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Components of Spatial Learning in the Rat

STEVEN LEE POULTER

Department of Psychology

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Abstract

A series of experiments were conducted to investigate the nature of how navigational systems interact in the rat (*Rattus norvegicus*) and the neural structures that support these interactions. The first set of experiments focused on geometry learning and how a reference frame based on the shape of the environment interacted with other non-geometric reference frames. The results revealed that rats were capable of rapidly integrating geometric cues with featural cues in only a single exposure to the cues in compound. This is a novel contribution to the current literature as it opposes the notion that featural information can only be 'pasted on' to a geometric reference frame over time. The effect of the rats' sex on their propensity to use geometric and landmark cues was also investigated. The findings are the first to reveal no difference between male and female rats in the extent to which landmarks overshadow geometry learning when *generalization decrement* is controlled for. However, in a separate task, male rats were able to use both relevant geometric and landmark information better than female rats following changes to the relative reliability of environmental cues. In a separate series of experiments, the navigational strategies rats rely upon and the neural substrates underpinning these strategies was investigated. In a task requiring rats to use the colours of the enclosure walls to locate a hidden goal, it was found that the performance of rats with hippocampal damage and rats with dorsolateral striatum damage was identical to that of normal rats, i.e. they all solved the task using an allocentric strategy over an egocentric strategy. Importantly, the findings revealed that the hippocampus is not required to learn the spatial relationship between differently coloured features. A separate task revealed that hippocampal damage enhanced landmark learning (egocentric), and dorsolateral striatum damage enhanced room cue learning (allocentric) suggesting that these two systems compete for behavioural control in normal rats. Finally, the last experiment revealed that, under certain training conditions, the hippocampus is not critical for the acquisition of a place solution but is more likely involved in a path integration process. This result holds important implications for the role of the hippocampus in 'knowing where' versus 'getting there'.

Declaration and Statement of Copyright

The work in this thesis is based on research carried out at the Department of Psychology, University of Durham, England, UK. No part of this thesis has been submitted elsewhere for any other degree or qualification and it is all my own work unless referenced to the contrary in the text. Anthony McGregor and Yutaka Kosaki conducted the surgical procedures described in Experiments 6, 7 (Yutaka Kosaki also sketched the histological reconstructions for Replication 2), 10 and 11. The remaining surgical and histological procedures described herein were conducted by the author.

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Part of the work presented in Chapter 2 has been published as follows:

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"Try not to become a man of success,
but rather try to become a man of value."

~ Albert Einstein

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1 Introduction

1.1 Motivation

Spatial memory in humans and other animals plays a crucial role in survival by supporting the encoding and retrieval of key locations where, for example, food and shelter can be found. The study of spatial memory in non-human animals has proved insightful to the understanding of human memory in general as the cognitive processes and spatial paradigms used between species are very similar. Thus, spatial navigation tasks, particularly in rodents, provide an experimental assay to investigate the behavioural changes in and neurobiology of learning, memory and cognition. However, despite a prevalence of tasks designed to investigate spatial memory, either on dry land or in water, it is still not known exactly how animals solve such tasks. On the face of it, experimental procedures such as the Morris water navigation task offer a relatively simple operational means to clear up such uncertainty. In reality however, a whole suite of interrelated functions, including the employment of motor, motivational and perceptual systems, must interact in order for an animal to acquire and express a learned spatial behaviour. Moreover, there is typically more than one system available for a navigating animal to use, which raises several important questions. Does one system take precedence over another during navigation? And, does learning based on one strategy compete with learning based on others, or do these learning processes progress independently? These questions form the basis of the current thesis. A series of experiments using rats investigated

the nature of how navigational systems interact and the neural structures supporting these interactions. The research methods, described in the experimental chapters (2-6) of this thesis, addressing this overarching theoretical theme are highlighted in the final section of the current chapter, but first, by way of background, a brief introduction to various aspects of the studies or concepts contained within this thesis is provided.

1.2 Background

1.2.1 Types of Navigational Strategy

O'Keefe and Nadel (1978) proposed that animals can use two separate navigational strategies. A taxon strategy involves the formation of stimulus – response (S-R) habits such as heading toward a beacon. A locale strategy involves the integration of several distal cues to learn the location of a goal. The authors argued that taxon learning is governed by ubiquitous associative rules, which have formed the bedrock of several contemporary learning theories (e.g. Rescorla & Wagner, 1972; see page 6 for a brief description), while a locale strategy involves the learning of places within the context of a 'cognitive map' and is not governed by these rules. Experiments demonstrating that animals can learn about spatial information by simply exploring an environment in which no reward is provided (Blodgett, 1929; Tolman & Honzik, 1930) and are capable of solving spatial tasks in novel ways, such as taking short cuts (Gould, 1986; Roberts, Cruz & Tremblay, 2007; Tolman, 1948; Tolman, Ritchie & Kalish, 1946) have been said to support the notion that locale learning is processed non-associatively. That locale learning does not require the strengthening of connections between a stimulus and response has led to claims that spatial learning holds a special status by defying the laws of classical

associative learning models. In an attempt to address this issue, a body of research focused attention on how environmental cues compete for behavioural control during navigation tasks, an issue discussed in due course.

Navigational strategies have also been classified by taking into account the body movements of the navigating animal. A distinction between egocentric and allocentric strategies has arisen based on the assumption that the former, which is a particular form of S-R strategy, is dependent on information pertaining to the position of the animal in relation to spatial locations, while the latter is independent of the animal's position in space and involves the processing of relations between environmental cues, or an allocentric reference frame. An egocentric strategy can, for example, involve learning a particular motor habit triggered by a specific cue or location, e.g. 'upon arrival at the junction take a left', which does not rely on a constellation of ambient cues. This sort of strategy is inflexible unlike an allocentric strategy that can be used to create a novel route to the goal using different, and perhaps new, egocentric responses. Allocentric and egocentric strategies are also referred to in the literature as place and response (or cue response) strategies, respectively. A further classification has identified a distinction between ideothetic and allothetic navigational strategies. Employment of an ideothetic strategy requires a navigating animal to keep track of its own body movements to calculate distances and orientation. Self-motion, or ideothetic, cues can be generated by an animal drawing on vestibular information, efference copies of motor commands and or changes in visual information corresponding to changes in the speed and direction of body movements. An allothetic strategy is another term for a previously described allocentric strategy. Classification of the aforementioned strategies is not only supported by behavioural observations but also by neurobiological evidence

demonstrating that different neural structures sub-serve these different navigational strategies (see section 1.2.4).

1.2.2 Type of spatial information – Landmarks and Geometry

An important question concerning the study of spatial learning in animals is how do animals represent space, or what type of spatial information do animals use when navigating to a goal location? In answering this question a clear distinction has emerged in the literature between the use of discrete visual elements (landmarks) and the metric properties of bounded surfaces (environmental geometry). Evidence has revealed that animals are capable of using a landmark as a beacon (Timberlake, Sinning & Leffel, 2007) or in conjunction with one or more other landmarks (Benhamou & Poucet, 1998; Skov-Rackette & Shettleworth, 2005) to locate a goal. Cheng (1986) was the first to demonstrate that animals can also use the geometric properties of the environment to determine direction. In his experiment rats were trained to locate food in one corner of a rectangular arena. During a retention interval, the arena was rotated to ensure that the absolute position of the food in the test room had changed and rats had to reorient themselves when they were reintroduced to the arena and allowed to search for the food. Despite the presence of visual or odour cues that could have been used to disambiguate geometrically equivalent corners, the rats consistently made rotational errors, searching in the corner that was featurally distinct from the correct corner, but which shared the same geometric properties.

To explain the rats' innate preference for geometric information and an apparent disregard for 'featural' or non-geometric information, Cheng (1986) and later Margules and Gallistel (1988) proposed that rats process cues pertaining to environmental geometry in an encapsulated 'geometric module' that is impenetrable

to, and operates independently of the processing of, non-geometric cues. Gallistel (1990) suggests that a geometric module makes sense from an evolutionary standpoint as the macroscopic shape of an animal's natural environment rarely changes, whereas other non-geometric features such as the colour of surfaces and smells change across seasons. A central prediction of the geometric module hypothesis is that learning based on geometric cues should progress independently of learning based on non-geometric cues, such as landmarks.

1.2.3 Spatial learning and associative cue competition

According to O'Keefe and Nadel's (1978), Cheng 's (1986), and Gallistel's (1990) theories, animals form a map-like representation of their environment or the geometric properties thereof, and the processing of such information is unaffected by the addition of other environmental cues. Put another way, the presence of one cue in a navigating animal's environment does not restrict what can be learned about a different cue, an assumption that violates the predictions of many associative learning theories (e.g. Rescorla & Wagner, 1972). Of course, this assumption was, and still is, met with scepticism from an associative learning viewpoint, which holds no justifiable reason to suppose that spatial learning has a special status and is acquired non-associatively rather than being governed by the associative rules ubiquitous in other types of learning (see Pearce, 2009). Thus, in order to clarify this contentious issue, the careful application of an associative framework to the mechanisms underlying spatial learning is required. To introduce this topic, a brief description of the basic principles underlying associative learning is provided.

Pavlov's (1926, 1927) classic experiments using salivating dogs formed the foundation for years of empirical research on associative learning. Pavlov (1927) demonstrated that when animals learn to predict a biologically significant event, the

behavioural control that a target cue, such as a tone, exerts over behaviour can be altered by the presence of another cue, such as a light. This phenomenon, known as cue competition, has been pivotal to the investigation, and used as a tool to determine the underlying mechanisms, of Pavlovian conditioning. One example of a cue competition effect, which Pavlov (1927) documented, is known as *Overshadowing*. If, during training, two cues are presented simultaneously so that both signal the presence of a reward then responding to one of these cues during test is less than if it had individually been paired with the reward during training. In other words, one cue overshadows learning about the other. Blocking, which refers to a reduction in responding during test to one cue (cue B) following a training schedule where cue B is trained in compound with a second cue (cue A) that previously predicted the presence of the current reward, i.e. cue A has blocked learning about cue B, is another example of cue competition (Kamin, 1969). Overshadowing and blocking have been found in a wide variety of tasks and species (Mackintosh, 1974).

After the discovery of such cue competition phenomena, several theories of associative learning were formulated. The most influential of these was proposed by Rescorla and Wagner in 1972. Despite several shortcomings of this theory (see Pearce & Bouton, 2001 for an evaluation of these shortcomings), it remains very popular as it is able to explicate a wide range of experimental findings observed in both human and animal conditioning studies. Rescorla and Wagner's (1972) theory can be viewed in terms of a formalised model that accounts for the strengthening of a connection between a conditioned stimulus (CS), such as a tone, and an unconditioned stimulus (US), such as food, or, put another way, the change in associative strength (V) that any given stimulus acquires, during conditioning. This change in associative strength is related to the maximal value that the US can support

(λ), or the limit of learning, and is modulated by two learning-rate parameters, α and β, which have fixed values determined by the physical attributes of the particular CS and US, respectively. Thus, on any given conditioning trial the current associative strength of the CS, or the combined associative strengths of several CS's if more than one is present, is compared to λ and this discrepancy is treated like an error that requires correction, which produces a change in associative strength (ΔV). An important assumption of the Rescorla-Wagner (1972) model is that associative connections are strengthened not because a CS and US are simply presented together but because this co-occurrence is in some way surprising on the basis of current associative strength. As a consequence, the model can readily explain associative phenomena such as blocking, which cannot be explained by the co-occurrence of stimuli. A second important feature of the Rescorla-Wagner model is the assumption that different CS's compete for a share of a finite amount of associative strength, which again allows the model to account for such effects as blocking and overshadowing.

Despite theories of associative learning, such as Rescorla and Wagner's (1972) and subsequent extensions to the Rescorla-Wagner model maintaining the central tenet of an error-correction rule (e.g. Van Hamme and Wasserman, 1994), providing an explanation for overshadowing and blocking, it must be acknowledged that there are alternative explanations. For example, it has been proposed that overshadowing and blocking may be the result of performance deficits rather than competition for learning. In the case of overshadowing, one explanation is that animals trained in an overshadowing group (AB+) and tested with A alone, experience a greater change from training to test, or more *generalisation decrement*, than those animals trained and tested with A alone (e.g. Pearce, 1994). Within the

spatial domain, studies specifically controlling for generalisation decrement have shown that associative cue competition is responsible for overshadowing (Leising, Garlivk & Blaisdell, 2011; Sánchez-Moreno, Rodrigo, Chamizo, & Mackintosh, 1999), while a recent study has revealed that overshadowing by generalisation decrement is also possible (Chamizo, Rodríguez, Espinet, & Mackintosh, 2012). In terms of blocking, one of the more influential hypotheses to highlight the importance of performance factors in associative learning is the *comparator hypothesis* proposed by Miller and Matzel (1988; see also a more recent extension of this model by Stout & Miller, 2007). Briefly, the control that a specific CS acquires over behaviour is not determined by the absolute strength of its association with the US, but by its association with the US relative to the associative strength to the US of other stimuli experienced during training. Importantly, this model predicts that competition-like processes occur at test based on prior training experience as opposed to competition for associative strength occurring during acquisition. Unlike the Rescorla-Wagner model (1972) and similar variants, this model assumes that associative phenomena such as blocking occur via the simple co-occurrence of cues.

Historically, cue competition effects have been investigated by measuring an animal's response whilst in a conditioning chamber which makes it relatively easy to control conditioned stimuli such as tones and lights. In spatial memory tasks however, stimuli are comparatively more complex and a mobile animal is able to exert a greater influence over the cues to which it is exposed. Nonetheless, several studies have reported the presence of overshadowing and blocking using spatial learning tasks (e.g. March, Chamizo, & Mackintosh, 1992; Roberts & Pearce, 1999), which appear to disconfirm O'Keefe and Nadel's (1978) theory that spatial learning is not governed by an associative, error correcting rule. That said, it certainly cannot

be claimed that cue-competition effects are omnipresent in all spatial learning tasks. Indeed, several spatial experiments have demonstrated a distinct absence of overshadowing or blocking (e.g. Hayward, McGregor, Good & Pearce, 2003). This raises the question of what factors influence the emergence of cue competition effects in spatial learning. One factor that has received close attention in the literature is the type of spatial information encoded and more specifically whether animals are required to use the shape, or geometry, of their environment to find a target location (see Cheng, 2008 for a review).

In keeping with Cheng (1986) and Gallistel's (1990) geometric module theory, a number of experiments failed to reveal cue-competition effects when animals were provided with both informative geometric and non-geometric cues (e.g. landmarks) in order to accurately locate a hidden goal (Hayward, Good, & Pearce, 2004; Hayward et al., 2003; McGregor, Horne, Esber, & Pearce, 2009; Pearce, Ward-Robinson, Good, Fussell, & Aydin, 2001; Wall, Botly, Black, & Shettleworth, 2004; for similar results in humans: Redhead & Hamilton, 2007, 2009; and chicks: Tommasi, Gagliardo, Andrew & Vallortigara, 2003). These findings support the notion that the processing of geometric information takes place in a geometric module within the brain that is impervious to the processing of non-geometric information, and have led to suggestions that it may be geometry learning that holds a special status because the underlying processes involved are not governed by an error correction rule. Recent reports have challenged this view, however, by showing that under certain testing procedures it is possible to observe associative competition between geometric and non-geometric cues in rats (Cole, Gibson, Pollack & Yates, 2011; Graham, Good, McGregor & Pearce, 2006; Horne & Pearce, 2009a, b, 2011; Kosaki, Austen & McGregor, 2013; Pearce, Graham, Good, Jones & McGregor,

2006; Rhodes, Creighton, Killcross, Good & Honey, 2009; Rodríguez, Chamizo & Mackintosh, 2011), humans (Wilson & Alexander, 2008) and birds (Gray, Bloomfield, Ferrey, Spetch, & Sturdy, 2005).

A key question to arise from this literature is why some geometry studies have provided evidence of cue competition and others have not. One issue with interpreting these varied findings is that the non-geometric cues, arena shapes and procedural details differed between experiments. Therefore, it is difficult to elucidate with any certainty the key factors determining when and how non-geometric learning competes with geometric learning. One explanation is that the presence of cue competition is dependent on the type of non-geometric cue used. This argument is supported by reports revealing that information provided by the colour of enclosure walls can compete with geometry learning (Horne & Pearce, 2011; Pearce et al., 2006;), while experiments using similar procedures failed to show cue competition when discrete landmarks were used (e.g. McGregor et al., 2009). Given that wall colour cues are inextricably bound to the surfaces forming the shape of the enclosure, it may be possible for this type of featural cue to permeate the geometric module. Evidence from a recent study has eroded this view, however, and revealed that in certain situations learning based on discrete landmarks is able to compete with learning based on geometry (Kosaki et al., 2013). Thus, an alternative explanation must be offered for the variable presence of cue competition involving geometry learning. Recent studies (Austen, McGregor & Kosaki, 2013; Horne & Pearce, 2011) have revealed that the presence of cue competition between landmarks and geometric cues may vary as a function of the relative salience of these cues; with the presence of *within-compound associations*, which are described in more detail in subsequent chapters, mitigating cue competition effects such as overshadowing.

An additional factor recently identified to influence cue competition effects in a spatial learning task using rats is sex (Rodríguez, Chamizo & Mackintosh, 2011). These findings build on previous evidence, both in humans and non-human animals, demonstrating sex differences in spatial cognition (see Jones & Healy, 2006 for a review). One notable sex difference is that males and females tend to use different cues to solve spatial tasks. Males rely on the Euclidean (geometric) properties of the environment, while females use more discrete visual elements such as landmarks (in humans: Chai & Jacobs, 2009; Saucier, Green, Leason, MacFadden, Bell & Elias, 2002; Sandstrom, Kaufman, & Huettel, 1998; in rats: Rodriguez et al., 2011; Rodriguez, Torres, Mackintosh & Chamizo, 2010; Roof & Stein, 1999; Williams, Barnett, & Meck, 1990). It is possible that this difference in the type of cues males and females use underlies the common observation, typically using tasks requiring a geometric solution, that males outperform females in tests of spatial cognition. There have been a number of hypotheses proposed to explain these sex differences in spatial abilities including seven evolutionary hypotheses (Jones & Healy, 2006), variation in sex hormones (reviewed in Williams & Meck, 1991), variation in stress levels (e.g. Bowman, 2005), adaptive differences in brain wiring (e.g. Saucier, Shultz, Keller, Cook & Binsted, 2008), and differential responsivity to pre-training (Perrot-Sinal, Kostenuik, Ossenkopp & Kavaliers, 1996) or appetitive motivation (Mishima, Higashitani, Teraoka & Yoshioka, 1986).

Rodriguez et al. (2011) tested whether the difference between the sexes in the type of cue that they use correlates with the amount of associative strength that these specific cues acquire. Accordingly, the authors predicted that when animals are trained in compound with a geometric and landmark cue, and subsequently tested with each cue in isolation, and this performance is compared to the relevant control

groups, the geometric cue should overshadow the landmark cue in male rats while the reverse pattern of overshadowing should occur in female rats. The results confirmed this prediction and have been interpreted to support the proposal that an animal's reliance on a cue can influence the direction of cue-competition effects observed in spatial learning.

1.2.4 Neural substrates of spatial learning

There is a growing body of evidence suggesting that the hippocampus (CA1, CA2, CA3), a component of a wider brain system known as the hippocampal formation, is heavily involved in spatial learning and navigation (e.g. Morris, Garrud, Rawlins & O'Keefe, 1982; O'Keefe & Nadel, 1978). The specific brain regions encompassed by the term hippocampal formation has been the subject of debate, but it is now widely, though not universally, accepted that the dentate gyrus, hippocampus, entorhinal cortex (EC), subiculum, presubiculum and parasubiculum make up the hippocampal formation (see Andersen, 2007 for a more detailed discussion and justification for this usage of the term). These regions form a highly interconnected network which, it is generally agreed, has evolved to organise spatial information within the brain. The EC, which is the main interface between the hippocampus and other cortical areas, provides the HPC with its major source of cortical and highly processed sensory information (see for example Jones, 1993). The subiculum receives many inputs from the hippocampus and distributes this information to various cortical regions. Thus, the subiculum is viewed as the primary output structure of the hippocampus (O'Mara, Commins, Anderson & Gigg, 2001; Witter, 1993).

Several lines of research support the contribution of the different subregions of the hippocampal formation in spatial cognition. For example, lesion studies in

rodents have shown that, in a similar vein to the hippocampus, selective damage to the entorhinal cortex (e.g. Galani, Jarrard, Will, & Kelche, 1997; Hunt, Kesner & Evans, 1994), subiculum (e.g. Galani et al., 1997; Morris et al., 1990), or the entorhinal and subicular cortices combined (Good & Honey, 1997; Oswald & Good, 2000) impairs spatial learning. Moreover, neurophysiological studies have revealed that the activity of individual cells within different regions of the hippocampal formation react to spatial-specific information when an animal is free to move through its environment. For example, *place cells* that only fire when an animal is in a specific location have been found in the hippocampus (e.g. O'Keefe, 1976) and subiculum (e.g. Barnes, McNaughton, Mizumori, Leonard & Lin, 1990), *grid cells* that provide a grid-like metric to the neuronal representation of space have been found in the EC (e.g. Hafting, Fyhn, Molden, Moser & Moser, 2005) and pre- and parasubiculum (Boccara et al., 2010), and *head direction cells* that fire when an animal points its head in a particular direction have also been found in the EC (Sargolini et al., 2006). Additional support for the notion that the regions of the hippocampal formation work in concert as an integrated navigational system comes from studies showing that by disabling one specific region, e.g. the entorhinal cortex, the functioning of cells in another region, such as place cells in the hippocampus, become disrupted (e.g. Brun et al., 2008).

As well as the hippocampus being critical for spatial cognition in general, it has also been proposed that this structure is critical to the encoding of shape-based, or geometric, information during navigation tasks. For example, rats with selective damage to the hippocampus are severely impaired at using the shape formed by the walls of their enclosure when navigating to a goal location (Jones, Pearce, Davies, Good & McGregor, 2007; McGregor, Hayward, Pearce & Good, 2004; Pearce,

Good, Jones & McGregor, 2004). One interpretation of the disruptive effect is that animals possessing such hippocampal lesions are impaired at judging lengths and distances, which would make the task of distinguishing long from short walls more difficult (Jones et al., 2007). Empirical evidence to support this notion that hippocampal lesions impair distance discrimination has also emerged (Sakamoto & Okaichi, 1996, 1997). Because the processing of distance information is critical in defining geometric relationships between environmental cues, or for constructing a cognitive map within the brain, it is not surprising that the hippocampus has been implicated as the key structure in this process (see O'Keefe & Nadel, 1978).

Evidence from electrophysiological studies has also demonstrated that place cells in the hippocampus are sensitive to environmental shape (Muller & Kubie, 1987; O'Keefe & Burgess, 1996; Quirk, Muller, Rubie & Ranck, 1992; Lever, Wills, Caccucci, Burgess & O'Keefe, 2002) and to the geometric configuration of cues (O'Keefe & Conway, 1978; O'Keefe & Speakman, 1987; Pico, Gerbrandt, Pondel & Ivy, 1985). For example, Lever et al. (2002) recorded the firing rate of place cells after transferring rats from a circular to square environment, and, over time, these cells differentiated between the two environments based on their geometric properties. A similar observation in grid cells, which are thought to impact heavily on the activity of hippocampal place cells, has also been reported by Barry, Hayman, Burgess, and Jeffery (2007) who showed that grid cells compress when the shape of a rat's environment is reduced from a square to, for example, a smaller square or rectangle. Finally, a study by Doeller, King and Burgess (2008) using human participants and functional MRI imaging revealed that a search strategy based on using the geocentric boundaries of the environment enhanced activity in the hippocampus.

A second region of the brain that has been the focus of study in spatial memory tasks is the striatum and more specifically the dorsal striatum. Unlike the hippocampus, which has been described above to be involved in forming a map-like representation of the arena walls and their metric relations, the dorsal striatum is implicated in learning an egocentric response rule. This egocentric response rule, formed through the formation of stimulus-response (S-R) habits (Guthrie, 1935; Hull, 1943), and described above as an egocentric, or cue response, strategy requires the animal to learn the relationship between an environmental cue and a rewarded response. Several studies have reported that lesions or pharmacological disruptions to the dorsal striatum impair the learning of such a relationship (Cook & Kesner, 1988; Devan, McDonald & White, 1999; McDonald & White, 1993; Packard, Hirsh, & White, 1989; Packard & McGaugh, 1996). At the cellular level, it has also been shown that brain activity associated with synaptic strengthening and long-term memory formation is sustained in the dorsal striatum after rats are required to form a memory for an egocentric response strategy but not a place strategy (Colombo, Brightwell & Countryman, 2003). Furthermore, Brightwell, Smith, Neve and Colombo (2008) demonstrated that when the aforementioned brain activity in the dorsolateral striatum was blocked by virus-mediated gene transfer, performance on a task requiring rats to adopt an egocentric response strategy was impaired.

1.3 Focus of Thesis

In order to investigate the interaction between different navigational systems in the rat and the neural structures supporting these interactions, a series of experiments employing a range of methods measured changes in the behaviour of a navigating animal, which served as an index of spatial learning. In order to dissociate

the different learning systems rats use during navigation, it is important to develop tasks that can be solved using these different components. As mentioned above, it has been suggested that a navigational system mediating learning based on geometric information operates independently to systems supporting the learning of non-geometric information (see Cheng & Newcombe, 2005 for a review). Therefore, this stance would predict that learning confined to a geometric system should not face competition or be used collaboratively with learning processed in a non-geometric system. This prediction is tested using several methods in chapters 2 – 4. In chapter 2 a spontaneous recognition memory task was devised to assess the capability of rats to recognise the novelty of a non-geometric feature (an object) with reference to the local geometric context. In this way, rats were required to integrate geometric with non-geometric information, which should not be possible if the processing of these different cue types occurs in systems that are impervious to one another. The role of the hippocampus and dorsolateral striatum in learning based on a configuration of visual features and geometry was also investigated in order to further understand the neural structures involved in an object-in-local geometry task.

Chapter 4 used an associative learning framework, described above, to investigate whether learning based on non-geometric information can compete with learning based on geometric information. Again, this competition between different learning systems should not emerge if a system supporting geometry learning progresses independently of systems supporting other forms of learning. All rats were trained to find a hidden goal in a distinctively shaped environment containing additional discrete landmarks. For one group, both geometric and landmark cues could be used to locate the goal, while for another group only the geometric cues were informative. At the end of training, the landmarks were removed from the

environment to establish how much animals had learned about geometry. By using this method it was possible to test whether the presence of informative landmarks restricted, or overshadowed, learning based on geometry. Moreover, both male and female rats were used in order to investigate the effect of sex on the extent to which landmark and geometry learning processes competed with one another. Based on the premise that male rats prefer to use geometric cues while female rats prefer to use landmarks (see discussion in section 1.2.3) and this cue preference can affect the direction of overshadowing between geometry and landmark learning, an examination of sex differences provided an additional method to test the presence of cue competition in geometry learning. The logic being that the predicted sex differences should not be observed if it is not possible for a geometric system to compete with a non-geometric system in the first place.

The primary focus of the final two experimental chapters (5 & 6) moved away from geometry learning and investigated the interaction between two different navigational systems; one mediating allocentric learning and the other mediating egocentric learning. To achieve this, rats were induced with lesions to specific neural structures of the brain. By disabling specific brain structures it is not only possible to identify the direct function that a particular structure is responsible for, but it is also possible to gain a better understanding of the interaction between brain processes and their behavioural outputs. In this way, the experiments in Chapter 5 were designed to identify a double dissociation between the hippocampus and dorsolateral striatum in processing place-based allocentric and response-based egocentric information, respectively. Finally, Chapter 6 investigated the contribution of ideothetic and allothetic cues to place learning with an emphasis on the role that the hippocampus plays when these different types of cue are learned about.

In summary, neurophysiological and neuropsychological data appear to support the existence, both in humans and non-human animals, of separable and independent navigational systems supporting different forms of learning. As mentioned, a key question is how these systems interact. The methods outlined above have been devised to establish whether or not various navigational systems work in a complementary fashion or whether they compete for behavioural control. According to the view that certain navigational systems process information independently, and are impervious to the activity, of others, it is anticipated that no interaction should be observed between the systems under investigation in the experimental chapters of this thesis. Within each empirical chapter, an introduction to the specific topics that will be studied, and a discussion of the findings, will be provided, before a final conclusions section discusses the overarching themes raised.

2

Object-in-Local Geometry Memory

2.1 Introduction

When animals are disoriented in such a way that they are unable to accurately track a sequence of their own body movements, a reorientation strategy must be employed that relies on the use of stable visual information present within the environment. As discussed in Chapter 1, Cheng (1986) was the first to demonstrate that rats could utilise the geometric properties of the walls of an enclosed arena to determine direction whilst apparently ignoring non-geometric cues that could have been used to disambiguate geometrically equivalent locations. These findings led Cheng (1986) and later Margules and Gallistel (1988) to propose the *geometric module* hypothesis, which posits that rats process cues pertaining to environmental geometry in an encapsulated module that is impenetrable to non-geometric information. A central prediction of the geometric module hypothesis is that learning based on geometric cues should progress independently of learning based on non-geometric cues, such as landmarks. This work and interpretation formed the basis of years of empirical research aimed at answering two questions. First, during reorientation in a navigation task, do animals rely on geometric information over non-geometric, or featural, information when both frames of reference are reliable? Second, is it possible for featural cues to interact or compete with learning based on geometric cues?

In answer to both of these questions empirical data has provided conflicting results. Evidence from young children (18-24 months) and adults in an adapted

version of Cheng's (1986) task suggested that young children reoriented in much the same way as Cheng's rats in that their reorientation strategy was impervious to all but geometric information. Adults, on the other hand, were able to integrate both featural and geometric information to aid navigation (Hermer & Spelke, 1994, 1996; Wang, Hermer & Spelke, 1999; see also Bek, Blades, Siegal & Varley, 2010 for a similar finding in adults). Benhamou and Poucet (1998), in a water maze task using rats, positioned an array of landmarks to form a triangular shape and demonstrated that it was much easier for rats to learn the location of a hidden goal using geometric rather than featural information (see also: Wall, Botly, Black, & Shettleworth, 2004 for a similar result in a rectangular enclosure on dry land).

In general, however, evidence from the animal literature weighs heavily against the notion that geometry comes to have primacy in navigation while predictive featural information is ignored. Studies using pigeons (Kelly, Spetch, & Heth, 1998), chicks (Vallortigara, Zanforlin, & Pasti, 1990), fish (Sovrano, Bisazza, & Vallortigara, 2002, 2003), ants (Wystrach & Beugnon, 2009) and rhesus monkeys (Gouteux, Thinus-Blanc, & Vauclair, 2001) have all shown that animals are capable of reorienting by using geometric information in conjunction with featural information. To focus on one of the aforecited examples, Gouteux et al. (2001) trained Rhesus monkeys to locate food in one corner of a large enclosed rectangular chamber comprising of one small blue wall and three white walls. This meant that the disoriented monkeys could use the colour of the walls to distinguish between the correct corner and its geometrical equivalent, and, unlike Cheng's (1986) rats, the results revealed that this is exactly what they did.

Potential reasons for the discrepancy between those studies that show competition between geometric and non-geometric cues and those that fail to do so

have been discussed in Chapter 1, but given that Gouteux et al. (2001) used a very similar design to Cheng (1986), it is appropriate to consider why Rhesus monkeys in the former study were able to integrate wall colour with geometric cues but rats in the latter study weren't. One obvious reason could be that primates are cognitively more advanced than rats and are, therefore, better at conjoining visual information. However, this explanation is not supported by the studies cited above showing that a range of species including ants and fish can incorporate featural and geometric cues, while young children fail to do so. One important finding from Gouteux et al.'s (2001) series of experiments was that the ability of monkeys to use distinctive corner panels in order to disambiguate geometrically equivalent locations varied according to the size of these panels, i.e. they were capable of using large landmarks but not smaller ones. The authors suggested that the monkeys may have considered the smaller proximal landmarks to be less stable than larger featural landmarks. Some support for this interpretation comes from a study in young children by Learmonth, Newcombe, and Huttenlocher (1998) demonstrating that more permanent looking, immovable landmarks such as doors or bookcases are more likely to aid reorientation than smaller landmarks. Thus, differences in the physical properties, or the perceived physical properties, of featural cues may offer some explanation for the convoluted results within this literature.

Another method, discussed in Chapter 1, to establish whether featural cues are able to interact or compete with the learning of geometric cues, is to adopt an associative learning approach and identify how stimuli compete for associative strength. Thus, if the geometric module is impenetrable to non-geometric processing, the encoding of geometric cues should be unaffected by the concurrent encoding of other featural cues. As discussed, a number of experiments support the view that

geometry learning is unrestricted by the presence of informative featural cues, while other experiments oppose this stance and provide evidence for associative competition between geometric and featural cues (see section 1.3.3 for references).

Thus, these conflicting results from several lines of research raise the question of what factors affect how humans and animals use and integrate geometric information. Several authors have suggested that the independent processing of and reliance on geometric cues varies as a function of disorientation (Cheng & Newcombe, 2005; Gallistel & Matzel, 2013; Sutton, 2009). For example, Cheng & Newcombe (2005) claimed that the inertial sense of the rats in Cheng's (1986) study was disrupted due to the environment being rotated between sample and test phases and this played a key role in their failure to discriminate the target corner from the geometrically identical, but featurally distinct, corner (Cheng & Newcombe, 2005). To add further weight to this conclusion, the authors highlight empirical evidence from a replication study by Margules and Gallistel (1988) demonstrating that when rats were further disoriented between the exposure and test phase by being placed in a holding cage and rotated, they made more rotational errors than the rats in Cheng's (1986) experiment. However, caution must be exercised when accepting this interpretation as Margules & Gallistel (1988) did not provide a control group matching the conditions of Cheng's (1986) original experiment so this comparison has been drawn from the results of separate experiments. Nonetheless, it raises the possibility that disorientation is critical to a rat's propensity to use the macroscopic shape of the environment over other cues.

To directly test this hypothesis, Batty, Hoban, Spetch and Dickson (2009) compared the performance of disoriented rats with that of oriented rats in a navigational task conducted in a rectangular enclosure. Like Margules and Gallistel

(1988), Batty et al. (2009) found that the disoriented-trained rats showed a lack of preference for featural cues and a strong reliance on geometric cues. Accordingly, Cheng and Newcombe (2005) suggest that two independent processes should be considered when dealing with geometry studies, one used by animals when reorienting and another used to locate a particular target location. However, as Sutton (2009) points out, both these processes are quantified using the same dependent measure, i.e. the proportion of exploration time directed to a specific location, therefore it is difficult to disentangle and consider separately these processes.

A second factor that could influence an animal's use of geometric cues is the type of learning paradigm used. Cheng's (1986) original study used a spontaneous, working memory task. It is described as a working memory task because the location of the food reward changes from trial to trial so that on any given trial an animal is expected to match the correct location in the test box with a recently stored memory trace within the brain. However, all but one of the subsequent studies cited above, which have questioned the geometric module hypothesis, used reference memory paradigms in which the animal was extensively trained over multiple trials. The one study (Rhodes et al., 2009) in which rats were not provided with extensive, rewarded training, demonstrated convincingly that these animals were able to form associations between geometric and featural (in this case wall colour) cues. However, a closer inspection of the design reveals that although rats did not receive a training schedule typical of a reference memory task, they were pre-exposed to the arena containing scattered food pellets for a 5 minute session each day for 8 days. Consequently, it could be argued that these rats associated reward with the overall context or numerous locations of the arena, which displayed a constant geometric –

featural arrangement over 8 trials. Therefore rats in this task could construct a memory trace analogous to those formed during reference memory tasks. Taken together, prior findings still leave the caveat that Cheng's (1986) and Gallistel's (1990) geometric module hypothesis only applies to working memory paradigms.

A final factor that could affect how geometric information is processed relates to the nature of the task conditions. The majority of work contradicting the original findings of Cheng (1986) comes from studies using a Morris water maze and it is possible that the aversive nature of these tasks altered the strategy used by rats to learn the location of the platform. Differences in reorientation strategies can occur between appetitive and aversive tasks (Dudchenko, Goodridge, Seiterle, & Taube, 1997; Golob & Taube, 2002; Whyte, Martin, & Skinner, 2009), possibly due to the disparity in training, motivation, visual cues and proprioception (see Hodges, 1996, for a review).

In addition to proffering the encapsulated 'geometric module' hypothesis, which extends on the modularity of mind concept (Fodor, 1983, 2001), Cheng (1986; see also Cheng & Spetch, 1998) and Gallistel (1990) also concluded, based on the findings of Cheng (1986), that the geometric representation of the environment held by the rat during navigation is global in nature. Gallistel (1990) proposed that this global, cognitive map-like, representation derived from metric distances and angles could be used in a matching process in order to reorient and determine the appropriate heading direction. Gallistel (1990) extended this global matching account to include a principal axes hypothesis, whereby an animal is able to extract an abstract representation of the global shape of the environment by computing the primary axes of the space it is navigating through. In the case of rats navigating in a rectangular environment, the two primary axes - a principal (long) axis and a minor

(short) axis - would run through the geometric centre of the arena and perpendicular to one another so that rats can match the alignment of these axes stored in memory with the perceived axes of the current search space. Gallistel also pointed out that as well as using these axes, the rats in Cheng's (1986) rectangular arena must have some sense of wall length configuration to distinguish the corners at each end of the primary axis.

By taking a global fix of the overall geometric layout of the environment, Gallistel (1990) and Cheng (2005) have argued that this is a computationally cheap way for the brain to capture a lot of information and it avoids expending neural activity on the encoding of less informative and more errorful featural information. Gallistel (1990) adds further weight to his global matching hypothesis by suggesting that in a natural habitat the macroscopic shape of an environment is rarely symmetrical and therefore the rotational errors witnessed in Cheng's (1986) experiment would not materialise. Empirical support consistent with the notion that animals form global representations of the shape of their environment soon emerged (Gouteux et al., 2001; Hermer & Spelke, 1994; Kelly et al., 1998; Sovrano et al., 2002; Vallortigara et al., 1990), which in turn acted as a catalyst for further studies investigating the exact nature of this global representation.

To test predictions based on the nature of this global representation, it is important that the definition of a 'global' match is clearly understood. Yet, it would seem that a 'global' representation is in itself open to interpretation. For example, Pearce et al. (2004) took this definition to mean the overall shape of the environment. However, logic would dictate that a rat placed into a distinctively shaped box would not view the overall shape of the environment in the same way that an experimenter would from a bird's-eye perspective. Gallistel and Cramer

(1996) subsequently took this perceptual nuance into consideration by suggesting that rats may construct a global map of the geocentric environment by integrating shape-based information derived from different views of the environment at different times. Cheng (2005) offered a more dilute 'global matching' process, to that which he offered in 1986 and Gallistel put forth in 1990, by conceding that a global process does not necessarily need to encode the entire shape of an environment. Despite this definitional ambiguity, however, the results and interpretations described (Cheng, 1986; Cheng & Spetch, 1998; Gallistel, 1990) offered a research question that could be tested empirically: If the overall shape of an environment changes, is it possible for an animal to locate a target area with respect to preserved, local geometric cues? An example of a local geometric cue could be one corner of a rectangular shaped arena where, for example, a long wall is to the left of a short wall.

To address this question, Pearce et al. (2004) trained rats to locate an invisible escape platform in one corner of a white, rectangular-shaped pool of water. After becoming proficient in finding this escape platform, rats were then trained in a white, kite-shaped pool of water where the lengths of the long and short walls were identical to those forming the rectangle. Whilst in the kite-shaped pool, rats focused their search in the corner that shared the same geometric properties as the corner in which they found the platform when trained in the rectangle. Pearce et al. concluded that this result provides evidence to contradict Cheng (1986) and Gallistel's (1990) global matching account and adheres more toward the notion that rats navigate with reference to local geometric cues present within their environment.

A similar transformation study, using hungry chicks on dry land (Tommasi & Polli, 2004), manipulated the dimensions and angles of a parallelogram-shaped arena to test the ability of animals to encode local geometric information relating to the

angular amplitude and arrangement of a short and long wall forming a designated corner of the arena. This study provided further evidence that animals are able to learn about individual geometric elements of an arena. Indeed, the authors claimed that the geometric arrangement of arena corners could be used by chicks as local cues much like objects, with highly distinctive acute corners acquiring greater control over behaviour than the less distinctive obtuse corners (see also Kosaki et al., 2013).

However, both Pearce et al.'s (2004) and Tomassi and Polli's (2004) study involved extensive training to locate a hidden goal. Therefore, it is possible that these animals exhibited symptoms of overtraining owing to the fact that their navigational strategy shifted from a place-based representation to a response strategy formed through stimulus-response associations (e.g., Tolman, Ritchie & Kalish, 1946, 1947). For example, Pearce et al. (2004) and Jones et al. (2007) showed that, following training, rats were more likely to swim directly to the corner containing a hidden platform from short walls than from long. Their analysis was consistent with the idea that rats developed a habit of swimming in a particular direction following their release from some walls. It is conceivable that these habits formed the basis for the pattern of behaviour observed by Pearce et al. (2004) and by Esber, McGregor, Good, Hayward, and Pearce (2005) in arenas in which the overall shape had been transformed but in which some of the local geometric cues were common to both. Therefore, the amount of training could have altered the way in which animals used geometric cues in these experiments compared to the rats in Cheng's (1986) study.

Given the foregoing discussion, the primary purpose of the current experiments was to assess whether reorienting rats were capable in a *non-aversive*, *untrained* task of navigating with reference to local geometric cues formed by the

arena walls. To achieve this, a spontaneous object recognition (SOR) experiment was conducted using two arenas with different global shapes to assess whether rats can recognise an object based on its location relative to local geometric cues after only a single exposure to the environment. The SOR task is frequently used to examine recognition memory in rodents. Ennaceur and Delacour (1988) first developed the task to take advantage of the natural tendency of rats to explore novel over familiar objects (Cowan, 1976). Typically, in a sample phase rats are presented with identical copies of an object, A, in a familiar open-field arena. In a test phase one copy of A is presented together with a novel object, B, and this is explored in preference to object A. Variants on the task have been used to examine the rat's spatial memory by presenting familiar objects in novel locations (Ennaceur, Neave, & Aggleton, 1997), and the role of spatial context has been examined by swapping a familiar object's location with that of another (e.g., Dix & Aggleton, 1999; Good, Barnes, Staal, McGregor, & Honey, 2007) or by presenting novel object-location combinations in featurally distinctive environments (e.g., Eacott & Norman, 2004). As well as the aforementioned objective, the current study provides an opportunity to better understand the mechanisms underlying spontaneous recognition of object-location combinations by testing whether animals can use the local geometric cues of the environment for this recognition or whether maintenance of the global geometric shape of the environment or distal spatial information is necessary.

In Experiment 1 rats were exposed in a sample phase to an object, A, in one right-angled corner (see *Figure 2.1*: Corner E, where the short wall is to the left of a long wall) of an arena with a distinctive shape, with a second object, B, in another corner (see *Figure 2.1*: Corner F, where the short wall is to the right of a long wall) that was also right-angled but which was the mirror-opposite of the corner containing

object A. Following a retention interval rats were placed in a new arena in a different room for the test phase. The second arena was a different overall shape to the first, but the local geometric properties of the right-angled corners in the two arenas were identical. A new copy of object A was placed in the corner with the same local geometric properties as in the sample phase, together with another copy in the mirror-opposite of that corner. Because animals were placed into a different room and the arena was positioned, pseudo-randomly, at a different orientation for the test phase, it was ensured that, in keeping with Cheng's (1986) experiment, rats were disorientated prior to testing. Should rats exhibit a preference for exploring the copy of object A in the novel geometric location then it would indicate that they are capable of remembering the location of an object with reference only to the local geometric cues provided by the arena's shape and that object-location memory is not dependent on the global shape of the arena, the absolute position of the object in a room, or its position relative to other objects. As such, it is predicted that rats should dishabituate to both objects in the test phase if they were to rely on any of these latter sources of information. In Experiment 2 the generality of the findings from Experiment 1 were extended by using different shapes for the sample and test phases.

Thus, the current experiments remove two inherent problems with previous studies claiming rats learn a location based on local geometric cues: training and the nature of the reinforcement. Should rats in this task be able to detect the object in the novel geometric location following transfer between arenas it would indicate that in a single exposure to the environment the rat combined the identity of the object and its location relative to the geometric properties of the arena, which would be the first demonstration of such a finding in the rat.

2.2 Experiment 1: Rectangle A-B to Kite A-A

2.2.1 Methods

2.2.1.1 Subjects

The subjects were thirty male Lister hooded rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England), twenty of which were experimentally naïve and ten with experience in an unrelated water maze task. The prior experience of the latter ten animals was counterbalanced. At the start of the experiment the twenty naïve animals were approximately 3 months of age and the remaining ten animals were approximately 5 months of age. All animals were provided with ad libitum access to food and water and were housed in pairs in a light-proof, temperature-controlled room in which the lights were turned on at 0700 hours and off at 2100. Testing was conducted at the same time each day, during the light phase. The upper shelves were occupied with empty cages to provide animals with equal light and cover. All experiments were performed in accordance with the Animals (Scientific Procedures) Act 1986 and associated guidelines.

2.2.1.2 Apparatus

Two separate testing rooms of similar dimensions (approximately 290 cm x 185 cm x 260 cm high) were used throughout. Each room contained a speaker for white noise positioned on the wall and a table in the corner on which rats were held. Upon entry into test room one, the speaker was affixed to and the table was touching the west wall, whereas in test room two, the speaker was affixed to and the table was touching the east wall. The holding cage when positioned on each table was bordered tightly by the two corner walls of each testing room and a third dividing cardboard

wall affixed to the tabletop, which ensured that each rat's line of sight was restricted to a view of the aforementioned walls, the ceiling, or out the front of the cage. With this arrangement it was not possible for animals to view the arena whilst in the holding cage. A desk lamp containing a Prolite plus SCR - 11 watt (50Hz) bulb was placed behind each arena facing the north wall to prevent shadows from being cast on the arena and to provide a low level of luminance. A wide-angled video camera was affixed to an overhead rail above each arena. Images from the camera were transmitted to a monitor (ZM-CR114NP-II) and HDD DVD recorder (Sony RDR-HXD890), which were located in an adjacent room.

Two medium-density fibreboard arenas each occupying separate testing rooms were used. The interior walls of each arena were painted light grey. One arena was rectangular and the other was kite-shaped; both were made up of two long walls and two short walls. The dimensions of the long and short walls were identical in each arena (100 cm or 50 cm long x 50 cm high). For the kite-shaped arena the angle of the apex corner was 55°, the angle of the opposite corner was 130° and the two remaining corners were each at an angle of 90°. The right-angled corners E and G in the rectangle were geometrically equivalent, with the long side to the right of the short side. Equally, corner J of the kite-shaped arena was the geometric equivalent of corners E and G in the rectangle, and corner L of the kite was the geometric equivalent of corners F and H in the rectangle (see *Figure 2.1*). Each arena was located on the floor in the same testing room throughout and could be rotated to occupy four different positions oriented along a north-south or east-west axis.

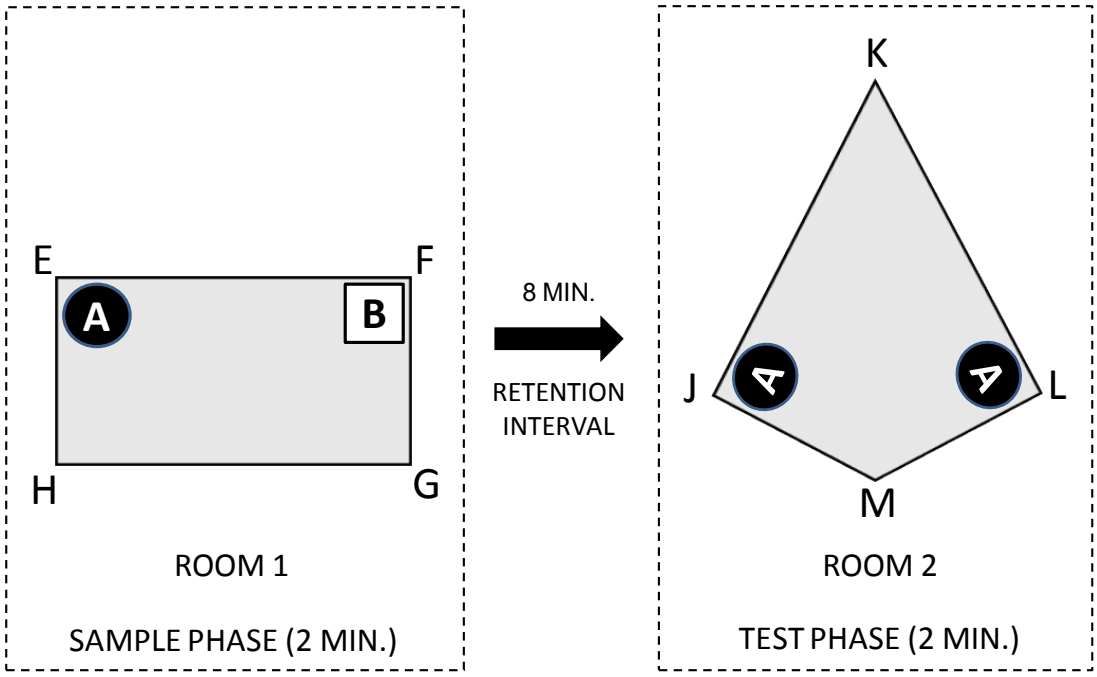

Figure 2.1. Schematic diagram showing the experimental design. Objects A and B are represented by black circular and white square symbols respectively. Preferential exploration of the object located in corner L of the kite indicates the animal's detection of its novelty with respect to the local geometric properties provided by the walls of the arena.

All objects presented in the experiment were trial unique, i.e. no animal experienced the same object on a different trial. Objects were junk objects including bottles, metal clips, ceramic ornaments, small toys, and combinations of objects. Where possible, the objects in each trial consisted of similar materials, to eradicate animal preferences for one material over another, and were of a similar height and volume. To prevent the objects from being knocked over, each object was affixed to the floor of the arena with Velcro. In each trial, an object was positioned tight against, but not touching, the two walls forming the appropriate corner of the arena. The centre of each object was located on an imaginary line that bisected the corner. For asymmetrical objects it was ensured that the orientation of each object within a specified corner remained constant from a rat's perspective when looking head on into that corner. For example, the handle of a cup always remained on the right-hand

side as the rat approached that corner, irrespective of which corner the cup was located.

2.2.1.3 Procedure

General Procedure

All behavioural testing was conducted at approximately the same time of day during the rats' light phase. Rats were transported into the test laboratory, four at a time, in a holding cage comprising of a Perspex bottom and wire top. Whilst transporting animals to and from the testing rooms a fleece cover was placed over the cage to minimise the stress caused by this movement. Throughout behavioural procedures, the holding cage and rats, when not being tested, resided on a table in the corner of the room. Each trial commenced with the experimenter, always approaching the arena from the same southerly direction, placing the rat gently into the centre of the arena. After the trial commenced the experimenter left the testing room and waited in an anteroom until the trial ended. Upon completion of the trial, the animal was removed from the arena and placed back into the holding cage.

Habituation

Rats received five sessions of habituation prior to beginning the experimental stage of the experiment. Habituation is important in order to acclimatise the animals to the arenas, the procedure and to being transported to and from the testing rooms. The first session of habituation consisted of animals being placed, in pairs for five minutes, into the rectangular arena, which was located in test room 1. After both pairs had completed exploration of the first arena they were then transported to test room 2 and left on the table for two minutes before being placed, in the same pairs

for five minutes, into the kite-shaped arena. Sessions two to five of habituation followed the same procedure as session one with the exception that animals were now allowed to explore each arena individually. Between each session of habituation each arena was rotated 90° anti-clockwise to ensure all rats explored the empty arenas in each of the four possible positions. Each session of habituation took place on a separate day and animals were run in the same order throughout. The arenas were wiped down with dry paper towelling prior to each animal or pair of animals beginning exploration. At the end of each testing day both arenas were cleaned with alcohol wipes.

Experimental Stage

Following habituation, animals received one object recognition trial per day for four days. In the sample phase, each rat was exposed to two different objects, A and B, in corners E and F of the rectangle, which was situated in test room 1, for two minutes. After a squad of four rats had completed this first sample trial they were then transported to an adjacent testing room (test room 2) for the test phase. In the test phase, each rat was placed in the kite-shaped arena in which two identical copies of one of one the objects were presented in the right-angled corners J and L. The retention interval between the sample and test phase for each rat was approximately 8 minutes. The orientation of the rectangle changed between days but remained constant for all animals on the same day. Only two of the four possible kite orientations were used on any given day, and although it was ensured that each orientation was counterbalanced equally between all animals, it was not possible to split each object subgroup (n = 15) exactly in half. For the test phase, animals were split into equal groups so that half received object A at test and the remainder object B, and, in so doing, ensured that the novel location (corner J or L of the kite) was

also assigned equally between animals. For each individual rat, the corner housing the novel object changed daily. Therefore, any preference for exploration of one object over another could not be explained by the positions of the objects with respect to generalization between extra-maze cues or by a preference for one right-angled corner over another. Upon completion of a trial by an animal and prior to the next animal beginning their trial, each object was thoroughly cleaned with alcoholic wipes and the arena was wiped down with dry paper towelling. At the end of each testing day both arenas were cleaned with alcohol wipes.

2.2.1.4 Performance Measures

Performance of rats in the test phase across four days was measured by recording the time that each animal actively explored or sniffed the objects head on from a distance of no greater than 2 cm. Brief whisking of or climbing on the objects was not recorded. Coding was conducted from video recordings and the scorer was blind to the conditions under which the rat was being tested. In addition, a second scorer, also blind, scored a subset of trials (40%) from the experiments. A Pearson product–moment correlation of exploration times between the two scorers was significant ($r = .90$, $p < .001$).

To accompany the manual scoring of exploration, Ethovision (version 3.1) software was used to track the movement of each animal in the test phase. With this program it is possible to overlay zones onto the recorded images so that the time a rat spent in a designated area could be objectively measured. For each 120 s test trial, this software was used to place a circular zone around each object so that there was a gap of approximately 5 cm between the edge of the object and the perimeter boundary of the zone. Thus, the time an animal spent within an area of 5 cm from the object could be recorded. Exploration was considered to have taken place if the rat's

head entered either of these circular zones.

From these object exploration times, a discrimination ratio, or a d2 ratio, was calculated. The d2 ratio for each rat was calculated by dividing the difference in time exploring the novel and familiar objects by the sum of these times. In this way, the d2 ratio better compensates for the variability in individual exploration times. As well as the aforementioned discrimination measure, the raw exploration time of each object was also used in the statistical analyses.

2.2.2 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied.

2.2.2.1 Stopwatch Scoring

The upper panel of Figure 2.2 shows the mean times animals spent exploring the novel and familiar location of objects, as defined by the local geometric properties of the corners, on each test trial. This figure illustrates that across all trials animals preferentially explored the novel object over the familiar object, with this preference being more marked on the first test trial. The lower panel of Figure 2.2 displays the mean d2 scores across four trials for each rat and it is clear from this figure that the columns are spread above the x-axis more than below, which suggests that, overall, animals preferred to explore the novel object location more than the familiar object location.

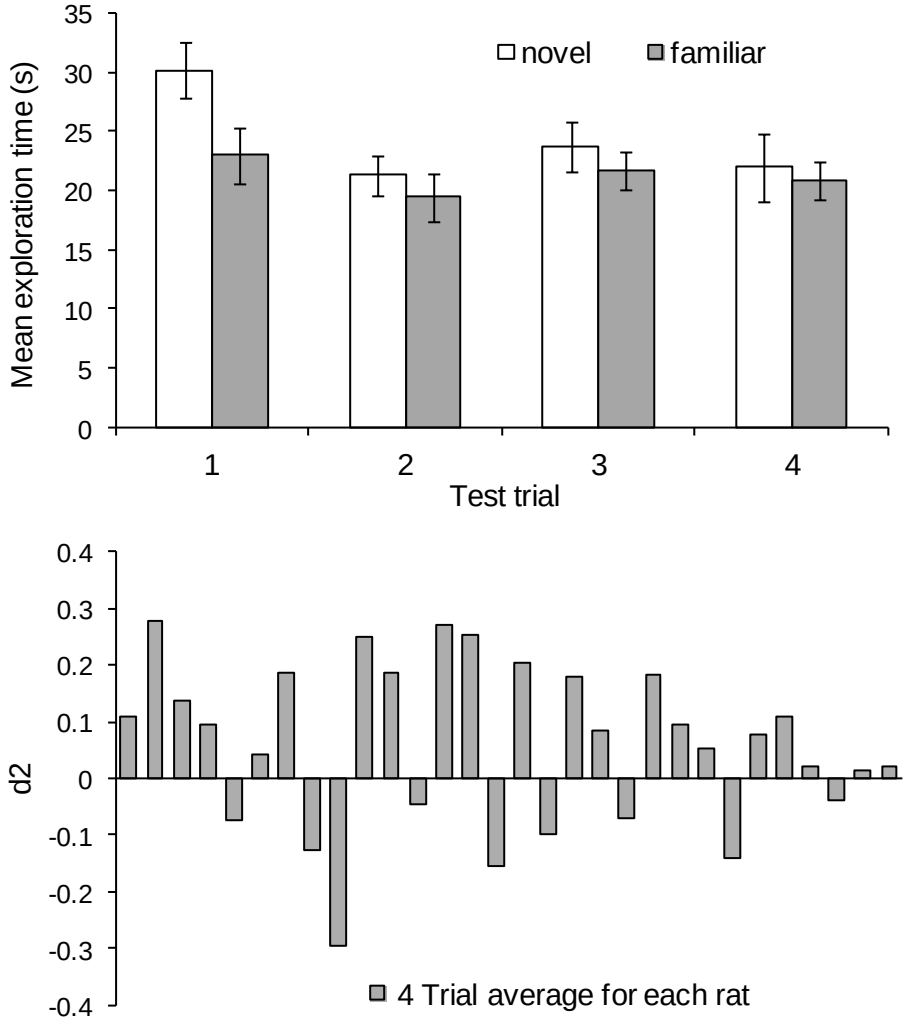

Figure 2.2. Upper panel: Mean exploration times (±SEM) for both novel and familiar object locations across four test trials. Lower panel: Mean d2 scores for each rat averaged across four test trials (chance = 0).

To confirm these observations statistically, a repeated measures analysis of variance (ANOVA) of individual object exploration times during the test phase with test trial (1-4) and object (novel vs. familiar) as repeated measures revealed that more time was spent exploring the novel than the familiar object, $F(1, 29) = 4.59$, $p = .041$. There was also a significant effect of test trial, $F(3, 87) = 5.85$, $p = .001$, but no interaction between the main effects, $F < 1$. Pairwise comparisons revealed that the effect of test trial was because of significantly higher exploration of objects in

Trial 1 than in Trial 2, while there were no differences among total exploration times for any of the other days. For the d2 scores, a one-sample t-test of the 4-day means of individual scores revealed that rats spent more time exploring the novel object than expected by chance, $M = .05$, $SEM = .02$, $t(29) = 2.29$, $p = .030$.

2.2.2.2 Ethovision – Time spent around objects

The upper panel of Figure 2.3 shows the mean time animals spent in each zone surrounding the novel and familiar object, as defined by the local geometric properties of the corners, on each test trial. This figure demonstrates that across the first three test trials animals spent more time exploring the area surrounding the novel object than the area surrounding the familiar object, with this pattern more marked in the first trial. However, statistical analysis revealed that this preference to spend more time in close proximity to the novel object was only marginally significant. A two-way ANOVA conducted on the time rats spent in zones placed around each object during the test phase with test trial (1-4) and object (novel vs. familiar) as repeated measures, revealed a significant main effect of test trial, $F(3,87) = 3.17$, $p = .028$, a marginally significant effect of object, $F(1, 29) = 3.41$, $p = .075$, and no test trial x object interaction, $F < 1$. The lower panel of Figure 2.3 depicts the discrimination ratio, or d2 score, for animals averaged across four test trials and, similar to the experimenter scores, it is clear from this figure that the columns are spread above the x-axis more than below, which suggests that, overall, animals preferred to explore the novel object location more than the familiar object location. A one-sample t-test conducted on the d2 scores, with an assigned test value of 0, confirmed this observation by revealing that rats spent proportionately more time in the zone placed around the novel object than expected by chance, $t(29) = 2.05$, $p = .050$.

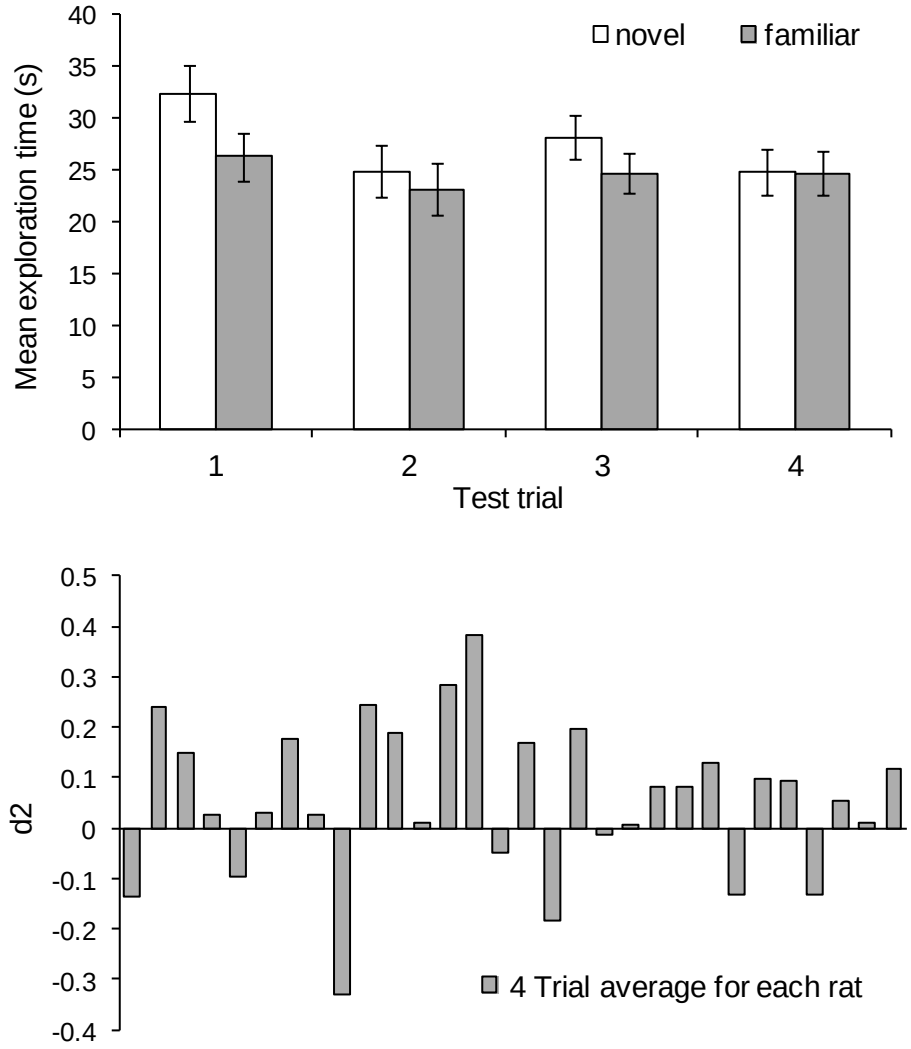

Figure 2.3. Upper panel: Mean time (±SEM) spent in areas around the novel (white bars) and familiar (grey bars) object locations across four test trials. Lower panel: Mean d2 scores for each rat averaged across four test trials (chance = 0).

Before considering the implications of the results for the hypotheses set out in the Introduction, a second experiment will be reported. The purpose of Experiment 2 was to confirm the results of Experiment 1, and also to extend the generality of the findings by transferring animals from a kite to a rectangle.

2.3 Experiment 2: Kite A-B to Rectangle A-A

2.3.1 Method

2.3.1.1 Subjects

The subjects were twenty experimentally naïve Lister hooded rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England). At the start of the experiment animals were approximately 3 months of age. Other details were identical to those described for Experiment 1.

2.3.1.2 Apparatus

The apparatus was identical to that used in Experiment 1.

2.3.1.3 Procedure

All aspects of the *General Procedure, Habituation, Experimental Stage* and *Performance Measures* were identical to Experiment 1, the only exception being the order in which each arena was presented to the animals. For the sample phase, animals were exposed to the kite-shaped arena in test room 2, before being transferred to test room 1 for the test phase conducted in the rectangular arena (see Figure 2.4).

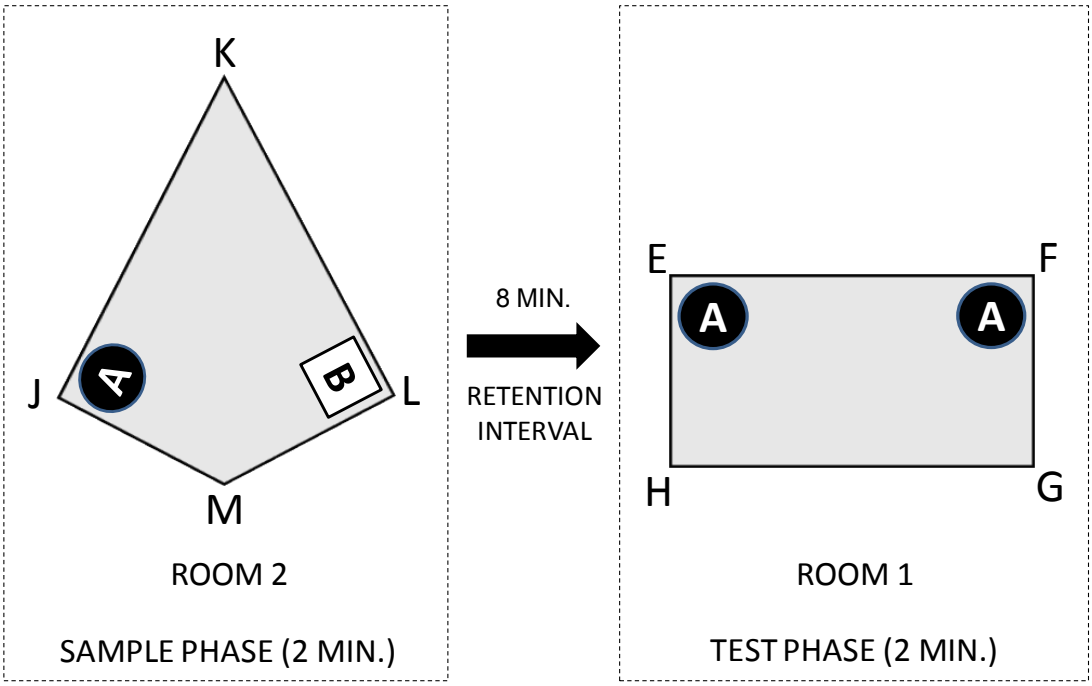

Figure 2.4. Schematic diagram showing the experimental design. Objects A and B are represented by black circular and white square symbols respectively. Preferential exploration of the object located in corner F of the rectangle indicates the animal's detection of its novelty with respect to the local geometric properties provided by the walls of the arena.

2.3.2 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied.

2.3.2.1 Stopwatch Scoring

The upper panel of Figure 2.5 shows the mean time animals spent exploring the novel and familiar objects, as defined by the local geometric properties of the corners housing the objects, on each test trial. It is clear from this figure that across all trials animals preferentially explored the novel object over the familiar object, with this preference being more marked on the last two test trials. The lower panel of Figure 2.5 displays the mean d2 score across four trials for each rat and an inspection

of this figure reveals that the columns are spread above the x-axis more than below, which suggests that, overall, animals preferred to explore the object located in a novel location more than the object located in a familiar location.

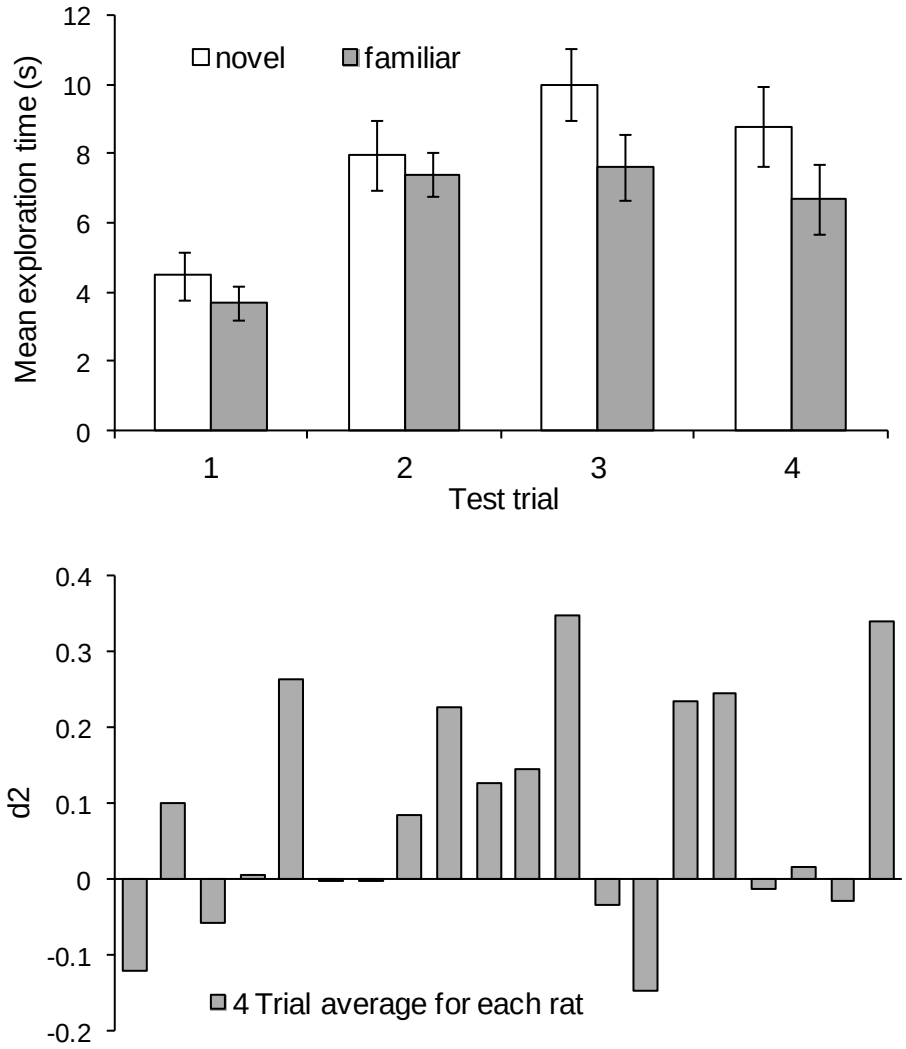

Figure 2.5. Upper panel: Mean exploration times (±SEM) for both novel and familiar object locations across four test trials. Lower panel: Mean d2 scores for each rat averaged across four test trials (chance = 0).

To confirm these observations statistically, a two-way ANOVA conducted on the object exploration times of each rat during the test phase with trial and object as repeated measures, revealed a significant main effect of test trial, $F(2.07, 39.4)$ =

11.82, $p < .001$, and object, $F(1, 19) = 8.74$, $p = .008$, but the test trial x object interaction was not significant, $F < 1$. Pairwise comparisons revealed that the effect of test trial was due to exploration of both objects being significantly lower in trial 1 than in the following three trials. For the d2 scores, which were averaged across four test trials, a one-sample t-test was conducted with a test value of 0. This analysis revealed that rats spent proportionately more time exploring the novel object than expected by chance, $t(19) = 2.6$, $p = .018$

2.3.2.2 Ethovision – Time spent around objects

The upper panel of Figure 2.6 shows the mean time animals spent in each zone surrounding the novel and familiar object, as defined by the local geometric properties of the corners housing the objects, on each test trial. This figure demonstrates that, with the exception of trial two, there appears to be a trend towards animals spending more time exploring the area surrounding the novel object over the area surrounding the familiar object. However, statistical analysis revealed that the time animals spent in a zone around the novel object was not significantly different to the time they spent in a zone around the familiar object, although the p value was $\leq .10$. This was revealed in a two-way ANOVA conducted on the time rats spent in zones placed around each object during the test phase with trial and object as repeated measures. There was a significant main effect of test trial, $F(1.89, 35.8) = 5.39$, $p = .010$, but the main effect of object, $F(1, 19) = 3.00$, $p = .10$, and the test trial x object interaction, $F(3, 57) = 1.73$, $p = .17$, was non-significant. The lower panel of Figure 2.6 depicts the discrimination ratio, or d2 score, for animals averaged across four days and similar to the experimenter scores, it is clear from this figure that the columns are spread above the x-axis more than below, which again suggests that, overall, animals preferred to explore the object in a novel location more than the

object in a familiar location. A one-sample t-test conducted on the d2 scores, with an assigned test value of 0, confirmed this observation, $t(19) = 2.34$, $p = .031$, with rats spending proportionately more time in the zone placed around the novel object than expected by chance,

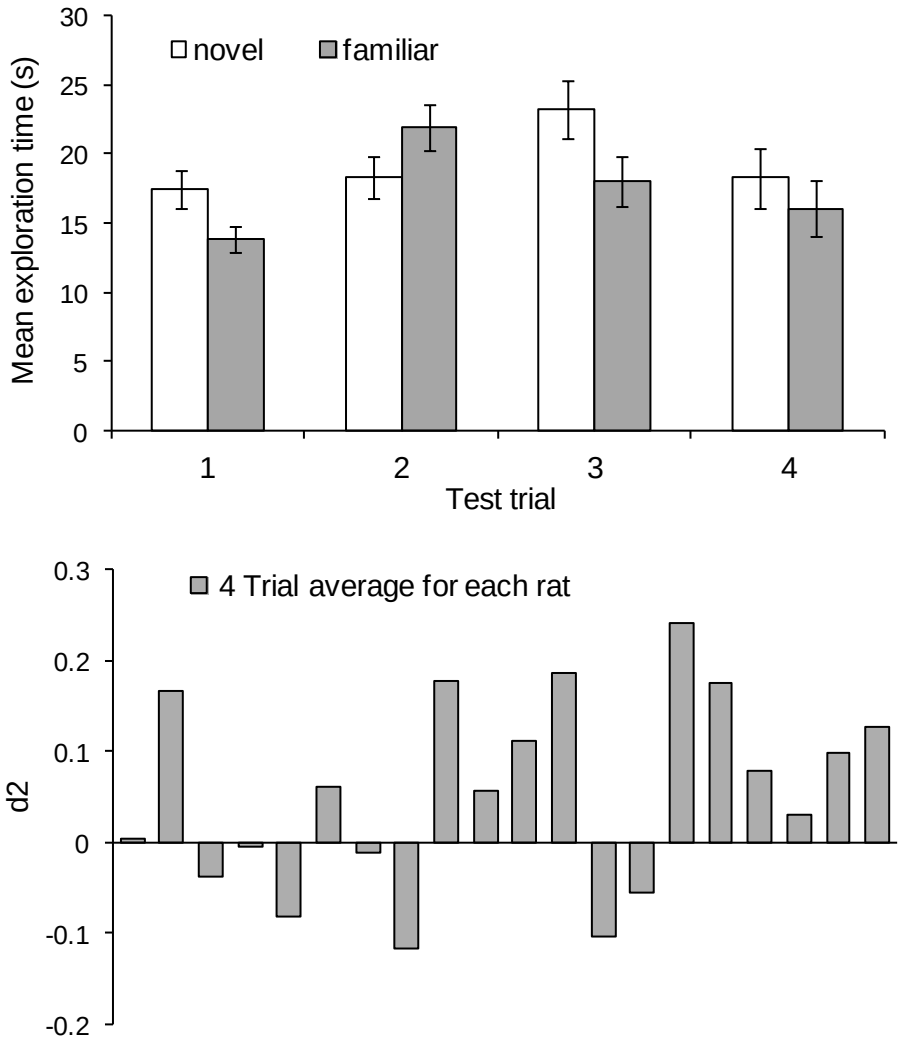

Figure 2.6. Upper panel: Mean time (±SEM) spent in areas around the novel (white bars) and familiar (grey bars) object locations across four test trials. Lower panel: Mean d2 scores for each rat averaged across four test trials (chance = 0).

2.4 General Discussion

The data from Experiments 1 and 2 demonstrate that in a spontaneous object recognition task rats are capable of remembering the location of an object with reference to the local geometric cues in which that object was first encountered. The corner containing the novel object-geometry combination varied among trials, so the recognition of familiarity with the previously encountered object-location combination occurred from a single exposure to the objects. The results are important both in terms of understanding the parameters of recognition memory as measured using spontaneous object recognition tasks, and in terms of the notion of a geometric module. For each experiment reported, two exploration measures were recorded: One manually by an experimenter using a stopwatch, which recorded the time rats actively explored each object, and a second by tracking software, which recorded the time rats spent in close proximity to each object. The tracking data was included to confirm the reliability of the experimenter's scoring. The results revealed that the tracking data was consistent with the stopwatch data when the discrimination ratios (d2) were compared, but, unlike the stopwatch data, the tracking data from the test phase did not reveal a significant difference in exploration of the novel and familiar object. This finding indicates that the manual scoring of exploration is more sensitive than the automated tracking of movement, but when the data is converted to a discrimination ratio (d2), and therefore corrected for differences in exploration, both measures offer consistent findings. One potential reason for the tracking data lacking sensitivity when compared to the experimenter scores is that the Ethovision software used in these experiments cannot discriminate between the animal actively exploring the object and simply being near the object.

With respect to recognition memory, the cues necessary to remember an

object's location have not been systematically studied. In the spatial context variants of the object recognition tasks, described in the Introduction (e.g., Dix & Aggleton, 1999; Good et al., 2007), both the relative positions of the moved objects (with respect to one another or to other objects in the array) and the absolute positions of the objects (with respect to cues outside the arena) could be used to define spatial location. Whether relative or absolute position is a prerequisite for spatial memory has not previously been tested. While context has been shown to be important in object-location memory (e.g., Dellu, Fauchy, Le Maul, & Simon, 1997), what aspects of a context are necessary to disambiguate occasions is not clear. The current results demonstrate that despite encountering objects in different rooms and in different arenas (meaning both the absolute and relative positions of the objects changed between phases), rats were able to detect an object that was novel with reference to some local contextual information provided by the shape of an arena.

The importance of the results of these experiments with respect to the geometric module is three-fold. First, since Cheng's (1986) seminal study, controversy has surrounded the issue focused on the existence in the rat brain of a dedicated 'geometric module' that is impenetrable to non-geometric information. Subsequent studies have questioned this hypothesis by showing that learning a location with reference to geometric information can be influenced by the presence of non-geometric features (e.g., Graham et al., 2006; Pearce et al., 2006; Horne & Pearce, 2009a; Rhodes et al., 2009). However, in the majority of these cases the non-geometric information in question has been provided by altering the colours of walls with different lengths, so it could be argued that the non-geometric cues are inextricably bound to the surfaces forming the geometric frame of reference and are thereby able to permeate the 'geometric module' in this way. Indeed, it has proven

difficult to demonstrate the influence of discrete landmarks on learning based on geometry (e.g., Hayward et al., 2003, 2004; McGregor et al., 2009; Pearce et al., 2001; but see Horne & Pearce 2009b, Kosaki et al., 2013). In the current experiments the objects can be regarded as discrete non-geometric cues, and the results provide unequivocal evidence that rats can integrate geometric information with these non-geometric cues on the basis of their familiarity in that geometric context.

Second, as discussed in the Introduction, Cheng (1986) and Gallistel (1990) proposed that when placed in a distinctively shaped arena, rats use a global representation of this shape as a metric frame of reference in order to find a goal. This proposal has been challenged by evidence from experiments showing that rats in a water maze are capable of transferring spatial behaviour between differently shaped arenas provided some local geometric information is common to both arenas (McGregor et al., 2006; Pearce et al., 2004; see also Tommasi & Polli, 2004). However, it is possible that the repeated reinforcement for heading to a particular set of cues led to a stimulus-response habit that was responsible for the transfer of behaviour between arenas. In Experiments 1 and 2, memory for the object's location based on local geometric cues occurred incidentally, without any obvious reinforcement and without repeated trials. Therefore, these are the first experiments to show learning based on local geometric properties and discrete objects in such a task.

Finally, Cheng (1986) suggested that rats rely primarily on the geometric properties of their environment to navigate, but that over time non-geometric cues could be 'glued on' to this metric frame (p.174). Cheng and Newcombe (2005) subsequently suggested that two processes should be considered in studies that involve learning about geometric cues: one used when reorienting, which is entirely

geometric, and another, which may include non-geometric cues, used to locate a particular target location (see also Sutton, 2009). However, in each of the above descriptions of how geometry may come to have primacy in navigation, it should be expected that in a single exposure to geometric and non-geometric cues, the geometric cues should be processed independently of the non-geometric features. While other studies discussed above suggest that non-geometric cues can interact with geometric cues over time, our experiments are the first to show that non-geometric cues are rapidly encoded along with geometric cues with only a single exposure to the cues in compound. Whether this is through a form of snapshot memory (see Cheng, 2008), an associative process (e.g., Whitt, Haselgrove, & Robinson, 2011), or because non-geometric cues integrate with geometry in some other way not expected by current versions of the geometric module hypothesis is open to question. Therefore, the following series of experiments in Chapter 3 seeks to investigate this line of inquiry by focusing attention on how objects and local geometric cues used in the current experiments are bound together. More specifically, it will be investigated whether this conjunctive processing is governed by associative rules.

3

Object-in-Local Geometry Representation: Associations & Neural Substrates

3.1 Introduction: *Within-Compound Associations*

The results of Experiments 1 and 2 provided unequivocal evidence that rats are capable of integrating local geometric cues (corners) with non-geometric cues (objects), but the nature of this integration remains unresolved. To encompass a range of associative phenomena observed in both animal conditioning (e.g. Miller & Matute, 1996) and human causal learning (e.g. Dickinson & Burke, 1996), several authors have suggested that when two different cues are presented in compound and paired with reward, associations form between these cues, which are commonly referred to as *within-compound associations* (Dickinson & Burke, 1996; Van Hamme & Wasserman, 1994). According to this hypothesis, the integration of geometric cues with featural cues, observed in Experiments 1 and 2, should involve the formation of within-compound associations. Conversely, animals may encode these cues non-associatively using an image matching or snapshot memory where a retinal snapshot is taken of the view from the goal location that can be used during subsequent navigation to match the current retinal view of the environment against it (e.g., Cartwright & Collett, 1982, 1983; but for applications in the rat see: Cheung, Sturzl, Zeil, & Cheng, 2008; Sturzl, Cheung, Cheng, & Zeil, 2008; Sheynikhovich, Chavarriaga, Strosslin, Arleo, & Gerstner, 2009). If rats encoded the object-corner configurations in this way during Experiments 1 and 2, i.e. as a single entity, then it is unlikely that the formation of within-compound associations would be observed.

The following series of experiments attempts to identify the presence of within-compound associations between local geometric cues and objects, but first, by way of background, a brief description of within-compound associations is provided.

Rescorla and Cunningham (1978) were among the first experimenters to show, in a taste aversion experiment, that when rats are presented with two different flavour cues simultaneously (e.g. salt and quinine) and one of these cues (e.g. quinine) is subsequently devalued, by pairing it alone with lithium chloride induced illness, an aversion to the other cue (e.g. salt), which had not been devalued, is also established. The interpretation of this finding offered by the authors was that although salt was never directly paired with the aversive outcome of illness, rats must have formed an associative link not only between quinine and lithium chloride but also between quinine and salt. Thus, rats associate quinine with illness through a direct CS-US link and also salt with illness through an indirect salt → quinine → illness associative chain, with the link between salt and quinine referred to as a within-compound association (WCA). A recent study by Rhodes et al. (2009) has provided evidence of WCAs between geometric and wall colour cues but, as mentioned in the discussion of Experiments 1 and 2, it may be the case that wall colour cues are inextricably bound to the surfaces forming the shape of the environment and are thereby able to permeate the geometric module. In fact, it has proven difficult to demonstrate the influence of discrete objects or landmarks on geometry-based learning. Thus, it remains unproven as to whether WCAs occur between geometric and discrete non-geometric cues in a non-reinforced, spontaneous task.

In the classic novel object recognition (NOR) task, animals are simply required to recognise the familiarity of a single object and therefore the formation of

conditioned associations between cues in the environment is not required. Conversely, variants of the standard NOR task such as object-place (e.g. Save, Poucet, Foreman & Buhot, 1992), object-context (e.g. Dix & Aggleton, 1999), object-place-context (Eacott & Norman, 2004) and temporal order (e.g. Hannesson, Howland & Phillips, 2004) tasks have demonstrated an apparent role for associative processes in recognition memory. Taking this support for associative processes in recognition memory one step further, Whitt et al. (2012) devised a task that provided evidence of 'indirect recognition' processes in rats, and demonstrated how the presence of one cue, such as an object or wall pattern, can evoke a memory for another absent object. The purpose of this study was to determine whether such an effect could explain the results of Experiments 1 and 2.

Brandon, Vogel and Wagner (2003; but see also the original *standard operating procedure* (SOP) model by Wagner, 1981) provide a model, capturing the activity dynamics of stimulus representations, to explain such findings as Whitt et al's (2012) indirect recognition. Briefly, this model supposes that a single stimulus, such as an object, comprises of constituent elements that are able to reside in one of three activity states. An animal's behavioural response to a stimulus is governed by the proportion of elements in each activity state. Elements may reside in: a primary ("A1") state, having been inactive, which is elicited by initial presentation of the stimulus, a secondary ("A2") state having passively decayed from A1, and an inactive ("I") state after passively decaying from A2. The model proposes that should the constituent elements of the stimulus be placed into the A1 state, then responding will be strong, however, once these elements have decayed into the A2 state, responding should diminish, before they revert back to the inactive state. The connecting pathways between each activity state, which form a closed loop, are such

that elements can only pass in one direction from A1 to A2 to I and back to A1 for the start of a new cycle. This unidirectionality means that elements cannot enter the A1 state directly from the A2 state.

In the current experiment, during the sample phase, animals were presented with two objects, A and B, positioned in opposite right angled corners of a kite-shaped arena (see Figure 3.1). Accordingly, animals should associate object A with the local geometric corner cue comprising of a short wall to the left of a long wall (corner J), and object B with the local geometric cue comprising of a short wall to the right of a long wall (corner L). After this sample phase, animals were placed in a holding cage for 8 minutes with a copy of one of the objects previously encountered, e.g. object A. By exposing the animal to object A for an extended period, the aim was to reduce the perceived novelty of, or devalue, this object. For the purposes of the following experiments the term *devalue* refers to this reduction in perceived novelty as opposed to any change in value related to a more conventional reward such as food. For the test phase, animals were placed into a rectangular arena, which maintained some of the local geometric cues provided by the kite. Therefore, should animals be capable of forming within-compound associations between a local geometric cue and an object, for example corner J and object A, the devaluation of object A should also devalue corner J despite the fact that corner J was never directly devalued. Because corners E and G of the rectangle are equivalent to corner J of the kite, according to relative wall length configuration, it was predicted that animals would spend less time in these devalued corners compared to corners F and H (See Figure 3.1).

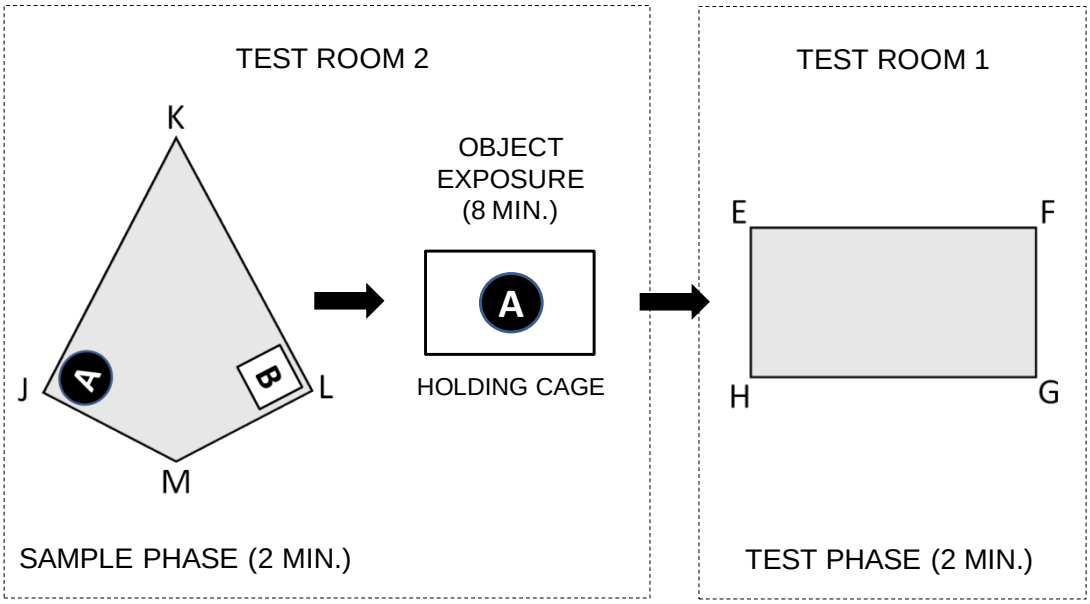

Figure 3.1. Schematic diagram showing the experimental design. Objects A and B are represented by black circular and white square symbols respectively. Following exposure to objects A and B in corners J and L of the kite, respectively, animals were habituated to object A in a holding cage for 8 minutes to devalue this object. If rats associate the devalued object A with corner J of the kite, then it was predicted that rats should be less inclined to explore the geometrical equivalent corners (E and G) of the empty rectangular arena during the test phase.

Using Brandon et al.'s (2003) mechanistic model to explain this behaviour, it was predicted that the recent exposure to object A prior to test, which would also indirectly activate a recent memory trace for corner J, will ensure both object A and corner J's elements remain stuck in a secondary (A2) state of activity. Corner L, on the other hand, was not activated associatively prior to the test phase and so its elements would remain in an inactive (I) state. Thus, during the test phase, corners E and G of the rectangle were, as already mentioned, geometrically equivalent to corner J of the kite, so their elements, like corner J's, would be in a secondary (A2) state of activity. Conversely, corner F and H's elements would be in an identical state to their geometric equivalent, corner L of the kite, i.e. in an inactive (I) state.

Therefore, because the model does not allow elements in a secondary (A2) state of activity, but only elements in an inactive (I) state, to enter directly back to a primary (A1) state of activity, it was predicted that during the test phase, rats will explore corners F and H of the rectangle more than corners E and G.

Before conducting the experiment proper, it is important to establish that exposing animals to an object in a holding cage for 8 minutes does indeed devalue this object. To this end, a pilot experiment was conducted in which animals were exposed to two objects, A and B, in opposite right-angled corners of a kite-shaped arena for two minutes before being transferred to a holding cage with a copy of one these objects, e.g. object A, for 8 minutes (sample phase). For the test phase, animals were placed back into the kite and exposed to the identical treatment they experienced during the sample phase except that now each object, A and B, was replaced with an identical copy. Should the devaluation procedure be effective, it is predicted that animals in this example will preferentially explore object B over the devalued object A. It is important to note that in the following analyses the devalued object or, in the case of Experiments 4 and 5, the corner predicted to form a within-compound association with the devalued object, is labelled as *familiar* and the non-devalued object or its associated corner cue is labelled as *novel*.

3.2 Experiment 3: Novel Object Devaluation Pilot

3.2.1 Methods

3.2.1.1 Subjects

The subjects were 16 male Lister hooded rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England) and were approximately 5 months of age at

the start of the experiment. The rats had previously participated in an unrelated water maze task and it was ensured that this prior experience was counterbalanced. Animals were housed in identical conditions to those in Experiment 1.

3.2.1.2 Apparatus

The apparatus was identical to Experiment 1 except that the rectangular arena located in test room 1 was not used in this experiment. There was also a new holding cage used in this experiment, which was placed on the table in test room 2 with cardboard dividers placed on either side of it so that from inside this cage an animal's view of the arena and the other holding cage, which contained the remaining animals, was obstructed.

3.2.1.3 Procedure

General Procedure

See Experiment 1 for procedural details.

Habituation

Four habituation sessions were conducted each consisting of the animal being placed individually into the kite shaped arena in test room 2 for 5 minutes before being taken out and immediately transferred to an empty holding cage for a further 3 minutes. All remaining details were identical to Experiment 1.

Experimental Stage

The experimental stage was identical to Experiment 1 with the following exceptions. In the sample phase, rats were exposed to two different objects, A and B, in corners J and L of the kite shaped arena situated in room 2 for two minutes before

being removed and placed into a holding cage containing a copy of object A for eight minutes. Subsequently, the rat was immediately transferred back into the kite-shaped arena, still in the same position and test room, and containing copies of objects, A and B, positioned in the same corners of the kite, J and L, as during the sample phase (see Figure 3.2). For each rat, the orientation of the kite changed between days but on the same day remained constant for both the sample and test phase. On each day, the orientation of the kite, the locations of objects in the sample and test phase and the identity of the object placed in the holding cage was counterbalanced between animals. For each animal it was ensured that the right-angled corner containing the novel object alternated between days.

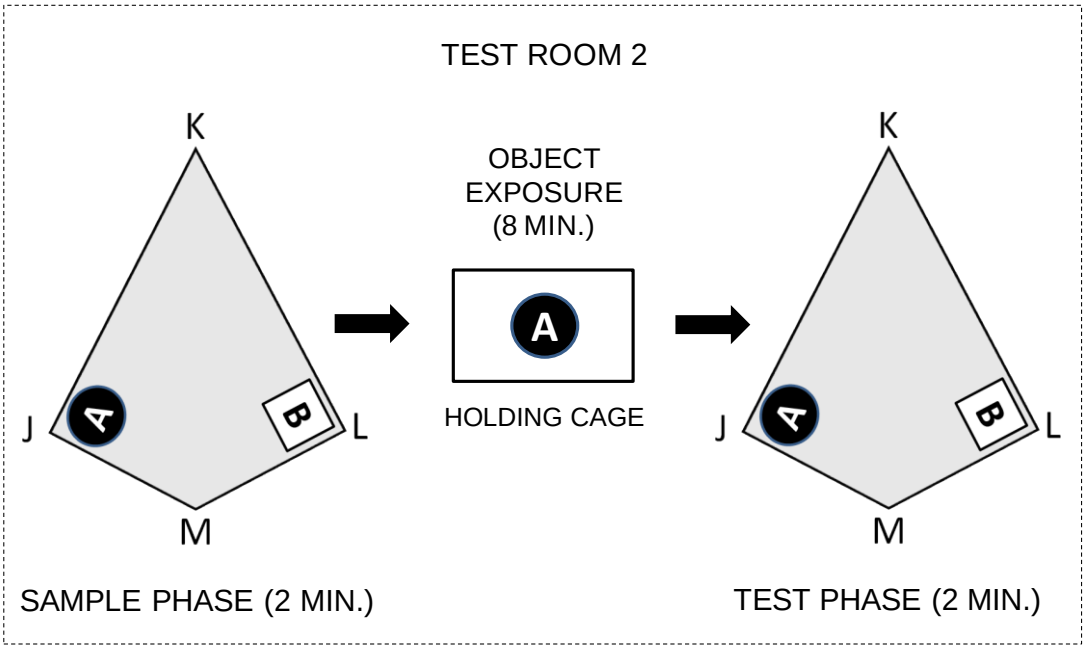

Figure 3.2. Schematic diagram showing the experimental design. Objects A and B are represented by black circular and white square symbols respectively. Preferential exploration of object B over object A in the test phase indicates that habituating rats to an object in a holding cage for 8 minutes reduces its perceived novelty, or devalues, it.

Performance Measures

The Performance Measures were identical to Experiment 1 except that Ethovision software was not used in this pilot study to track animals.

3.2.2 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied.

Stopwatch Scoring

The upper panel of Figure 3.3 shows the mean time animals spent exploring the novel and familiar objects on each test trial. It is clear from this figure that across all trials animals preferentially explored the novel object over the familiar object, with this preference being more marked on the first two test trials. The lower panel of the same figure displays the mean d2 score across four test trials for each rat and shows that the d2 scores for thirteen out of sixteen rats were above zero and so the majority of animals spent proportionately more time exploring the novel over the familiar object.

To confirm these observations, a two-way ANOVA conducted on the object exploration times of each rat during the test phase with test trial and object as repeated measures, revealed a significant main effect of object, $F(1, 15) = 11.62$, $p = .004$, and test trial, $F(3, 45) = 3.63$, $p = .029$, but the test trial x object interaction was not significant, $F < 1$. Pairwise comparisons revealed that the effect of test trial was due to a significant difference in overall exploration of objects in test trials 1 and 4, while there were no differences among total exploration times for any of the other days. For the d2 scores, which were averaged across the four test trials, a one-sample

t-test was conducted with a test value of 0. The analysis revealed that rats spent proportionately more time exploring the novel object than expected by chance, $t(15) = 3.81$, $p = .002$.

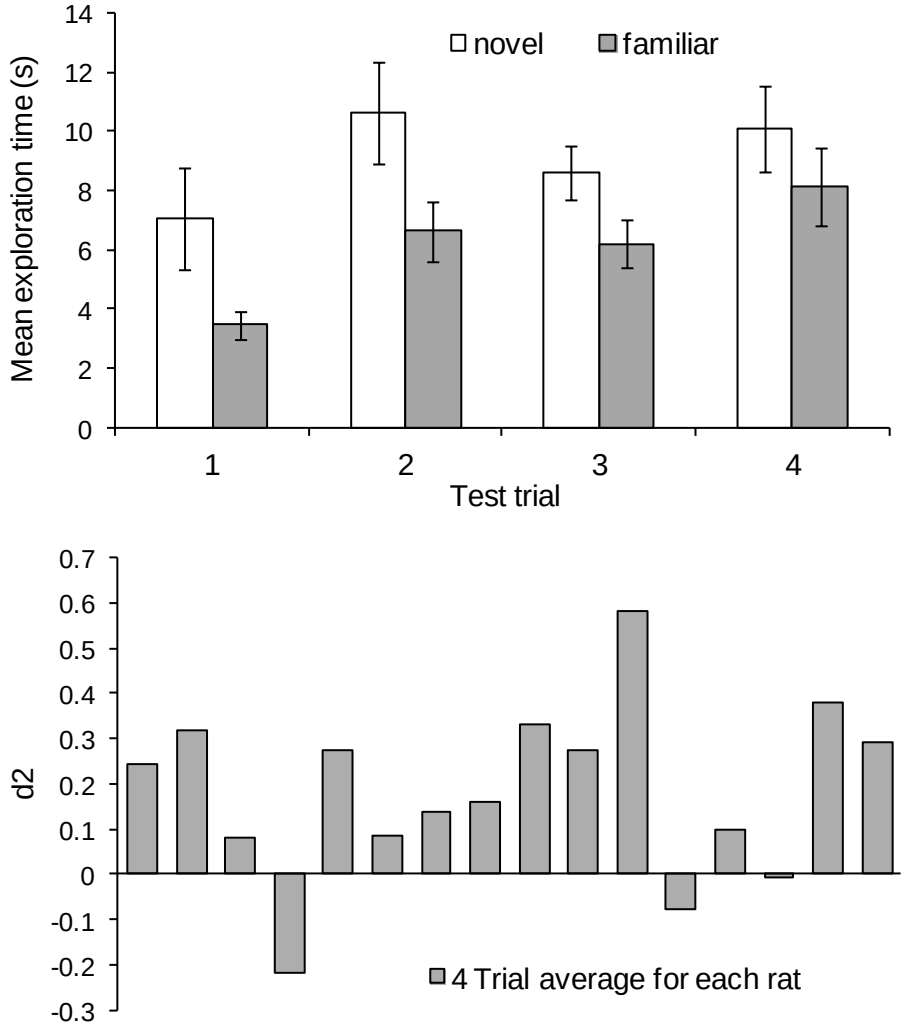

Figure 3.3. Upper panel: Mean exploration times (+SEM) for both novel (white bars) and familiar (grey bars) objects across four test trials. Lower panel: Mean d2 scores for each rat averaged across four test trials (chance = 0).

3.2.3 Discussion

The results of the pilot study were conclusive in that across all test trials animals spent more time exploring the novel, or non-devalued object, more than the over familiar, or devalued, object. Therefore, one can conclude with confidence that by exposing animals to an object in a holding cage for 8 minutes, the perceived novelty of this object is reduced, or the object is devalued.

3.3 Experiment 4: Indirect Geometry Devaluation (No Object at Test)

3.3.1 Method

3.3.1.1 Subjects

The subjects were 16 male Lister hooded rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England) and were approximately 7 months of age at the start of the experiment. The rats had previously participated in an unrelated water maze task and it was ensured that this prior experience was counterbalanced. Animals were housed in identical conditions to those in Experiment 1.

3.3.1.2 Apparatus

See Experiment 3 for details of the apparatus used.

3.3.1.3 Procedure

General Procedure

See Experiment 1 for procedural details.

Habituation

The first three habituation sessions were identical to those described in Experiment 3. However, on the morning of the fourth session of habituation, rats were subjected to the same procedure as the previous three sessions but four hours later were transported from the holding room, for a second time, to test room 1 in which they were individually exposed to the rectangular arena for 2 minutes. On the fifth day of habituation each animal was exposed to the rectangular arena in test room 1 for 2 minutes.

Experimental Stage

The experimental stage was identical to Experiment 1 with the following exceptions. In the sample phase, rats were exposed to two different objects, A and B, in corners J and L of the kite situated in room 2 for two minutes before being removed and placed into a holding cage containing a copy of object A for eight minutes. Subsequently, the rat was immediately transferred to test room 1 for the test phase where it was placed in the rectangular arena containing no objects for 2 minutes (see Figure 3.1 above in Introduction to chapter). The orientation of the kite changed between days but remained constant for all animals on the same day. The rectangular arena was positioned in four different orientations each day and these orientations were counterbalanced among animals. The locations of objects in the sample phase and the identity of the object placed in the holding cage was also counterbalanced between animals each day.

Performance Measures

The performance measures were identical to Experiment 1 with the following exceptions. Object exploration was not recorded manually by the experimenter as there were no objects present during the test phase. Thus, exploration by rats in this experiment was exclusively measured using Ethovision (version 3.1) software, which tracked the movement of each animal in the test phase. Using this software, the rectangular arena was divided into four equal quadrants and the time rats spent in each quadrant was recorded. Because the local geometric properties of corners diametrically opposite each other in the rectangular arena were identical (e.g. corners E and G or F and H), the time spent in these corners was combined to provide a single value of the time spent in the novel geometric corners versus the familiar geometric corners. As well as using quadrant zones in Ethovision, smaller circular zones (approximately 24cm in diameter) were also used, which were individually positioned in each corner of the rectangular arena so that the centre of each zone corresponded to where the centre of an object would have been had there been one present. Similar to the quadrant data, the time animals spent in diametrically opposite zones was combined.

3.3.2 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied.

3.3.2.1 Ethovision – Quadrant Zones

The upper panel of Figure 3.4 shows the mean time animals spent in the novel and familiar quadrant zones, as defined by their local geometric properties, on

each test trial. It is clear from this figure that across all test trials animals did not spend more time in the novel quadrants of the arena. In fact, on the first two test trials rats spent slightly more time in the familiar quadrants. The lower panel of Figure 3.4 displays the mean d2 scores across four test trials for each rat and it is apparent that animals did not spend proportionately more time in the novel quadrants than the familiar quadrants as evidenced by the fact that half of the d2 scores fell below zero.

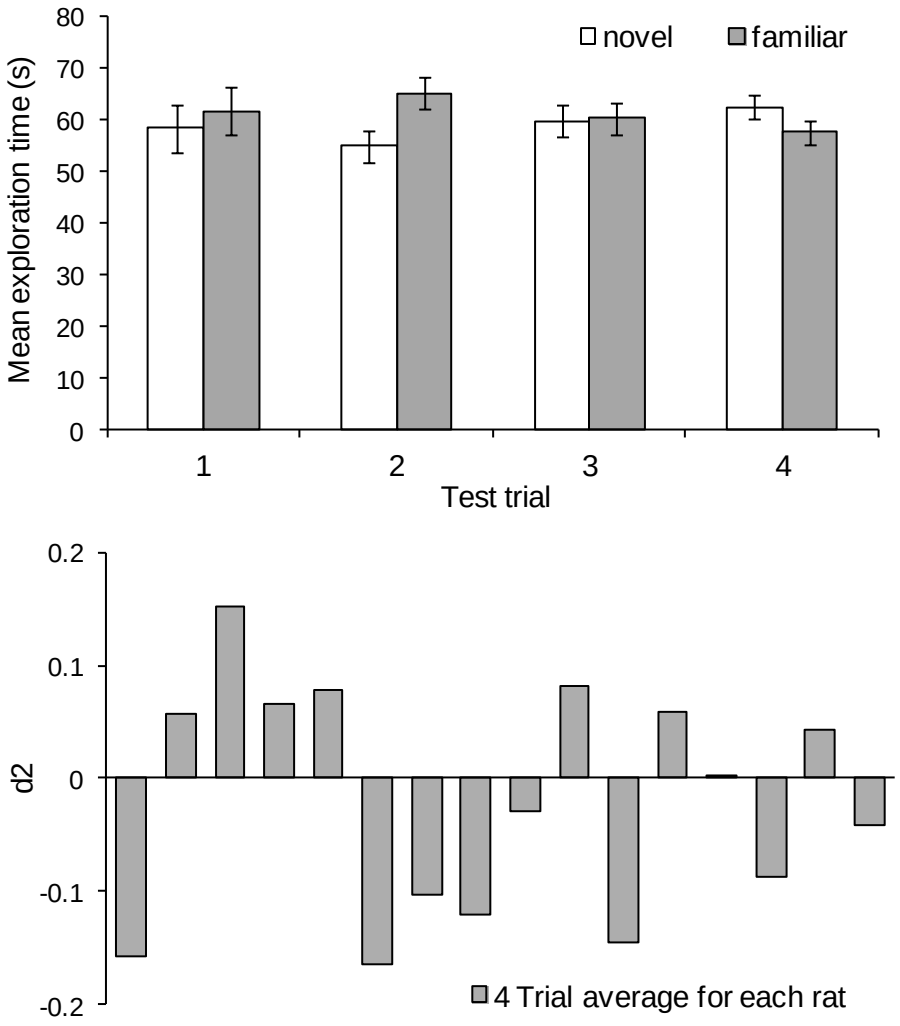

Figure 3.4. Upper panel: Mean time (±SEM) spent in novel (white bars) and familiar (grey bars) quadrants of the rectangular arena across four test trials. Lower panel: Mean d2 scores for each rat averaged across four test trials (chance = 0).

To validate these observations statistically, a two-way ANOVA, conducted on the time each rat spent in quadrants of the arena during the test phase with test trial and quadrant zone as repeated measures, revealed no significant main effects or interactions, *F*s < 1. For the d2 scores, averaged across four test trials, a one-sample t-test was conducted with a test value of 0. The analysis revealed that rats did not spend proportionately more time in the novel quadrants than expected by chance, $t(15) = -.77$, $p = .45$.

3.3.2.2 Ethovision – Circular zones

The pattern of statistics was the same for the smaller circular zones as it was for the quadrant zones. An ANOVA conducted on time in zone with test trial and zone as repeated measures revealed that rats did not spend significantly longer in the two novel zones (*M* = 10.2, *SEM* = .93) than the two familiar zones (*M* = 11.5, *SEM* = .91), $F(1, 15) < 1$. The d2 scores (*M* = -.06, *SEM* = .06) also followed the same pattern of statistics as reported for the quadrant zones. The performance of rats across different time frames within each test trial was also analysed but the pattern of statistics did not deviate from those reported above.

3.3.3 Discussion

It was postulated in the Introduction that should Cheng's (1986) geometric module hypothesis stand true, animals should not be capable of integrating geometric information with non-geometric information in a spatial memory task. More specifically, using a design employed in non-spatial tasks (e.g. Rescorla & Cunningham, 1978), it was investigated whether rats were capable of forming within-compound associations between a local geometric cue (a right-angled corner

defined by its long and short wall properties) and an object. Having been exposed to two different object-corner pairings during the sample phase (Object A + Corner J & Object B + Corner L), one of these objects, e.g. object A, was devalued. Should animals have formed a within-compound association between object A and corner J, it was expected that corner J would also be indirectly devalued. Therefore, at test when no objects were present, it was predicted that animals should spend less time exploring the corners of the arena which shared the same local geometric properties as corner J. Unfortunately the results of this experiment were not consistent with this prediction. Rats did not spend any more time in the corners of the arena which were associated with a comparatively novel object than in the corners associated with a devalued, familiar object. Therefore, this experiment has failed to substantiate the notion that rats are capable of forming within-compound associations between a local geometric cue and an object.

The aim of this experiment was to build on the results of Experiments 1 and 2 in order to determine the presence of and mechanism underlying the associative links formed between a corner of an arena and an object. However, in Experiments 1 and 2 and also in Whitt et al.'s (2012) experiment, behaviour was measured by recording the amount of time animals spent exploring objects during the test phase. In the current experiment, the objects were removed at test and so behaviour was measured by recording the amount of time animals spent in each corner of the arena. This testing procedure, in which the objects were removed, could be a potential reason why rats did not perform as predicted. Recording an animal actively exploring an object is somewhat different to recording the time it spends in a particular area of the arena. Even if rats in this experiment were capable of forming within-compound associations between an object and a particular corner of the arena, it is possible they

were not motivated to explore this corner as it was obvious to visualise from some distance away that there was no interesting stimulus, such as an object, present to explore. Thus, this lack of motivation could have masked any potential preferences to spend time in one corner over another and explain the random behaviour observed. With this potential design flaw in mind, Experiment 5 was designed to counteract this problem.

3.4 Experiment 5: Indirect Geometry Devaluation (With Object X Present)

3.4.1 Introduction

The current experiment was conducted using an identical design to Experiment 4 with the exception that a common object, X, was used during the sample and test phase. Thus, each object in the sample phase sat on top of an upturned, beige, ceramic pot, which was termed object X. During the test phase, the objects were removed and identical copies of object X were individually positioned in corners E and F of the rectangular arena (see Figure 3.5). By presenting animals with a common stimulus, object X, the aim was to provide an object during the test phase towards which animals could focus their search. The predictions for this experiment are identical to those for Experiment 4. So, during the sample phase, should animals have formed an associative link between an object, the corner it is positioned in, and object X, e.g. object A → Corner J → object X, it was expected that animals during the test phase would spend less time exploring the copy of object X positioned in the corner associated with a recently devalued object, e.g. corner E and object A, respectively (see Figure 3.5).

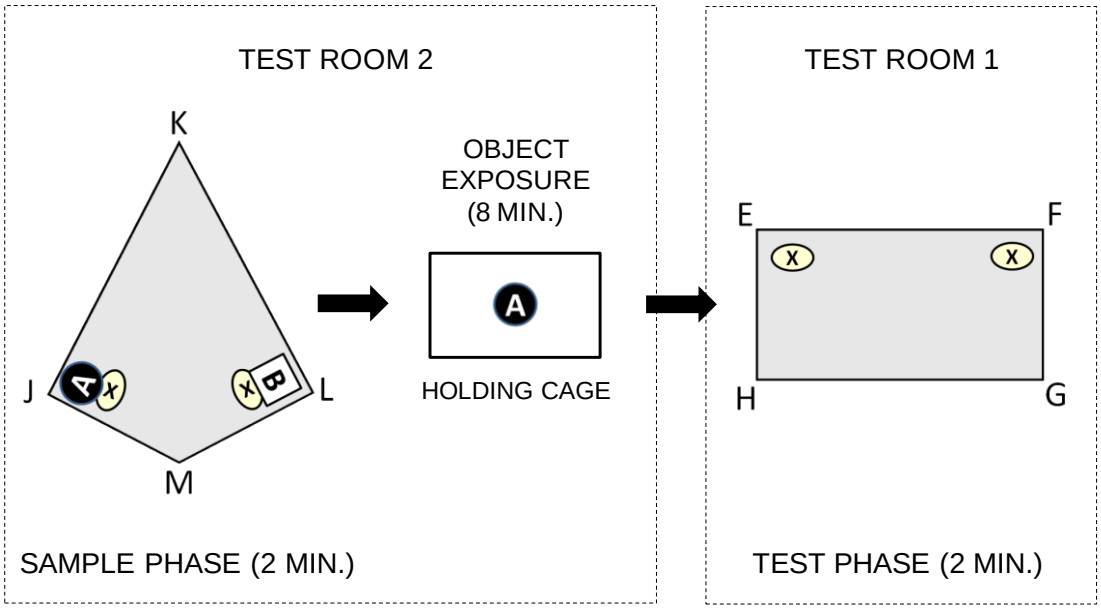

Figure 3.5. Schematic diagram showing the experimental design. Objects A, B and X are represented by black circular, white square and yellow oval symbols respectively. Following exposure to objects AX and BX in corners J and L of the kite, respectively, animals were habituated to object A in a holding cage for 8 minutes to devalue this object. If rats associate the devalued object A with corner J and object X of the kite, then it was predicted that animals should be less inclined to explore the copy of object X at test that was placed in corner E of the rectangle, which is the geometrical equivalent of corner J.

3.4.2 Method

3.4.2.1 Subjects

The subjects were 20 male Lister hooded rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England) and were approximately 6 months of age at the start of the experiment. The rats had previously participated in an unrelated water maze task and it was ensured that this prior experience was counterbalanced. Animals were housed in identical conditions to those in Experiment 1.

3.4.2.2 Apparatus

The apparatus used was identical to Experiment 4 except for the addition of four identical copies of a new object, object X, which was an upturned, circular, beige pot 9 cm in diameter and 5 cm deep. A single copy of object X resided in corners J and L of the kite and corners E and F of the rectangle (see Figure 3.5) throughout Habituation and the Experimental stage.

3.4.2.3 Procedure

General Procedure

The procedural details were identical to Experiment 4.

Habituation

All aspects of the habituation procedure were identical to Experiment 4 except that a single copy of object X was located in each right-angled corner of the kite and in corners E and F of the rectangular arena.

Experimental Stage

The experimental stage was identical to Experiment 4 with the following exceptions. During the sample phase, each object, A and B, was affixed centrally on top of a copy of object X in corners J and L of the kite, respectively, as opposed to each object being affixed to the arena floor. For the test phase, instead of the rectangular arena not containing any objects as in Experiment 4, in this experiment a single copy of object X was positioned in corners E and F of the rectangular arena (see Figure 3.5).

Performance Measures

The Performance Measures were identical to Experiment 1.

3.4.3 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied.

3.4.3.1 Stopwatch Scoring

The upper panel of Figure 3.6 shows the mean time animals spent exploring the novel and familiar objects on each test trial and it is evident that rats did not preferentially explore the novel object over the familiar object. In fact, on trial 3 it appears that rats spent more time exploring the familiar object, although variance in exploration of this object was high. The lower panel of Figure 3.6 displays the mean d2 scores averaged across four test trials for each rat and an inspection of this figure reveals a sporadic spread of d2 scores with no discernible pattern, which is indicative of the fact rats did not spend proportionately more time exploring one object over the other. To test statistically the above observations a two-way ANOVA conducted on the object exploration times of each rat during the four test trials with test trial and object as repeated measures revealed no significant effects or interactions, $Fs(1, 19) \leq 1.59$, $p \geq .22$. For the d2 scores, which were averaged across four test trials, a one sample t-test was conducted with a test value of 0. The analysis revealed that rats did not spent proportionately more time exploring either object than expected by chance, $t(19) = -.60$, $p = .55$.

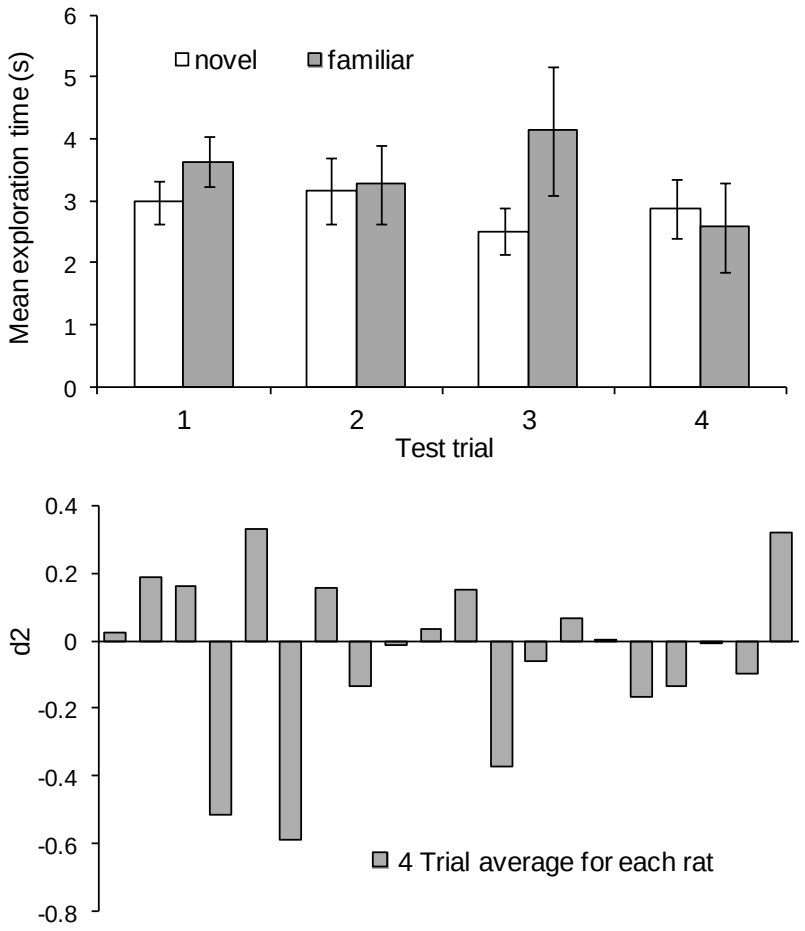

Figure 3.6. Upper panel: Mean exploration times (±SEM) for novel (white bars) and familiar (grey bars) objects across four test trials. Lower panel: Mean d2 scores for each rat averaged across four test trials (chance = 0).

3.4.3.2 Ethovision – Time spent around objects

The time spent in zones around the novel and familiar objects followed the same pattern of statistics as the stopwatch exploration scores with no significant effects or interactions emerging, *Fs* < 1. This was also the case for the d2 scores, $t(19) = -.82$, $p = .42$, which were non-significant. A range of time bins were analysed, for both the stopwatch scores and Ethovision recordings, to investigate the performance of rats across different time frames within each test trial but the pattern of statistics did not deviate from those reported above.

3.4.4 Discussion

The results of Experiment 5 were inconsistent with the predictions offered in the Introduction. To counteract the issue of animals not having objects to explore during the test phase in Experiment 4 the current experiment provided a common stimulus, object X, which was paired with each object during the sample phase. For the test phase, only copies of object X were presented in mirror opposite corners of the rectangle (E and F). Even though, at test, animals were provided with an object to explore in each of these corners, a comparative decline in exploration to object X positioned in a corner associated with a devalued object was not observed. One potential reason for this lack of discriminatory exploration during the test phase could be because animals were exposed to object X on every trial. Therefore, this prolonged exposure to object X across trials may have rendered this object very familiar, and so animals were less motivated to explore either copy during the test phase let alone discriminate between them. If this argument was valid it could be suggested that animals may perform better on earlier trials of the experiment when over familiarisation to object X was less prominent, but this was not the case.

The design of the current experiments and Whitt et al.'s (2012) study were very similar but the results were inconsistent. Whitt et al. provided evidence of associative processes in an object recognition task and the present experiments did not. Thus, it is worth addressing differences between the current procedure and that of Whitt et al. One obvious difference is the type of cues that were paired with the objects. Whitt et al. used object-object (Experiment 1) and object-wall pattern (Experiment 2) pairings while the current experiments used object-local geometry pairings. Whether rats were able to associate the cues used in the former experiment more readily than those in the current experiment is open to question. A second

difference is the duration of exposure times, with Whitt et al. providing 5 or 10 minutes (depending on group) for exploration during the sample phase, and the present experiments providing 2 minutes. Perhaps longer exposure times allowed rats to form a stronger association between cues in Whitt et al.'s study. Finally, the revaluation, or over familiarisation, procedures differed between each study. In the current experiments, after being exposed to object-corner pairings in the sample phase, rats were removed from the arena and placed in an entirely different context - an empty holding cage - in which they were exposed to a copy of one of the objects experienced during the sample phase. Whitt et al. used a similar procedure but the devaluation stage was conducted in an arena identical in shape and position to the arenas used during the sample and test phases. The similarity of contextual information between stages in Whitt et al.'s study may have facilitated the formation of associations between cues.

Despite the aforementioned procedural differences, however, it is important to point out that Experiments 1 and 2 used the same object-geometry pairings and exposure times as the present experiments and were effective enough to produce a robust preference for the object in a novel geometric location. Additionally, the results of Experiment 3 (pilot study) demonstrated that the devaluation procedure was effective in reducing the novelty of an object in a standard object recognition task. Therefore, the logic behind the procedural details of the current experiment was supported by previous results. However, given the failure to provide evidence that rats can form links between an object and geometric information, it is important to consider potential future avenues for this work. One possibility could be to use a different method of revaluation, or indeed invoke an upward shift in the value of the target object rather than devalue it. This could be done, for example, using a

conditioned place preference (CPP) task in which the rat is placed in a small chamber with the target object after receiving a drug with reinforcing properties. Alternatively, the target object could be reinforced with food, a method used by Rhodes et al. (2009) who succeeded in showing that rats can form within-compound associations between wall colour and corners. Another potential design could be to revalue the geometric cue rather than the object. Finally, the pilot study (Experiment 3) confirmed that the object devaluation procedure was effective when animals were placed into a kite shaped arena. However, it has not been confirmed whether or not animals show the same devaluation effect in the rectangle. Although, this procedural detail may sound trivial, when predicted outcomes stop working it is important to investigate where they stopped working. Therefore, it would be advisable to run another pilot study showing the effects of object revaluation in the rectangle.

In summary, Experiments 4 & 5 did not provide evidence for the presence of within-compound associations between a local geometric cue and a discrete object. Whether these findings lean toward the view that rats in Experiments 1 and 2 were solving the task non-associatively, synonymous with an image matching account, or whether the design of these experiments has in some way failed to demonstrate the predicted behaviour is open to debate. The null results reported here certainly cannot be used as evidence to support one account over the other. Notwithstanding these findings, Experiments 1 and 2 still provide evidence that local geometric cues were used to identify the novel over the familiar object. The purpose of remaining experiments in this chapter is to address the question of which neural structures are involved in such processing.

3.5 Introduction: *Neural Substrates of an Object-in-Local Geometry Representation.*

The results of Experiments 1 and 2 provided strong evidence that rats learned about the location of an object with reference to geometric information provided by the walls of the enclosure. Therefore, in a broad sense, it is useful to establish which brain structures are involved in the processing of an object-in-local geometry configuration. Several rodent studies have shown that the hippocampus is required during recognition tasks in which an animal must integrate objects with contextual information, which is either spatial (Bussey, Duck, Muir & Aggleton, 2000; Good et al., 2007; Save et al., 1992), featural (Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002), or temporal (Good et al., 2007; Hannesson et al., 2004) in nature. Conversely, numerous studies have demonstrated that rodents with hippocampal lesions are not impaired in standard object recognition tasks (Ainge, Heron-Maxwell, Theofilas, Wright, de Hoz & Wood, 2006; Good et al., 2007; Mumby et al., 2002; but also see, for example, Broadbent, Squire & Clark, 2004; Clark, Zola & Squire, 2000; Gaskin, Tremblay & Mumby, 2003). As well as considering the brain structures involved in the integration of different types of contextual cues, as far as the spatial aspects of an object recognition task are concerned, it is also important to consider how this spatial information is represented and how this in turn impacts on the brain structures required.

For example, rats in Experiments 1 and 2 could use a snapshot memory (see Introduction at start of chapter for a description), or an *individual corner solution* (Pearce et al., 2004) where a configuration of elements is learned in an associative manner. In this case, the elements would be walls of differing lengths forming a particular corner with, for example, the short wall to the left of a long wall.

Alternatively, rats in Experiments 1 and 2 could be employing an egocentric response strategy, which requires the animal to learn the relationship between an environmental cue and a rewarded response. For example, rats could be using a single wall of a given length as a cue to implement a specific motor response. Of course Experiments 1 and 2 were not designed to investigate which, if any, of the aforementioned strategies rats employed, but it is worth considering the possibility that they may have been using an egocentric response strategy given the findings of Pearce et al. (2004) who used the same shaped arenas and a very similar design.

In Pearce et al.'s (2004) experiment, rats were trained to locate an escape platform in one corner of a rectangular pool before being tested in a kite-shaped pool. The results, described in the introduction to Experiments 1 and 2, revealed that rats could distinguish the correct from the opposite right-angled corner in the kite using local geometric knowledge acquired during training in the rectangle. However, the results of this study also revealed that an equal number of rats swam directly to the apex corner of the kite as swam to the correct right-angled corner. The authors interpreted this result as evidence of rats performing a stimulus-response, or *single wall*, strategy in which they find a long wall and swim along its length in a given direction as opposed to distinguishing corners by other geometric differences.

Given, then, the possibility that local geometric cues can be used to support different navigational solutions it is important to consider the brain structures required for each. It was argued in Chapter 1 that geometry learning (e.g. individual corner solution) and an egocentric response strategy (e.g. single wall solution) is sub-served by the hippocampus and dorsal striatum respectively. This is supported by empirical spatial learning studies demonstrating that the hippocampus is critical to the processing of shape-based, or geometric, information (Jones et al., 2007; Lever et

al., 2002; Pearce et al., 2004; McGregor et al., 2004; Sakamoto & Okaichi, 1996) and the dorsal striatum plays a vital role in the learning of an egocentric response rule (e.g. Packard & McGaugh, 1996). With this in mind, the purpose of the following experiments was to examine the effect of hippocampal (*HPC*) and dorsolateral striatum (*DLS*) lesions on the ability of rats to recognise the novelty of an object with reference to the local geometric properties of arena walls, i.e. subject these lesioned animals to the same experimental treatment as Experiments 1 and 2. Should animals form a map-like representation of the arena walls and their metric relations during this task, it is predicted that *HPC* lesions but not *DLS* lesions will impair performance. However, should rats employ an egocentric response strategy, it is predicted that *DLS* and perhaps *HPC* lesions will impair performance, with the latter case dependent upon whether distance discrimination is disrupted.

Finally, although the focus has been on specific deficits that *HPC* and *DLS* lesions may induce, it is possible that by eliminating one of these structures it may facilitate or enhance learning by the other. To explain this phenomenon, it has been argued that the hippocampus and striatum exert simultaneous control over navigation by sub-serving different strategies, and in certain situations competition for behavioural control emerges (Chavarriaga, Strosslin, Sheynikhovich & Gerstner, 2005; Kosaki, Poulter, Austen, McGregor, in prep.; Lee, Duman & Pittenger, 2008; White & McDonald, 2002). Therefore, when compared to control (*Sham*) animals it is possible that animals subjected to lesions of the *DLS* will be unhindered by competition from an egocentric response strategy and may exhibit better shape or place learning, which is processed by the hippocampus. To date, this is the first experiment in rats to investigate the effect of *DLS* lesions on the learning of enclosure shape. Conversely, for rats with *HPC* lesions, removal of competition

between navigational strategies should not be observed as in order to perform a striatal-based single wall solution, animals would require the ability to discriminate long from short walls. Accordingly, the prediction for animals subjected to *HPC* lesions is that performance should be at chance level irrespective of the navigational strategy employed.

Before investigating the effect of the aforementioned lesions on an object-in-local geometric context task it is important to establish that any lesion-induced deficits are not due to some general motor, perceptual or motivational deficits. Therefore Experiment 6 provides animals with a standard object recognition task prior to Experiment 7.

3.6 Experiment 6: Lesion Effects in Standard NOR

3.6.1 Method

3.6.1.1 Subjects

The experiment was conducted in two replications, with 32 animals in the first and 35 animals in the second replication. Animals from the first replication were approximately 8 months of age and had been used in two prior experiments: one comprising of an object recognition task (Experiment 7) and the other an unrelated navigation task in a Morris water maze. It was ensured that this prior experience was counterbalanced. Animals in the second replication were experimentally naive and approximately 6 months of age at the start of the experiment. Given that there was a significant effect of replication on the mean object exploration times for the four test trials combined, $F(1, 52) = 4.36$, $p = .042$, the data from the two replications were analysed and presented separately. Accordingly, at the start of the experiment, subjects were 32 and 35 male Lister hooded rats (*Rattus norvegicus*) supplied by

Charles River (UK) and housed in identical conditions to those described in Experiment 1. The first replication contained 12 rats in each hippocampal (*HPC*) and dorsolateral striatum (*DLS*) lesion group and 8 sham-operated rats and the second replication contained 12, 10 and 13 rats in each *HPC*, *DLS* and *Sham* group respectively.

3.6.1.2 Surgical Procedure

Each animal was placed into a Perspex anaesthetic chamber, which was filled with a mixture of isoflurane (5%) and oxygen (2L/min). Once deeply anaesthetised, the experimenter removed the animal from the chamber, shaved its head and then secured it into a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). A plastic pipe was positioned close to the rat's snout, which fed a constant supply of isoflurane and oxygen. At this stage, the anaesthetic was reduced to a maintenance concentration (1-2% isoflurane at 0.8L/min) and it was ensured that the animal's heart rate and reflexes were closely monitored throughout to make sure the rat remained at the appropriate level of anaesthesia.

During surgery the rat was wrapped in cotton cloth and placed on a heat mat. A digital thermometer probe was placed under the animal's body so that the experimenter could monitor its temperature. Eye ointment was placed over the eyes of the rat and saline solution was constantly applied to the surface of the brain to retain moisture. An incision was made, with a scalpel, along the midline of the scalp then the bone covering the neocortex on either side was removed using a dental drill and burr cutter. An arm comprising of a 2-µl Hamilton syringe and electronic microdrive (model KDS 310, KD Scientific, New Hope, PA) was then mounted on to the stereotaxic frame. Once attached, it was possible to manoeuvre the needle of the syringe to the appropriate coordinates and, with the electronic microdrive,

administer the desired quantity (.05 - .10 µl) and rate of infusion (.03 µl/min) of excitotoxin (Ibotenic acid).

There were 28 and 12 injection sites for each bilateral hippocampal and dorsolateral lesion, respectively (see Table 3.1 for the coordinates and volume of infusions). Ibotenic acid (Biosearch Technologies, San Rafael, CA), dissolved in phosphate-buffered saline (pH 7.4) to produce a 63-mM solution, was infused at each injection site with the needle left in place for 2 minutes to permit thorough diffusion of the amino acid into surrounding tissue. Prior to penetrating the dura with the Hamilton syringe needle, a finer gauge needle was used to create a small surface slit at the point of entry to facilitate passage. Each time the Hamilton syringe needle was removed from the brain it was thoroughly cleaned using two cotton buds soaked with 70 % alcohol. Sham animals underwent a similar surgical procedure, except that after having the dura perforated with a standard needle, the subsequent insertion of the Hamilton syringe needle was not performed.

After surgery, sutures (Mersilk 3-0, Ethicon Inc.) bound the wound of each animal before it was placed into a Thermacage maintained at a temperature of 40°C where it was allowed to recover. All animals were administered subcutaneously with Buprenorphine (.01-mg/kg, pre and post operation) to provide analgesia, and a saline and glucose solution (10-ml, post operation) to facilitate rehydration. Once the rats had sufficiently recovered, they were placed, alone for the first couple of days, back into their home cages where they were provided with soaked chow and a hydrogel pack. All animals were given a minimum of 14 days postoperative recovery time prior to commencement of training.

Table 3.1

The injection sites and rate of infusion for ibotentic acid (IBO) administration. Bregma was used as a reference point for anteroposterior (AP), mediolateral (ML) and dorsoventral (DV) coordinates. To determine an accurate DV reference point, the depth of dura was measured at AP -4.8 and ML ±4.1. Table *a.* displays the coordinates for bilateral hippocampal lesions and table *b.* for bilateral dorsolateral striatum lesions.

a) Hippocampal lesions

AP (-)	ML	DV (-)	IBO (µl)
5.4	-5.0	6.1	0.08
	-5.0	5.3	0.08
	-5.0	4.5	0.09
	+5.0	6.1	0.08
	+5.0	5.3	0.08
	+5.0	4.5	0.09
	-4.2	3.9	0.10
	+4.2	3.9	0.10
4.7	-4.5	6.5	0.05
	+4.5	6.5	0.05
	-4.0	7.2	0.10
	-4.0	3.5	0.05
	+4.0	7.2	0.10
	+4.0	3.5	0.05
3.9	-3.5	2.7	0.10
	+3.5	2.7	0.10
	-2.2	3.0	0.10
	-2.2	1.8	0.10
	+2.2	3.0	0.10
	+2.2	1.8	0.10
3.1	-3.0	2.7	0.10
	+3.0	2.7	0.10
	-1.4	3.0	0.10
	-1.4	2.1	0.10
	+1.4	3.0	0.10
	+1.4	2.1	0.10
2.4	-1.0	3.0	0.05
	+1.0	3.0	0.05

b) Dorsolateral striatum lesions

AP	ML	DV (-)	IBO (µl)
+1.6	+3.0	4.2	0.25
	-3.0	4.2	0.25
+0.8	+3.7	4.6	0.25
	-3.7	4.6	0.25
-0.5	+4.5	4.6	0.25
	-4.5	4.6	0.25

Upon completion of behavioural procedures, rats were injected with a lethal dose of sodium pentobarbitone (*Euthatal*) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde solution (0.1M phosphate-buffered). Each

brain was removed from the animal, placed in a jar filled with 4% paraformaldehyde solution (0.1M phosphate-buffered solution) for several days and then transferred to a second jar filled with 25% sucrose (in 0.1M PBS) for another day. Using a cryostat set to -19°C the brains were frozen and sliced into coronal sections (40-µm thick), which were placed onto positively charged slides (Thermo Scientific Superfrost Plus). The sections were stained with cresyl violet and analysed using a microscope and brain atlas (Paxinos & Watson, 1998). Reconstructions of the brain sections were created and these images were processed in Matlab® to determine the percentage of tissue damage either in the hippocampus or dorsolateral striatum.

3.6.1.3 Apparatus

See Experiment 1.

3.6.1.4 Procedure

The *General Procedure*, *Habituation*, *Experimental Stage* and *Performance Measures* were identical to Experiment 2 with the following exceptions. First, the animals in Replication 1, which had already been subjected to the original habituation schedule prior to Experiment 7, were given one refresher habituation session prior to commencement of the current experiment, which involved animals spending five minutes in their holding cage in each testing room. Second, for the *Experimental Stage*, the design was identical to Experiment 2 with the exception that in this experiment all animals were presented with two copies of object A in corners J and L of the kite during the sample phase in test room 2 and subsequently presented with a copy of object A in corner E and a new object, object B, in corner F of the rectangular arena during the test phase in test room 1 (see Figure 3.7). For ease of elucidation, the aforementioned method assumes that two copies of object A were

presented in the sample phase, and a copy of object A and object B were presented in corners E and F of the rectangle, respectively, during the test phase, but in reality on each object recognition trial, half the animals were exposed to two copies of object A and half were exposed to two copies of object B during the sample phase, and for the test phase the location of each object (A and B), housed either in corner E or F of the rectangle, was counterbalanced among animals.

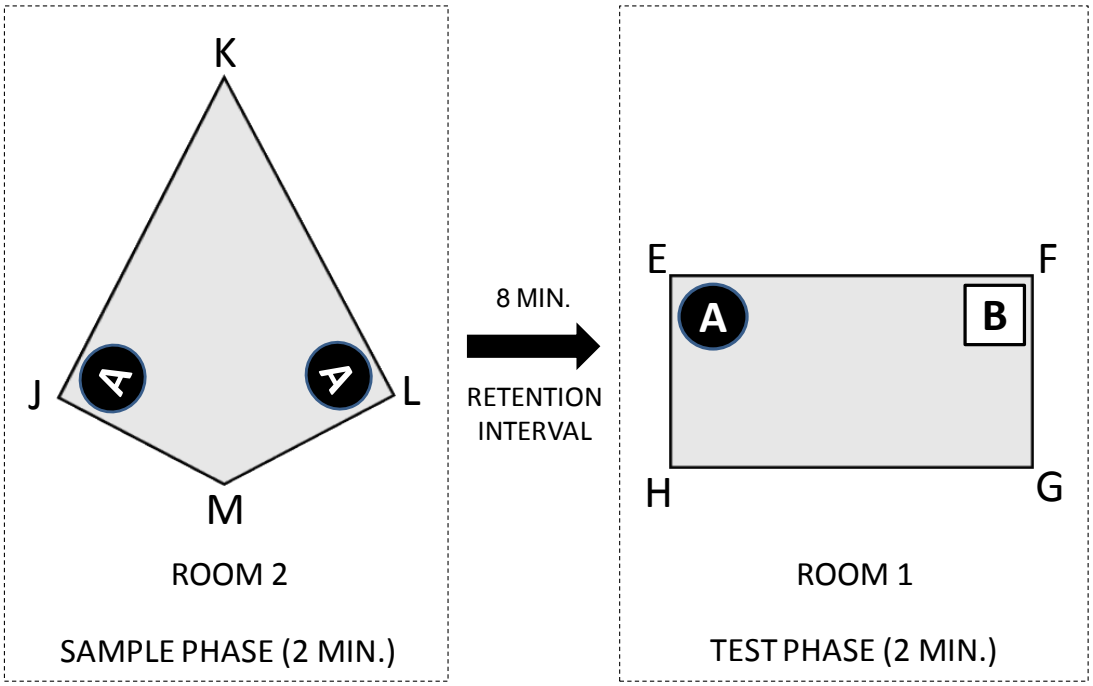

Figure 3.7. Schematic diagram showing the experimental design. Objects A and B are represented by black circular and white square symbols respectively. Preferential exploration of object B over object A at test indicates the animal's detection of its novel identity despite the fact both of these objects were placed in a differently shaped arena in a different room.

3.6.2 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied.

3.6.2.1 Histology

Figure 3.8a (Replication 1) and 3.8b (Replication 2) depicts reconstructions of the minimum (black shading) and maximum (grey shading) extent of hippocampal (panel A) and dorsolateral striatum (panel B) lesions on a series of coronal sections (see also Figure 3.9 for representative micrographs). For Replication 1, rats in Group *HPC* all sustained bilateral damage to the dorsal and ventral hippocampus (CA fields 1-4), the dentate gyrus and the subicular cortices. Analysis of total hippocampal tissue loss revealed a mean of 90.2% (range 85.7% - 93.6%) with a median of 90.4%. The main sparing of hippocampal tissue was observed in the most medial areas of the dorsal hippocampus. One rat received lateral damage in both hemispheres that extended into the lateral entorhinal, perirhinal and ectorhinal cortices, so this animal was excluded from the analysis. In the majority of the remaining 11 rats there was damage to the cortical area overlying the dorsal hippocampus. This typically included partial damage to motor, visual, somatosensory, parietal and retrosplenial agranular cortices (for reports of similar extrahippocampal damage in hippocamptomized rats see: Albasser, Amin, Lin, Iordanova & Aggleton, 2012; Iordanova, Burnett, Aggleton, Good & Honey, 2009). Similar to Albasser et al. (2012), the partial cortical damage described above left plenty of sparing in each of these areas. For rats in Group *DLS* visible widening of the lateral ventricles was observed in all cases owing to tissue shrinkage caused by the lesion. Inspection of the stained tissue revealed that the intended lesion site was

off target in three rats. In these cases, which were excluded from subsequent analysis, there was significant extra-striatal damage to cortical areas adjacent to the *DLS*. In the remaining rats, cell loss and modest gliosis was found in the targeted area. Thus, for Replication 1, there were 11, 9 and 8 rats included in the behavioural analyses for Group *HPC*, *DLS* and *Sham* respectively.

For Replication 2, rats in Group *HPC* sustained similar hippocampal damage to that described for Replication 1. Analysis of total hippocampal tissue loss revealed a mean of 82.2% (range 70.4% - 90.4%) with a median of 82%. The cortical damage above the dorsal hippocampi was similar to that described for Replication 1 although less extensive. One rat received extensive extra-hippocampal damage and was excluded from subsequent analysis. For rats in Group *DLS* the histology was very similar to that described for Replication 1. In total, four rats were excluded due to extraneous damage, typically into the cortical areas ventrolateral to the DLS: the granular and agranular cortices as well as the piriform cortex. Accordingly, for Replication 2, there were 11, 6 and 13 rats included in the behavioural analysis for Group *HPC*, *DLS* and *Sham*, respectively.

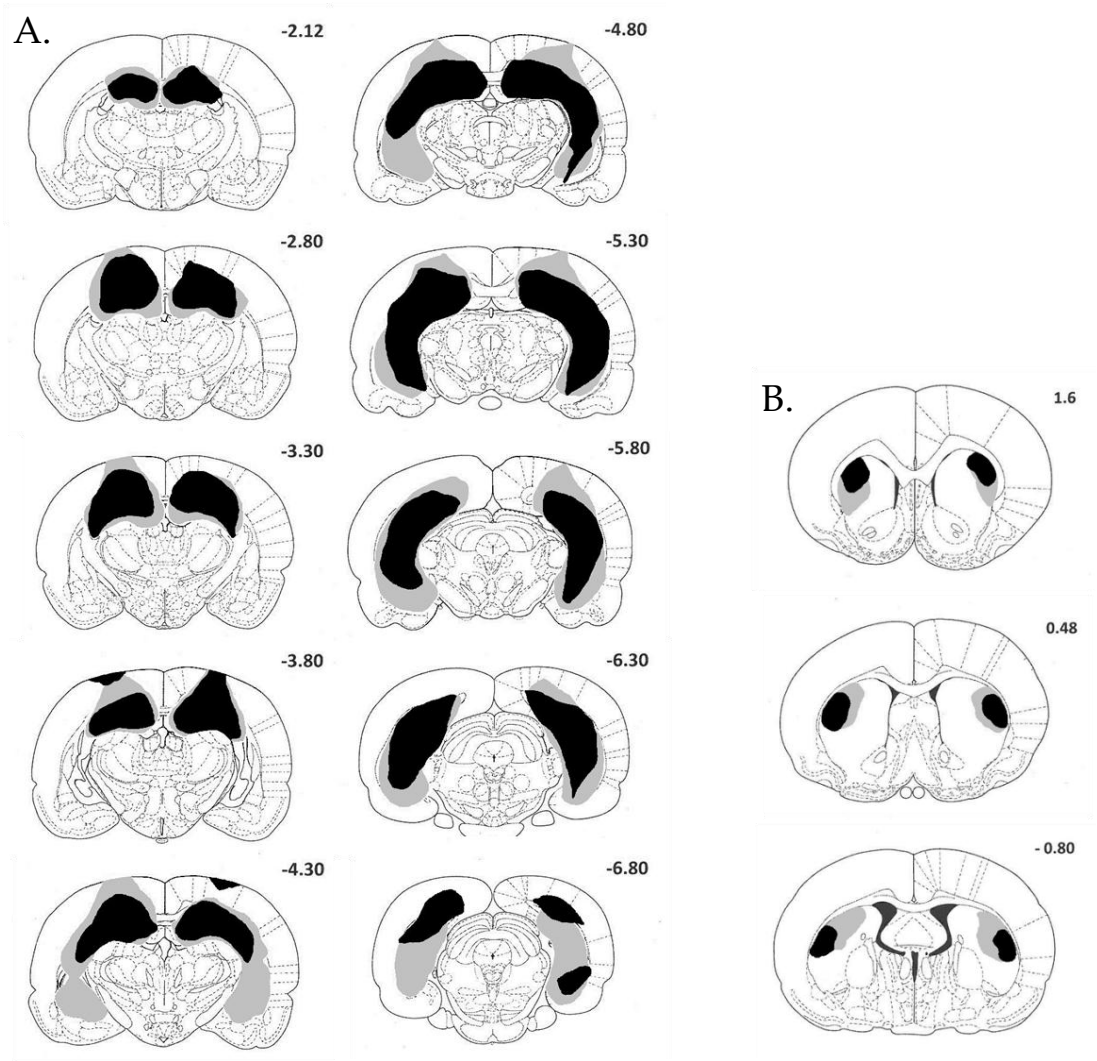

Figure 3.8a. Coronal sections displaying the extent of hippocampal damage (A) and dorsolateral striatum damage (B) in rats from Replication 1. The case with the largest (grey shading) and smallest (black shading) amount of tissue loss is represented for each lesion group. The numbers refer to the distance behind bregma for each section. (For Replication 2's histological reconstructions: see *Figure 3.8b* on next page).

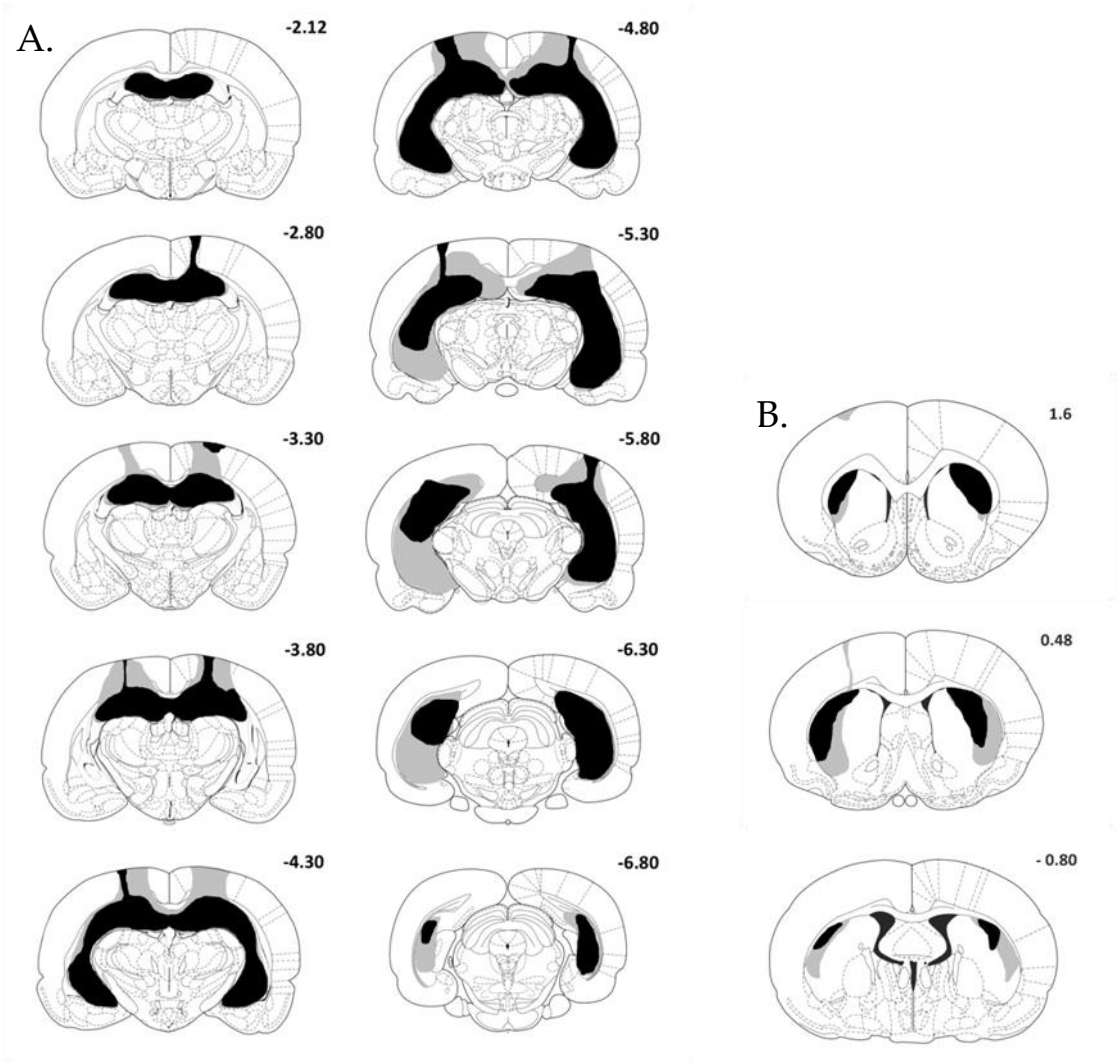

Figure 3.8b. Histological reconstructions for Replication 2. See *Figure 3.8a* for a description.

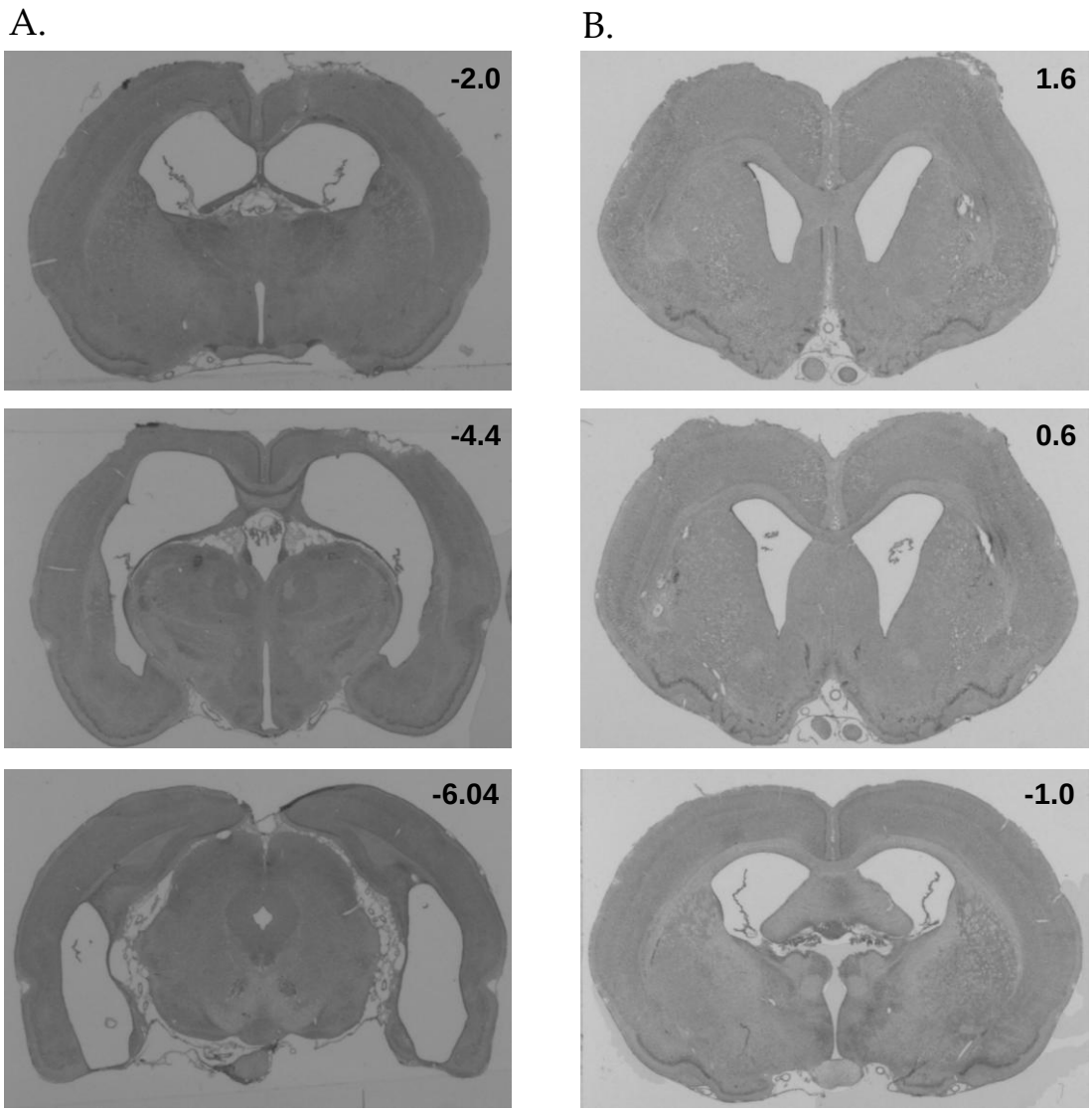

Figure 3.9. Photomicrographs of three coronal sections from a representative hippocampal lesion (A) and dorsolateral striatum lesion (B). The numbers refer to the distance behind bregma for each section (40 µm thick).

3.6.2.2 Stopwatch Scoring

Figure 3.10 shows the mean time animals spent exploring the novel and familiar object, averaged across four test trials. It is clear from both panel A and B that animals in all groups preferentially explored the novel object over the familiar object. For Replication 1 in the upper panel (A), object exploration times are very

similar between groups, whereas for Replication 2 in the lower panel (B), sham animals appear to spend slightly more time exploring the novel object than animals in the other lesion groups.

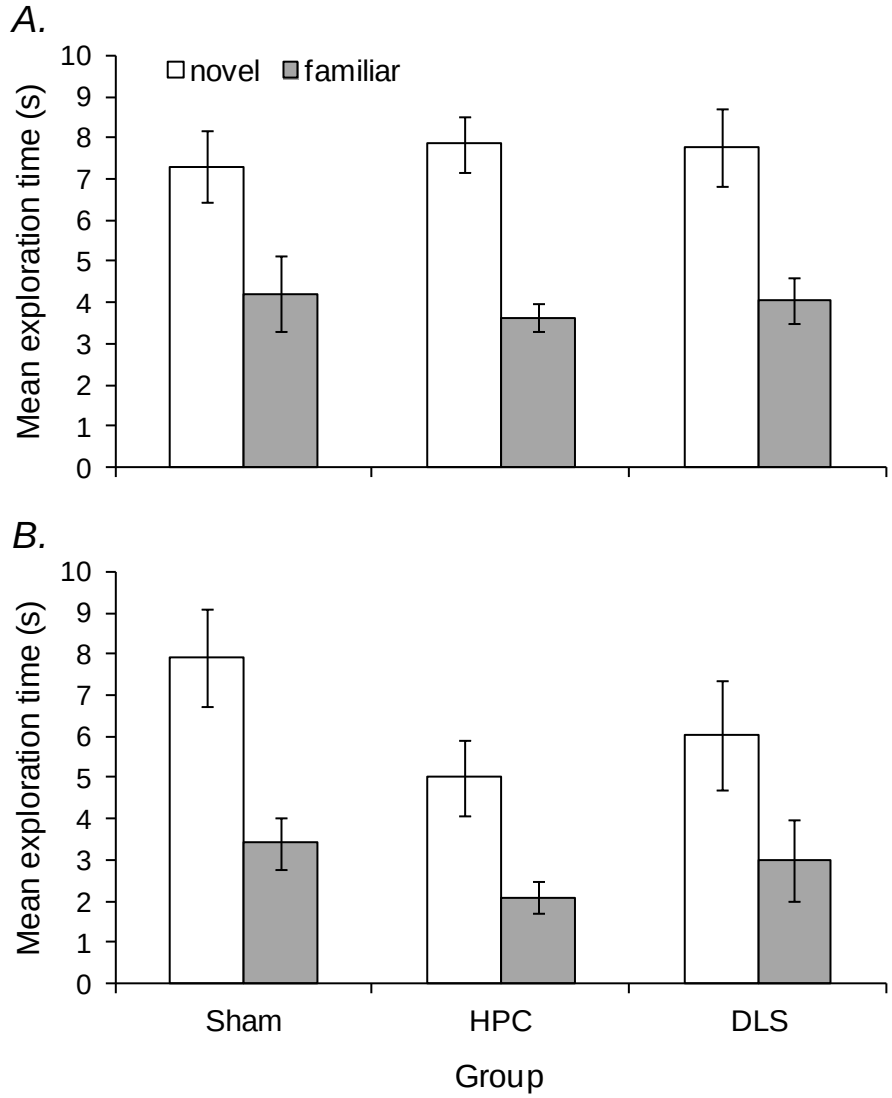

Figure 3.10. Mean time (±SEM) spent exploring the novel (white bars) and familiar (grey bars) objects averaged across four test trials for each group in Replication 1 (Panel A) and Replication 2 (Panel B).

To validate these observations statistically, a two-way ANOVA conducted for each replication on the object exploration times of each rat averaged across four

test trials with lesion group as the between-subjects variable and object the repeated measure revealed that for Replication 1 all animals explored the novel object more than the familiar object, $F(1, 25) = 82.4$, $p < .001$, but the remaining effects and interactions were non-significant, Fs < 1. For Replication 2, all animals explored the novel object more than the familiar object, $F(1, 27) = 27.05$, $p < .001$, but again, the remaining effects and interactions were non-significant, Fs ≤ 2.37, ps $\geq .11$. A comparison between replications revealed that exploration of both objects was higher for Replication 1 when compared to Replication 2, $F(1, 52) = 4.36$, $p = .042$.

The left-hand (Replication 1) and right-hand (Replication 2) panels of Figure 3.11 display the mean d2 scores for each rat (symbols) and each group (bars) averaged across four test trials. First of all, an inspection of the group mean d2 scores in both replications reveals that animals across all groups were preferentially exploring the novel over the familiar object, as illustrated by d2 values above 1 (range 0.23 – 0.39). It is also clear from the spread of individual mean d2 scores that, with the exception of four rats from Group *HPC* in Replication 2, all animals achieved a d2 score above 0, which again lends support to the notion that animals were preferentially exploring the novel object. To confirm these observations, one-sample t-tests were conducted, with a test value of 0, on the individual d2 scores for each group, which were averaged across four test trials. For Replication 1, the analysis revealed that animals from all groups spent proportionately more time exploring the novel object than expected by chance, ts ≥ 5.38, ps $\leq .01$. In a similar analysis for Replication 2, the same pattern of results emerged, ts ≥ 2.65, ps $\leq .024$. Although there was little difference in the mean d2 scores between replications for each group, it appears that the spread of d2 scores is larger for Group *HPC* in Replication 2. This variability in behaviour could be correlated with lesion size, i.e.

rats with larger lesions acquired lower d2 scores. To assess this possibility, rats from each group were ranked according to lesion size and a Pearson product-moment correlation was computed to assess the relationship between d2 score (4 day average) and lesion size. For Replication 1 there was no significant correlation between these two variables for Group *HPC*, $r = .293$, $p > .05$, or for Group *DLS*, $r = -.228$, $p > .05$. For Replication 2 there was also no significant correlation for Group *HPC*, $r = -.342$, $p > .05$, or for Group *DLS*, $r = -.628$, $p > .05$.

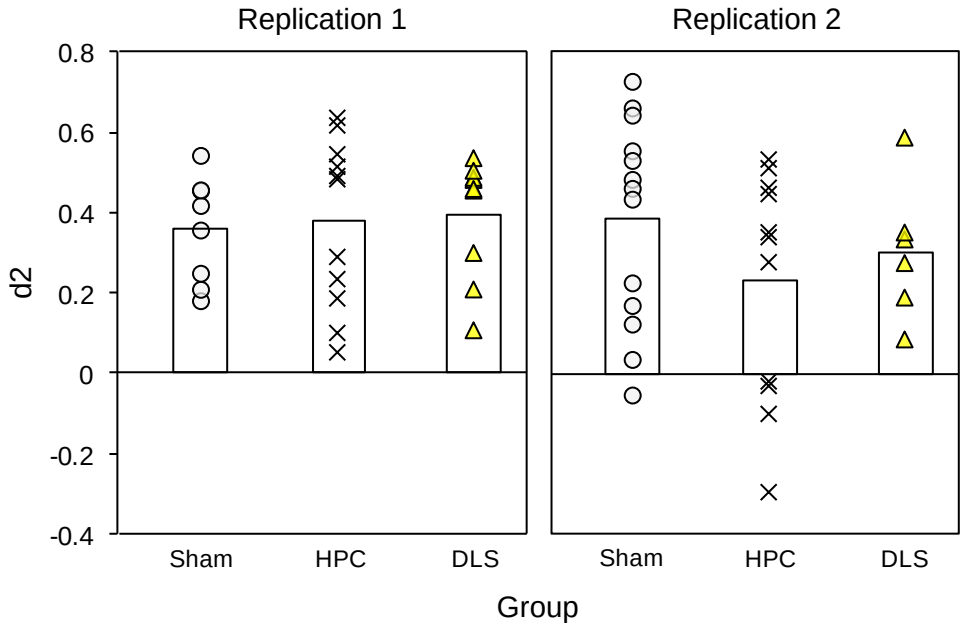

Figure 3.11. Mean d2 scores for groups in each replication averaged across four test trials. Individual symbols represent the mean for each rat and the bars represent the mean for each group. A value of zero on the y axis is chance level, a value closer to 1 indicates preferential exploration of the novel object and a value towards -1 indicates preferential exploration of the familiar object.

3.6.3 Discussion

The results of Experiment 6 demonstrated that both rats with *HPC* lesions and rats with *DLS* lesions were capable of recognising objects based on their familiarity. Therefore, any lesion deficits observed in the subsequent object-in-local geometry task cannot be attributable to general perceptual, motor or motivational factors.

3.7 Experiment 7: Lesion effects on Object-in-Local Geometry memory

3.7.1 Method

3.7.1.1 Subjects

The experiment used the same subjects as Experiment 6 and was again conducted in two replications, with 32 animals in the first and 35 animals in the second replication. All animals from the first replication were experimentally naive and approximately 4 months of age at the start of the experiment. Animals in the second replication were approximately 8 months of age and had been run in a prior object recognition task (Experiment 6). It was ensured that this prior experience was counterbalanced. As with Experiment 6, there was a significant effect of replication on the mean object exploration times for the four test trials combined, $F(1, 52) = 48$, $p < .001$, so the data from the two replications were analysed and presented separately. Accordingly, subjects were 32 and 35 male Lister hooded rats (*Rattus norvegicus*) supplied by Charles River (UK) and housed in identical conditions to those in Experiment 1. At the start of the experiment the first replication contained

12 rats in each hippocampal (*HPC*) and dorsolateral striatum (*DLS*) lesion group and 8 sham-operated rats. In the second replication there were 12, 10 and 13 rats in each *HPC*, *DLS* and *Sham* group, respectively.

3.7.1.2 Surgical Procedure

Refer to Experiment 6 for the surgical procedure.

3.7.1.3 Apparatus

See Experiment 1.

3.7.1.4 Procedure

All aspects of the *General Procedure*, *Habituation*, *Experimental Stage* and *Performance Measures* were identical to Experiment 2 (see Figure 3.12 for a recap of the design).

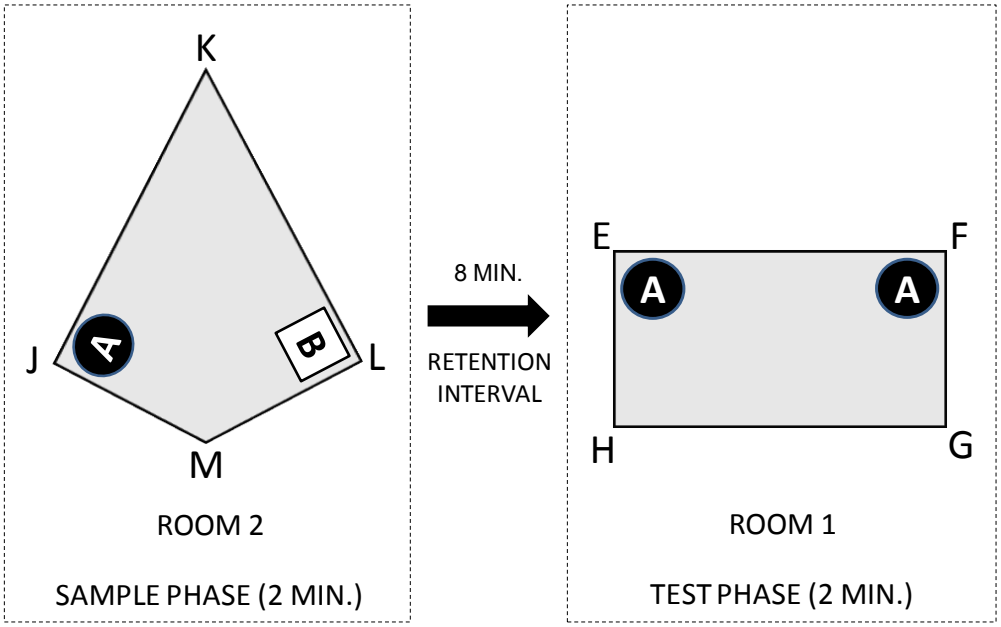

Figure 3.12. Schematic diagram showing the experimental design. Objects A and B are represented by black circular and white square symbols respectively. Preferential exploration of the object located in corner F of the rectangle indicates the animal's detection of its novelty with respect to the local geometric properties provided by the walls of the arena.

3.7.2 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied.

3.7.2.1 Histology

Refer to Experiment 6 and Figures 3.8 and 3.9.

3.7.2.2 Stopwatch Scoring

The upper panel (A) (Replication 1) and lower panel (B) (Replication 2) of Figure 3.13 shows the mean time animals spent exploring the novel and familiar objects, as defined by the local geometric properties of the corners housing them, averaged across four test trials. Because there were no significant effects or interactions involving day, $Fs \leq 2.09$, $p \geq .121$, for ease of elucidation, the data for each group was collapsed across the four test trials. The upper panel of Figure 3.13 suggests that sham-operated animals in Replication 1 preferentially explored the novel object over the familiar object, whereas for Group *HPC* and *DLS* this preference did not emerge. For the animals belonging to Replication 2 (panel B) there was no object preference in any group. It is also interesting to note from the two panels of this figure that across all groups total object exploration time was markedly lower in animals from Replication 2, which were older and had had prior experience in an object recognition task (Experiment 6), than in animals from Replication 1. A two-way ANOVA, conducted separately for each replication, of individual object exploration times averaged across four test trials with lesion group as the between-subjects variable and object as the repeated measure revealed no

significant between- or within-subjects effects or interactions for either Replication (Replication1: Fs < 1, Replication 2: Fs ≤ 2.07, ps ≥ .15).

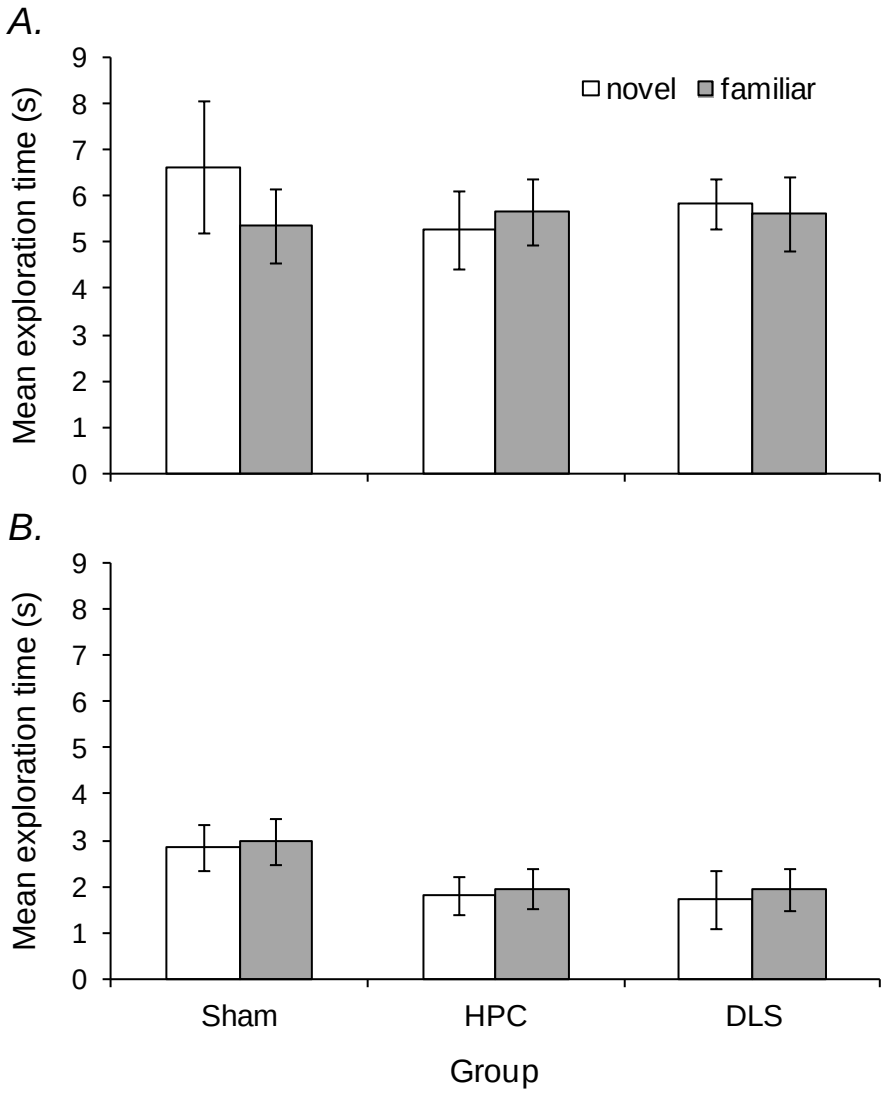

Figure 3.13. Mean time (+SEM) spent exploring the novel (white bars) and familiar (grey bars) objects averaged across four test trials for each group in Replication 1 (Panel A) and Replication 2 (Panel B).

The left-hand (Replication 1) and right-hand (Replication 2) panels of Figure 3.14 display the mean d2 scores for each rat averaged across four test trials. For Replication 1 the figure indicates that for Group *Sham* there appears to be more data

points lying above the chance level (dashed line), which shows that these rats spent proportionately more time exploring the novel object. Object exploration for Group *HPC* and *DLS* appears to be at chance. Turning to the right-hand panel of this figure, there is no discernible pattern of object preference in any group belonging to Replication 2. To confirm these observations, one sample t-tests were conducted, with a test value of 0, on the d2 scores for each group, which were averaged across four test trials. For Replication 1, this analysis revealed that each group did not spend proportionately more time exploring the novel object than expected by chance, *t*s ≤ 1.63 , *p*s ≥ .15. In a similar analysis for Replication 2, the same pattern of results emerged, *t*s < 1.

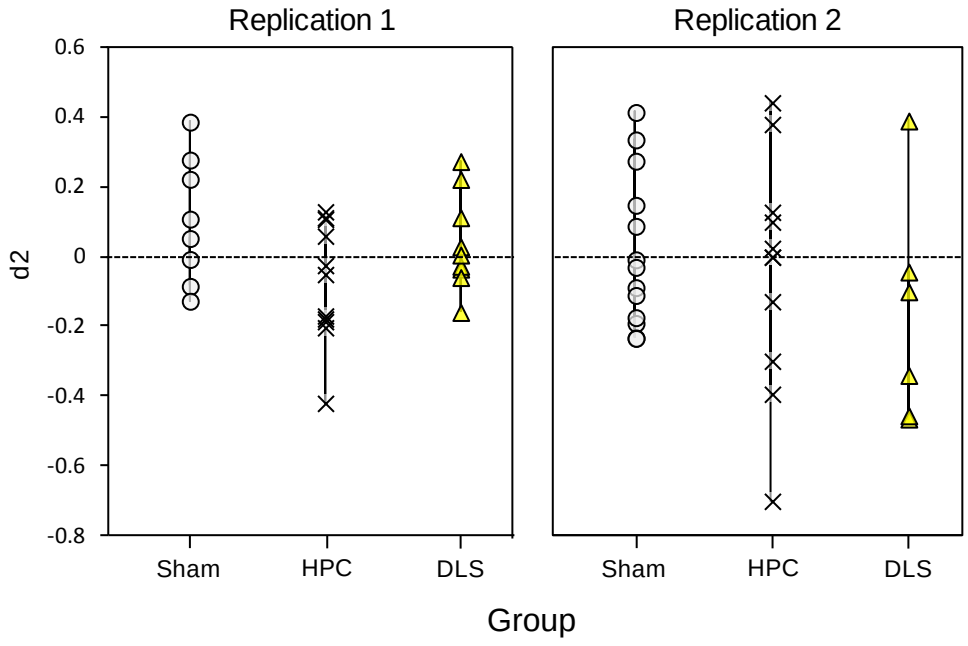

Figure 3.14. Mean d2 scores for groups in each replication averaged across four test trials. Individual symbols represent the mean for each rat. A value of zero on the y axis is chance level, a value closer to 1 indicates preferential exploration of the novel object and a value towards -1 indicates preferential exploration of the familiar object.

3.7.2.3 Ethovision Scoring

The stopwatch data for Replication 1 showed a trend towards Group *Sham* exploring the novel object more than the familiar object but this was not statistically significant. Therefore, the tracking data were analysed to investigate whether animals spent more time in a zone close to the novel object than the familiar object. A similar two-way ANOVA to that described for the experimenter scores was conducted for each replication using the individual times in each object zone. The pattern of results for Replication 2 was no different to that reported for the experimenter scores (*F*s < 1). However, the exploration times for Replication 1, averaged across the four test trials, are displayed in Figure 3.15.

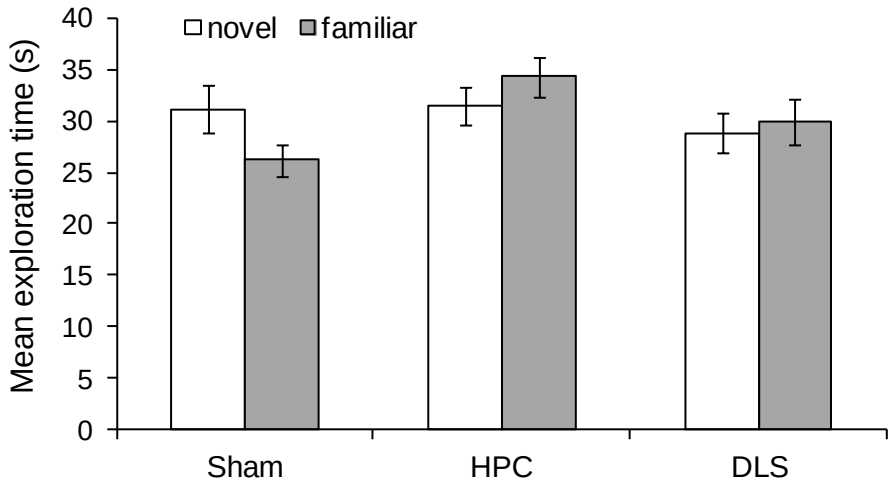

Figure 3.15. Mean time (±SEM) spent in areas around the novel (white bars) and familiar (grey bars) object locations averaged across four test trials for Replication 1.

What is clear from this figure is that Group *Sham* spent more time searching close to the novel object than the familiar object, while Group *HPC* and *DLS* did not discriminate between the objects. To confirm this characterisation of the data, the

ANOVA revealed a significant object x lesion group interaction, $F(2, 25) = 3.36$, $p = .050$, with tests of simple main effects revealing that Group *Sham* spent significantly more time near the novel object ($M = 31.2$, $SEM = 2.21$) than the familiar object ($M = 26.2$, $SEM = 2.15$), $F(1, 25) = 4.44$, $p = .045$, while Group *HPC* and *DLS* did not, $Fs(1, 25) \leq 2.06$, $p \geq .16$.

3.8 General Discussion

The main finding from Experiment 6 was that rats from all groups, i.e. Group *Sham*, *HPC* and *DLS*, were capable of recognising a novel object during the test phase despite the fact that the test arena comprised a different global shape and was situated in a different room. This finding is important as it eliminates the possibility that any impairments in rats with lesions subsequently observed during Experiment 7 were the result of procedural differences between the variant of the design used in the current series of experiments and other more standard versions of the novel object recognition task (e.g. Ennaceur & Delacour, 1988). The absence of any effect of bilateral hippocampal lesions on standard object recognition memory, observed in Experiment 6, replicates the results of other studies (e.g. Barker & Warburton, 2011; Bussey et al., 2000; Mumby et al., 2002; Forwood, Winters, & Bussey, 2005; Good et al., 2007; Langston & Wood, 2010: Winters, Forwood, Cowell, Saksida, & Bussey, 2004). However, the finding that bilateral dorsolateral striatum lesions produce no deficit in standard object recognition memory is a novel contribution to the current literature.

Overall, the results of Experiment 7 using the experimenter stopwatch scores were equivocal. As predicted, during the test phase, rats with hippocampal lesions were unable to discriminate between two identical objects based on the corner in

which they were positioned. It was suggested in the Introduction that rats with *DLS* lesions and an intact hippocampus may be capable of encoding an object-corner configuration but the results of the current experiment did not support this assertion. Whether the chance performance observed in rats with *DLS* lesions was due to a disruption in the formation of a response strategy, e.g. a single wall response, or a place representation, e.g. a single corner solution, is open to question. Unfortunately, it was also found that sham-operated animals did not discriminate between the novel and familiar objects during the test phase when the stopwatch scores were analysed. However, sham rats from Replication 1 did discriminate between each object-corner configuration at test when the Ethovision scores were analysed. Thus, potential reasons why the performance of sham rats in Experiment 7 failed to replicate the stopwatch results reported in Experiments 1 and 2, and was at chance level for Replication 2 but not Replication 1 when the Ethovision data was used, must be considered.

As mentioned, novel object preference during the test phase was more marked in the sham animals from Replication 1 than Replication 2. This argument is supported by the Ethovision data which shows that sham animals from Replication 1 spent significantly more time in a zone surrounding the novel object than the familiar object whereas sham animals from Replication 2 spent an equal amount of time in each object zone. Although, statistically, the experimenter stopwatch scores for sham animals in Replication 1 did not reveal a significant preference for the novel over the familiar object during the test phase there was a trend towards this result, but the variance in exploration of the novel object was high and the sample size was small (n=8).

Unlike in standard object recognition tasks, e.g. Experiment 6, the object preference effects witnessed in object-in-local geometry recognition tasks, e.g. Experiments 1, 2 and the current experiment (Experiment 7), are far more subtle. Therefore, because the behavioural response is weak and the variance large, a larger sample size is required. Prior to the current experiment it was expected that the data from both replications could be pooled providing a sample size of 20 sham animals, which, in our lab, has been the minimum number of animals necessary to produce a significant effect in an object-in-local geometry task of this nature (e.g. Experiment 2). Thus, if the argument is accepted that the pattern of behaviour observed in Replication 1, using the experimenter stopwatch scores, lies in the correct direction but lacks statistical power due to a small sample size, the question still remains why the sham animals in Replication 2 (n = 12) were operating completely at chance.

One potential reason is that these animals were twice the age of those used in Replication 1 and Experiments 1 and 2. Empirical evidence indicates that rats' performance on cognitive tasks requiring the use of spatial learning and memory deteriorates with age (e.g. Cavoy & Delacour, 1993; Sofie, Buhot & Poucet, 1992; for review see: Barnes, 1988; Ingram, Jucker, & Spangler, 1994). Furthermore, the extent to which animals exhibit exploratory behaviour is reduced in aged rats (Rowe, Spreekmeester, Meaney, Quirion, & Rochford, 1998; Soffie, Buhot, & Poucet, 1992; Shukitt-Hale, Casadesus, Cantuti-Castelvetri & Joseph, 2001). However, like these previous studies, showing a reduction in locomotor activity in older rats, animals in this experiment, irrespective of their age, were not impaired at a standard object recognition task, which they participated in during the previous experiment (Experiment 6).

Thus, although one cannot attribute any deficit in spatial recognition during

the current experiment to some overall impairment of locomotor or sensorimotor capabilities, or to a general reluctance to respond to novel objects, there is still a plausible explanation as to how lower object exploration times observed in Replication 2 during Experiment 7 could impact on performance in the test phase. To explain, it should be expected that the preference for one object over an identical copy in the rectangle (test phase) is proportional, or at least related, to the degree of learning that took place in the kite (sample phase); it should be related to the strength of association between one corner in the kite (to-be-novel corner) and object B, and between the other corner (to-be-familiar corner) and object A, and it should also be related to the level of discrimination between objects A and B. Thus, if, during the sample phase in the kite, overall exploration of objects A and B is low for Replication 2, one should expect the preference shown in the test phase to also be low.

A second reason why performance may have differed between the two replications in Experiment 7 is that rats from Replication 2 had already participated in an object recognition task, (Experiment 6), whereas rats from Replication 1 were experimentally naive. To appreciate the implications of this order effect, first consider the design of Experiment 6 in which the sample phase consisted of rats being presented with two identical copies of an object, e.g. object A, positioned in mirror opposite right-angled corners of the kite, corners J and L, before being tested with a copy of object A and a new object, object B, positioned in mirror opposite corners of the rectangle, E and F (Figure 3.7). For Replication 2, this prior experience could have increased the similarity between the two object-corner compounds in the kite based on the following mechanism. Suppose animals learned [Object A – Corner J] and [Object A – Corner L] associations in the previous

experiment, and then went on to learn [Object C – Corner J] and [Object D – Corner L] associations in the current experiment. Now, to the extent animals still remembered the items learned in the previous experiment, the representations of the associations acquired in the current experiment should be expressed as [Corner J – Object A – Object C] and [Corner L – Object A – Object D], which should be more similar to each other than [Corner J – Object C] and [Corner L – Object D], due to the presence of the common element, object A. Consequently, in the current experiment if preference during the test phase in the rectangle is determined by the level of discrimination between the two corner-object compounds formed during the sample phase in the kite then it is expected that the preference for the novel object-corner compound over the familiar object-corner compound is smaller in Replication 2.

The experiments reported in Chapters 2 and 3 investigated the interaction between geometric and non-geometric cues and the neural systems involved in encoding this information. However, this series of experiments utilised a novel object recognition task which involves no external reward and relies on a rats' natural propensity to explore novelty. Therefore, it is possible for extraneous factors beyond the control of the experimenter, such as prolonged exposure to objects over time, to reduce motivation in rats and ultimately lead to noisy data. Taking this into consideration, the following series of experiments, once again designed to investigate the interaction between discrete visual and geometric cues, used a water maze paradigm to ensure that rats remained motivated to locate a goal location.

4 Sex Differences in Spatial Learning

4.1 Experiment 8: Overshadowing of Geometry Learning

4.1.1 Introduction

The proposal by Cheng (1986) and Gallistel (1990) that animals possess a geometric module predicts that cue competition effects, such as overshadowing, which are commonly observed in many forms of associative learning, should not materialise between geometric and non-geometric cues when both signal the location of a goal. Conflicting empirical evidence from a range of studies, summarised in Chapter 1, has provided support both for and against this proposal. In the majority of these experiments rats were provided with useful information from landmarks placed inside the arena in order to determine what effect this had on learning based on the geometry of the enclosure. In some experiments the landmark disrupted learning about geometry (Horne & Pearce, 2009b, 2011; Kosaki et al., 2013); in others the landmark had no effect (Hayward et al., 2003, 2004; McGregor et al., 2009; Pearce et al., 2001); and finally, in others the landmark facilitated, or potentiated, the learning of geometry (Graham et al., 2006; Horne & Pearce, 2011; Pearce et al., 2006). As Horne and Pearce (2011) point out, because the aforementioned experiments used different landmarks and arena shapes it is difficult to ascertain the underlying factors that affect cue competition effects between geometric and non-geometric cues in spatial memory tasks. However, one factor that has recently been

implicated as important is sex (Rodriguez et al., 2011).

Cognitive sex differences in spatial memory tasks both in animals and humans have been studied extensively (e.g. Maccoby & Jacklin, 1974; Epting & Overman, 1998; Forcano, Santamaria, Mackintosh, & Chamizo, 2009). Of particular relevance to the current discussion is the emergence of evidence suggesting that, broadly speaking, males tend to rely on an allocentric strategy, while females use a more egocentric approach in order to solve spatial problems (Lawton, 1994). More specifically, it has been found during navigation tasks that males rely more on geometric cues, whereas females rely more on landmark cues; a finding supported in both the human (Chai & Jacobs, 2009; Saucier, Green, Leason, MacFadden, Bell & Elias, 2002; Sandstrom, Kaufman, & Huettel, 1998) and animal (Rodriguez et al., 2010, 2011; Roof & Stein, 1999; Williams, Barnett, & Meck, 1990) literature. A consequence of this differential reliance on geometric and landmark cues between the sexes is that the relative salience of these cues should be affected, with males perceiving geometric cues to be more salient than landmark cues and females perceiving landmark cues to be more salient than geometric cues. Thus, it has recently been possible to investigate the influence of sex differences in rodents on the effects of cue competition between geometric and landmark cues (Rodriguez et al., 2011).

Rodriguez et al.'s (2011) first experiment, in a Morris water maze, compared the performance of male and female rats in a navigation task which used a classic overshadowing design. Overshadowing, described in Chapter 1, refers to the finding that when two cues simultaneously signal reward, the presence of one cue restricts, or overshadows, what can be learned about the other (Pavlov, 1927). Moreover, Mackintosh (1976) revealed that it is usually the more salient cue that overshadows

the less salient cue. Accordingly, Rodriguez et al. (2011) predicted that during a spatial task, in which informative geometric and landmark cues were presented concurrently, the geometric cue should overshadow the landmark cue in male rats and the reciprocal overshadowing effect should occur in female rats. At first glance, the results of this experiment concurred with the authors' prediction. However, closer scrutiny of the design of this experiment reveals, as the authors themselves acknowledge, that the observed reciprocal overshadowing effect could in fact be due to a phenomenon known as *generalization decrement* as opposed to any associative competition.

Consider Rodriguez et al.'s (2011) Experiment 1, in which the two control groups were each provided with only a single informative cue during training, i.e. a shape cue for one group and a landmark cue for the other, and were trained and tested under identical conditions. The two experimental groups, on the other hand, were trained identically to one another with an informative geometric and landmark cue presented in compound, but tested differently with one group tested in an environment with the geometric cue removed and the landmark cue remaining, and the other group tested with the landmark cue removed and the geometric cue remaining. Consequently, those animals in the experimental groups experienced a greater change from training to test than the animals belonging to the control groups. Thus, if one remains with the stance that males are more reliant on geometric cues and females on landmark cues, it follows that removal of the more salient cue during the test trial, i.e. the geometric cue for males and the landmark cue for females, will induce a greater perceptual change than removal of the less salient cue. Therefore, without appealing to any cue competition effects, an explanation of the reciprocal overshadowing observed by Rodriguez et al. can be provided (see Wagner &

Brandon, 2001; Pearce, 1994 for formalised models of how this pattern of overshadowing occurs).

Rodriguez et al. (2011) concluded that this alternative generalization decrement account does not detract from the finding that the sex of a rat affects the direction of overshadowing between a geometric and landmark cue. However, even if this line of argument is accepted, it certainly cannot be claimed that this experiment provides unequivocal evidence for sex differences in the outcome of associative competition between a geometric and landmark cue. In fact, convincing evidence from a series of experiments conducted by the same laboratory (Chamizo, Rodriguez, Espinet & Mackintosh, 2012; see also Pearce et al., 2001 for similar evidence), using a similar water maze paradigm but investigating the effects of overshadowing between different landmarks, actually indicated that generalization decrement, as opposed to cue competition effects, was responsible for the observed overshadowing effect. Although one cannot directly apply the results of Chamizo et al. (2012) to the previous experiment (Rodriguez et al., 2011) given that different cues were used, if nothing else, this most recent finding lends support to the possibility that the results from Rodriguez et al.'s (2011) overshadowing experiment were the consequence of generalization decrement. With this in mind and in the same vein as Rodriguez et al. (2011), the current spatial learning experiment used a classic overshadowing design to investigate the effect of a rat's sex on associative competition between an informative geometric and landmark cue. However, unlike Rodriguez et al.'s (2011) design, the current experiment eradicated the potential for generalization decrement to affect any differentiation in behaviour between control and experimental groups. To achieve this, it was ensured that any differences between the training and test environments were matched across all groups.

4.1.2 Method

4.1.2.1 Subjects

The subjects were 20 male and 20 female experimentally naive hooded Lister rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England). At the start of the experiment rats were approximately 3 months of age and were housed, with male and female rats in separate rooms, in identical conditions to those described in Experiment 1.

4.1.2.2 Apparatus

The experiment took place in a white, circular, fibre glass pool with a diameter of 200 cm and a depth of 60 cm. The pool was filled to a depth of 30 cm with water, which was warmed to a temperature of 25°C (± 2°C), rendered opaque with the addition of 500 ml of white opacifier (OP303B, supplied by Rohm and Haas, UK) and changed daily. The pool was elevated 40 cm off the ground on a secure platform positioned in the centre of a laboratory (465 cm x 395 cm x 230 cm high). A white, circular, perspex ceiling (200 cm in diameter and 0.5 cm thick) was suspended directly above the pool at a distance of 108 cm from the uppermost edge of the pool walls. Recessed into this suspended ceiling were eight 45-W spotlights, each 18.5 cm in diameter and arranged equidistantly from one another in a 1 m, centred circle. These spotlights, as well as two 35-W, 1.5 m strip lights individually placed on the east and west walls (68 cm above and parallel to the floor with the midpoint on the east-west axis of the pool) and four 50 cm^2 ceiling lights each housing four 14-W tubes 50 cm in length and positioned in each corner of the room (60 cm from each wall comprising the corner) illuminated the testing room during

the experimental period. There was a hole, 35 cm in diameter, cut out of the centre of the suspended ceiling which allowed a wide-angled video camera to be positioned centrally on a tripod 5 cm above. A HDD DVD recorder (Sony RDR-HXD890) and monitor (ZM-CR114NP-II) were located on a table in the southwest corner of the room where images from the video camera were transmitted. The recorded video files were subsequently analysed using Ethovision software (EthoVision, Noldus, NL) to measure the swim path of each rat. Other features of the room included a table for the holding box to sit on in the southeast corner, a door in the centre of the south wall and a boiler and water tank in the northwest corner. A circular curtain rail was affixed to the ceiling so that a light grey, 150 cm-high curtain could be drawn throughout the experiment to fully surround the pool and hang at a distance of 25 cm from the pool's edge.

The escape platform, which stood 2 cm below the surface of the cloudy water, was constructed of clear Perspex and comprised of a circular disc with concentric grooves machined into it (10 cm in diameter, 1 cm thick) sat atop a cylindrical rod (1.5 cm diameter x 26 cm long) which was itself attached to a square base (25 cm^2, 1 cm thick). Throughout experimentation, the shaped arenas were manufactured by placing polyurethane boards into the pool. They were 58 cm high, 0.5 cm thick, with the length being dependent on the shape that was created. Each board had attached to it a hollow, square aluminium rail (1.5 cm^2) that ran parallel to and sat flush with one of the long sides. This rail protruded from each end of the board and sat on the top lip of the pool wall so that the board could be suspended vertically into the pool.

Throughout the current experiment, two white, polyurethane boards were used. They were positioned inside the circular pool to form an isosceles-like,

triangular shaped pool. Each aforementioned board was 176 cm in length and had an aluminium rail 200 cm in length attached to one of the long sides. These inserted boards formed the two straight walls of the triangle with the third base wall, which was curved and 188 cm long, formed by the white perimeter wall of the circular pool. All three corners were touching the wall of the circular pool (see Figure 4.1). The angle of each base corner was 115° and the apex was 56°. Throughout training, the centre of the escape platform was 25 cm from the point at which the two walls of the appropriate corner met, on a trajectory which split this corner in half. Two types of landmark were used, a sponge ball, 9.5 cm in diameter and painted matt black, and a hollow, octagonal prism, constructed of white polystyrene with each rectangular panel measuring 9.5 cm x 4 cm wide x 1 cm thick. The top edge of the prism had a white, octagonal plastic lid glued onto it whereas the bottom edge was painted black so that from underneath, the hollow white cavity of the prism was visible along with a black, octagonal-shaped underside edge (1 cm wide, with sides measuring 4 cm). The prism also had two centred, horizontal black stripes (2.5 cm band width) with a gap of 2.5 cm between them, painted around the entire perimeter of its outer surface. Each landmark was suspended in position above the triangular arena at a height of 26 cm from the surface of the water. This was achieved by fixing each landmark to a horizontal clear Perspex rod (1 cm in diameter), which was then attached at its other end to the aluminium rail that ran along the top end of the wall. The centre of each landmark was positioned directly above the centre of the escape platform if it had been present in that corner.

4.1.2.3 Procedure

Assignment of groups

Animals in each sex were randomly and equally assigned to two groups before commencement of the experiment. The control group, referred to as Group ***Geometry*** (10 males & 10 females), were trained to locate the escape platform in one of the two base corners of the isosceles-like triangle with a solitary, identical landmark suspended above each of these corners. The experimental group, referred to as Group ***Geometry + Landmark*** (10 males & 10 females), were also trained to locate the escape platform in one of the two base corners of the isosceles-like triangle but the identity of the landmark suspended above each base corner was different. Throughout training, after being assigned to the relevant condition, each animal experienced the hidden platform in a fixed location with respect to the shape of the pool and arrangement of landmarks. The positions and identity of landmarks also remained constant for each animal. Conditions were counterbalanced so that half the animals in each group were trained to find the platform under the black ball landmark and half under the striped prism; these subgroups (n=5 for each sex) were then split again so that approximately half the animals were trained to find the platform in one base corner of the triangular pool and half in the other (these subgroups of 5 could not be split equally but across the 10 animals in each experimental and control group an equal number was assigned to each corner) (see Figure 4.1).

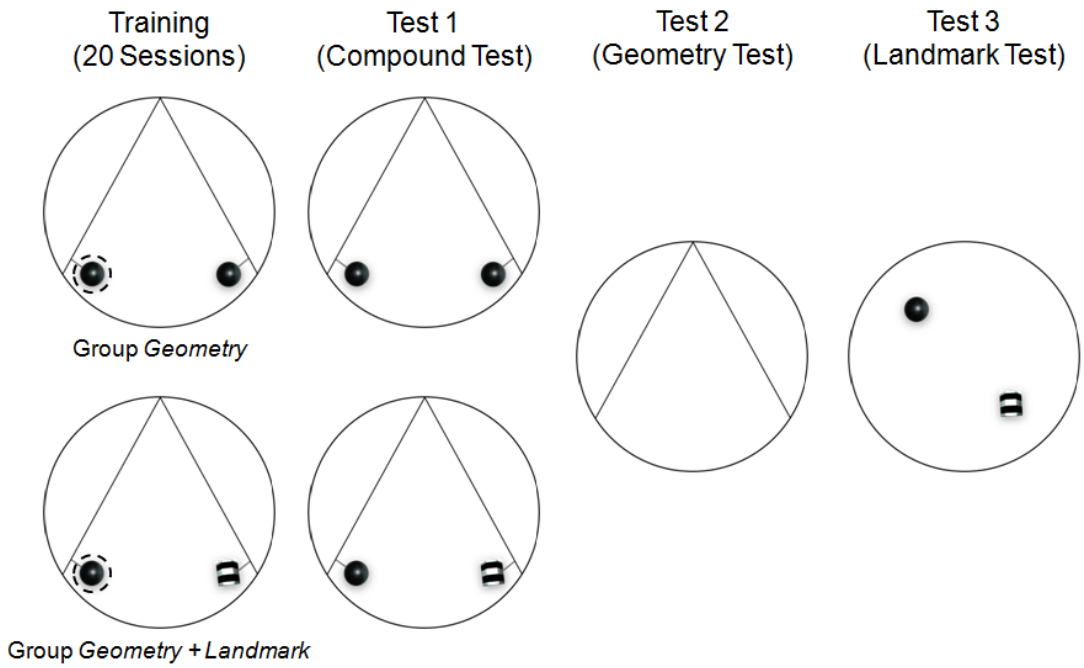

Figure 4.1. Schematic diagram showing the experimental design. Rats were placed in a triangular or, for the landmark test, circular pool of water. Black filled circles and striped prisms represent different types of landmarks, whereas the circle comprising of a dashed line represents a submerged platform. The platform was placed in one of the base corners of the triangular pool throughout training. For each test trial the platform was removed.

General Procedure

Rats were transported into the test laboratory, five at a time, in a Perspex carrying box, which housed each animal in a separate compartment. Throughout testing, the carrying box and rats resided on a table in the south east corner of the room. The trial commenced with the experimenter, ensuring that the rat's head faced the wall, placing the rat gently into the pool and ended when the hidden platform was located. If the animal failed to find the platform within sixty seconds, the experimenter entered the curtained area surrounding the pool and guided the rat to the platform by holding out a hand in front of its nose. The rats were left on the platform for 20 seconds before the experimenter removed the animal from the pool,

dried it with a towel and placed it back into the holding box, where it remained until the remaining four animals had each completed a trial. This cycle was repeated until all five rats had received four trials. The midpoints of the three walls of the triangular arena were designated as the points of release into the pool from which an animal could start the trial. The arena was rotated between each trial and could be oriented in four positions with the apex of the triangular pool pointing north, east, south or west. The release points and arena positions were assigned randomly for each trial with the constraint that three different release points and four different orientations were used within a session. These manipulations ensured that rats could not learn the absolute position of the platform within the testing room or use a fixed strategy from a constant release point. For extinction test trials the escape platform was removed from the pool, animals were released from a novel location in the centre of the arena and allowed to swim for thirty seconds before being removed. At the end of each day all arena walls were cleaned with disinfectant spray and thoroughly rinsed with clean water.

Training

Rats received twenty sessions of training with four trials to a session except for sessions 16, 20 and 21 that comprised of three training trials followed by a thirty second extinction test.

Extinction Tests

On the fourth trial of session 16 the first extinction test (*Compound Test*) took place with the shape of the arena and position of the landmarks remaining identical to that which the rats experienced during training. This test trial was carried out to offer a behavioural measure to accompany the training data of how well

animals could discriminate the correct from incorrect base corners when landmarks were present. Because it wasn't clear from the training data whether male and female rats had learned to discriminate the corners equally well, this *Compound* Test offered an additional measure. After this first extinction test, animals received four sessions of retraining before a second extinction test (*Geometry Test*) was conducted on the fourth trial of session 20. This geometry test was conducted with the triangular shape of the arena remaining as it had during training but now the platform and both landmarks were removed. Animals then received one final session of retraining before the final *Landmark Test*, which was conducted on the fourth trial of session 21. The landmark test took place in the circular pool (200 cm in diameter) with each of the two landmarks positioned 130 cm apart and 35 cm from the edge of the pool along a north northwest – south southeast axis. To achieve this, each landmark was attached to thin soldering wire, which could be hung from hooks affixed to the circular ceiling above the pool. It was ensured that the landmarks were suspended at the same height above the surface of the water as they were during training. The striped prism landmark was placed in the southeast quadrant of the pool and the black ball landmark in the northwest quadrant.

4.1.2.4 Performance Measures

For each training trial, acquisition rate was measured by recording escape latency and first choice. Both measures were recorded live by the experimenter, who watched images of the test arena on a monitor situated in the southwest corner of the testing room. Escape latency, or the time taken for a rat to reach the platform after being released into the pool, was recorded by the experimenter using a stopwatch. The first choice measure was established by recording which of three circular corner zones the rat first visited after it had been released into the pool. These corner zones,

drawn on the monitor screen, were approximately 30 cm in diameter with the centre of each zone corresponding to the centre of the potential escape platform position for that corner. This raw first choice data could then be used to calculate two behavioural measures: *percentage of correct first choices*, i.e. the percentage of trials within each session that an animal visited the correct corner zone first, and *percentage of same direction turns*, i.e. the maximum percentage of trials in a session that animals turned in the same direction after being released into the pool. This *percentage of same direction turns* measure, recorded to assess whether animals had adopted a habit-based response over a goal-directed response, was calculated in three session blocks ensuring each of the three release points had been used an equal number of times, which eliminated the possibility that any observed turn biases were simply an experimental artefact based on where the animal had been released from.

For the extinction tests, the recorded footage of each rat's swim path could be tracked using Ethovision (version 3.1) software. With this program, it was possible to overlay zones onto the recorded images so that the time a rat spent in a designated area could be objectively measured. For each thirty second extinction test trial, this software was used to manufacture two zones each measuring approximately 25 cm in diameter, or approximately six times the area of the escape platform, which were individually positioned so that the centre of each zone corresponded to where the centre of the escape platform would have been if it had been paired with that particular cue during training. Thus, for the compound and geometry tests, one zone resided over the correct geometric corner and the other zone over the incorrect geometric corner, and for the landmark test, one zone resided over the correct landmark and the other over the incorrect landmark. Exploration was considered to have taken place if the rat's head entered either of these circular zones.

To determine the thigmotactic tendencies of animals during each extinction test, Ethovision was again used to place a zone (20 cm wide) around the perimeter of the pool, which could record the amount of time that animals spent close to the walls of the arena.

4.1.3 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied.

4.1.3.1 Training

Figure 4.2 shows the mean escape latency (upper panel) and the mean percentage of correct first choices (lower panel) for each group across 20 sessions (in five four-session blocks) of training. This figure demonstrates that acquisition of the task, both in terms of escape latency and choice accuracy, was better for male and female rats in Group *Geometry + Landmark* than in Group *Geometry*. It is also clear that, overall, male animals acquired the task more rapidly than female rats. The percentage of correct first choice data demonstrate that choice accuracy was superior in males when compared to females for Group *Geometry* but this difference was not evident for Group *Geometry + Landmark*.

To support this description of the data, three-way ANOVAs were conducted separately on mean individual escape latencies in each four-session training block and mean individual percentages of correct first choices in each block. The between-subject variables were sex (male, female) and training condition (*Geometry*, *Geometry + Landmark*) and the repeated measure was session block. The ANOVA of escape latencies revealed a significant main effect of training condition, $F(1, 36) =$

22.1, $p < .001$, session block, $F(2.03, 72.9) = 491$, $p < .001$, and a marginally significant effect of sex, $F(1, 36) = 4.00$, $p = .053$. There was also a significant session block x sex interaction, $F(2.03, 72.9) = 4.36$, $p = .016$. Tests of simple main effects to examine this interaction revealed that males located the platform significantly quicker than females during session blocks 1 and 4, Fs$(1, 36) \geq 6.24$, ps $\leq .017$. All remaining effects and interactions were non-significant, Fs ≤ 2.07, ps $\geq .13$.

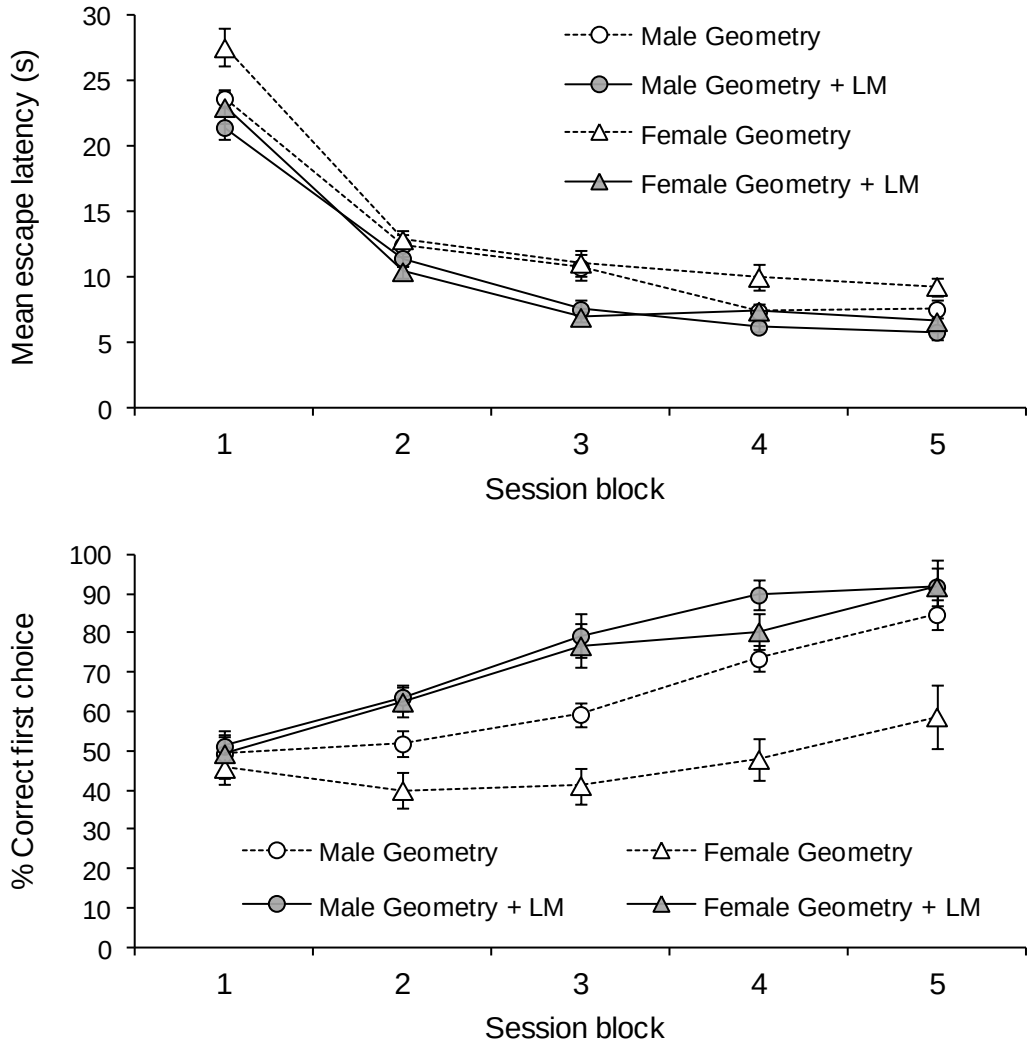

Figure 4.2. The mean (±SEM) escape latencies (upper panel) and percentage of correct first choices (lower panel) across 20 sessions of training, which are presented in five session blocks with four sessions in each block. The groups are split by sex and training condition (*Geometry* and *Geometry + Landmark*).

For the percentages of correct first choices, the ANOVA revealed a significant main effect of sex, $F(1, 36) = 9.68$, $p = .004$, training condition, $F(1, 36) = 32.7$, $p < .001$, and session block, $F(3.02, 109) = 48.4$, $p < .001$. There was also a significant sex x training condition interaction, $F(1, 36) = 4.76$, $p = .036$, and session block x training condition interaction, $F(3.02, 109) = 6.31$, $p = .001$. Tests of simple main effects to investigate the interaction between sex and training condition revealed that for Group *Geometry*, males first visited the correct corner on significantly more occasions than females, $F(1, 36) = 14.0$, $p = .001$, but this sex difference was not apparent for the experimental group, $F(1, 36) < 1$. The interaction also revealed that for both sexes, Group *Geometry + Landmark* made significantly more correct first choices than Group *Geometry*, with this training condition effect more marked in females, $F(1, 36) = 31.2$, $p < .001$, than in males, $F(1, 36) = 6.25$, $p = .017$. All remaining within-subjects effects and interactions were non-significant, $Fs \leq 2.19$, $p \geq .09$.

Figure 4.3 displays the percentage of same direction turns for each session block. It is clear from this figure that, overall, females were more inclined to turn in the same direction, as on previous trials within a session, after release into the pool. However, this inclination was particularly prominent for female rats trained in the *Geometry* condition. To validate this characterisation of the data, an ANOVA of percentage of same direction turns on the session block mean (there were 3 sessions to a block for this measure, the reason for this is given in the *Performance Measures* section) for individual rats, with sex and training condition as between-subjects factors, revealed a significant main effect of sex, $F(1, 36) = 4.52$, $p = .040$, and training condition, $F(1, 36) = 6.75$, $p = .014$, and a significant interaction between these two variables, $F(1, 36) = 5.09$, $p = .030$. There was also a significant effect of

session block, $F(3.85, 139) = 10.4$, $p < .001$, and a significant session block x sex, $F(3.85, 139) = 7.24$, $p < .001$, and a session block x training condition, $F(3.85, 139) = 3.32$, $p = .014$, interaction.

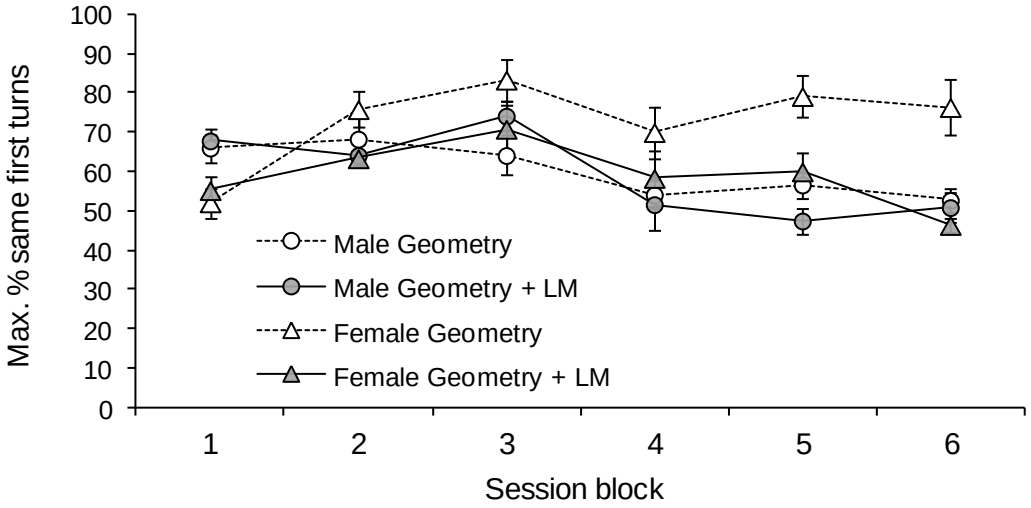

Figure 4.3. The maximum percentage of same direction turns following release into the pool within a session (averaged across session blocks with 3 sessions to a block). The groups are split by sex and training condition (*Geometry* and *Geometry + Landmark*).

Tests of simple main effects to examine the significant sex x training condition interaction on percentages of same direction turns revealed that after being released into the pool, females turned in the same direction more than males, but this effect was only apparent for Group *Geometry*, $F(1, 36) = 9.60$, $p = .004$, and not Group *Geometry + Landmark*, $F(1, 36) < 1$. This interaction also revealed a main effect of training condition on percentage of same direction turns for females, $F(1, 36) = 11.77$, $p = .002$, with Group *Geometry* turning in the same direction more frequently, but not for males, $F(1, 36) < 1$. Tests on the interaction between session block and sex revealed that females made significantly more turns in the same direction across the last two session blocks, Fs$(1, 36) \geq 4.88$, ps $\leq .034$. All remaining effects and interactions were non-significant, Fs$(3.85, 139) \leq 1.86$, ps

≥.12.

4.1.3.2 Extinction Tests

Compound Test

Figure 4.4 shows the result of the compound extinction test and it is clear that rats from both sexes and training conditions discriminated the correct from incorrect corner. However, a more critical finding is that this discrimination is more marked in Group *Geometry + Landmark* for both males and female rats.

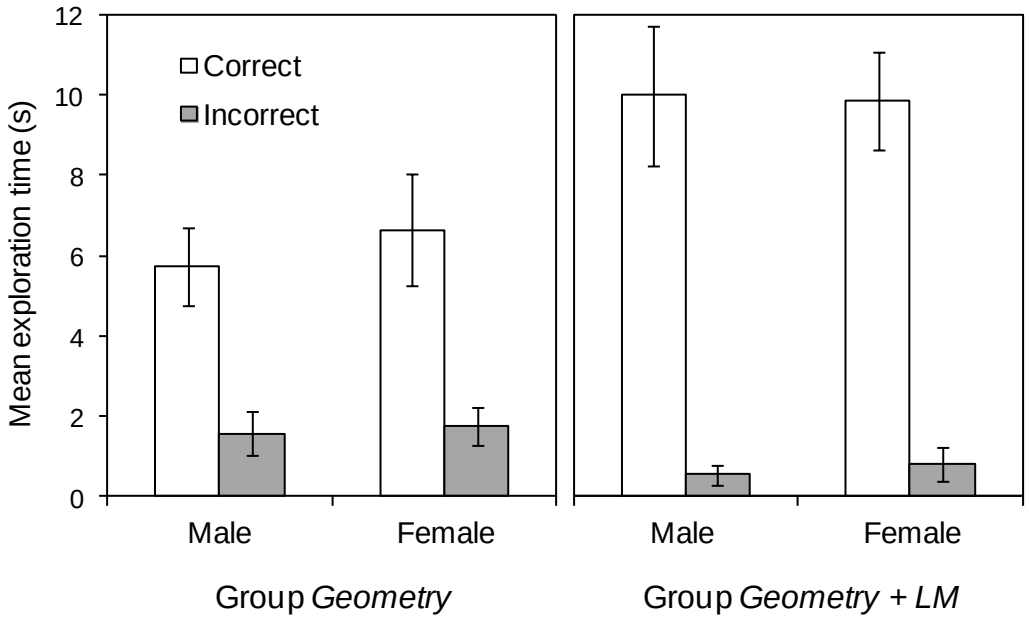

Figure 4.4. The mean time (± *SEM*) spent in the correct (white bars) and incorrect (grey bars) corner zones during the *Compound* test for each sex and training group.

To confirm this characterisation of the data, a three-way mixed model ANOVA, conducted on exploration times, with sex and training condition as between-subjects variables and corner zone (correct and incorrect) as the repeated measure revealed a significant main effect of training condition, $F(1, 36) = 4.94$, $p =$

.033, zone, $F(1, 36) = 74.0$, $p < .001$, and a significant zone x training condition interaction, $F(1, 36) = 8.66$, $p = .006$. Tests of simple main effects to examine this interaction revealed that, irrespective of sex, both Group *Geometry* and Group *Geometry + LM* were able to discriminate the correct from incorrect base corners of the triangular pool, $F(1, 36) = 16.02$, $p < .001$, and $F(1, 36) = 66.7$, $p < .001$, respectively, with Group *Geometry + Landmark* spending significantly more time in the correct zone, $F(1, 36) = 7.50$, $p = .010$, and significantly less time in the incorrect zone, $F(1, 36) = 5.01$, $p = .031$, than Group *Geometry*. All remaining between- and within-subjects effects and interactions were non-significant, Fs < 1.

Geometry Test

Figure 4.5 shows the result of the geometry test and it is clear from this figure that, once again, rats from both sexes and training conditions discriminated the correct from incorrect base corner of the triangular arena. However, a more critical finding is that discrimination of the correct from incorrect corner was more marked in Group *Geometry + Landmark* when compared to the Group *Geometry*, which is the opposite pattern of results to that predicted if landmarks had overshadowed learning based on geometry. This finding was evident in both males and females. To confirm this interpretation statistically, a three-way mixed model ANOVA, conducted on time spent in the correct and incorrect zones with sex and training condition as between-subjects variables revealed a significant main effect of training condition, $F(1, 36) = 9.23$, $p = .004$, and zone, $F(1, 36) = 54.7$, $p < .001$, and a significant interaction between these two variables, $F(1, 36) = 7.06$, $p = .012$. Tests of simple main effects to examine this interaction revealed that, irrespective of sex, both Group *Geometry* and Group *Geometry + Landmark* were able to discriminate the correct from incorrect base corner of the triangular pool, $F(1, 36) = 11.22$, $p =$

.002, and $F(1, 36) = 50.5$, $p < .001$, respectively, with Group *Geometry + Landmark* spending significantly more time in the correct corner than Group *Geometry*, $F(1, 36) = 9.15$, $p = .005$. All remaining between- and within-subjects effects and interactions were non-significant, Fs ≤ 2.46, ps $\geq .13$.

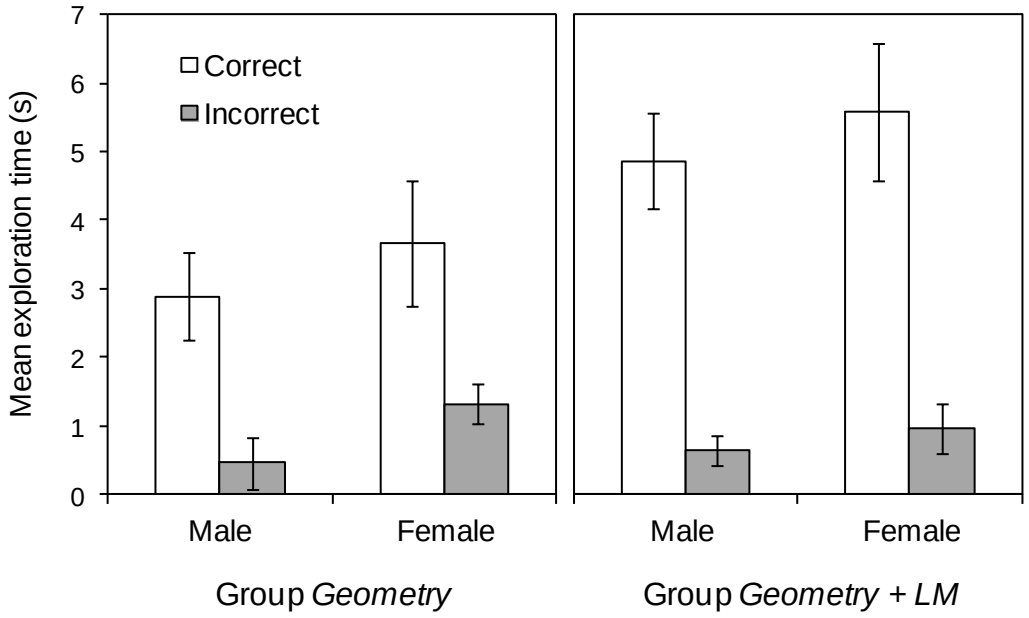

Figure 4.5. The mean time (± *SEM*) spent in the correct (white bars) and incorrect (grey bars) corner zones during the *Geometry* test for each sex and training group.

Landmark Test

Figure 4.6 illustrates the result of the landmark test and, as expected, it shows that Group *Geometry* was unable to discriminate the correct from incorrect landmark. However, there is a trend towards rats in this group exploring the incorrect landmark more than the correct landmark. This could be because the incorrect landmark is not technically incorrect for these rats as they had never encountered it before. Therefore, the novelty of this object could be driving the observed preference. The performance of Group *Geometry + Landmark* revealed that animals

from both sexes were able to discriminate the correct from incorrect landmark. A three-way mixed model ANOVA, conducted on time spent in the correct and incorrect zone with sex and training condition as between-subjects variables revealed a significant effect of zone, $F(1, 36) = 7.16$, $p = .011$, and a significant zone x training condition interaction, $F(1, 36) = 28.1$, $p < .001$. Tests of simple main effects to examine this interaction revealed that Group *Geometry + LM* was able to discriminate the correct from incorrect landmark, $F(1, 36) = 31.8$, $p < .001$, but Group *Geometry* was not, $F(1, 36) = 3.44$, $p = .072$. All remaining between- and within-subjects effects and interactions were non-significant, Fs$(1, 36) \leq 2.41$, ps $\geq .13$.

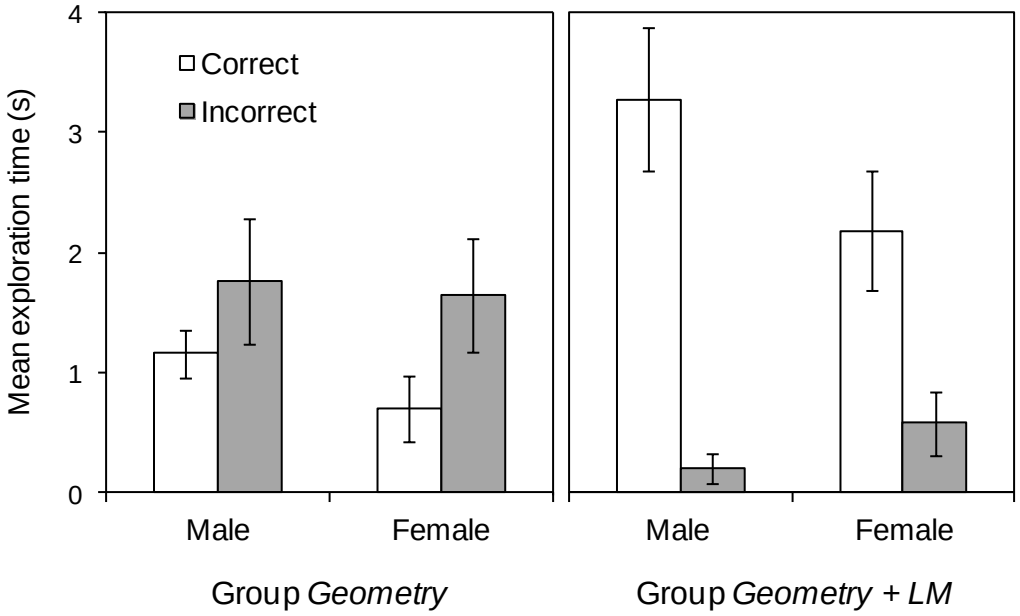

Figure 4.6. The mean time (± *SEM*) spent in the correct (white bars) and incorrect (grey bars) landmark zones during the *Landmark* test for each sex and training group.

4.1.3.3 Thigmotaxis

Figure 4.7 displays the mean time male and female rats spent close to the arena walls during each extinction test. With the exception of Group *Geometry +*

Landmark during the landmark test, the figure shows that female rats spent more time close to the arena walls than male rats in each extinction test. This heightened thigmotaxis in females when compared to males was particularly marked in Group *Geometry*.

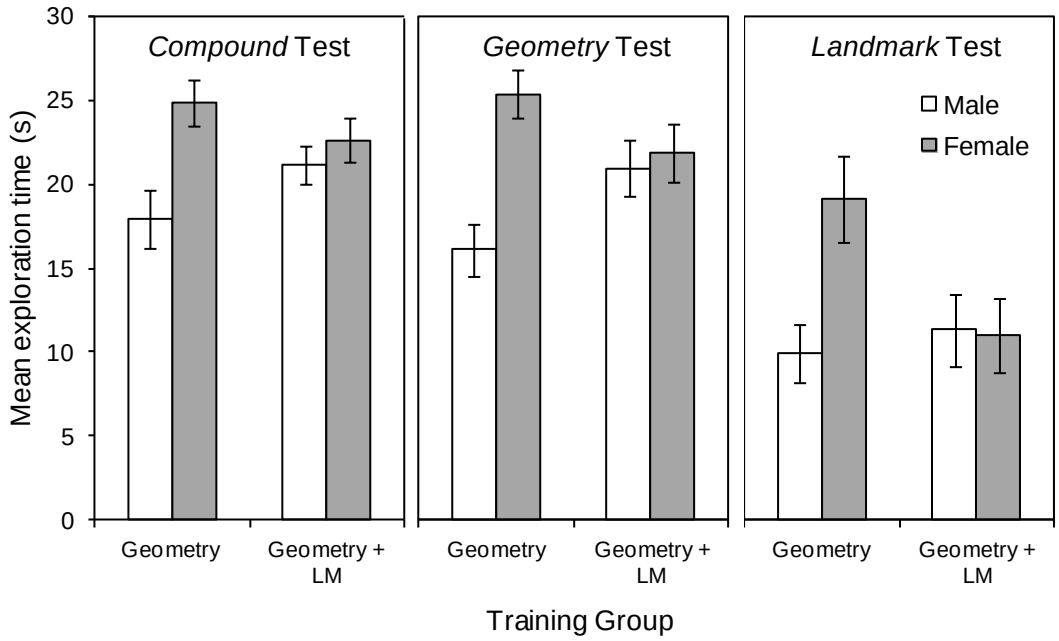

Figure 4.7. The mean time (± *SEM*) spent close to the boundary walls during each extinction test for male (white bars) and female (grey bars) rats in each training group.

To validate these observations an ANOVA of mean time spent close to the arena walls for individual rats during each extinction test (*Compound*, *Geometry* & *Landmark*) with sex and training condition as between-subjects factors and extinction test as the repeated measure was conducted. The ANOVA revealed a significant main effect of sex, $F(1, 36) = 10.3$, $p = .003$, extinction test, $F(1.65, 19.7) = 59.9$, $p < .001$, and significant sex x training condition interaction, $F(1, 36) = 7.43$, $p = .010$. Tests of simple main effects to investigate this interaction revealed a significant overall main effect of sex on thigmotaxis for rats in Group *Geometry*,

$F(1, 36) = 17.6$, $p < .001$, but not for rats in Group *Geometry + Landmark*, $F(1, 36) < 1$. This interaction also revealed a significant main effect of training condition for female rats, $F(1, 36) = 5.23$, $p = .028$, with Group *Geometry* more thigmotactic than Group *Geometry + Landmark*, but not for male rats, $F(1, 36) = 2.46$, $p = .13$.

4.1.4 Discussion

In an experiment designed to reveal differential effects of overshadowing on the sexes, rats of both sexes were assigned to two separate training conditions. Rats in Group *Geometry* (control group) were trained to locate a hidden goal by reference to the shape of their environment, which contained uninformative landmark cues. Rats in Group *Geometry + Landmark* (experimental group) were trained to locate a hidden goal that could be located by reference to two sources of information: the shape of the environment and landmark cues. A subsequent geometry test was conducted with the hidden goal and landmarks removed from the environment to assess how much animals in each group had learned about the geometric properties of the arena. The performance of Group *Geometry* provided a measure of how much could be learned about the geometric properties of the arena when this was the only source of informative information available. Accordingly, if the findings from the geometry test revealed that Group *Geometry + Landmark* had learned less about the shape of the environment than Group *Geometry* this would have provided evidence that the presence of informative landmark cues had restricted learning based on geometry.

The results revealed that in both male and female rats, learning based on the shape of the environment was more marked for Group *Geometry + Landmark* than for Group *Geometry*. Thus, instead of the landmark cues overshadowing shape-based

cues, they did in fact facilitate, or potentiate, shape learning (as in Pearce et al., 2006). This result is the first demonstration of discrete landmarks potentiating geometry in both male and female rats and opposes the predictions of several theories of learning that apply a rule incorporating a global error term (e.g. Rescorla & Wagner, 1972). These theories would predict that the two sources of information available to the experimental group in the current experiment should compete for a finite amount of associative strength and, as a consequence, less should be learned about geometry in rats belonging to this group than the control group.

It must be pointed that, although rare, several studies have provided evidence, at least in male rats, of potentiation of geometry learning (Graham et al., 2006; Horne & Pearce, 2011; Pearce et al., 2006), but it could be argued that the non-geometric cues used in these previous experiments did not constitute discrete landmarks since they were integrated with the geometric cues. The current finding that landmarks potentiated geometry learning in female rats contrasts starkly with the result of a very similar experiment conducted by Rodriguez et al. (2011). Rodriguez et al. predicted, based on previous evidence that landmarks are more salient than geometric cues for female animals and vice versa for male animals, that landmarks would overshadow geometry in females and vice versa for males. However, as discussed in the Introduction, one interpretation of Rodriguez et al.'s (2011) finding of differential overshadowing effects in male and female rats is that, without appealing to any cue competition effects, this pattern of behaviour was the result of generalization decrement. The present results support this interpretation by demonstrating that when the effects of generalization decrement were controlled for, by ensuring the perceptual change in training and test environments was matched for both experimental and control groups, the overshadowing of geometry learning by

landmarks was not observed. The results of the landmark test in the current experiment provided further support for a lack of sex differences in cue preference, as males and females discriminated between the correct and incorrect landmark in equal measure. So, a critical question to arise from this potentiation result is: why was the associative competition between cues, ubiquitously observed in classic conditioning experiments (e.g. Pavlov, 1927), not apparent in the current experiment when animals were provided with geometric and landmark cues, which were both informative in allowing rats to escape from a pool of water?

One explanation, discussed in Chapter 1, is that the processing of information pertaining to the shape, or geometry, of an animal's environment takes place in a dedicated geometric module (Cheng, 1987; Gallistel, 1990), which prevents non-geometric information from entering. Accordingly, the processing of geometric and non-geometric information occurs independently and so competition between these cues does not materialise. This theory is not, however, consistent with the present finding that the presence of an informative landmark cue enhances learning about a geometric cue. That said, Cheng (1986), in proposing his modular theory, did point out that animals will first and foremost establish a geometric framework of their environment and over time featural cues, such as landmarks, can be 'pasted on' to this geometric frame in order to further facilitate navigation. This kind of theory could account for the findings in this experiment although caution should be exercised when comparing the performance of animals in water to on dry land during navigation tasks (Dudchenko et al., 1997; Golob & Taube, 2002).

A second explanation for the present failure to observe overshadowing is related to the relative salience of competing cues. Evidence suggests that a cue of weak salience will be overshadowed by a cue of stronger salience (Mackintosh,

1976). Accordingly, it could be argued that the salience of the landmark cues in the present experiment was low relative to the salience of the geometric cues and therefore it was not possible for the former to overshadow the latter. An extreme stance to this argument would be that, under the present treatment, rats did not notice the landmarks at all. However, this interpretation is unlikely for two reasons. First, the results of the landmark test revealed that rats had learned to discriminate between the landmarks. Second, the fact that landmarks produced a potentiation effect indicates that they were of sufficient salience to affect learning based on geometry. Indeed, several studies have revealed that for potentiation to occur the relative salience of the potentiating cue must be high relative to the to-be-potentiated cue (e.g. Slotnick, Westbrook & Darling, 1997).

A third explanation for the present lack of associative cue competition or the emergence of potentiation relates to the mechanisms underlying cue interactions. One proposed mechanism that can account for the facilitatory effect informative landmarks have on geometry learning involves the formation of within-compound associations (Horne & Pearce, 2009a; Rhodes et al., 2009; see also the Introduction to Chapter 3 for a description). In context of the current experiment, if within-compound associations had formed between the correct landmark and geometric cue (corner) during training, it was anticipated that during the geometry test, in which the landmarks were removed, the presence of the correct geometric cue would evoke a memory for the correct landmark cue and promote an approach response toward this corner. Of course, this phenomenon would only emerge in Group *Geometry + Landmark* as rats in Group *Geometry* experienced landmarks that were an unreliable predictor of the platform's position and so any within-compound associations formed between the landmark and correct geometric cue during training would be

extinguished when these rats entered the incorrect base corner of the triangle, which contained an identical landmark to the correct corner. Thus, it is possible that informative landmarks in Group *Geometry + Landmark* did restrict what was learned about geometric cues, to the extent that there was a weak association between the correct geometric cue and the escape platform, but this effect may have been attenuated by the indirect geometric ↔ landmark → platform associative link.

Another mechanistic explanation for the observed potentiation result involves a process referred to as *feature enhancement* (Miller & Shettleworth, 2007). Simply put, this process of *feature enhancement* takes place when a cue of high predictive value, which exerts strong control over an animal's approach to a specific location, results in other coincidental, contiguous cues acquiring greater associative strength than they would have done otherwise. According to this model, rats in Group *Geometry + Landmark* in the present experiment could be guided to the correct corner more than Group *Geometry* based on the use of relevant landmark information, which would lead to the correct corner gaining more associative strength.

Instructive to this explanation is an assessment of rats' performance during training. Group *Geometry + Landmark* displayed superior performance when compared to Group *Geometry*, both in terms of choice accuracy and latency to find the platform, which is in keeping with the predicted pattern should feature enhancement occur. However, in the last session block of training prior to the critical geometry test, which it could be argued is the most indicative session block to analyse given the likelihood of rats transferring their most recently acquired behaviour to the test, there was no difference in correct first choice accuracy between Group *Geometry + Landmark* and Group *Geometry* for male rats, however for

female rats, Group *Geometry + Landmark* made more first visits to the correct corner than Group *Geometry*. Whether this pattern of behaviour in female rats is evidence of feature enhancement is open to debate, and certainly the current dataset cannot confirm this assumption. However, the training data suggests that male and female rats may have developed different strategies in order to solve the task.

In summary, this experiment has shown that during a geometry test, male and female rats were equally adept at using the shape of their environment to locate a hidden goal. Moreover, geometry learning in both sexes was potentiated by the presence of reliable landmark cues. Despite a distinct lack of sex differences during probe trials, performance during training suggested that male and female rats may have been relying on different strategies to acquire the task. Thus, the following experiment was conducted to investigate sex differences in the use of geometric and landmark cues when, during training, one cue type was rendered irrelevant and the other remained a reliable predictor of the platform's location.

4.2 Experiment 9: Changes to the Reliability of Different Types of Cues

4.2.1 Introduction

The results of Experiment 8 demonstrated no effect of sex when rats were required to learn the location of a hidden goal by reference to two sources of information: the shape of the environment and landmark identity. However, although the results of the test trials indicated that male and female rats arrived at a very similar learning endpoint, the training data indicated that the processes by which rats arrived at this point may have differed between the sexes. Thus, it may be the case

that the behavioural index of learning during a final test trial, i.e. how long rats spend in a given location, does not necessarily reflect the sex differences that take place in how rats acquire the task. Therefore, in order to tease apart any sex differences in the way males and females use geometric and landmark cues during acquisition, it is necessary to investigate if male and female rats are differentially affected by changes to the validity of these cues during training.

In a study on human participants, Sandstrom, Kaufman and Huettel (1998) designed an experiment with such an investigation at its core, in which males and females were trained in a virtual Morris water maze task. In this task the training stage consisted of participants being provided with informative geometric and landmark information to facilitate their search for a hidden escape platform. For the testing stage the training environment could be manipulated to provide three conditions, *stable landmark*, *geometric*, and *random landmark*. The *stable landmark* condition rendered the geometric information uninformative whilst the informative landmark cues, available during training, remained identical. The *geometric* condition retained the identical geometric information provided during training but removed the landmarks. Finally, the *random landmark* condition retained the identical geometric information provided throughout training but provided uninformative landmarks which moved around the environment randomly. Participants from each sex were randomly and equally assigned to one of these testing conditions in which additional training trials were provided so that performance could be measured. The authors found no sex difference in performance in the *stable landmark* condition. However, when informative landmark information was not available, i.e. in the *geometric* and *random landmark* conditions, the performance of males was superior to females. Or, put another way, females were

unable to accurately utilise the remaining geometric information and / or were more adversely affected by the removal or change in predictability of landmarks.

Sandstrom et al.'s (1998) results serve to underline the different extent to which males and females rely on landmark and geometric cues during training trials. However, the result from Experiment 8 suggests that this differential reliance during training does not necessarily map directly onto behaviour during a final test trial. Thus, the current experiment, using rats and an actual Morris water maze environment in place of humans and a virtual environment, sought to replicate the design of Sandstrom et al. (1998) to investigate differences in cue use by male and female rats during training. Furthermore, and unlike Sandstrom et al.'s (1998) study, this experiment provided test trials after all training stages were complete to assess the impact of specific modifications to the training environment on the overall learning of particular cues.

Male and female rats were trained identically to Group *Geometry + Landmark* in the previous experiment, in an environment in which informative geometric and landmark cues signalled the location of an escape platform. Following the training stage, a test stage was conducted in which rats received additional training trials but with some aspects of the environment modified. During this test stage, half the rats in each sex were trained in the *Geometry Relevant* condition and the remaining rats were trained in the *Landmark Relevant* condition. In the *Geometry Relevant* condition, the geometric cues continued to reliably signal the location of the hidden platform, while the landmark cues were rendered uninformative. Conversely, in the *Landmark Relevant* condition, the landmark cues continued to reliably signal the location of the platform, while the geometric cues were rendered uninformative. The prediction based on Sandstrom et al.'s (1998) finding was that

male rats should outperform female rats in the *Geometry Relevant* test condition but performance should be similar for both male and female rats in the *Landmark Relevant* test condition. Following training and test stages, extinction tests were conducted to assess how much male and female rats had learned about the geometric and landmark cues (see Figure 4.8).

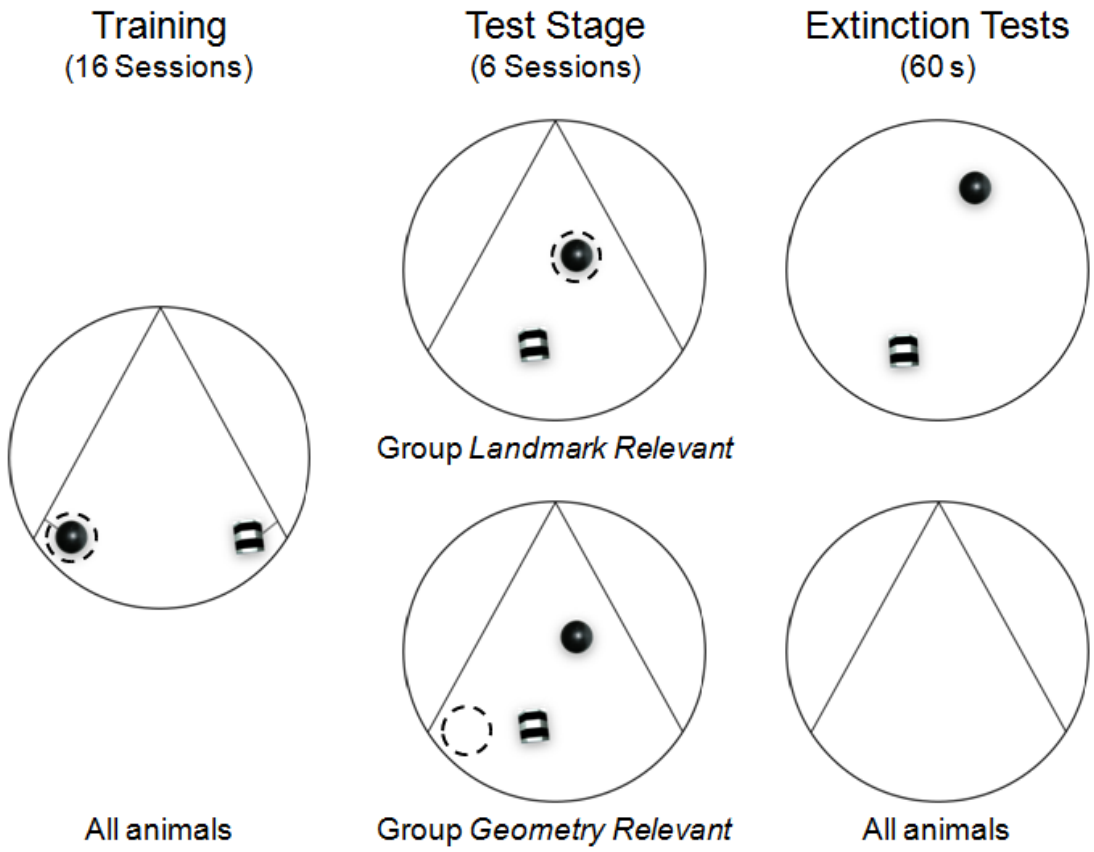

Figure 4.8. Schematic diagram showing the experimental design. Black filled circles and striped prisms represent different types of landmarks, whereas the circle comprising of a dashed line represents a submerged platform. During Training, rats could use two sources of information (geometry and landmarks) to locate the platform in a triangular pool. For the Test Stage, the two landmarks moved around the pool from trial to trial and depending on the group the platform was now found either under the previously rewarded landmark (Group *Landmark Relevant*) or the previously rewarded corner (Group *Geometry Relevant*). Final extinction tests measured how much had been learned about each cue type following Training and the Test Stage.

4.2.2 Method

4.2.2.1 Subjects

The subjects were 20 male and 20 female experimentally naive hooded Lister rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England). At the start of the experiment they were approximately 3 months of age. All animals were housed in identical conditions to those in Experiment 8.

4.2.2.2 Apparatus

See Experiment 8 for the apparatus used.

4.2.2.3 Procedure

Training

All aspects of the training procedure in the current experiment were identical to those described for Group *Geometry + Landmark* in Experiment 8 except that rats were trained for 15 sessions in this experiment. The counterbalancing of conditions was identical to Group *Geometry + Landmark* in Experiment 8 except that there were twice as many animals used in the present experiment. Therefore, for half the rats in each sex (n=10) the platform was found under the black ball landmark, and for the other half the platform was found under the striped prism. For half the rats in each of these landmark sub-groups (n=5) the platform was in found in one base corner of the triangle, and for the remaining rats the platform was found in the opposite base corner.

Test Stage

On the day after training was complete, rats began the Test stage. The Test stage took place in the same triangular arena, with curtains surrounding it, as training, but now rats were required to locate the escape platform whilst the two landmarks moved around the pool pseudo-randomly from trial to trial. For rats assigned to the *Geometry Relevant* test condition, the escape platform remained in the same corner of the triangular pool as during training. For rats assigned to the *Landmark Relevant* test condition, the escape platform remained under the same landmark cue as during training (see Figure 4.8). Rats were tested in one of these conditions for 6 sessions (4 trials to a session). It was ensured that prior training experience of landmark and corner was counterbalanced for each test condition. Several procedural aspects of the Test stage remained identical to training. For example, the counterbalancing of release walls and arena orientations during the Test stage was identical to that described during training. However, the designated release points for the animals were not at the midpoints of the three walls of the triangular arena, as was the case during training, but rather at a point on the wall that was equidistant between the landmark and corner that had previously been rewarded during training. In this way, the choice rats made between the previously rewarded corner and landmark was not biased by where the rat was released from.

As mentioned, during the Test stage, the landmarks moved around the pool from trial to trial. To achieve this, each landmark was attached to thin soldering wire, which could be hung from hooks affixed to the circular ceiling above the pool. It was ensured that the landmarks were suspended at the same height above the surface of the water as they were during training. The hooks on the ceiling were arranged symmetrically and equidistantly in four circles. Within any of the four orientations

that the triangular pool could be positioned, there were 32 possible positions for the landmarks to be located. As the triangular pool was rotated between trials the 32 landmark positions remained constant relative to the geometric frame of the pool. For the first three sessions of training in the Test stage, the landmark positions were assigned randomly using a random number generator with the constraint that each landmark could not be used in the same position twice and there was a minimum distance between the two landmarks of 30 cm. For the final three sessions of the Test stage, the landmark positions were assigned in a pseudorandom fashion taking into account the positions used in the previous three sessions to ensure that when averaged across all six sessions the correct and incorrect landmarks held a similar relationship to the previously rewarded corner, i.e. one of the landmarks was not found on more occasions to be closer to the corner where the platform had been located during training.

Extinction Tests

On the day after the final session of the Test stage, rats received a single *Geometry* and *Landmark* extinction trial, one shortly after the other. As during Training and the Test stage, curtains surrounded the pool and rats were run in squads of five which meant that each animal remained in the holding box for approximately twelve minutes between the first and second extinction test trial. For rats that were previously tested in the *Landmark Relevant* condition, the *Geometry* extinction trial preceded the *Landmark* extinction trial, and for those rats that were previously tested in the *Geometry Relevant* condition, the reverse order of extinction trials was presented. The *Landmark* extinction trial took place in a circular pool (200 cm in diameter) with each of the two landmarks positioned 135 cm apart and 32.5 cm from the edge of the pool along a north northeast – south southwest axis. The striped

prism landmark was located in the southwest quadrant of the pool and the black ball landmark in the northeast quadrant. The *Geometry* extinction trial took place in the same triangular shaped arena used throughout training and the Test Stage but now the landmarks were removed (see Figure 4.8). The location of the release point, in the centre of the pool for both *Landmark* and *Geometry* extinction trials, ensured that animals commenced the trial at a location equidistant from each landmark and base corner, respectively.

4.2.2.4 Performance Measures

The performance measures during training were identical to Experiment 8. For the Test Stage, as well as the experimenter recording escape latency, the recorded swim path of each rat was tracked for every trial using Ethovision (version 3.1) software. Tracking commenced when the animal was released into the pool and ended when it had found the platform. The tracking data provided an objective measure of which of the two previously rewarded cues rats first swam to (correct first choice). Two circular zones, each approximately 37 cm in diameter, or approximately fourteen times the area of the escape platform, were individually positioned over the previously rewarded corner and landmark so that the centre of each zone corresponded to where the centre of the escape platform would have been had it been paired with that cue during training. These zones were labelled as geometric zone and landmark zone. Entry was considered to have taken place if the rat's head entered either of these circular zones.

It is noteworthy to mention that it was also possible to set up zones during the Test stage around the two cues that were previously unrewarded during training. However, due to the confinement of space afforded by the arena and the design of the experiment dictating that each animal should be released from a wall midway

between the corner and landmark that had previously been paired in compound with the platform during training, on certain trials it was unavoidable that rats had to be released into one of the remaining two zones surrounding a cue not previously rewarded during training. Therefore, a substantial number of trials were polluted when analyses focused on exploration of all cues. Accordingly, the following analyses only include the data from the two cues that, as part of a compound, signalled the location of the platform during training. The critical comparison focused on the differential extent to which male and female rats chose a previously rewarded geometric cue over a previously rewarded landmark cue.

For each sixty second probe trial, Ethovision software was used to manufacture two zones each measuring approximately 50 cm in diameter, or approximately twenty five times the area of the escape platform, which were individually positioned so that the centre of each zone corresponded to where the centre of the escape platform would have been if it had been paired with that particular cue. So, for the *Landmark* extinction trial, one zone resided over the correct landmark and the other over the incorrect landmark, and similarly for the *Geometry* extinction trial, one zone resided over the correct geometric corner and the other over the incorrect geometric corner.

4.2.3 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied.

4.2.3.1 Training

Figure 4.9 shows the mean escape latency (upper panel) and the mean percentage of correct first choices (lower panel) for each sex across 15 sessions (in five blocks of three sessions) of training. A correct first choice was defined as a first entry to the corner zone containing the platform and therefore chance level was at 33%. This figure shows that acquisition of the task, reflected in escape latency, is equal for both sexes. However, the first choice data demonstrate that male rats swam directly to the correct corner more frequently than female rats, particularly during the last three session blocks.

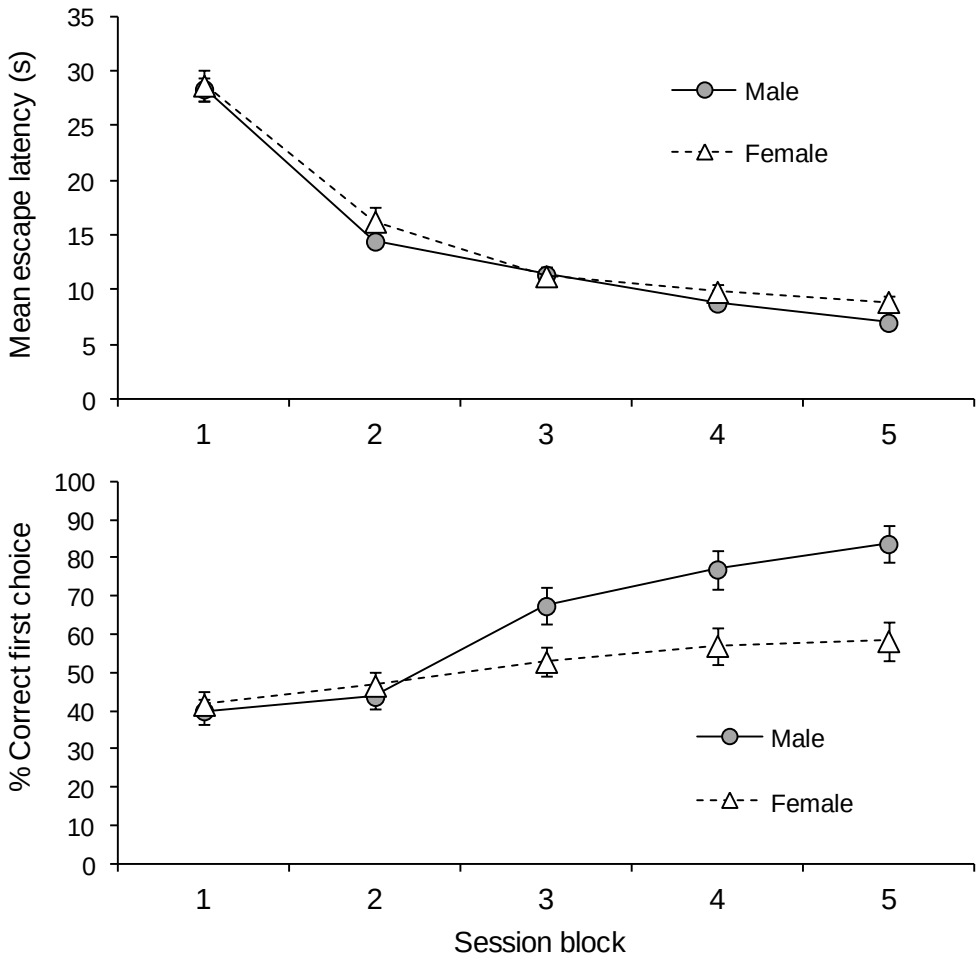

*Figure 4.9***.** The mean (± SEM) escape latencies (upper panel) and percentage of correct first choices (lower panel) across 5 session blocks of training (3 sessions to a block) for male and female rats.

To validate this interpretation of the data, two-way ANOVAs were conducted separately on the session block mean escape latencies and percentages of correct first choices of individual rats. The between-subject variable was sex and the repeated measure was session block. The ANOVA conducted on escape latency revealed a significant main effect of session block, $F(2.51, 95.3) = 337$, $p < .001$, but all remaining between- and within-subjects effects and interactions were non-significant, Fs ≤ 1.09, ps $\geq .30$. For percentages of correct first choices, the ANOVA revealed a significant main effect of sex, $F(1, 38) = 6.36$, $p = .016$, session block, $F(2.96, 113) = 28.7$, $p < .001$, and a significant interaction between these two factors, $F(2.96, 113) = 4.52$, $p = .005$. Tests of simple main effects to examine this interaction revealed that the percentages of correct first choices was significantly higher in males in each of the last 3 session blocks of training, Fs$(1, 38) \geq 4.49$, ps $\leq$ .041.

Figure 4.10 displays the mean percentages of same direction turns across 15 sessions of training for each sex (in 5 blocks of 3 sessions), and it is clear from this figure that the percentage of same direction turns was higher in females in the final two session blocks. A similar ANOVA conducted on the session block mean percentages of same direction turns (see Experiment 8 for a description) of individual rats revealed a significant main effect sex, $F(1, 38) = 6.36$, $p = .016$, (Males: $M = 55.7$, $SEM = 3.41$; Females: $M = 67.9$, $SEM = 3.41$), session block, $F(3.19, 121) = 9.05$, $p < .001$, and a significant interaction between these factors, $F(3.19, 121) = 3.93$, $p = .009$. Tests of simple main effects to examine this interaction revealed that the percentage of same direction turns was higher in females but only in the last two session blocks (6 sessions) of training, Fs$(1, 38) \geq 7.06$, ps $\leq$ .011.

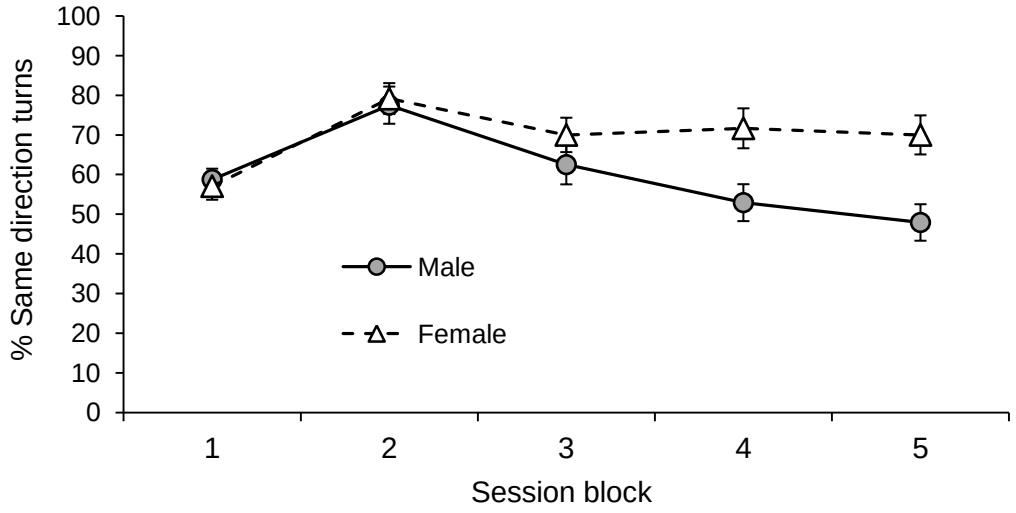

*Figure 4.10***.** The mean (± SEM) percentage of same direction turns across 5 session blocks of training (3 sessions to a block) for male and female rats.

4.2.3.2 Test Stage

The latency and accuracy to locate the escape platform after changes to the spatial contiguity of environmental cues was recorded. The following behavioural measures of escape latency and percentage of correct first choice were analysed by combining the session mean data for each rat into session blocks (3 blocks comprising of 2 sessions each). The upper panel of figure 4.11 illustrates that for rats tested both in the *Geometry Relevant* and *Landmark Relevant* condition, latencies were marginally shorter for males than for females. The lower panel of the same figure displays the percentages of correct first choices (chance = 50%) and it is clear that in the *Landmark Relevant* condition, males were more accurate in their search accuracy than females. In fact, performance of female rats in the *Landmark Relevant* condition did not progress beyond a level expected by chance. Search accuracy in the *Geometry Relevant* condition was more evenly matched between the sexes with female rats slightly outperforming males.

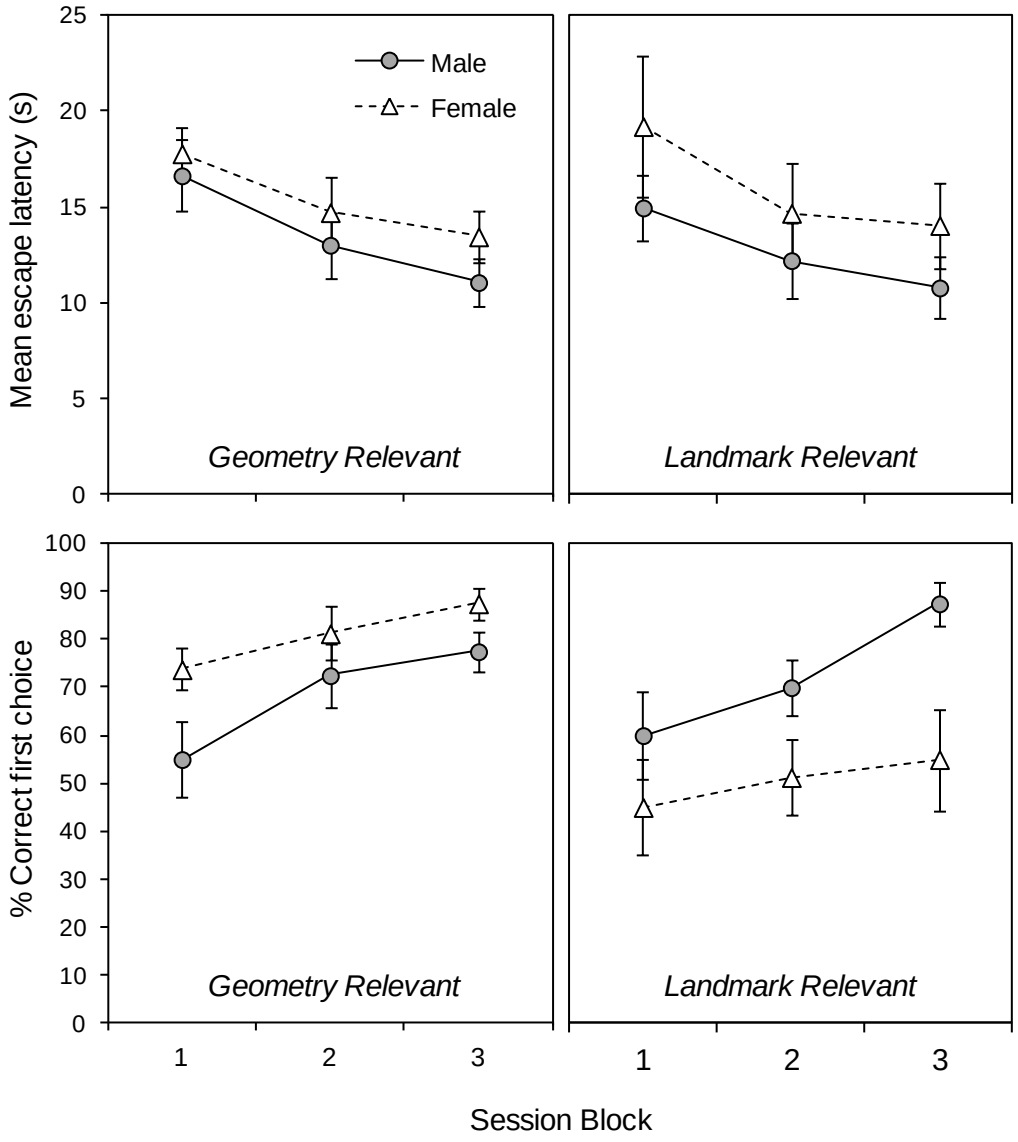

Figure 4.11. The mean (± SEM) escape latencies (upper panel) and percentage of correct first choices (lower panel) during the Test stage for male and female rats. In the *Geometry Relevant* test condition, the target geometric cue remained reliable while the target landmark cue was rendered unreliable and in the *Landmark Relevant* condition, the target landmark cue remained reliable while the target geometric cue was rendered unreliable. Training data is presented in 3 session blocks (2 sessions to a block).

To confirm these observations statistically, a three-way ANOVA of individual mean escape latencies with sex and test condition (*Geometry Relevant* & *Landmark Relevant*) as between-subjects variables and session block as the repeated measure, revealed a significant main effect of session block, $F(2, 72) = 15.9$, $p <$

.001, but no remaining significant main effect or interactions, *F*s ≤ 1.96, *p*s ≥ .17. A similar ANOVA conducted on individual mean percentages of correct choices revealed a significant main effect of test condition, $F(1, 36) = 8.20$, $p = .036$, and session block, $F(1.65, 59.2) = 17.0$, $p < .001$, and a significant sex x test condition interaction, $F(1, 36) = 8.20$, $p = .007$. Subsequent analyses of simple main effects showed that the effect of sex was significant in the *Landmark Relevant* test condition, $F(1, 36) = 6.69$, $p = .014$, but not in the *Geometry Relevant* test condition, $F(1, 36) = 2.14$, $p = .15$. There was also a significant effect of test condition for female rats, $F(1, 36) = 12.7$, $p = .001$, with choice accuracy higher in females tested in the *Geometry Relevant* condition than the *Landmark Relevant* condition, but not for male rats, $F < 1$. Mean correct first choice performance for the 6 test sessions combined was compared for each group against chance performance. Only females tested in the *Landmark Relevant* condition failed to first visit the correct location more than expected by chance, $t(9) < 1$, unlike the remaining groups, *t*s(9) ≥ 3.28, *p*s ≤ .010.

4.2.3.3 Extinction Tests

Geometry Test

Figure 4.12 shows the result of the *Geometry* extinction test and it is clear that both male and female rats previously trained in the *Geometry Relevant* test condition discriminated the correct from incorrect base corner of the triangular arena. For rats trained in the *Landmark Relevant* test condition, discrimination was less marked, especially for female rats whose performance was at chance. It also clear from this figure that across both conditions the time spent in the correct geometric corner was higher for males when compared to females.

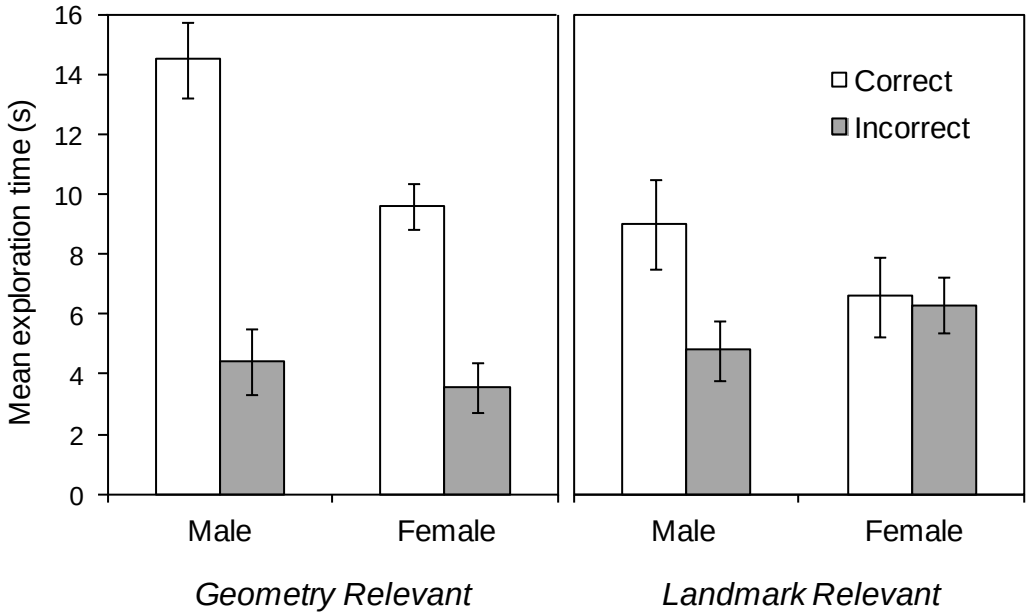

Figure 4.12. The mean (± *SEM*) time spent in the correct (white bars) and incorrect (grey bars) corner zones during the sixty second *Geometry* test for male and female rats in each training condition.

To support this characterisation of the data, a three-way ANOVA with test condition (*Geometry Relevant* and *Landmark Relevant*) and sex as between-subjects variables and zone (correct and incorrect) as a repeated measure revealed a significant main effect of sex, $F(1, 36) = 5.14$, $p = .029$, zone, $F(1, 36) = 38.5$, $p < .001$, and a significant sex x zone, $F(1, 36) = 5.74$, $p = .022$, and test condition x zone interaction, $F(1, 36) = 12.2$, $p = .001$. Simple main effects analyses to examine the sex x zone interaction revealed that, overall, males spent significantly more time in the correct corner of the triangular pool than females, $F(1, 36) = 8.65$, $p = .006$, but no more time in the incorrect corner, $F(1, 36) < 1$. Subsequent tests also revealed that rats tested in the *Geometry Relevant* condition spent more time in the correct corner than rats tested in the *Landmark Relevant* condition, $F(1, 36) = 11.6$, $p = .002$. The effects of sex within each test condition were analysed using planned comparisons. For the *Geometry Relevant* condition, males spent significantly more

time than females in the correct corner, $F(1, 36) = 7.75$, $p = .008$, but not in the incorrect corner, $F(1, 36) < 1$. However, for those rats previously trained in the *Landmark Relevant* condition, there were no sex differences in time spent either in the correct corner, $F(1, 36) = 1.89$, $p = .18$, or the incorrect corner, $F(1, 36) = 1.26$, $p = .27$.

Landmark Test

Figure 4.13 displays the mean time animals spent in zones surrounding the correct and incorrect landmark. This figure shows that both male and female rats trained in the *Geometry Relevant* test condition did not discriminate between landmarks. For rats trained in the *Landmark Relevant* test condition, it is clear that males spent more time searching under the correct landmark than females.

To confirm these observations statistically, an ANOVA of individual exploration times with sex and test condition (*Geometry Relevant* and *Landmark Relevant*) as between-subjects variables and zone (correct and incorrect) as the repeated measure revealed a significant main effect of test condition, $F(1, 36) = 102$, $p < .001$, and zone, $F(1, 36) = 58.1$, $p < .001$. There were also the following significant interactions: sex x zone, $F(1, 36) = 4.83$, $p = .035$, test condition x zone, $F(1, 36) = 41.0$, $p < .001$, and sex x test condition x zone, $F(1, 36) = 5.50$, $p = .025$. Subsequent analyses of simple main effects to investigate the main effects of sex within each test condition revealed that for rats trained in the *Geometry Relevant* test condition there was no difference between male and female rats in exploration of the correct and incorrect landmark, $Fs(1, 36) < 1$. However, for rats trained in the *Landmark Relevant* test condition, males spent more time searching under the correct landmark, $F(1, 36) = 7.72$, $p = .009$, and less time searching under the incorrect landmark, $F(1, 36) = 5.72$, $p = .022$, than females.

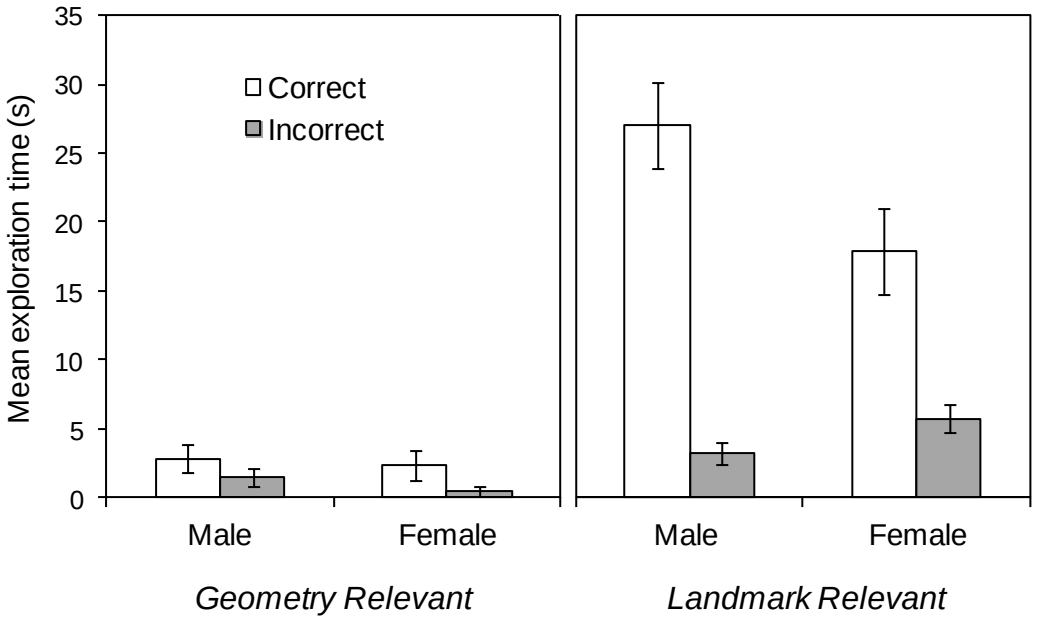

Figure 4.13. The mean (± *SEM*) time spent in the correct (white bars) and incorrect (grey bars) zones during the sixty second *Landmark* test for male and female rats in each training condition.

4.2.3.4 Thigmotaxis

Table 4.1 displays the mean time rats spent within 20 cm of the arena walls during each extinction test trial. An inspection of this table shows that during both extinction tests, but particularly during the landmark extinction test, rats of both sexes spent more time close to the arena walls after being trained in the *Geometry Relevant* test condition. An inspection centred on sex differences within each test condition and each extinction test shows that females spent more time close to the arena walls than males during the geometry extinction test having been trained in the *Geometry Relevant* test condition. Similarly, females appear to have spent more time close to the walls during the landmark extinction test having been trained in the *Landmark Relevant* test condition.

Table 4.1

Mean time in seconds (SEM) spent within 20 cm of the arena walls during each 60 second extinction test.

	Geometry Extinction		Landmark Extinction	
	Geometry Relevant	*Landmark Relevant*	*Geometry Relevant*	*Landmark Relevant*
Male	44.8 (2.7)	41.7 (2.0)	46.2 (2.8)	14.5 (3.0)
Female	52.8 (1.1)	44.3 (1.3)	49.7 (2.9)	21.9 (2.8)

To support this description of the data, a three-way ANOVA of individual times spent within 20 cm of the arena walls during each extinction test with sex and test condition as between-subjects variables and extinction test (geometry and landmark) as a repeated measure revealed that, overall, females spent more time close to the arena walls than males, $F(1, 36) = 6.51$, $p = .015$, and rats trained in the *Geometry Relevant* test condition directed their search close to the arena walls more than rats trained within the *Landmark Relevant* test condition, $F(1, 36) = 71.3$, $p < .001$. The ANOVA also revealed a significant sex x test condition x extinction test interaction, $F(1, 36) = 3.84$, $p = .050$. Tests of simple main effects to investigate this interaction revealed that female rats spent significantly more time close to the arena walls than male rats during the Geometry extinction test after being trained in the *Geometry Relevant* test condition, $F(1, 36) = 9.02$, $p = .005$, and during the Landmark extinction test after being trained in *Landmark Relevant* condition the same sex effect was close to significance, $F(1, 36) = 3.37$, $p = .075$.

4.2.4 Discussion

The results of Experiment 8 indicated that although male and female rats were able to learn about geometric and landmark cues to a similar level they may differ in the extent to which they rely on particular types of cues during training. The purpose of the present study was to identify differential cue use by male and female rats when tested in a series of training trials. Rats were required to find a hidden goal in a location defined by two sources of information: the geometric properties of one corner of the triangular pool and a beacon suspended above the platform displaying unique visual properties. For the Test stage, rats of both sexes were trained either in the *Geometry Relevant* condition or the *Landmark Relevant* condition. For rats trained in the *Geometry Relevant* condition, in which landmark information was rendered unreliable but geometric information remained predictive, no sex differences emerged during acquisition of the task. For rats trained in the *Landmark Relevant* condition, in which geometric information was rendered unreliable but landmark information remained predictive, male rats chose to visit the reliable landmark cue over the unreliable geometric cue more than female rats.

Following training in the Test stage, all rats received a geometry and landmark extinction test to assess the amount learned about each cue type. The results revealed that for both sexes, performance differed as a function of previous training condition during the Test stage. As expected, rats trained in the *Geometry Relevant* condition performed better in the geometry extinction test than rats trained in the *Landmark Relevant* condition. Conversely, rats trained in the *Landmark Relevant* condition outperformed rats trained in the *Geometry Relevant* condition during the landmark extinction test. A more critical finding, however, was that when rats were tested in an extinction trial with the cue type that during training remained

a reliable predictor of the platform's position, male rats outperformed females. Thus, when cues remained predictive, the ability of male rats was superior to female rats in the use of both geometric and landmark cues.

The findings from the Test stage do not support previous evidence that males are more likely to use informative geometric cues to solve a spatial task and females are more likely to use landmarks (Sandstrom et al., 1998; Jones & Healy, 2006; Rodríguez et al., 2010). In the Sandstrom et al. (1998) study it was found that performance of female participants was more disrupted than the performance of males when landmark information was rendered unreliable and the geometric information remained predictive. However, the present findings show that both male and female rats quickly developed an effective strategy to locate a hidden goal based solely on geometric cues when landmarks moved around the environment in an unreliable fashion. Also opposing previous findings is the results of the *Landmark Relevant* test condition revealing that female rats were less able to make use of landmark information when geometric information was rendered unreliable.

However, the poorer performance of female rats trained in the *Landmark Relevant* condition during the Test stage was only reflected in their first choice accuracy, and it is noteworthy to point out that this performance measure could have been affected by the rats' thigmotactic tendencies. The results of the geometry extinction test revealed that for those rats trained to locate the platform in one corner of the triangular pool both during training and the Test stage, females spent more time close to the arena walls than male rats. Whether this pronounced thigmotaxis in female rats was related to heightened stress or anxiety (Treit & Fundytus, 1988; Beiko, Lander, Hampson, Boon, & Cain, 2004) or was symptomatic of females having formed a habit-based (S-R) response during training that involved swimming

close to the walls of the triangular arena is open to question. Certainly, the results of the *percentage of same direction turns* measure, which assessed whether rats were making the same responses after being released into the pool, revealed, both in this experiment and Experiment 8, that during training, female rats were more predisposed to employ habit-based responses than male rats.

Based on this evidence, then, it is possible that female rats in the current study, prior to locating the platform during training, acquired a habit of swimming close to the walls of the triangular pool. If these heightened thigmotactic tendencies continued during the Test stage, female rats would have been more likely to swim through the correct corner before the correct landmark as the random movement of landmarks meant that on the vast majority of trials the landmark cues were positioned away from the walls of the enclosure. Therefore, the findings reported that female rats chose the correct geometric cue over the correct landmark cue in the *Geometry Relevant* condition, but never chose the correct landmark cue over the correct geometric cue in the *Landmark Relevant* condition could have been an artefact of thigmotaxis.

Despite this argument, it is important to mention that after all the training trials were complete (training and Test stage), the results of the extinction tests revealed no sex differences in thigmotaxis between males and females trained in the *Landmark Relevant* condition, which suggests that the conditions under which these animals were trained, i.e. follow the landmark and not the corner, eventually attenuated any thigmotactic tendencies in females. Yet, the rewarded landmark cue in the *Landmark Relevant* training condition never exerted strong enough control over behaviour so that female rats chose the correct landmark over the correct corner, which contrasts with the performance of male rats and previous evidence in

female rats (Rodriguez et al., 2010). It is also interesting that during the geometry extinction test, despite females trained in the *Geometry Relevant* condition exhibiting greater thigmotactic tendencies than males trained in the same condition, these animals were still proficient at discriminating the correct from incorrect corner. Therefore, it was not the case that female rats were simply circling the edges of the pool in a random fashion but rather they directed their search towards the correct location whilst remaining closer to the walls when compared to males. That said, the results of the current experiment and Experiment 8 showing that female rats are generally more thigmotaxic than male rats may pose a problem for any attempt to examine cue competition effects as exposure to the target cues could be sampled differently by males and females.

As mentioned, the findings from the final extinction tests revealed that, following changes to the training environment, both male and female rats could discriminate between corners during the geometry extinction test and between landmarks during the landmark extinction test if these particular cue types had continued to be a reliable source of information during training. It is a somewhat surprising result that female rats trained in the *Landmark Relevant* condition were able to discriminate the correct from incorrect landmark during the extinction test despite their first choice performance remaining at chance level across all training sessions of the Test stage. Thus, even though the predictive landmark cue did not gain sufficient control over behaviour to markedly improve their first choice accuracy during the Test stage, performance in the landmark test belied this inability. Perhaps, as discussed, during training in the Test stage after being released from certain release points into the pool female rats developed a habit of swimming through a base corner of the arena before locating the landmark. Although this is not

perhaps the most efficient strategy to locate the platform and defies certain principles of behaviour (Thorndike, 1911), the lack of sex differences in escape latencies indicates that it is of no great behavioural cost to employ a search strategy that incorporates a habit-based behaviour. Thus, in this instance, the first choice measure during training may not be as sensitive an indicator of learning as an analysis of search profiles during extinction tests.

The extinction tests also revealed that following changes to the training environment, in which one cue type remained a reliable predictor of the location of a hidden goal and the other cue type did not, male rats were more proficient at using the reliable cue type on its own than female rats. However, the results of Experiment 8 revealed that when male and female rats were trained in an unchanging environment with predictive geometric and landmark cues, the results of subsequent extinction tests revealed that learning of each cue type was at a very similar level. Taken together, then, these results indicate that male rats adapt better to changes in the reliability of cues within their training environment, irrespective of whether geometric cues continue to be predictive and landmark cues rendered unreliable or vice versa.

To conclude, the present results do not bolster the proposal that the potentiation of geometry learning by landmarks observed in Experiment 8 was mediated by different processes for males and females. One interpretation of the findings from the training data in Experiment 8 was that female rats in Group *Geometry + Landmark* were more accurate in their search behaviour than rats in Group Geometry who were not provided with informative landmarks. However, for male rats search behaviour was just as accurate irrespective of whether informative landmarks were present. Based on this evidence, it was predicted in the current

experiment that by rendering landmark information unreliable, female rats would struggle more than males to locate the platform by reference to geometric cues, whereas when landmark information continued to be reliable and geometric information was rendered unreliable, the performance of male rats would be more disrupted than the performance of females. The current findings showed that performance during training in the Test stage was matched for males and females in the *Geometry Relevant* condition and females were less accurate than males in their search accuracy in the *Landmark Relevant* condition. However, caution must be exercised when interpreting these findings as the development of habit-based behaviour may have impacted on the first choice performance measure.

All the experiments described so far have focused attention on how rats process shape-based information within their environment. The presence of sex differences and the identification of neural structures involved in such processing have formed part of this investigation. However, as discussed in Chapter 1, one aim of this thesis is to attempt to dissociate different navigational strategies, and associated neural structures, used during spatial learning tasks. However, for reasons documented in the following chapter, it is sometimes difficult to dissociate certain navigational strategies, such as place and response learning, when only geometric information is provided within a rat's environment. Therefore, Experiments 10 and 11 move away from the learning of geometry and instead focus on the strategies rats use when provided with informative wall colour cues and landmarks, respectively.

5

Dissociating Navigational Strategies

5.1 Experiment 10: Allocentric vs. Egocentric Learning

5.1.1 Introduction

It was discussed in Chapters 1 and 3 that two brain systems, the hippocampus and striatum, play important roles in spatial learning and memory. Furthermore, experimental evidence indicates that these two neural systems mediate different forms of learning and memory during navigation. The hippocampal system is thought to be pivotal in tasks requiring the flexible use of cues to construct an allocentric, cognitive map-like representation (see O'Keefe & Nadel, 1978). The striatum, on the other hand, has been implicated in tasks in which navigation requires the formation of an egocentric response rule (Packard & McGaugh, 1996). Some evidence has also indicated that allocentric and egocentric response learning can occur in parallel (e.g. Chang & Gold 2003; White & McDonald 2002). To demonstrate this, a task must be devised that can be solved using either mechanism. However, studies investigating the conditions under which one form of learning is expressed behaviourally over the other has, to a certain extent, been inconclusive (Tolman et al. 1946, 1947; for a review see Restle, 1955).

One factor proposed to influence the relative expression of an allocentric or response solution is the amount of training administered to the animal, with rats typically exhibiting a hippocampal allocentric solution early in training and a striatal response solution later (Packard & McGaugh, 1996). A second factor is the nature of

the testing environment (Restle, 1957; Packard & White, 1987) with animals tested in open mazes and granted access to varied extra-maze cues more likely to exhibit an allocentric solution (e.g. Tolman et al., 1946, 1947; Blodgett & McCutchan, 1947), while animals placed in closed mazes more likely to exhibit a response solution (Thompson & Tompson, 1949; Blodgett & McCutchan, 1948). Traditionally, however, experiments in closed mazes investigating egocentric response strategies have used T-mazes which force animals to make a body turn at a particular choice point. Whether animals can flexibly employ a similar egocentric response to particular cues in a distinctively shaped arena, which provides more open space, is still open to debate. Pearce et al. (2004) suggested that rats may be capable of forming an egocentric response rule with respect to cues provided by wall length in an arena. Based on this assumption, Experiment 7 in the current thesis attempted to identify if rats were using either an allocentric or egocentric strategy.

Recall Experiment 7 in which the performance of *HPC* and *DLS* lesioned rats was assessed to investigate their ability to recognise the novelty of an object based on the local geometric context in which it was placed. The objective of this experiment was to assess whether lesions to the *HPC* and *DLS* would disrupt the encoding of geometric information provided by the arena walls. However, the design of this experiment did not allow for a double dissociation between an allocentric and response solution because the only informative environmental cues available to solve the task were geometric in nature. Therefore for those animals with damage to the hippocampus it was not possible to perform a striatal-based, egocentric response rule, such as to find a long wall and turn right because, as mentioned earlier, hippocampal lesions impair distance discrimination (e.g. Jones et al., 2007). Therefore, if one aims to identify this double dissociation in a single task, it is

necessary to devise a task that does not require rats with hippocampal lesions to establish an egocentric response rule using only the geometric properties provided by the test environment.

One source of information that can be utilised by rats with lesions to the hippocampus in enclosed environments is wall colour. Pearce et al. (2004) and McGregor et al. (2004) demonstrated that rats with these lesions were capable of accurately locating an invisible escape platform placed in one corner of a rectangular or square swimming pool with alternating black and white walls. However, when rats with hippocampal lesions were required to perform the same task in a rectangular pool comprising of four white walls, so that only informative geometric cues were available, they were severely impaired. The authors concluded that rats with hippocampal lesions were capable of using wall colour cues and incapable of using shape-based cues in a navigation task. However, there still remained the question of how these wall colour cues were being exploited.

The results from a second stage of training in Pearce et al.'s (2004) experiment were informative in answering this question. After being trained in a rectangular pool with long black walls and short white walls, both rats with hippocampal lesions and sham-operated controls were transferred to a kite-shaped pool with long black walls and short white walls. Animals were then trained to locate the escape platform in the corner of the kite that was geometrically equivalent with the corner that contained the platform in the rectangular pool. Therefore two types of cue, geometry and wall colour, were predictive in signalling the location of the platform. At the beginning of this stage 2 training all rats chose the correct right-angled corner and the apex corner equally. One interpretation of this is that rats were using a response solution presumably by selecting a wall, based either on its length

or colour or both, and responding by swimming in a particular direction and distance from it. A more interesting finding, however, was that as stage 2 training progressed sham-operated animals became progressively proficient at discriminating the correct from apex corner of the kite while rats with hippocampal lesions continued to choose the apex as frequently as the correct right-angled corner. This improvement in discrimination by sham animals indicates that they were able to switch their behavioural response from a habit-based, egocentric rule to a strategy informed by another source of information such as the geometric or colour configuration of the walls that made up the correct right-angled corner. In contrast, it would appear rats with hippocampal lesions were impaired at switching to a more efficient solution. So, what possible reasons are there for these lesioned animals being less flexible in their use of wall colour cues than sham animals?

First, and least interesting, is based on evidence showing that rats with hippocampal lesions can become impaired at withholding responses to a previously rewarded cue (see Douglas, 1972; Isaacson, 1974, for a review). Therefore, rats with hippocampal damage in Pearce et al.'s (2004) experiment may have struggled to withhold a previously rewarded response to, for example, a black wall. Second, it could be the case that hippocampal lesions impair the learning of wall colour cues because the employment of an allocentric solution is disrupted. For example, hippocampal damage could prevent rats from identifying the spatial relationship between black and white walls, i.e. the correct corner consists of a black wall to the left of a white wall. Finally, and as previously mentioned, hippocampal damage can disrupt the formation of a geometric representation. Therefore, the impairment in discrimination between the apex and correct right-angled corner observed in rats with hippocampal lesions could be because these rats can only make use of

informative wall colour cues while sham animals can use both wall colour and geometric cues.

In summary, Pearce and colleagues identified that rats with hippocampal lesions could use the colours of enclosure walls effectively to aid navigation. However, this finding raises two critical questions: how are these lesioned animals able to represent wall colour cues; and is it possible to identify a double dissociation between the hippocampus (*HPC*) and dorsolateral striatum (*DLS*) in processing place-based allocentric and response-based egocentric information, respectively? With these questions in mind, the current experiment was designed using a square swimming pool so that there were no informative geometric cues that could confound any results when investigating differences in performance between rats with hippocampal lesions and sham-operated rats. The pool was constructed of alternating black and white walls and rats were trained to locate the escape platform 25 cm from one corner. Based on the evidence described above it is predicted that sham animals should be able to locate the escape platform by either the spatial relationship between the walls in the correct corner (e.g. black wall to the left of a white wall), or an egocentric response rule such as "select a wall of a certain colour and swim to the right-hand end of it". For rats with hippocampal lesions, given their inability to make use of the spatial (allocentric) relationships between different coloured walls, it is predicted that they will be forced to adopt an egocentric response rule. Conversely, for rats with lesions to the *DLS* that are unable to make use of the egocentric response rule it is predicted that they should use only the spatial (allocentric) relationship between different coloured walls.

To test these predictions, Group *Sham*, *HPC* and *DLS* were trained for 14 sessions in a square pool with alternating black and white walls. Following this

Stage 1 training, a test trial was conducted with the walls of the pool transformed so that walls of the same colour were adjacent to each other. Group *DLS* should continue to search for the platform in the corner with the same spatial colour arrangement as the corner in which they found the platform during training. Group *HPC* would be expected to search in the same corner as that described for Group *DLS,* and also in the all-black and all-white corners (or either one of these), if during training they learned to identify the position of the platform by a response rule based on the colours of the individual walls (see Figure 5.1). The interaction of egocentric and allocentric strategies will be assessed in the performance of the sham-operated control group. If their performance resembles that of Group *DLS* then it may be concluded that the allocentric solution took precedence over the egocentric solution. If however, they show the same pattern of behaviour as Group *HPC* this may be taken as evidence that the egocentric response rule was dominant. Alternatively, it is possible that in some sham-operated rats an egocentric solution is observed and in others an allocentric solution is evident.

The results of the test trial revealed that all groups directed their search towards the corner where the platform had been located during training, i.e. all groups were capable of learning the spatial (allocentric) relationship between different coloured walls. As there were no differences in behaviour between groups, further tests were conducted in an attempt to identify any differences in the use of colour cues. For Stage 2 training, the arrangement of coloured walls remained identical to the *Transform* test (same coloured walls adjacent to each other). Rats were given 6 sessions of this training with the platform positioned in the corner comprising of the same spatial colour arrangement as during Stage 1 training. This stage of training was implemented to investigate any between-group differences that

the preceding test trial failed to capture. The predictions for this stage followed the same principles described above for the *Transform* test. Stage 3 training followed (four sessions), before a final test trial. During this stage, the colours of the walls forming the square arena were altered so that each wall was halved vertically with one half black and one half white (see Figure 5.1). This final stage was to investigate the behavioural control by the spatial configuration of coloured walls only (e.g. where black was to the left of white) in the absence of a uniformly coloured wall (i.e. a wall of a single colour). If performance in Stage 2 was based on both the colour configurations (at corners) and the S-R habits based on uniform-coloured walls, then the aim of Stage 3 was to remove the influence of the uniformly coloured walls (i.e. single wall S-R), and test the effect of colour configurations. For this stage, it was predicted that Group *DLS*'s performance should fall to chance if in the previous stages they were locating the correct corner based on an egocentric response rule informed by uniform-coloured walls. For Group *Sham* and *HPC* it was predicted that animals would use the spatial configuration of coloured walls and search at the correct corner and correct boundaries according to the relative position of the black and white sections (e.g. where black was to the left of white).

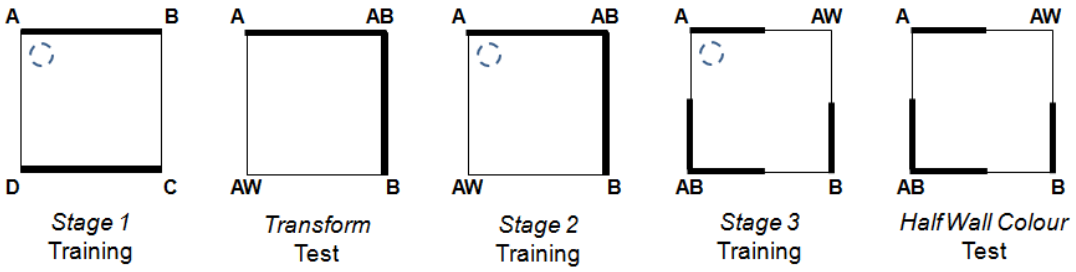

Figure 5.1. Schematic diagram showing the experimental design for Stages 1-3. All stages were conducted in a square pool constructed of black and white walls. The dashed line circle represents a submerged escape platform. In all stages, corner A = black wall to the right of a white wall, corner B = black wall to the left of a white wall, corner AW = all white, corner AB = all black.

5.1.2 Method

5.1.2.1 Subjects

The subjects were 32 male Lister hooded rats (*Rattus norvegicus*) supplied by Charles River (UK), which were approximately 6 months of age at the start of the experiment. All animals had previously participated in an unrelated object recognition task (Experiment 7) and it was ensured that this prior experience was counterbalanced. At the start of the experiment there were 12 animals with bilateral lesions of the hippocampus (*HPC*), 12 animals with bilateral lesions of the dorsolateral striatum (*DLS*) and 8 sham-operated animals. Rats were housed in identical conditions to those in Experiment 1.

5.1.2.2 Surgical Procedure

Refer to Experiment 6 for surgical procedure.

5.1.2.3 Apparatus

The apparatus was identical to Experiment 8 except for the following differences.

Throughout the current experiment, four polyurethane boards were used. For Stage 1 and 2 of training, two of these boards were black and two were white and for Stage 3 of training all four boards were coloured identically with one half of each board black and the other half white (split vertically). For all stages, the four boards, which were each 137 cm in length, were positioned centrally inside the circular pool to form a square pool (137 cm^2). Once all the boards were suspended vertically from aluminium rails in the correct position, Velcro was used to fasten the boards together to ensure that they abutted one another tightly and there were no gaps in any of the

corner joints.

Throughout training, the escape platform was placed in the appropriate corner of the pool with its centre 25 cm from the point at which the two walls of the corner met on a trajectory which split this corner in half.

5.1.2.4 Assignment of groups

For the three groups: *Sham*, *HPC* and *DLS*, the schedule for all stages of training and testing was identical.

5.1.2.5 General Procedure

All aspects of the general procedure were identical to Experiment 8 except that in this experiment there were four designated points of release into the pool, still at the midpoint of each wall, as opposed to three in the triangular pool. This meant that although the release points were still assigned randomly for each trial the constraint now was that four different release points were used within a session. The arena was rotated between trials as described in Experiment 8.

5.1.2.6 General Performance Measures

The recording of escape latency and first choice measures by the experimenter were identical to Experiment 8. Based on the corner zone that animals first visited after being released into the pool, a *percentage of same direction turns* measure was calculated. Swim turns were categorised into four types: near left, near right, far left and far right. From these turn data it could be established if, after release, animals were repeatedly making the same type of turns. Unlike Experiment 8 it was not necessary to analyse this data in three session blocks as each release point was used an equal number of times within a session. Additional measures were

also investigated using the raw first choice data, which included the number of *near vs. far turns* animals made after immediate release into the pool. A near turn was defined as a first entry into a corner zone located at either end of the release wall and a far turn defined as a first entry into a corner zone located either end of the wall opposite the release wall. It was also investigated whether or not the colour of release wall affected the aforementioned behavioural responses.

Specific performance measures applicable to each training stage and test are described in the relevant sections below.

5.1.2.7 Training

Pre-training

Rats received two sessions of pre-training in the circular pool in which a stick, 1 cm in diameter, was attached to the submerged escape platform. The stick was painted with black and white horizontal stripes (band width 1 cm) and stood vertically to a height of 15 cm above the surface of the water. The purpose of this pre-training was to ensure the animals were able to climb onto the escape platform and avoided adopting a strategy where they circled around the edge of the pool. The position of the platform was moved randomly across trials with the constraint that it was a minimum of 25 cm from the edge of the pool and each of the four quadrants of the pool housed the platform at least once within a session. The four release points (north, south, east and west) were also randomised with the constraint that rats were released once from each point within a session.

Stage 1 Training

Rats received 14 sessions of Stage 1 training, which involved locating the escape platform in one corner of a square pool with alternating black and white walls. The platform position remained constant with respect to the colours of the walls for each animal across all sessions but was counterbalanced between animals in each group so that half the animals were trained to locate the escape platform with the black wall to the left of a white wall (corner B & D) and half were trained with the white wall to the left of a black wall (corner A & C) (see Figure 5.1).

Because session 14 of Stage 1 training comprised of only three trials due to the final trial being an extinction test, analyses investigating the type of turns rats made following release into the pool used only the first 13 sessions. Ethovision (version 3.1) software was used to track four trials of session 13, i.e. the last full session of Stage 1 training. Rectangular wall zones (25 cm wide) were placed parallel to and along each wall and ended 30 cm before they reached an adjoining corner wall. Therefore, the time each rat spent within 25 cm of each perimeter wall, but not in areas close to the corners of the pool could be recorded. These four wall zones were defined by their colour and release wall symbol to give: *white wall BC* zone, *white wall AD* zone, *black wall AB* zone and *black wall CD* zone. In a separate analysis of the same swim paths, four circular corner zones (each 58 cm in diameter) were constructed, which were positioned individually in each corner so that the centre of each zone corresponded to the centre of the escape platform had it been paired with that corner. These zones were defined as corner zones *A*, *B*, *C* and *D* (see Figure 5.1).

Stage 2 Training

Rats received 6 sessions of Stage 2 training in which the coloured walls of the square pool had been transformed so that the two black walls adjoined to form one corner, and the two white walls adjoined to form the opposite corner. For each rat in this stage of training the escape platform remained in the same corner with respect to the wall colour configuration as Stage 1 of training (see Figure 5.1).

Stage 3 Training

In Stage 3 of training, which consisted of four sessions, the colours of the walls forming the square arena were changed so that each wall was halved vertically with one half black and one half white. These walls of the square pool were arranged in such a way that one corner comprised of two boards where the white section of each board adjoined (corner All White, or AW), and the opposite corner comprised of two boards where the black section of each board adjoined (corner All Black, or AB). The remaining two corners of the square, corners A and B, were each presented with the exact coloured wall configuration displayed during the previous two stages of training. For each rat the escape platform remained in the same corner, with respect to wall colour configuration, as it was positioned in during Stage 1 and 2 of training (see Figure 5.1).

5.1.2.8 Extinction Tests

Transform Test

The first extinction test (*Transform test)* took place on the fourth trial of session 14 following three Stage 1 training trials. The black and white coloured walls of the square pool were transformed so that the two black walls adjoined to form one

corner and the two white walls adjoined to form the opposite corner (see Figure 5.1). To measure behaviour, circular corner zones (40 cm in diameter) were constructed using Ethovision and positioned individually in each corner so that the centre of each zone corresponded to the centre of the escape platform had it been paired with that corner. These zones were defined as corner zones *A, B, AB* (all black) and *AW* (all white) (see figure 5.1). The time rats spent in each zone was recorded.

Half Wall Colour Test

The second test (*Half Wall Colour test*), which took place the day after the final session of Stage 3 training with no training trials preceding it, was carried out in the square pool constructed of walls identical in colour configuration to those experienced by rats during Stage 3 of training. To measure performance, eight identical square zones (each 35 cm^2) were formed and positioned using Ethovision so there was a single zone placed in each corner of the square pool (two sides of each corner zone lining up with the two corner walls of the pool) and a single zone placed at the midpoint of each wall of the pool (with one side of each zone placed in line with and parallel to the appropriate pool wall and centred so that its midpoint corresponded to where the boundary between the black and white coloured sections of the pool wall met). As the vertical black and white sections of the pool walls formed different coloured boundaries, there were two mid-wall coloured boundaries forming an identical colour configuration to corner A (with a white wall to the left of a black wall) and two mid-wall coloured boundaries forming an identical colour configuration to corner B (with a white wall to the right of a black wall). Accordingly, the 8 zones were categorised as: *correct corner zone, incorrect corner zone, correct mid-wall barrier zone 1, correct mid-wall barrier zone 2, incorrect mid-wall barrier zone 1, incorrect mid-wall barrier zone 2, all black corner zone,*

and *all white corner zone*. Again, the time rats spent in each of these zones was recorded.

5.1.3 Results

A Type I error of $p < .05$ was adopted throughout. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was applied. When pairwise comparisons were required to further investigate the significant main effect of *lesion group*, post-hoc tests using the Bonferroni correction were conducted.

5.1.3.1 Histology

The histological analysis for the animals used in this experiment is reported in Experiment 6 (Replication 1), which was conducted after the current experiment. Thus, there were 8, 11 and 9 rats included in the behavioural analyses for Group *Sham*, *HPC* and *DLS* respectively.

5.1.3.2 Pre-training

A two-way ANOVA, which was conducted on the session mean latencies of individual rats for the two pre-training sessions with lesion group as the between-subject variable and session as the repeated measure revealed no significant effect of lesion group, $F(2, 25) < 1$, (*Sham*: $M = 17.7$, $SEM = 2.26$; *HPC*: $M = 20.6$, $SEM = 1.93$; *DLS*: $M = 21.2$, $SEM = 2.13$) and no significant session x group interaction, $F(2, 25) < 1$. Due to this lack of difference in the ease at which rats from different lesion groups were able to vacate the perimeter walls of the pool and climb onto the escape platform, the main stage of training commenced after these two pre-training sessions.

5.1.3.3 Stage 1 Training

Figure 5.2 shows the mean escape latency (upper panel) and the mean percentage of correct first choices (lower panel) for each lesion group across 14 sessions of Stage 1 training. A correct first choice was defined as a first entry to the corner containing the escape platform or the diametrically opposite corner comprising of an identical coloured wall configuration (chance = 50%). Both panels of the figure demonstrate that acquisition of the task, both in terms of escape latency and choice accuracy was slower for Group *HPC* compared to Group *Sham* and *DLS*. Towards the end of Stage 1 training (last 3 sessions), however, it appears that choice accuracy was equal across all three groups, whereas the escape latencies remained slower for Group *HPC* throughout.

The descriptions were supported by a two-way ANOVA conducted separately on the individual mean escape latencies and percentages of correct first choices for each of the 14 sessions with lesion group as the between-subjects variable and session as the repeated measure. The ANOVA of escape latencies revealed a significant main effect of lesion group, $F(2, 25) = 8.59$, $p < .001$, and session, $F(4.73, 118) = 29.6$, $p < .001$, however, the lesion group x session interaction was non-significant, $F(9.45, 118) = 1.07$, $p = .39$. Pairwise comparisons revealed that the significant effect of lesion group was due to the fact that Group *HPC* ($M = 16.42$, $SEM = 0.68$) took significantly longer, across the whole of Stage 1 training, to locate the escape platform when compared to Group *DLS* ($M = 13.46$, $SEM = 0.75$), $p = .022$, and Group *Sham* ($M = 12.30$, $SEM = 0.80$), $p < .01$. A similar ANOVA of percentages of correct first choices revealed a significant main effect of lesion group, $F(2, 25) = 3.38$, $p = .05$, and session, $F(13, 325) = 6.67$, $p < .001$, but the session x lesion group interaction was not significant, $F(26, 325) = 1.15$, $p = .284$. Pairwise

comparisons revealed that Group *HPC* (*M* = 91%) visited the correct corner on almost significantly fewer occasions than Group *Sham* (*M* = 97%), p = .079, but not Group *DLS* (*M* = 96%), *p* = .169, there was no significant difference between Group *Sham* and *DLS*, *p* = 1.

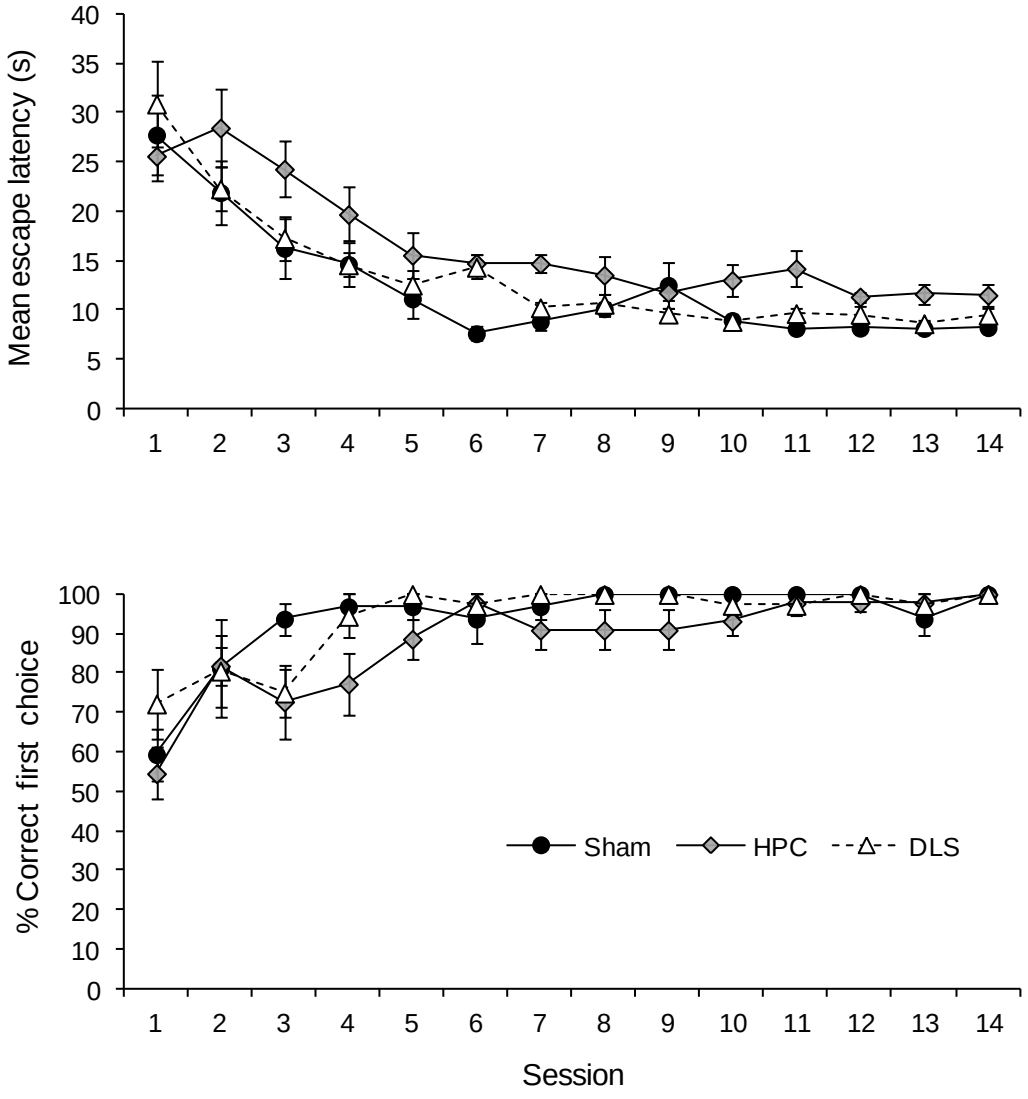

Figure 5.2. The mean (±*SEM*) escape latencies (upper panel) and percentages of correct first choices (lower panel) for each group across 14 sessions of Stage 1 training.

Although there was no significant session x lesion group interaction, which suggests that first choice performance was less accurate for Group *HPC* throughout the whole

of Stage 1 training, an inspection of Figure 5.2 indicates that first choice accuracy was more evenly matched towards the end of training. More specifically, it appears the acquisition rate for Group *HPC* was slower but that an asymptotic level was reached at the end of Stage 1 training. Thus, a two-way ANOVA conducted on the mean percentages of correct first choices for only the last 5 sessions of Stage 1 training revealed no significant between- or within- subjects effects or interaction, Fs ≤ 1.91, ps $\geq .15$.

The effect of the colour of release wall on escape latencies and correct first choices was also examined but a three-way ANOVA with lesion group as the between-subjects variable and wall colour (black or white) and session as repeated measures revealed no significant main effects or interactions involving wall colour on latencies, Fs < 1, or correct first choices, Fs < 1.

The raw first choice data were also used to establish if rats were employing the same turns after being released into the pool. However, ANOVAs conducted on the mean percentage of same direction turns from any coloured wall (Fs ≤ 1.69, ps $\geq .20$) or when wall colour was included as a factor (Fs ≤ 2.91, ps $\geq .071$) revealed no significant main effects or interactions. It was also calculated for each session whether at the start of each trial rats made a near or far turn, i.e. after being released into the pool did the rat first enter a corner situated at either end of the release wall (near turn) or a corner situated at either end of the wall opposite the release wall (far turn). Figure 5.3 displays the mean discrimination ratio for each group averaged across the first 13 sessions of Stage 1 training. Chance level (dashed line) was 0.5, any value below this level indicates that rats made a near turn on proportionately more occasions than a far turn, and any value above this level indicates that rats made a far turn on proportionately more occasions than a near turn. Examination of

this figure reveals that Group *Sham* and Group *HPC* were performing at chance level with no preference to turn to a near or far corner zone. However, the mean discrimination ratio score for Group *DLS* is above chance level, which indicates that animals in this group preferentially turned into a far corner zone on more occasions than a near corner zone.

The descriptions were supported by one-sample t-tests, conducted on the 13 session mean discrimination ratios for each group with a test value of 0.5, which revealed that Group *HPC*, $t(10) = -.08$, $p = .94$, and Group *Sham*, $t(7) = 1.07$, $p = .32$, were making near and far turns at a level expected by chance, whereas Group *DLS*, $t(8) = 2.27$, $p = .05$, made proportionately more far turns than expected by chance.

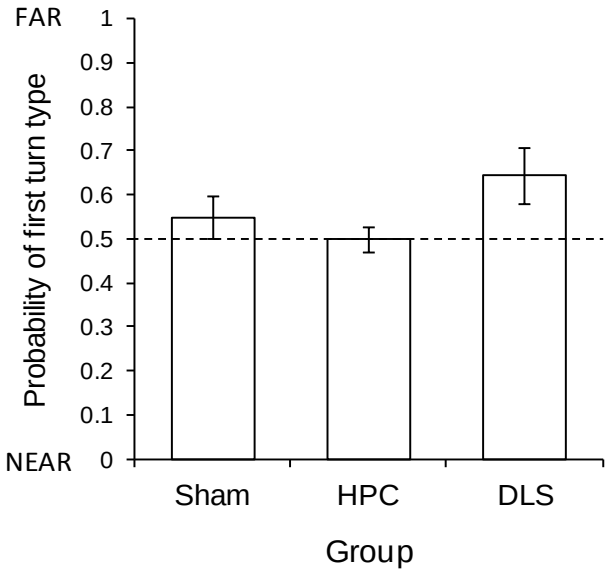

Figure 5.3. Mean discrimination ratio of near vs. far first turns for each group averaged across 13 sessions of Stage 1 training.

To accompany the manual scoring of escape latency and first choice by the experimenter, the last full session of Stage 1 training (session 13) was tracked using Ethovision software in an attempt to establish an explanation for why Group *HPC*

were consistently slower to locate the escape platform than the other two groups. Thus, swim speed and swim path profiles were analysed. As described in the performance measures section, wall zones and corner zones were set up to look at the time animals spent close to the perimeter walls of the pool and which corner zone they spent their time in. For all behavioural measures subsequently reported, the data were averaged across four training trials of session 13 for each rat.

To ascertain the swim speed of each rat during a trial, Ethovision software was used to calculate the session mean velocity for each rat. A one-way ANOVA was conducted with lesion group as the between-subjects variable. Levene's test indicated unequal variances, $F(2, 25) = 5.98$, p .008, so Welch's ANOVA was used and revealed no significant main effect of lesion group, $F(2, 15.5) < 1$, (Group *Sham*: $M = 63.9$, $SEM = 2.04$; *HPC*: $M = 61.7$, $SEM = 2.26$; *DLS*: $M = 66.8$, $SEM = 4.30$). The mean percentages of exploration times spent close to the perimeter walls of the arena were calculated along with the percentages of times that animals spent swimming close to a black or white wall. A two-way ANOVA conducted on these data with lesion group as the between-subjects variable and wall colour zone as a repeated measure revealed no significant between- or within-subjects effects or interactions, Fs ≤ 1.52, ps $\geq .24$. This analysis has eliminated the possibility that Group *HPC* were slower to locate the platform due to being slower swimmers or more thigmotaxic. Therefore, attention will now focus on exploration in each corner zone of the pool.

The time rats spent in circular corner zones was recorded and from these data it was possible to calculate the time it took for each rat to visit a correct corner zone. A two-way ANOVA, conducted on the mean latencies of first visits with lesion group as the between-subjects variable and corner zone (correct and incorrect corner

zones) as the repeated measure revealed a significant main effect of corner zone, $F(1, 25) = 525$, $p < .001$, with animals taking significantly less time to visit the correct corners than the incorrect corners. All remaining between- and within-subjects effects and interactions were non-significant, Fs ≤ 1.05. Thus, rats in Group *HPC* visited a correct corner as quickly as the remaining groups but, once there, they took longer to locate the platform than rats in Group *Sham* and *DLS*. This could be due to several reasons, which are considered in the following analyses.

First, it has been shown that rats with *HPC* lesions are impaired at withholding a response to a rewarded cue. Thus, Group *HPC* may have spent longer searching for the escape platform in the correct opposite corner despite the platform being absent. To assess this possibility a two-way ANOVA of individual exploration times with lesion group as the between-subjects variable and corner zone as the repeated measure was conducted and revealed a significant corner zone x lesion group interaction, $F(4.22, 52.8) = 3.27$, $p = .016$. Pairwise comparisons to investigate this interaction revealed that Group *HPC* spent more time in the correct corner, $F(2, 25) = 4.08$, $p = .029$, and the correct opposite corner, $F(2, 25) = 7.46$, $p = .003$, than Group *Sham* and *DLS*. However, there were no group differences in time spent in the incorrect corners, Fs < 1. Therefore, the longer latencies observed during Stage 1 training for Group *HPC* cannot be attributed to these rats spending longer only in the correct opposite corner and not the correct corner.

Second, because Group *HPC* spent more time, overall, in the pool searching for the platform (i.e. their escape latencies were higher) it is important to establish if these longer search patterns were confined to the correct corners of the pool or if Group *HPC* also spent longer searching away from the corners. Thus, a one-way ANOVA was conducted on the session mean times spent in the pool excluding the

corner regions. This analysis revealed no significant effect of lesion group, $F(2, 25) = 2.14$, $p = .14$. Therefore, Group *HPC* spent more time confined to the correct corners of the pool attempting to locate the escape platform when compared to Group *Sham* and *DLS*.

5.1.3.4 Transform Test

As described in the performance measures section, Ethovision was used to create four corner zones: *correct* zone, *incorrect* zone, *all black* zone and *all white* zone. Examination of Figure 5.4 reveals that each group spent a similar amount of time in each corner of the pool.

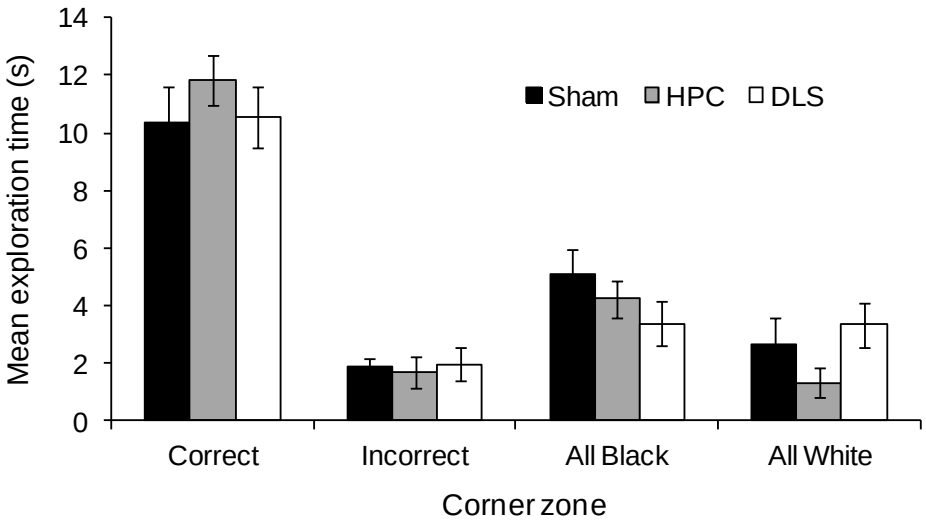

Figure 5.4. The mean (± *SEM*) time spent in each corner zone by each group during the *Transform* test (same coloured walls adjacent).

To test this observation statistically, a two-way ANOVA of individual times with lesion group as the between-subjects variable and corner zone as the repeated measure, revealed a significant main effect of corner zone, $F(2.07, 51.7) = 65.4$, $p < .001$, but no significant effect of lesion group, $F(2, 25) < 1$, or corner zone x lesion

group interaction, $F(4.14, 51.7) = 1.20$, $p = .32$. For the main effect of zone, pairwise comparisons revealed that rats spent more time in the correct zone compared to the remaining zones and more time in the all black zone when compared to the incorrect and all white zones. The time rats spent within 20 cm of the walls of the arena was also recorded. This was to assess if any group exhibited a higher level of thigmotaxis during the *Transform* test trial. A one-way ANOVA conducted on these data with lesion group as the between-subjects factor revealed no significant main effect of lesion group, $F(2, 25) < 1$, (Group *Sham:* $M = 33.7$, $SEM = 2.24$, *HPC*: $M = 31.2$, $SEM = 1.93$, *DLS*: $M = 30.7$, $SEM = 2.07$).

5.1.3.5 Stage 2 Training

In the event that any effects of the transformation from opposite to adjacent same coloured walls were not detected in the *Transform* test, training was continued in Stage 2 with the walls in the same arrangement as during the *Transform* test to investigate whether any group differences would emerge. Figure 5.5 displays the mean escape latencies (upper panel) and the mean percentage of correct first choices (lower panel) for each lesion group across 6 sessions of Stage 2 training. A correct first choice was defined as a first entry to the corner containing the escape platform, so chance level was 25%. Examination of this figure suggests that rats in Group *HPC* were consistently slower to locate the escape platform when compared to Group *Sham* and *DLS*, but no group differences were apparent in terms of first choice accuracy.

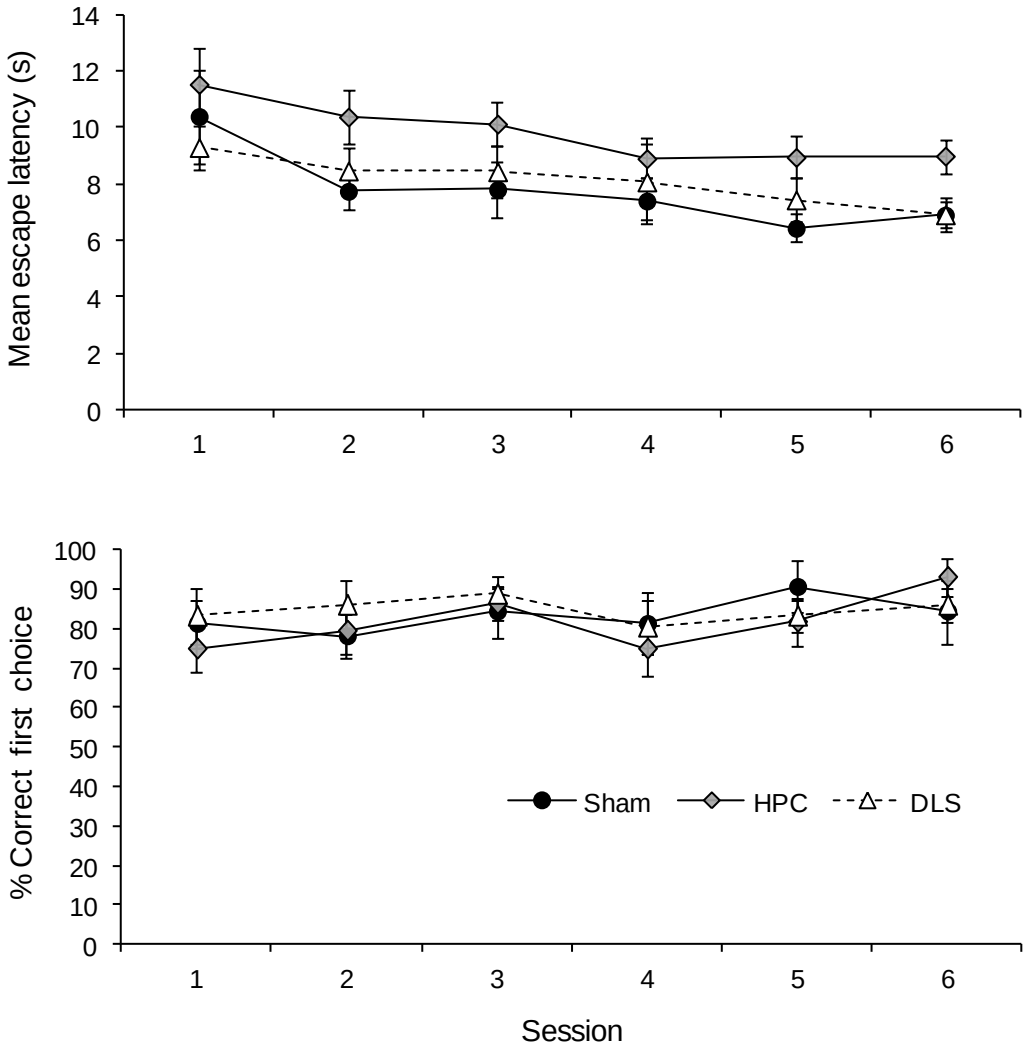

Figure 5.5. The mean (± *SEM*) escape latencies (upper panel) and percentages of correct first choices (lower panel) for each group across 6 sessions of Stage 2 training.

To test these observations statistically, a two-way ANOVA with lesion group as the between-subjects variable and session as the repeated measure was conducted on the session mean latencies for each rat and revealed a significant main effect of lesion group, $F(2, 25) = 5.98$, $p = .008$, with Group *HPC* slower than Group *Sham* and *DLS*, and a significant main effect of session, $F(5, 125) = 4.61$, $p = .001$, but the session x lesion group interaction was non-significant, $F < 1$. A similar ANOVA was conducted on the mean percentage of correct first choices for individual rats on each session. This analysis, however, revealed no significant between- or within subjects

effects or interactions, *F*s ≤ 1.48, ps ≥ .20. From the first choice data, the percentage of same direction turns and the frequency of the type of turn (near vs. far turns calculated as a discrimination ratio: 1 = all far turns) employed by each rat was calculated. A two-way ANOVA conducted separately for each measure on the individual session means with lesion group as the between-subjects variable and session as the repeated measure revealed no significant effects or interactions, *F*s ≤ 1.49, *p*s ≥ .20 (Group *Sham*: *M* = 38.0, *SEM* = 2.65, *HPC*: *M* = 39.4, *SEM* = 2.26, *DLS*: *M* = 38.4, *SEM* = 2.50), and *F*s ≤ 1.24, *p*s ≥ .30 (Group *Sham*: *M* = .516, *SEM* = .026, *HPC*: *M* = .523, *SEM* = .022, *DLS*: *M* = .491, *SEM* = .024), respectively.

5.1.3.6 Stage 3 Training

Stage 3 training was to determine whether a disruption to the uniformity of wall colouration affected each group differentially. Figure 5.6 presents the mean escape latency (upper panel) and the mean percentage of correct first choices (lower panel) for each lesion group across 4 sessions of Stage 3 training. An inspection of this figure suggests that acquisition of the task both in terms of escape latency and choice accuracy was matched across lesion groups. There is a hint that Group *HPC* was slower to locate the escape platform on the final 3 sessions of training but this difference when compared to the other two groups is minimal.

To confirm these observations, a similar two-way ANOVA was conducted on the session mean latencies for each rat and revealed a significant main effect of session, $F(1.83, 45.8) = 11.38$, $p < .001$, but no other significant main effects or interactions emerged, *F*s ≤ 1.20, *p*s ≥ .33. A similar ANOVA, conducted on the mean percentage of correct first choices revealed no significant between- or within-subjects effects or interactions, *F*s < 1.

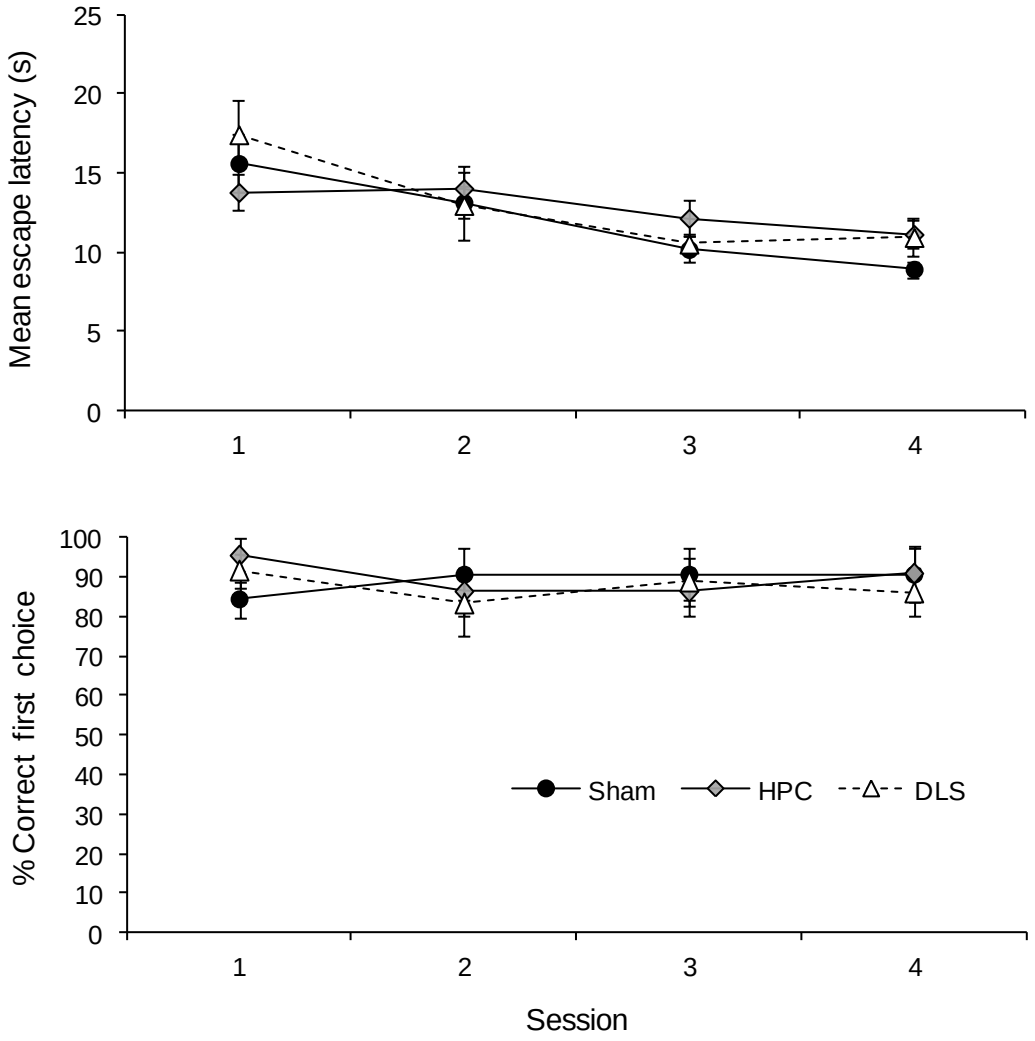

Figure 5.6. The mean (± *SEM*) escape latencies (upper panel) and percentage of correct first choices (lower panel) for each group across 4 sessions of Stage 3 training.

5.1.3.7 Half Wall Colour Test

Ethovision was used to create six zones categorised as: *correct corner* zone, *incorrect corner* zone, *correct mid-wall barrier* zone, *incorrect mid-wall barrier* zone, *all black corner* zone, and *all white corner* zone. For the mid-wall barrier zones an average was taken of the two correct and incorrect zones to give one value for each. Figure 5.7 shows that all groups spent more time in the correct corner and correct wall barrier zones when compared to the remaining zones. It is also apparent

from this figure that rats spent more time in the correct corner zone than the correct mid-wall barrier zone. The group differences for time spent in each zone appear minimal.

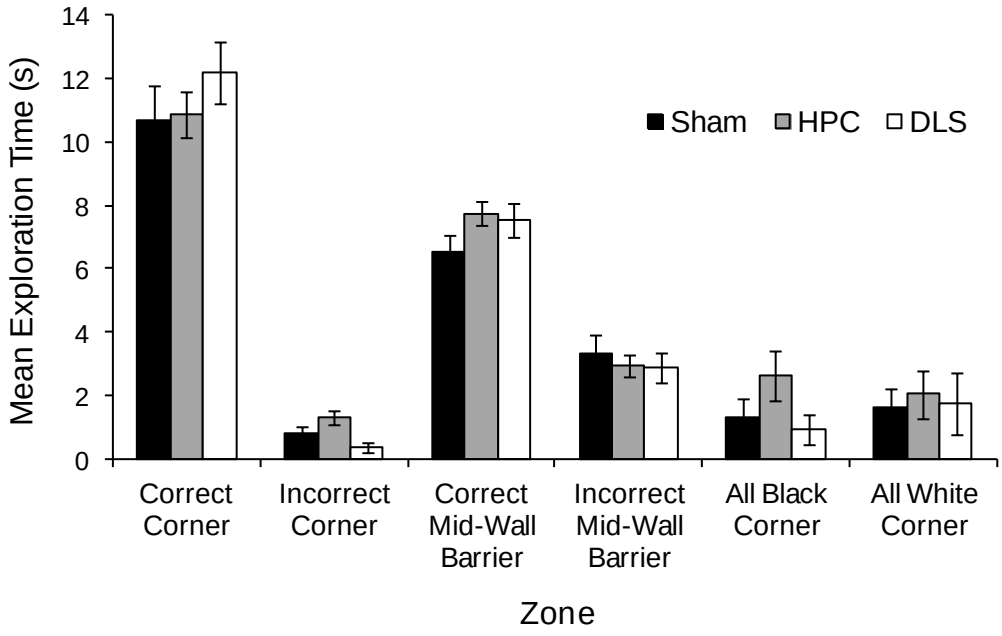

Figure 5.7. The mean (± *SEM*) time spent in each zone by each group during the *Half Wall Colour* test.

To test these observations statistically, a two-way ANOVA conducted on the exploration time for each rat with lesion group as the between-subjects variable and zone as the repeated measure revealed a significant main effect of zone, $F(3.23, 80.7) = 110$, $p < .001$, with animals spending significantly more time in the *correct corner* zone when compared to the remaining zones, and more time in the *correct mid wall barrier* zone than the *incorrect mid wall barrier* zone. The ANOVA also revealed no significant effect of lesion group, $F(2, 25) = 2.89$, $p = .07$, or lesion group x zone interaction, $F(6.46, 80.7) < 1$.

5.1.4 Discussion

Several studies have identified a double dissociation between the hippocampus and dorsal striatum in processing place-based allocentric and response-based egocentric information, respectively (Packard & McGaugh, 1996; Packard et al., 1989; Devan & White, 1999; McDonald & White, 1993). A demonstration of how localised lesions impair performance in one task, while leaving another task unaffected supports the notion of multiple memory systems in the brain (see White & McDonald, 2002). Whether a double dissociation of the nature described can be observed when rats are required to use colour cues in a distinctively shaped arena remains undetermined. In the current experiment rats were trained to locate a hidden goal in one corner of a square arena with alternating black and white walls. In a test trial, the walls of the pool were transformed so that walls of the same colour were adjacent to each other. If rats with hippocampal lesions were impaired at making use of the allocentric relationship between coloured walls, and therefore obliged to identify the position of the hidden goal by an egocentric response rule based on the colour of individual walls, then they would be expected to search in the same corner in which the platform was found during training, and also in the all-black and all-white corners. Rats with lesions to the dorsolateral striatum should have continued to search for the platform in the corner with same allocentric colour configuration as the corner in which they found the platform during training. Behaviour in sham animals could be assessed to establish which strategy took precedence in such a task. The results of the *Transform Test* revealed that all animals focused their search in the corner in which the platform was located during training. Performance did not differ between groups and showed that the allocentric solution took precedence over the egocentric response strategy. This result also demonstrated that damage to the

hippocampus did not impair the ability of rats to identify a corner by the spatial relationship between the black and white walls creating it, or to use an allocentric solution.

The results of Stage 1 oppose suggestions that the hippocampus is critical in the encoding of structural relations between items, should swimming to the corner with the correct arrangement of black and white walls involve learning their structural relations (Aggleton & Pearce, 2002; Eichenbaum, Otto & Cohen, 1994; Sanderson, Pearce, Kyd, & Aggleton, 2006; see Pearce et al., 2004 for discussion), and are also at odds with Pearce et al.'s (2004) findings. As described in the Introduction, in Pearce et al.'s (2004) Experiment 2 the pattern of behaviour suggested that sham-operated animals were capable of switching from an egocentric response strategy to an allocentric strategy, whereas rats with hippocampal lesions continued to exhibit a response solution. It appeared from this evidence that for rats with hippocampal lesions, navigation based on an egocentric rule was obligatory and that information provided by the configuration of wall colours could not to be utilised. Evidence from the current experiment, however, indicates that for Group *HPC* not only was this response rule non-obligatory but these rats were competent in using the configuration of wall colours. The findings at hand also oppose the behaviour observed by sham animals in Pearce et al.'s experiment. As already mentioned, during the transform test, sham animals in the present experiment exhibited behaviour tantamount to an allocentric solution, while in Pearce et al.'s first two transform test sessions sham animals appeared to be using a response solution.

One key difference between this and Pearce et al.'s (2004) experiment that could provide a potential reason for the disparity in results is that rats were tested in

a square and kite-shaped pool, respectively. Thus, it could be the case that frequent visits to the all-black, apex corner of the kite observed in Pearce et al.'s experiment were due to an unconditioned response rather than a reflection of an egocentric response rule. In fact, in a prior experiment (1A) Pearce and colleagues (2004) observed the same pattern of behaviour when rats were transferred from a white rectangle to a white kite-shaped arena. The explanation by the authors for this result was that rats were navigating with reference to a single long wall rather than the metric layout of each corner. However, it remains unproven whether rats, when placed into a kite-shaped arena, are using a single wall solution or are showing an unconditioned response by seeking out a confined, dark place. The findings from the current experiment lend support to the latter alternative.

Having established no group differences during the *Transform* test, rats were given six sessions of Stage 2 training. This training was conducted with the walls of the square pool arranged in an identical layout (same coloured walls adjacent) as during the *Transform* test. This stage was provided to see if any group differences in navigational strategy emerged as training in the transformed pool progressed. With the exception of Group *HPC* being slower to locate the escape platform, for reasons discussed below, there were no other group differences in acquisition of Stage 2 training. This result supports the findings of the *Transform* test and suggests any absence in group differences cannot be due to the original test trial lacking behavioural sensitivity.

For Stage 3 training, rats were trained for four sessions in a square pool modified so that the walls were halved vertically with one half black and the other white. This modification was implemented to assess the effect of removing the influence of uniformly coloured walls, which rats could use to form an egocentric

response, and test the ability of rats to use the boundaries formed when a white section of wall meets a black section. The results revealed that from the outset of training all groups were capable of discriminating the correct from incorrect corners of the pool. An extinction test (*Half Wall Colour Test)* conducted at the end of stage 3 training provided further confirmation that animals were capable of discriminating the correct from incorrect corners, evidenced by the fact that animals spent more time in this corner than in any other. The results of the extinction test also revealed that animals spent more time in the correct mid-wall barrier zones than the incorrect mid-wall barrier zones. Importantly, there were no group differences in behaviour throughout the training sessions or extinction test in Stage 3 of the experiment. If it were the case that certain animals were using an egocentric solution, which is unlikely based on the results from Stage 2, this strategy did not appear to be disrupted by removing the uniformity of wall colour. Rather, the results lend further support to the notion that all groups were capable of forming an allocentric representation of wall colour cues.

Although the critical test trials described in the current experiment did not reveal any behavioural differences between groups, there were a few lesion specific impairments observed during training. For Stage 1 training, rats with hippocampal lesions were initially less accurate in locating a correct corner of the pool, but this impairment disappeared with more sessions of training. These rats also took longer than rats belonging to Group *Sham* and *DLS* to locate the escape platform, with this mild impairment persisting throughout all stages of training. To gain a better understanding as to why Group *HPC* was slower to locate the escape platform, a computerised tracking system recorded the swim path of each rat during the final session of Stage 1 training. The results of the tracking data eliminated the possibility

that longer latencies observed in rats with hippocampal lesions were due to slower swim speeds or enhanced thigmotaxis. Further analysis revealed that rats in Group *HPC* swam directly to a correct corner of the pool as quickly as rats in the remaining groups but they remained in this area in search of the platform for longer. An obvious reason for this behaviour, previously mentioned, is that rats with hippocampal lesions are impaired at judging distances and, therefore, having located the correct corner, rats in Group *HPC* were impaired at judging the distance between the platform and arena walls (e.g. Jones et al., 2007). This finding holds important implications for studies using escape latency as an index of learning in rats with hippocampal lesions because any impairment observed may be less about knowledge of the target cue and more related to distance discrimination.

The results of Stage 1 training also revealed that after being released into the square pool from the midpoint of any given wall, rats with dorsolateral striatal lesions exhibited a tendency to first visit a far corner of the pool over a near corner. Despite this habit, these rats quickly learned to choose the correct far corner. It is difficult to argue that this habit was driven by an egocentric response rule based on responding to a wall of a particular colour given that animals headed directly to a far corner irrespective of the colour of the wall. Furthermore, these animals could not have used a fixed response, ignoring wall colour, such as "turn to the far right corner after being released" as the location of the correct corner on the far side of the pool alternated between trials. It would seem more plausible, then, that the behaviour of always swimming to the opposite side of the pool is related to motor function rather than memory. It has been proposed that the dorsal striatum can act as an inhibitory system and that lesions to this structure impair the ability to suppress forward motion. Indeed, it has been reported that these animals have a strong tendency to

move forward, resist attempts to thwart this movement, and push stubbornly against immovable objects (Fox, Kimble & Lickey, 1964; Mettler & Mettler, 1942; Thompson, 1959). Thus, this behaviour of swimming to the furthest correct corner of the pool without encountering an obstructive wall may reflect this tendency. It must be noted, however, that this obstinate forward movement did not emerge when animals with *DLS* lesions were sitting on the escape platform, i.e. there was no tendency for these rats to jump off.

The tests previously described failed to identify any behavioural deficits in the learning of colour cues when animals were induced with lesions to the *HPC* or *DLS*. Accordingly, Experiment 11 was conducted to try to determine whether the same animals used in Experiment 10 were impaired in their use of room cues or landmark cues when both types of cue were learned about in compound.

5.2 Experiment 11: Place vs. Landmark Learning

5.2.1 Introduction

It was described in Chapter 1 that navigational strategies can be dissociated according to whether rats employ a 'place' strategy, involving the processing of relations between environmental cues (O'Keefe & Nadel, 1978), or a 'cue' response strategy requiring acquisition of a fixed response to discrete cues (Hull, 1943). Tasks designed to investigate place and cue response strategies measure the same allocentric and egocentric forms of learning described in Experiment 10. Accordingly, rats either with lesions to the hippocampus or dorsal striatum can be used in an attempt to identify a double dissociation between place and cue response learning, respectively. For example, experiments in which place and cued learning

can be assayed in parallel have shown that rodents with hippocampal lesions are impaired at place learning and unimpaired at cued learning and for those animals with dorsal striatum lesions the opposite behavioural effects are observed (Packard & McGaugh, 1992; Lee et al., 2008).

Both Packard & McGaugh (1992) and Lee et al. (2008) used a water maze task in which rats and mice, respectively, were assigned to either a place condition or a cued condition. Rats in both conditions were trained to locate a hidden goal positioned under one of two visible landmarks in a circular pool of water, which was located in a room containing various extra-maze cues. For rats trained in the place condition the landmark signalling the location of the platform remained in the same position with respect to room cues, while the visual pattern on the landmark varied from trial to trial. For the cued condition, the landmark signalling the location of the platform had a specific visual pattern, while the spatial locations of landmarks varied from trial to trial. For both conditions, the performance of animals either with hippocampal or dorsal striatum lesions was compared to sham-operated animals. Although, the pattern of results, described above, demonstrated a double dissociation between place and cue learning, this double dissociation has yet to be observed in a single water maze task that can be solved using either a place solution or a cue response solution. Therefore, it is not possible to assess in sham animals whether one form of learning dominates over the other, and whether both forms of learning can be acquired in parallel but compete for behavioural control (Chavarriaga et al., 2005; Kosaki, Poulter, et al., in prep.; Lee et al., 2008; White & McDonald, 2002).

Two water maze studies attempting to investigate this line of inquiry used a design in which cue and place training was given to rats concurrently (room cues + visible platform) interspersed with single sessions of place only training (room cues)

before a final competition test, which pitted responding to room cues against the visible platform (Devan & White, 1999; McDonald & White, 1994). These experiments demonstrated that hippocampal damage prevented place but not place + cue learning and produced a preference for the cue response during the competition test. Rats with dorsal striatum damage, on the other hand, acquired both the place + cue and place only versions of the task but displayed a preference for the place response during the competition test. Sham animals acquired both versions of the task but showed an equal preference for each response during the competition test. These findings provide a double dissociation between lesion-specific response preferences but the extent to which cue learning occurred is difficult to ascertain as there was no test to measure the control that the visible platform exerted over behaviour in the absence of competing room cues. Therefore, it could be argued that cue response tendencies during a competition test are not a direct index of learning.

Thus, given the foregoing discussion and lack of impairment in the use of wall colour cues by rats with either hippocampal (*HPC*) or dorsolateral striatum (*DLS*) lesions in Experiment 10, the current experiment attempts to identify a double dissociation between the *HPC* and *DLS* in processing place-based allocentric and cue-based egocentric information, respectively. Additionally, as a final behavioural deficit test, it was investigated whether *HPC* and *DLS* lesions impair the processing of geometric information in a distinctively shaped arena. As discussed in Chapter 1, there is a growing body of evidence supporting the notion that the hippocampus is critical for the processing of shape-based information. Therefore, an assessment of geometry learning can be used as further behavioural histology that the hippocampal lesions were effective. Moreover, the effect of *DLS* lesions on geometry learning has yet to be investigated, so the results will prove insightful to this line of inquiry.

During the *Room Cue + Landmark* task, rats were trained to locate a hidden goal positioned under one of two visually distinct landmarks in a circular pool of water, which was located in a room containing various extra-maze cues. Thus, the position of the hidden goal could be located with reference to room cues or by approaching the correct landmark based on its visual properties. On the basis of the previous discussion it was anticipated that after training, performance in rats with *HPC* lesions would be impaired during a room cue test but spared during a landmark test. In contrast, it was expected that rats with *DLS* lesions would be impaired during a landmark test but unimpaired in a place test. The performance of sham-operated rats in each test should give an indication as to whether they have acquired a place or cue response, or both in parallel. A final competition test was conducted in which the landmark and place cues that signalled the location of the platform during training were no longer spatially contiguous to each other. In this way, the previously rewarded place and landmark cues could be pitted against each other so that the relative responses to each cue could be measured for each group. In a subsequent *Shape* task, rats were trained to locate a hidden goal in one corner of a rectangular shaped pool. After this training, a probe trial was conducted to assess the amount each group learned about the shape of the arena.

5.2.2 Method

5.2.2.1 Subjects

The subjects were 32 male Lister hooded rats (*Rattus norvegicus*) supplied by Charles River (UK), which were approximately 8.5 months of age at the start of the experiment. All animals had previously participated in an unrelated object recognition task (Experiment 7) and a water maze task in a square shaped arena

(Experiment 10). It was ensured that this prior experience was counterbalanced. To achieve this, animals were grouped according to the object that was assigned as novel on the first day in Experiment 7, and it was ensured for Experiment 10 that half the animals from each of these object subgroups had the platform paired with one corner of the pool (i.e. black to the left of white), and the remainder of the animals had the platform paired with the other corner (i.e. black to the right of left). For the current experiment, it was ensured that half the animals from each correct corner subgroup in Experiment 10 were trained with the black ball and the remainder with the striped prism landmark. Finally, from these landmark subgroups it was ensured that half the animals found the escape platform in the northwest quadrant of the pool and the remainder in the southeast quadrant.

At the start of the experiment there were 12 animals with bilateral lesions of the hippocampus (*HPC*), 12 animals with bilateral lesions of the dorsolateral striatum (*DLS*) and 8 sham operated animals. Rats were housed in identical conditions to those in Experiment 1.

5.2.2.2 Surgical Procedure

Refer to Experiment 6 for surgical procedure.

5.2.2.3 Apparatus

The apparatus was identical to Experiment 8 except for the following differences.

For the *Room Cue + Landmark* task, the curtain around the pool was opened and tied up in the northeast corner of the room, which provided animals with unrestricted access to extra-maze visual cues, or room cues. These room cues included, on the north wall of the laboratory, a white gas boiler unit, which resided in

the northwest corner of the lab close to the ceiling and west wall, a black and white striped poster positioned horizontally central and 50 cm down from the ceiling, a pale yellow pentagonal shaped poster located close to the ceiling in the northeast corner and a curtain, which was tied up in the northeast corner of the laboratory, and on the east wall, a large poster with six gold 10 point stars painted on it located centrally on the upper half of the wall, a tall grey table positioned in the southeast corner of the lab where the animals remained in a carrying box and were dried during the experiment, and on the south wall, a white board, with aluminium trim, placed on the upper section of the wall in the southeast corner of the room, a large white double door situated horizontally central with the top of the frame 30 cm from the ceiling, a black and white circular poster (65 cm in diameter) affixed to the back of the door, and in the south west corner of the room there was a low table with a monitor screen sat on top of it and a chair where the experimenter sat throughout testing, above this table in the corner of the lab there were two large, black, equilateral triangles, one on the south wall and one on the west wall, with one point of each triangle touching the ceiling and the other point touching the other triangle, and on the west wall, a multicoloured picture poster placed left of centre and 50 cm from the ceiling. Aside from the aforementioned room cues, the walls and ceiling of the laboratory were predominantly white.

The two landmarks used in the *Room Cue + Landmark* task (identical to those described in Experiment 8) were each attached to thin soldering wire, which could be hung from hooks affixed to the circular ceiling above, in order to suspend each landmark 27 cm above the surface of the water from its lowest vertical point.

For the *Shape* task, conducted in a white rectangular pool, the two short boards were 90 cm and the two long boards were 180 cm. Refer to Experiment 8 for

details of how the boards were suspended in pool. Curtains surrounded the pool during this stage so that room cues were no longer visible from inside the pool.

5.2.2.4 Procedure

General Procedure

The general procedure was identical to Experiment 8 except for the details concerning the designation of release points and arena orientations, which are described for each stage below. For extinction test trials the escape platform was removed from the pool, animals were released from a novel location which, unless otherwise stated, was in the centre of the pool and allowed to swim for sixty seconds before being removed.

Room Cue + Landmark Training

Animals received 17 sessions of *Room Cue + Landmark* training. On each of the four sessions that ended with an extinction test, animals received only two trials of training as opposed to the usual four. Two discrete, visually distinct landmarks were each positioned 110 cm apart and 35 cm from the edge of the pool along a northwest-southeast axis. For each animal the array of room cues and the location of each landmark remained constant. The centre of the escape platform was positioned directly below and in line with the centre of one of these landmarks and remained in the same position for each animal throughout training. Landmark identity and platform position were counterbalanced so that within each group half the animals were trained with the platform under the black ball and half under the striped prism. These landmark subgroups were split further so that half the animals were trained with the platform in the northwest quadrant of the pool and the remaining half with

the platform in southeast quadrant. The four release points (north, south, east and west) were randomised with the constraint that rats were released once from each point within a session (see Figure 5.8).

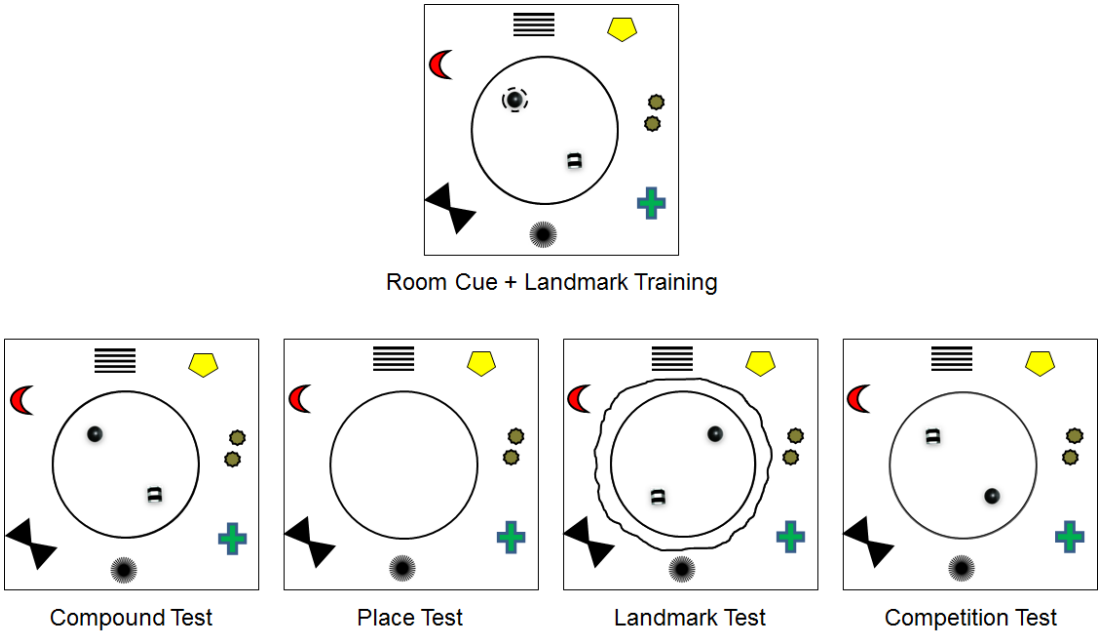

Figure 5.8. Schematic diagram of the experimental design for the *Room Cue + Landmark task*. All training and tests were conducted in a white, circular swimming pool (large white circle), which was situated in the centre of a laboratory (large square). The dashed line circle represents a submerged escape platform. Small black filled circles and striped prisms represent different types of landmarks suspended above the pool. Various symbols scattered outside the pool represent ambient room cues situated around the laboratory. The squiggly line surrounding the pool in the *Landmark* test represents a curtain, which obstructed any room cues from view when rats were swimming in the pool. Between each test, rats received *Room Cue + Landmark* retraining. The order of the *Place* and *Landmark* test was counterbalanced between animals.

Room Cue + Landmark Extinction Tests

Rats received four extinction tests in the *Room Cue + Landmark* task. The first test (*Compound Test*), which took place after 10 sessions of training, was

conducted in the circular pool with the landmarks and room cues arranged identically as they were during training. This test was conducted to provide a behavioural measure, alongside the training data, of how much rats had learned about the landmark and room cues in compound. Rats were then provided with two and a half sessions of *Room Cue + Landmark* retraining before receiving their second extinction test, the type of which was counterbalanced so that half the animals from each group were given a *Place Test* first and the other half were given a *Landmark Test* first. For the *Place Test*, the room cues remained identical to training but the landmarks were removed and animals were released from the southwest release point of the circular pool. For the *Landmark Test*, a curtain was drawn around the full circumference of the pool, so that animals were denied access to extra-maze room cues. Each landmark remained suspended above the pool, still positioned the same distance apart and from the edge of the pool as during training, but on a northeast-southwest axis as opposed to a northwest-southeast axis. Subsequently, rats received one and a half sessions of *Room Cue + Landmark* retraining before being presented with their third extinction test. For half the animals in each group who received the *Place Test* first they were now given a *Landmark Test* and for the remaining animals the reverse applied. Rats then received one and half sessions of *Room Cue + Landmark* retraining before the final *Competition Test.* This test provided each animal with the identical room cues and landmarks as those presented during training, however, the fixed position of each discrete landmark was swapped (see Figure 5.8).

Shape Task

Animals received 4 sessions of *Shape* training conducted in a white rectangular pool. Throughout this training and the subsequent test trial, the curtains

were drawn around the pool so that rats could only use shape-based information provided by the walls of the pool, e.g. the platform was always found in a corner where a short wall was to the left of a long wall. For each rat within a session the platform was located in one corner of the rectangular pool for a randomly selected two trials and in the diametric opposite corner for the remaining two trials. Technically, the diametrically opposite corners of the white rectangular pool should look identical to a rat but the escape platform was oscillated between these corners to minimise the chance that rats could use some local cue, odour or otherwise, to aid their search for the platform. The escape platform was placed in the designated corner with its centre 25 cm from the point at which the two walls of the corner met on a trajectory which split this corner in half. The midpoints of each wall were designated as the points of release into the pool. The arena was rotated between each trial and could be oriented in four positions through a north-south or east-west axis. The release points and arena positions were assigned randomly for each trial with the constraint that the four different release points and orientations were used within a session.

After four sessions of *Shape* training, rats received their final extinction test (*Shape Test*) in the white rectangular pool oriented in a novel position along a north-east – south-west axis.

5.2.2.5 Performance Measures

All measures were identical to Experiment 8 except for the following. For *Room Cue + Landmark* extinction tests, Ethovision was used to create 2 circular zones each 25 cm in diameter. The two zones were positioned in opposite quadrants of the pool so that the centre of each zone corresponded to where the centre of the

escape platform would have been if it were paired with the relevant cue for that quadrant. These two zones were defined as *correct* zone and *incorrect* zone. For all tracking analyses exploration was considered to have taken place if the rat's head entered any zone. For the *Shape Test*, circular zones (33 cm in diameter) were placed in each corner of the pool, centred on the potential platform position, and categorised as *correct* and *incorrect* zones.

5.2.3 Results

5.2.3.1 Room Cue + Landmark Training

Figure 5.9 presents the mean escape latency (upper panel) and the mean percentage of correct first choices (lower panel) for each lesion group across 17 sessions of *Room Cue + Landmark* training. It is clear from this figure that Group *HPC* was consistently slower to locate the escape platform, although there were no differences between groups in terms of choice accuracy. To test statistically the above observations, two-way ANOVAs were conducted separately on the session mean escape latencies and percentage of correct first choices for each rat with lesion group as the between-subjects measure and session as the repeated measure. The analysis conducted on escape latency revealed a significant main effect of lesion group, $F(2, 25) = 10.32$, $p = .001$, and session, $F(5.14, 129) = 53.7$, $p < .001$. However, the session x lesion group interaction was non-significant, $F(10.3, 129) = 1.30$, $p = .24$. Post-hoc tests to examine the lesion group differences revealed that Group *HPC* was significantly slower to locate the platform than Group *Sham* ($p = .001$) and *DLS* ($p = .014$), but there was no significant difference between Group *Sham* and *DLS* ($p = .625$). The percentage of correct first choice data revealed a significant main effect of session, $F(7.9, 198) = 11.4$, $p < .001$, but no significant

effect of lesion group, $F(2, 25) < 1$, or session x lesion group interaction, $F(15.8, 198) = 1.32$, $p = .19$.

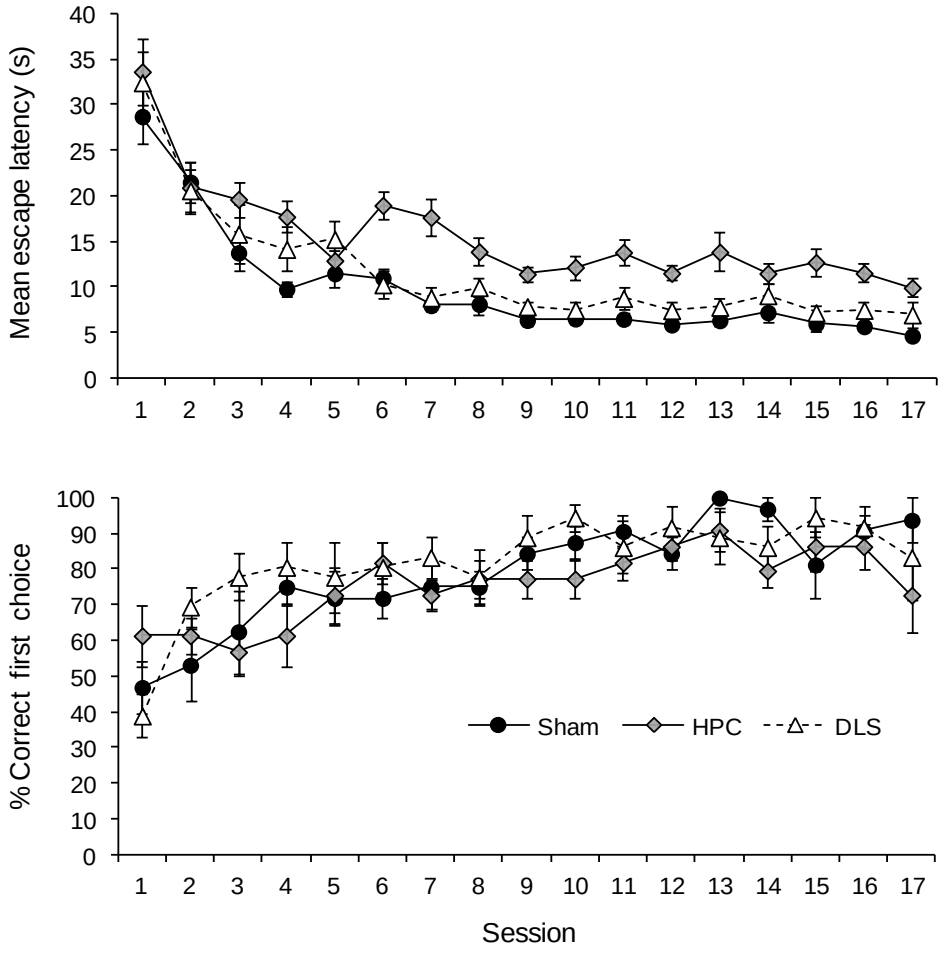

Figure 5.9. The mean (± *SEM*) escape latencies (upper panel) and percentage of correct first choices (lower panel) for each group across 17 sessions of *Room Cue + Landmark* training.

5.2.3.2 *Compound Test*

Figure 5.10 displays the results of the *Compound* test and it is clear that discrimination between the correct and incorrect zone was more marked for Group *Sham* than for the remaining groups with Group *HPC* spending least time in the correct zone.

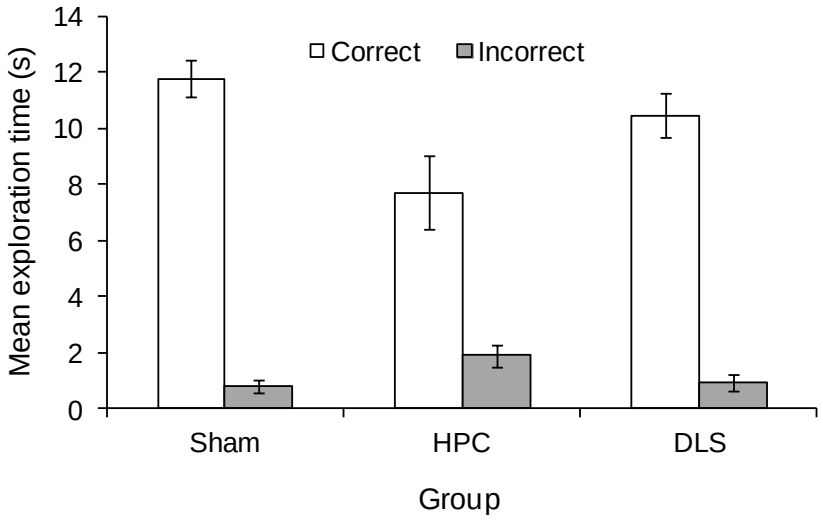

Figure 5.10. The mean time (± *SEM*) spent in the correct (white bars) and incorrect (grey bars) zones during the *Compound* test for each group.

To validate these observations, a lesion group x zone (correct vs. incorrect) ANOVA of individual exploration times revealed a significant main effect of zone, $F(1, 25) = 162$, $p < .001$, and a significant zone x lesion group interaction, $F(2, 25) = 5.26$, $p = .012$. Tests of simple main effects to investigate this interaction revealed a significant effect of lesion group on time spent in the correct zone, $F(2, 25) = 4.02$, $p = .031$, with subsequent post-hoc tests revealing that Group *HPC* searched for significantly less time than Group *Sham* ($p = .035$) but for a similar amount of time to Group *DLS* ($p = .204$), there was no significant difference in exploration between Group *Sham* and *DLS* ($p = 1$). For the time spent in the incorrect zone, there was no significant effect of lesion group, $F(2, 25) = 3.15$, $p = .060$. Simple main effects tests on the zone x lesion group interaction also revealed that all groups discriminated between zones, with post-hoc tests revealing that more time was spent in the correct than incorrect zone for all groups, Fs$(1, 25) > 28.4$, ps $< .001$. The main effect of lesion group on time spent in both zones combined was non-significant, $F(2, 25) = 2.17$, $p = .14$.

5.2.3.3 Place Test

For the *Place* and *Landmark* tests the order of testing was counterbalanced so that half the animals in each group received the *Place* test first and the remaining animals received the *Landmark* test first. Unless stated otherwise, the following behavioural measures were analysed using a three-way repeated measures ANOVA with lesion group and test order (*Place* test 1st and *Place* test 2nd) as the between-subjects variables and zone (correct vs. incorrect) as the repeated measure.

Inspection of Figure 5.11 reveals that all groups could discriminate the correct from incorrect place zones, and this discrimination was evenly matched across all groups. To confirm this assertion statistically, an ANOVA of individual exploration times revealed a significant main effect of zone, $F(1, 22) = 27.1$, $p < .001$, with all animals spending more time in the correct than incorrect zone, but no remaining significant between- or within-subjects effects or interactions, Fs < 1.

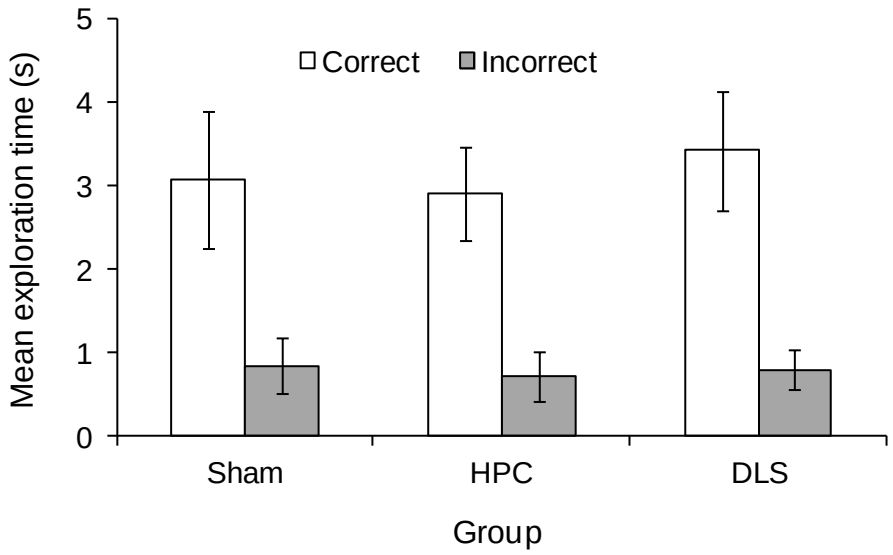

Figure 5.11. The mean time (± *SEM*) spent in the correct (white bars) and incorrect (grey bars) zones during the *Place* test for each group.

5.2.3.4 Landmark Test

Figure 5.12 illustrates the results of the *Landmark* test and it is clear that Group *HPC* spent more time searching under the correct landmark than the remaining groups. For rats belonging to Group *Sham* and even more so for rats belonging to Group *DLS*, discrimination between the correct and incorrect landmark was poor.

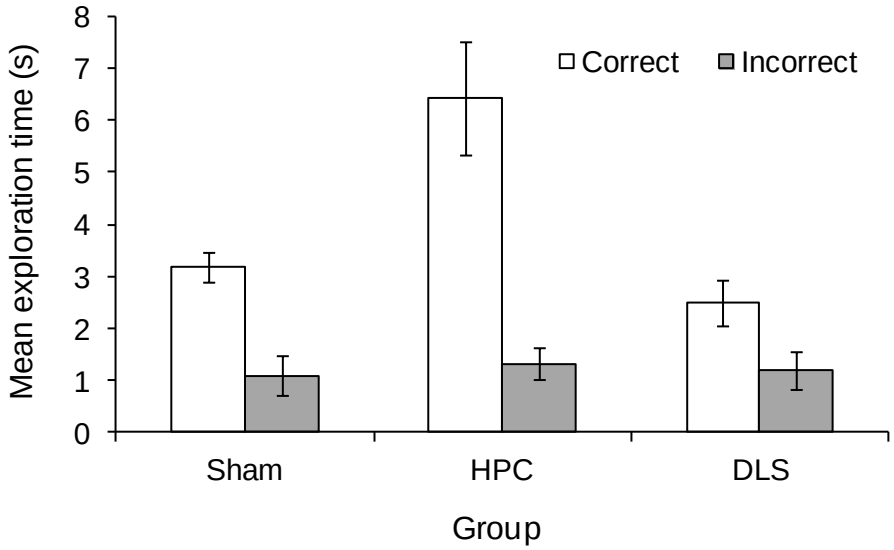

Figure 5.12. The mean time (± *SEM*) spent in the correct (white bars) and incorrect (grey bars) zones during the *Landmark* test for each group.

To confirm this characterisation of the data, an ANOVA of exploration times for individual rats revealed a significant main effect of lesion group, $F(2, 22) = 9.85$, $p = .001$, and zone, $F(1, 22) = 22.3$, $p < .001$, and a significant interaction between these factors, $F(2, 22) = 4.14$, $p = .03$. Subsequent analyses of simple main effects to investigate this interaction revealed a significant main effect of lesion group on time spent in the correct zone, $F(2, 22) = 7.00$, $p = .005$, with post-hoc tests revealing that Group *HPC* spent more time searching under the correct landmark than Group *Sham*

(p = .034) and *DLS* (p < .01), and Group *DLS* searched for an approximately equal time to Group *Sham* (p = 1). There was no effect of lesion group on time spent under the incorrect landmark, $F(2, 22) < 1$. For the main effect of zone in this interaction, pairwise comparisons revealed a significant main effect of zone for Group *HPC*, $F(1, 22) = 29.0$, $p < .001$, but only a marginal significant effect for Group *Sham*, $F(1, 22) = 3.67$, $p = .069$, and no significant effect for Group *DLS*, $F(1, 22) = 1.44$, $p = .24$.

5.2.3.5 Competition Test

Figure 5.13 shows the results of the *Competition* test. All groups spent more time searching at the previously rewarded place with respect to room cues than under the previously rewarded landmark. It would also appear that there was a stronger preference for place in Group *HPC* than in the remaining groups. However, an ANOVA of exploration times for individual rats revealed a significant main effect of lesion group, $F(2, 22) = 14.8$, $p < .001$, with pairwise comparisons revealing that Group *HPC* explored both zones for longer than Group *Sham* ($p < .001$) and *DLS* ($p < .001$), and a significant main effect of zone, $F(1, 22) = 12.4$, $p = .002$, with all animals spending more time in the place zone than the landmark zone, but there was not a significant lesion group x zone interaction, $F(2, 22) = 1.50$, p = .25. All remaining main effects and interactions were non-significant, Fs ≤ 2.59, ps ≥ .12.

Despite the above ANOVA revealing that the response biases displayed both by rats with *HPC* lesions and rats with *DLS* lesions during the *Competition* test did not differ significantly from those observed in controls, it is difficult to assess the impact of prior tests on the performance of sham animals. Therefore, it is insightful to assess the relative response preference for each group separately, especially as Figure 5.13 appears to illustrate a clear place response preference in Group *HPC*,

which is less marked in the remaining groups. Accordingly, a paired-samples t-test was conducted for each group with the paired variables of time in correct place vs. correct landmark. This analysis revealed no significant difference between times for Group *Sham*, $t(7) = 1.05$, $p = .33$, and Group *DLS*, $t(8) = 1.70$, $p = .13$, while Group *HPC* spent significantly more time in the correct place zone than the correct landmark zone, $t(10) = 3.24$, $p = .009$.

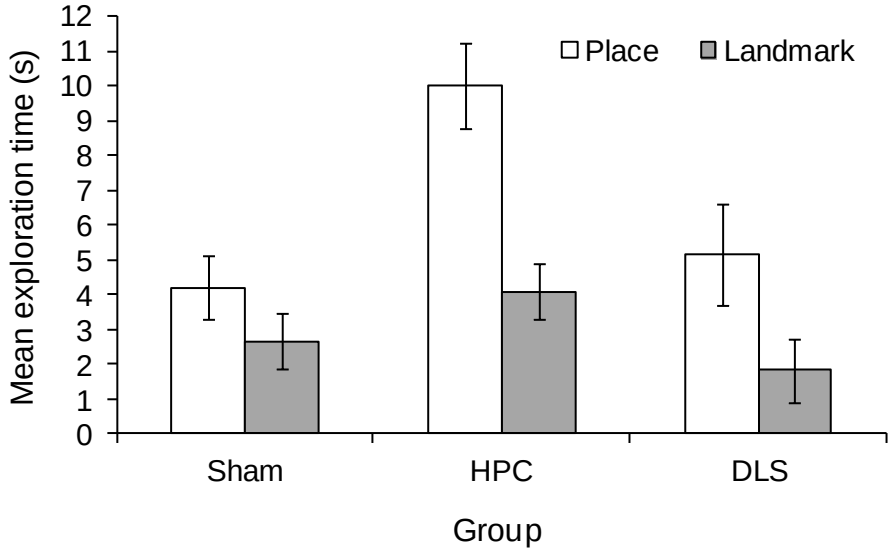

Figure 5.13. The mean time (± *SEM*) spent in the previously rewarded place (white bars) and landmark (grey bars) zones during the *Competition* test for each group.

A frequency analysis was also conducted to establish the number of place responders versus the number of cue responders in each group (the same as that devised by Devan & White, 1999). Responses were categorised according to which zone (place or landmark) animals first visited. Table 5.1 displays the number of animals in each group that first swam to each of these zones during the competition test. In support of the zone search times above, the table shows that rats from all groups showed a preference for place over cue responses. However, there is a hint that proportionately more animals first chose a cue response in Group *HPC*. To

validate this characterisation of the data these frequencies were analysed using χ^2 tests. These tests revealed no significant difference in frequencies between Group *Sham* and *HPC* ($\chi^2 = 1.36$, $p = .24$), Group *Sham* and *DLS* ($\chi^2 < 1$), or Group *HPC* and *DLS* ($\chi^2 < 1.7$, $p = .2$).

Table 5.1

Number of rats that swam to the previously rewarded place (place responders) versus the previously rewarded landmark (cue responders) during the Competition test

	Sham	HPC	DLS
Place Responders	7	7	8
Cue Responders	1	4	1

5.2.3.6 Shape Training

Figure 5.14 shows the mean escape latency (upper panel) and the mean percentage of correct first choices (lower panel) for each lesion group across 4 sessions of *Shape* training. Examination of this figure reveals that Group *HPC* were slower and less accurate at locating the platform than Group *Sham* and *DLS*, while Group *Sham* was most proficient in acquiring the task. Despite these differences in acquisition, performance improved in all groups as training progressed.

To validate this description, a two-way ANOVA of individual escape latencies with lesion group as the between-subjects measure and session as the repeated measure revealed a significant main effect of lesion group, $F(2, 25) = 7.54$, $p = .003$, with post-hoc tests revealing that Group *HPC* took significantly longer to locate the escape platform than Group *Sham* ($p < .01$), but a similar amount of time as Group *DLS* ($p = .190$), and that Group *Sham* and *DLS* exhibited similar latencies

(p = .205). There was also a main effect of session, $F(3, 75) = 25.6$, $p < .001$, but a non-significant session x lesion group interaction, $F(6, 75) = 1.68$, $p = .14$. A similar ANOVA conducted on individual percentages of correct first visits revealed a significant main effect of lesion group, $F(2, 25) = 4.09$, $p = .029$, with post-hoc tests revealing that the correct first choice percentage of Group *HPC* was significantly lower than that of Group *Sham* ($p = .03$), but there were no other significant lesion group differences in choice accuracy (ps > .254). There was also a significant main effect of session, $F(3, 75) = 5.15$, $p = .003$, but a non-significant session x lesion group interaction, $F(6, 75) = 1.89$, $p = .09$.

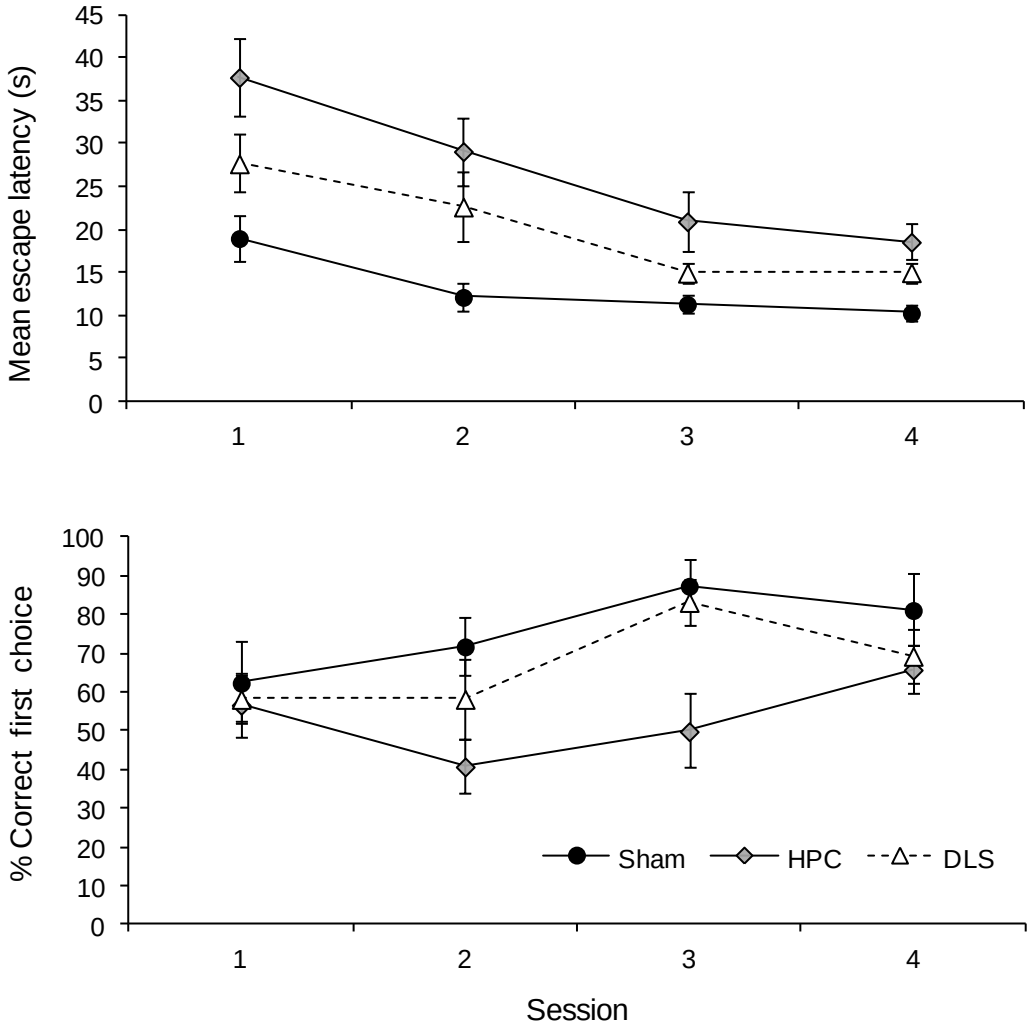

Figure 5.14. The mean (± SEM) escape latencies (upper panel) and percentage of correct first choices (lower panel) for each group across 4 sessions of Stage 2 training.

5.2.3.7 Shape Test

The results of the *Shape* test are presented in Figure 5.15. It is clear that all groups were able to discriminate the correct from incorrect corners of the rectangle but this discrimination was less marked in Group *HPC* when compared to the remaining groups.

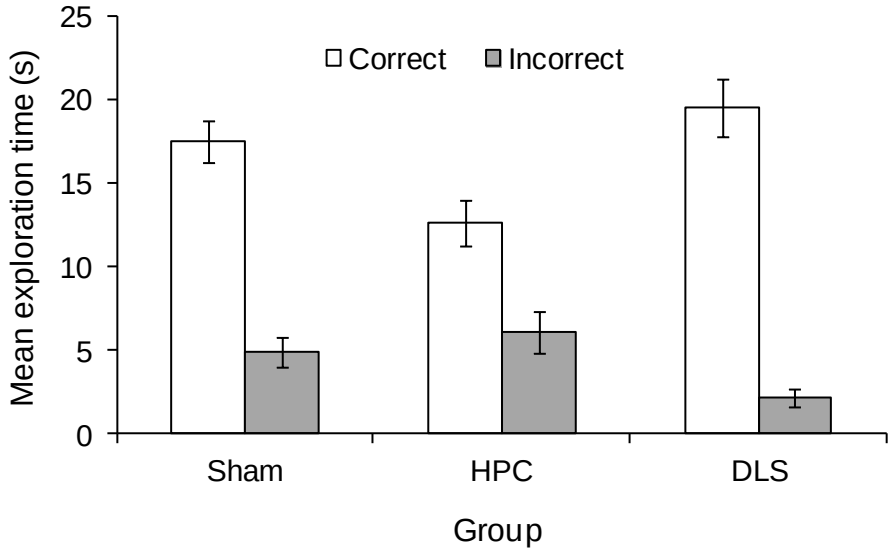

Figure 5.15. The mean time (± *SEM*) spent in the correct (white bars) and incorrect (grey bars) corners of the rectangular pool during the *Shape* test for each group.

To validate this characterisation of the data, a two-way ANOVA of individual times spent in the correct and incorrect zones of the rectangular pool, with lesion group as the between-subjects measure and zone (correct zone vs. incorrect zone - calculated by combining the time spent in the appropriate corner and the corner diametrically opposite) as the repeated measure, was conducted. This ANOVA revealed a significant main effect of zone, $F(1, 25) = 130$, $p < .001$, and a significant zone x lesion group interaction, $F(2, 25) = 9.35$, $p = .001$. A simple effects analysis to investigate the interaction between zone and lesion group revealed

a significant main effect of lesion group on time spent in the correct zone, $F(2, 25) = 6.5$, $p = .005$, with post-hoc tests revealing that Group *HPC* spent significantly less time in this zone than both Group *Sham* ($p = .024$) and *DLS* ($p < .01$), and also on time spent in the incorrect zone, $F(2, 25) = 3.79$, $p = .036$, with post-hoc tests revealing that Group *DLS* spent significantly less time in this zone than Group *HPC* ($p = .012$) but not Group *Sham* ($p = .090$). This analysis also revealed a significant effect of zone for all groups, $Fs(1, 25) \geq 15.0$, $ps \leq .001$.

5.2.4 Discussion

In the *Room Cue + Landmark* task, rats were trained to locate a hidden goal that remained in a fixed position with respect to distal room cues (place response) and under one of two visually distinct landmarks (cue response). Because the task could be solved using either a place response or a cue response, the aim of the experiment was to identify a double dissociation between the hippocampus (*HPC*) and dorsolateral striatum (*DLS*) in processing place and cue information, respectively. Thus, following training, rats were subjected to three tests: a place test, a cue test and a competition test. Based on previous evidence (Packard & McGaugh, 1992; Lee et al., 2008) it was predicted that damage to the *HPC*, but not the *DLS*, would impair place learning and damage to the *DLS*, but not the hippocampus, would impair cue response learning. For the *Competition* test it was predicted that rats with *HPC* damage would show a preference for the cue response and rats with *DLS* damage would show a preference for the place response. Performance of sham animals would be instructive as to the relative contribution of each learning mechanism during such a task.

The pattern of results observed did not identify the predicted double

dissociation between place and cue learning. During the place test when the landmarks were removed all animals were able to discriminate the correct from opposite areas of the pool. The performance of both rats with lesions to the *HPC* and rats with *DLS* damage was unimpaired when compared to sham animals. The results of the cue response (*Landmark*) test showed that hippocampal damage facilitated landmark learning, while *DLS* damage did not impair performance relative to sham-operated controls. However, although a direct comparison revealed no difference in discrimination between rats in Group *DLS* and *Sham*, examination of performance for each group individually indicated that sham rats could almost discriminate the correct from incorrect landmark, while Group *DLS* couldn't. The fact that both of these groups failed to significantly discriminate between landmarks when place cues were removed suggests that the presence of place cues during training was critical for successful completion of the task. The findings of the *Competition* test did not reveal the pattern of response tendencies observed in previous studies (Devan & White, 1999; McDonald & White, 1994). There was no difference in response bias between Group *Sham*, *HPC* and *DLS*. All rats expressed a preference for the place response over the cue response which, contrary to previous evidence, was more marked for rats with hippocampal damage.

The results of the place test were surprising in that rats with *HPC* damage did not exhibit an impairment in their ability to use room cues. A key objective for conducting this experiment was to determine if rats with hippocampal lesions were impaired at using room cues given that they failed to show an impairment when using colour cues in Experiment 10. This failure to observe an impairment is at odds with a substantial body of work reported in previous studies (Morris et al. 1982; Morris, Anderson, Lynch & Baudry, 1986; Sutherland, Kolb & Whishaw, 1982;

Whishaw, 1987; see also Devan & White, 1999; Lee et al., 2008; McDonald & White, 1994; Moser, Moser & Anderson, 1993; Pearce, Roberts & Good, 1998). However, a fundamental difference between this and the vast majority of the aforecited experiments is that in the current experiment, along with room cues, rats were provided with informative landmark cues, one of which acted as a beacon, whereas only room cues were provided in the other experiments. Admittedly, Devan & White (1999) and McDonald & White (1994) trained *HPC* rats with both room cues and a beacon but their test of place learning comprised of single interspersed training sessions in which the beacon was removed from the pool. The index of learning in these experiments was escape latency. However, it has already been observed in the current experiment and Experiment 10 that escape latency during training may be longer for rats with hippocampal lesions for non-mnemonic reasons (see Discussion of Experiment 10 for a more detailed explanation). Therefore, it is prudent to compare experimental findings using the same index of learning.

Interestingly, in a similar experiment in which rats with hippocampal lesions were trained to locate an escape platform with reference to room cues and a beacon it was also found that these animals were as proficient at searching for the platform during a place test with the beacon removed as sham-operated animals (Whishaw & Jarrard, 1996). The design of this experiment, like the current experiment, ensured that, during training, rats were placed into the pool from different start points which should disrupt the formation of a simple egocentric response rule. Moreover, both experiments showed that rats with hippocampal lesions were able to swim in different directions and distances to accurately locate the platform. Whishaw & Jarrard interpreted their finding as evidence that the hippocampus in the rat is not responsible for spatial learning, per se, but is critically involved in the integration of

movements when searching for a goal location. The results of the place test in current experiment lend support to this claim and demonstrate that under certain training conditions rats with lesions to the hippocampus are capable of acquiring a place solution using distal room cues.

A second reason which could influence the level of place memory attained by rats with *HPC* lesions is the amount of training animals receive. For example, Morris, Schenk, Tweedie & Jarrard (1990) showed in a water maze task that rats with lesions to the *HPC* displayed impaired levels of place memory after 7 sessions of training but after being trained for a further 7 sessions, performance of lesioned rats matched that of sham animals. Given that rats in the current experiment also received 14 sessions of training prior to the *Place* test, it is possible that this extended training contributed to the ability of rats with *HPC* lesions to express a similar level of place memory as sham-operated controls.

There are a number of alternative explanations for the current finding that rats with hippocampal lesions are capable of using distal room cues to solve a navigation task. Firstly, it could be argued that these lesioned animals simply learned a set of egocentric motor response rules commencing from each start point and ending with locating the platform. However, should these strategies have formed during training they would not help in the test trial when rats were released from an entirely novel start point. Furthermore, because there were no local cues to mark the location of each start point, rats would need to make use of distal cues in order to distinguish between the multiple start points. The fact that rats were also provided with a beacon above the platform meant that the task did not require them to incorporate distance and direction information from each start point. A second argument could be that rats were simply using a salient distal feature in the room as a

polarising cue. However, the precision with which Group *HPC* searched for the escape platform during the test trial indicated the use of at least two distal cues in order to gauge the relative distance and direction of said polarising cue from each start point. Thus, the results suggest Group *HPC* had formed a place solution.

The findings from the landmark test did not fully support the proposal that damage to the *DLS* impairs a cue response strategy. A direct comparison of landmark discrimination between Group *DLS* and Group *Sham* did not reveal any difference in performance. The disparity in results between this experiment and those reporting a cue response impairment in rats with dorsal striatum damage (e.g. Packard & McGaugh, 1992; Lee et al., 2008) could again be due to differences in the training conditions. In these previous experiments, which similarly tested cue response learning in a probe trial, lesioned rats were trained in a landmark discrimination task in which room cues were irrelevant. In the current experiment, animals were trained in a landmark discrimination task in which room cues could also be used to locate the hidden goal. Therefore, the landmark test in the current experiment, although designed to measure the amount rats learned about landmark cues, could also reflect the extent to which rats were disrupted after room cue removal. Accordingly, it is possible that the present training and test procedure disrupted the performance of sham animals more than a task in which room cues were irrelevant during training. If this were the case then any failure to identify an impairment in rats with *DLS* lesions could be more a function of the disruption to baseline performance in control rats. The results of the competition test are insightful to this discussion as they show that Group *Sham* preferred a place response over a landmark response which makes it all the more plausible that removal of place cues, which exerted strong control over behaviour, would disrupt performance during the landmark test.

The results of the *Landmark* test also revealed that Group *HPC* were able to utilise landmark cues better than sham-operated controls. Or, the ability of Group *Sham* to use landmark cues was more markedly disrupted by the removal of distal room cues. Because the level of performance by Group *Sham* was better in the *Compound* test (room + landmark cues), equal in the *Place* test, and worse in the *Landmark* test when compared to Group *HPC*, the results indicate that sham rats were more able to utilise room and landmark cues additively. More specifically, it would appear Group *Sham* developed a navigation strategy that was more effective at incorporating both landmark and room cues but more disrupted by the removal of room cues. It is possible that sham rats used room cues to establish heading and approximate the location of the platform, and landmark cues to more accurately pinpoint the exact location of the platform. For Group *HPC* however, their strategy may have been more cue based, concentrating on landmark approach. The current finding that hippocampal lesions facilitate a cue-based, or response strategy, supports previous studies demonstrating that rodents with hippocampal lesions perform better than controls when required to employ a response strategy, such as a landmark discrimination, when extra-maze cues are available (Bussey et al., 1998; Lee et al., 2008; Saksida et al., 2007; Sanderson et al., 2012). This facilitation may occur because cue response learning in rats with *HPC* damage is not as affected by interference from place learning processes as it is for sham animals. This present finding that striatum-based learning is enhanced when rats are induced with hippocampal lesions supports a multiple memory systems theory (Warrington, 1979; White & McDonald, 2002; Poldrack & Packard, 2003) by showing that these neural structures have distinct functions that can, under certain circumstances, compete for control of behavioural expression during a learning task.

One question to arise from the interpretation of the present results that rats with *HPC* damage were more predisposed to use a cue-based response strategy from the outset of training is why were these animals not impaired during the *Place* test if landmark cues exerted more control over behaviour when compared to controls? One explanation could be that rats with *HPC* damage learned about the room cues incidentally after being guided to the hidden goal with reference to landmarks (e.g. Whishaw & Jarrard, 1996). Secondly, and as mentioned above, the extended training rats received may have allowed rats with hippocampal damage to build an accurate allocentric representation of room cues (e.g. Morris et al., 1990).

The results of the *Competition* test did not support previous findings that relative to controls, rats with *HPC* damage express a preference for the cue response and rats with *DLS* damage show a preference for the place response (e.g. Devan & White, 1999). All rats expressed a preference for the place response over the cue response with this preference more marked in Group *HPC*. Furthermore, when compared to sham-operated controls, Group *HPC* spent more combined time in the place and landmark zones, which may have been reflective of the fact that these rats shuttled between the correct and incorrect landmark cues. However, it is difficult to interpret the results of the final competition test as rats had already received three extinction tests, which could have caused differential extinction effects among groups. For example, during the *Landmark* test, Group *HPC* spent more time than Group *Sham* under the correct landmark, which may have resulted in the correct landmark losing more associative strength for Group *HPC* than for Group *Sham*. Consequently, during the *Competition* test the correct landmark may have exerted less control over approach behaviour for Group *HPC*.

The *Shape* task in the current study was conducted to test the ability of each

group to use geometric cues when animals were trained to locate the escape platform in one corner of an all-white rectangular shaped pool. When compared to the performance of Group *Sham*, the results revealed an impairment during acquisition of the task, both in terms of escape latency and choice accuracy, in Group *HPC* but not in Group *DLS*. Furthermore, the results of the test trial conducted at the end of training with the platform removed revealed that when compared to Group *Sham*, Group *HPC* spent significantly less time searching in the correct corners of the pool, while Group *DLS* spent a similar amount of time searching in the correct corners. Such a result is in keeping with the findings of previous experiments demonstrating that hippocampal lesions impair rats' ability to use geometric cues (Pearce et al., 2004; McGregor et al., 2004). This finding provides further evidence that the lesions induced upon rats in Group *HPC* were effective in producing a behavioural deficit.

In summary, a key finding from the present experiment is that under certain training conditions rats with complete bilateral lesions to the hippocampus are capable of acquiring a place solution using distal room cues. This finding holds important implications for the function of the hippocampus and questions whether this neural structure is critical for the encoding of an allocentric representation. If the hippocampus is not the sole locus of a 'cognitive map' then the question arises of what specific role this structure plays in navigation. The following experiment was designed to explore this line inquiry.

6 The Hippocampus: Getting There or Knowing Where

6.1 Experiment 12: Active vs. Passive Learning

6.1.1 Introduction

It is a commonly held theory that the hippocampus plays an important role in spatial learning and memory. Convincing evidence from a broad range of species including humans (Astur, Taylor, Mamelak, Philpott, & Sutherland, 2002; Bohbot et al., 1998; Burgess, Maguire, & O'Keefe, 2002; Goodrich-Hunsaker, Livingstone, Skelton, & Hopkins, 2010; Gomez, Rousset, & Charnallet, 2012), primates (Beason-Held, Rosene, Killiany, & Moss, 1999; Hampton, Hampstead, & Murray, 2004; Lavenex, Amaral, & Lavenex, 2006; Murray, Baxter, & Gaffan, 1998), birds (Colombo & Broadbent, 2000; Hampton & Shettleworth, 1996; Watanabe & Bischof, 2004) and rodents (Cassel et al., 1998; Morris et al., 1982; Pearce et al., 1998; Sutherland et al., 1982) supports this theory by demonstrating that selective damage to the hippocampus causes spatial learning, memory and navigation deficits. However, further evidence has shown that some spatial abilities in humans and rats with hippocampal damage can be spared (Corkin, 2002; Rosenbaum et al., 2000; Whishaw & Jarrard, 1996), a finding also supported by the results of Experiment 11. For example, modifications to the training procedure used during conventional Morris water maze (MWM) tasks can facilitate rats with hippocampal damage in learning to find an invisible escape platform occupying a fixed location in relation to

ambient room cues, or allothetic cues. Such modifications include additional training (Bast, Hannesson & Skelton, 1998; Morris et al., 1990), cueing or shaping of the target location (Whishaw, Cassel, & Jarrard, 1995; Whishaw & Jarrard, 1996; Whishaw & Tomie, 1997; also see Experiment 11), or starting with a large target area and gradually reducing its size over training (Day, Weisend, Sutherland, & Schallert, 1999). Given this pattern of spared and impaired spatial abilities following hippocampal damage and the suite of motor, motivational and perceptual brain processes involved during navigation tasks, the specific nature of the role that the hippocampus plays in spatial learning and memory remains unclear.

One theory proposes that the hippocampus, via the formation of neural signatures, constructs and stores maps of the surrounding physical environment, or the spatial relations among cues (O'Keefe & Nadel, 1978; Tolman, 1948). As discussed in Chapter 1, strong support for this 'cognitive map' theory is provided by the existence of 'place cells' in the rat hippocampus (O'Keefe, 1976; O'Keefe & Dostrovsky, 1971), which fire only when the rat occupies a specific location in its environment. Similar neuronal properties in the hippocampus of humans (Ekstrom et al. 2003) and primates (Ludvig, Tang, Gohil, & Botero, 2004; Nishijo, Ono, Eifuku, & Tamura, 1997; Ono, Nakamura, Fukuda & Tamura, 1991; Rolls, Robertson, & GeorgesFrancois, 1997) have also been identified. Evidence from a range of studies implies that the space-specific signal observed in place cells is controlled by distal allothetic cues (Miller & Best, 1980; Muller & Kubie, 1987; O'Keefe, 1991; O'Keefe & Speakman, 1987; Speakman & O'Keefe, 1990). However, should the hippocampus be the exclusive locus of a cognitive map of the surrounding environment, and therefore the seat of spatial learning, results revealing spared place learning in rats with hippocampal lesions should not be possible. Moreover, the

specific spatial function of hippocampal place cells has been questioned via evidence showing that these cells can be selectively tuned to non-spatial information (Dudchenko, Wood, & Eichenbaum, 2002). Thus, it is possible that the hippocampus is not responsible for spatial memory, per se, but a broader memory system, which is critical in, but not exclusive to, spatial learning.

In opposition to the notion that the hippocampus is essential during navigation tasks for "knowing where", a second theory proposes that this structure is instead critical for "getting there" (Whishaw, Cassel, & Jarrard, 1995). More formally, it has been argued that the hippocampus mediates a process known as path integration (PI), a method that a navigating organism relies upon by keeping track of its own body movements to calculate distances and orientation. Self-motion, or ideothetic cues, can be generated by drawing on vestibular information, efference copies of motor commands and or changes in visual information corresponding to changes in the speed and direction of body movements. Evidence to support the role of the hippocampus in ideothetic navigation has been provided by experiments demonstrating that rats with hippocampal damage deprived of visual allothetic cues and required to rely solely on ideothetic cues cannot navigate successfully. However, their ability to use allothetic cues accurately, when available, was spared (Maaswinkel & Whishaw, 1999; Wishaw & Gorny, 1999; Wishaw & Maaswinkel, 1998, but see also Aylan & McNaughton, 1999 for evidence that rats with hippocampal lesions are capable of using path integration). A separate analysis at the cellular level has provided evidence that neuronal activity in the hippocampus is tightly tuned to ideothetic cues (Blair & Sharp, 1996; Golob & Taube, 1997; Jeffery & O'Keefe, 1999; McNaughton et al., 1996; McNaughton, Battaglia, Jensen, Moser, & Moser, 2006; O'Mare, Rolls, Berthoz & Desner, 1994; Sharp, Blair, Etkin, &

Tzanetos, 1995; Taube & Burton, 1995; Wiener, 1996). For example, hippocampal pyramidal cells exhibit place specificity even in the absence of allothetic cues (Markus, Barnes, McNaughton, Gladden, & Skaggs, 1994; O'Keefe & Speakman, 1987; Quirk, Muller, & Kubie, 1990). Additionally, the contribution of motor action to neuronal activation in the hippocampus has been emphasised by an experiment in which place cells were virtually silent when a rat was trained to tolerate restraint and unable to move voluntarily through its environment (Foster, Castro, & McNaughton, 1989).

Thus, the foregoing discussion has suggested that a navigating organism can use either an integration of ideothetic cues or the spatial relationship between allothetic cues. In each case, there is evidence that the hippocampus is heavily involved. However, to what extent each strategy is used and the hippocampus required (for one or both strategies) is difficult to ascertain in any given circumstance. To dissociate these strategies, an experimental design must be employed that renders one type of cue uninformative. For example Whishaw and colleagues removed all allothetic cues by testing rats with blindfolds on or in darkness so that only ideothetic cues could be relied upon for successful navigation and found that rats with damage to the hippocampus were impaired at this task (Maaswinkel & Whishaw, 1999; Wishaw & Gorny, 1999; Wishaw & Maaswinkel, 1998). Presently, however, there has not been an experiment to investigate the effect of hippocampal damage when, during training, rats are deprived of ideothetic cues and must rely only on allothetic cues.

The current experiment sought to examine this line of inquiry by using an adapted MWM procedure. Two groups of rats with hippocampal lesions were trained to locate an invisible platform in a circular swimming pool using extra-maze room

cues (*Room Cue* task). One group was required to swim to the platform from different points at the edge of the pool (Group *Active*). The other was placed directly on the platform, similarly from different points, without being required to swim (Group *Passive*). Two groups of sham-operated controls received similar training. Following training, a probe trial was conducted, with the platform removed, during which all rats were required to swim in search of the platform. At the end of this *Room Cue* task, the room cues were hidden from view and all animals were trained to swim to the platform in one corner of a white rectangular pool (*Shape* task). As discussed in Chapter 1, it has been demonstrated on several occasions (e.g. Jones et al., 2007) that hippocampal lesions impair rats' use of shape-based information. Therefore, this *Shape* task was included to provide further behavioural histology that the hippocampal lesions were effective.

During acquisition of the *Room Cue* task, it was anticipated that passive placement on the platform would deprive rats of self-motion feedback, or ideothetic, cues, and so animals would be required to encode the spatial relationship between allothetic cues. The cognitive map theory would predict that damage to the hippocampus should impair this allothetic navigation, irrespective of whether rats learn the task actively or passively. In contrast, if the hippocampus' primary role during navigation is to integrate and monitor motion information and is not concerned with encoding allothetic information, then the prediction would be that hippocampal damage would produce a subtle or no impairment in rats receiving passive placement training, while lesioned rats allowed to swim in search of the platform should display more marked impairments compared to non-lesioned control rats.

In addition, the current experiment may also be insightful for separate reason.

It has been argued that as opposed to the hippocampus being essential for the encoding of place-based information, it is in fact required for the flexible integration of non-spatial information during navigation tasks (Day et al., 1999; Ramos, 2002, 2010; Whishaw & Jarrard, 1996). For example, rats with hippocampal damage may struggle to elucidate the purpose of a specific task and perseverate with inappropriate behaviours, which would emerge as an acquisition deficit. This argument is supported by the work of several authors demonstrating that rats with hippocampal lesions exhibit far superior place learning abilities when facilitatory training conditions minimize the disruptive effects of non-spatial deficits during acquisition (Ramos, 2002, 2010; Whishaw & Jarrard, 1996; Weisend et al., 1999). In accordance with this argument it was predicted that rats with hippocampal lesions in the current experiment that were passively placed onto the escape platform, which should reduce any non-spatial memory demands, should acquire the task more readily than those rats required to swim in search of the platform.

6.1.2 Method

6.1.2.1 Subjects

The subjects were 25 experimentally naive male Lister hooded rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England). At the start of the experiment rats were approximately 4 months of age. 13 animals underwent surgery to create bilateral lesions of the hippocampus (*HPC*) and 12 animals underwent sham operations. Rats were housed in identical conditions to Experiment 1.

6.1.2.2 Surgical Procedure

Refer to Experiment 6 for the surgical procedure.

6.1.2.3 Apparatus

The apparatus used was identical to Experiment 11 except for the following changes.

Throughout the *Room Cue* task there was no curtain surrounding the pool, but instead the curtain was tied up in the northeast corner of the room, so that animals were provided with unrestricted access to extra-maze visual cues, or room cues. These room cues were identical to those described in Experiment 11 with a number of modifications made in an attempt to heighten their salience. The north wall of the laboratory was entirely covered with black wallpaper except for a vertical white stripe (25 cm wide) positioned horizontally central and spanning the height of the wall, and the white boiler unit, which now had a poster displaying a black and white head affixed to it. A piece of dark blue material, 25 cm wide and 100 cm long, was suspended vertically from the ceiling at a distance of 30 cm above and 40 cm outward of the pool's southeast edge. In the southeast corner of the laboratory, a white air conditioning unit (37 cm long x 116 cm wide) was installed on the east wall close to the ceiling and south wall. A free standing white board (122 cm long x 81 cm wide) was positioned 54 cm outward from the southwest edge of the circular pool, which acted as a screen to conceal the experimenter who sat in the southwest corner of the lab during trials. On the front face of the white board (angled towards the pool) a black paper circle (25 cm in diameter) was affixed in-between two black crosshatched semi circles drawn in pen (40 cm in diameter), which were positioned horizontally central on the upper section of the white board. There was also a thin black stripe (13 cm wide x 150 cm long) positioned diagonally on the upper right section of the west wall.

For *pre-training* (described below) and the *Shape* task, four white

polyurethane boards were used to construct a rectangular shaped pool (90 x 180 cm long), which was surrounded by curtains so that rats could use only a beacon or shape information respectively to locate the escape platform.

6.1.2.4 Assignment of groups

HPC lesioned and sham-operated animals were randomly split into two groups so that 7 *HPC* animals were assigned to Group *Passive* and 6 *HPC* animals were assigned to Group *Active*, and for sham animals there were 6 animals assigned to each of the aforementioned groups. These *Active* and *Passive* groups were divided again according to escape platform position during the *Room Cue* task so that half the animals in each group were trained to locate the platform in the northwest quadrant of the pool and half trained with the platform in the southeast quadrant of the pool (for Group *HPC Passive* 4 animals were assigned to the southeast quadrant and 3 to the northwest quadrant).

During the *Shape Task*, half the animals in each group found the escape platform in a corner of the rectangular pool where the short wall was to the right of a long wall and the remaining half experienced the platform in a corner where the short wall was to the left of a long wall.

6.1.2.5 Procedure

General Procedure

Rats were transported into the test laboratory and kept in the carrying box during behavioural procedures in the same manner as described in Experiment 8. All animals received one training session, consisting of four trials, per day with the exception of those days when a test trial was conducted. At the end of each day all

arena walls were cleaned with disinfectant spray and thoroughly rinsed with clean water.

Pre training

Rats received two sessions of *Pre-training* in the rectangular pool where they were trained to locate a visible escape platform (striped stick attached), which changed location across trials. The purpose of this *Pre-training* was to familiarise animals with climbing onto the escape platform and encourage rats to avoid adopting a strategy of repeatedly circling around the edge of the pool. The trial commenced with the experimenter, ensuring that the rat's head faced the wall, placing the rat gently into the pool and ended when the platform was located. If the animal failed to find the platform within sixty seconds, the experimenter entered the curtained area surrounding the pool and guided the rat to the platform by holding out a hand in front of its nose. Rats were left on the platform for 20 seconds before the experimenter removed the animal from the pool, dried it with a towel and placed it back into the holding box, where it remained until the remaining four animals had each completed a trial after which the cycle was repeated until all five rats had received four trials. The midpoints of the four walls of the rectangular arena were designated as the points of release into the pool from which an animal could start the trial. The release points were assigned randomly for each trial with the constraint that four different release points and were used within a session. The orientation of the pool remained constant across all trials of *Pre-training*. The platform was moved pseudo-randomly across trials with the constraint that its position varied according to where the rat was released from, i.e. rats could not adopt a fixed motor response after release, and its centre was a minimum of 25cm from the edge of the pool.

Room Cue Task

Following *Pre-training*, rats received 14 sessions of *Room Cue* training conducted in the circular pool with unrestricted access to ambient room cues. Animals were trained to swim to (*Active*) or were placed (*Passive*) on an invisible escape platform occupying a fixed location. The platform was positioned so that its centre was 50cm from the edge of the pool along a northwest-southeast axis, either in the northwest or southeast quadrant of the pool depending on which position had been assigned to that particular animal. For rats in *Group Active* the trial commenced and terminated in a similar manner as described for *Pre-training* but animals were released from a designated point at the edge of the circular pool. For rats in *Group Passive*, however, the experimenter carried the animal to the appropriate release point, held it just above the surface of the water, and passively placed it from the point of release to the escape platform on a straight-line trajectory (see Figure 6.1). All rats were left on the platform for 30 seconds before being removed from the pool. Once removed and quickly dried, the animal was given a 30 second intertrial interval (ITI), starting immediately after removal from the platform, before commencing its second trial. This cycle was repeated until the animal had completed four trials after which it was dried and placed back into the holding box so that the next rat could begin its four trials. The experimenter continued in this fashion until all five rats had received four trials. The circular pool was divided equally into eighths so that eight cardinal compass points could be used as the points of release into the pool. The release points were assigned randomly for each trial with the constraint that eight different release points were used across two sessions (8 trials). This manipulation ensured that rats could not learn a fixed strategy from a constant release point. It was also ensured that the assignment of release points was identical

for Groups *Active* and *Passive*.

After 14 sessions of *Room Cue* training, rats received the *Room Cue* test trial on day 15, which was preceded by two additional training trials (using the south and north release points). For the test trial the platform was removed and animals were placed in the centre of the pool (from southwest release point) for 60 seconds.

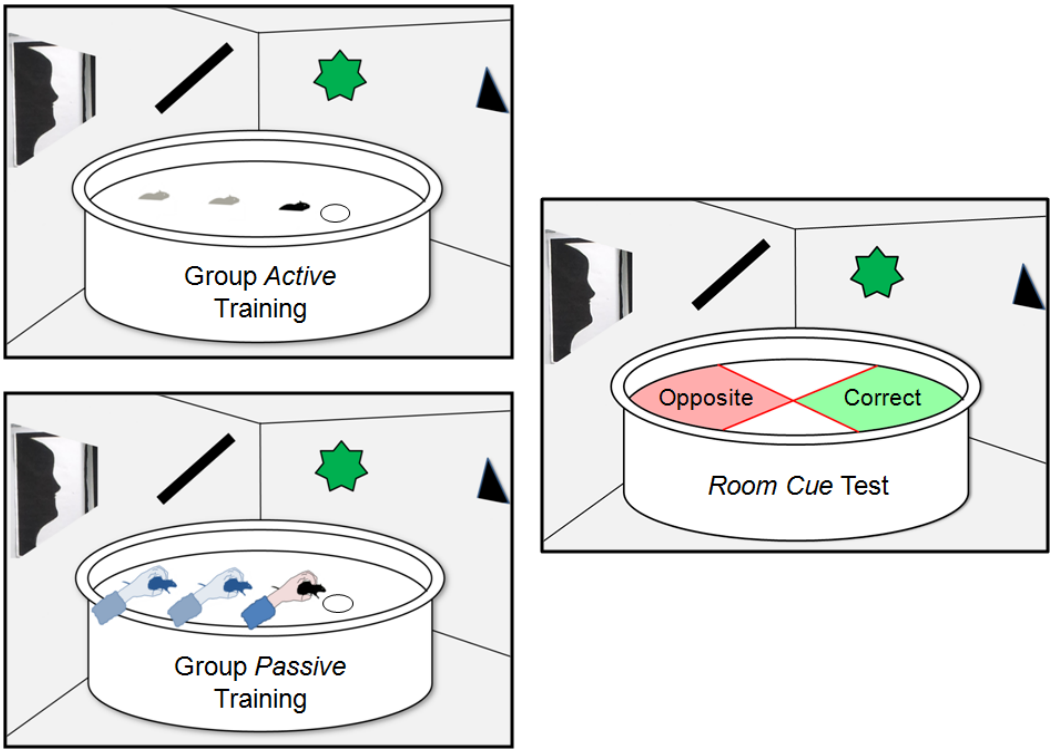

Figure 6.1. Schematic diagram of apparatus for the *Room Cue* task. A large water tank was positioned in the centre of a laboratory, which contained numerous ambient cues, such as posters and shapes on the wall. The small black circle represents an invisible escape platform. The quadrant sections depicted in the *Room Cue* Test represent notional zones used for analysis of exploration times.

Shape Task

Following the *Room Cue* task, rats received 6 sessions of *Shape* training that involved locating a hidden escape platform in one corner of the white rectangular pool. This task required rats to use the metric layout of the pool walls to locate the

platform, e.g. the platform was always found in a corner where a short wall was to the left of a long wall. The procedure was identical to that described for Stage 2 training in Experiment 11 except that rats were trained for 6 sessions.

After the 4th training trial of session 6, rats received a test trial with the platform removed. For this test, with the arena oriented in a novel position (along a north-east - south-west axis), animals were placed into the centre of the pool and allowed to swim for 30 seconds.

6.1.2.6 Performance Measures

Training

For *Room Cue* training, latencies to locate the escape platform of Group *Active* were recorded by the experimenter using a stop watch. For *Shape* training, as well as escape latencies, a first choice measure was recorded. The first choice measure was established by recording which of four circular corner zones the rat first visited after it had been released into the pool. These corner zones, drawn on the monitor screen, were approximately 30cm in diameter with the centre of each zone corresponding to the centre of the potential position of the escape platform for that corner of the rectangle. This raw first choice data could then be used to calculate the percentage of trials within a session that an animal visited the correct or rotationally opposite zone first (chance was 50%).

Extinction Tests

As described in Experiment 8, the recorded footage of each rat's swim path was tracked using Ethovision (version 3.1) software. For the *Room Cue* task, the pool was divided into equal quadrant search zones and the time rats spent in the

quadrant zone that previously contained the escape platform during training (correct quadrant) and the diametrically opposite quadrant zone (opposite quadrant) was recorded (see Figure 6.1). The time in quadrant zone data were also used to determine the ratio of time in the correct quadrant to time in the opposite quadrant. This was achieved by calculating a discrimination ratio (time in correct quadrant / time in correct + opposite quadrant) for each rat. Ethovision was also used to record rats' mean proximity to the former platform location and mean swim velocity across the entire test trial. Finally, a 'heading error' measure was recorded by using Ethovision to set up a circular zone (central exit zone) approximately 65cm in diameter, which was positioned in the centre of the circular pool. Two straight lines were then drawn, one beginning at the centre of the pool (point of release for each animal) and ending at the centre of the former platform position, and the second line also beginning at the centre of the pool and passing through the exact point where the rat departed the central exit zone. In this way, the heading error was established by calculating the angular difference between the straight line trajectory to the former platform position and the trajectory of the rats' swim path after being released.

For the *Shape* test, four circular search zones each measuring approximately 25 cm in diameter, or approximately six times the area of the escape platform, were individually positioned so that the centre of each zone corresponded to where the centre of the escape platform would have been if it had been paired with that corner. For this test, the time rats spent in the two correct corner zones, according to the geometric layout, was calculated and compared to the time rats spent in the remaining two corner zones.

6.1.3 Results

A Type I error of $p < .05$ was adopted throughout.

6.1.3.1 Histology

Figure 6.2 depicts reconstructions of the minimum (black shading) and maximum (grey shading) extent of hippocampal damage on a series of coronal sections (see also Figure 6.3 for photomicrographs of a representative hippocampal lesion). All rats belonging to Group *HPC* sustained extensive bilateral damage to the dorsal and ventral hippocampus (CA fields 1-4), the dentate gyrus and the subicular cortices. Analysis of total hippocampal tissue loss revealed a mean of 86.4% (range 80.4% - 90.3%) with a median of 86.5%. The main sparing of hippocampal tissue was observed in the most medial areas of the dorsal hippocampus. In the majority of rats there was damage to the cortical area overlying the dorsal hippocampus. This typically included partial damage to motor, visual, somatosensory, parietal and retrosplenial agranular cortices (for reports of similar extrahippocampal damage in hippocamptomized rats see: Albasser, Amin, Lin, Iordanova & Aggleton, 2012; Iordanova, Burnett, Aggleton, Good & Honey, 2009). As Albasser et al. (2012) report, the partial cortical damage (described above) left plenty of sparing in each of these areas. Following histological examination, all 13 rats were considered acceptable for inclusion in subsequent behavioural analyses.

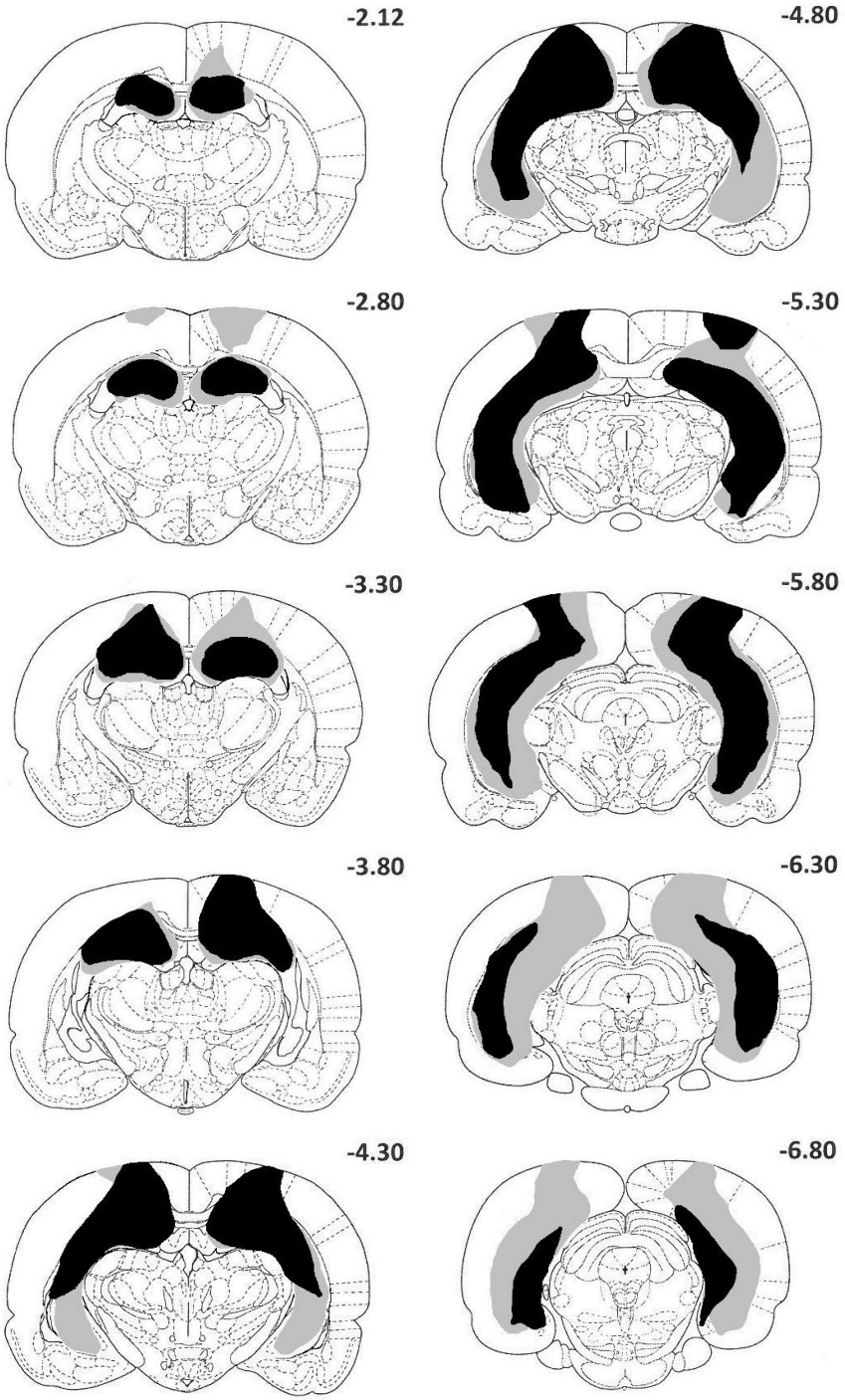

Figure 6.2. Coronal sections displaying the largest (grey shading) and smallest (black shading) amount of hippocampal tissue damage. The numbers refer to the distance behind bregma for each section.

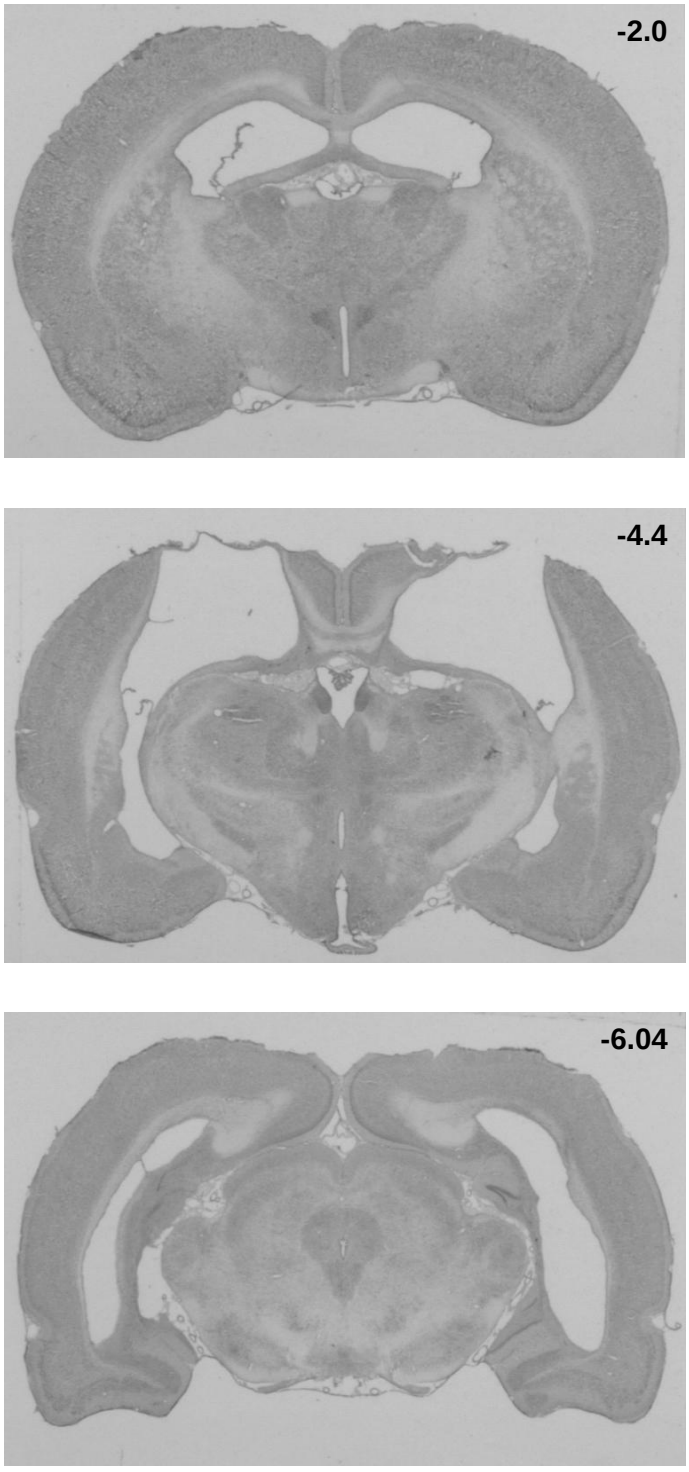

Figure 6.3. Photomicrographs of three coronal sections from a representative hippocampal lesion. The numbers refer to the distance behind bregma for each section (40 μm thick).

6.1.3.2 Room Cue Training

Figure 6.4 shows the mean escape latency for sham rats and rats with hippocampal lesions trained in the *Active* condition across 14 sessions of *Room Cue* training. An inspection of this figure reveals that Group *HPC* appeared to be consistently slower to locate the escape platform than Group *Sham*. However, a two-way ANOVA of session mean escape latencies for each rat with lesion group as the between-subjects factor and session as the repeated measure did not substantiate this observation. There was a significant main effect of session, $F(13, 130) = 5.28$, $p < .001$, but no remaining significant between- or within-subjects effects or interactions, $F\text{s} \leq 2.39$, $p\text{s} \geq .15$.

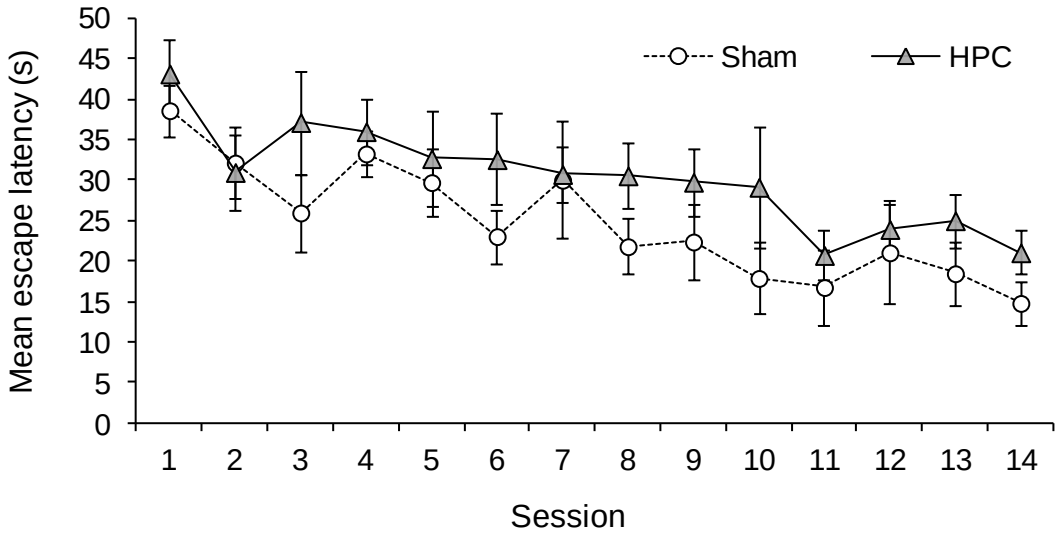

Figure 6.4. The mean (±*SEM*) escape latencies for sham operated controls (*Sham*) and rats with hippocampal lesions (*HPC*) during the *Active* training condition.

6.1.3.3 *Room Cue Test*

The upper panel of Figure 6.5 displays two exemplar swim paths for each lesion group trained in the *Active* and *Passive* condition. The former platform

position is displayed as a small circle with a dashed line. The lower panel of the same figure shows the time rats spent exploring the correct and opposite quadrants of the pool.

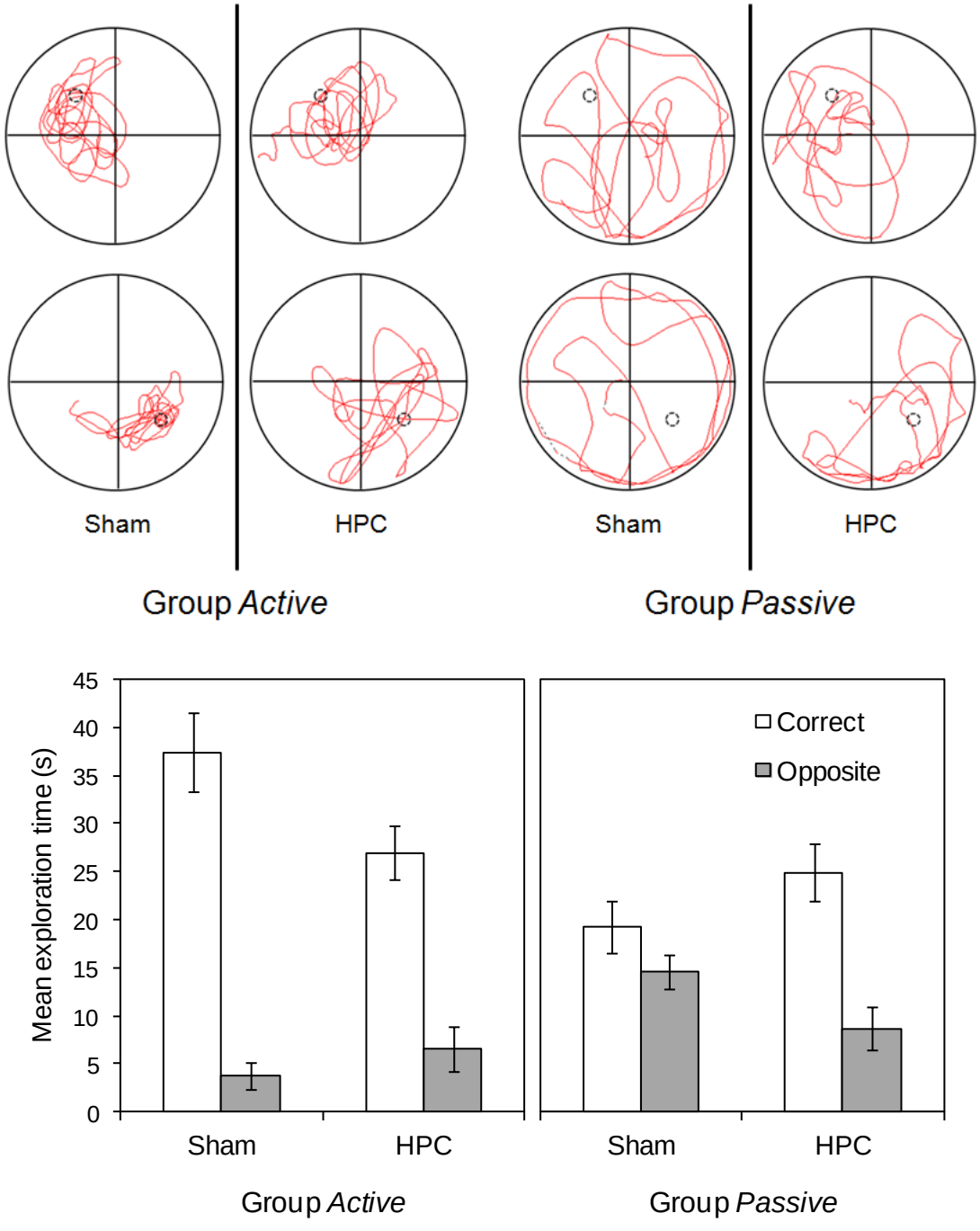

Figure 6.5. The results of the *Room Cue* test, in which the escape platform was removed. The upper panel shows two typical swim paths (one for each platform position) for each group. The lower panel illustrates the mean time (±*SEM*) rats spent in the correct (white bars) and opposite (grey bars) quadrants of the pool.

It is clear from both of these panels that all groups were able to discriminate the correct from opposite quadrant except for Group *Sham* trained in the *Passive* condition. Examination of this figure also reveals that for those animals trained in the *Active* condition, Group *Sham* spent more time in the correct quadrant than Group *HPC*. Comparing lesion groups across training conditions, it is clear that for Group *Sham*, rats trained in the *Active* condition outperformed those trained in the *Passive* condition. However, for Group *HPC* performance is very similar for rats across both training conditions.

To confirm these observations statistically a three-way ANOVA of individual times spent in each quadrant zone with lesion group (*Sham* vs. *HPC*) and training condition (*Active* vs. *Passive*) as between-subjects variables and quadrant (correct vs. opposite) as the repeated measure was conducted. The ANOVA revealed a significant main effect of training condition, $F(1, 21) = 12.10$, $p = .002$, and quadrant, $F(1, 21) = 61.8$, $p < .001$, and significant quadrant x training condition, $F(1, 21) = 14.82$, $p = .001$, and quadrant x lesion group x training condition, $F(1, 21) = 6.47$, $p = .019$, interactions.

Tests of simple main effects were conducted to examine the significant interaction between quadrant, lesion group and training condition. To achieve this, simple effects tests of each individual variable for each combination of levels of the other IV's was conducted, which used the pooled error term from the original three-way ANOVA. Because the error variance of the DV was equal across groups (Fs < 1.84, ps > .170) it was considered appropriate to use the aforementioned pooled error term. To account for the multiple comparisons, the following p values were adjusted using the Bonferroni correction. Thus, this simple effects analysis revealed a significant main effect of quadrant (time in correct > opposite quadrant) for all

groups, Fs(1, 21) ≥ 10.9, ps ≤ .003, except for Group *Sham* trained in the *Passive* condition, $F(1, 21) < 1$. As such, only Group *Sham* trained in the *Passive* condition was unable to discriminate the correct from opposite quadrant of the pool. This interaction also revealed a significant main effect of training condition for *Group Sham* on time spent in the correct, $F(1, 21) = 21.6$, $p < .001$, and incorrect, $F(1, 21) = 11.0$, $p = .003$, quadrants, with animals trained in the *Active* condition spending more time in the correct quadrant and less time in the opposite quadrant than animals trained in the *Passive* condition. There was no significant main effect of training condition for *Group HPC* on time spent in either quadrant, Fs(1, 21) ≤ 1.33, ps ≥ .26. Therefore, it would appear that during the probe trial prior training condition (*Active* vs. *Passive*) impacted heavily on the performance of sham operated rats but did not affect performance in the same way for rats with hippocampal lesions. Indeed, the performance of Group *HPC* was very similar across both training conditions. The interaction also revealed a significant main effect of lesion group on time spent in the correct quadrant (*Sham* > *HPC*) for rats trained in the *Active* condition, $F(1, 21) = 5.68$, $p = .027$, but not for the remaining quadrants and conditions, Fs ≤ 3.83, ps ≥ .064.

Table 6.1 displays the results of a training condition x lesion group ANOVA of discrimination ratios, in an attempt to make clearer the results of the test trial. Inspection of both tables, one showing the main effects and interactions (table A) and the other showing the simple main effects for the interaction (table B), reveals no significant difference in discrimination ratio scores when rats were trained actively but a significant difference for rats trained in the *Passive* condition, with Group *HPC* discriminating better than Group *Sham.* It is also apparent for Group *Sham* that discrimination ratio scores were far higher for rats trained actively than

those trained passively. The means for each group marked with subscript asterisks are significantly above the level expected by chance and the table shows that all groups' discrimination ratio scores were above chance except for sham animals that were trained passively.

Table 6.1

A. Training Condition x Lesion Group Factorial Analysis of Variance for Discrimination Ratio Scores

Source	*df*	*F*	η^2	*p*
(A) Lesion Group	1	0.45	0.01	0.511
(B) Training Condition	1	10.52	0.29	0.004
A x B (interaction)	1	4.83	0.13	0.039
Error (within groups)	21			

B. Tests of Simple Main Effects. Discrimination Ratio Scores for Lesion Groups

Training Condition	Lesion Group		Simple Effects: *F*
	Sham	*HPC*	*df* (1, 21)
Active	0.9_{**} (0.09)	0.8_{**} (0.17)	1.13
Passive	0.56 (0.15)	0.74_{*} (0.19)	4.26**
Simple Effects: *F* *df* (1, 21)	14.27**	0.57	

Note. ** $p < .01$, * $p < .05$. Standard deviations appear in brackets below means. Means with subscript asteriks are significantly above the level expected by chance (0.5)

Table 6.2 displays the results of a training condition x lesion group ANOVA of individual *mean proximity to the former platform position* scores during the test

trial. An examination of the top table (A) reveals a significant main effect of training condition and a significant lesion group x training condition interaction on mean proximity scores. Tests of simple main effects displayed in the lower table (B) shows that proximity scores were markedly lower for rats trained in the *Active* condition, or these rats searched in closer proximity to the former platform position, than rats trained passively. This table also shows that for rats trained in the *Passive* condition, *Group HPC* searched in closer proximity to the former platform location than *Group Sham*.

Table 6.2

A. Training Condition x Lesion Group Factorial Analysis of Variance for Proximity to Former Platform Position

Source	*df*	*F*	η^2	*p*
(A) Lesion Group	1	.135	0.00	0.717
(B) Training Condition	1	62.637	0.69	0.000
A x B (interaction)	1	6.391	0.07	0.020
Error (within groups)	21			

B. Tests of Simple Main Effects. Proximity Scores for Lesion Groups

Training Condition	Lesion Group		Simple Effects: *F*
	Sham	*HPC*	*df* (1, 21)
Active	70.65 (30.07)	92.85 (18.58)	2.25
Passive	177.89 (12.54)	148.16 (33.54)	4.35*
Simple Effects: *F* *df* (1, 21)	52.58**	15.06**	

Note. ** $p < .01$, * $p < .05$. Standard deviations appear in brackets below means.

In order to establish if any difference in performance between lesion groups was attributable to differences in motor function, or swim speed, an analysis of rats' mean swim velocity during the test trial was conducted. A one-way ANOVA of individual mean velocities with lesion group as the between-subjects factor revealed no significant effect of lesion on swim speeds, $F(1, 23) < 1$, (*Sham*: $M = 67.7$, $SD = 10.6$, *HPC*: $M = 70.2$, $SD = 11.1$).

Figure 6.6 displays the heading error for each group after being released into the centre of the pool. The striking finding from this figure is that rats with hippocampal lesions trained passively initially headed on a more direct trajectory towards the former platform position than the remaining groups. Sham animals trained passively and *HPC* animals trained actively exhibited the greatest heading error.

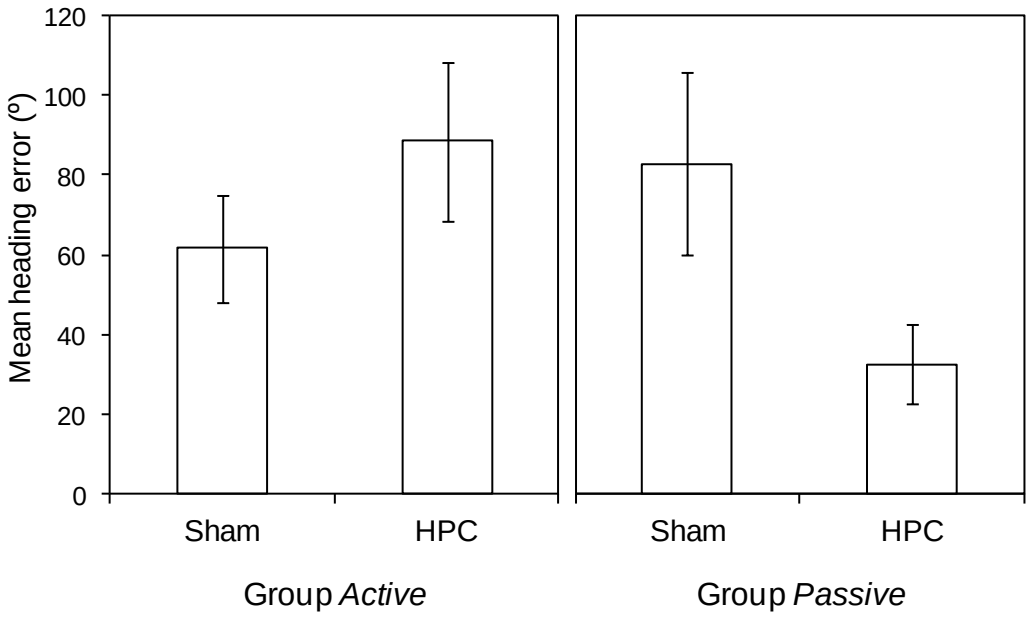

Figure 6.6. The mean heading error (±*SEM*) after release into the centre of the pool during the *Room Cue Test* for each lesion group trained actively and passively.

A two-way ANOVA conducted on individual heading error angles with lesion group and training condition as between-subjects variables revealed a significant lesion group x training condition interaction, $F(1, 21) = 5.44$, $p = .030$, but no significant main effects, $F < 1$. Tests of simple main effects to investigate this interaction revealed a significant main effect of lesion group for animals trained in the *Passive* condition, $F(1, 21) = 5.33$, $p = .031$, with greater heading error for Group *Sham* than Group *HPC*, but not for those trained in the *Active* condition, $F(1, 21) < 1$.The interaction also revealed a marginally significant effect of training condition for Group *HPC*, $F(1, 21) = 3.92$, $p = .061$, with rats trained in the *Active* condition displaying greater heading error than rats trained in the *Passive* condition, but not for Group *Sham*, $F(1, 21) = 1.77$, $p = .20$.

6.1.3.4 Shape Training

Figure 6.7 shows the mean escape latencies (upper panel) and the mean percentage of trials in a session when animals first visited a correct corner (lower panel) across 6 sessions of *Shape* training. An inspection of this figure reveals that throughout *Shape* training the escape latencies were shorter for sham rats than for rats with hippocampal lesions in both Group *Active* and Group *Passive*. It is also clear from this figure that, overall, the latencies were shorter for rats in Group *Passive* than Group *Active*. The percentage of correct first choice data shows that sham rats were more accurate in discriminating the correct from incorrect corners of the rectangle than *HPC* rats if these animals had previously been trained in the *Active* condition during the *Room Cue* task. For rats previously trained in the *Passive* condition, there was no difference in choice accuracy between sham rats and rats with hippocampal lesions. To confirm this characterisation of the data, a lesion group x training condition ANOVA, with session as the repeated measure, was conducted

on the session mean latencies for each rat and it revealed a significant main effect of lesion group, $F(1, 21) = 16.2$, $p = .001$, training condition, $F(1, 21) = 8.61$, $p = .008$, and session, $F(5, 105) = 19.5$, $p < .001$.

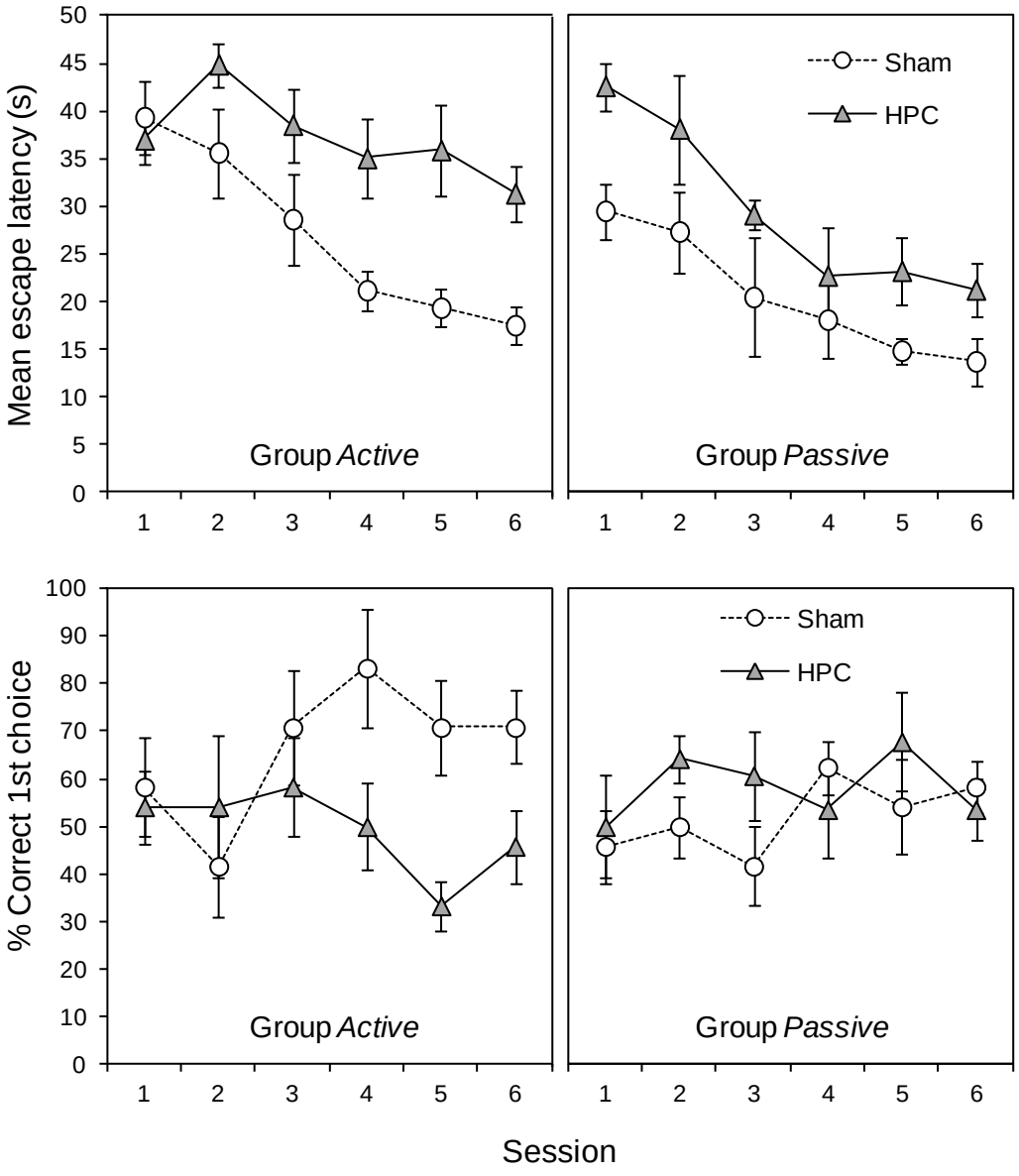

Figure 6.7. The mean (+*SEM*) escape latencies (top panel) and percentage of correct first choices (bottom panel) for sham rats (white circles) and rats with hippocampal lesions (grey triangles) previously trained either in Group *Active* (left panels) or Group *Passive* (right panels) across 6 sessions of *Shape* training in a white rectangle.

A similar ANOVA of percentage of correct first choices using the session means of individual rats revealed a significant lesion group x training condition interaction, $F(1, 21) = 4.72$, $p = .041$, and a significant session x lesion group interaction, $F(5, 105) = 2.39$, $p = .043$. Subsequent simple main effects analysis to investigate the interaction between lesion group and training condition revealed a significant main effect of lesion group for those rats previously trained in the *Active* condition, $F(1, 21) = 4.82$, $p = .040$, with choice accuracy higher for Group *Sham* than Group *HPC*, but not for those rats previously trained in the *Passive* condition, $F(1, 21) < 1$. There was no significant effect of training condition for either Group *Sham* or *HPC*, $Fs(1, 21) \leq 3.35$, $p \geq .082$.

6.1.3.5 Shape Test

Figure 6.8 shows the mean time each lesion group spent in the correct and incorrect corner zones of the rectangular pool during the test trial. It is clear from this figure that sham rats discriminated the correct from incorrect corners better than rats with hippocampal lesions in both Group *Active* and Group *Passive*. This figure also shows that sham rats previously trained in the *Active* condition during the *Room Cue* task performed considerably better in the *Shape* test than sham rats previously trained in the *Passive* condition. To confirm these observations a lesion group x training condition ANOVA of individual exploration times with corner zone as the repeated measure (correct vs. incorrect) revealed a significant main effect of corner zone, $F(1, 21) = 45.6$, $p < .001$, and significant corner zone x lesion group, $F(1, 21) = 6.15$, $p = .022$, and corner zone x training condition, $F(1, 21) = 4.37$, $p = .049$, interaction.

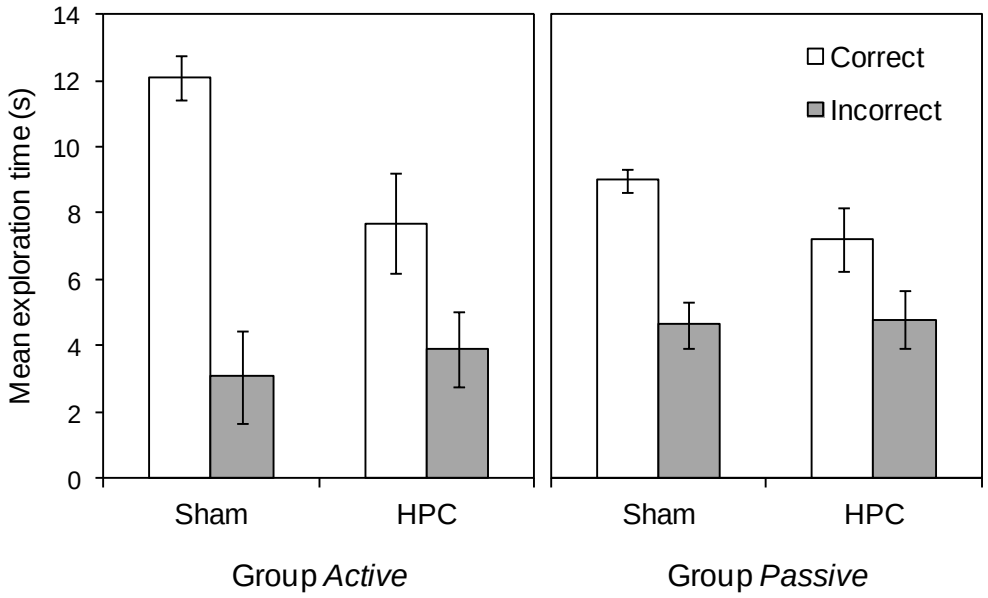

Figure 6.8. The mean time (±*SEM*) rats spent in the correct (white bars) and incorrect (grey bars) corners of the rectangular pool during the *Shape Test*.

All remaining main effects and interactions were non-significant, *F*s ≤ 3.29, *p*s ≥ .084. Subsequent tests of simple main effects revealed that sham rats spent more time in the correct corners of the rectangle than rats with hippocampal lesions, $F(1, 21) = 9.94$, $p = .005$, but there were no lesion group differences in time spent in the incorrect corners, $F(1, 21) < 1$. Similar tests to examine the corner zone x training condition interaction revealed no significant difference in exploration between Group *Active* and *Passive* in either the correct or incorrect corner zones, *F*s$(1, 21) \leq 3.35$, *p*s ≥ .081, but discrimination, or time spent in the correct vs. incorrect zones, was more marked for Group *Active*, $F(1, 21) = 37.7$, $p < .001$, than for Group *Passive*, $F(1, 21) = 11.3$, $p = .003$. Thus, from these results it can be concluded that, irrespective of prior training condition, sham rats outperformed rats with hippocampal lesions in a *Shape* task. However, prior training experience in a separate *Room Cue* task affected performance, particularly for sham-operated animals.

6.1.4 Discussion

The results of the current experiment demonstrate in a standard MWM task that rats with bilateral hippocampal lesions are capable of learning the location of an escape platform with reference to ambient room cues. This finding is consistent with reports from other studies, mentioned above, of spared place learning in rats with hippocampal damage following certain training procedures. For example, Morris et al. (1990) using a similar MWM task, demonstrated that rats with hippocampal lesions were impaired in their search accuracy after 7 sessions of training but that this impairment disappeared after a further 7 sessions of training (see also Bast, Wilson, Witter & Morris, 2009 for a similar result). In the current experiment animals were trained in a similar fashion (*Active* condition) for 14 sessions and although rats with hippocampal lesions could discriminate the correct from opposite quadrants of the pool, which indicates a place response was acquired, they were impaired relative to sham-operated control rats. In a second condition, a separate group of rats was trained in a modified version of the MWM task that involved animals being placed passively onto the escape platform (*Passive* condition). The present findings are the first to demonstrate spared place learning in rats with hippocampal lesions following passive training. Indeed, the results of a probe test revealed that hippocampal lesions significantly enhanced accurate search behaviour following passive training compared with shams. These results hold important implications for the function of the hippocampus in spatial learning.

Given that rats with hippocampal lesions, trained in either the *Active* or *Passive* condition, were capable of using allothetic room cues to accurately locate a target area, the current study opposes cognitive map theories suggesting that the hippocampus is the sole locus for allothetic processing (e.g. O'Keefe & Nadel,

1978). Thus, if the hippocampus is not critical for allothetic processing, it raises the question of what it is responsible for during navigation. One proposal, discussed in the Introduction, is that it is critical for path integration (PI) (Whishaw, 1998). The current results indirectly lend support to this notion but it must be acknowledged that many processes are involved during navigation, and without further experimentation any interpretation is somewhat speculative. In both training conditions, rats were provided with allothetic room cues to aid navigation, but only rats trained in the *Active* condition could potentially draw upon ideothetic cues. Therefore, according to PI theory, it is predicted that any impairments in navigation produced by hippocampal damage will only materialise in a task requiring the use of ideothetic cues. Consistent with this prediction, hippocampal lesions impaired rats' use of room cues in the *Active* condition only.

A second prediction posited by the PI theory is that performance by rats with hippocampal lesions should be relatively unaffected by the removal of ideothetic cues given that the path integrator, which would normally process ideothetic input, has been removed. The present results are also consistent with this prediction as the probe trial revealed that navigational performance in rats with hippocampal damage was very similar across both training conditions despite those animals trained in the passive condition being deprived of ideothetic cues. However, it must be pointed out that the results did provide several indicators, such as mean proximity to the former platform position, that navigational performance by rats with hippocampal damage was more accurate in the *Active* than *Passive* condition. It is difficult, though, to pinpoint a reason for this finding given the fundamental differences between each training condition. One exemplar reason could be that rats in the *Active* condition suffered less from generalization decrement during the probe trial. The most striking

finding to be observed, however, was the total inability of control rats trained in the *Passive* condition to exhibit any search preference during the probe trial.

This inability in sham animals and the fact that hippocampal lesions significantly enhance navigational performance following passive training provides evidence for the emergence of competition between navigational systems in their control over behavioural expression (Poldrack & Packard, 2003). More specifically, the results indicate that in a latent learning MWM task the hippocampus can inhibit processing by another navigational system. If one remains with the stance that the path integrator is located within the hippocampus, as evidence highlighted above suggests, rats with complete removal of the hippocampus will be uninhibited by a PI system in their processing of allothetic cues, while sham animals could, potentially, be faced with competition between a PI system and an allothetic system. For example, during training, rats in the *Passive* condition were carried to various locations within the room and placed onto the escape platform from one of eight release points spaced equally around the circumference of the pool wall. It is possible that this procedure had a more disruptive effect on the learning of room cues for sham-operated rats, with an intact path integration system, than for rats with hippocampal lesions. However, if this were the case it could be argued that sham rats in the *Active* condition would also be more disrupted by this release procedure relative to rats with hippocampal lesions, but the training data suggests that, if anything, sham rats perform better than rats with hippocampal lesions. Admittedly, though, this argument must be treated with caution given the suite of potential factors that could affect navigational performance in Group *Active* aside from those related to differing release points (see Experiment 10 for example).

A second interpretation for the poor navigational performance in sham rats

trained passively is that competition between navigational systems occurred during the probe test. Bearing in mind that this probe trial was the first opportunity rats had to actively explore the environment, it is possible that the stream of ideothetic input, generated by the animals own movements, competes for behavioural expression with the previously learned allothetic information. Indeed, several studies, including this one, have emphasised the predominant role swimming plays in place learning during a MWM task, compared to the modest contribution of passive placement on the platform (Chew et al., 1989; Sutherland & Linggard, 1982, Sutherland et al., 1987; Whishaw, 1991). Therefore, it is conceivable that any newly created ideothetic cues dominate behavioural control over previously learned allothetic cues. The enhanced performance by rats with hippocampal lesions could again reflect the lack of interference from a path integration system, but in this case during the probe test trial.

One explanation for the present finding that rats with hippocampal lesions exhibit spared spatial learning abilities is that the navigational performance observed does not reflect true place learning, but rather a learned set of egocentric response rules. Specifically, rats could have learned a specific motor pattern from each of the eight release points. However, the results of this experiment do not support this explanation for a number of reasons. First, had animals developed a habit unique to each release point it would have been impossible to use one of these fixed responses from a novel release point such as the centre of the pool, which was used during the probe trial. However, the angle of departure measure revealed no impairment by rats with hippocampal damage in heading to the former platform position. Indeed, for rats trained passively, hippocampal lesions significantly reduced heading error. Second, there were no local cues to mark the position of each release point so

animals would still need to rely on distal room cues in order to accurately distinguish one release point from another, which in some cases were close together, before employing an egocentric response rule. Third, for rats with hippocampal lesions trained passively it would be extremely difficult if not impossible to acquire a set of egocentric response rules when deprived of any ideothetic cues during training and, yet, these rats still exhibited accurate search behaviour during the probe trial. A second explanation for the place learning observed in rats with hippocampal lesions is that these animals simply used a salient, polarising room cue to base their response upon. However, although in theory possible, the results of the probe trial revealed that the search behaviour of rats with hippocampal lesions was finely calibrated, which would require the use of at least one other room cue to accurately triangulate the target location. In essence, the present findings, along with the findings from Experiment 11, indicate that rats with hippocampal lesions acquired a place response.

The results reported highlight the importance of ideothetic, or motion, cues in the normal processing of spatial information. Previous evidence attempting to demonstrate that rats are capable of learning the location of an escape platform following passive placement training is convoluted with a number of studies reporting modest latent learning abilities (Jacobs, Zaborowski & Whishaw, 1989a; Keith & McVety, 1988; Sutherland & Linggard, 1982; Whishaw, 1991). However, even this scant evidence lacks reliability due to design flaws, insensitive behavioural measures and speculative interpretation. To further compound matters, Jacobs, Zaborowski, and Whishaw (1989b) later retracted their stance that rats were capable of latent learning after replicating the original study in a series of experiments, all of which revealed no evidence of place learning following passive training. Recently,

however, Horne, Gilroy, Cuell, & Pearce, (2012) provide convincing evidence that animals are able to discriminate between corners of a distinctively shaped pool following passive placement training. Thus, it appears that the reliance on ideothetic cues by rats varies, but whether as a function of procedural aspects, such as the stability of the location from which the rat is passed onto the platform, or the type of spatial information encoded is open to debate.

On a separate note, it has been suggested that hippocampal lesions produce deficits during navigation tasks that are not necessarily related to the processing of spatial information. That is, rats with hippocampal damage are unable to flexibly integrate non-spatial information. However, if the non-spatial requirements of the task are made easier, then these rats show evidence that they have formed a place response (Day et al., 1999; Ramos, 2002, 2010; Whishaw & Jarrard, 1996). Using this logic, it was predicted that rats with hippocampal lesions in the current experiment would be less impaired at forming a place response if they were passively placed onto the escape platform as opposed to having to find the platform of their own accord. However, the present results do not offer any reliable evidence in support of this argument. Rats with hippocampal lesions trained actively were impaired in their search behaviour but these animals still exhibited proficient spatial abilities. However, for rats trained in the *Passive* condition it is difficult to compare performance among groups given that sham rats performed so poorly. It is not so much of a case that passive placement eradicated any non-spatial deficits in lesioned animals, but rather eradicated any spatial abilities in sham-operated controls.

Finally, the results of the *Shape* task demonstrated that rats with hippocampal lesions were impaired at using the geometric properties of a distinctively shaped pool in order to locate an escape platform. A finding consistent with previous experiments

demonstrating that the hippocampus is critical to the encoding of such geometric information (Jones et al., 2007; Lever et al., 2002; McGregor et al., 2004; Pearce et al., 2004; Sakamoto, & Okaichi, 1996). This impairment in shape-based learning by rats with hippocampal damage also provides a behavioural measure of the effectiveness of the lesions.

A second finding from the *Shape* test was that prior training experience in the *Room Cue* task severely affected performance, with this effect particularly prominent in sham rats. This result is surprising as, even though rats were provided with 24 trials of active *Shape* training prior to the *Shape* test, the training conditions that Group *Passive* experienced in a separate *Room Cue* task still disrupted performance. One explanation could be that during the *Room Cue* task, the act of swimming was never paired with the escape platform for Group *Passive* and this disrupted subsequent acquisition of the *Shape* task in which rats were required to swim to the platform. This result, once again, underlines the importance of movement in acquisition of a navigation task, but in this instance, during a previous task. As with the findings of the *Room Cue* task, it would appear that poor performance during spatial memory tasks may not necessarily reflect an inability to learn about spatial cues, per se, but is closely connected to the procedural aspects of the task.

7 Conclusions

The series of experiments reported in this thesis were designed to investigate various components of spatial memory in the rat. Given that rodents lack the ability to use a language but have been shaped by Natural Selection to encode and recall spatial information, tasks measuring their spatial memory abilities provide a powerful analytical tool to heighten our understanding of general learning mechanisms in both animals and humans. However, to use this tool effectively it is important to understand the strategies and neural substrates involved during such tasks. The research undertaken within this thesis attempted to contribute to this understanding by adopting a framework in which the analysis of behavioural and brain systems was integrated. The key objective was to assess whether different navigational strategies or the use of different frames of reference during spatial memory tasks were learned about independently or whether one strategy or cue type interacted with others.

7.1 Is Geometry Learning Special?

It has been proposed that for rats to reorient, or establish heading, the use of geometric information is obligatory, while the use of informative non-geometric information is ignored (Cheng, 1986; Margules & Gallistel, 1988). Although subsequent evidence questioned this primacy for geometry by demonstrating that non-geometric cues could interact with geometric cues over time, Experiments 1 & 2 are the first to demonstrate that rats are able to rapidly encode discrete non-

geometric cues in conjunction with geometric cues. This finding is important as it weakens the argument that geometry may only come to have primacy in navigation, and be processed independently of non-geometric information, when animals first reorient themselves (e.g. Cheng & Newcombe, 2005; see also Sutton, 2009). The results of Experiments 1 & 2 alongside recent evidence that the learning of discrete landmark cues can compete with the learning of geometric cues (Kosaki, Austen & McGregor, 2013) opposes several theories, such as Wang and Spelke's (2000), claiming that environmental geometry is processed in a different fashion to learning based on other visual features.

If the argument stands that the principles underlying geometry learning are no different to those observed in other forms of learning, then it should be possible to observe associative cue competition between geometric and non-geometric cues. Experiment 8 failed to reveal overshadowing of geometry learning by discrete landmarks. However, the results clearly demonstrated that predictive landmark cues facilitated learning based on geometry. This finding contradicts the proposal by Cheng (1986) and Gallistel (1990) that geometry learning is impervious to other visual cues but, placed alongside the results of various other studies, also raises the question of what determines whether visual features in the environment facilitate or encumber learning based on geometry. Two recent studies have addressed this question and provided evidence that associative cue competition effects, such as overshadowing, vary as a function of the relative salience of competing cues (Mackintosh, 1976). Both Kosaki et al. (2013) and Horne and Pearce (2011) showed that highly salient landmarks overshadowed learning about geometry, while landmarks of lower salience either failed to overshadow or actually potentiated learning about geometry.

A second approach to investigate whether animals are capable of integrating geometric with non-geometric information is to identify evidence of an associative link (within-compound association) between these different frames of reference. In this way, Experiment 7 exposed animals to an object-corner configuration during a sample phase, and in a subsequent phase devalued (via extended habituation) only the object that was previously presented as part of a compound. If, during the sample phase an associative link had formed between the object and the corner in which it was placed, then it was predicted that by devaluing the object, the corner it had previously been associated with would also be devalued, even though the corner cue had not been directly devalued (Rescorla & Cunningham, 1978). The results showed no evidence for the presence of within-compound associations (WCAs) between a geometric and non-geometric cue. Potential reasons for this failure to observe WCAs in Experiment 7 have already been discussed with the nature of novel object recognition tasks and the rats' prior experience likely influencing the results. It is interesting to note that a recent study using a very similar design to investigate the presence of WCAs between corners and discrete landmarks in a water maze task, which promotes high motivation and rapid learning in animals (Hodges, 1996), revealed that the revaluation of landmark cues affected learning based on geometric cues following a training schedule in which both cue types were presented in compound (Austen, Kosaki & McGregor, 2013; see also Whitt et al., 2012 for a similar finding using wall patterns and objects in a NOP task similar to that used in Experiment 7).

Overall, the results reported in this thesis and other recent studies indicate that geometry learning is governed by the same universal principles observed in other forms of learning and across a wide range of species. Moreover, learning based

on geometric information interacts with learning based on other discrete visual features such that animals can integrate these different frames of reference to facilitate navigation. This certainly does not rule out the possibility that, under certain testing conditions, animals come to rely more on geometric information than other visual features, but the view that geometry learning is processed independently and is impervious to learning based on non-geometric information is weakened by recent findings.

7.2 Sex Differences in Spatial Learning

As described above, one approach to test whether animals acquire information based on different frames of reference independently and in parallel is to examine whether or not different types of cues compete for associative strength. It has also been described that one critical factor contributing to the emergence of cue competition effects is the relative salience of competing cues. Recent evidence has suggested that sex differences in cue preference, which is equated to a sex difference in the perceived salience of different cues, affects the degree to which these different cues interact (Rodriguez et al., 2011). Experiment 8 investigated this line of inquiry by looking at whether landmark cues would overshadow learning based on geometry to a different extent in male than in female rats. The results revealed that geometry learning was potentiated by the presence of informative landmarks, the extent of which was equal for both male and female rats. This result opposes a separate finding that landmarks overshadow geometry learning in female rats but fail to do so in male rats (Rodriguez et al., 2011). One potential reason for this disparity in results is that the design of Experiment 8 ensured that any perceptual changes to the test environment, not necessarily related to memory function, were matched for both

experimental and control groups, while in Rodriguez et al.'s (2011) experiment this was not the case. It is difficult to argue with certainty that this generalization decrement was the sole reason for the contradictory results but these findings serve to underline the importance of ensuring that any extraneous variables are controlled for.

Despite Experiment 8 revealing no sex differences in the overall amount learned about geometric and landmark cues, there was a suggestion that for female rats, acquisition of the task was facilitated when animals were trained in compound with predictive landmarks and geometric cues compared to animals that were trained with only predictive geometric cues. Therefore, Experiment 9 examined whether changes to the relative validity of target geometric and landmark cues would differentially affect performance of male and female rats during training trials. It was predicted that male rats would perform better than female rats in a condition in which the target geometric cue continued to reliably signal reward while the target landmark cue was rendered unreliable. Conversely, it was predicted that females would perform better in a condition in which the target landmark cue continued to be reliable and the target geometric cue was rendered unreliable. The results did not follow expectation and revealed no sex differences in performance when the geometric cue remained reliable, while males outperformed females when the landmark was the only reliable frame of reference. However, the results indicated that the inability of female rats to swim directly to the correct landmark cue could have been an artefact of thigmotaxis. The critical finding from the final test trials was that males were superior to females both in their use of geometric cues and landmark cues following a training procedure involving a change to the reliability of one of these cues.

In summary, the results of Experiment 8 provided no support for the notion that innate sex differences in the perceived salience of geometric and landmark cues altered the degree to which these different cues interact and control spatial behaviour. In a broad sense, one interpretation of the results of Experiment 9 is that male rats were more flexible in responding to relevant cues following changes to the training environment and / or the relative validity of different cues. This lack of flexibility in female rats could be related to these animals adopting more habit-based behaviours, a claim supported by the fact that female rats tended to turn in the same direction after being released into the pool when compared to male rats. It is noteworthy that evidence both in rodents (Kim, Lee, Han & Packard, 2001) and humans (Schwabe et al., 2007; Scwabe & Wolf, 2009) suggests that habit-based behaviour is modulated by stress levels, with high levels of anxiety leading to a habit-based strategy at the expense of goal-directed learning. This would certainly fit with the current results as it was found both in Experiment 8 and 9 that female rats displayed higher levels of thigmotaxis which has been shown, through pharmacological (Treit & Fundytus, 1988) and hormonal (Beiko et al., 2004) studies, to be a reliable indicator of anxiety.

Beiko et al. (2004) also found greater thigmotaxis in female rats when compared to male rats during a Morris water maze task and suggested that sex differences in navigational performance during such a task could be more related to differential stress responses rather than any difference in spatial cognitive abilities. The authors also suggested that sex differences in performance may only emerge early on in training when enhanced stress responses are at their greatest in naive female rats. The results of Experiments 8 and 9 do not support this argument as females displayed a proclivity for thigmotaxis during the test trials at the end of

training. Whether a change of context and absence of the platform during the test trial or the development of a more habit-based strategy is responsible for the heightened thigmotaxic tendencies observed in female rats is open to debate but the current findings revealed that irrespective of any sex differences in swim patterns, both male and female rats could accurately locate a hidden goal in various tasks using both the shape of their environment and the landmarks contained therein.

7.3 The Neural Substrates of Spatial Learning Components

7.3.1 Egocentric vs. Allocentric Strategies

A large body of evidence supports the assertion that animals are able to navigate by reference to either an egocentric reference frame or an allocentric reference frame depending on whether a point of interest is anchored to the animal's body or to external environmental cues. However, as Burgess (2006) points out, it is difficult to dissociate the contribution of these two frames of reference using only behavioural studies because it can often be argued that the presence of an allocentric representation can equally be accounted for by appealing solely to the formation of an egocentric representation (Bennett, 1996). Much debate has also surrounded the issue of how egocentric and allocentric navigational strategies interact. Does each frame of reference work together in a cooperative manner? Do these strategies simply switch from one to the other depending on the sensory information available, or is this switch dependent on the time course of training? (see Wang, 2012; Gramann, 2013; Burgess, 2006, for a more thorough discussion).

Given the drawbacks of a purely behavioural approach to dissociating navigational strategies, several studies, both in humans and non-human mammals,

have adopted a neurobiological approach and demonstrated that egocentric and allocentric strategies are sub-served by distinct neural structures (Bohbot, Iaria & Petrides, 2004; Iaria, Petrides, Dagher, Pike, & Bohbot, 2003; Packard & McGaugh, 1996; White & McDonald, 1993). More specifically, these studies showed that the hippocampal and striatal system are critical for allocentric and egocentric learning, respectively. To investigate this line of inquiry further, Experiments 10 & 11 were designed with the aim of providing a task that rats could solve using either an allocentric or egocentric reference frame. The performance of rats with lesions to the hippocampus and of rats with lesions to the dorsolateral striatum was compared to that of sham-operated controls. If, during these experiments, damage to the hippocampus impaired allocentric learning but spared egocentric learning, and damage to the dorsolateral striatum impaired egocentric learning but spared allocentric learning (double dissociation), it would be logical to conclude that these two frames of reference were processed independently by distinct areas of the brain and rely on different sources of information.

In Experiment 10 rats were required to use the colour of the walls to successfully locate the hidden goal. This could be achieved by using the structural relationship between black and white walls (allocentric solution), i.e. the platform is located in a corner where the black wall is to the right of a white wall, or by forming a response rule to a single wall of a particular colour (egocentric solution), i.e. the platform is to the left-hand end of a black wall. A test was conducted which pitted the allocentric solution against the egocentric solution. The results suggested that rats either with damage to the hippocampus or dorsolateral striatum searched for the platform by reference to the structural relationship between black and white walls (allocentric solution). Critically, there was no difference in performance between rats

with either type of lesion and sham-operated controls. Thus, the findings suggest that an allocentric strategy, if it can be defined as such, took precedence over an egocentric strategy in normal animals and damage to the hippocampus did not impair rats' ability to discriminate the structural properties of a coloured pattern, which is contrary to the claims of certain authors (Aggleton & Pearce, 2002; see also Kroll, Knight, Metcalfe, Wolf, & Tulving, 1996).

The task reported in Experiment 11 required rats to locate a hidden goal by either discriminating between two beacons (cue response solution) and /or by using a constellation of room cues (allocentric solution). Following training, rats received two separate tests to assess how much they had learned about the cue response and allocentric solutions. It was expected that rats with damage to the hippocampus would be impaired at using room cues (allocentric solution) but not beacons (cue response solution) and rats with dorsolateral striatum damage would display the reverse pattern of impaired and spared abilities. The results revealed no impairment of either strategy following lesions to the hippocampus or dorsolateral striatum. However, any impairments in lesioned rats may have been masked by a disruption to the performance of control rats. If, for example, control animals had adopted a dual strategy that involved the integration of both allocentric and landmark reference frames, then it is difficult to compare the performance of these animals with lesioned rats during a test trial in which one reference frame remained and the other was removed. For example, during the landmark test trial (cue response), rats with damage to the dorsolateral striatum could have been impaired at using landmarks, but the performance of control animals could have been disrupted following the removal of room cues even though these rats were more than capable of using landmark information on its own. The findings supported this interpretation by

revealing that control rats were more accurate than rats with either hippocampal or *DLS* damage at locating a hidden goal when both landmarks and room cues could be used in conjunction with one another. However, when only landmark cues could be used, hippocampal damage enhanced performance, and when only room cues could be used, dorsolateral striatum damage enhanced performance. Taken together, these results suggest that normal functioning rats integrate proximal landmark and allocentric reference frames in a cooperative fashion, but these reference frames, which are processed by distinct neural structures, can, at some level, compete for control over behavioural expression (White, 2009; White & McDonald, 2002).

7.3.2 The Hippocampus: Getting There or Knowing Where

An important finding from Experiment 11 was that when rats with hippocampal damage were trained to locate a hidden goal, from various starting positions, by reference to both proximal landmarks and distal room cues, they were as proficient as control rats at locating the target location when the proximal landmarks were no longer present in the environment. This finding, which is in keeping with several other studies showing that, after certain manipulations to the training schedule, rats with hippocampal damage can acquire a place response (e.g. Day et al., 1999; Morris et al., 1990; Whishaw & Jarrard, 1996), raises the important question of what role the hippocampus plays in navigation. Given the complexity of the hippocampus and its vast array of associated projections it is not surprising that this structure has been implicated in many cognitive functions. In regard to navigation, a popular view is that the hippocampus is critical for the formation of an allocentric reference frame, or a cognitive map (O'Keefe & Nadel, 1978), while others have suggested that this structure is less important for cognitive mapping and

more involved in the processing of an egocentric strategy known as path integration (e.g. Whishaw, Cassel & Jarrard, 1995). However, as has previously been discussed, it is difficult to determine from behavioural observations which navigational strategy animals are employing and so it is therefore difficult to infer which strategy has been impaired following brain damage. One illuminating study that attempted to address this issue conducted a task in which rats were blindfolded so that the use of an allocentric strategy based on visual cues would have been impossible and, therefore, a strategy based on the rats' own prior movements (path integration) was required. The results revealed that, unlike control rats, rats with hippocampal damage were unable to use a path integration strategy to return to a target location (Whishaw & Maaswinkel, 1998). This is an important finding, but it has yet be investigated what effect hippocampal damage has on navigational performance when allocentric information is available but movement cues, essential for path integration, are removed. Accordingly, Experiment 12 was designed to investigate this line of inquiry.

In Experiment 12, rats were trained to locate a hidden platform occupying a fixed position with respect to distal room cues. For all animals a trial commenced from one of eight different starting positions. In the *Active* condition, rats were required to swim to the hidden platform, and in the *Passive* condition, rats never moved to the platform of their own accord but were passively placed onto it. At the end of training, a probe trial was conducted with the hidden platform removed and all rats were required to swim in search of the platform. If the hippocampus is critical for the encoding of distal room cues it was expected that rats with severe damage to this structure would be impaired at a standard room cue task irrespective of whether self motion cues were available. However, if the hippocampus is predominantly

involved in path integration, it was expected that rats would be impaired in a task requiring the encoding of movement cues (*Active* condition) but not in a passive learning task (*Passive* condition).

The results revealed that hippocampal damage impaired performance of rats trained in the *Active* condition but enhanced performance of rats trained in the *Passive* condition. These findings hold important implications for the function of the hippocampus and support the idea that this brain region is not solely responsible for encoding a representation of place based on distal cues (cognitive map), but is heavily involved in a path integration process. Like other empirical evidence, these data also highlight the importance of mobile animals being allowed to explore their environment in order to accurately process spatial information (e.g. Sutherland et al., 1987; Sutherland & Linggard, 1982). The inability of control rats and the ability of rats with hippocampal damage to latently learn about distal room cues appeals to the existence of competition in normal functioning animals between navigational strategies that are based on different sources of information. However, it is not possible to infer from the present results when and how this competition takes place. Are sham animals that are passively trained receiving interference during acquisition of the task or during the probe trial?

One potential explanation for the poor performance of sham rats that were trained passively is that these animals were more disrupted during training by being carried to various starting points and passed onto the platform than rats with hippocampal damage. Or, put another way, perhaps hippocampal damage reduced the interference during acquisition of the task. A second explanation is that sham rats were more disrupted during the probe trial, which was the first opportunity they had to move around the environment, than rats with hippocampal damage. If both control

and lesioned animals had passively acquired an allocentric representation of the room, but control rats received ideothetic input, generated from swimming around during the probe test, then it is possible for competition between allothetic and ideothetic systems to occur. If the argument stands that the path integrator resides in the hippocampus and is therefore disabled in rats with hippocampal lesions, then any competition between ideothetic and allothetic inputs during the probe trial may have been eradicated for these animals.

In an attempt to test which explanation is most valid, a follow up experiment is required which seeks to attenuate the interference normal rats receive either during training or during the probe trial. The training conditions would be identical to the *Passive* condition in Experiment 12 except that one group of animals (Group *Box*) would be passed onto the platform in a light tight box in order to reduce any visual interference caused by being carried to the different starting positions around the room. A second group (Group *Experience*) would receive normal passive training but at the end of every third session be allowed to swim around the pool for thirty seconds with the escape platform removed. Finally, Group *Control* would receive standard passive training as described for Experiment 12. At the end of training, all animals would receive a probe trial with the platform removed.

If the disruption to the performance of control rats during Experiment 12 was due to interference during acquisition it is expected that Group *Box* should outperform Group *Control* during the probe trial. The performance of Group *Experience* will be insightful to examine the extent to which interspersed swim trials, which allow voluntary exploration of the environment, influence how rats form a place solution. An associative learning view would predict that these swim trials would extinguish any response to the target location as the platform was always

absent. However, several cognitive mapping theories (e.g., Nadel, 1991) claim, based on previous empirical evidence (e.g. Blodgett, 1929; Tolman & Honzik, 1930), that animals can construct a map of their environment in the absence of reward, while other authors claim that this map must integrate both ideothetic and allothetic cues in order for it to be accurately constructed (Jacobs & Schenk, 2003; McNaughton et al., 1996). If this cognitive map stance is accurate, it is predicted that Group *Experience* should exhibit better navigational abilities than Group *Control*. On a separate note, interspersed swim trials could habituate rats to the sensory input from motion cues generated by swimming around the environment, which would result in Group *Experience* receiving less interference, relative to controls with no prior swimming experience, from ideothetic input during the final probe test. The performance of Group *Experience* could be measured across all stages of training to examine how learning progressed or declined.

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