The Role of S-S* Associations in Spatial Learning

This thesis is presented for the degree of Doctor of Philosophy

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Declarations

No portion of the work presented in this thesis has been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

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Large parts of Chapter 3 of this thesis appear in

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Data used in Experiments 2 and 3 have also been reported in

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Summary

In this thesis I have addressed two questions of particular interest regarding spatial learning. The first question is concerned with the nature of the associations formed during spatial learning; do animals form S-R or S-S* associations? Evidence that animals acquire S-S* associations which can then be used to locate a hidden goal was provided by Experiment one using placement training to produce latent spatial learning. Experiments 2 – 8A produced results consistent with this finding. Experiment 8B provided evidence that animals also use S-R associations.

The second question concerned the type of cues that enter into S-S* associations with the goal. Experiments in Chapter 3 provided evidence that animals can use features of the environment to locate the goal, but no evidence of a global representation was found.

Chapter 4 explored the role of the hippocampus in the acquisition of S-S* associations, and demonstrated that the hippocampus is required for the learning based on spatial S-S* associations, but not for S-R associations.

Contents

Page

Chapter 1 - Introduction

Spatial learning is the process which allows an animal to find a hidden goal by reference to landmarks that are some distance from it. It is an essential skill found among most species and allows an animal to learn the position of food sources, and nests as well as other important goals. From the point of view of associative learning theory, at least two important questions, which have been considered for more than 70 years, are raised by successful demonstrations of spatial learning. The first question concerns the nature of the associations that are formed during spatial learning. Do the animals acquire a stimulus-response (S-R) association, which allows a given set of cues to elicit a particular action? Alternatively, the animal may acquire a stimulus-stimulus (S-S), or what I shall call stimulus-goal (S-S*) association, whereby 'S' refers to the available cues and 'S*' refers to the goal. Of course, it could be the case that spatial learning involves both kinds of association.

The second question concerns the nature of the stimulus that forms the initial link in both of the above associations. Is it based on particular features of the environment, or is it based on a more global pattern of stimulation provided by the landmarks surrounding the goal?

The nature of the stimulus in spatial learning

Much of the research into spatial learning has been guided by the claim that animals possess a 'cognitive map' (see Gallistel, 1990). The definition of a cognitive map is not entirely clear since the specifics of the definition have generally not been formally identified, and when a formal definition is given it often differs between researchers (see Bennett, 1996 for a review). However, broadly speaking a cognitive map can be considered to be a mental representation of the entire environment, which provides information about the spatial relationships among important goals and the landmarks that surround them. As a cognitive map incorporates the entire environment into a single representation, I will use the terms ‘cognitive map’ and ‘global representation’ synonymously. In the first part of this section of the chapter I shall evaluate in some detail the findings from three experiments that have been influential because they have been said to show that animals acquire cognitive maps.

There are many other studies which have been said to demonstrate the existence of cognitive maps (e.g. Brown, Rish, Vonculin, & Edberg, 1993; Chapuis, Durup, & Thinusblanc, 1987; Chapuis, Thinusblanc, & Poucet, 1983; Cheng, 2000; Collett, 1987; Menzel, 1973; Van Beusekom, 1948), but they share many of the weaknesses of those that I consider.

Tolman, Ritchie and Kalish (1946). If an animal is able to form a cognitive map, then it should be the case that when a familiar route is blocked, the animal is able to use the map to find an alternative route. Tolman, et al. (1946) attempted to demonstrate this ability in rats. They conducted an experiment in which rats were trained in an arena (Figure 1) to run from the starting box across a circular table to a corridor that made several turns and then ended in a food box. After four sessions of training, the rats would run immediately across the table and along the corridor to reach the food. They were then transferred to a second, similar arena (Figure 2). In the second arena, the corridor along which the animals had been trained to run was blocked and a number of other corridors were added. The corridors radiated from the circular centre such that none of them was oriented in the same direction as the original corridor.

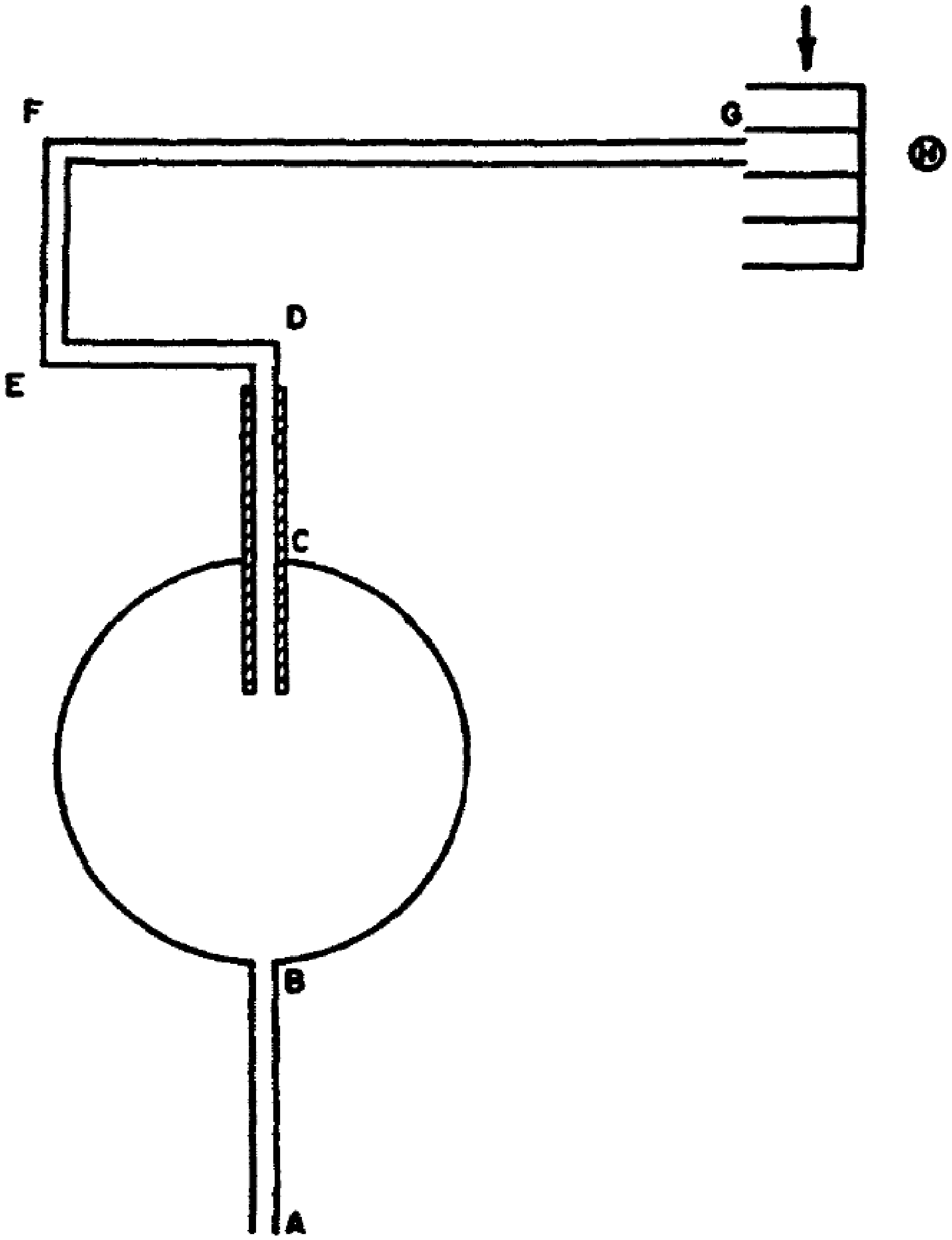


Figure 1. The apparatus used to train rats (Tolman, et al., 1946). A represents the release point and G represents the food box. H is the location of the light over the food box.

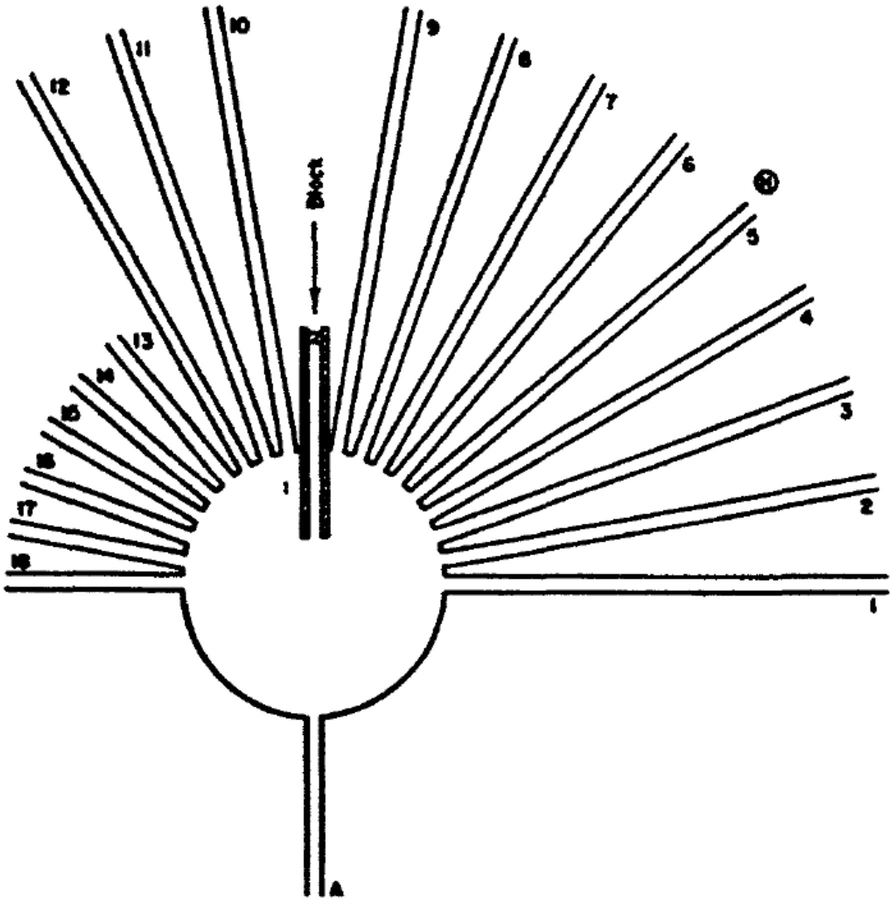


Figure 2. The apparatus used to test rats (Tolman, et al., 1946). A is the release point. The thick lines show the corridor that was previously used to get to the food box, but was blocked on the test trial. H represents the light which previously hung over the food box.

The rats were unable to travel along the route they had previously used to find the food and so had to choose an alternative route. There were twelve possible paths to choose from, and the majority of rats chose the path that ran in such an orientation that it stopped 4 inches from the position of the goal box in the previous arena. As such, these animals chose the path that would take them directly to where the goal box would have been if they were being tested in the original arena. This behaviour appears to indicate that the animals did not simply learn the route to the goal, but were aware of the goal's position relative to other parts of the maze and were able to select a route that took them to approximately the same point. In other words, this behaviour is consistent with the claim that animals acquire cognitive maps.

The most obvious flaw of Tolman et al.'s (1946) experiment is that there was a light hanging above the goal box at all times. The light remained a constant cue as to the location of the goal box and could have acted as a 'beacon'. A beacon is a landmark above or beside the goal, and when it is used to navigate, the animal simply needs to move towards it (Gallistel, 1990). This is known as 'beacon homing' and does not require the animal to understand the spatial relationships between the goal and the other landmarks within the environment. As such, it would not be considered to be spatial learning. Instead the animal simply needs to head in the direction of the beacon until the distance between itself and the beacon is reduced sufficiently that the animal has located the goal. In a more recent study, Muir and Taube (2004) attempted to replicate Tolman et al.'s experiment and found that if a beacon was not present near the goal, then the animals did not demonstrate the same preference for running down the arm leading to the location of the goal box in the previous arena. This finding suggests that there is merit in the argument that Tolman et al.'s results occurred because the animals were displaying beacon homing rather than the use of a cognitive map.

Alternatively, it is possible that the rats had acquired a cognitive map, but that the test arena used by Muir and Taube (2004) was sufficiently different to the one used during training, that the change resulted in a generalisation decrement. As such, the animals were unable to use the previously acquired cognitive map in the new environment to enable them to locate the goal.

Gould (1986) presented a similar set of data to that of Tolman et al. (1946) in which he demonstrated that bees took a novel short cut between two foraging sites (see Figure 3). Previously, bees had been trained to travel from the hive to site A to find food. They were then captured while making the journey between the hive and site A and moved, in darkness, to site B. Rather than travel back to the hive and continue to use the learned route to site A, they travelled to A directly along a novel, shorter route. This ability to take a novel short cut was seen as evidence that the bees had acquired a cognitive map of their environment. However, as with Tolman et al.'s experiment, other groups of researchers found that they could not replicate Gould's findings unless there were prominent landmarks around the two foraging sites (Dyer, 1991; Menzel, Chittka, Eichmuller, Geiger, Dagmar, Knoll, 1990; Wehner, Bleuler, Nievergelt, & Shah, 1990; Wehner & Menzel, 1990).Unlike the design devised by Tolman et al., the failure to replicate this effect cannot be attributed to a generalisation decrement as the bees were not required to navigate in a new environment.

Morris (1981). Morris (1981) claimed to have found evidence that rats use cognitive maps, suggesting that vertebrates possess a global representation of the environment surrounding them.

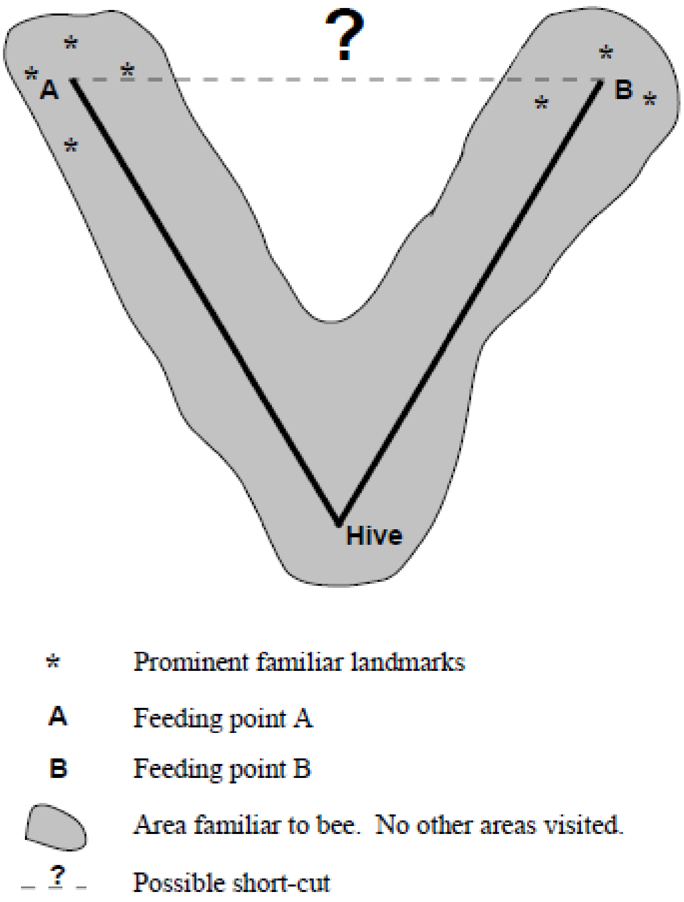


Figure 3. A plan of the environment used by Gould (1986) to test whether bees acquire cognitive maps. Taken from Bennett (1996).

Morris also made use of a design in which there was no generalisation decrement between the training and the test environments. Morris used a pool of water (1.3 m in diameter) in which a platform was placed to act as a goal for rats since they prefer to stand on it than be in the water. The pool was surrounded by a number of cues on the walls of the room. Morris trained rats to find either a visible or invisible platform. For those animals in the visible-platform group, the platform stood on top of a vertical pole tall enough that the platform itself stood just above the surface of the water. For an invisible-platform group, a shorter pole was used, such that the platform itself sat just below the surface of the water. The water was made white and opaque so that when the platform was below the surface of the water it was not visible to the rats. For both groups the platform was always placed in the same position in the pool. Each rat was lowered into the water from one of four possible start locations and was allowed to swim to find the platform. After a number of sessions the rats were given a test trial in which the platform was removed. They were released into the pool and given 60 seconds to swim freely, while the swim path that they took was recorded. Morris compared the amount of time spent in each quadrant of the pool by each group in order to determine whether or not they searched for the platform in the correct area of the pool. If an animal spent significantly more time in the correct quadrant of the pool than in any other quadrant then it would suggest that the animals knew where to look for the platform and therefore had learned about its location relative to the room cues. The group for which the platform was previously visible did not spend more time in the correct quadrant than would be expected by chance. The group for which the platform had not been visible during training spent significantly more time in the correct quadrant than the visible platform group. The results indicate first, that the animals in the invisible-platform group could not see the platform under the water and had not simply learned to swim towards it. Had this been the case then they would have been effectively lost when the platform was removed and would have behaved in much the same way as the animals that had been able to see the platform during training. Thus, unlike Tolman et al.'s (1946) results, the tendency of the rats to move towards the location of the platform during training cannot be explained in terms of travelling towards a beacon. Secondly, the results suggest that the animals had identified the position of the platform with reference to landmarks outside the pool. Thus the experiment provides a clear example of successful spatial learning.

Having established this result, Morris (1981) conducted a second experiment, which was designed to show that rats in the previous experiment were able to locate the platform through the use of a cognitive map. As a cognitive map allows a mental representation of the entire environment, they should be equally able to find the goal regardless of where in that space they are released. Conversely, the animals may simply have learned a route to the goal. That is, they may have repeatedly headed in the same direction in relation to the cues throughout training. Learning a route is a relatively inflexible method compared to a cognitive map, since it requires the animal to be in a certain position relative to the cues in order to follow the learned route. Thus, if animals simply learn the correct route to the goal, then they should find it more difficult to find the platform when released from a novel start point. Using a cognitive map, however, the animal should be able to locate the goal easily from any point in the environment since the flexibility of the map would allow for novel routes to be taken. In the second experiment, Morris trained a cohort of rats using the submerged platform. However, in this experiment, each rat was released from the same place throughout training. The platform was always placed in the same position. At test, the animals were then released from a novel start point in the pool and were allowed to swim freely for 60 seconds. The results indicated that the rats did not take longer to find the platform when they were released from the novel starting point than when they were released from the point used during training. Morris concluded that this provided evidence of a cognitive map, since the results suggest the animals had not just learned the correct path from the release point to the goal. Had they only learned a path from the start point to the goal, then they would have found it considerably more difficult to find the platform when released from a novel start point.

The problem with the results of Morris's (1981) second experiment, however, is that the animals did not initially head in the correct direction when they left the release point during the test trial. Morris reported the swim paths of the rats, and only one of the six rats headed directly to the platform. The other five rats initially swam in an incorrect direction before correcting themselves and swimming towards the platform. One of these rats swam around a considerable distance before eventually finding the platform. There is no obvious reason why the rats should not head directly to the platform if they possess a cognitive map. Instead, the animals may have learned a number of different routes to the goal, using cues outside the pool as landmarks. On the test trial they may then have moved around the pool at random until they recognised cues from a learned route. Morris' experiment provides a useful method for studying spatial learning, but does not demonstrate unequivocally that animals possess cognitive maps.

Furthermore, Sutherland, Chew, Baker, & Linggard (1987) provided evidence that animals that were forced to take a novel route to a hidden platform in a circular pool were not as accurate as animals who had previously taken that route to the platform.

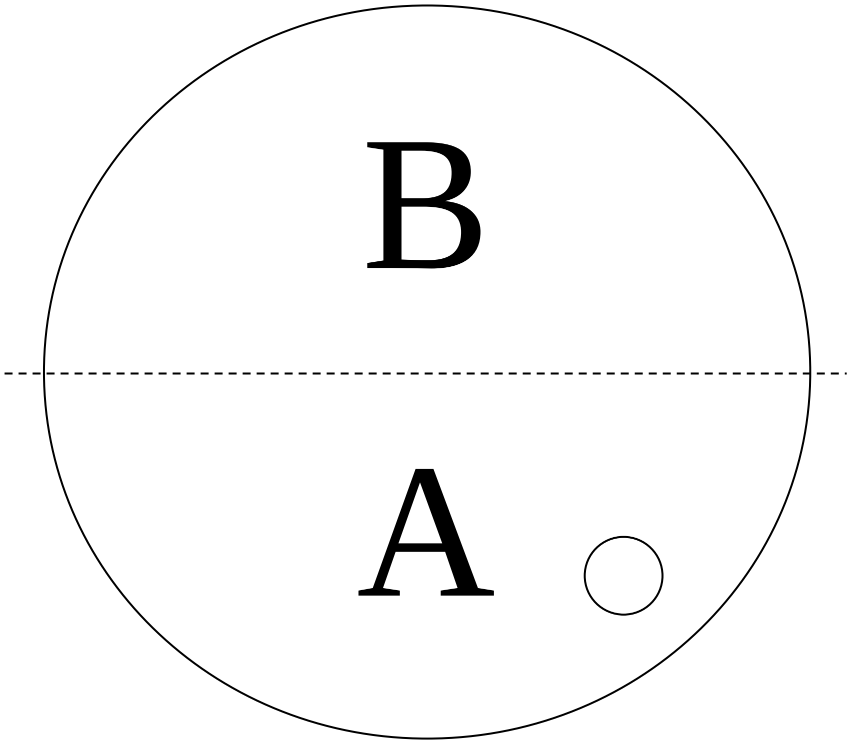


Figure 4. The apparatus used by Sutherland, Chew, Baker and Linggard (1987). The large circle represents the circular pool of water and the dashed line represents the clear partition creating sides A and B. The small circle represents the platform.

As with Morris's (1981) study, animals were trained to locate a hidden platform which was always in the same place in a circular pool. In the Sutherland et al. study, the pool was divided into two halves - side A and side B (see Figure 4). The platform was always placed in the same location in side A of the pool. Some animals had access to the entire pool during training, while another group of animals was given experience of swimming in both halves, but was prevented from travelling between them by a Perspex barrier separating the two sides. Importantly, the animals could view all of the cues from either side of the pool as the barrier was transparent; the barrier did not affect the animals' ability to see side A from side B and vice versa. Thus, the latter group had the same exposure to each side of the pool as the animals that were given access to all of the pool, but they were not given experience of swimming from side B to the platform. The rats were all given eight trials of training daily for three days in which they were trained to swim to the platform. For those that were given separate training in sides A and B, the animals were placed in side B immediately after the training trial. The amount of time spent in side B was yoked such that it was equal to the amount of time spent in side B by the group that had access to both sides. To yoke the time spent in side B between groups, the time spent on that side was recorded for each animal that had access to both sides A and B at the same time. For each of these rats a counterpart in the group which had access to only one side at a time was given the same amount of time in side B. They were then given four test trials in which they were released from the edge of side B of the pool. The latency to find the platform was significantly longer for the animals that had been exposed to side B, but had not been allowed to swim between the two sides than the group which had been given access to both sides at the same time. Sutherland et al. argued that this result demonstrates an inability in animals to take a novel route to the goal. These results cast further doubt over the claims by Morris (1981) that the novel routes in rats demonstrate the use of cognitive maps.

Cheng (1986). The third influential experiment to consider was devised by Cheng (1986) who trained rats to find food in a rectangular arena with landmarks placed in the corners. For reasons to be made clear shortly, Cheng argued that this training results in animals acquiring a representation of the entire shape of the arena which is stored in a dedicated geometric module. He likened this representation to a cognitive map.

In his extremely influential study rats were placed in a dry, rectangular arena in which a card was placed in each corner that acted as a visual stimulus. Each of the cards had a different pattern on them and as such the corners could be identified by referring to the cards. Food was hidden in only one corner, and during training the animals began reliably searching in the correct corner for food. During a test trial, with the food removed, the rats searched in the correct corner before any other corner on 71% of the trials, as would be expected if the animal was referring to the stimulus card in the corners. However the animals also showed a preference for searching in the corner diagonally opposite the correct corner on 21% of the trials. They chose the remaining two corners first on 8% of the trials. Cheng argued that the preference for the corner diagonally opposite the correct corner, referred to as a rotational error, over the remaining incorrect corners, could only be explained in terms of the shape of the arena. He argued that the animals had a global representation of the rectangular shape of the environment, and that using this information alone it is not possible to discriminate between the correct corner and the corner diagonally opposite. Although the animals did appear to be using the cards in the corners to distinguish the two corners from each other, the number of errors that occurred in the diagonally opposite corner over the two remaining corners was used as evidence that animals had a mental representation of the entire shape of the environment. Furthermore, in a later stage of the experiment, the cards in the correct corner and the diagonally opposite corner were removed, and the animals spent approximately equal amounts of time in each of these corners, and considerably more time than was spent in either of the incorrect corners. These results further suggest that all of the animals had learned about the entire shape of the arena, and not just the stimuli within the corners themselves.

The methodology devised by Cheng has proven extremely influential (see Cheng, Huttenlocher, & Newcombe, 2013). For example, experimental investigations of the geometric module have been conducted with species such as pigeons (Kelly, Spetch, & Heth, 1998), fish (Valeria Anna Sovrano, Bisazza, & Vallortigara, 2007), chicks (Vallortigara, Zanforlin, & Pasti, 1990), rhesus monkeys (Gouteux, Thinus-Blanc, & Vauclair, 2001), and also young children (Hermer & Spelke, 1994). The concept of a geometric module has become so influential that it was suggested that "the ability to use purely geometric information for reorientation is widespread among vertebrates, likely representing an evolutionarily ancient trait and a very basic cognitive mechanism" (Sovrano, Bisazza, & Vallortigara, 2002, p. 56-57.)

Evidently, the findings presented by Cheng (1986) have been taken as evidence by a large number of researchers that animals possess a global representation, or cognitive map of their environment. Pearce, Good, Jones, & McGregor (2004), however, proposed that the results could be explained in more simple terms by assuming that animals navigate with reference to components or features of the arena. For instance, if the rats were trained to find food in Corner A of a rectangular arena (Figure 5), they may not have learned its location in relation to the entire environment, but instead learned that it is at the left hand end of a long wall, or at the right hand end of a short wall. Alternatively they may have learned to look for a corner with a long wall to the right and a short wall to the left. Not only do these alternatives explain how the rats would be able to find the correct corner using features of the environment, rather than a global representation of the environment, but also predicts the rotational errors that Cheng described. If the animal were to search for a long wall and turn left then this would lead them to the diagonally opposite corner as often as it would the correct corner. The same is true if the animal searched for a short wall and turned right, or if they travelled towards a corner with a long wall to the right and a short wall to the left.

Pearce et al. (2004) devised a way of determining whether animals made use of the shape of the entire environment, or specific features of its shape when navigating in a rectangle. Rats were initially trained to locate a platform a little below the surface of the water in a rectangular swimming pool. The platform was always placed in the same corner of the rectangle. As with Morris (1981), the water was made opaque so that the platform could not be seen. The rat was released from the middle of one of the four walls and allowed to swim for a maximum of 60 seconds to find the platform.

The corner that the animal entered first was recorded, as measured by a notional 40 cm radius zone in each of the four corners of the rectangle. The centre of the zone was where the two walls making the corner met. Once an animal entered one of these zones it was considered to have entered that corner and made a ‘choice’. Consistent with Cheng’s research, the rats entered the zones in the correct and the diagonally opposite corners first on more trials than the remaining two corners, but did not differ in their preference for either of these zones.

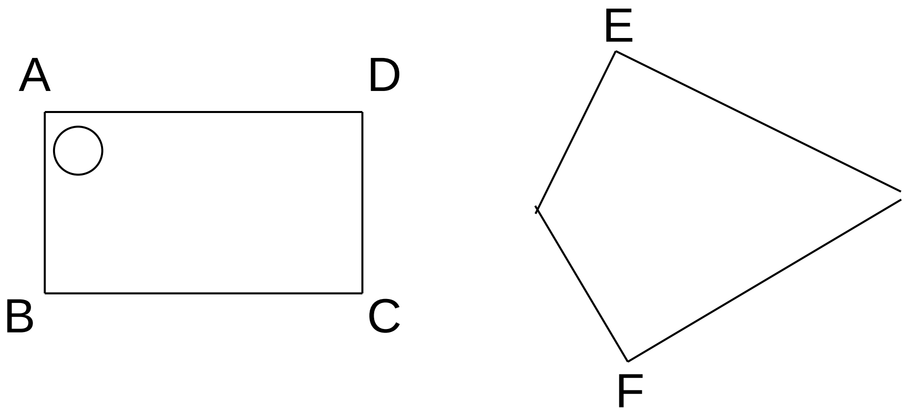


Figure 5. Apparatus used by Pearce et al., (2004). The circle shows the position of the platform in the rectangle. Corners A and C in the rectangle are identical to corner E in the kite in terms of the local geometric configuration. Corners B and D in the rectangle are identical to corner F in the kite.

To test whether the tendency to go to the correct and diagonally opposite corners equally was due to navigation based on the shape of the arena, or features of the shape, the rats were then given a transfer test. The animals were tested in a kite-shaped arena (Figure 5). The kite was made up of the same four walls as the rectangle. As a consequence the arrangement of the two walls creating the correct corners in the rectangle, Corners A and C, was the same as for Corner E in the kite. Likewise the arrangement of the two walls creating an incorrect corner in the rectangle, Corners B and D, was the same as for Corner F in the kite. If the animals were using a global representation to navigate in the rectangle then it would be expected that they would not show a preference for Corner E over Corner F in the kite. As the shape of the environment had changed the animals would effectively be lost in the new environment. Instead, when the animals were released into the kite-shaped pool they expressed a stronger preference for corner E than corner F, as measured by the corner that they entered first, using the same criteria as the test in the rectangle. They also showed a slight preference for the apex. These results suggest that the animals were not using a global strategy. However, the results can be explained if the animals were using a local feature strategy to navigate. If the animals were looking for a corner with a short wall to the left of a long wall, for instance, then they would show a preference for corner E which had these geometric properties, over corner F in the kite. Alternatively if the animals moved to a long wall and turned left, or a short wall and turned right then they would also have gone to corner E more so than corner F. In fact, the long wall strategy can also explain the preference for apex as it was also at the left hand end of a long wall.

The results therefore raise the possibility that the behaviour demonstrated by the rats in Cheng’s (1986) experiment was due to them navigating with reference to features rather than to a global representation. In other words, his experiments fail to demonstrate unambiguously that animals navigate with reference to an overall representation of their environment. One of the main aims of the present thesis will be to determine what information is acquired during spatial navigation and the relative influence of local and global cues during spatial learning in a variety of arenas.

The three experiments described in detail in this section were all originally presented as evidence that animals develop a global representation of the environment which they then use to navigate. However the first two are easily dismissed when it is appreciated that a beacon was available for the rats in Tolman et al.'s (1946) study, and the animals in Morris's (1981) may have learned to find the goal by following routes based on individual landmarks. Cheng (1986) provided particularly influential evidence, but his results can easily be explained by assuming that animals referred to features of the shape rather than the shape itself. Having said that, it remains possible that animals learn about both global cues and local features of the environment, but that the experiment by Pearce et al. (2004) did not permit rats to demonstrate their knowledge about the global properties of the training environment. It is possible that given a test which is more sensitive to any tendency by the animals to use global cues, it would be possible to demonstrate that they do learn about both feature and global information. Accordingly, some of the experiments in the following chapters were designed with the aim of providing rats with an adequate opportunity to display knowledge of global spatial information, should it animals use it to indicate where a goal is situated.

The nature of the associations in spatial learning

The first part of this introduction was concerned with whether animals use a global representation or distinct features of the environment during spatial learning. The second consideration, which will now be discussed, concerns the nature of the associations that contribute to spatial learning. That is, what is the nature of the information about the environment, whether it be global or feature cues, that enters into an association that enables the animal to locate the goal.

One possibility is that animals form a stimulus-stimulus (S-S), or stimulus-goal (S-S*) association. An S-S* association is an appreciation of the spatial relationship between the location of the goal (S*) and the cues provided by the environment (S). For instance, in the Pearce et al. (2004) experiment, the animals may have learned that the platform was in a corner with a long wall to the right of a short wall, or they may have learned that the platform was at the left hand end of a long wall. In these examples, a feature of the environment enters the initial element of the S-S* association. Alternatively, if it is accepted that animals form cognitive maps, then it is conceivable that a map in its entirety will enter into an S-S* association with the goal. Cheng (1986), for example, suggested that individual landmarks become pasted onto the geometric representation of the shape of the training environment, and it is quite possible that the same is true for goals such as food and escape platforms.

It has long been appreciated that one problem with an S-S* analysis of spatial learning is that it is not clear how an S-S* association would lead the animal to the goal. One solution to this problem is provided by Cartwright & Collett's (1982) snapshot model of animal navigation. The model states that when an animal is at the goal, it takes a mental ‘snapshot’ of the surrounding landmarks. Later, when the animal is trying to locate the goal, it compares its current retinal image with the remembered snapshot. The animal attempts to reduce the disparity between the two and in doing so moves closer to the goal until the images match up. Thus, this account explains both how an animal represents the S-S* association and how it uses it to navigate to the goal.

An alternative account of how an animal may use the available cues in the environment states that rather than forming S-S* associations, the animal acquires a stimulus-response (S-R) association. An S-R association forms when an animal performs a certain response that results in a positive outcome. In the case of the study by Pearce et al. (2004) considered above, animals may acquire an S-R association where the stimulus is, for instance, the long wall of the training arena. If the platform was always placed in corner A of the rectangle (Figure 5), the rats may have learned to swim to a long wall and turn left. Alternatively, they may have learned to swim to a corner made up of a short wall to the left of a long wall.

Unlike S-S* learning, S-R accounts do not assume that the animal appreciates the location of the goal in relation to the surrounding cues. Rather, they assume that animals learn a particular habit in response to the cues which results in finding the goal.

From as early as 1898 (e.g. Thorndike, 1898, 1911; Hull, 1943) it has been argued that S-R associations are the basis of all learning and there have been a number of studies demonstrating the use of S-R associations in spatial tasks. For instance, Restle (1957) used apparatus similar to that shown in Figure 6 which was made up of four arms. One arm was allocated as the starting location, and the arm opposite the starting location was blocked, effectively creating a T-shaped arena. Rats were released at the start location and were able to explore both of the adjacent arms of the arena. Food was always placed in the arm to the right of the start location, and so animals showed a preference for searching there over the arm to the left of the starting location during the course of training. There were a number of cues around the room that were visible to the animal during training, and so it is possible that they learned about the position of the food relative to these cues. Alternatively, the animals may have learned to turn right when leaving the starting location, which would constitute an S-R association. To determine which strategy the animals were using, the arm that originally acted as the starting location was blocked, and the previously blocked arm was used as the new starting location. Upon being released from the new location, the rats still turned right into the adjacent arm, despite this being the arm which had never contained food. This result demonstrates the use of an S-R association as the animals had clearly learned a habit which they used to locate the food. This was true even though the animals could have used the cues around the room, which would have taken them to the arm that was then on the left of the new starting point, but was the arm which had previously contained food (see also Packard and McGaugh, 1996).

Although Restle (1957) successfully demonstrated the use of S-R associations, attempts to demonstrate the use of S-S* associations in spatial learning have been met with mixed success. In order to demonstrate the learning of spatial S-S* associations, a number of studies have attempted to demonstrate a phenomenon that I shall refer to as latent spatial learning. The majority of such studies have been conducted in a swimming pool, which I shall review, but first an attempt to demonstrate latent learning using appetitive conditioning warrants discussion. White (2008) described experiments in which rats were confined to one of two arms of a radial maze. In one of the arms food was always present, and in the other arm food was never present. At test, the animals were released into the centre of the maze and were able to access either arm. The animals showed a preference for entering the arm which had previously contained food over the one which had not. As the animals were always confined to either arm, they were never given experience of heading into the rewarded arm from the centre of the radial maze, and so were unable to form a suitable S-R association. Thus, it was argued that the animal's preference for entering the arm at test was a result of latent spatial learning. However, it should be noted that a light was present in the correct arm throughout the training and test procedures. Although the animals did not have experience of heading towards the arm with a light in it from the centre of the radial maze, experience of travelling towards the light while in the correct arm cannot be ruled out. The animals may have travelled around the arm while confined to it, and so it is not possible to say with certainty that an S-R response involving the light was not acquired. This S-R association could then have been used when the animal was placed in the centre of the maze to enable them to find the food.

I shall now review the evidence for latent spatial learning in the swimming pool. In a typical latent spatial learning experiment in a swimming pool rats receive placement training which involves placing the rat on a platform in a pool of water, but never allowing the animal to swim around the training arena. As such, it can never develop an S-R association as it is not given the chance to move around the arena in order to acquire a response that will reliably lead it to the goal. Tests for latent spatial learning using placement training have been met with mixed success.

For instance, Sutherland & Linggard (1982) conducted a study in which they tested the possibility that animals learn about the position of a platform with reference to distal cues whilst on the platform.

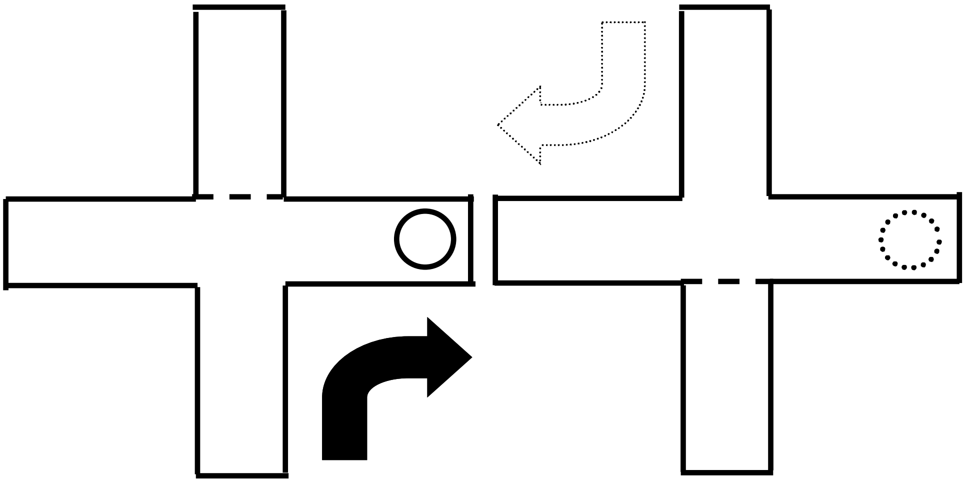


Figure 6. Apparatus used by Restle (1957). The left hand panel shows the apparatus during the training stage. The straight, dashed line represents the barrier blocking one of the arms. The circle represents the location of the food and the arrow represents the direction that the animals travelled from the starting location to the food in the arm to the right. The Right hand panel shows the apparatus as it was during the test. The dotted circle represents the arm in which food had previously been placed and the dotted arrow represents the direction that the rats would travel had they been using S-R associations. The dashed line represents the barrier across the original starting location.

To test this proposal, they conducted an experiment with two groups: ‘correct’, and ‘incorrect’. The correct group received placement training on a platform, in which the rats were placed on the platform for ten seconds at a time, five times a day, for a total of eight days.

The platform was always in the same place in the pool. This treatment was intended to encourage the development of S-S* associations which would provide information about the position of the goal with reference to landmarks outside the pool. The incorrect group received similar placement training, but during the training stage the platform was in a different place to where it was placed during a subsequent test session. As the incorrect group received misleading information while on the platform, any S-S* associations that might be acquired would be expected to hinder later navigation. After placement training was complete, the rats were given a number of test trials in which they were required to swim to the platform after being placed in the water. They were released from one of four different locations in the pool and the time taken to reach the platform was recorded.

As predicted, the correct group initially showed significantly shorter latencies to find the platform than the incorrect group. The length of the swim path was also shorter for the correct than the incorrect group. Similarly, the heading error was significantly smaller for the correct group than the incorrect group. The heading error was measured as the angle between two straight lines. One line was drawn between the rat’s start point and the centre of the platform. The other line was drawn between the start point and the direction in which the rat was swimming after it had travelled a full body length from the start point. Thus, heading error is a measure of how accurately the animals headed towards the platform.

The most relevant finding of the experiment is that exposure to the environment from the platform in the correct location reduced the time to find the platform at test. The learning which occurred while the animal was on the platform cannot have been based on S-R associations since, at the first test trial, none of the animals had previously been given the opportunity to swim to the platform. As such it would appear that learning based on S-S* associations had occurred during placement training.

Keith & McVety (1988) argued that Sutherland and Linggard (1982) may have underestimated the effects of placement training and that latent spatial learning may be even more apparent if the animals were given experience of the procedural aspects of the test trial prior to being tested. They conducted an experiment that was very similar to the one just described but they trained the rats to swim to find a platform prior to placement training. They argued that rats may perform better on placement training tasks, if they are familiar with the requirement to swim to the platform prior to receiving the test. The first stage of the training, in which they learned to swim to a platform, took place in a different pool to the placement training. The placement training was conducted in a novel environment to ensure that there was no exposure to the surrounding cues other than during the placements. Therefore, any subsequent navigation can only have occurred through latent learning. They found that animals that had been trained to search for a platform took less time to find the platform on the test trial in the novel environment, than those which had not received this training.

Jacobs, Zaborowski, & Whishaw (1989a) conducted a similar experiment; however, as will be explained shortly, it was later retracted. They initially conducted a study in which animals received placement training and were then able to locate the platform more quickly than animals that received the same training, but for which the platform was in a different place during the test. However, in a second study aimed at determining how long the animals retained the knowledge of the position of the platform, they found that the animals quickly forgot what they had learned. Delays of varying length were given between the last training trial and the test session. The rats were assigned to one of the following groups: ‘not-placed’, ‘immediate’, ‘2H’, ‘6H’, ‘12H’, ‘24H’. Those in the not-placed group did not receive any placement training. Instead, they were given a single test trial in the testing pool in which they were released into the water from the edge of the pool and allowed to swim for up to 120 seconds to find the platform. For the remaining rats, the training consisted of 15 placements on the platform. Those in the immediate group then received the test trial, which was identical to the test given to the not placed group, immediately after the training. The remaining groups received the test trial after a delay of either 2,6,12, or 24 hours (H).

The mean latencies to find the platform by each group were then calculated. The results showed that the immediate group had the shortest latencies to find the platform. The latencies for the groups with a 2-, 6-, or 12- hour gap between the final training trial and the first test trial were all equal, but longer than for the immediate group. However for those in the 24 H group, for reasons that are not clear, the latencies were significantly longer than for those animals that had received no prior placement training. The lack of successful navigation after a 24-hour delay suggests that S-S* associations may not be very important in navigation since they are not retained for long periods of time. If an animal really does navigate with reference to S-S* associations, then this representation would presumably have to last for more than 24 hours to be of vital use to the animal.

Jacobs, Zaborowski, & Whishaw (1989b) later retracted the foregoing paper as they were unable to replicate the effects of their placement learning. They conducted seven separate attempts to replicate their original findings. In each experiment a placement group received 15 placements on a platform which was always in the same position. This treatment was conducted in exactly the same way as for the experiments described by Jacobs et al. (1989a). A second group, the ‘no placement’ group, received no placement training. In total 52 rats received placement training and 42 received no placement training. After the latencies to find the platform were averaged over all of the rats, there was no significant effect of the two treatments. Jacobs et al. suggested that there was at least one methodological flaw that may have accounted for the apparent effect in the original paper. In the original studies the rats were released from the north of the pool and had to swim to the southwest quadrant of the pool to find the platform. The tester was always waiting to pick the rat up from this quadrant and, since the rat was always picked up by the tester during training, the rats may simply have headed towards them, and inadvertently found the platform, rather than having learned its location.

A number of other studies that demonstrated learning during placement training also warrant mention (Pearce, Roberts, Redhead & Prados, 2000; Prados, Chamizo & Mackintosh, 1999: Prados, redhead & Pearce, 1999), although they were not intended to demonstrate that placement training is sufficient for animals to learn the location of the platform. Experimenters in each of these papers gave animals exposure to cues surrounding a circular pool of water by repeatedly placing them on a platform. The animals then received a number of test sessions during which they were required to swim to the platform, which was in a different place to where it had been during the placement training stage of the experiment. The time taken to reach the platform during the test sessions was recorded. The time taken to reach the platform was taken as a measure of how quickly the animals learned the location of the platform. Exposure to the landmarks during placement training was shown to facilitate or inhibit learning during the test stage of the experiments depending on whether or not the platform remained in the same place during the entire exposure stage (Pearce et al., 2000; Prados et al., 1999). The number of landmarks that were visible to the animal during placement training was also shown to affect how quickly the animal located the platform during the swimming stage of the experiment (Prados et al., 1999). Although these experiments demonstrate that animals are able to learn about the surrounding cues during placement training, these experiments do not provide any indication of whether animals also learn about the location of the platform in relation to the cues.

There is some suggestion that animals do use S-S* associations in evidence presented by Sutherland and Linggard (1982) and by Keith and McVety (1988). However, the finding by Jacobs et al. (1989a) that these associations do not last as long as 24 hours after training, casts some doubt as to the extent to which they influence spatial learning. Similarly, the fact that Jacobs et al. (1989b) later retracted their findings in placement training further suggests that evidence for S-S* associations in spatial learning may not be reliable. The repeated failure to reproduce latent spatial learning through placement training by Jacobs et al., combined with a plausible account of how the results of their original paper may not truly have demonstrated spatial learning, casts serious doubt over the results of the other experiments described in this review. It is possible that the results presented by Sutherland et al. and Keith and McVety were also produced through similar methodological flaws. It is therefore necessary to conduct further experiments, in order to determine whether or not it is possible to produce reliable, long lasting latent spatial learning using placement training.

The experiments reviewed in this chapter raise two questions. The first question is: Do animals rely on features or global representations when navigating towards a hidden goal surrounded by landmarks that are some distance from it? The second question is: How do animals navigate with reference to landmarks surrounding the goal? Do they serve as the stimulus component of S-R associations, or S-S* associations? Experiments in Chapter 2 will address the second question by examining whether it is possible to demonstrate reliable latent spatial learning. The experiments were successful in this respect and thus lend support to the claim that spatial learning depends in part upon the acquisition of S-S* associations. The experiments in Chapter 3 make use of the methodology described in Chapter 2 to explore the extent to which the stimulus component of the S-S* association is based on features of the environment, or on a global pattern of stimulation provided by the environment. Chapter 4 of the thesis explores the role of the hippocampus in the development of S-S* associations. An interesting outcome of these experiments is they provide novel evidence for the role of S-R associations in spatial learning.

Chapter 2

The aim of the present chapter is to deal with the question raised in the Chapter 1 of whether or not animals acquire S-S* associations which they use to navigate. As described in the first chapter, previous research that attempted to demonstrate these associations, by giving rats placement training, had mixed results.

The purpose of the first experiment was to determine whether or not placement training is effective at producing latent spatial learning. The method of training given in the following experiment was created in an attempt to address some of the reasons why placement training may have previously produced mixed results.

First, it was noted that in each of the latent spatial learning experiments described in Chapter 1, the cues that were available to the animals were all outside the arena in which they were trained. Perhaps the effects of placement training were so variable because these cues were not salient enough. If the rats failed to pay attention to the cues outside the pool used for the placement training, for example, then the failure to find any evidence of successful latent spatial learning by Jacobs et al. (1989b) is hardly surprising.

As an alternative method for investigating latent spatial learning, it was therefore decided to use a rectangular swimming pool with rats placed on a platform situated in one of the corners. As each location of the platform within the rectangle can be identified with reference to the length of the walls of the arena, it was hoped the walls would provide more salient information than cues outside the arena. Experiments have already shown that rats can readily learn to identify the correct corner, that is the corner containing the goal and the diagonally opposite corner, in a rectangle, when they must make their own way to the goal on every trial (e.g. Cheng, 1986; Pearce et al., 2004). Moreover, because the platform is situated near to the corner of the pool it is reasonable to expect that the subjects will pay considerable attention to the cues relevant for successful latent learning during their placement training. For both these reasons, therefore, it seems likely that placement training with this methodology will be more successful than that used by Jacobs et al. (1989b).

Throughout the entire experiment the pool was surrounded by curtains to ensure that the only cues available to the animal were elements of the arena itself. The use of a curtain also provided a solution to the suggestion by Jacobs et al. (1989b) that one of the reasons why animals in their previous paper (1989a) had been able to find the platform was because the rats learned to travel towards the experimenter. As suggested in Chapter 1, it is possible that the animals discovered during placement training that proximity to the experimenter resulted in being taken out of the pool since the experimenter picked up the animal at the end of each trial. When the animals were then released into the pool at test to find the platform, they may have simply headed towards the experimenter and found the platform by chance as it was more or less situated on a straight line between the release point and where the experimenter waited. During all stages of the following experiment, the experimenter stood on the outside of the curtain to ensure they were not visible to the rats in the pool. It was therefore hoped that the location of the experimenter relative to the pool would not be responsible for the rats’ ability to find the platform.

Experiment 1

In the first experiment the animals received training in which a hidden platform was always in the same corner of a rectangular pool. All of the animals received placement training in which they were placed on the platform during training, and had no experience of swimming in the rectangular pool. The purpose of this experiment was to establish whether or not it is possible for animals receiving placement training to learn about the position of the platform relative to the available cues. After twelve sessions of training, the rats received a single test trial in which they were given 60 seconds to swim around the pool in the absence of the platform. The amount of time they spent in each corner of the rectangle was recorded during the test trial. The ‘correct’ corners were defined as the corner in which the platform had been placed during placement training, and the diagonally opposite, and therefore geometrically identical, corner. The remaining two corners were considered the incorrect corners.

During placement training, any animal that launched itself from the platform was removed from the experiment as it was not possible to determine what the animal had learned whilst not on the platform. That is, time in the water may have resulted in the opportunity to develop S-R associations that would help the animal find the platform during the test trial. By ensuring that none of the animals had experience of swimming in the pool before the test trial, it was reasonable to suppose that their behaviour during testing was controlled solely by S-S* associations. In an attempt to encourage the animals to stay on the platform during placement training, all of the animals received three sessions of pre-training prior to the first training trial. They were placed on a platform in a circular pool for 30 sec at a time. If they left the platform then they were guided back and left there for the remainder of the 30 seconds. By the end of the final trial of three sessions of pretraining, all of the rats remained on the platform for the entire 30 seconds.

Method

Subjects. The rats were 8 male, hooded-Lister rats purchased from Harlan Olac Ltd (Bicester, Oxford, England) weighing between 250 to 300 g at the start of the experiment. The rats were housed in pairs in a room with a 12-hr/12-hr light/dark cycle, with the lights being turned on at 07:00. Subjects were naive to the swimming pool but had previous behavioural training in Skinner boxes during which they received appetitive conditioning and were reduced to no more than 80% of their free-feeding weights. Prior to the start of the present experiment they were allowed access to unrestricted food for one week, and continued to receive unrestricted feeding throughout the course of the experiment.

Apparatus. A white fibreglass pool of 2 m diameter, 0.6 m deep and raised 0.6 m off the floor was filled with water 27 cm deep. The water was maintained at a temperature of 25°C (± 2°C), and was changed daily. The water was made opaque with 0.5 L of white opacifier E308 (Rohm & Haas, U. K. Ltd. , Dewsbury). A circular platform marked with concentric grooves, which was mounted on a pole, was 10 cm in diameter. The upper surface of the platform was 2 cm below the surface of the water. Grey Perspex boards were used to make the walls of the rectangle. Two long boards were 1.8 m long and 0.59 m high. Two short boards were 0.9 m long and 0.59 m high. All boards were 2 mm thick. Attached to the top of the boards were bars that extended over the edges of the pool so that the walls could be arranged in a rectangle and suspended from the top of the pool edge.

A suspended circular ceiling above the pool was 2 m in diameter. It was suspended 1.75 m above the floor of the pool. At the centre of the ceiling was a hole of 30 cm diameter in which was placed a camera with a wide-angled lens. The camera was suspended 25 cm above the hole and was connected to a video recorder and a screen in an adjoining room. Water maze software (Morris & Spooner, 1990) was used to track the animals movements from this camera. There were eight 45-W lights in the ceiling above the pool which were equally spaced in a circle of 1.6 m diameter, which was concentric with the centre of the circular ceiling. The lights were 22.5 cm in diameter the pool was surrounded by a white curtain which hung from the edge of the circular ceiling. It was 1.5 m high and fell to 25 cm below the top of the pool edge. The curtain was drawn around the pool all times.

The pool was in a room that was 4 m x 3 m and was 2.3 m high. The training room was lit by 1.53 m strip lights, with two on both the East and West walls, and were 75 cm above the floor. The door was 1.75 m x 2 m and was in the centre of the South wall which led into the adjoining room containing the monitor on which the rats could be observed. This room was also where the rats were held whilst waiting to be put in the swimming pool.

The training was counterbalanced so that for half of the animals the correct corners were those with a long wall to the left of a short wall and for the other half the correct corners were those with a long wall to the right of a short wall.

Procedure

Pre-training. Rats were taken from the holding room to the testing room eight at a time in individual boxes. The rats received three pre-training sessions in which they were placed on the platform in a circular pool for 30 sec. If they swam away from the platform, they were guided back to the platform without being taken from the water. The experimenter guided it back to the platform by placing a finger in front of its nose and leading it towards the platform. Occasionally a rat would swim away from the experimenter when they tried to guide the rat in this way, and so the experimenter would place a finger on top of the platform, which the rat could then swim towards. Using one of these two methods every rat that left the platform was eventually led back to it. They were left on the platform for the remainder of the 30 sec and were then removed from the pool. They were never taken out of the pool from the water; they were always guided back to the platform first so as to avoid the acquisition of an association between leaving the platform and being taken out of the water. There were four trials in each pretraining session; the platform was placed in a different area of the circular pool for each trial, but was always placed on a notational line that bisected the quadrant. The position of the platform was randomised between trials with the restriction that each area was used once and was placed 25 cm from the edge of the pool on two of trials and 50 cm from the edge on the remaining two trials. After the rat received one trial, it was dried with a towel and each of the other rats received that trial before the first rat began the second trial and so on.

Training. Each rat received twelve training sessions in the rectangular pool. There were four trials in each session. For each of these trials the rat was placed on the platform, which was 25 cm from the corner, placed on a notational line bisecting the corner. For the purposes of counterbalancing, for half of this group the platform was placed in corner A and for the other half it was placed in corner B (see Figure 5). During each training trial the rats were placed on the platform for 30 sec. They were then removed and dried with a towel. Each of the other rats then received the same trial before the first rat received the next trial. The orientation of the rectangle within the pool was randomised between trials to ensure that no extra-maze cues could be used to indicate the location of the platform. The possible orientations were for corner A to be at the N, S, E or W edge of the pool, with the north most point of the pool being the furthest from the adjoining room.

Testing. The last trial of the twelfth session was a test trial. The rats were released from the centre of the pool and allowed to swim freely for 60 sec in the absence of the platform. The rats were viewed on the screen in the adjoining room and their paths were recorded using the Watermaze software. The software recorded the amount of time spent in each corner, which was defined as a notational circular zone of 30 cm diameter the centre of which was 25cm from the respective corner. (That is, where the centre of the platform was originally located in the correct corner and in the equivalent place in the other corners). After 60 sec, the rat was removed from the pool, dried and placed back in the box whilst each of the other rats was tested. The amount of time spent in each zone was expressed as a percentage of the total time spent in the arena.

The ‘first choice’ of each rat was also recorded. This was the first corner that the rat entered on the test trial. The rat was considered to have entered a corner once it was within a notational arch created by a circular zone of 40 cm diameter, the centre of which was located at the corner.

Results

The results from the test trial can be seen in Figure 7. The amount of time spent in the two correct corners, that is the corner in which the platform was placed and diagonally opposite corner, were combined into a single figure, giving the percentage of time spent in the correct zone. The same method was used for the remaining two corners, creating a single figure stating the percentage of time spent in incorrect zone. The results were simplified by normalising the data so that the two counterbalanced groups could be referred to as one group. The figure shows the average time spent in correct zone, indicated by the grey square, and the incorrect zone by all eight animals. The figure shows that the animals spent more time in the two correct than the two incorrect corners. A 2-way ANOVA was conducted comparing the time spent in the correct and the incorrect zones. For this analysis and for all other analyses in this thesis, a *p* value above .05 was not considered significant. The ANOVA revealed a significant effect of zone *F*(1,6) = 7.24, *p* = .04, *MSE* = 71.18, but the effect of counterbalancing (whether the platform was in corner A or B) was not significant, *F*< 1. Similarly, the interaction between the two was not significant, *F*(1,6) = 1.41, *MSE* = 47.0. The significant effect of zone indicates that placement training resulted in successful learning about the location of the platform within the rectangle. As the animals were not given the opportunity to swim around the arena prior to placement training, they can only have developed S-S*, and not S-R associations during the training stage. Figure 7 also shows that on being released into the pool six out of eight rats swam directly to a correct corner. Although this finding adds further support to the claim that the placement training resulted in successful latent spatial learning, it was not statistically significant using a binomial test, *p* = .11. However, sample sizes that are as low as this would require all but one of the rats to head directly to the correct corner to reach statistical significance. It should therefore perhaps not be assumed that given a greater number of subjects, a significant number of them would not choose to enter a correct corner before an incorrect corner.

To return to the ANOVA, as there was no effect of counterbalancing, it will be assumed that this is true of all of the following experiments in which animals were trained in a rectangle, and so the analysis will not be considered again.

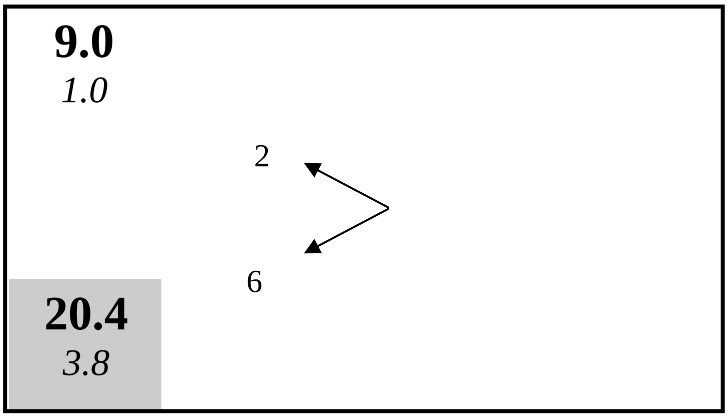


Figure 7. Group mean percentages (in bold) and standard errors (in italics) of the time spent in the correct and incorrect corners during the test trial by the animals in Experiment 1. The numbers at the end of the arrows indicate how many rats headed directly to each corner at the outset of the test trial. Values across both correct corners were combined to provide a single figure. Values for the incorrect corners were also combined. The grey square indicates the correct corner.

The current study confirms that animals can form S-S* associations during placement training, which they can then use to navigate to a hidden goal. As such, the remainder of this thesis will make use of placement training in order to study the role and content of S-S* associations in spatial learning. However, prior to using this design to determine the type of information that form the content of these associations, the remaining experiment in this chapter will confirm the reliability of my method of placement training by assessing how its outcome is affected by manipulating different training parameters. The practical reasons for doing so were to ensure that the most effective training was given to produce the most successful spatial learning in the subsequent experiments. Jacobs et al. (1989a) demonstrated that the latency to find the platform during the test trials decreased as the number of placements increased. One purpose of the following experiment was to determine if a similar manipulation could also result in an improved performance on test trials such as the one used in the current experiment.

Experiment 2

Experiment 2 was designed to determine how many placements were required for the animal to demonstrate successful latent spatial learning. Jacobs et al. (1989a) conducted a similar experiment, which examined how the effectiveness of placement training was influenced by the number of training trials. They gave rats either 5, 10 or 15 placements on the platform. During the test trials, in which rats were required for the first time to swim to the platform, the animals given 15 placements were significantly quicker to find the platform than those which received 10 placements, who in turn were significantly quicker than those who received 5 placements. However the design of Experiment 1 was different to that of Jacobs et al., and so it is necessary to determine how many placements in the arena used in Experiment 1 are required for the rats to demonstrate a preference for the correct corners during the test trial. The rats were given either 2, 5, 9 or 13 sessions of placement training in one corner of the rectangle. They were then given a test trial in the same manner as described in Experiment 1.

A second purpose of Experiment 2 was to determine whether a preference for the correct corner was retained after a delay of 24 hours. As noted in Chapter 1, Jacobs et al. (1989a) found that although they could demonstrate successful latent spatial learning with as few as 5 placements, even with 15 placements, this training was ineffective with a gap of 24 hours between the final placement trial and the test trial. The second purpose of the current experiment was therefore to determine whether the current method of placement training would result in sufficiently robust latent spatial learning that its effects would remain evident 24 hours after the final training trial. Often animals are required to remember the whereabouts of a goal, such as the location of hidden food, for a much longer period of time than five minutes. For instance, the Clark's nutcracker buries seeds in a large number of 'caches' throughout the autumn and then revisits the caches and collects the seeds throughout the winter and spring. The nutcracker is throughout to revisit up to 3750 caches in a year (Vander Wall, 1982). If the S-S* associations formed by the rats in the previous experiments do not last as long as 24 hours they are unlikely to play an important part in spatial learning. After the animals were given the first test session, they all received a further session of training the following day and were tested again 24 hours later.

Method

Subjects and apparatus. All of the 64 rats were from the same supplier and were housed in the same way as the rats in Experiment 1. They were randomly assigned to one of the four groups. Originally, there were16 animals in each group, but this number was reduced during the course of the experiment. Four rats were removed because they left the platform and swam during training. A further four were removed from the first test trial because there was a problem with tracking their movements during the test. For the same reason, a single rat was removed from the second test trial. As a result, for Test 1 there were 15 rats in the 13-session group, 14 in the 9-session group, 16 in the 5-session group and 12 in the 2-session. For Test 2 there were 14 rats in the 13-sessions group, 14 in the 9-session group, 16 in the 5-session group, and 13 in the 2-session group.

The apparatus was the same as was used in Experiment 1.

Procedure. The pretaining, training and testing were exactly the same as for the single group of Experiment 1, with the exception that the rats received varying numbers of placement training sessions. The number of sessions of training received by each rat is indicated by the name of the group (13-, 9-, 5-, 2-session). Each rat received a test trial on the fourth trial of the final session of training – Test 1. The rats all received another full session of training the day after the first test session and then, 24 hours later, they received a further test trial – Test 2.

Results and Discussion

The results of Test 1 can be seen in Figure 8, which shows that all four groups spent more time in the two search zones in the correct corners than in the search zones in the two incorrect corners during the test in the rectangular pool. A two-way

ANOVA comparing the amount of time spent in each zone by each of the four groups revealed a significant effect of zone, *F*(1,53) = 25.07, p <.01, *MSE* = 26.51, but no significant effect of group, *F*<1, and no interaction, *F*<1. The amount of time spent in the correct and incorrect corners were compared using t-tests to provide a more specific analysis of whether or not each group showed a preference for the correct corners. All four groups spent a significantly greater amount of time in the correct corners than the incorrect corners, *t*(14) = 2.68, *p* = .02, *t*(13) = 2.74, *p* = .02, *t*(15) = 2.15, *p* > .05, *t*(11) = 2.97, *p* = .01 for groups 13-, 9-, 5- and 2-session respectively. These results demonstrate that with even as little as two sessions of training, the rats showed a preference for the correct corners over the incorrect corners. 6 of the 15 rats in the 12-session group, and 11 out of the 16 animals in the 5-session group entered a correct corner before an incorrect corner. Using a binomial test, this preference is not significant, *p* = .15 and *p* = .06 respectively. However, 12 of the 14 in the 9-session group, and 10 of the 12 in the 2-session group entered a correct corner before entering an incorrect corner. Using a binomial test, this preference is significant, *p* <.01 and *p* = .02 respectively. Moreover, if the rats are combined, then 39 of the 57 rats entered the correct corner first, which is significant using a binomial test, *p* <.05.

The results from the second test trial can be seen in Figure 9. Even after a 24-hour delay, all of the groups spent more time in the correct corners than the incorrect corners. A two-way ANOVA comparing the amount of time spent in each zone by the four groups showed a significant effect of corner, *F*(1,53) = 19.79, *p*<.01, *MSE* = 26.12, but no effect of group, *F*(3,53) = 1.67, *MSE*=11.81, and no interaction, *F*(3,52) = 1.69, *MSE* = 26.12.

A series of t-tests was conducted to examine whether each group showed a significant preference for the correct over the incorrect corner. The difference was significant for groups 9-session, *t*(13) = 4.27, *p*<.01, and 5-session, *t*(15)= 3.29, *p*<.01. However the difference for groups 13-session, *t*(13) = 1.55, and 2-session, *t*(12) = .69, were not significant. None of the groups chose to enter the correct corner first on significantly more occasions than the incorrect corner. In both the 13- and 9-session groups, 7 of the 14 rats entered the correct corner first, *p* = .21, 10 of the 16 in the 5-session group, *p* = .12, and 7 of the 13 in the 2-session group, *p* = .21, entered a correct corner before entering an incorrect corner. When all four groups are combined, 31 out of 57 rats entered the correct corner first, which is also not significant using a binomial test, *p* = .08.

Jacobs et al. (1989a) found that although rats could learn where the platform was situated in a circular pool with as few as five placements, they also noted that the time to reach the platform during the test trial significantly decreased with a greater number of placements. Specifically, they found that 10 placements produced significantly shorter latencies to find the platform than 5 placements, and 15 placements produced significantly shorter latencies than 10 placements. These findings are contradictory to the current results which show that there was no significant effect of being given different amounts of training. While it is possible that stronger associations were created with greater amounts of training in my experiment, there are a number of reasons why the results suggest that this is not the case. First, in each training session there were four trials, and therefore four placements. Jacobs et al. looked at the effect of the amount of training given, up to 15 placements in total.

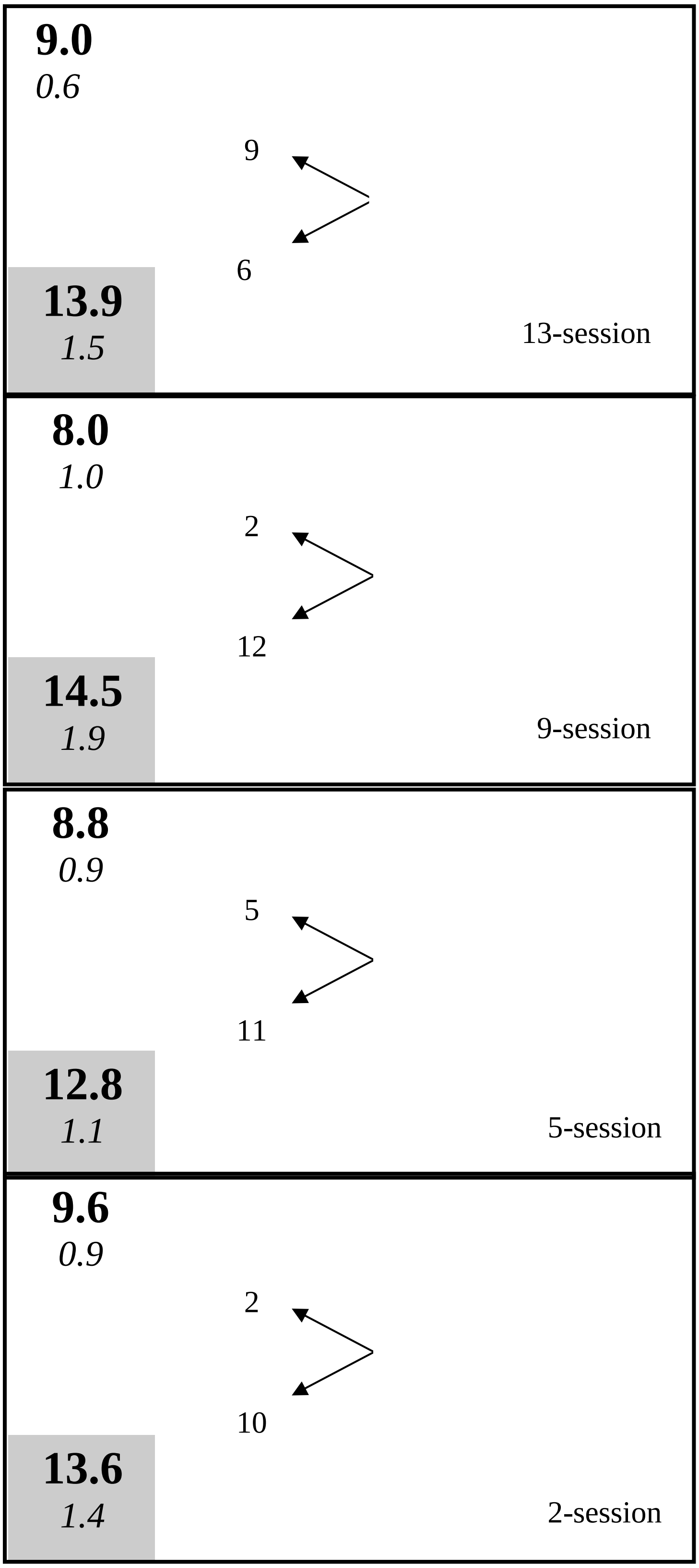


Figure 8. Group mean percentages (in bold) and standard errors (in italics) of the time spent in the correct and incorrect corners for the test 1 of Experiment 2. From the top the panels show the results for the 13-, 9-, 5- and 2-session groups. The numbers at the end of the arrows indicate how many rats headed directly to each corner. Values across both correct corners were combined to provide a single figure. Values for the incorrect corners were also combined. The grey square indicates the correct corner.

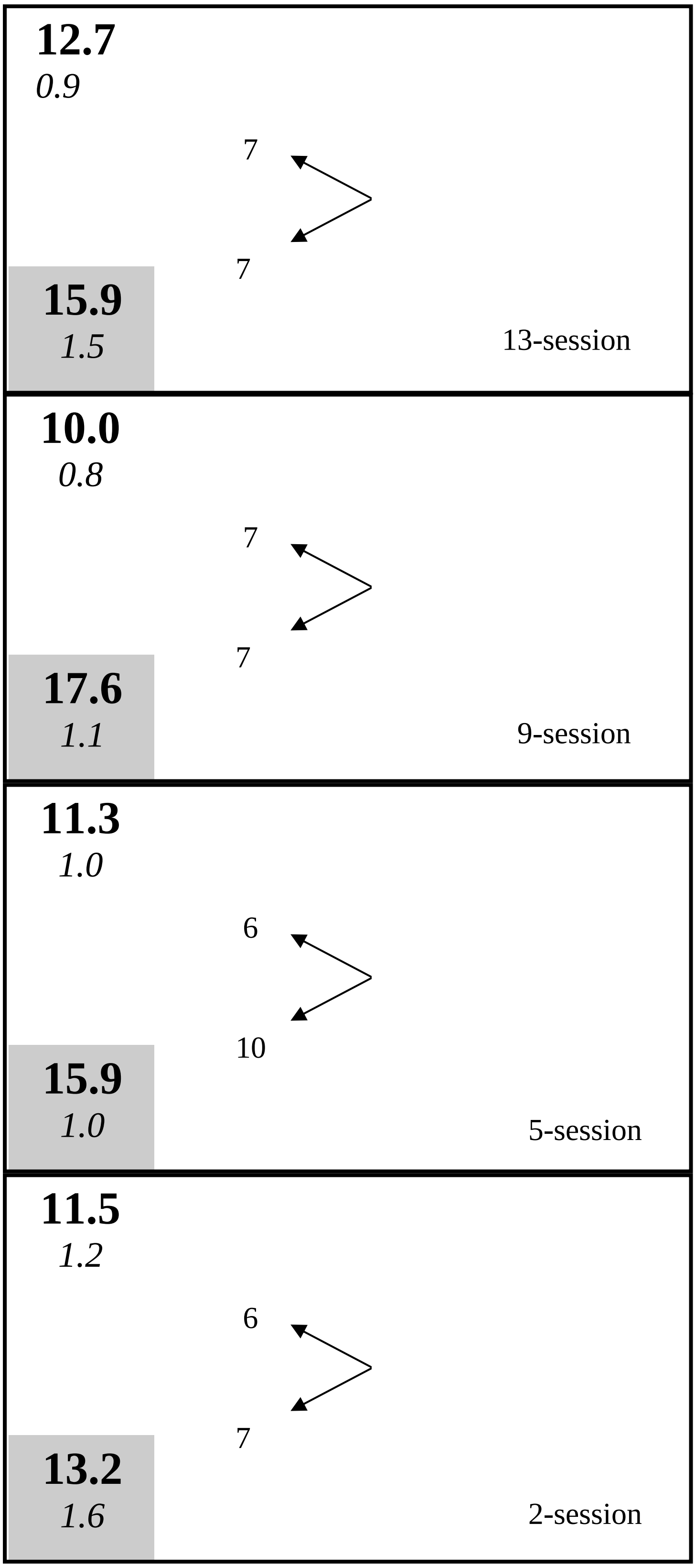


Figure 9. Group mean percentages (in bold) and standard errors (in italics) of the time spent in the correct and incorrect corners for test 2 of Experiment 2. From the top the panels show the results for the 13-,9-,5- and 2-session groups. The numbers at the end of the arrows indicate how many rats headed directly to each corner. Values across both correct corners were combined to provide a single figure. Values for the incorrect corners were also combined. The grey square indicates the correct corner.

This number was exceeded by all of the four groups in the current experiment except the 2-session group. It is possible that the learning of S-S* associations reaches asymptote around 15 placements, and so there was no room for the animals to learn more beyond this point. As such, differences between the 5-, 9- and 13-session groups would not be expected, however the animals in the 2-session group might be expected to show a significantly weaker preference for the correct corner than the other groups as they only received seven placements in total, which they did not.

An alternative explanation is that the available cues were more salient in the present experiment than in the study by Jacobs et al. (1989a). As explained above, unlike any of the previous research described, the current apparatus provided cues which were a part of the arena itself, rather than outside the arena. The possible increase in salience of the cues in the present study may mean that spatial learning occurred at a more rapid rate than in the study by Jacobs et al. Consequently, only a few sessions of training would be required for the animal to learn the location of the platform with reference to these cues. It is not possible from the current experiment to determine how accurate either of these accounts is, however it is clear that rats require very few sessions of training before they develop a strong preference for the correct over the incorrect corners at test. It is also important to note that a non-significant effect of the number of sessions of training does not mean that there is no effect of the number of placements. A more sensitive test may have revealed an effect of greater amounts of training, however it is apparent that the rats can develop S-S* associations in the current spatial task with relatively little training.

Test 2 was conducted to see if the rats could retain the S-S* association that they had acquired during the initial training over a delay of 24 hours. The first point to note from the current results is that both the 5-session and 9-session groups continued to show a preference for the correct corners over the incorrect corner after a delay of 24 hours since the most recent training trial. The result are contradictory to Jacobs et al. (1989a) who found that given placement training, animals could not retain latent spatial learning after 24 hours. As explained, there are at least two differences between the current methodology and that used by Jacobs et al. that may account for the difference in performance in the two experiments. First, the rats were given more training in the current experiment. Whereas animals in the current experiment were given a minimum of 11 placements (the 2-session group received a total of 7 placements before Test 1, and 4 between Test 1 and Test 2), the rats in the Jacobs et al. experiment were only given 15. The greater amount of exposure to the cues through an increased number of placements may explain the more successful navigation in the current experiment. The fact that animals in the 2-session group did not show a significant preference for the correct over the incorrect corners provides some weight for this explanation, as they received fewer placements than the animals used by Jacobs et al. In keeping with this explanation, the animals in the 5-session and 9-session group who received 23 and 39 placements respectively did show a significant preference for the correct corner. These results suggest that given a sufficient number of placements, rats can retain latent spatial learning after a delay of 24 hours. This is not true of the 13-session group; this point will be returned to later in the discussion.

As noted, a further difference between the design used by Jacobs et al (1989a) and the current design was the location of the available cues. Unlike Jacobs et al., where the cues were outside the experimental arena, the cues in the current study were contained in the arena itself. It was hoped that the arena used in the current experiment would therefore produce more salient cues than those provided in previous experiments. The salience of the cues may provide a further explanation as to why, contradictory to Jacobs et al. experiment, animals in Experiment 2 were able to retain latent spatial learning over a delay of 24 hours. Perhaps more salient cues meant that the animals could acquire more robust S-S* associations during placement training, which were therefore not forgotten as quickly as they were by the animals used by Jacobs et al. In reality, it is probably a combination of the number of placements and the salience of the cues that meant that the current design produced sufficiently robust latent spatial learning that it was remembered 24 hours later.

A preference for the correct over the incorrect corner was not evident for the 2-session and the 13-session groups. The reason for this result by the 2-session group has already partly been dealt with. It is quite plausible that while animals with only three sessions of training are able to acquire S-S* associations, this is not enough training for them to develop sufficiently robust associations that they are retained for 24 hours. The results of the 13-session group are a little more difficult to explain as they received more training than the two groups which showed an ability to retain this information over a delay of 24 hours. One possibility is that there was simply a sampling error, and that for some reason independent of the experimental methods, this group of rats was less able than the others to retain information acquired during placement training. There is some evidence that this may be the case as the same group performed worse numerically during Test 1 than the 9-session group which received four fewer training sessions. Alternatively, it may be that increased training beyond a certain amount of placements results in a poorer performance. A possible explanation for this effect is that rats which have received more training and, therefore, more exposure to the environment are less anxious than those which have received fewer training trials. Those rats which are less anxious may spend more time exploring the pool and be more willing to enter areas to which they had not already been exposed. Therefore, while the 13-session group continued to show a preference for the correct over the incorrect corners, this preference might have been weakened by a strong tendency to explore regions of the pool other than the correct corner. At this point, it is not possible to determine whether the reduced preference for the correct corner by the 13-session group was a result of sampling error or to an increased tendency to explore the environment. This issue is dealt with again in Experiment 4 (see Chapter 3).

It is important to note that there was some variability between the groups as to which corner the rats entered first during the first test of Experiment 2. However, when all four groups were combined at Test 1, the preference for entering the correct corner before an incorrect corner was significant. At Test 2, the preference was not significant even when all four groups were combined. Although the corner that the animals enter first does not appear to be as sensitive a measure as the percentage of time spent in each zone, it appears to be an additional indication of whether placement training has been effective, especially when the group size is large.

General Discussion

The experiments in this chapter were conducted primarily to confirm that the adopted method of placement training results in latent spatial learning. Experiment 1 confirmed the effectiveness of the placement training. It is not possible to explain this result in terms of S-R associations because the placement training was designed specifically to prevent the formation of such an association. Experiment 1 therefore demonstrates that S-S* associations alone can be used to learn about the position of the platform within the arena through placement training.

Test 1 of Experiment 2 then demonstrated that placement training is extremely effective, since a preference for searching in the correct corner could be produced with as few as two sessions of training and there was no significant benefit of providing the animals with additional training trials.

Test 2 of Experiment 2 demonstrated that the learning which occurred during placement training was still effective 24 hours after the final placement trial for those animals which had received either six or ten sessions of training. This is a particularly important finding since Jacobs et al. (1989a) were unable to demonstrate that placement training resulted in any long-term changes in behaviour. To my knowledge this experiment is the only example of placement training resulting in associations that were retained for at least 24 hours, and provides support for accounts which argue that animals use S-S* associations to navigate (e.g. Keith and McVety, 1988).

Chapter 1 outlined the varied results produced by placement training in previous experiments. The experiments described in this chapter provide the first examples of consistently successful placement training. Furthermore this chapter demonstrates the S-S* associations which develop during placement training are robust enough to influence the animals behaviour at least 24 hours later. The reliability of the effect of placement training demonstrated in these experiments lends considerable support to theories of spatial learning based on S-S* associations. For instance, Tolman et al. (1946) and O’Keefe and Nadel (1978) suggest that spatial relationships in an environment are represented by a cognitive map and are independent of behaviour. That is, animals should not have to rely on S-R associations to learn the location of the goal within the arena. Clearly if animals were to make use of S-R associations alone to navigate, then placement training would not be successful in producing a preference for searching in the correct corner of the arena at test. Thus, the rats must have acquired and made use of S-S* associations. This finding raises the question of whether spatial learning, in an arena with a distinctive boundary, involves any S-R associations at all. If the results demonstrate that the learning while on the platform is sufficient to produce successful navigation then it is feasible that this is the only type of information that is gathered during spatial navigation, even when animals are permitted to explore the environment. This possibility is addressed again in Chapter 4.

Having established that that the adopted placement procedure is an effective way of producing spatial learning based on S-S* associations, the next chapter will investigate the nature of the information encoded in these associations.

Chapter 3

The experiments in Chapter 2 demonstrated that animals that receive placement training in a rectangular pool show a preference for searching in the corners with the same geometric properties as the corner where that training took place. This finding was taken as evidence that rats can develop S-S* associations which allow them to learn the spatial relationship between the platform and the cues provided by the environment. The purpose of the current chapter was to determine the type of cues which enter into the S-S* associations developed during placement training. As outlined in Chapter 1, animals may form their spatial memory based on global cues or on a certain feature of the environment. Global representations, or cognitive maps, include information about the entire environment. If animals navigate with reference to global cues, then in the rectangular arena used in the previous chapter, their mental representation would be of the rectangle as a single shape. Alternatively, the animals might be using specific features of the environment to form an association. That is, they may only refer to particular elements of the arena, rather than the entire environment. Feature cues can be further divided into local or distal cues. For instance, in the example given in Figure 5 (Page 16) they may have learned that the platform was at the left hand end of a long wall. This would constitute a local cue as it is proximal to the goal. Alternatively, the animals may have learned that the correct corner was diagonally opposite a corner with a long wall to the right of a short wall. This would constitute a distal cue as it is a feature of the environment, but is not proximal to the goal. For the purposes of this thesis, the local cues are defined as the corner in which the platform is placed and the two walls that make that corner. The distal cues are defined as the three corners in which the platform is not placed and the two walls that do not make up the local corner. It is not possible to determine which cues the animals make use of when they are trained and tested in the same arena, because all of the cues are available during both stages of the experiment. Instead, a transfer test is required, in which one or more of the cues is altered.

Experiment 3

The purpose of Experiment 3 was to determine whether or not rats rely on global cues to learn the position of a goal within the environment by using a transfer test. The design of the experiment was based on that of Pearce, Good, Jones & McGregor (2004), but rather than swimming to the platform, rats were placed on it. Rats received placement training in corner A of the rectangle and were then transferred to a kite-shaped arena that was made up of the same four walls (Figure 5). As with the apparatus used by Pearce et al. (2004) corner E in the kite was geometrically identical to corner A in the rectangle. Similarly, corner F in the kite is geometrically identical to corner B in the rectangle. If the animals use local cues to navigate then they should show a preference for corner E over corner F, because corner E is made up of the same local features as corner A, the correct corner. However, if the animals were using global cues then they would be lost in the kite, as the shape of the test environment is different to that used for training, and so would not show a preference for corner E in the kite over corner F. Similarly, the animals would not be expected to show a preference for corner E if the S-S* associations in the rectangle were based on distal cues, since the distal cues from corner E in the kite were not the same as those viewed from corner A in the rectangle.

Animals were divided into three groups, the 4-session, 8-session and 16-session group, which denoted the number of placement training sessions in the rectangle that they received prior to being released into the kite-shaped arena. The animals were given varied amounts of training since the results of Test 2 of Experiment 2 in the previous chapter suggested that the amount of training given may affect the robustness of the acquired S-S* associations. As it is quite possible that a generalisation decrement may occur as a result of the transfer for the test trial, the extent to which the latent spatial learning is robust may affect the animal’s ability to navigate in the new arena. There was also some suggestion from the 13-session group in Experiment 2, that greater amounts of training may reduce the animal’s preference for the correct corner. For these reasons, the animals were given different amounts of training before being tested in the kite.

Method

Subjects and apparatus. Forty-eight animals were from the same stock as those used in Chapter 2 and were maintained in the same way. The same boards as were used in Experiments 1 and 2 were used to create the rectangle. They were then rearranged to form a kite during the test trial. All other details of the apparatus were the same.

Procedure. The rats were evenly divided into three groups. Three rats were removed as they left the platform during placement training, resulting in 16 animals in the 4-session group, 15 in the 8-session group, and 14 in the 16-session group.

The experiment started with three sessions of pretraining, which were conducted in the same manner as for the experiments in Chapter 2. The animals then received either 4, 8 or 16 sessions of placement training in the rectangle which was identical to the placement training given in the previous two experiments, with four trials in each session. The platform was always placed in the same corner and the animal was placed on the platform for 30 seconds at a time. On the final trial of the final session, the rats received a single test trial in the kite-shaped arena. As with the tests in the rectangle in the previous chapter, a notional zone with a 30-cm diameter was placed in each of the four corners of the kite. The rats were released into the middle of the pool and allowed to swim for 60 sec. Their movements were tracked using the tracking software and the time spent in each of the zones was recorded. The corner that each rat entered first during the test trial was also recorded.

Results and discussion

The results of the experiment can be seen in Figure 10. The upper panel shows that the animals in all three groups spent more time in the correct corner than the incorrect corner during the test in the kite. An ANOVA comparing the amount of time spent in each of these corners by the three groups shows a significant effect of zone, $F(1,42) = 14.37$, $p < .01$, $MSE = 63.38$. However, there was no effect of group, $F(2,42) = 2.57$, $MSE = 63.38$, and the interaction was not significant $F < 1$. Individual t-tests were conducted comparing the time spent in each corner for each group. For the 8-session and 16-session groups, the preference was significant, $t(14) = 3.22$, $p = .03$, and $t(13) = 2.42$, $p < .01$, respectively. However, the preference for the correct corner over the incorrect corner was not significant for the 4-session group, $t(15) = 1.37$. None of the three groups swam directly to the correct corner significantly more often than would be expected at chance level. Of the 4-session group, 9 of the 16 animals entered the correct corner first, $p = .17$ as did 8 of the 11 animals in the 8-session group, $p = .08$, and 10 of the 14 in the 16-session group, $p = .06$. However, with the three groups combined, 30 out of the 45 animals entered the correct corner before the incorrect corner significantly more often than would be expected by chance, $p = .01$.

In order to determine whether there were significant preferences for any other corner, the results from the three training groups were combined, and all four of the corners in the kite were compared. The results can be seen in the bottom panel of Figure 10. A one-way ANOVA showed an effect of zone, *F*(3,132) = 10.36, *p* <.01, *MSE* = 110.59. A series of t-tests revealed that the animals spent significantly more time in the correct corner than any other, *ts*(44) > 2.98, *p* <.01 but all other possible comparisons were not significant, *ts* (44) < 1.39, *p* >.17.

The results indicate first that animals that were transferred to the kite-shaped arena after placement training showed a preference for the corner with the same geometric properties as the corner in which the platform had been placed in the rectangle. This preference was not significant for the 4-session group. However, these results can easily be understood when it is considered that these animals may simply not have had enough training to form sufficiently robust learning that they were able to act upon it in the new environment. The results from the remaining two groups however, provide clear evidence that during placement training, animals learn about feature cues that are located close to the goal. If the animals had been relying on a global representation of their environment, then they would not have shown a preference for the correct corner because they would have been lost in an environment of a different shape to the one in which they had been trained. Similarly, had the animals used distal feature cues to learn about the location of the platform, they would not have shown a preference for the correct corner over any other. None of the distal cues that were present during the training stage in the rectangle were available during the test in the kite. Thus, if the animals had been relying on distal cues, then they would have been unable to locate the correct corner in the test arena.

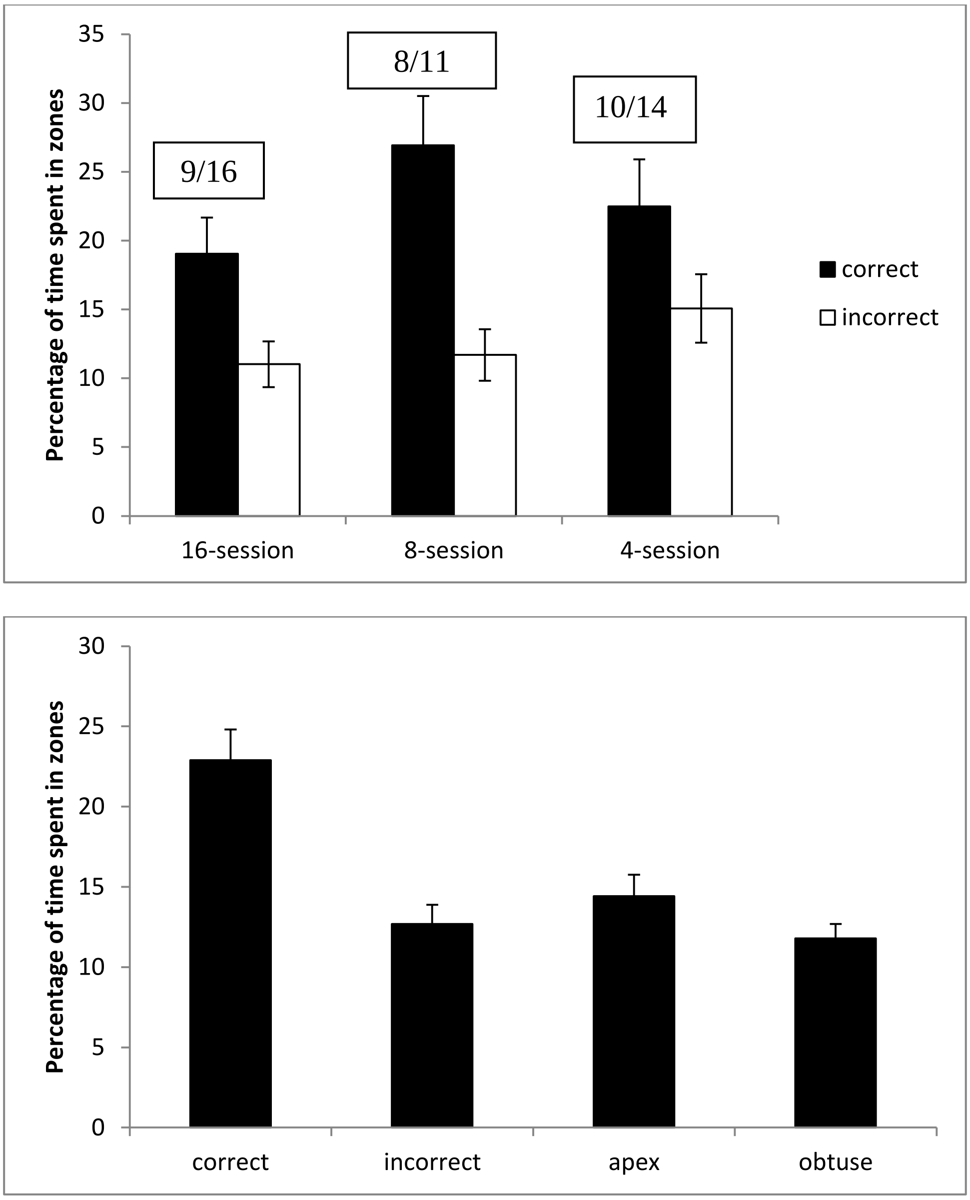


Figure 10. The results of Experiment 3. The top panel shows the percentage of time spent in the correct and incorrect corners. The numbers in the boxes above show the number of animals in the group that went to the correct corner before the incorrect corner. The second number in the box indicates the number of animals in that group. The bottom panel shows the percentage of time spent in each corner of the kite-shaped arena by all of the animals combined. Error bars refer to +/- 1 SEM.

As with the design used by Pearce et al. (2004), upon which the current experiment was based, the result does not preclude the possibility that animals also learn about global cues, or distal cues. The test trial only serves to demonstrate that animals can learn about the local features of the environment, and that they do not rely solely on a global representation of the training arena, or solely on individual distal cues. It is possible that animals learn about all three kinds of cue, but that the test trial that they received did not provide an opportunity to demonstrate knowledge of any cues, other than local ones.

The remaining experiments in this chapter were designed with the intention of giving animals sufficient opportunity to demonstrate the use of both feature and global cues, should they possess such representations. In order to do so, squares with distinctively coloured walls were used. As will become evident in the following experiments, using such an arena provides more opportunity to study the effects of varying different types of cues independently than is possible using walls of different lengths.

Experiment 4

The principal reason for conducting Experiment 4 was to confirm that latent spatial learning can be successful in a square arena in which some of the walls are different to the others. One group of rats was repeatedly placed on a platform that was situated in one corner of a square arena with three white walls and one black (B) wall – the 1-B group. The platform was always in the same corner at one end of the black wall (see upper left-hand panel of Figure 11). Towards the end of the placement training, the rats received a single trial in the pool in which they had been trained.

They were then given a second test in a new arena, comprising alternating black and white walls (see upper right-hand panel of Figure 11). On the basis of the results of the previous experiments, it was expected that rats would show a preference for the corner where the platform was previously situated over any other corner in the familiar arena. For the test in the new arena, it was not clear what the outcome would be. The training in the original arena might result in the position of the platform being identified by local cues as was seen in Experiment 3. In the case of the current experiment, the local cues were the black and white walls creating the corner. For example, given the arrangement shown in the upper left hand panel of Figure 11 rats might learn that the platform is situated at the left-hand end of the black wall, or in the corner where a white wall is to the left of a black wall (e.g. George, Ward-Robinson, & Pearce, 2001). On this basis, a clear preference for the corners that match the one where placement training took place should be observed for the test in the new arena. Alternatively, rats might identify the position of the platform in the familiar arena with reference to distal or global cues. Because the changes required to create the new test environment will modify both kinds of cue, any control they acquire should then be weakened, and result in animals displaying rather little interest in the corners that are common to those used for placement training.

A second group of rats was included in the experiment – the 2-B group. This group received the same placement training as the 1-B group, but in the arena with alternating black and white walls. The group also received the same two test trials as the 1-B group. Despite the use of a different training environment, the predictions made for the 2-B group are the same as for the 1-B group. Thus, if the corner where the platform was situated is identified by local cues, then the 2-B group will show a strong preference for searching in the corners made from these cues in both arenas.

If, however, the position of the platform is originally defined relative to distal or global cues, then replacing a black wall with a white wall in the new arena is likely to disrupt the influence of these cues and result in a rather weak preference for one kind of corner over the other.

Method

Subjects and apparatus. The rats were 36 male, hooded-Lister rats from the same stock and maintained in the same way as for the previous experiments.

All aspects of the room and pool were identical to the previous experiments, only the walls of the arena were different. The two square arenas were constructed from four white Perspex walls measuring 141.0 cm long, 59.0 cm high, and 22 mm thick. Three of these walls also served as black walls by covering with matte black paint, on one side only, the region between the upper edge of the board and a horizontal line that was 24 cm above the surface of the water. The board was white below this line in order to reduce an unconditioned preference for black over white walls that was found in preliminary studies. One side of these three walls could thus serve as a white wall while the other could serve as black wall. Each wall was placed vertically in the pool and suspended from square aluminium bars (1.0 cm x 1.0 cm) that extended beyond the edge of the pool. The walls were attached to the bars by nuts and bolts which enabled either side of each wall to face into the pool, with the bars outside the pool. Depending on the arrangement of the bars supporting the walls, the upper edge of the wall was either 48 or 49 cm above the surface of the water. The sequence in which individual boards were combined to create the arena varied randomly from session to session.

Procedure. All aspects of the pretraining and training procedure were the same as with the previous experiments.

The ten sessions of placement training took place in the square pool with three white walls and one black wall for the 1-B group, and alternating black and white walls for the 2-B group (see Figure 11). Each rat was repeatedly placed on the platform for 30 sec facing the corner, in a corner created by a black and a white wall. For half the rats in each group the platform was always situated in a corner where a black wall was to the left of a white wall. The placement training for the remaining rats took place in the corner where the black wall was to the right of the white wall. Within each session for the 2-B group, the platform was situated in one of the two possible corners for two trials, and in the other possible corner for the remaining two trials. The arena was rotated within the pool by 90, 180 or 270 degrees from one trial to the next in a random sequence, with the constraint that any given corner occupied four different locations, with reference to the experimental room, within each session.

The test trials differed slightly from the previous experiments in that both the orientation of the arena and the position of the experimenter were randomised independently of each other between every subject. This strategy was used in case the rats, during the test trial, adopted the strategy of swimming towards where the experimenter had been standing when they were released. If the experimenter had been standing beside a correct corner during the test trial then the rat's strategy would misleadingly give the impression of successful latent spatial learning. This aspect of the method was adopted for all of the remaining experiments.

The two test trials of the experiment were conducted on Trial 4 of Sessions 8 and 10 of the placement training. For half the rats, the first test trial took place in the arena that was used for placement training, and the second test trial took place in the new arena. The remaining rats received the opposite sequence of test trials. The rats were released into the centre of the pool, facing the experimenter. The experimenter always stood beside the centre of a wall when releasing the rat, and then moved to the adjoining room to observe the rat on the monitor. The test trials were conducted in the absence of the platform, and rats were allowed to swim in the pool for 60 sec.

As with the previous experiments, the time spent in each corner was recorded.

Results and Discussion

For this experiment, and the two immediately following it, the corner used for placement training was constructed from a black and a white wall. Any corner that is identical to this corner will be referred to as a correct corner. Corners that are the mirror image of the correct corner will be referred to as an incorrect corner. Moreover, to simplify their presentation, the results from the test trials have been normalised by ignoring the counterbalancing of the locations of the correct and incorrect corners in each experiment.

The group mean percentages of time spent in the corners of the arena for the test trials with the 1-B group are displayed in the upper row of Figure 11 and the results for the 2-B group are shown in the lower row. For both groups, the results in the left-hand column are from the test in the familiar arena, which was used for training, and the results in the right-hand column are from the test in the new arena. The figure shows that both groups spent more time in the correct than incorrect corners during the test in both the familiar and new arena, but the extent of this preference was greater for both test trials in the 1-B than the 2-B group. This observation was supported by the results of a four-way ANOVA with the within-group factors of zone (correct or incorrect) and arena (familiar or new), and the between-group factors of group (1-B or 2-B) and location (whether the platform was in a corner with a black wall to the left of a white wall, or in a corner with a white wall to the left of a black wall). The analysis revealed a significant Location x Zone interaction, $F(1, 32) = 4.74$, $p = .04$, but the main effect of location, and all the remaining interactions were not significant, Fs(1, 32) < 1.35. Subsequent investigation of the significant interaction, using tests of simple main effects, revealed a significant effect of zone for both locations, Fs(1, 32) > 4.54. In view of this pattern of results, the effect of location was ignored in the following analysis, and was not considered again for the remaining experiments in this chapter.

As can be seen in Figure 11, in each test for both groups, more time was spent in the correct corners than in any other corner, which confirms the effectiveness of the placement training. However, the discrimination between the correct and incorrect corners was more marked for both test trials with the 1-B group than with the 2-B group. This observation suggests that the critical influence on the outcome of the test trials was where training took place, and not where testing took place. To simplify the statistical analysis, a discrimination ratio was calculated for each of the two test trials for every rat. The ratio was of the form $C/(C+I)$, where C is the percentage of time spent in the correct corners, and I the time spent in the other black and white corners – the incorrect corners. There were two correct and two incorrect corners for the 2-B group and one of each of kind of corner for the 1-B group, which makes it meaningful to compare the two ratios directly. The results displayed in Figure 12 support the foregoing observation by showing that the discrimination ratios for the two test trials with the 1-B group are similar, and considerably greater than for the 2-B group, which are also similar.

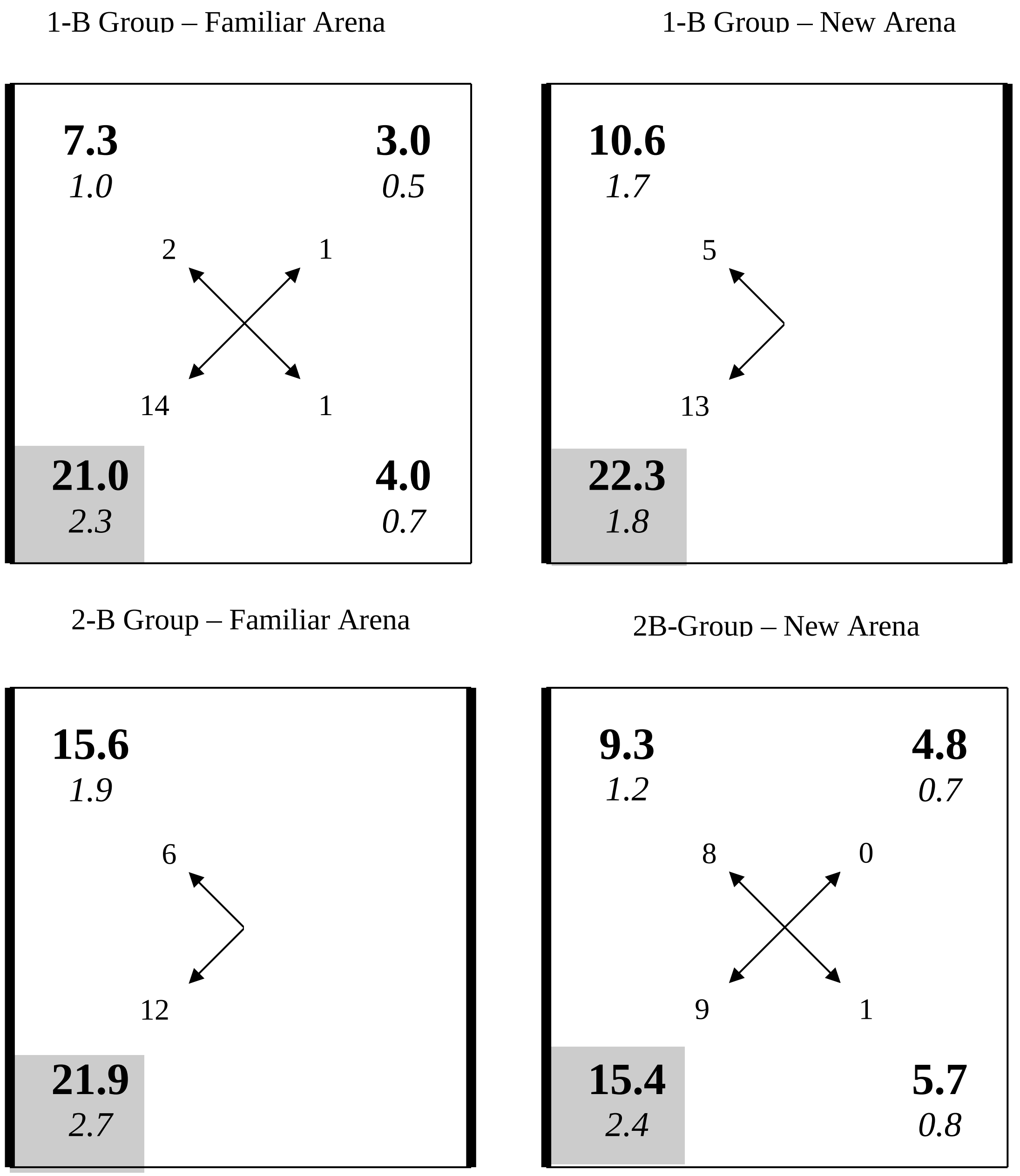


Figure 11. Group mean percentages (in bold) and standard errors (in italics) of the time spent in the correct and incorrect corners during the test trial by the animals in Experiment 4. The numbers at the end of the arrows indicate how many rats headed directly to each corner. Values across both correct corners were combined to provide a single figure both group tested in the arena with two black walls. Values for the incorrect corners were also combined to create a single figure in the arena with two black walls. The grey square indicates the correct corner.

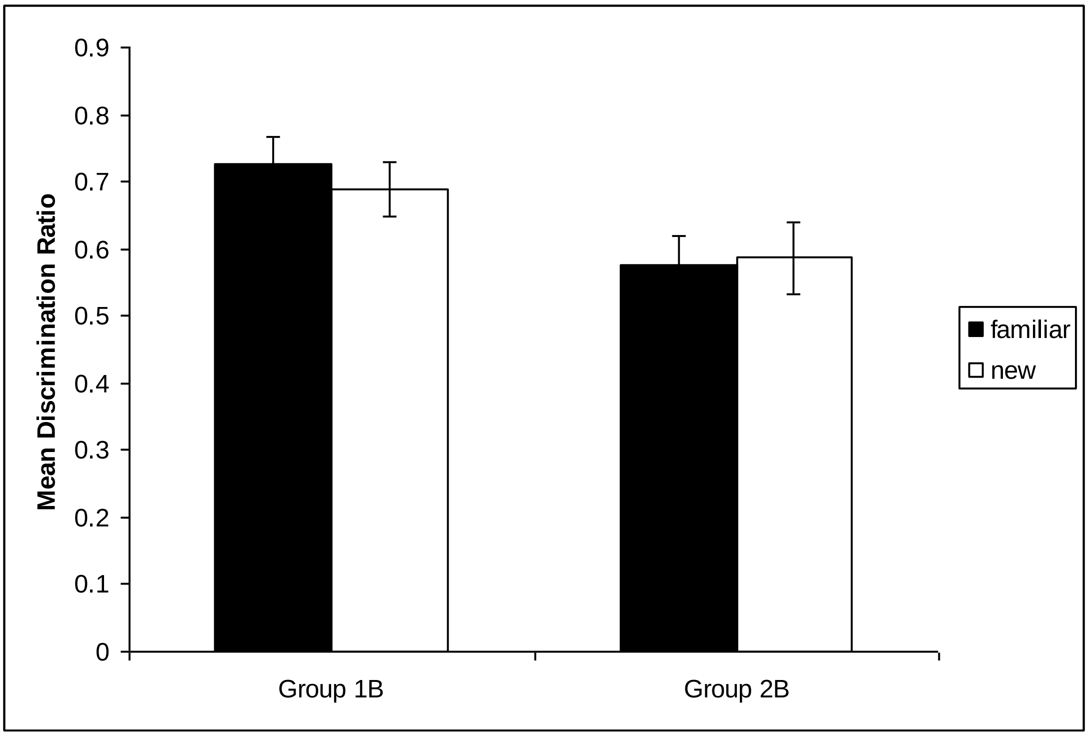


Figure 12. Discrimination ratios for Experiment 4. The bars show C/(C+I) for each group, where C is the amount of time spent in the correct corner and I is the amount of time spent in the incorrect corner. Error bars show +/- 1 SEM.

A two-way ANOVA of individual discrimination ratios revealed a significant effect of group, *F*(1, 34) = 6.58, *p* < .05, *MSE = .043*, but the effect of whether the test context was familiar or new, and the interaction were not significant, *F*s < 1, *MSE*s =.029.

Further support for the conclusion that placement training is more effective in an arena with one rather than two black walls comes from two sources. First of all, a series of *t*-tests revealed that the 1-B group spent significantly more time in the correct than the incorrect corners in the arena with one black wall, *t*(17) = 4.82, *p* < .01, and in the arena with two black walls, *t*(17) = 4.00, *p* < .01; whereas the 2-B group did not spend significantly more time in the correct than incorrect corners in either the arena with two black walls, *t*(17) = 1.49, *p* > .10, or one black wall, , *t*(17) = 1.91, *p* > .10. The second source derives from the analysis of the corners which rats headed to first on being released from the middle of the pool for a test trial. Focussing on just those rats that headed directly for a corner at either end of a black wall, one-tailed binomial tests revealed that the number selecting the correct corner was significantly above chance for the 1-B group when tested in the arena with one black wall, *p* <.01, or two black walls, *p* <.05. Equivalent analyses for the 2-B group failed to reveal a significant effect for the test in the arena with one black wall, *p* = .50, or two black walls, *p* = .12.

To return to the analysis of discrimination ratios, the absence of a two-way interaction indicates that being tested in a new environment did not disrupt performance, relative to that seen by each group in the familiar environment. In order to assess further the effect of the transition from one environment to the other, a Bayesian analysis was performed, together with a standard paired t-test, based on the discrimination ratios described above for every subject in the familiar arena and the new arena. The Bayesian analysis tells us whether the data favour more the null hypothesis (there being no difference between the discrimination ratios for the two environments) or the alternative hypothesis (there being a difference between the two sets of ratios). The Bayes factor is the relative probability of the null hypothesis to the alternative hypothesis such that a value of 1 would mean that each is just as likely. A value of 3 would mean that the null hypothesis is 3 times more likely than the alternative hypothesis given the data and the priors, and has been suggested as a cut off when deciding that data substantially favour the null hypothesis (see Rouder, Speckman, Sun, Morey & Iverson, 2009, for more details). Analysis of the pairs of ratios found in favour of the null hypothesis, $t(35) = 0.33$, $p > .05$, Bayes factor = 7.41.

An implication of this conclusion is that the global properties of the training arenas did not form part of the spatial memory that was used to identify where the platform could be found. If these properties had been important, then changing them should have resulted in a reduction in the amount of time that was spent in a correct corner. The obvious caveat to this conclusion is that it is based on the failure to detect a drop in performance by each group when they were tested in the new rather than familiar arena (see Figure 12). Perhaps evidence of control by global cues would have been revealed with a different method of training, or testing. That said, the experiment revealed that the placement training was highly effective when the 1-B group was tested in the new arena, which provided ample scope to observe a decrement in performance during this test trial.

For similar reasons, it is unlikely that the position of the platform was identified by means of distal cues in the 1-B group. These cues would be based on the two white walls that were opposite the correct corner, and would include the three corners that were not used for placement training. If they were used to identify where the platform was situated, then the replacement of one of these walls by a black wall to create the new test arena would be expected to remove some of the distal cues, and result in less time being spent in the correct corner during the test in the new than the familiar arena, which was not the case. Of course, it remains possible that for some reason rats relied on the distant white wall that was present throughout both tests as the distal cue for identifying where the platform was situated. Although there is a degree of special pleading with this account, it is not possible to rule it out.

Finally, it seems most likely that rats identified the position of the platform with reference to local cues provided by the walls creating the corner where the platform was located for placement training. These cues were unaffected by the transformation of the familiar arena to the new arena for the 1-B group, which would then explain why performance was similar in both tests for this group. Many experiments have shown that rats can use a local cue to find a goal (e.g. Cheng, 1986; Pearce, Graham, Good, Jones, & McGregor, 2006), but it is possible this ability was based on previously acquired stimulus-response associations. The novel contribution of the present experiment, together with that of the previous experiment, is that the successful performance on the test trials was due to the influence of S-S* associations involving the local cue. Moreover, the experiment shows for the first time that latent spatial learning can be effective in an environment composed of black and white walls.

The relative lack of success with the placement training for the 2-B group was not expected. The failure of this group to show a clear discrimination between the correct and incorrect corners in the familiar environment might be interpreted as evidence of a performance deficit, which was brought about by having to swim to a correct corner in the presence of two correct corners. The stumbling block for this suggestion is that the 1-B group showed a clear discrimination between the correct and incorrect corners in the arena with alternating black and white walls, which points to a failure of the 2-B group to acquire a spatial memory during the placement training. Placement training, therefore, may be effective in some arenas, that used for the 1-B group, but not others, that used for the 2-B group. Before taking this conclusion seriously, however, it should be noted that both tests with the 2-B group revealed a numerical, but not a statistically significant preference for the correct over the incorrect corner. The possibility arises therefore that with a different and, perhaps, larger sample of rats, placement training would be effective in an arena with alternating black and white walls.

In order to test the foregoing possibility, a single group of rats in Experiment 5 received placement training and a test trial in the same environment as the 2-B group of Experiment 4. The size of the group was marginally larger than for its counterpart in Experiment 4. It was hoped that this factor, together with the change brought about by use of a different sample, would allow the experiment to reveal a clearer outcome to that seen in Experiment 4.

A second test trial was included in the experiment which involved a square arena constructed from two adjacent black walls, and two adjacent white walls (see the right-hand panel of Figure 13). The purpose of this test relates to a further question posed by the above experiment. After placement training in an arena with one black wall and three white walls, the 1-B group showed a significant preference for the correct over the incorrect corners when it was tested in an arena with alternating black and white walls. I argued that this preference probably reflected the influence of spatial learning based on local cues, but the possibility that this preference was based on learning about the position of the platform with reference to a distal cue could not be ruled out. The test in the new arena in the present experiment involves a change to both of the distal walls that were present during placement training. If placement training is effective because it results in distal cues being used to indicate where the platform can be found, then the present test in the new arena will fail to reveal a preference for the correct over the incorrect corners. On the other hand, if the placement training encourages spatial learning based on local cues then, because these cues will be present during the test in the new arena, a significant preference for the correct over the incorrect corners will be observed.

Experiment 5

Method

Subjects and apparatus. The 24 rats were from the same stock and housed in the same manner as for the previous experiments. Two of these were discarded from the experiment because they launched themselves into the pool from the platform during placement training, and one rat was discarded from the experiment because of a failure in the recording equipment during a test trial. The apparatus, including the four walls, was the same as for Experiment 4.

Procedure. The details of the pretraining, the placement training, and the two test trials were the same as for the 2-B group of Experiment 4, except that the rats were given a total of 12 sessions of training and the tests took place on the final trial of sessions 10 and 12. The apparatus that was used for the test in the new arena was different only in that it consisted of a square pool with two adjacent white walls, and two adjacent black walls (see right-hand panel of Figure 13).

Results and Discussion

The left-hand panel of Figure 13. shows the group mean percentage of time spent in the correct and incorrect corners of the square pool during the test in the training arena. The results for the test in the new arena are shown in the right-hand panel of the figure. In both cases, the results have been normalised so that the correct corner, which is identified by the grey square, is created by a white wall to the left of a black wall. As with the previous experiment, the time spent in the correct zone was made up of the total time spent in the two correct corners, this was also true of the time spent in the incorrect zone. More time was spent in the correct black and white corner than the incorrect black and white corner in both arenas, with the extent of the preference being similar in both environments. In support of these observations a two-way ANOVA based on individual percentages of time spent in the correct and incorrect corners revealed a significant effect of corner (correct or incorrect), $F(1, 20) = 34.92$, $p < .001$, $MSE = 31.0$, but the effect of arena (familiar or new), $F < 1$, $MSE = 19.22$, and the interaction were not significant, $F(1, 20) = 1.35$, $MSE = 41.68$.

A striking result from the experiment is that during the test in the new arena more time was spent in the corner created from two black walls than any other corner, although this difference was small for the comparison with the correct corner. A one-way ANOVA based on individual percentages of time spent in each of the four corners of the new arena revealed a significant effect, of corner, $F(3, 60) = 15.1$, $p < .001$, $MSE = 53.1$. Paired *t*-tests then revealed that significantly more time was spent in the corner created from two black walls than either the corner created from two white walls $t(20) = 6.77$, $p < .001$, or the incorrect corner, $t(20) = 4.25$, $p < .002$, but the comparison with the correct corner was not significant, $t(20) = 1.00$, $p > .25$.

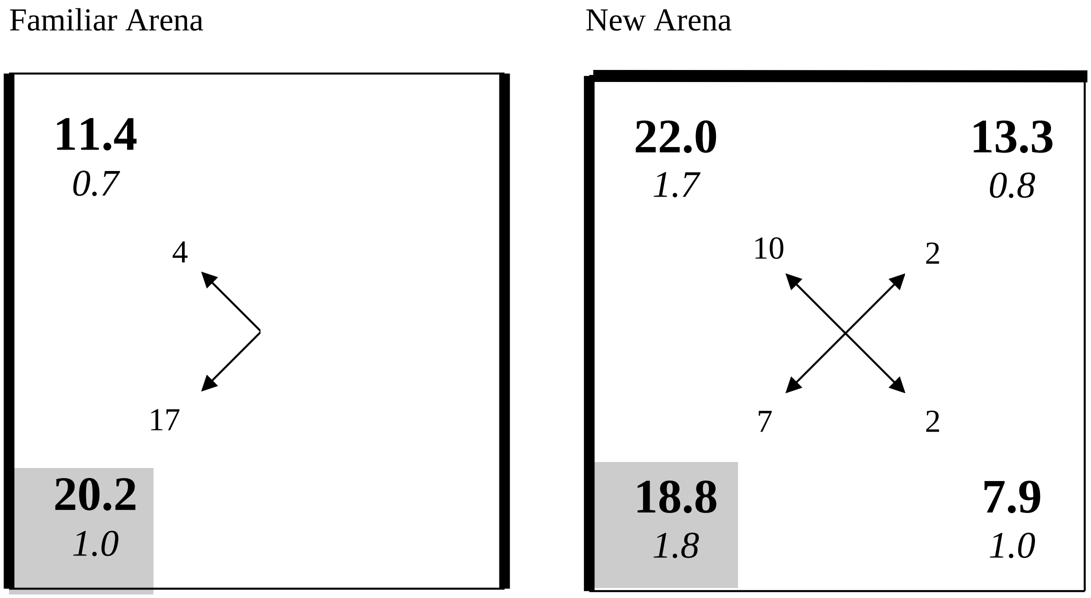


Figure 13. Group mean percentages (in bold) and standard errors (in italics) of the time spent in the correct and incorrect corners during the test trial by the animals in Experiment 5. The numbers at the end of the arrows indicate how many rats headed directly to each corner. Values across both correct corners were combined to provide a single figure in the arena with two black walls. Values for the incorrect corners were also combined to create a single figure in the arena with two black walls. The grey square indicates the correct corner.

The left-hand panel of Figure 13 shows that 17 out of 21 rats headed for a correct corner after being released for the test trial in the familiar pool, which was significant with a binomial test, $p < .01$. In the new arena, only 7 rats headed directly for the correct corner, with 10 heading straight for the corner with two black walls.

The results from the test trial in the arena with alternating black and white walls revealed a clear preference for the correct over the incorrect corners. This effect was evident in the strong tendency for rats to head directly for a correct than an incorrect corner, on being released into the pool. It was also evident in the greater amount of time that was spent in the correct than the incorrect corners throughout the 60-sec test trial. When they are taken together, the results from Experiments 4 and 5 indicate that it is not easy for rats to benefit from placement training in a square arena with alternating black and white walls, but it is not impossible. In support of this last conclusion, we can note that unpublished experiments from our laboratory have revealed successful placement training in an environment with alternating black and white walls.

The placement training also resulted in significantly more time being spent in the correct than the incorrect corner in the arena with two adjacent black walls and two adjacent white walls. The transformation to the training arena for this test makes it unlikely that placement training was effective through the location of the platform being identified with reference to a global representation of the training arena. Likewise, because the transformation involved a change to the two distal walls, and not the two local walls, relative to where the platform was originally situated, it is unlikely that the observed preference was due to the platform’s location being identified with reference to distal cues. Instead, the results in the new arena provide strong evidence that placement training encourages spatial learning based on local cues.

A further finding from the test in the new arena was that rats spent a large proportion of the trial in the corner created by two black walls. One explanation for this outcome is that during their placement training, rats identified the position of the platform as being at a particular end of a black wall. For the arrangement shown in Figure 13 it would be the left-hand end of the black wall. On being tested in the new arena, this information would then lead rats to search either in the correct corner, or in the corner with two black walls. An alternative explanation is that rats on being released into an unfamiliar pool exhibit an unconditioned tendency to head for the darkest region. Experiment 6 was conducted in order to evaluate these rather different explanations.

Experiment 6

A single group of rats received placement training in the same manner as for the previous experiments, but in an arena with three white walls and one wall comprising vertical black and white stripes. The platform was situated in a corner created by a white wall and the striped wall. A test trial was then conducted in the familiar arena, in order to confirm the effectiveness of the placement training. A further test trial was conducted, which was based on the test in the new arena in Experiment 5, but with two striped walls rather than two black walls (see Figure 14). The vertical edges of the striped walls were both white, so that the corner created by the two striped walls was also white and thus lighter than the black corner in the new arena of Experiment 5. If the preference for the black corner in the previous experiment was due to an unconditioned preference for searching in dark corners, then conducting the equivalent test with striped walls should reduce this preference. Alternatively, if the preference for the black corner occurred because rats identified the position of the platform as being at a particular end of distinctive wall, it is possible that the present training will result in similar strategy being adopted. On this basis, the test in the new arena will reveal a strong preference for searching in the correct corner, and in the corner made from two striped walls.

Of course, it is conceivable that the experiment will reveal a stronger preference for the correct corner over any other corner in the new arena. A straightforward explanation for this outcome is that during placement training rats will learn that the platform is in a corner where the striped wall is, say, to the right of a white wall. That is, the spatial memory may encode structural information based on the spatial relationship between the two walls (e.g. Aggleton & Pearce, 2002; George et al., 2001).

Method

Subjects and apparatus. The 12 rats were from the same stock and housed in the same manner as for the previous experiments. One rat was discarded from the experiment for failing to remain on the platform during one of the placement trials. The apparatus was the same as for the previous experiments, with the addition of two striped walls. The striped walls were made by attaching seven vertical strips of black plastic adhesive film (Deco d-c-fix) to white walls. The stripes were 10 cm wide, separated by a gap of 10 cm, and extended from the top of the boards to below the surface of the water. There was a white stripe of between 5 and 6 cm width at each edge of the striped walls.

Procedure. The details of the pretraining, placement training, and testing were the same as for the previous experiment except that the animals received a total of 11 sessions of training and the tests took place on the final trial of sessions 8 and 11. During the placement training the pool was constructed from three white walls, and one striped wall. For half the rats the platform was in the corner with a white wall to the left of the striped wall, and for the remaining rats the platform was situated in the corner at the other end of the striped wall. The new arena for the second test trial was built from two adjacent white walls, opposite two adjacent striped walls.

Results and Discussion

The mean percentages of time spent in each of the four corners during the test trial in the training arena can be seen in the left-hand panel of Figure 14, while the results for the test in the new arena can be seen in the right-hand panel. The results have again been normalised, so that for all rats the correct corner is depicted as the one where a white wall is to the left of the striped wall. It is evident that the tests in both arenas resulted in a stronger preference for the correct corner than any other corner. In order to compare performance in the two arenas, a two-way ANOVA was conducted using individual percentages of time spent in the correct and incorrect corners. There was a significant effect of corner, $F(1, 10) = 66.67$ $p < .001$, $MSE = 44.02$, but the effect of arena, $F(1, 10) = 4.13$, $p > .05$, $MSE = 18.5$, and the interaction, $F(1, 10) = 4.06$, $p > .05$, $MSE = 76.0$, were not significant. In order to compare the time spent in the four corners of each arena, separate one-way ANOVA were conducted. Analysis of individual percentages of time spent in each corner revealed a significant effect in the original arena, $F(3, 30)= 38.20$, $p < .001$, $MSE = 32$, and the new arena, $F(3, 30)= 9.41$, $p <.01$, $MSE = 40.53$. Paired comparisons then revealed that significantly more time was spent in the correct corner than any other corner in the original arena, *t*s(10) > 5.86, *p* <.001, and the same was true for the new arena, *t*s(10) > 2.84, *p* <.05. Further paired comparisons, based on the time spent in each of the three corners other than the correct corner, failed to reveal any significant differences for the original arena, *t*s(10) < 1.16, *p*s >.20, or the new arena, *t*s(10) < 1.82, *p*s >.05 .

The placement training resulted in a clear preference for the correct corner over any other corner in both the familiar and the new arena. The result of the test trial in the new arena are of particular interest, as they have important implications concerning the nature of the local cues that were used to identify where the platform was situated. I noted in the introduction to the experiment that if the position of the platform is defined as being at a certain end of a striped wall, then rats will spend a similar amount of time in the correct corner and the corner with two striped walls. Moreover, more time will be spent in these corners than the remaining two corners. The pattern of results failed to confirm this prediction. Subjects, of course, might have identified the correct corner as being at a certain end of a white wall. If this were the case, then they should have spent a considerable amount of time in the corner created by two white walls in the new arena, as well as in the correct corner. Once again, the results failed to confirm this prediction. The possibility now remains that the rats adopted both of the above strategies, which would then result in them spending more time in the correct corner than in the corners created either by the two striped walls or by the two white walls. An implication of this proposal is that more time should have been spent in corners built from either two striped walls or two white walls than the incorrect corner created from a striped and a white wall. As can be seen in Figure 14, the results from the experiment did not support this prediction.

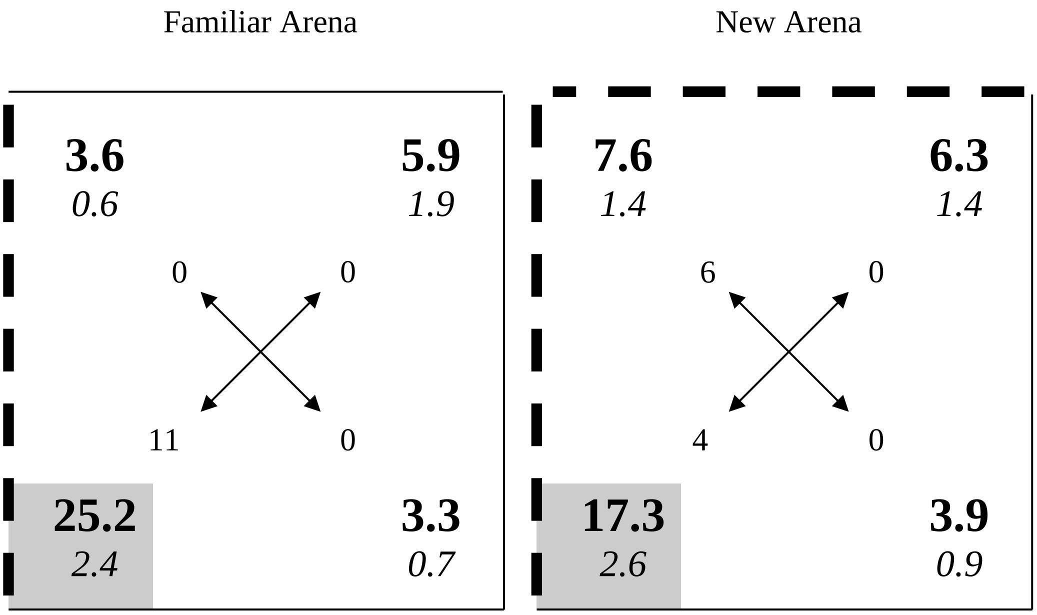


Figure 14. Group mean percentages (in bold) and standard errors (in italics) of the time spent in the correct and incorrect corners during the test trial by the animals in Experiment 6. The numbers at the end of the arrows indicate how many rats headed directly to each corner. The grey square indicates the correct corner.

Therefore, if the position of the goal was identified with reference to local cues, then the most likely interpretation of our results is that the spatial relationship between the walls creating the correct corner was used to identify this corner.

The relatively small amount of time spent by subjects in the corner made from two striped walls stands in stark contrast to the amount of time spent in the equivalent corner for the test in the new arena in Experiment 5. Although this difference between the outcomes of the two experiments is consistent with the possibility that an unconditioned attraction to dark corners was responsible for the strong preference for the black corner in Experiment 5, it can be explained in other ways. The different rats that were used in the two experiments, or slight differences in the way they were trained might also have been responsible for the stronger preference that was seen to the corner created from two black rather than two striped walls.

The left-hand of Figure 14 shows that during the test in the familiar arena, all 11 rats headed directly to the correct corner on being released into the pool. A binomial test revealed that this preference for heading towards the correct than the incorrect corner was statistically significant, $p < .001$. The right-hand panel of the same figure shows that approximately half of the rats headed directly to the correct corner during the test in the new arena, and the remainder headed directly to the corner created by the two striped walls. Given that rats spent rather little time in this corner of the pool, it is unlikely that the tendency to head directly towards it was a consequence of what was learned during the placement training. Perhaps, therefore, rats were attracted to the corner created by two striped walls for no reason other than its novelty.

Experiment 7

The results thus far can be explained by assuming that placement training results only in local cues being used to identify where a platform is situated. The purpose of Experiment 7 was to determine if distal cues can also be used to reveal where a goal is hidden. The experiment involved two groups that were trained and tested in exactly the same way, but in different arenas, in order to confirm the reliability and generality of any effect that was found. For the striped-corner group, three walls of the square arena were striped, and one was white. For the black-corner group three walls were black and one was white (see Figure 15). Placement training was conducted in a corner created by two walls of the same type – either two striped walls, or two black walls. As the arenas contained another corner that was identical to the correct corner, it would not be sufficient for subjects to rely just on local features to identify where the platform was situated. To identify the correct location of the platform, the animal would have to know about its relationship with the white wall on the far side of the arena from the corner where placement training was conducted. In other words, for placement training to be effective, it would be necessary to make use of distal cues. The experiment concluded with a single test trial in the same arena that was used for placement training.

Method

Subjects and apparatus. The 24 rats were from the same stock and housed in the same manner as for the previous experiments. At the start of the experiment they were randomly assigned in equal numbers to the two groups. One rat from the black-corner group was discarded from the experiment for gaining experience of swimming in the pool during the placement training. One arena was constructed from one white wall and three black walls. In keeping with Experiment 4, the region of the wall that was painted black extended from the top of the board to 24 cm above the water. The other arena was constructed from one white wall and three striped walls, which were made in the way as for Experiment 6.

Procedure. The details of the pretraining and placement training were the same as for the previous experiments, with the following exceptions. The placement training took place in an arena with three striped walls and one white wall for the striped-corner group, and in an arena with three black walls and one white wall for the black-corner group. Six rats from the striped-corner group received placement training in one of the corners created by two striped walls, and the remaining rats were trained in the other corner created by two striped walls. A similar method of counterbalancing was used for the black-corner group. There were eight sessions of placement training, with a single test trial occurring on Trial 4 of Session 8

Results and Discussion

In keeping with the previous experiments, the results from the counterbalanced conditions have been normalised to simplify their presentation. The right-hand panel of Figure 15 shows the results from the test trial with the striped-corner group, and the left-hand panel shows the equivalent results for the black-corner group. Both groups spent more time in the correct corner than any other corner during the test trial, and there was little difference between the performance of the two groups. In support of these observations, a two-way ANOVA, using individual percentages of time spent in each of the four corners, revealed a significant effect of corner, $F(3, 60) = 9.17$, $p <.01$, $MSE = 70.16$, but the effect of group, $F(1, 20) = 2.17$, $p >.10$, $MSE = 14.04$, and the interaction, $F<1$, were not significant. Paired comparisons, using the results from both groups combined, revealed that significantly more time was spent in the correct corner than in any of the other corners, *t*s(21) > 2.93, *p*s <.01. It is also evident from Figure 15 that the amount of time spent in the incorrect corner composed of identical walls was similar to the amount spent in the remaining two corners. Further paired comparisons, using the results from both groups combined, revealed that the mean amount of time spent in the two corners created from two different walls did not differ significantly from the amount of time spent in the incorrect corner created from two identical walls, $t(21) = 1.42$, $p > .15$.

In contrast to the previous experiments, there is no convincing evidence that rats had a preference for heading directly for a particular corner on being released into the pool. Analysis of the number of rats heading for each of the four corners, for the two groups combined, failed to reveal a significant preference for one corner over the others, χ^2s(3) < 4.54, $p > .20$.

The stronger preference, in terms of the amount of time spent in the correct corner over any other corner by both groups confirms that rats relied on a distal cue in order to identify where the platform was situated. If they had relied solely on local cues then they would have been unable to tell the difference between the correct corner and the corner which, in terms of local features, was identical to the correct corner. Having established that animals can navigate with reference to distal cues, it becomes relevant to ask about the nature of these cues. Inspection of Figure 15 shows there were several cues that could have been used to identify where the platform was situated. Its location could have been defined, for example, as being diagonally opposite a corner where the white wall was in a particular spatial relation to the black (or striped) wall or, perhaps in a particular position with reference to the white wall.

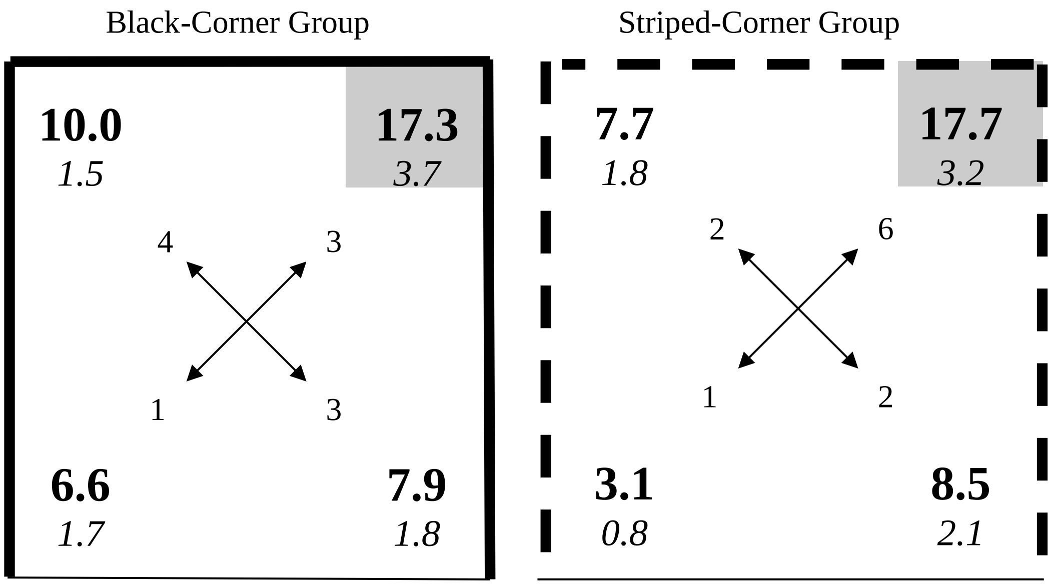


Figure 15. Group mean percentages (in bold) and standard errors (in italics) of the time spent in the correct and incorrect corners during the test trial by the animals in Experiment 7. The numbers at the end of the arrows indicate how many rats headed directly to each corner. The grey square indicates the correct corner.

There is nothing in the present data that allows a choice to be made between these alternatives. It is also possible that rats acquired a global representation of the entire arena which was then used to find the platform on the test trial. This possibility will be considered further in the General Discussion of this chapter.

General Discussion

The five experiments in this chapter have shown that being placed on a platform in a corner of a pool with distinctive walls is a reliable method for demonstrating latent spatial learning. Such learning was effective when the distinctive walls were different lengths, and when the walls were white, black or striped.

The main concern of the present chapter was to identify the type of cues that enter into S-S* associations during placement training, as with the experiments in Chapter 2, learning during placement training can only be based on S-S* and not S-R associations.

The first three experiments of this chapter demonstrate that animals are able to identify the location of the goal with reference to local cues after receiving placement training. They are able to do this in an arena with walls of different lengths, and in an arena where the walls are made distinctive by their colour. Experiment 6 further suggests that the representation of these cues is based on structural information of the sort “white wall to the left of black wall” (Aggleton & Pearce, 2002; George, Ward-Robinson, & Pearce, 2001). It is possible that animals also learn that the platform can be found at a certain end of a particular wall, but the results from Experiment 6 revealed no support for this possibility.

Experiment 7 demonstrated that in addition to local cues, animals can use distal cues to identify the correct corner of an arena after placement training. An important issue that is raised by this finding concerns the manner in which animals treat local and distal information, when both can be used to indicate where a platform is located. It is tempting to suggest that animals will rely on both kinds of cue at the same time, but one aspect of the results from Experiment 7 contradicts this suggestion. The test trials revealed a clear discrimination between the two corners that were identical in terms of their local cues, which must be attributed to rats making use of distal cues. If they also learned about the significance of local cues during placement training, then they should have expressed some interest in the incorrect black corner during the test trial. There was no evidence of this being case. Perhaps, therefore, subjects learned about the significance of both local and distal cues during pretraining, and chose to ignore the former during the test trial because they could be found in two different corners. Alternatively, the local cues provided by the incorrect black corner may have been ignored during the test trial, because they were in conflict with the information provided by the distal cues about where the platform was situated.

Another possible explanation for the results from Experiment 7 is that rats acquired a global representation of the entire arena which incorporated both local and distal information. There is something to be said in support of this possibility. The experiments have shown that rats make use of both local and distal cues, which would be expected if they have a global representation. The experiments have also shown that animals appreciate the spatial relationship between adjacent walls in the arena, which would be expected if they have a global representation of the arena. Furthermore, if rats acquired a global representation of the arenas in Experiment 7 then during the test trial they should be able to identify the correct corner, and treat the three remaining corners as being incorrect.

The results from Experiment 4, however, are not so compatible with the idea that animals acquire a global representation of their environment during placement training. Rats were trained in one environment, and then tested in a different environment which contained the same local cues that defined the correct corner in the original environment. Despite the overall differences between the two environments, there was no evidence that performance was disrupted by the transition from the familiar to the new arena. If knowledge about the location of the platform is based on a global representation, then changing the layout of the training arena should have affected performance on the test trial. The results of Experiment 4 thus make it hard to accept that that latent spatial learning depends upon the development of global spatial representations.

One response to the failure of Experiment 4 to reveal any evidence of the influence of a global representation is to argue that the test was not sufficiently sensitive, or that the choice of arenas was not appropriate for some reason. While this may be true, even if Experiment 4 had revealed a disruptive effect of testing rats in a new arena, such an outcome would not confirm that animals acquire global spatial representations. Instead, during training animals may identify the platform’s location with reference to one or more individual distal cues. The transformation of the arena for the test trial would then affect the ability to identify the correct corner by removing at least some of these cues. In other words, if any experiment should reveal that a change to an environment disrupts the search for the correct corner, then this finding can be attributed to the removal of a distal cue that was used to identify the correct corner. It is hard to know what evidence could be sought in order to go one step further and argue that the distal cue belonged to a global representation.

In summary, the experiments in this chapter have demonstrated that latent spatial learning results in spatial memories based on local and distal cues. No compelling evidence has been found to show that latent spatial learning also results in global spatial memories.

Chapter 4

The purpose of the current chapter is to determine the role of the hippocampus in latent spatial learning. The hippocampus is of theoretical importance since it is the region of the brain in which the cognitive map is believed to be represented (O'Keefe and Nadel, 1978; Jacobs & Schnek, 2003; Sheynikhovich, Chavarriaga, Strösslin & Gerstner, 2009). The reason is that 'place-cells', which are found primarily in the hippocampus, fire maximally depending on where the animal is within its environment. Based on Tolman's (1948) claim that animals possess cognitive maps of their environment, it was argued that place cells in the hippocampus are responsible for the encoding of the cognitive map. As explained in Chapter 1, a cognitive map can be thought of as a global representation of the enviornment. Chapter 2 demonstrated that animals which are repeatedly placed on a platform in one corner of a rectangular arena learned about the location of the platform in relation to the surrounding cues, based on S-S* associations alone. Although Chapter 3 then demonstrated that these associations appear to be based on local and distal feature cues, as opposed to a global representation, or a cognitive map, it is still of interest to determine what part the hippocampus plays in navigation based these associations. A cognitive map is assumed to be a representation of the animal's environment which is independent of any behaviour on the part of the animal, meaning it is not based on S-R associations. This is also true of latent spatial learning which occurs during placement training. It is therefore possible that although there is very little evidence for a cognitive map, as explained in Chapter 1 and as demonstrated throughout this thesis, spatial S-S* associations based on feature cues may involve the hippocampus. If this is the case, then it would be expected that animals with hippocampal lesions would not show a preference at test for the corner in which the platform had been placed during placement training.

The role of the hippocampus in the type of latent learning tasks described throughout this thesis is also of interest, because the ability to discriminate between the correct and incorrect corner may require an intact hippocampus for reasons that are not related to S-S* associations alone. As Experiment 6 demonstrated, rats appear to learn about the ‘structure’ of the corner in which the platform is placed. That is, rather than learning that the platform is at the left-hand end of a striped wall, for instance, they appear to learn about the relationship between the two walls. A number of studies suggest that learning about a combination of stimuli and the relationship between them requires an intact hippocampus (Aggleton & Pearce, 2002; O’Reilly & Rudy, 2000, 2001; Rudy & Sutherland, 1989). For example, Sanderson, Pearce, Kyd & Aggleton (2006) conducted a study in a rectangular swimming pool in which an opaque partition protruded from the centre of one of the walls, effectively creating two sections at one end of the pool. A stimulus card was placed at the end of each section. From the opposite side of the pool, the end of both sections, and therefore both stimulus cards could be viewed from the same position. The stimulus cards were of the sort shown in Figure 16. They were made of the same two components, but were the mirror image of each other. A platform was always placed under one of the stimulus cards, but not the other. The rats simply had to discriminate between the two stimuli based on the orientation of the components of the stimuli, and travel down the relevant section to find the platform. It is important to note that steps were taken to ensure that the task could not be solved just by looking to one side of the pattern. That is, the rats could not simply look at the left side of the stimulus card to see if it was black or white and discriminate between them on that basis.

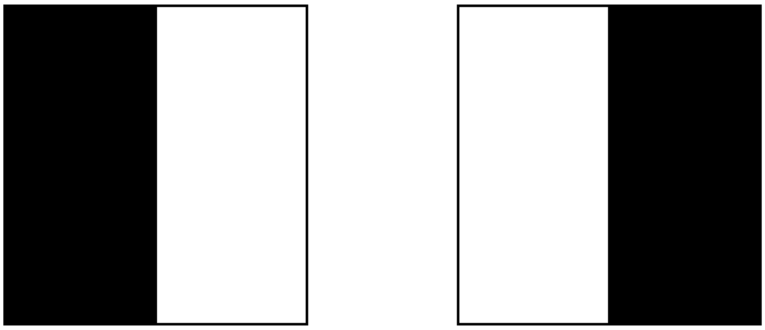


Figure 16. An example of the type of stimuli used by Sanderson et al. (2006). The two stimuli are cards made up of the same two components, in two different orientations.

Instead, to solve this problem rats had to appreciate the spatial relationship between the two sides of the cards. While sham-operated animals were able to solve this task, animals with hippocampal lesions were not. There are very clear similarities between the stimuli used by Sanderson et al. and the appearance of a corner made up of a black wall and a white wall, such as those used in the experiments in this thesis. If the animals in the previous experiments formed associations based on the structure of the correct corner, animals with hippocampal lesions might be expected to find the tasks described in this thesis very difficult.

Experiment 8A was intended to determine the role of the hippocampus in latent spatial learning in the tasks described throughout this thesis, whether it be that the animals can no longer learn about structure, or whether they suffer a complete deficit in the ability to develop spatial S-S* associations.

Experiment 8A

In an experiment based on the arena used in Experiment 6, both rats with hippocampal lesions and sham-operated rats were given placement training in a square made up of two adjacent white walls and two adjacent striped walls. Half of the animals with lesions of the hippocampus (HPC) received placement training in a corner made up of one white wall and one striped wall, and were referred to as the ‘HPC-Structure’ group. As noted, a deficit in the ability to locate the correct corner during the test trial by hippocampal animals in this condition may be due to an inability to form S-S* associations, or an inability to learn about the structure of available stimuli.

Therefore, a second group with lesions of the hippocampus, referred to as the ‘HPC-Stripes’ group received placement training in the corner made up of two striped walls. Should the HPC-Stripes group show a preference for the striped corner at test, then it could be concluded that the deficit in the hippocampal animals in the HPC-Structure group in locating the correct corner occurred as a result of the role of the hippocampus in processing structural information. However, if the HPC-Stripes group also showed a deficit by revealing a preference for the correct corner, then this would suggest that the hippocampus is responsible for forming S-S* associations, since learning about the all-striped corner would not require an understanding of structure.

Method

Subjects and apparatus. The 48 male Lister-hooded rats were from the same stock and maintained in the same way as with the previous experiments. Twenty-four of the rats received hippocampal lesions, and the remaining rats were sham-operated such that they received the same surgical treatment as the rats receiving hippocampal lesions, but without any damage to the hippocampus. The rats were divided into four groups; HPC-structure, Sham-structure, HPC-stripes, and Sham-stripes, where the first part of the name referred to the lesion and the second part to the training condition. The rats had all previously received prior experience with a trace conditioning task involving visual, auditory stimuli and footshock. However, they were all naive to the swimming pool and any spatial learning tasks. Two rats were removed from the experiment because they left the platform during placement training. One swam in the pool and the other repeatedly jumped onto the walls of the arena, leaving 12 animals in the HPC-structure and Sham-Stripes groups, and 11 in the two remaining groups. The apparatus was the same as that used for Experiment 6.

Procedure.

Surgical procedure. The surgical procedure was very similar to that described by Marshall, McGregor, Good and Honey (2004). Isoflurane was administered to anaesthetise the animals, who were then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and the head was held at -3.3mm. An incision was made into the scalp and bone from the skull was removed in order to expose the neocortex above the location of the hippocampus. Ibotenic acid (Biosearch Technologies, San Rafael, CA) was dissolved in phosphate-buffered saline [pH 7.4], producing a solution of 63 mM concentration. The ibotenic acid was administered using a 2-μl Hamilton syringe, which was placed in the stereotaxic frame, into the hippocampus. 28 sites were injected with 0.05-0.10 μl solution using a KD Scientific electronic pump (Model 5000; Boston, MA), at a rate of 0.05 μl/min. the needle remained in position for 2 min following every injection to allow full diffusion and to limit the spread of the ibotenic acid into the surrounding cortical area.

The surgical procedure for the sham-operated animals was identical to that given to the animals receiving lesions to the hippocampus, except that instead of receiving ibotenic acid to any region of the brain, the dura was pierced with a 25-gauge Microlance3 needle (Becton Dickinson, Drogheda, Ireland).

The rats received standard post-operative care in line with the relevant regulations. The experiment commenced more than eight weeks after the rats received surgery.

Experimental procedure: All aspects of the pre-training, training, and test were the same as for Experiment 6, except that only the two Structure groups received placement training in a corner made up of one striped wall and one white wall. The rats in the Stripes groups received placement training on a platform that was always placed in the corner made up of two striped walls. The rats received eight sessions of placement training in the appropriate corner, as dictated by their assigned group. There were four trials in each session, in which the rat was placed on the platform for 30 sec before being removed from the pool. The swimming paths of each of the animals, the percentage of time spent in each search zone and the corner that they entered first during the test trial were recorded.

Results and Discussion

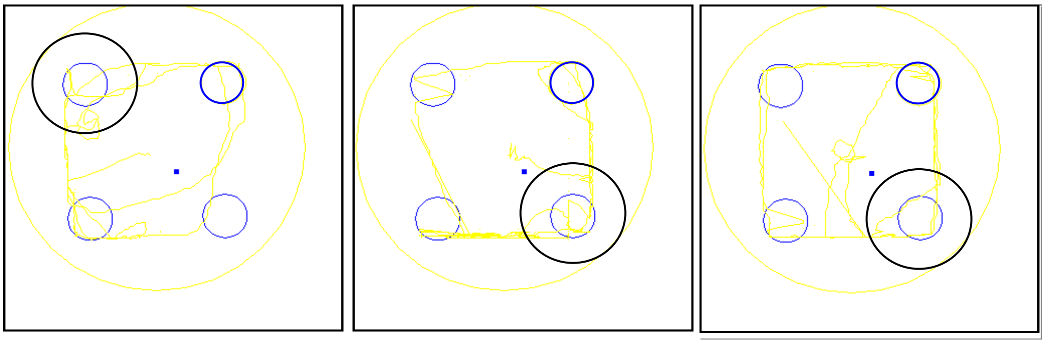
One animal was removed from the following analysis due to insufficient tissue loss in the hippocampus. See the Discussion section of Experiment 8B for an explanation of the exclusion criteria.

The swim paths of three animals selected at random from each of the four groups can be seen in Figure 17 which shows that all of the animals spent most of the time swimming around the edge of the pool during the test trial. Importantly, the swim paths of animals with hippocampal lesions and sham-operated animals was very similar, other than sham-operated animals spent more time in the correct corner than the other corners. It would therefore seem reasonable to assume that differences in the amount of time spent in each zone by the different groups was not a result of different swimming behaviour between the zones.

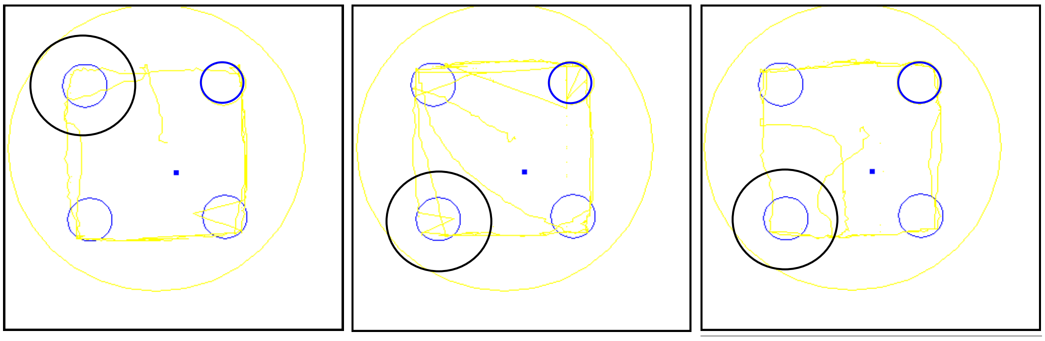
The manner of presentation of the remaining results from the test trial was the same as for the previous experiments. These results can be seen in Figure 18, which shows that the Sham-Structure and Sham-Stripes group spent more time in the corner in which they had received placement training than in any other. This is not true of the two groups with hippocampal lesions. An ANOVA comparing the amount of time spent in the correct corner by the sham-operated group and the group with hippocampal lesions in both conditions (Structure or Stripes) showed that there was a significant effect of lesion, $F(1,41) = 9.82$, $p < .01$, $MSE = 47.03$, whereby the animals with hippocampal lesions spent less time in the correct corner than the sham-operated animals. There was no significant effect of condition, $F<1$, and there was no significant interaction $F<1$. These results point to the important, novel conclusion that lesions of the hippocampus impair latent spatial learning in the swimming pool. The results therefore extend the generality of the effects shown by Chai and White (2004) and confirm the reliability of their results. Chai and White demonstrated the effect of hippocampal lesions on latent spatial learning in a radial maze, however a radial maze is much more restrictive of an animal's movements than a swimming pool since they must travel down one of the available arms. In the swimming pool, the animal is able to travel freely in any direction. In order to understand the reasons for this impairment, additional analyses were conducted on pairs of groups separately.

First, as with the previous experiments, the time spent by the two Structure groups in the correct corner was compared with the time spent in the incorrect corner - that is the corner that is the mirror image of the correct corner. A t-test comparing the amount of time spent in the two corners by the group Sham-Structure, showed a significant preference for the correct corner $t(10)= 4.54$, $p<.01$. However the HPC-Structure group did not show a significant preference for the correct corner, $t(11) = .79$, demonstrating that while the sham-operated animals were able to learn about the location of the platform in relation to the available cues, the animals with lesions to the hippocampus were not.

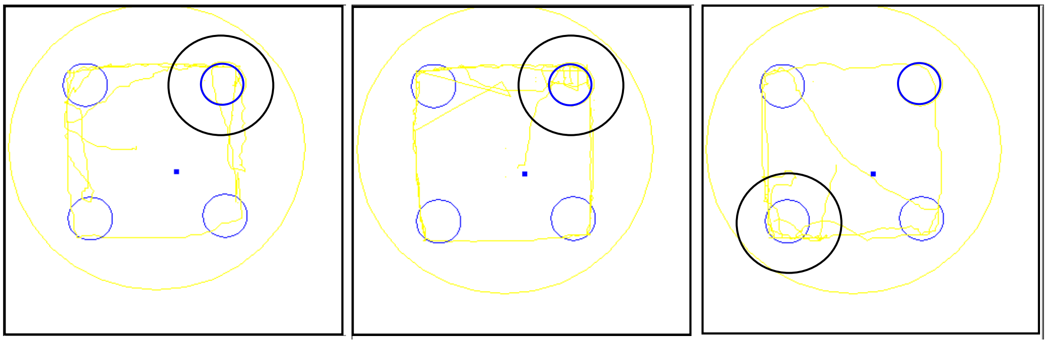
HPC-Structure



HPC-Stripes



Sham-Structure



Sham-Stripes

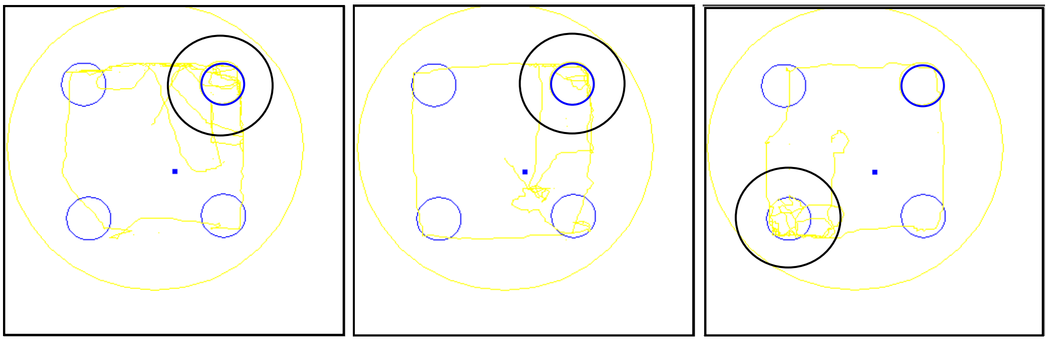


Figure 17. The swim paths of three animals from each group during the test trial of Experiment 8a. The four small dark circles represent the four designated search zones in the square and the largest dark circle shows the correct corner for that animal. The light grey lines show the swim path of the animal.

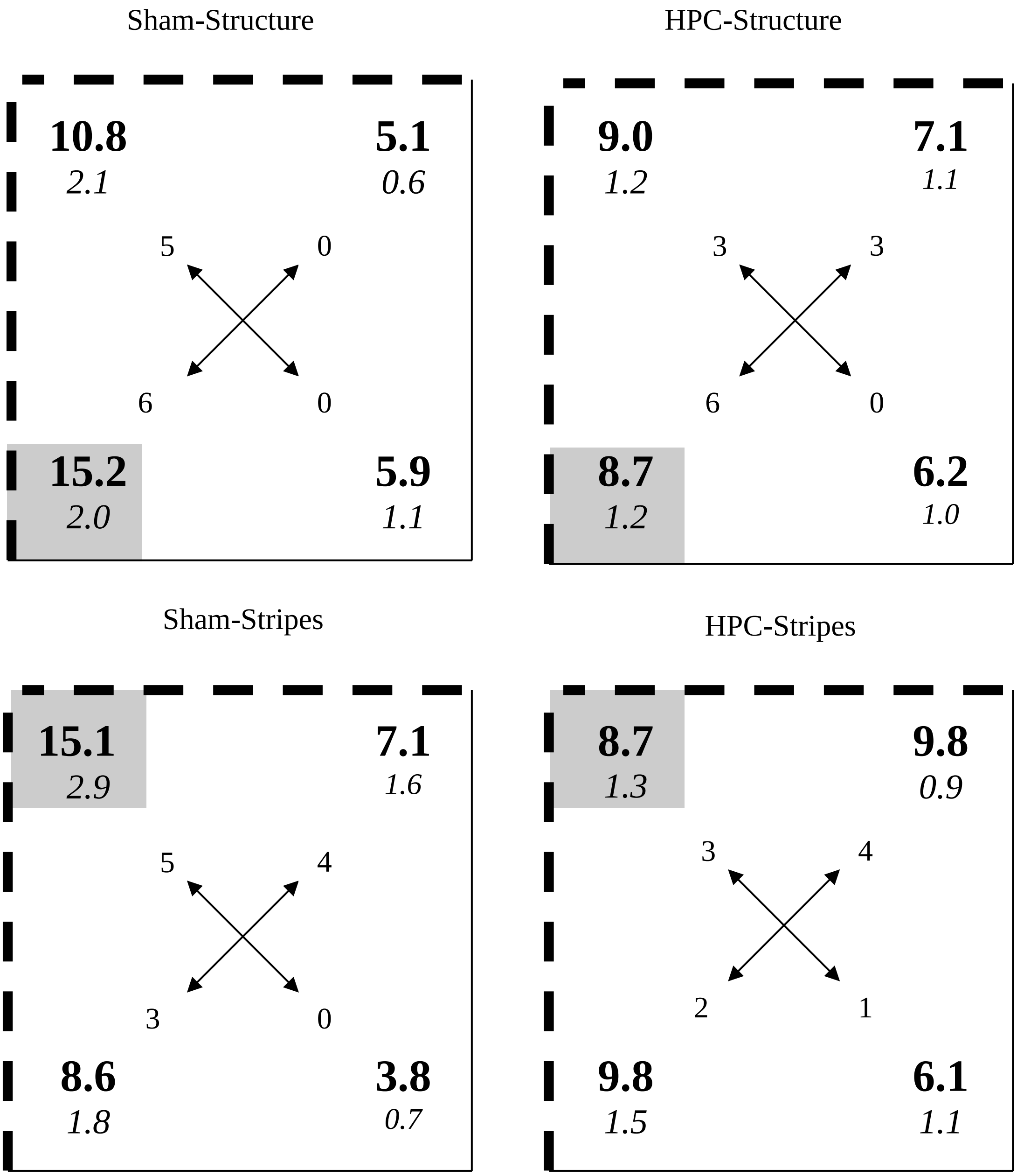


Figure 18. The results of Experiment 8A. The dashed lines represent the striped walls, and the thinner, solid line, the white walls. The Grey square represents the correct corner. The numbers at the end of the arrows represent the number of animals that went to that corner first. The larger, bold numbers are the percentage of time spent in each corner, and the smaller, italicised numbers are the standard error means.

The implication of this finding is that damage to the hippocampus either prevents animals from learning about the significance of the structural relationship between the walls creating the correct corner, or that they disrupt latent spatial learning in general. Analysis of the results from the remaining two groups suggests the second of these interpretations is more likely to be correct.

If hippocampal lesions are effective only because they disrupt the ability to acquire structural information, then the HPC-Stripes group should have spent more time in the correct corner of the square during the test trial than any other corner, because there is no need to rely on structural information to identify this corner. All that is required is that the animal appreciates the correct corner is composed entirely of striped walls. In contrast to this prediction, a one-way ANOVA of the time spent in each of the four corners by the HPC-Stripes group during the test trial failed to reveal a significant effect of zone, $F(3,27) = 1.94$, $MSE = 15.72$. On the other hand, a similar one-way ANOVA for the results from the Sham-Stripes group revealed a significant effect zone, $F(3,33) = 5.0$, $p < .01$, $MSE = 53.37$. Subsequent paired comparisons showed that this group spent significantly more time in the correct corner than in the opposite corner created from two white walls, $t(11) = 3.53$, $p < .01$, thus confirming the effectiveness of the placement training. A surprising finding was that the Sham-Stripes group did not spend significantly more time in the correct corner than either of the remaining two corners $ts(11) < 2.05$, $ps > .13$. The reasons for this outcome are not clear. The results in Figure 18 clearly show a strong preference for the correct corner. The number of subjects in the Sham-Stripes group is relatively low compared to some of the other experiments in this thesis, and perhaps a greater number would have produced a significant result. Alternatively, it is possible that the animals only referred to one of the walls that made up the correct corner during the test trial. If some of the animals searched for a corner with a striped wall to the left and some for a corner with a striped wall to the right, then it would be expected that much of the time would be spent in the two corners adjacent to the correct corner. This account contradicts the findings in Experiment 6 which showed that animals learned about both of the walls that made up the correct corner. Perhaps the extent to which the animals learn about both of the walls is dependent upon factors which are not apparent at this time. Whatever the merit of this suggestion, the results from the experiment indicate that hippocampal lesions disrupt latent spatial learning, regardless of whether or not it is based on structural information.

Further evidence that animals with hippocampal lesions were unable to acquire S-S* associations during placement training can be seen in the corner that the rats entered first on the test trial. For the animals in the two Structure conditions the number of animals that went to either of the two corners made up of a striped and a white wall were analysed. Of the animals in the HPC-Structure group, nine animals went to one of these two corners before either of the other corners, six of which went to the correct corner first, which is not significant using a binomial test, $p = .16$. Of the animals in the Sham- Structure group, six animals went to one of the striped and white corners before any other. All of these animals went to the correct striped and white corner, $p = .02$. For the animals in the Stripes condition, as there was no clear corner to which the correct corner could be compared, binomial analyses were conducted on which of the four corners the rats entered first. Of the ten animals in the HPC-Stripes group, three entered the correct corner before any other and of the twelve animals in the Sham-Striped condition, five entered the correct corner first, neither of which is significant, $p =.25$ and $p = .1$, respectively.

The foregoing results imply that the hippocampus is important for latent spatial learning. That is, it is important for the development of S-S* associations. If this interpretation is correct, then it is conceivable that if rats were required to swim to a platform situated in a corner in a square arena composed of two adjacent white walls and two adjacent striped walls, then their performance might not be disrupted by lesions of the hippocampus. It is conceivable that this training will allow the animals to find the platform by means of S-R associations, and there is considerable evidence that such associations are not affected by lesions of the hippocampus (e.g White, 2008). In order to test this prediction, the four groups in the previous experiment were used for a further study. Rather than being given placement training, each of the four groups received eight further sessions of training in which they were required to swim to the platform, which was situated in the same place as for the placement trials. The groups then received a final test trial in which they were released into the pool in the absence of the platform. Assuming that the animals were then able to use S-R associations, on this occasion it was expected that all four groups would show a preference for searching in the correct corner.

Experiment 8B

All of the animals that were tested in Experiment 8A were given ‘active’ training in the same arena as that used for the previous training. During active training, rats were released from the edge of the arena and allowed to swim to find the platform, in order that they may develop S-R associations. For each rat the platform remained in the same place as it had been during the previous placement training. After eight sessions of active training, the rats were given a test trial identical to the one that they received after placement training. A preference for the correct corner was expected for all of the sham-operated animals, since they had already learned its location in the previous experiment. A preference for the correct corner over any other corner by the rats with hippocampal lesions would make the important point that damage to this region does not result in a general impairment of spatial behaviour. Instead, this result would lend support to the claim that the effects of the hippocampal lesions in the previous experiment were due to an impairment in the capacity for acquiring spatial S-S* associations.

Method

Subjects and apparatus. The subjects and apparatus were the same as in Experiment 8A. The position of the platform was consistent with each animal's previous training.

Procedure.

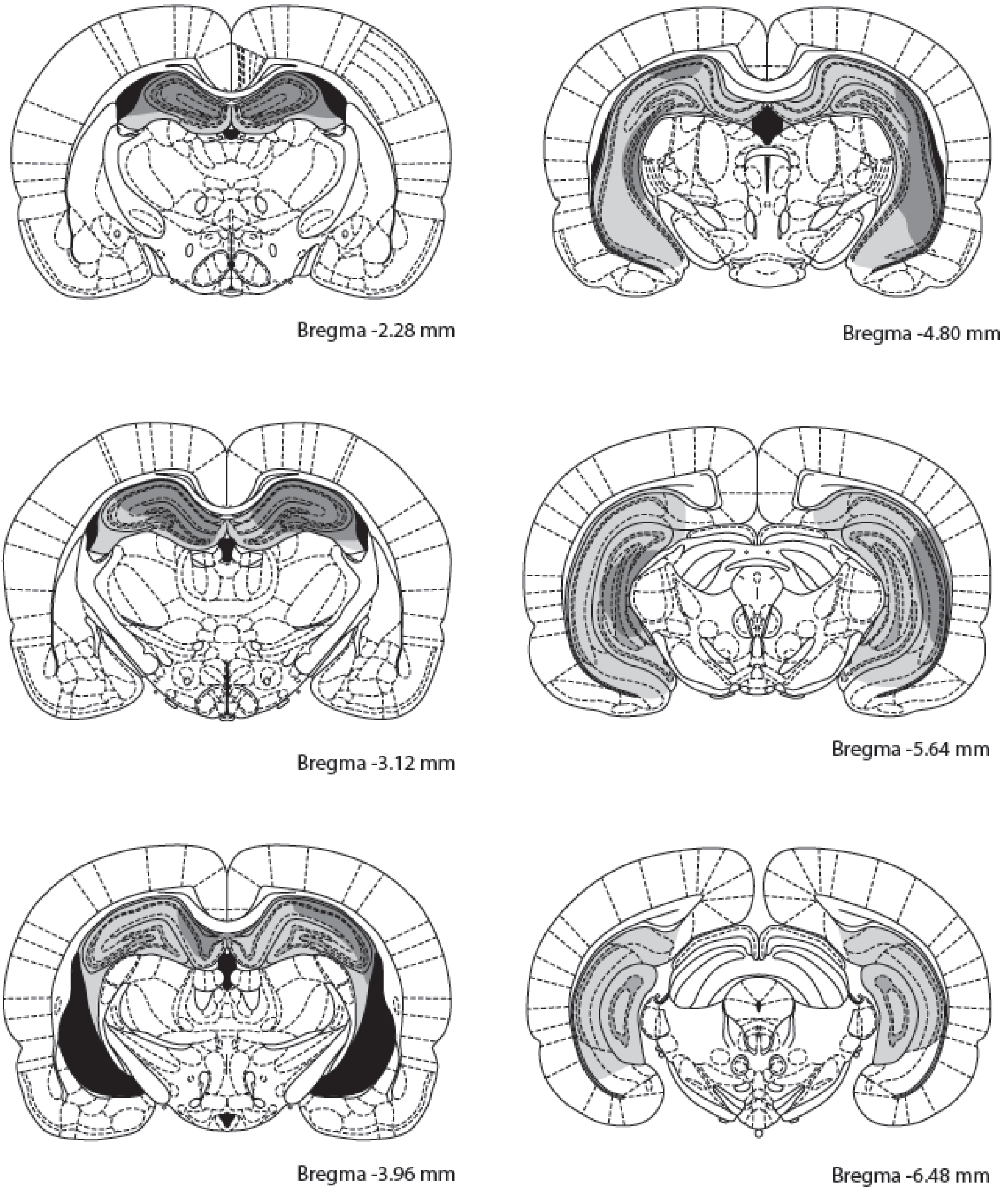
Training. The rats were given eight sessions of active training, with four trials in each session. The rats were released from the centre of one of the walls, facing the wall, and were given 60 sec to find the platform. If the animal did not find the platform in the given time, then they were guided to it by the experimenter. The wall from which the animal was released was randomised between trials with the restriction that each wall was used once in a session. The orientation of the arena in relation to the room was also randomised so that each 90 degree rotation was used once in a session.

Test. On the final trial of the seventh session, the animals were given a single test trial, which was identical to the test trial given in the previous experiment.

Histology: After the behavioural procedure was concluded, the animals were administered a lethal dose of sodium pentobarbitone (Euthatal). They were transcardially perfused with 0.9% saline, and then with 10.0% formal-saline. The brains were then postfixed for 24 hours, and then placed in phosphate-buffered (0.1 M) 30.0% sucrose solution for a further 24 hours. They were then frozen in a -20 °C cryostat before they were sliced along the coronal plane creating 40-mm sections. These were placed on gelatine-coated slides which were left to dry for 24 hours at room temperature. After they had been dried they were stained with cresyl violet and examined under a microscope, and the extent of the damage to the hippocampus was established using the boundaries defined by Paxinos and Watson (1998).

Results

Hippocamal lesions. Using sections adapted from Paxinos & Watson (2005) the extent of the hippocampal damage was plotted at six, equally spaced coronal sections (Bregma -2.28, -3.12, -3.96, -4.80, -5.64, -6.48). The total tissue loss across all six sections for all 23 animals ranged between 14.35 and 98.16%. However, the criteria for excluding an animal due to insufficient tissue lost was based on the damage to the dorsal hippocampus. Given the spatial nature of the behavioural tasks given to the animals, it was considered that significant damage to this area of the hippocampus was the minimum requirement for inclusion in the final analysis (see Bannerman, Yee, Good, Heupel, Iverson & Rawlins, 1999). That is, only animals with >50% tissue loss between Bregma -2.28 and -3.96 were included in the final analysis. One rat was removed from the HPC-Stripes group as there was only 46.31% tissue loss in this area. 20 out of the remaining 22 animals had tissue loss in this area of > 90%. Figure 19 shows the overall tissue loss based on the animals with the largest (light grey) and smallest (dark grey) lesions of the dorsal hippocampus in the 22 animals that were included in the behavioural analysis.



Bregma -2.28 mm

Bregma -4.80 mm

Bregma -3.12 mm

Bregma -5.64 mm

Bregma -3.96 mm

Bregma -6.48 mm

Figure 19. The largest (light grey) and smallest (dark grey lesions) in animals used in experiments 8A and 8B at six different coronal slices.

The rat with the largest dorsal hippocampal tissue loss also had the largest overall tissue loss (98.16%). There was one rat with less overall tissue loss than the rat with the smallest dorsal hippocampal tissue loss (the total loss was 22.34%). Of the 22 rats, 20 had overall hippocampal tissue loss of >50%.

Behaviour. The time taken to find the platform by each group during active training can be seen in Figure 20. Although the sham-operated animals were quicker to find the platform than the animals with hippocampal lesions throughout training, all four groups became quicker over time, suggesting that they were all able to learn about the location of the platform. A three-way ANOVA comparing the latencies over each session by each of the groups shows that there was a significant effect of lesion $F(1,41) = 21.09$, $p < .01$, $MSE = 217.65$, and a significant effect of session, $F(6,246) = 25.71$, $p < .01$, $MSE = 79.52$. However the effect of training condition was not significant, $F<1$. The lesion by training condition interaction was not significant, $F<1$, neither were the remaining two-way interactions, or the three-way interaction, $Fs(6, 246) <1.01$, $p > .48$, $MSEs = 79.34$. The results of the test trial can be seen in Figure 21, which shows that all four groups spent more time in the correct corner than any other corner. An ANOVA comparing the amount of time spent in the correct corner by each group revealed a significant effect of lesion, $F(1,41) = 7.49$, $p < .01$, but there was no significant effect of condition, $F(1,41) = 3.02$, $p=.09$, and there was no significant interaction between the two, $F(1,41) = .02$, $MSEs = 54.75$. It is not surprising that there was a significant effect of lesion, as the sham-operated animals had already learned about the location of the platform during placement training, which the animals with hippocampal lesions had not. As the differences in the latencies to find the platform during the training suggest, the associations developed during placement training by the sham-operated animals in both conditions gave them an advantage over the animals with hippocampal lesions in finding the platform.

In order to provide a more detailed analysis of the animals' behaviour, the amount of time spent in the correct and incorrect corners was compared for the HPC-Structure and Sham-Structure groups separately. As is consistent with Experiment 8A, the Sham-Structure group showed a significant preference for the correct over the incorrect corner, $t(10) = 11.77$, $p < .01$. However, unlike the first test trial, during the current test the HPC-Structure group also showed a preference for the correct corner over the incorrect corner, $t(11) = 3.19$, $p = .01$.

As with Experiment 8A, the time spent in all four corners was compared for animals trained in the Sham-Stripes group. As with the previous test trial, there was a significant effect of corner, $F(3,33) = 88.4$, $p < .01$, $MSE = 15.31$. A series of t-tests revealed that there was a significant difference between the time spent in the correct corner and each of the other corners $ts(11) > 9.04$, $ps < .01$. An ANOVA comparing the amount of time spent in each of the four corners by the HPC-Stripes group also showed an effect of zone, $F(3,27) = 24.81$, $p < .01$, $MSE = 20.9$. A series of t-tests showed that there was a significant preference for the correct corner over all of the other corners, $ts(10) > 4.92$, $ps < .01$.

The corner that each animal entered first was analysed in the same way as it was in Experiment 8A. For the animals in the Structure condition, the analysis focused on the corner that was entered first by animals that entered one of the two white and striped corners. In the HPC-Structure group, nine of the animals went to one of the two white and striped corners first, and six of these went to the correct one of these two corners first, $p = .16$. Of the animals in the Sham-Structure group, seven went to one of the two white and striped corners first, and of these all seven went to the correct corner, which is significant using a binomial test , *p* <.01. As with Experiment 8A, binomial tests were used to analyses the number of animals that went to the correct corner out of any of the possible four corners for the two Stripes groups. In the HPC-Stripes group eight out of ten rats went to the correct corner first, and in the Sham-Stripes group ten out of twelve rats went to the correct corner first.

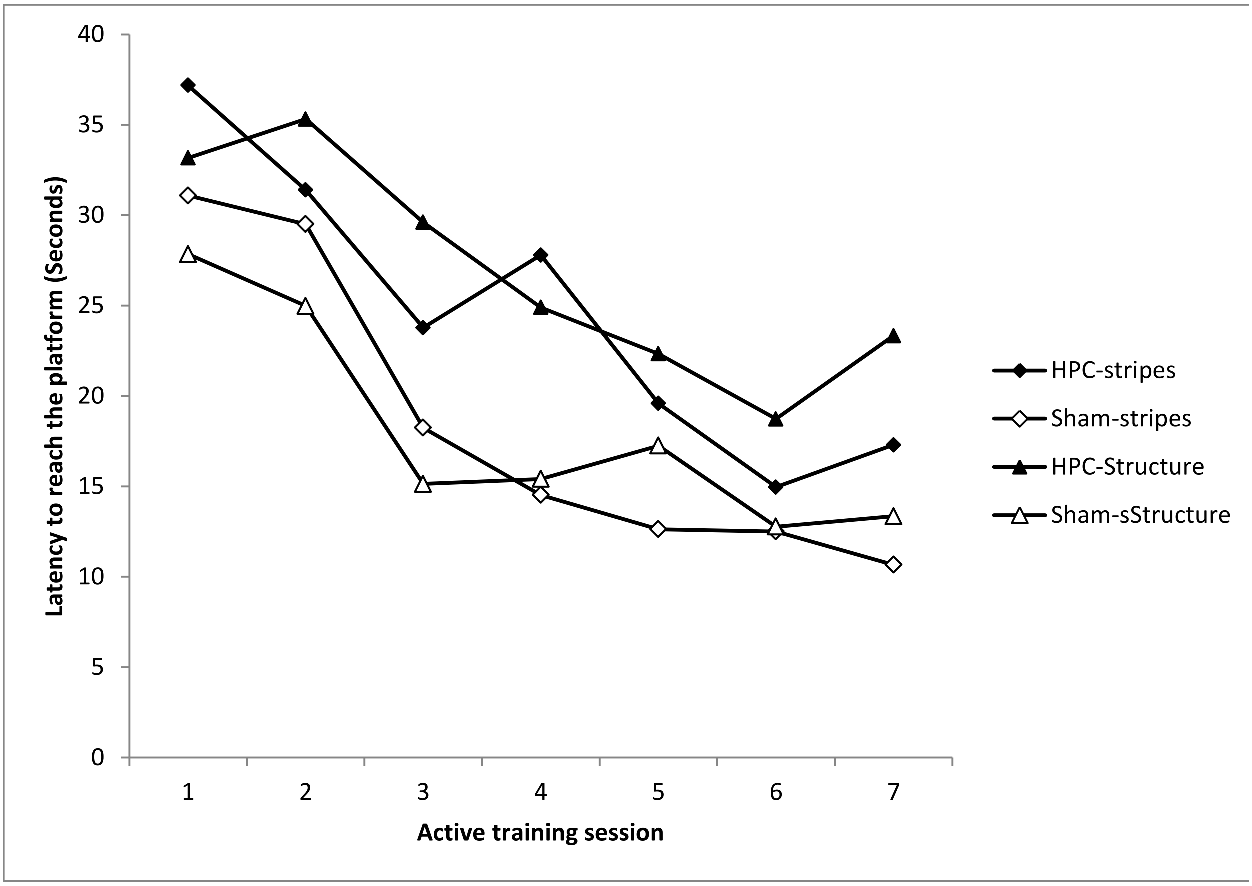


Figure 20. Session mean latencies to reach the platform during training for each group during the active training in Experiment 9B. The open shapes represent the two Sham-operated groups and the closed shapes represent the two groups with lesions of the hippocampus.

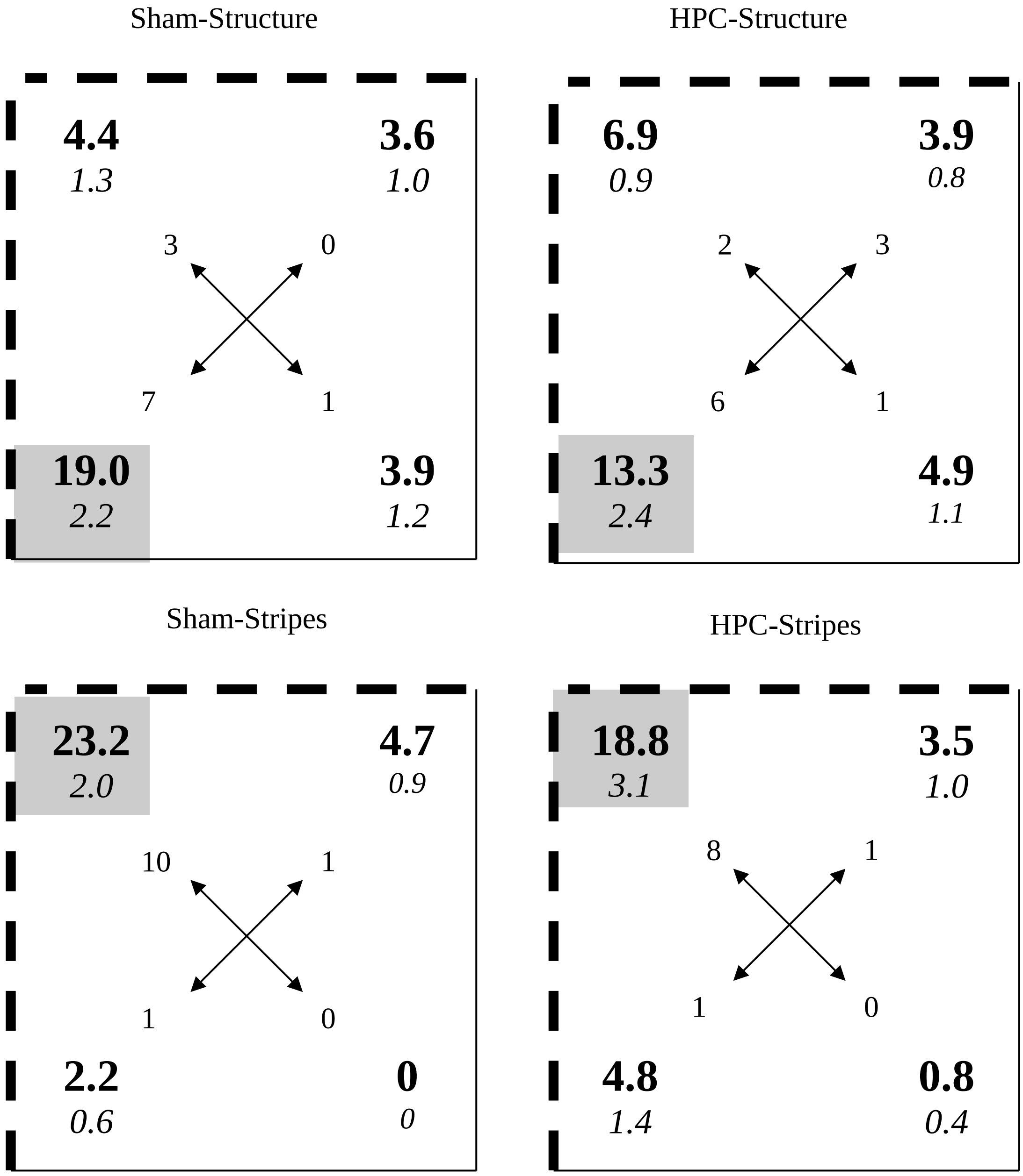


Figure 21. The results of Experiment 8B. The dashed lines represent the striped walls, and the thinner, solid line, the white walls. The Grey square represents the correct corner. The numbers at the end of the arrows represent the number of animals that went to that corner first. The larger, bold numbers are the percentage of time spent in each corner, and the smaller, italicised numbers are the standard error means.

For both groups the result was significant using a binomial test, *p* =.04, and *p* <.01, respectively. These results demonstrate that in keeping with Experiment 8A, the Sham- Stripes group entered the correct corner before any other corner on a significant number of occasions. This is also true of the Sham-Structure group. This result is not surprising for the sham-operated animals as they went to the correct corner on a significant number of trials prior to the active training. Of greater interest, are the results of the two groups with hippocampal lesions. The animals in the HPC-Stripes group went to the correct corner before any other on a significant number of occasions, demonstrating the effectiveness of active training. While the HPC-Stripes group did not enter the correct corner before the incorrect corner on a significant number of occasions, it should not be assumed that active training was not effective for this group. Referring to the number of animals that went to the correct corner first in the Sham-Structure group, compared to the Sham-Stripes group, there is some suggestion that learning about the correct corner is more difficult for animals in the Structure condition than in the Stripes condition. Perhaps if the animals in the HPC-Structure group were given more sessions of active training, a preference for heading to the correct corner first would be evident.

Returning to the analysis concerning the percentage of time spent in each corner, the preference for the correct corner by sham-operated animals in both conditions is not surprising, as these animals demonstrated such a preference with only placement training experience. The subsequent active training would only have added to the learning which had already occurred. Of greater interest, is the results of the rats with lesions of the hippocampus, which showed a preference for the correct corner in both the Structure and Stripes condition after receiving active training. If it is accepted that being required to swim to the platform allowed animals to find it by means of S-R associations, then the results imply that the hippocampus is not important for the development of spatial S-R associations.

General Discussion

The purpose of the current chapter was to determine the role of the hippocampus in latent spatial learning. Experiment 8A demonstrated that animals with hippocampal lesions could not learn about the location of the platform with reference to the available cues when they were placed upon a platform that was situated in either a corner made up of two identical walls or two non-identical walls. The results demonstrated further that the role of the hippocampus is not limited to learning about the spatial relationship between cues. The results of Experiment 8B, in which animals with hippocampal lesions were able to learn about the location of the platform after being given active training, demonstrated that the animals with hippocampal lesions did not have a general spatial learning deficit. These results in combination clearly demonstrate that the hippocampus is responsible for the development of S-S*, but not S-R associations.

The results from Experiment 8A are consistent with the assumption that cognitive maps are represented in the hippocampus. If animals navigate with reference to cognitive maps, then damage to the hippocampus would be expected to impair the animal’s ability to locate the goal. However, the results from Chapter 3 clearly demonstrate that animals do not appear to rely on a global representation of their environment. Lesions of the hippocampus resulted in an inability to acquire the S-S* associations which have been shown to develop during placement training in animals with an intact hippocampus. The results of Chapter 3 suggest that only certain features of the environment enter into these S-S* associations. It is therefore concluded that while S-S* learning depends upon the hippocampus, there is little reason to assume that these associations involve a complex global representation.

One point that needs to be addressed is the difference in preference for the two incorrect corners made up of a striped and a white wall by the Sham-Stripes group in part A and part B of the experiment. At the first test trial, this group showed a significant preference for the correct corner, the corner made up of two striped walls, over the corner made up of two white walls, but not over the two remaining corners. It was suggested in the discussion of that test that this was because the animals referred to only one of the striped walls when trying to locate the correct corner, and so swam to the two other corners which also contained a striped wall. At the second test, the same group preferred the correct corner over all three of the other corners. There are at least two explanations for this difference. This first explanation is that given more training, the animals began to attend to both of the striped walls and so had no reason to enter a corner in which there was also a white wall. The second explanation is that the S-S* associations which developed between only one striped wall and the platform were ‘corrected’ when rats swam around the pool. If the animals were to learn that there was a striped wall to the left while on the platform, for instance, this association alone would only take the rat to the platform in 50% of the corners which satisfied this rule. During active training the animals have adequate opportunity to learn more accurate associations, than during placement training where such an association would not be corrected.

There is at least one study with which the current results conflict. White and McDonald (1993) placed rats in two arms of a radial maze. In one arm food was always present, while in the other arm it was not. The food reward is considered to be the S* of the S-S* association that was assumed to be necessary if the animals were to learn the significance of the different arms. The animals were placed, after a number of training trials, in the centre of the maze. Animals with lesions to the amygdala did not show a preference for entering the arm which had previously contained food, while control animals, and animals with lesions of the fimbria fornix were able to solve this task. White (2008) therefore argued that S-S* associations are not part of the role of normal hippocampal activity. Chai and White (2004) conducted a similar experiment, but the two arms were adjacent to each other. In this case, it was found that animals with hippocampal lesions, but not amygdala lesions, could not solve the task. White argued that in this case, as the two arms were next to each other, the views at the end of the arms would be very similar. The animals therefore, had to learn about the spatial relationship between the two arms. White argued that this was an S-S association, and that this was the domain of the hippocampus. However, it is not entirely clear how the S-S associations described by White differ to the S-S* associations that have been referred to throughout this thesis, and which he claimed were formed when placement training took place in opposite arms of the radial maze.

The results of these two studies do not comply with the findings in this chapter, since the results of Experiment 8A clearly demonstrate an inability of animals with hippocampal damage to learn S-S* associations. Similarly, if the animals in the Chai and White (2004) study could not learn which arm was correct because an intact hippocampus is required to learn about the spatial relationship between two stimuli, then it might be expected that only the HPC-Structure, but not the HPC-Stripes group would be unable to solve the placement training task. As demonstrated, this was not the case.

There are however, a number of differences between the two studies described and the experiment conducted in this chapter. For instance, the experiments in this chapter made use of a platform in an aversive environment rather than a food reward, the animals in the two studies by White and his colleagues had to learn about two locations rather than just one, and so on. It is therefore difficult to draw meaningful comparisons between my experiments and those performed by White, and a discussion of the differences between the results would be somewhat speculative.

It should also be noted that unlike the current experiments, a variety of studies have shown that animals with hippocampal lesions are unable to locate a hidden goal, even when they are allowed to move around the environment during training. For instance, Morris, Garrud, Rawlins & O'Keefe (1982) found that animals with hippocampal lesions could not locate a hidden platform in a circular pool with cues on the walls of the room. Similarly, Pearce et al. (2004) found that animals with hippocampal lesions could not locate a hidden platform in a rectangular pool, such as the one used in Chapter 2. Since it should be possible to find the goal in both of these studies by means of S-R associations, and since I have followed White (2008) in assuming that an intact hippocampus is not necessary for the formation of S-R associations, then it follows that hippocampal lesions should not disrupt spatial behaviour in experiments such as those just described. The obvious conclusion to draw is that the hippocampus is not just involved in the formation of spatial S-S* associations, but also has other functions. One possibility is that the hippocampus is important for forming representations of combinations of cues (Aggleton & Pearce, 2002; O'Reilly & Rudy, 2000, 2001: Rudy and Sutherland, 1989; Sanderson et al., 2006)), another possibility is that it is important for making judgements based on relative length or distance (e.g. Pearce et al., 2004). A disruption in either of these functions would make it difficult for animals to find accurately a hidden goal in either of the tasks just described.

It thus appears that the hippocampus influences spatial behaviour in a variety of ways. However, the assumption that the hippocampus is responsible for all forms of spatial learning is clearly incorrect. The experiments described here provide clear evidence that while S-S* learning is hippocampal-dependent, S-R learning is not, and that animals are able to learn the location of a hidden goal without an intact hippocampus when they are given an opportunity to actively explore the environment.

Chapter 5 – General Discussion

As outlined in Chapter 1, there were two questions that the current thesis aimed to answer. The first concerned the type of associations that animals acquire during spatial learning; do animals use S-S* or S-R associations to locate a hidden goal that they have previously visited? The second question concerned the type of cue which entered into these associations; do animals make use of global cues, or more local feature cues, or do they use of combination of both? The following discussion will describe how the experiments reported in this thesis answered these two questions. At least one role of the hippocampus in spatial learning tasks will then be considered. The implications of the findings in this thesis will then be discussed.

Animals receiving placement training can develop S-S* associations

Chapter 2 demonstrated that animals that had received placement training in a rectangular swimming pool, were able to locate the corner in which the platform had been placed during training. While a number of researchers (Jacobs et al. 1989a; Keith & McVety, 1998; Sutherland and Linggard, 1982) have shown that placement training can produce successful navigation when animals are later allowed to swim to the platform, Jacobs et al (1989b) later retracted their paper demonstrating the effect. The reasons that they gave for being unable to replicate what had initially appeared to be a robust effect threw into doubt similar findings by the other researchers. For instance, Jacobs et al suggested that the position of the researcher during the test stage may have meant that the animals found the platform incidentally, whilst heading towards the experimenter, rather than deliberately attempting to locate it there. The experiments in Chapter 2 conducted placement training in such a way as to control for these extraneous factors. For example, a curtain always surrounded the pool so that the animals could not see the experimenter while looking for the platform. After being repeatedly placed on the platform the rats in Experiment 1 showed a preference for the correct corners, the corner in which they had been trained, and the corner diagonally opposite, over the incorrect corners. Thus Experiment 1 provides clear evidence that animals receiving placement training are able to learn about the location of the platform, as demonstrated during a subsequent test trial. Animals were not able to develop S-R associations during placement training because they were never given an opportunity to swim to the platform, it follows by default that their ability to identify where the platform was located depended upon S-S* associations

Experiment 2 was conducted to determine how many sessions of training were required for the animals to develop these S-S* associations through placement training, and whether or not the associations were retained over a delay of 24 hours. The results indicate that S-S* associations can be acquired with as few as two sessions of training and, with as few as six sessions of training, these associations can be retained for 24 hours. Chapter 2 provides the first demonstration of how readily S-S* associations are learned, and how robust the learned associations are. The Experiments in Chapter 3 demonstrated the generality of the effects of placement training, since the animals were also able to develop S-S* associations when placed on a platform in a square arena with distinctively coloured walls.

S-S associations are based on local and distal features

The second question raised in Chapter 1 concerned the type of cues that enter into S-S* associations. Pearce et al. (2004) previously demonstrated that animals that were trained to swim to a platform in a rectangular swimming pool were able to do so with reference to local cues alone. However, the animals in the Pearce et al. study may have solved the task using S-R associations as they were able to swim around the pool and the current thesis is concerned with the nature of cues entering into S-S* associations. As such, Experiment 3 was based on the design used by Pearce et al., in which animals were trained in a rectangular pool but tested in a kite-shaped pool. Unlike the Pearce et al. study, the animals in Experiment 3 were given placement training so that they could acquire only S-S* associations. When they were tested in the kite-shaped arena, as with the Pearce et al. animals, they were able to locate the corner with the same local features, defined by the two walls that made up the corner, as the corner in which they had received placement training in the rectangle. The next two experiments of Chapter 3 (Experiments 4 and 5) also demonstrated that animals can navigate with reference to local cues, but failed to find any indication that animals had any reliance on global cues. Experiment 7, the final experiment of Chapter 3, demonstrated that animals can also learn about the location of the platform during placement training with reference to distal cues, as defined by the three corners in which the platform was not placed and the two walls furthest from the correct corner. Thus, Chapter 3 demonstrated that S-S* associations can be based on both local and distal cues.

It is necessary to consider whether a representation of both the local and distal features of the environment constitutes a global representation. While the animal clearly has an appreciation of the location of the goal in relation to both the local and distal features, a global representation would suggest that the animals have an appreciation of the spatial relationship between each of the features of the environment. In this way, all of the features enter into a single representation. However, in each of the first three experiments of Chapter 3, the arena used in the test trial was substantially different to the arena used for the training trials. If navigation at the time of testing was based on a global spatial relationship acquired during training, then the change in the arena would be expected to disrupt severely the animal’s ability to locate the correct corner. There is in fact, very little evidence from the experiments in Chapter 3 that a change in arena affected the animal’s ability to identify the correct corner. It is therefore concluded, that it is very unlikely that spatial S-S* associations are based on global representations of the environment.

Furthermore, it is very difficult to think of an experiment that would unequivocally demonstrate the use of global cues since it is not possible to retain the global features of the environment while removing the local and distal cues. Evidently animals will refer to feature cues whenever they are present and so it is not possible to isolate the influence of a global representation on the animal’s spatial behaviour from the influence of feature cues. However, it can be said with some certainty that animals do not rely solely on global cues alone, since they can make use of local feature cues when their global arrangement has changed. The lack of a generalisation decrement when the animals were transferred to a new environment seems to provide the strongest suggestion that animals do not rely on global cues at all. It is hard to imagine how the animals would not be affected by a change in the global representation if it had previously entered into an S-S* association with the platform.

The role of the hippocampus in the acquisition of S-S* associations

The purpose of Chapter 4 was to understand the role of the hippocampus in the development of S-S* associations. Previously it has been suggested that the hippocampus is the brain structure responsible for a global representation of the environment, or a cognitive map (O’Keefe and Nadel, 1978; Jacobs & Schnek, 2003; Sheynikhovich, Chavarriaga, Strösslin & Gerstner, 2009). While Chapter 3 found no evidence that animals possess a global representation of their environment, however, the hippocampus may still be the region responsible for representation of S-S* associations. Experiment 8A demonstrated that animals with lesions of the hippocampus were unable to locate the correct corner of a square arena with striped and white walls after receiving placement training. This was true of both the group repeatedly placed in a corner with one white wall and one striped wall, and the group repeatedly placed in a corner made up of two striped walls. Animals that had not received lesions to the hippocampus were able to locate the correct corner at test when trained in either corner, whereas both groups with lesions of the hippocampus failed to show any benefit of the placement training The animals then received active training in Experiment 8B, in which they were able to swim around the pool and so were given the opportunity to develop S-R as well as S-S* associations. After receiving active training, both groups with lesions to the hippocampus were now able to locate the correct corner. These results suggest that animals with lesions to the hippocampus are not able to develop S-S* associations, but are able to develop S-R associations, indicating that an intact hippocampus is required for animals to learn S-S* associations in a spatial task, but is not required for all forms of spatial learning. This finding is also in keeping with findings that S-R associations develop in a separate region of the brain to S-S associations, specifically they are considered to be the domain of the caudate nucleus (see White, 2008 for a review).

It should be noted that if S-R associations are not the domain hippocampus, then it should follow that animals with hippocampal lesions should not be impaired in any active training task. There are at least two exceptions to the rule (eg Morris et al, 1982; Pearce et al, 2004) which were discussed in Chapter 4. As previously noted, the results of these experiments can be understood with reference to a number of other roles that the hippocampus is believed to play that do not include S-R learning. Clearly, the acquisition of S-S* associations is not the only role that the hippocampus plays in spatial tasks, however the evidence from Experiment 8a strongly suggests it is not responsible for the acquisition of spatial S-R associations.

Do animals use S-R associations in a spatial task?

The results of the experiments in Chapters 2 and 3 raise the question of whether all spatial learning is based on S-S* and not S-R associations. The finding in Chapter 2 that animals can navigate with S-S* associations alone may suggest that animals do not need S-R associations to navigate. Furthermore, the finding that S-S* associations are based on local and distal, but not global cues suggests that the learning that occurred while animals received placement training does not differ from the learning that occurs during active training. To clarify, Pearce et al (2004) successfully showed that animals that had received active training were able to locate a corner which had previously contained a platform based on local cues alone. The results of Chapter 3 demonstrate that this is also the case for animals that had received placement training. The results combined may lead to the suggestion that animals that receive active training in the pool do not learn anything different about their environment compared to animals which received only placement training. If this is the case, then it may suggest that animals do not use S-R associations in spatial tasks at all, since the successful location of the correct corner can be explained in terms of S-S* associations alone.

At least two results suggest that animals receiving active training do learn about S-R associations as well as S-S* associations. The first being the results of the experiments in Chapter 4, which demonstrate that animals that with hippocampal lesions are unable to acquire S-S* associations, but are able to locate a hidden goal when given the opportunity to swim to the platform repeatedly. The ability to locate the platform without the use of S-S* associations indicates that the animals must be using S-R associations in order to reach the platform.

Furthermore, Restle (1957), as described in Chapter 1, provided clear evidence that animals do use S-R associations in a spatial task when they are able to explore the environment. Animals that were trained in an arena where food was always placed in the an arm to the right, still turned to the right even when the arena was rotated and the extramaze cues indicated that the correct arm was on the left. The results of Tolman et al., Pearce et al., and Chapter 4 taken in combination strongly suggest that animals that are able to explore the environment develop S-R associations which allow them to locate the goal.

Implications for Cognitive Maps

By demonstrating the acquisition of S-S* associations, Chapter 2 provided evidence which is in keeping with the assumption that animals possess cognitive maps. A cognitive map is generally understood to be a global representation of the environment that is not dependent on behaviour. That is, the representation does not include any learned habit or response. If animals possess cognitive maps then it should be the case that they do not need to learn a route or response in order to locate the corner in which the animals received placement training. The fact that animals that have received placement training, during which they are not able to learn a response or habit, are able to locate the correct corner during a test trial may provide some support for cognitive maps. Similarly, the finding in Chapter 4 that the animals with lesions to the hippocampus are unable to locate the correct corner after receiving placement training satisfies the assumption that cognitive maps are represented in the hippocampus by place cells which fire maximally depending on the animal's position within their environment (O'Keefe and Nadel, 1978; Jacobs & Schenk, 2003; Sheynikhovich et al., 2009).

However there are number of results throughout this thesis that suggests that animals do not possess cognitive maps. As explained, Chapter 3 demonstrates that there is no evidence that S-S* associations are based on the global properties of their environment. This finding, in combination with previous research such as Pearce et al. (2004) which finds that S-R associations do not appear to be based on a global strongly suggests that animals do not possess such a map.

There is at least one other implication of the results of this thesis for O'Keefe and Nadel's (1978) account of the hippocampus in spatial behaviour, in particular. O'Keefe and Nadel distinguish 'taxon' from 'locale' learning. They considered locale learning to be true spatial learning, the use of a cognitive map, which they considered to be the domain of the hippocampus. Taxon learning was not considered to be true spatial learning and could simply be based, for instance on a local beacon. Taxon learning is not considered to include the use of a cognitive map and is not considered to involve the hippocampus. If animals were able to solve a spatial task using taxon learning, which they should be able to do without the need for an intact hippocampus, then animals trained in the striped condition in Experiment 8a should be able to locate the correct corner during the test trial, even if they had received a lesion to the hippocampus. They were not required to learn about the spatial relationship between two non-identical walls, instead they simply had to head towards a corner made up of two striped walls. This task could have been solved with taxon learning, based on O'Keefe and Nadel's definition, but animals with hippocampal lesions were unable to. This finding poses a further problem for O'Keefe and Nadel's account of spatial learning, and the role of the hippocampus in spatial learning.

How do animals navigate with reference to S-S* associations?

While it is evident that animals can acquire and make use of S-S* associations in a spatial task, one of the main problems with S-S* accounts of spatial learning, is that they do not explain the mechanism by which the association allows the animal to navigate to the goal. In the case of S-R associations, the learned habit is performed and therefore leads the animal to the goal, but the way in which S-S* associations lead the animal to the goal is not clear.

A 'snapshot' or 'template' account of spatial learning may explain how animals can use S-S* associations to reach the goal (e.g. Cartwright & Collett, 1982; Cheung et al, 2008; Haselgrove, George, & Pearce, 2005) The model proposed by Cartwright & Collett stated that animals take a mental 'snapshot' of the view when they are at a goal. Later, when they are trying to find the goal again, they compare what they could see at the goal with what they can currently see and try to reduce the disparity between the two. In doing so, they gradually move closer to the goal until the two views match up. If the animal forms an association between the platform and the view of the surrounding walls from the platform, then a snapshot account of navigation may explain how an S-S* association leads an animal to the goal (see also, Sheynjkhovich et al., 2009; Stürzl, Cheung, Cheng, & Zeil, 2008).

There may be some support for this proposal in the fact that six out of the eight experiments described provided evidence that animals entered the corner in which they had received placement training before entering an incorrect corner. This tendency suggests that the animals made a deliberate attempt to head to the correct corner as soon as they were released into the pool. If they had, instead, travelled around the pool until they entered the correct corner, and then stayed there, then they would not have headed directly towards the correct significantly more often than would be expected by chance. According to the snapshot model of spatial learning, the animal should be able to head towards the goal as soon as they are able to view the landmarks, or in this case walls, that surround it. From the centre of the pool all four corners are visible, and so the tendency to head towards a correct corner first by the animals in the majority of the experiments described is in keeping with the predictions of a snapshot model. In the few examples where a significant number of animals did not enter the correct corner first, it was generally the case that the group size was fairly small, and so achieving a significant effect using a binomial test is more difficult. In these cases, increasing the group sizes may have produced a significant result. In the case of Test 2 of Experiment 2, whereby animals were tested 24 hours after the final training session, a large group size did not produce a significant number of animals entering a correct corner before an incorrect corner. As can be seen from the results, while two of the training groups spent significantly more time in the correct corners than the incorrect corners, the preference for the correct corner by the four groups combined was not as strong as it had been when there was no delay between the final training trial and the test trial. Given the nature of the first choice as a potentially less sensitive measure than the recordings of the time spent in each zone, the non-significant number of animals entering a correct corner first is perhaps not surprising. Similarly, the first choice of the animals in Experiment 7, in which a distal cue identified the correct corner, did not show a significant number of animals entering the correct corner first. However it was possibly more difficult for these animals to select the correct corner initially, as they were required to swim away from the identifying cue to do so. Therefore, using a snapshot to guide their behaviour would not necessarily have taken them to the correct corner first. In summary, aside from the exceptions described, the instances of animals heading to the correct corner before an incorrect corner is in keeping with the predictions of a snapshot model.

While the current data are consistent with a snapshot account of spatial learning, there are at least two problems with it. First, such an account cannot readily explain the effect of potentiation (eg Pearce et al, 2006) or superconditioning (Horne & Pearce, 2010) that has been found in a rectangular or kite-shaped pool by the removal of a feature cue in the corners of the arena. The influence of the shape of the environment on the animal's behaviour should not be strengthened by the removal of a feature cue if the animal is referring to a mental snapshot to locate the correct corner.

Second, Iordonova, Burnett, Aggleton, Good and Honey (2009) demonstrated that animals with lesions to the hippocampus are not impaired on a non-spatial S-S task (see Holland & Bouton, 1999, for a review of the effects of hippocampal lesions in classical conditioning). If animals navigate with reference to a mental snapshot which does not encode anything more specific to the spatial aspects of the task, such as distance or direction, than the view from the goal, then it is hard to understand how this information differs from non-spatial tasks. Evidently there appears to be a difference between spatial S-S* associations and a non-spatial S-S associations since damage to the hippocampus affects them differently. However, a snapshot model does not provide an adequate explanation of what these differences might be.

Implications for spatial learning: Summary

The experiments from the current thesis clearly demonstrate that animals are able to develop S-S* associations during placement training that allow them to locate a hidden goal, which are encoded in the hippocampus. While this may be in keeping with cognitive map accounts of spatial learning, there is no evidence from the behavioural and lesion experiments described that animals possess a global representation of their environment. Instead S-S* associations appear to be based on local and distal cues within the environment. What is not clear is how animals are able to use these associations to locate the goal. While a snapshot model may go some way to providing an explanation of how spatial S-S* associations are used, it cannot account for a number of cue competition effects that have been demonstrated in spatial tasks. Similarly, it cannot explain how a spatial S-S*association differs from a non-spatial S-S association. It is therefore very difficult to draw any firm conclusion about exactly what a spatial S-S*association is.

Based on the evidence that the hippocampus is responsible for spatial S-S* associations, but not non-spatial S-S associations it is clear that there is a distinct difference between the two. More work is required to establish exactly what constitutes a spatial association, and how this information is encoded. Similarly, more work is required to understand the mechanism by which an S-S* association allows an animal to locate a hidden goal.

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