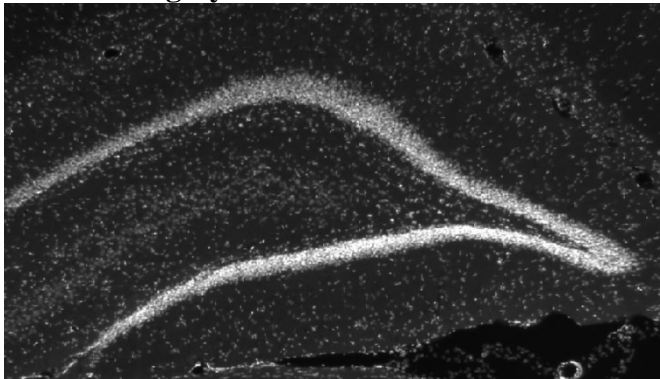


Figure 8. Representative section of the superior blade of the dentate gyrus (DG) taken from successful adrenalectomized (**A**), incomplete adrenalectomized (**B**) and sham surgery (**C**) rats. In picture A, the white arrows are pointing to the granule cell layer of the DG, which is substantially lower in volume.

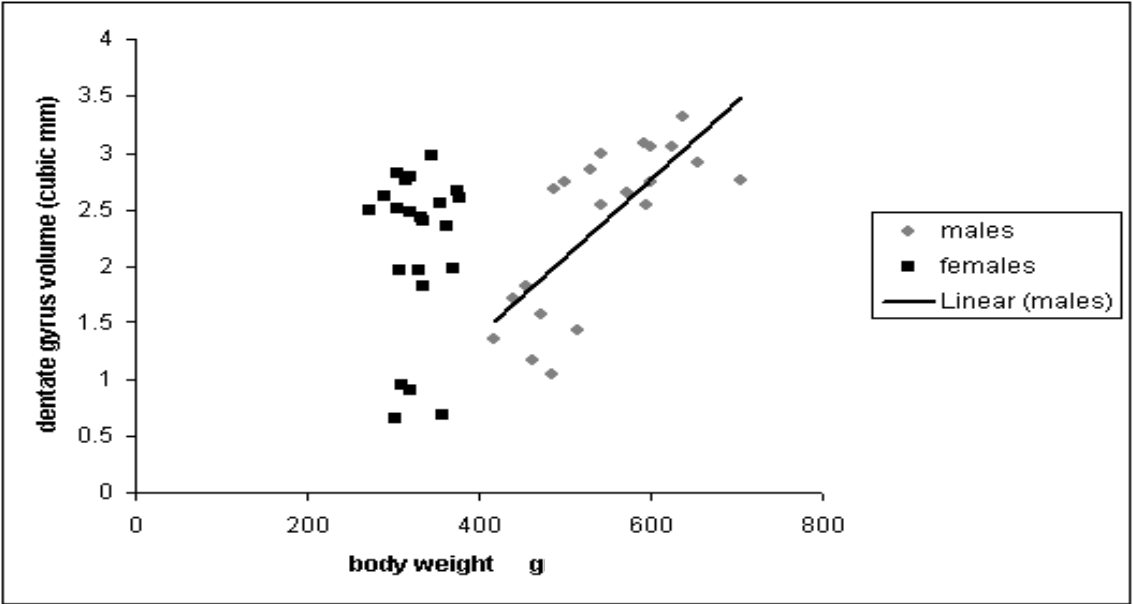


Figure 9. Body weight was a significant predictor for the volume of the dentate gyrus (DG) in males, but not in females. Line represents the linear trend line for males, $y = 0.0069x - 1.3865$.

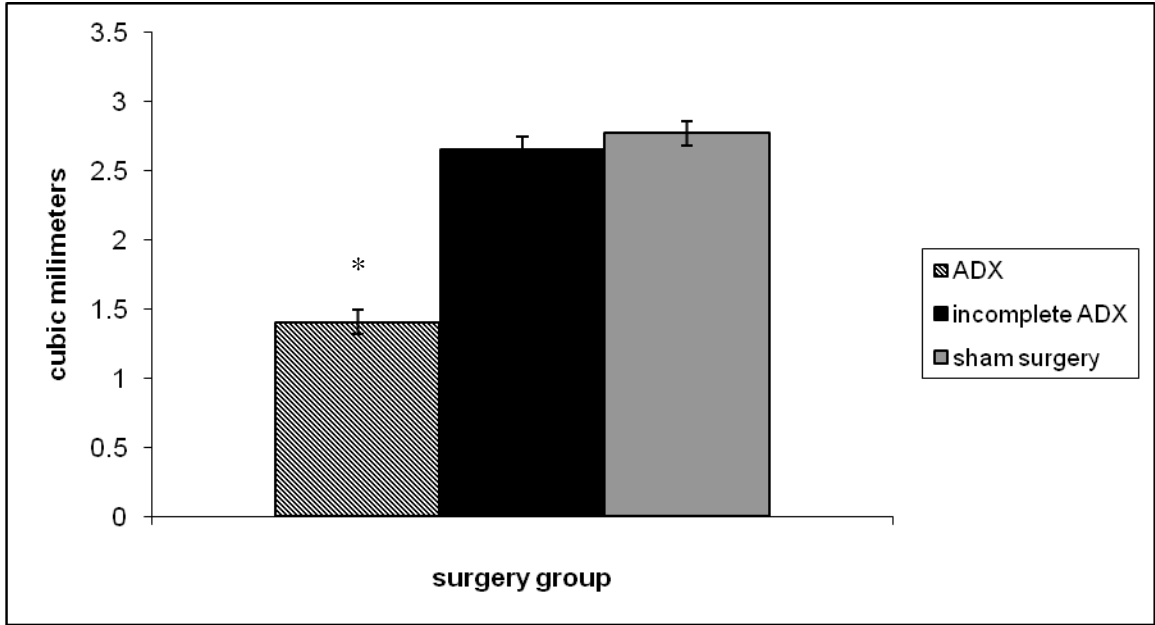


Figure 10. Mean volumes (mm³) of the dentate gyrus (DG) in the surgery groups. Error bars represent the standard error of the mean.

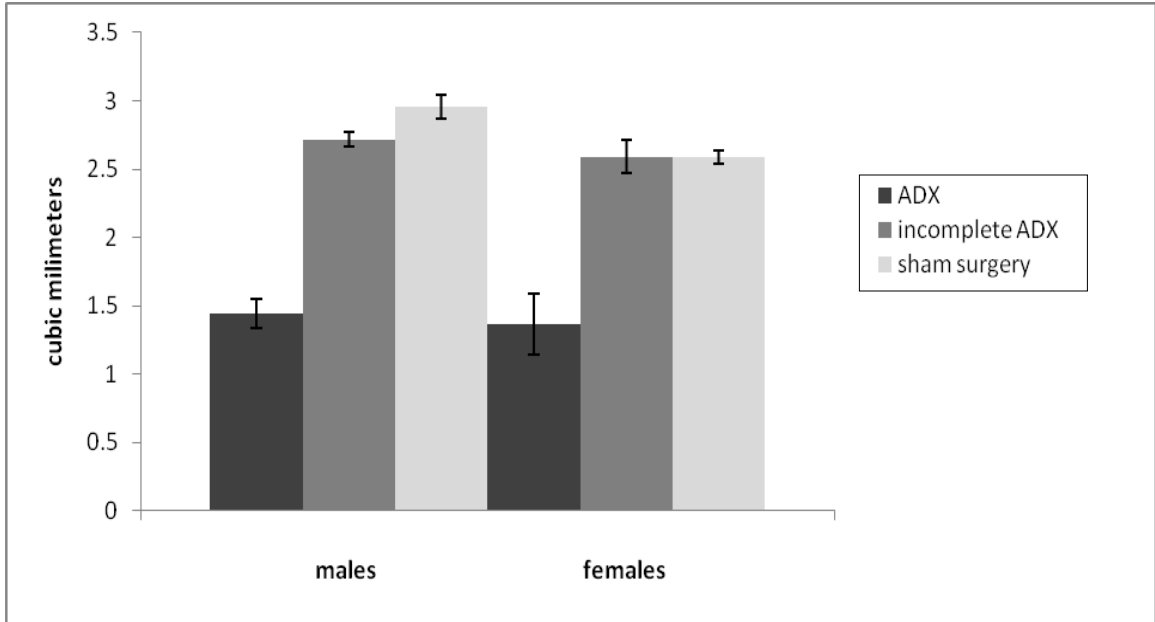


Figure 11. Volume (mm³) of the dentate gyrus (DG.) Male rats have a significantly larger DG volume compared to females in sham surgery and incomplete adrenalectomy (ADX) rats. No significant difference was observed between the male ADX and female ADX rats. Error bars represent the standard error of the mean.

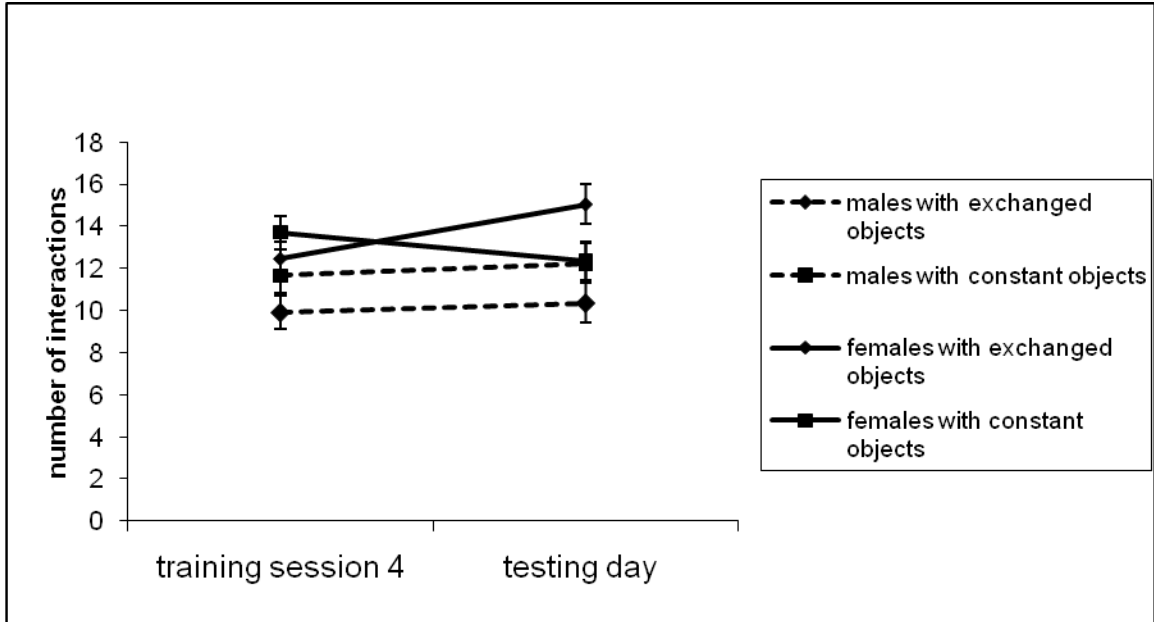


Figure 12. Sum of the total interactions with the objects. Female rats had significantly more interactions with the exchanged objects on the testing day compared to the interactions with constant objects, and compared to the number of interactions with the exchanged objects during training session four. There were no significant differences in behaviour for males. Error bars represent the standard error of the mean.