

**THE ROLE OF THE HIPPOCAMPUS  
IN CONFIGURAL LEARNING**

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**PhD (2009)**

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### **Publications and Presentations**

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## **Abstract**

Contemporary theories of animal learning propose that memory for a specific event can be based upon either an elemental network of associations, a configural associative network or a hybrid of these possibilities. The two aims of this thesis were (1) to assess whether rats form configural representations of the spatiotemporal features of specific cues, and (2) to test the hypothesis that the hippocampus plays a critical role in configural representations that encode the spatiotemporal properties of an event, more commonly known as episodic memory. Chapter 2 investigated rats' ability to represent the spatiotemporal context in which objects were presented. These experiments failed to find robust evidence for such an ability. Chapter 3 discusses the development of a novel task, based on a sensory preconditioning procedure, that demonstrated configural memory for the spatiotemporal features of auditory cues in normal rats. In addition, it was shown that excitotoxic lesions of the hippocampus disrupted such configural memories. The experiments reported in Chapter 4 used the procedure developed in Chapter 3 to show that temporary inactivation of the hippocampus during memory retrieval disrupted configural, but not elemental memory retrieval. The results presented in this thesis support the hypothesis that normal rats are able to form elemental and configural representations involving the spatiotemporal properties of cues, and that the hippocampus has a role in configural but not elemental associative memory.

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*Table 1:* The total number of infusions with associated coordinates and volumes of ibotenic acid administered. All coordinates are relative to bregma (bregma = 0.0). AP = Anterior-posterior. ML = Medio-lateral. DV = Dorso-ventral. IBO = Ibotenic acid (volume in microlitres,  $\mu$ l).

## **Chapter 1**

### **1.0. General introduction**

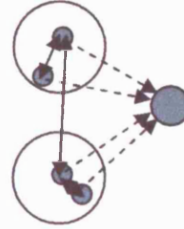
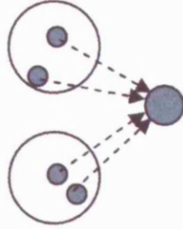
The aim of this thesis is to investigate the role of the rat hippocampus in processing and retrieving configural information in rats. The emphasis here is on memory for patterns of stimulation with episodic content (i.e., what, where and when), which can be construed as either being supported by simple elemental associative structures or configural structures. More specifically, research will be presented that evaluates the hypothesis that the rat hippocampus contributes to the formation and retrieval of configural memories formed as the result of simple exposure to patterns of relatively neutral stimuli. Chapter 1 begins by providing a brief overview of the main theories of animal learning and memory that are relevant to the issue of how animals learn about patterns of stimulation in general. This is followed by an evaluation of recent research on the behavioural and neural systems that underpin memory for patterns in animals. The dominant perspective within the field of animal learning is an associative one, and the chapter begins with an outline of the standard associative explanations for learning and memory.

### **1.1. Associative learning**

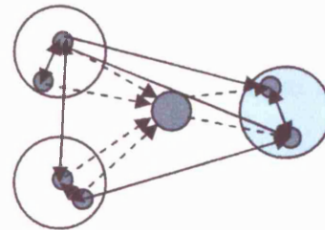
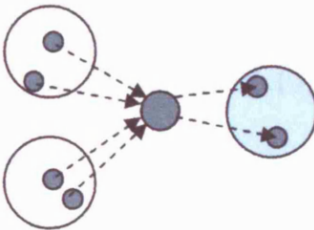
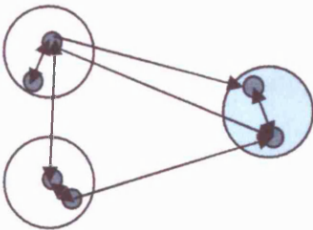
The fundamental proposition of associative learning theory is that pairing two stimuli will lead to a connection or association to form between the internal representations of these stimuli (see, for example, Hall, 1991). The precise nature of the associative structures involved remains a contentious issue, but three general classes of theory have been suggested and these are depicted in Figure 1.

**Elemental****Configural****Hybrid**

Simple exposure to a pattern consisting of two stimuli

**Elemental****Configural****Hybrid**

Pairing a two-stimulus pattern with a third stimulus



**Figure 1.** Alternative patterns of connections created within elemental, configural and hybrid associative networks by simple exposure to a pattern consisting of two stimuli (upper row) and after pairing that pattern with another stimulus (e.g., during conditioning). Small dark circles represent elements/micro-features of each stimulus (represented by large white circles). Medium dark circles denote a configural representation activated by a pattern. Large grey circles represent another stimulus with which the pattern was paired (e.g., an unconditioned stimulus in Pavlovian conditioning).

Figure 1 illustrates the candidate associative structures that might be acquired during simple exposure to a pattern of stimulation consisting of two stimuli (upper row), and the structures that might result from pairing this pattern with another stimulus (lower row). The candidates can be divided according to whether they involve direct links



forming between the elements of the patterns (left), the elements coming to activate a separate configural unit (centre), or both (right).

One of the most influential elemental theories of associative learning was developed by Rescorla and Wagner (1972). The long-standing influence of this form of model reflects the fact that it allowed the formation and direct assessment of novel empirical predictions, and that it can accommodate a broad range of behavioural phenomena (see also McLaren & Mackintosh, 2000). However, there are aspects of associative learning that cannot be accommodated readily by elemental theories such as the Rescorla-Wagner model (1972). These include one-trial overshadowing (e.g., James & Wagner, 1980; Mackintosh & Reese, 1979; Pearce, 2002) and negative patterning (e.g., Alvarado & Rudy, 1995; Rudy & Sutherland, 1995). Overshadowing refers to the observation that where a less salient conditioned stimulus (e.g., a quiet tone) is accompanied by a more salient stimulus (e.g., a bright light) it acquires less ability to provoke conditioned responding than if it has been conditioned in isolation. The Rescorla-Wagner model (1972) predicts that such an effect will be evident after several conditioning trial, because after the first compound conditioning trial the associative strength of the overshadowing stimulus (the bright light in this case) will constrain the associative strength acquired by the target stimulus (the tone). However, it does not predict that overshadowing will occur on the first trial, when the light will have no associative strength and cannot constrain that acquired by the tone. In contrast to this prediction, tests have shown that overshadowing does occur on the first conditioning trial (Mackintosh & Reese, 1979; Pearce, 2002). Attempts to incorporate this observation with elemental learning models have suggested the involvement of an attentional mechanism, where the subject's attention is directed toward the overshadowing stimulus more than the target stimulus, and thus the overshadowing stimulus acquires greater

associative strength. However, one-trial overshadowing, without the inclusion of attentional factors, is consistent with a configural analysis in which, following compound conditioning, responding to the target is determined by its similarity to the configural representation formed on the compound conditioning trial (Pearce, 1987, 1994).

In a negative patterning discrimination, the presentation of a compound of two stimuli (e.g., a tone and light) signals one outcome (e.g., no food) whereas the presentation of either one of the stimuli alone signals a different outcome (e.g., food). According to an elemental model of the type developed by Rescorla and Wagner (1972), the presentation of the compound should be especially likely to elicit conditioned responding as both of its components are associated with the same reinforcer. This state of affairs should result in summation of the associative strengths of the tone and light and result in an increased conditioned response. However, animals can learn such patterning discriminations - withholding responding on compound trials and responding whenever the elements are presented in isolation (e.g., Grand & Honey, 2008; Rescorla, 1972; Woodbury, 1943).

In contrast to elemental models, configural theories propose that associative learning involves the development of links between configural representations of the patterns of stimulation present on a given trial and the outcome of that trial (e.g., Pearce, 1994). For example, during a patterning discrimination, separate configural representations of the elements and the compound become linked to no food and food, respectively (i.e., tone+light→no food, tone→food and light→food). As indicated above, configural analyses also provide a simple account for other phenomena that have proven difficult for elemental analyses to explain (e.g., one-trial overshadowing; for a full discussion, see Pearce, 2002).

Finally, it must be noted that elemental and configural learning processes need not be viewed as mutually exclusive. Several hybrid models have been developed in the context of behavioural evidence (e.g., Kehoe, 1986), and have been prompted by neuroscientific dissociations of simple discrimination learning and configural discriminations (see, for example, Rudy & O'Reilly, 2001). It should, however, be acknowledged that some of these neuroscientific dissociations have proven difficult to interpret and indeed to replicate (e.g., compare, Rudy & Sutherland, 1989, with Davidson, McKernan, & Jarrard, 1993). The next section will now consider in detail the evidence that the hippocampus, a brain region linked closely with memory processes in human and non-human animals, makes a specific contribution to episodic memory processes. Given the information presented discussed in this section, it seems equally likely that this form of memory could be supported by either elemental or configural associative structures. This idea will be further developed in the sections that follow.

## **1.2. The role of the hippocampus in episodic memory**

The study of medial temporal lobe resection patients such as HM led to the proposal that the hippocampus plays a critical role in memory processes (e.g., Milner, Corkin & Tueber, 1968). Although characterisation of the role of the hippocampus in humans remains controversial, there is evidence that the hippocampus contributes to episodic memory processes (e.g., Aggleton & Brown, 1999; Eichenbaum & Fortin, 1993; Hwang & Golby, 2006; Nyberg, 1998; Tulving, 2002). The concept of episodic memory was first developed by Tulving (1972). This term refers to memories that place events in specific spatiotemporal context (often referred to as what, where and when memory). It has been argued that episodic memory is distinct from memory for factual information (i.e., semantic memory, Wheeler & McMillan, 2001; c.f., Tulving 2002). The extent to

which episodic memory is distinct from other forms of memory, with its own separate underlying psychological and neural mechanisms, has yet to be determined. In the context of this discussion, it could be argued that the formation of an integrated memory for what happened where and when might be supported by elemental or configural associative structures. That is, the same general theoretical analysis could be applied to the content of episodic memory as has been applied to memory involving other types of information. For example, Rudy and Sutherland (1995) draw comparisons between their configural theory, and the theory proposed by Gaffan (1991), who suggested that the hippocampus supports memory for the “whole scene” in which an event takes place. The idea that nonhuman animals might also be capable of forming episodic memories has also gathered pace since Clayton and Dickinson (1998) published their seminal work on the behaviour of the western scrub jay (see Eacott & Norman, 2004; Eichenbaum & Fortin, 2003). The next section considers the evidence that nonhuman animals are able to form integrated memories that encode the spatiotemporal context of an event, followed by a discussion of the evidence that hippocampal damage may disrupt this form of memory in animals.

### **1.3. Episodic memory in nonhuman animals**

Tulving (1972, 2002) identified conscious recollective processes, such as *cronoaesthesia* and the possession of an *autonoetic* consciousness (Eichenbaum & Fortin, 2003; Tulving, 2002) as defining features of human episodic memory. Any assessment of episodic memory in non-verbal animals clearly precludes immediate access to this feature of episodic memory. Therefore the premise for much of the animal work in this area is based on a more restricted definition of episodic memory - one that emphasises memory for the spatiotemporal context in which an event occurred. This more restricted definition

is often referred to “what”, “where” and “when” memory or “episodic-like” memory (Clayton, Griffiths, Emery & Dickinson, 2001a; see also Clayton, Yu & Dickinson, 2001b; Eacott & Norman, 2004; Eichenbaum, Fortin, Ergorul, Wright & Agster, 2005; Rudy & Sutherland, 1995; Tulving, 1972; Wright & Agster, 2005).

#### **1.4. Models of episodic-like memory in animals**

The following sections critically discuss some of the recent attempts to assess episodic-like memory in animals, with particular focus on the extent to which these demonstrations show that animals can form an integrated memory of what happened where and when; and whether they can be explained by supposing that the animals have encoded the information elementally, configurally or in a manner that utilises both.

##### **1.4.1. California scrub jays**

Clayton and colleagues have produced some of the most compelling evidence for episodic-like memory in their studies of the behaviour of food storing birds (Clayton & Dickinson, 1999a; Griffiths, Dickinson & Clayton, 1999; Clayton *et al*, 2001a, Clayton *et al*, 2001b; Griffiths & Clayton, 2001; Raby, Alexis, Dickinson & Clayton, 2007). Food storing birds, such as scrub jays, spontaneously store seeds over a wide territory for later collection; and scrub jays can recall what food item they stored, the time at which that item was stored, and its spatial location. In one of the later, more complex experiments, Clayton *et al*. (2001b) supplied scrub jays with two food types, peanuts and mealworms, and allowed the birds to bury the items at specific feeding tray locations before retrieving the items after intervals of 4, 28 and 100 hours. Half of the birds (the degrade group) were trained that, over specific intervals, the preferred reward, mealworms, degraded, rendering them inedible. For the remaining birds (the replenish group) the food items

were replaced with fresh stock. Clayton *et al.* (2001b) hypothesised that, if the birds in the degrade group were able to form an integrated memory for what type of food had been cached, where and when (length of interval since caching), they would favour retrieval of peanuts over mealworms (the preferred food item) at later but not earlier time points. The results were very clear: scrub jays in the degrade group searched for mealworm caches after the shorter interval (4 hours) when they were still edible, but searched for peanuts after the longer intervals (28 hours, 100 hours). This pattern of results is consistent with the view that the birds had encoded and integrated information about what food was buried where and when (Clayton *et al.*, 2001a; 2001b; Eacott, Easton & Zinkivskay, 2005; Zentall, 2005). This conclusion is supported by the fact that birds that received no degradation training showed no indication of preferring peanuts at any stage of testing.

The findings described in the previous paragraph, together with additional control manipulations incorporating caches of crickets (more palatable than peanuts but less so than mealworms, with degradation times that differed from both) supported the conclusion that the scrub jays were able to remember the contents of the caches and their individual rates of decomposition. The way in which birds and other animals recall temporal information, the ‘when’ element of episodic memory, is unclear. Eichenbaum and Fortin (2003) argue that scrub jays can use decaying memory traces as a method for determining interval length (Friedman, 1993). Although utilising the different decay rates of memories would allow scrub jays to successfully complete the test, Eichenbaum and Fortin (2003) argue that this method does not constitute episodic-like memory as there is no ‘when’ information integrated into the memory representation. However, Clayton *et al.* (2001b) address this concern by highlighting the fact that retrieval behaviour does not noticeably decline over longer intervals, pointing out that using the differing trace

strength of memories to successfully complete the test, depends on the unlikely assumption that progressively weaker memory traces would still result in equivalent levels of exploration. Clayton *et al* (2001b) argue that this process is unlikely to provide a complete explanation of their results. However, they do not provide conclusive evidence to rule it out completely; consequently, it remains a possible, if not a plausible, explanation of their results. The latter observation highlights the issue that the uncertain character of the ‘when’ representations encoded by nonhuman animals means that, at present, there is no general consensus as to what does or does not represent ‘when’. This issue will be discussed in further detail later in this chapter.

The work carried out by Clayton, Dickinson and their colleagues has provided evidence that scrub jays are able to form integrated representations that include the identity, location and (features associated with) the time of storage of an item. However, their procedure has limited use in terms of exploring the anatomical substrates of this form of memory (Eacott *et al*, 2005). Furthermore, it is possible that caching behaviour may be a highly specialised form of behaviour resulting from the development of specialised brain systems that are not evident in other animals. Thus, the generality of these behavioural observations remains to be determined. Moreover, while the results from scrub jays are consistent with them exhibiting “episodic-like” memory (cf. Clayton *et al*, 2001b), the theoretical and neural bases of this memory are not known. As previously discussed, the scrub jays’ behaviour may be supported by an integrated memory that consists of a pattern of elementary associations. Indeed, as part of the discussion regarding the nature of the memories used by scrub jays in their procedure, Clayton *et al* (2001b) propose a memory structure that requires elemental associations to be formed between the internal representations of the experimental stimuli. The

alternative explanation is that scrub jays acquire a configural representation for the combinations of food item, cache location and interval presented in the training trials.

#### **1.4.2. Experiments in rodents**

There have been several attempts to extend the findings from scrub jays to rodents. Bird, Roberts, Abroms, Kit and Crupi (2003) developed a procedure that was closely modelled on that used by Clayton and colleagues. This procedure also involved two food types: cheese and the less preferred pretzels. Rats were trained to remove the foods from the centre of the maze and cache them in boxes at the end of the arms of a maze. Tests confirmed that the rats searched the arms in which the preferred foods were stored and thus demonstrated memory for 'what' they had stored and 'where'. In order to assess memory for temporal interval, the preferred food items were spoiled (by treating it with quinine) at a specific interval after storing. It was predicted that rats would show a decreased preference for searching in the locations containing the preferred food item at the interval associated with the reduced palatability of the reward. Bird *et al.* (2003) found that rats failed to change their search preferences to reflect the change in food palatability after specific intervals, and concluded that rats were unable to form integrated memories for what-where-when in this procedure. However, it is perhaps noteworthy that the failure of rats to respond on the basis of the interval between storing and retrieving the food items may reflect the fact that the caches were spoiled after a moderate length of time but remained edible after longer interval, a manipulation that does not occur in nature, and thus may prove more difficult for rats to learn. Alterations to the procedure might yield positive results, but at present the absence of sensitivity to manipulation of the 'when' elements render the procedure unsuitable for investigating hippocampal function.

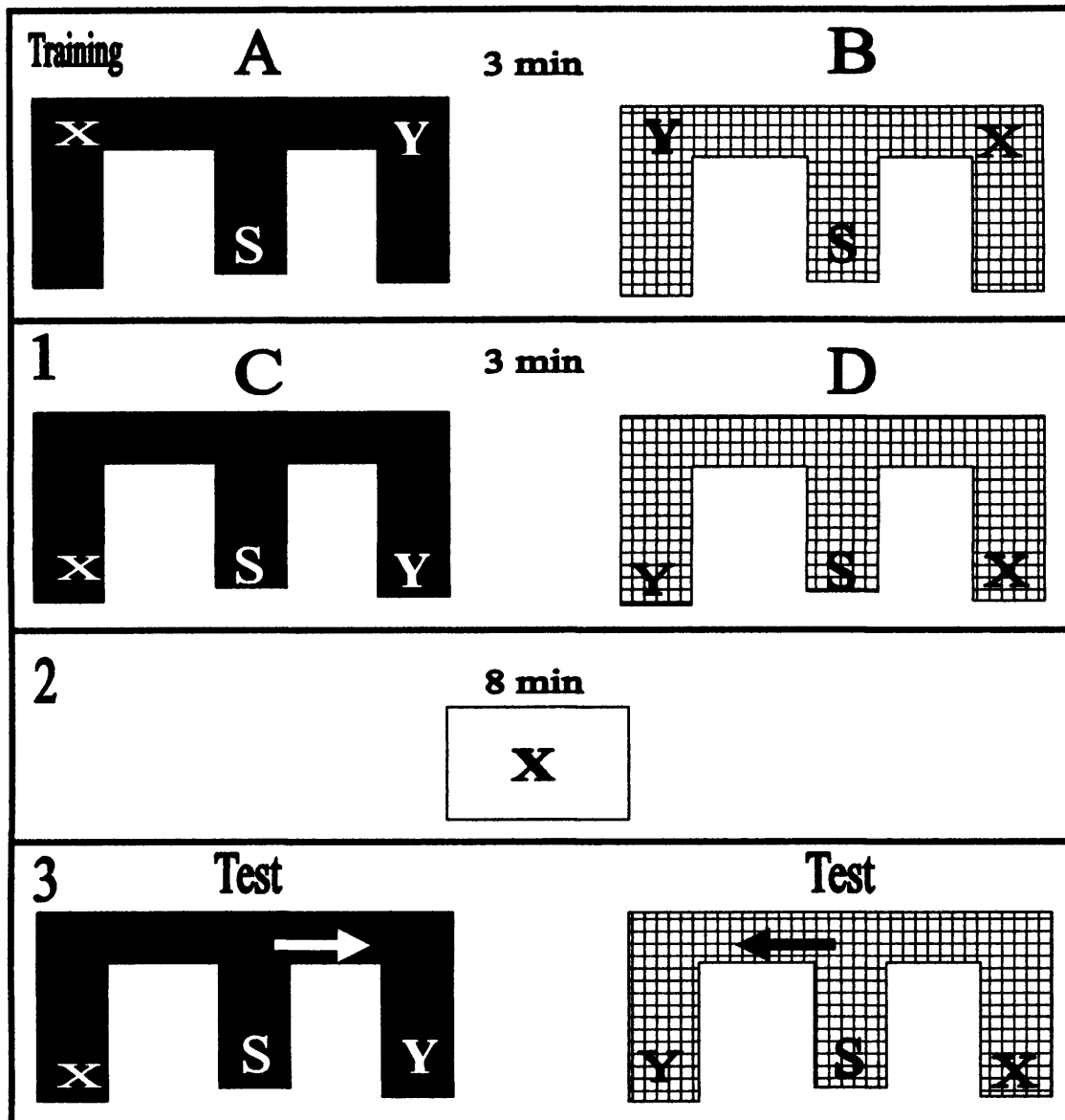


Babb and Crystal (2006a) trained rats in an eight-arm radial maze in which all arms contained food. This was followed by a forced choice phase, where four of the eight arms were accessible (entry into the remaining arms was blocked) and contained food but only one arm contained a preferred food item (chocolate). Rats were trained that the arms containing food reward varied after short and long retention intervals (RI). After a one hour RI all eight arms were accessible, with the four arms previously blocked containing food. After a twenty-five hour RI, the same four arms previously blocked contained food, plus the arm that contained chocolate in the forced choice phase contained chocolate once again. The rats demonstrated sensitivity to these manipulations by showing a preference for the arm that contained the chocolate reward after the long RI. A revaluation test where chocolate was paired with lithium chloride before testing resulted in the rats showing a decreased preference for the arm containing the chocolate reward after the long RI. This finding demonstrated that the rats had encoded the sensory properties of the rewards contained in each arms, rather than exploring the arms based on memories that specific arms contained more palatable rewards. Taken together, the results suggest rats were able to form an integrated memory that specified the spatiotemporal features of food storing. A similar experiment conducted by the same researchers (Babb & Crystal, 2006b) including two palatable flavours (one of which is devalued) also revealed that rats encode the sensory properties of the stimuli, which is more suggestive of an integrated memory for what, where and when.

Unlike Bird *et al.* (2003), Babb and Crystal (2006a, 2006b) showed that rats correctly modified their exploratory behaviour in response to variations in the interval length (one or twenty-five hours). Although Babb and Crystal (2006a) suggested that their results are consistent with the hypothesis that rats are able to form a configural memory of the time of day and place at which a food item was stored, the results are also

amenable to interpretation in terms of simple elementary associations (cf. Figure 1). For ease of explanation, assume that the short and long intervals are represented by  $I_1$  and  $I_2$ , respectively, the eight arms of the maze are represented by letters A to G, and that chocolate and less-preferred food reward by X and Y respectively. During training, X, the preferred food, is always presented in the same arm (A) after the same interval ( $I_2$ ). This could lead to an associative link between X and A, and between X and  $I_2$ . If the rat is placed in the maze at time point  $I_2$  this will activate a representation of X. Activation of a representation of X will then, in turn, activate a representation of A (i.e., a memory for the arm containing the preferred reward). Changing the incentive motivation properties of X will weaken the approach response to A (c.f., Balleine & Killcross, 2006). In conclusion, although the results reported by Babb and Crystal (2006a) appear to demonstrate memory for spatiotemporal context, it is unclear whether the effects are based upon elemental or configural integration. Although the associated experiment (Babb & Crystal, 2006b) establishes that rats are capable of differentiating between two palatable flavours, the very similar nature of the procedures means that this experiment also susceptible to the same criticism. Therefore, the use of this procedure as an assay of hippocampal function is limited by the fact that no strong *a priori* predictions can be made.

#### 1.4.3. An alternative rodent procedure: what, where and which.



**Figure 2.** 'What-where-which' procedure. Rats were trained in two E-mazes, each with a distinct pattern on the walls that provided two contexts. Before testing, rats received training sessions in which they were placed in the maze for 3 min. in arrangements A and B, where the locations of the stimulus objects (X and Y) alternated between contexts. This procedure was repeated in Stage 1 of the test in arrangements C and D, with objects placed at the bottom of the external arms. In Stage 2, rats were exposed to one stimulus object in a neutral context for 15 min. In Stage 3, rats were returned to the E-mazes in the same configuration as stage 1, and the first arm they chose to explore was recorded. S = Constant starting position where rats began training/test; →, ← = predicted first choice

Eacott and Norman (2004) have argued that the ‘when’ component of episodic-like memory can be replaced by a physical context (or ‘which’ component) that, like when, could serve as an occasion specifier (cf. Clayton & Dickinson, 1999b; Clayton *et al*, 2001b). Eacott *et al* (2005) utilised an object novelty preference paradigm (Dix & Aggleton, 1999; Ennaceur & Delacour, 1988). The general features of the procedure are shown in Figure 2. In this procedure, rats were first exposed to the two contexts each containing two different objects (X and Y). However, the location of the objects (left or right) differed as a function of the context (see Figure 2). In the next (exposure or habituation) stage, the rats were placed in a neutral context with one of the objects (e.g., X) for a period of eight minutes. This exposure phase was designed to reduce the novelty of the object and leave the rat with a preference for exploring Y and for searching for Y within the maze. In the critical test stage, the rats were given an opportunity to visit both locations of the maze. The hypothesis was that the rats would choose to avoid the arm that contained the object presented recently during the habituation stage, and to instead search for object Y in the context+arm configuration in which that object had been presented. To show this pattern of exploration they would need to have represented where the objects had been in which context. Eacott *et al* (2005) reported that the rats displayed a significant preference (65.2%) for the arm that housed the relatively novel object in each context. Rats therefore appeared to have acquired an integrated ‘what-where-which’ memory. As a tool for examining configural memory during unsupervised training, (where a stimulus or pattern of stimuli is presented in the absence of an associated stimulus of motivational significance, see Figure 1; cf. Rudy & Sutherland, 1995) this general procedure has much potential. Successful performance on the test suggests that rats are forming configural representations of the what, where and which stimuli presented during training sessions, without reinforcement (see Figure 1). Although rats

may be forming simple elemental associations between the individual pairs of stimuli, the structure of the training trials means that such elemental associations could not provide a basis for the rats preferences: the objects are paired equally often with the left and right arms, and with two contexts. However, the combinations of context+arm does provide (configural) information about the location in which the preferred object (i.e., the object that has not undergone further habituation) will be found. Furthermore, the procedure could be modified to include a temporal component, in order to demonstrate the integration of temporal with either contextual or spatial information. This issue will be directly investigated in Chapter 2 of the thesis.

#### **1.4.4. Odour sequence memory in rats**

A novel approach to investigating episodic-like memory in rats has been used by Eichenbaum and colleagues (Eichenbaum & Fortin, 2003; Eichenbaum *et al*, 2005; Ergorul & Eichenbaum, 2004; Eichenbaum & Fortin, 2005). This approach involved assessing rat's memory for sequences of odours. For example, in Ergorul and Eichenbaum (2004) rats with hippocampal lesions were able to discriminate between more or less recently presented odours, but perhaps unsurprisingly showed no evidence of spatial memory for the locations in which the odours had been presented. In a related experiment, Eichenbaum and Fortin (2005) used receiver-operating characteristics (ROC) signal detection analysis in the context of a similar odour recognition paradigm. This procedure involved analysing the number of true positives (i.e., correctly identified familiar target odours) and false positives (i.e., incorrectly identifying a novel odour as familiar). Hippocampal damage in both rats and humans resulted in performance that preserved the familiarity component of the ROC curve, but disrupted the all-or-nothing recollective component of recognition memory. The latter component is thought to

involve the retrieval of the spatiotemporal context in which an item was presented and these results provide converging support for the role of the hippocampus in episodic memory. However, it should be noted that interpretation of ROC in behavioural studies remains controversial due to the uncertain variables involved; e.g. it has been shown, in human studies, that ROCs can be influenced by the perceived requirements of the test, rather than providing a reliable measure of different components of recognition memory (Rotello, Macmillan, Reeder & Wong, 2005). In the light of this observation, it is rather difficult to know what to make of the similarity between ROCs in animal studies and human studies (Eichenbaum & Fortin, 2005). The use of word-recall tests (cf. Eichenbaum & Fortin, 2005), to demonstrate episodic memory in humans, has also been questioned (Tulving, 2002).

Returning to Ergorul and Eichenbaum (2004), the procedure incorporated stimuli with what, where and when properties by presenting rats with a sequence of individual odours, each of which was placed in a unique spatial location in the experimental arena during the training trials. As the stimuli each had a unique combination of odour, location and relative recency, each odour could be viewed as having specific spatiotemporal properties. The rats also received training sessions where they were rewarded for exploring less recently encountered odours. During test trials, rats were simultaneously presented with two familiar odours in their associated locations, and rewarded for exploring the odour that was encountered first during training trials. Ergorul and Eichenbaum (2004) report that rats showed a significant preference to explore the less recent odour, and argued that this behaviour was supported by the rats recalling spatiotemporal information about the odours.

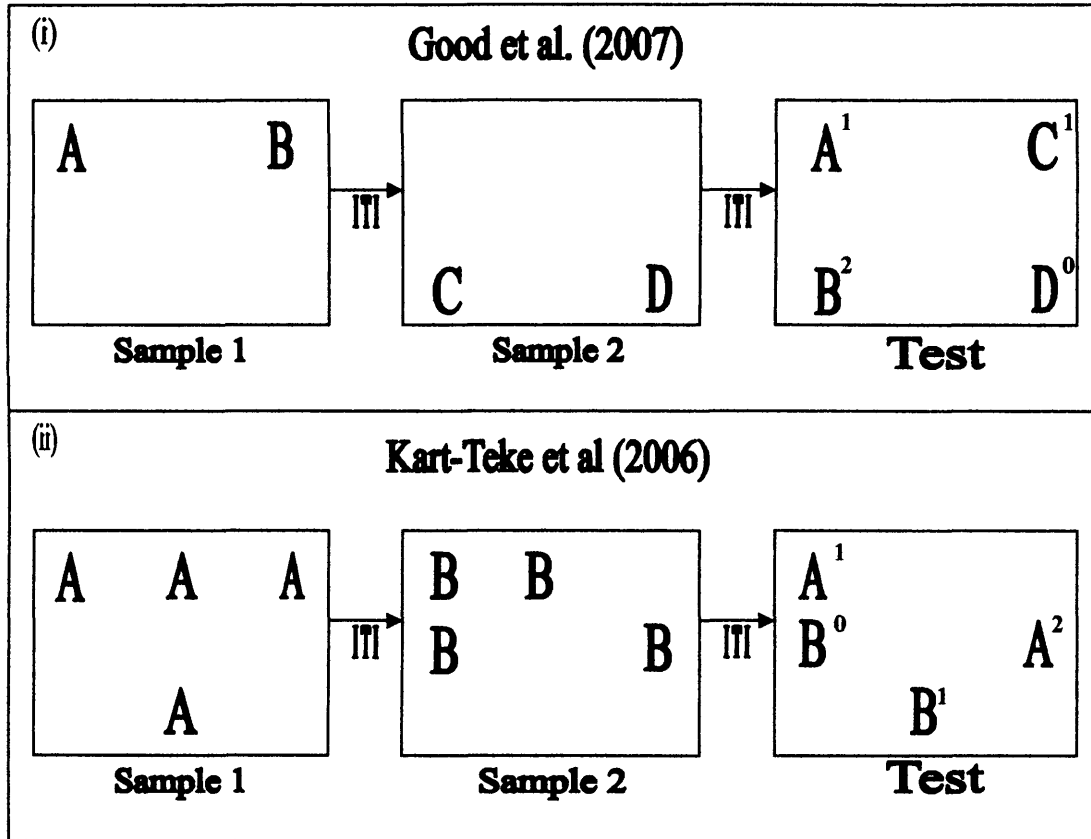
It is possible to generate both configural and elemental analyses for the results reported by Ergorul and Eichenbaum (2004; see Figure 1). If specific odours are viewed

as the ‘what’ element, the spatial location in the experimental environment as the ‘where’ element, and the relative recency of the odours presented in the test as the ‘when’ element, then there are two ways in which the rats may be able to correctly determine the difference in recency of exposure between the stimuli present at test. First, by retrieving the specific spatiotemporal properties of each odour via an integrated configural representation; second, by the odours retrieving their spatial and temporal properties by elemental associations. These possibilities are not explicitly discussed in the paper itself, but Ergorul and Eichenbaum (2004) performed supplementary tests where rats were presented with familiar odours in novel spatial locations (what-when) and with unfamiliar (where-when) stimuli in familiar locations. The results of these tests suggest that the spatial information alone is not a sufficient basis upon which to generate the critical test results.

Ergorul and Eichenbaum (2004) also show that rats with hippocampal lesions performed poorly on the what-where-when test, but had no influence on the what-when test. It could be argued that the what-where-when test is more likely to be based upon configural memory than is the what-when test (cf. Rudy & Sutherland, 1995). However, there is insufficient data to rule out the possibility that rats are relying on a series of linked elemental representations when performing the what-where-when test: the test does not require configural memory. Moreover, Ergorul and Eichenbaum (2004) also report that rats with hippocampal lesions also perform poorly on the where-when test; so it is possible that the poor performance on the what-where-when test is due to hippocampal lesions disrupting the ability of rats to form encode where *per se*. Such a disruption would almost inevitably interfere with performance on the what-where-when test, whether it were dependent on a configural or elemental system. The results of this procedure are therefore of limited use in assessing whether the hippocampus is involved

in elemental or configural processing of the components of episodic memory (i.e., what, where and when).

#### 1.4.5. Episodic-like memory in object novelty tests



**Figure 3.** Experimental designs used by (i) Good *et al.* (2007) and (ii) Kart-Teke *et al.* (2006). Both procedures included two sample stages and a test stage. In both procedures each stage lasted 5 min. Each test stage includes one static object, one spatially changed object, one temporally changed object and one spatiotemporally changed object. Numbers beside stimulus objects in the test stages denote the total number of spatial/temporal changes that the object has undergone since the sample stages (ITI = 2 min. in Good *et al.*, 2007, and 50 min. for Kart-Teke *et al.*, 2006)

There are two studies that have used object novelty preference to assess memory for the spatiotemporal context in which an object has been placed: Kart-Teke, De Souza Silva, Huston and Dere (2006), and Good, Barnes, Staal, McGregor and Honey (2007).



Although the two experimental paradigms differ in some ways, they both make use of the well-documented tendency of rats to explore novel objects (e.g., Dix & Aggleton, 1999).

The design of the Good *et al.* (2007) study is summarised in the top panel of Figure 3. Rats received two consecutive exposure sessions in which they were placed in a square arena with two different objects in unique locations. Following the second exposure period, the rats were given a test in which all four objects were presented again, with the exception that two of the objects (one from the first and one from the second exposure period) switched locations. Items encountered less recently were considered to have undergone a greater change in temporal context than an object presented more recently. Previous research has shown that rats will explore recently presented items less than items presented more remotely, and will explore objects re-presented in the same location less than those re-presented in a different location (e.g., Dix & Aggleton, 1999). Good *et al.* (2007) showed that rats were more likely to explore an object that had been seen less recently and in a different location (i.e., object B in Figure 3) than the remaining objects that had undergone either a temporal (object A) or a spatial shift (objects C) or the object that had not undergone either form of shift (object D). In addition, Good *et al.* (2007) found that rats with hippocampal lesions did not exhibit a preference for object B over object A, or object C over object D. That is, the spatial shift did not influence the pattern of exploration in rats with hippocampal lesions. However, these results do not require the assumption that there is anything other than two independent effects operating at test (one spatial and the other temporal) and that hippocampal damage has an effect on the spatial but not the temporal effect. That is, this procedure does not allow a choice between elemental and configural contributions to representing patterns of information.

In the Kart-Teke *et al.* (2006) study, rats received two exposure trials before a test phase (see lower panel of Figure 3). On the first exposure trial, the rats were presented with four identical objects arranged in four (out of eight possible) locations. For the second exposure trial, another set of four identical novel objects were used. Two objects were placed in locations previously occupied by an object in the first trial, and the remaining two objects were placed in novel locations. On the test trial, two objects from the first sample stage and two objects from the second sample trial were presented to the rats. One of each object pair was placed in a location previously occupied by that object type, and the remaining item of each pair was placed in a location novel for that object type. The results from the test stage of Kart-Teke *et al.* (2006) differ from those of Good *et al.* (2007). The rats showed a marked avoidance of the object that had undergone a spatiotemporal change and a preference for the remaining objects that had changed either their spatial location or had been presented in the first exposure trial. Kart-Teke *et al.* (2006) argue that the avoidance of the object that had undergone a spatiotemporal shift must reflect memory for the spatiotemporal features of the object. Why such a shift should result in avoidance under some conditions (Kart-Teke *et al.*, 2006) and approach under others (Good *et al.*, 2007) remains unclear. However, the fact that spatial and temporal manipulations influence object exploration suggest that the spontaneous exploration procedure might be further developed to provide a suitable vehicle for assessing hippocampal function. This was the initial approach adopted in Chapter 2.

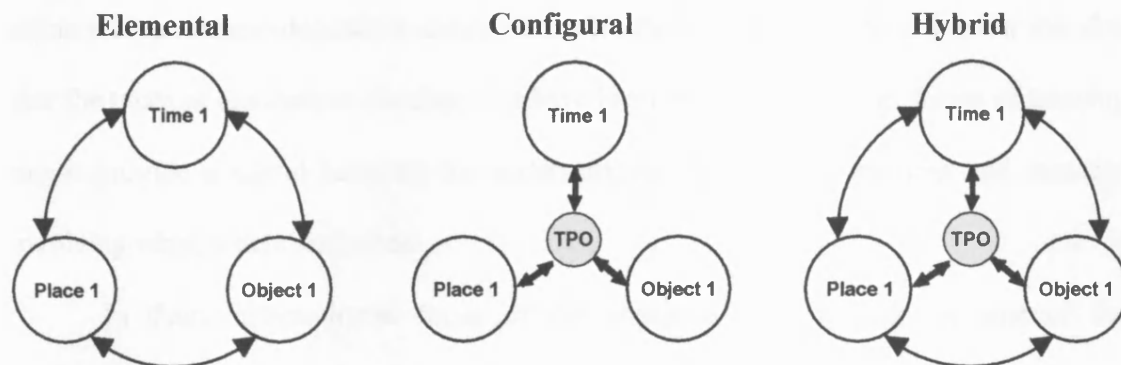
### **1.5. Episodic memory and the problem of ‘when’**

The importance of ‘when’ in episodic-like memory has led to some debate in the literature, with some claiming that it is the most important component of episodic-like memory (Clayton *et al.*, 2001a) and others claiming that its role can be taken by any other

occasion specifier, like “which” (Eacott & Norman, 2004; Zentall, Clement, Bhatt & Allen, 2001). Clayton *et al.* (2001a) argue that ‘when’ is the most important component as it is the only component that is unique to every episode. However, if commonly used experimental variables such as time of day, sequence, or interval length are accepted as representing and being encoded as ‘when’ elements, then an animal may encounter the same ‘when’ more than once. As already indicated, Eacott and Norman (2004) have argued that the ‘when’ aspect of episodic-like memory can be substituted by other occasion specifier such as the physical context in which other stimuli (e.g., objects) are presented. the argument concerning the importance of the 'when' element in episodic or episodic-like memory is secondary to the general aim of this thesis, which is, in general, concerned with the role of the rat hippocampus in processing and retrieval of memories for patterns of neutral stimulation, with particular reference to whether elemental or configural processes are involved. The choice of stimuli, however, was motivated by recent interest in episodic memory in nonhuman animals. Accordingly, the experiments use procedures in which the stimuli are, at an operational level, what, where and when, with the intention of developing a configural learning model with the potential to facilitate future study of episodic-like memory. It is also worth mentioning that there are numerous potential cues that could be used by rats to represent a specific when variable such as time of day (e.g. circadian rhythms, thirst, laboratory background noise etc.) Although the research conducted in this thesis was originally inspired by the episodic memory literature, given the theoretical issues involved and difficulty in establishing the nature of specific 'when' stimuli, the debate concerning the importance of ‘when’ in episodic-like memory will not be addressed in the following empirical chapters, except for instances when the experimental results obtained have some specific bearing on this issue; the focus of the thesis remains the role of the rat hippocampus in the processing

and retrieval of configural memory. For the time being, further references to episodic-like memory can be interpreted as involving an integrated memory (elemental or configural) for a specific combination of ‘what’, ‘where’ and ‘when’.

### 1.6. Configural memory and episodic-like memory



**Figure 4.** Elemental, configural and hybrid associative structures in which the elements of an exposed pattern of stimuli (i.e., ‘what’, ‘where’ and ‘when’; e.g. Time 1, Place 1 and Object 1) become directly linked to one another by elemental associations, indirectly linked to one another through a shared capacity to activate an independent, configural unit (TPO; for Time 1+Place 1+Object 1), or a hybrid of both types of links are acquired.

As stated in the previous section, the aim of this thesis is to investigate the role of the rat hippocampus in processing and retrieval of memories for patterns of stimulation. The hippocampus has been subject to a great deal of study with regards to its role in memory function, particularly with regard to configural learning (Alvarado & Rudy, 1995; O’Reilly & Rudy, 2001; Rudy & Sutherland, 1989, 1995; Wishaw & Tomie, 1991), and there is also a large body of literature that emphasises the importance of the hippocampus in episodic memory processing (Aggleton & Brown, 1999; Eichenbaum & Fortin, 2003; Milner *et al*, 1968; Tulving, 2002). Comparisons have been made between the putative configural and episodic functions of the hippocampus (e.g. Rudy & Sutherland, 1995). Previously in this chapter, recent experimental attempts to

demonstrate episodic-like memory in animals were discussed, and it has been shown that each example can be explained in terms of an elemental and/or configural learning system. These possibilities, together with their hybrid, are depicted in Figure 4 in order to make concrete the theoretical alternatives that are to be explored in this thesis. Although it remains a possibility that episodic memory is a distinct form of memory supported by its own hippocampal-dependent mechanism, this thesis takes as a starting point the idea that the types of associative structure that have been implicated in other forms of learning might provide a useful heuristic for understanding the basis of learning and memory involving what, where and when.

In short, although the focus of this thesis is investigating the role of the hippocampus in encoding and retrieving patterns of stimulation in general, the stimulation used will be episodic-like in nature. In this thesis the patterns of stimulation used include temporal information (i.e., relative recency in Experiment 1; time-of-day in Experiments 2-9), spatial information (i.e., spatial location in Experiments 1 and 2; contexts in Experiments 3-9), and either objects (Experiments 1 and 2) or auditory stimuli (Experiments 3-9). As it transpired, following the development of appropriate behavioural tasks in Chapters 2 and 3, the results of principal interest relate to the use of variants of a novel task that assessed whether the retrieval of elementary and configural memories involving these types of information are differentially reliant on the hippocampus.

## **1.7. Summary**

Chapter 1 has evaluated evidence from studies with animals that have assessed the proposition that they can form integrated memories that have an episodic-like character.

That is, they can form memories that bind a stimulus (e.g., an object or odour) to the spatiotemporal context in which it was presented.

It was hypothesised that rats would be able to demonstrate integrated configural memories for patterns of stimulation that were episodic-like in nature, and that this ability would be dependant on the hippocampus, whereas tests that could be supported by the use of elemental associations would not be affected by hippocampal lesioning. To this end, investigations began by drawing on existing behavioural procedures (Chapter 2) and when these proved to be less fruitful than had been anticipated (Experiments 1 and 2), it resulted in the development of some novel assays of mnemonic integration (Chapter 3) that were based on the phenomenon of sensory preconditioning (e.g., Brogden, 1939; Rescorla & Cunningham, 1978). This procedure has been used previously to show that animals can link representations of neutral stimuli that have co-occurred. For example, after exposure to one pattern (e.g., tone+light) establishing a conditioned response to one component of the pattern (e.g., the light) results in the remaining component (e.g., the tone) also eliciting conditioned responding. This robust finding indicates that a memory of the pattern has been formed, but does not allow one to conclude whether a configural memory of the pattern or simple chains of elementary associations are mediating test performance (e.g., tone→light→shock). Indeed, this ambiguity might help to explain why some have found that sensory preconditioning is disrupted by hippocampal lesions and others have not (see Ward-Robinson, Coutureau, Good, Honey, Killcross, & Oswald, 2001). In Chapter 3, following the unsuccessful procedures used in Experiments 1 and 2, an alternative procedure that had proved to be successful in other experiments was adopted. As a result, a sensory preconditioning procedure was developed that shows that rats can form configural memories that represent which auditory stimulus was presented in which context and at what time of day (Experiments 3-5). This procedure was then

used to examine whether this form of mnemonic integration was disrupted in rats given excitotoxic lesions of the hippocampus prior to behavioural training (Experiment 6). Finally, in Chapter 4, temporary inactivation procedures were used to contrast the role of the hippocampus during the retrieval of such configural memories (Experiment 7) and other memories (involving the same content) that could be supported by elementary associations (Experiments 8 and 9).

### **1.8. Aims of thesis**

As discussed earlier in the chapter, the process of binding elements for a spatiotemporal context could be based upon simple elemental associations, configural associations or both (see Figures 1 and 4). In evaluating the available literature it becomes clear that there is little unambiguous evidence that animals are capable of forming configural representations of such a pattern of stimulation and, therefore, no extant way of assessing the role of the hippocampus in this form of binding (e.g., Rudy & O'Reilly, 2001). What is needed, therefore, are behavioural assays that allow the psychobiological models of hippocampal function to be appropriately evaluated. The aim of this thesis is to provide such assays and use them to evaluate the hippocampal role in the processing of configural memories for stimuli that have episodic-like properties.

## **Chapter 2**

### **Assessment of two potential assays of configural integration**

*Summary.* The two experiments reported in this chapter investigate the ability of rats to show configural integration of episodic-like information using two variants of existing object recognition paradigms. Experiment 1 employed the procedure developed by Good *et al.* (2007), who reported that rats showed greater exploration of an object that had undergone a spatiotemporal context change than objects that had undergone either a spatial or a temporal context change. The results of this study were not sufficiently clear-cut to provide a basis for further investigation. Experiment 2 employed a novel procedure that made use of an E-maze (Eacott *et al.*, 2005). In this procedure, the contexts (A or B) in which two objects (X and Y) were presented was dependent upon the time of day (morning or afternoon): In the morning object X was presented in context A and Y was presented in B, and in the afternoon this arrangement was reversed. Subsequently, X was paired with food and Y was not. If rats acquired configural memories of the context, time of day and object, then it was anticipated that this revaluation procedure would result in rats preferring to approach context A in the morning and context B in the afternoon.

#### **2.0. Introduction**

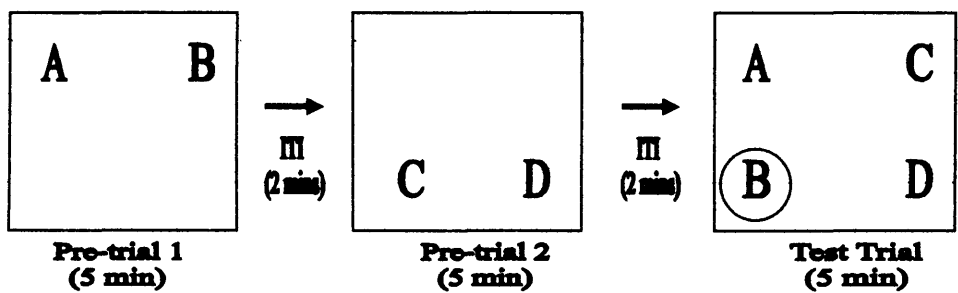
Rats display a preference for exploring novel objects, familiar objects that have been encountered less recently than others (change in temporal context) and familiar objects that have undergone a spatial location change (see Dix & Aggleton, 1999; Eacott *et al.*, 2004; Ennaceur & Delacour, 1988; Good *et al.*, 2007; Karte-Teke *et al.*, 2006).



Good *et al.* (2007) showed that rats are especially likely to explore an object that has undergone a spatiotemporal change; an effect that is at least consistent with the rats having formed an integrated representation involving the components associated with episodic-like memory (Clayton *et al.*, 2001b; Eichenbaum & Fortin, 2003; Rudy & Sutherland, 1995). However, as previously discussed, the results could also be explained by the combined, but separate influences of spatial and temporal changes on exploratory behaviour. The aim of Experiment 1 was to replicate the findings of Good *et al.* (2007), with the intention of then modifying the procedure to allow the observed behaviour to be explored both behaviourally and neurally (i.e., by assessing the role of the hippocampus using both lesion and inactivation techniques). For example, it was the intention to proceed by developing a test in which pairs of objects were presented in each corner. In one corner, the pair would consist of one object that had undergone neither a change in spatial or temporal context and one object that had undergone a change in both. The other corner would contain one object that had undergone a spatial change and one that had undergone a temporal change. In this way, the pairs of objects in both corners would be equated in terms of the number of changes; if rats showed a preference for exploring the corner containing the object that had undergone a combined spatiotemporal change, it would suggest that this procedure reflected more than the simple summation of effects caused by spatial and temporal changes in isolation. The results of Experiment 1 and of other pilot studies, however, did not provide compelling grounds for pursuing this approach. The possible reasons for the failure to replicate the effects reported by Good *et al.* (2007) are discussed later in the chapter.

**2.1. Experiment 1: Arena study**

The design of Experiment 1 is closely modelled on that described by Good *et al.* (2007) and is depicted in Figure 5. Briefly, rats first received exposure to one pair of objects (A and B) in separate corners of a square arena, and shortly after they received exposure to a second pair of objects (C and D) in the remaining corners. During the final test that followed, all four objects were presented, and while objects A and D were presented in the same corners as they had occupied during exposure, the spatial positions of objects B and C were swapped. The test includes objects that had undergone a spatial change (C), a temporal change (A), both changes (B) or neither change (D). On the basis of the results reported by Good *et al.* (2007) rats should be most likely to explore object B and least likely to explore object D.



**Figure 5.** Replicated from figure 3i. A schematic representation of the procedure used in Experiment 1. A, B, C and D denote objects; the squares represent the experimental arena; ITI = Inter-trial interval; and the circle indicates the object that has undergone a spatiotemporal shift.

## 2.2. Method

*Subjects.* Experiment 1 used 22 naïve male hooded Lister rats (*Rattus norvegicus*; supplied by Harlan Olac Ltd., UK; *ad libitum* weight range: 350-450 g) that were approximately 7 months old. Rats were housed in pairs and maintained in a temperature and humidity controlled room (20-22°C) on a standard 12 hour light-dark cycle. The rats had free access to food and water in their home cages. All experiments were covered by a home-office licence and complied with Home-Office regulations, in accordance with the “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) and the UK Animals (Scientific Procedures) Act (1986).

*Apparatus.* All trials took place in a wooden arena that was painted matt dark grey (W x L x H: 100cm x 100cm x 60cm) and filled with sawdust to an average depth of approximately 1cm. The arena was placed in the centre of an experimental room that was illuminated by standard overhead fluorescent room lights. The walls of the room were decorated with a variety of extramaze cues (e.g. black and white posters). The objects were obtained from a variety of commercial sources, all were constructed from durable nonporous materials that could not be easily gnawed or damaged by the rats during exploration (e.g., glass bottles, tin cans, ceramic ornaments and glassware). The objects were, approximately, matched in terms of size. Each item was weighted or secured to the floor of the arena with adhesive to prevent toppling when the rats explored them. The objects were cleaned with alcohol wipes before and after use on all trials. A video camera was mounted on the ceiling (approx 250cm above centre point of arena), and connected to a DVD recorder to record the exploratory behaviour for live and possible off-line scoring. A reduced instruction set computer (RISC PC) was placed alongside the arena,

and the scoring program 'EthoVision' was used to manually score the behaviour from direct observations, and to allow scoring from recordings if deemed necessary.

*Procedure.* The rats received one day of acclimatization to the experimental arena, in which they were placed individually in the centre of the arena with no stimulus objects and allowed to explore for 5 min. On the next two days this procedure was repeated, but with the addition that a novel object was placed in the centre of the arena to reduce general neophobia associated with the presence of an object. This object was not used during the later stages of the experiment. The experimenter was present during habituation, remaining stationary at the workstation used for scoring exploration behaviour.

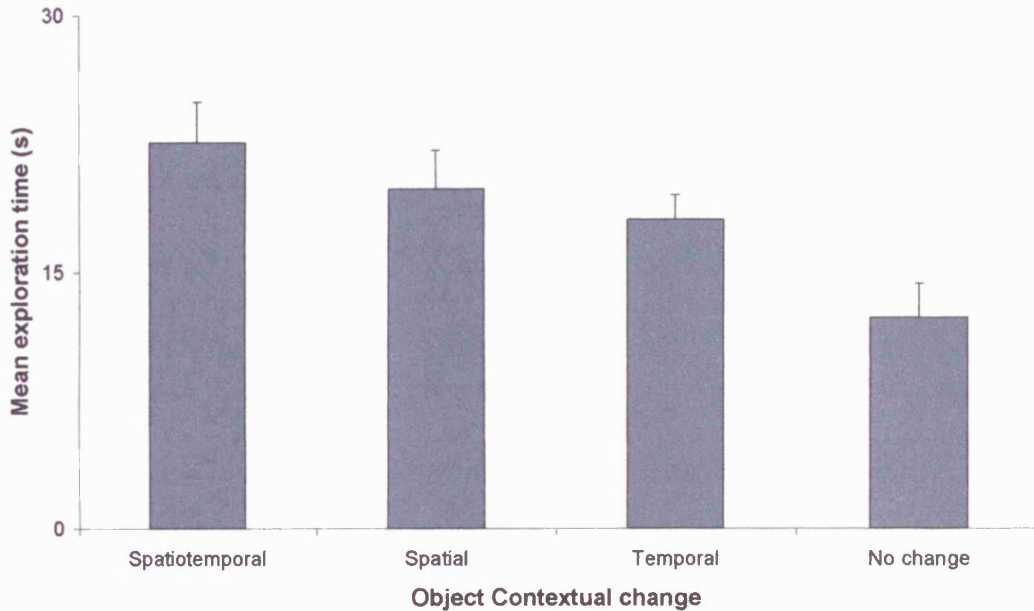
The critical experimental procedure consisted of 3 parts: 2 pre-trials and a test trial. During pre-trial 1, each rat was presented with two novel objects (A and B). These were placed in adjacent corners of the arena (see Figure 4), equidistant between arena centre and corners themselves. The rats were allowed to explore the objects for 5 min. before being removed from the arena and returned for a 2 min. ITI to their covered home cage. During the ITI the walls of the arena and objects were cleaned with alcohol wipes to remove odour cues. For pre-trial 2, two new objects (C and D) were placed in the remaining corners of the arena at the same distance from the walls as A and B. Rats were again allowed to explore for 5 min. before being returned to the home cage for the second ITI. The arena and objects were again cleaned with alcohol wipes. For the test trial, identical copies of the 4 objects were placed in the arena in the following manner: Objects A and D were presented in the same corners as they had been on pre-trials 1 and 2, respectively; whereas the corners in which objects B and C were presented at test were

exchanged with respect to those that they occupied on Pre-trials 1 and 2, respectively. Thus, D was tested under conditions that most closely matched those that prevailed during exposure (same corner, recently observed); object A and C were tested under conditions that did not match those of training because they had either been presented less recently (A) or in a different place (C); and object B had both been presented less recently and in a different corner. Each rat was tested twice with two different stimulus objects sets, with a one-day break between first and second tests. The objects that served as A, B, C and D were counterbalanced as was the adjacent pairs of corners in which the pairs of objects (AB/CD) were placed.

*Behavioural and statistical measures.* Object exploration was recorded during the test and was defined as the amount of time a rat spent actively sniffing, with front paws resting on or head pointed towards an object at a distance no greater than 2cm (see Good *et al.*, 2007). If the rat was within 2 cm of the object, but facing away from it, then it was not scored as exploring the object. Similarly, if the rat was on top of an object it was not considered to be exploring the object.

ANOVA and other parametric statistical analyses were used in this experiment and throughout the thesis.

### 2.3. Results



**Figure 6.** Experiment 1: Mean exploration times (in seconds) of rats in the test session. Error bars show standard error of the mean (+SEM).

One rat contracted an illness between testing stages and thus could only be tested once, therefore any results obtained from this subject were excluded from the analysis. Figure 5 shows the mean exploration times for each of the four objects pooled across both tests. Inspection of this figure suggests that object B (that had undergone a spatiotemporal shift) elicited the most exploration (22.58s) and that object D (that had undergone the least change) elicited the least exploration (12.38s). Object C (that has been presented recently, but had changed location) and object A (that was presented in the same location, but had not been presented recently) elicited intermediate amounts of exploration (19.25s and 18.05s respectively). Statistical analysis provided partial support for this description of the results presented in Figure 5. Analysis of variance (ANOVA)

with stimulus (A-D) as the within-subjects factor revealed an effect of stimulus,  $F_{(3,60)} = 6.626, p < .05$ . Pairwise comparisons between exploration scores for individual stimuli revealed that A, B and C each elicited greater exploration than D (smallest  $t_{(20)} = 2.679, p < .05$ ), but that there were no significant differences between exploration of A, B and C (largest  $t_{(20)} = 1.869, p > .05$ ).

## 2.4. Discussion

The results of Experiment 1 replicate the overall pattern of behavioural results reported by Good *et al.* (2007), but failed to yield the pattern of statistical significance found in that paper. In particular, while rats in Experiment 1 were numerically, at least, more likely to explore an object (B) that had undergone the greatest change in spatial and temporal context than objects (A and C) that had only undergone one of these changes, this difference was not statistically significant. The reason for this failure to replicate is puzzling given the similarity between the two experiments. However, there are several seemingly minor differences that might have contributed to the difference patterns of statistical significance. These differences will be considered, in detail, below.

Before the start of the Experiment 1, pilot studies (not reported here) were performed in order to ensure that there were a sufficient number of available stimulus objects that elicited sufficient levels of exploration. These pilot studies were very closely modelled on the procedure used by Good *et al.* (2007), including the same selection of stimuli and the same arena. However, the preliminary tests revealed that rats persistently attempted to, and in some cases succeeded in, escaping from the arena used in the original experiment. Therefore, one difference between the Good *et al.* (2007) study and

Experiment 1 is that the walls of the area were higher in Experiment 1 (60 cm) than in the arena used by Good *et al.* (2007; 50 cm). This difference might have reduced the ability of rats to encode the spatial layout of the objects with respect to the extra-maze (room) cues and thereby reduced the likelihood of observing the effect of interest; and rats might have been more reliant on the spatial cues provided by the arrangement of stimulus objects themselves. This state of affairs might have increased the exploration directed towards each object and effectively masked differences in levels of exploration determined by spatial shifts (O'Keefe, 1991). This possibility is supported by the fact that Good *et al.* (2007) reported that rats tended to spend more time exploring the temporally changed object than the spatially changed object, whereas in Experiment 1 the opposite tendency was apparent (see Figure 6).

An alternative possibility can be derived from the observation that although the timing of the trials were *nominally* the same in Experiment 1 and Good *et al.* (2007), there might have been minor difference between the timing of the trials across the two studies. However, this possibility seems implausible given the fact that rats were exploring the test objects (spatiotemporal and temporal) that had been presented less recently than the remaining objects (see Figure 6).

Finally, a task that relies on spontaneous exploration, during both training and testing, might inevitably produce results that are inherently variable. The data obtained from the experiment is insufficient to determine the validity of these issues raised. The fact that data is collated from several repetitions of the procedure suggests that the lack of significance is not a result of low power, and that the underlying problems with the procedure were persistent. Although the procedure was designed to be as close as



possible to that used by Good *et al.* (2007), circumstances and resources prevented an identical replication of the Good *et al.* (2007) procedure, meaning a direct comparison between this procedure and that used in Experiment 1 was unavailable. It was with the latter considerations borne in mind, that it was decided to adopt a procedure that was less reliant upon spontaneous exploration.

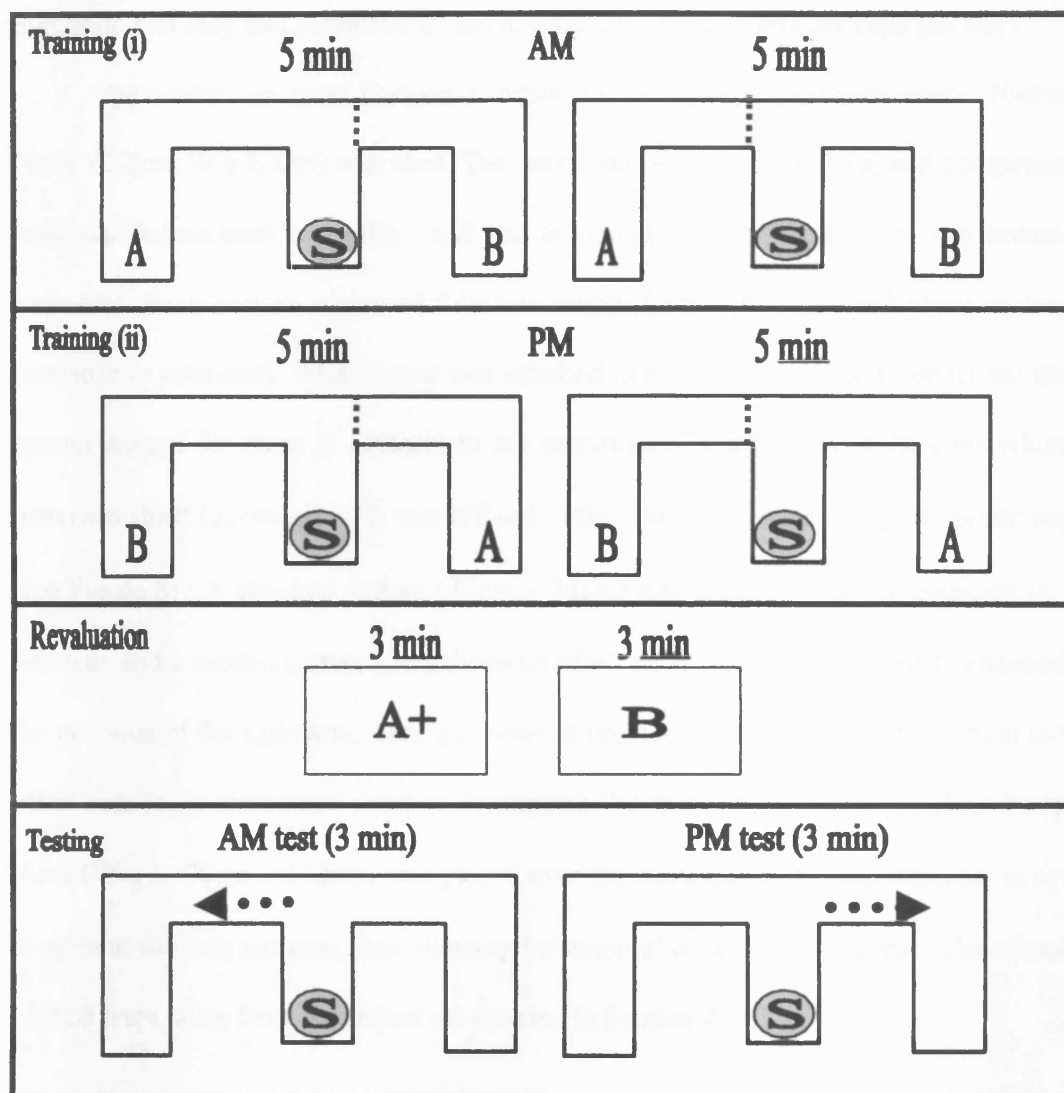
## **2.5. Experiment 2: E-maze study**

The failure of the object recognition procedure used in Experiment 1 to produce any significant results raised questions as the suitability of the procedure for investigating the ability of rats to form hippocampal dependent configural memories for spatiotemporal context, which is the aim of this thesis. As a result, Experiment 2 adapted the E-maze apparatus used by Eacott *et al.* (2005) in an attempt to demonstrate integration of what object was presented where and when in a way that is beyond simple binary (i.e., what-where and what-when) associations. The design of the experiment is summarized in Figure 7. The original Eacott *et al.* (2005) procedure demonstrated configural memory for object, location and context in rats. However, in order to investigate to the aims of this thesis, the procedure was modified in order to test for configural memories for what-where-when. This required several modifications to the training and testing elements of the procedure, which will be described in the method section (for details regarding the original procedure, see Eacott *et al.*, 2005).

During the first stage of the experiment, rats received two sessions each day; one in the morning (AM) and one in the afternoon (PM). In the AM sessions, rats were released from the central arm of the maze and upon entering the left arm would encounter

object A, whereas upon entering the right arm they would encounter object B. In addition to the extra-maze cues associated with each arm, the entrance to the left and right arms were discriminated by the presence of different intra-maze cues. In the PM sessions, the position of the objects with respect to the left and right arms was reversed. In the second stage of the procedure, rats were placed in a square arena at midday where object A was paired with food and object B was not. Finally, rats were placed in the central start arm of the maze in the morning and afternoon and were allowed to explore both of the remaining arms. The question of interest was whether the rats would approach the arms in which object A had been presented at the appropriate time of day: In the current example, would the rats approach the left arm in the morning and the right arm in the afternoon? Given the fact that the left and right arms were equally often paired with objects A and B and that the morning and afternoon were also equally frequently paired with A and B, then simple binary associations could not provide the basis for any arm preference. Instead, a preference to explore the left arm in the morning and right arm in the afternoon must be based on an integrated, configural memory that codes for what happened where and when.

## 2.6. Method

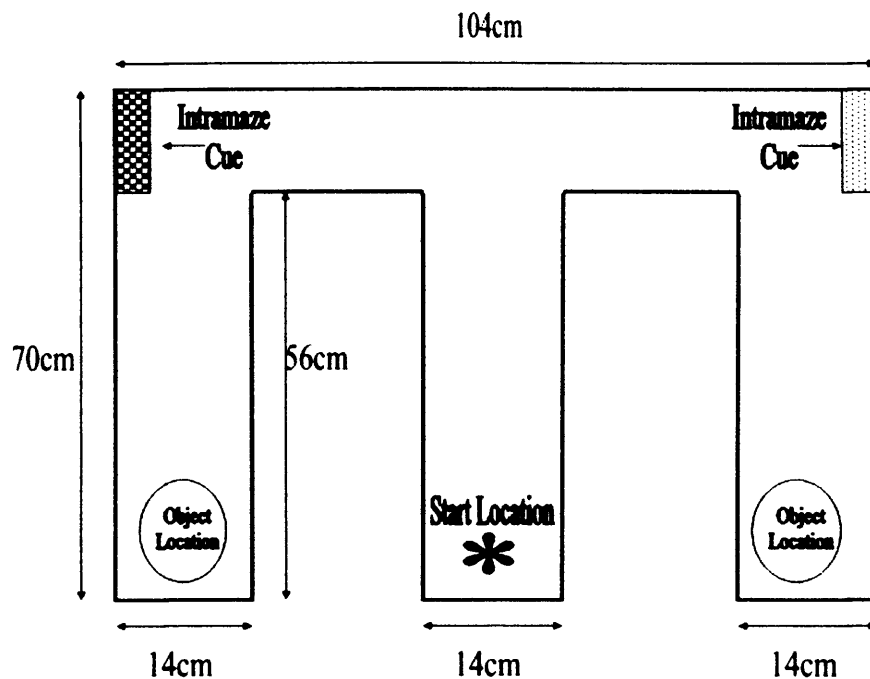


**Figure 7** A schematic representation of the E-maze procedure. S represents starting location; broken lines signify that an entrance to an arm is blocked; 'A' and 'B' represent stimulus objects; '+' represents food. Arrows represents arm predicted to be chosen by rats during the test.

**Subjects.** The subjects were 12 naïve male hooded Lister rats (*ad libitum* weight range: 350-450 g; approximately 6 months old). They were from the same supplier as

Experiment 1 and housed and maintained in the same way as that experiment with the exception that they had restricted access to food (30g food pellets per cage per day).

*Apparatus.* A clear Perspex E-maze (overall external measurements: 104cm x 70cm x 22cm; W x L x H) was used. The maze was placed on the floor, and composed of three parallel sections, or 'arms', and one adjoining section that allowed rats access to each arm. Each section measured 14cm in width. Each arm measured 56cm in length (entrance to terminus). White paper was attached to the external walls to render the maze opaque except for areas at adjacent to the entrances of the left and right arms where a patterned sheet (22cm x 24cm) was affixed to the outer wall instead of plain white paper (see Figure 8). A checked pattern (22cm x 24cm) was attached near the entrance of the left arm, and a spotted pattern (2cm diameter black dots, 7cm centre to centre) attached to the entrance of the right arm. This was done to provide intramaze cues, in addition to the other cues (e.g., extramaze cues) to distinguish the arms of the maze. A clear Perspex sheet (W x L: 70cm x 110cm) was placed over the maze during testing sessions, in order to prevent subjects escaping, but allowing behavioural observation to occur. The stimulus objects were taken from the object set detailed in Section 2.2.



**Figure 8.** The internal layout of E-maze, measurements, and the position of the experimental components. Patterned sections represent visual cues affixed to outer walls to provide intramaze cues that differentiate left and right arms.

A video camera was mounted on the ceiling (approximately 200cm above centre point of arena), and connected to a video recorder in order to record the exploratory behaviour for live and possible off-line scoring. A RISC PC was placed alongside the arena, and the scoring program 'EthoVision' was used to manually score the behaviour from direct observations. A second matt grey square arena, as described in Experiment 1 (Section 2.2), was used as during the revaluation stage (involving Coco pops, Kellogs) and to assess the efficacy of the revaluation procedure.

*Procedure.* The rats received 2 days of habituation in the E-maze. Each rat was placed in the centre of the empty E-maze at the constant start position (see Figure 8) and allowed to explore for 10 min. at, approximately, midday for two successive days. Rats

were allowed unrestricted access to all arms of the maze during habituation sessions. The experimenter was present during habituation, sat stationary at the workstation used for scoring exploration behaviour. Rats also received 2 days of habituation to the arena used for the revaluation stage of the experiment, where they were placed in the centre of the arena and allowed to explore for 5 min. before being removed and returned to the home cage. Habituation again took place over two days, with each session occurring around midday.

The remainder of the experiment lasted nine days. On the first 4 days, rats received two training sessions on each day, one in the morning (9.30am onwards) and one in the afternoon (2.30pm onwards). Each session consisted of two parts. If objects are represented by A and B, and outer arms of the maze are represented by X (nearest to the checked intra-maze cue) and Y (nearest to the spotted intra-maze cue), then in each part rats had access to one object-arm (what-where) combination. In the morning session, access to one arm was restricted, allowing the rats 5-min access to only one combination (e.g., AX), before being returned to the covered home cage in a nearby holding room for a 5 min. ITI. The rat was then returned to the E-maze for the second part, in which the alternate arm was blocked, allowing 5-min access to the second combination (i.e., BY). In the afternoon, the objects occupied the opposite arms, (i.e. AY and BX; see Figure 6). The second day of the training trials followed the same procedure, with the difference that the order in which the arms were blocked was reversed. For example, if on Day 1 the rats were exposed to AX, BY in the morning session, and AY, BX in the afternoon, then on Day 2 the rats were exposed to BY, AX in the morning and BX, AY in the afternoon. This sequence was repeated on days 3 and 4, and the object location and order of

presentation of stimulus combinations was counterbalanced between subjects. E-maze and objects were wiped down with alcohol wipes each time a subject was removed from the apparatus, in order to remove odour cues.

Revaluation sessions began at midday. Each object was placed at the centre of the square arena. On a rewarded trial, 2.5g of Coco Pops was placed around object A at a distance of no more than 2cm from the base of the object. Rats were put in the arena and allowed to consume the reward for 3 min. and were then removed from the arena. On a nonrewarded trial, the rats were placed in the arena with object B for 3 min., but there were no Coco pops. Half of the rats received a rewarded trial that was followed by a nonrewarded trial on Day 1 and the reverse on Day 2; and the remainder received the opposite sequences of trials. The rats were returned to their home cages in the 3-min ITI between the two trials on each day and during this period objects A and B were cleaned with alcohol wipes.

Rats received two test sessions on each of two days, one in the morning and one in the afternoon, which began at approximately the same time of day as the training trials. Each test consisted of a rat being placed in the E-maze at the same start location. The E-maze contained no objects, and all areas of the maze were accessible. Rats were allowed to explore for 3 min. The amount of time that rats spent exploring the two outer arms was measured. A rat was said to be exploring an arm when their head and forelimbs were beyond the entrance to that arm. The rear half of the body crossing the entrance with the head and forelimbs outside of the arm was not scored as exploratory behaviour. Exploration was scored online or from recording where required in three successive blocks of one min. The E-maze was cleaned with alcohol wipes after each trial to

remove odour cues. It was predicted that the subjects would initially spend more time exploring the arm that had housed the revalued object during the training trials, and that this arm preference would depend upon the AM and PM session. Objects were not present in the E-maze during the test trials, nor were they visible during the initial stages of training trials when subjects were placed in the start location, meaning successful performance would imply the use of configural what-where-when memories.

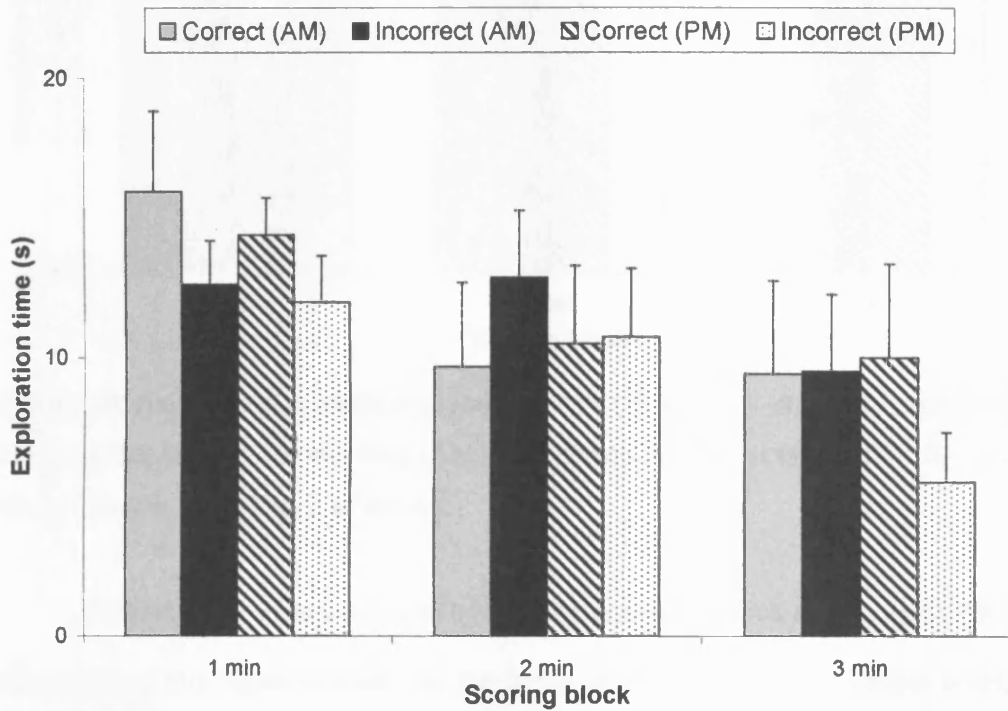
A test to assess the effectiveness of the revaluation procedure was conducted in the square arena at midday. Objects A and B were both placed in adjacent quadrants of the arena and rats were allowed to explore for 3 min. The amount of time that the rats spent in the quadrants occupied by one of the objects was assessed in consecutive 1-min blocks. The quadrant each object occupied was counterbalanced between subjects. After each session, rats were returned to the home cages. If the revaluation trials had been successful, then the rats were expected to spend significantly more time in proximity to the revalued object, A, than the non-revalued object, B.

## **2.7. Results**

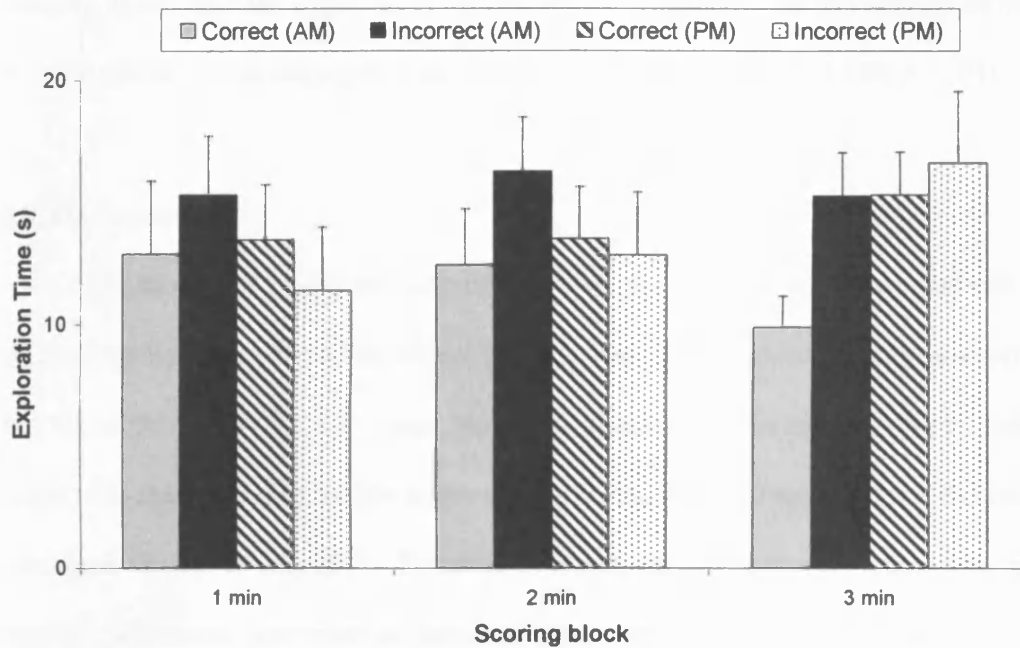
Figure 9 shows the results from the first testing day. In particular, it shows the mean amounts of time rats spent in the arms associated with object A in the morning and afternoon (the correct arms) and the arms associated with object B in the same sessions (the incorrect arms). Inspection of this figure suggests that, at least in the first minute of testing, there was a tendency for rats to spend more time in the correct than the incorrect arms. However, exploration of the objects decreased across blocks and any consistent sign of a preference to explore the correct arms had disappeared by the second and third



minutes of testing. ANOVA with arm (correct versus incorrect), test (morning or afternoon) and block (1-3) as factors revealed no effect of arm or test ( $F_s < 1$ ), but there was an effect of block ( $F_{(2,66)} = 5.726, p < .05$ ). There were, however, no interactions between these factors (largest  $F_{(2,66)} = .860, p > .05$ ).



**Figure 9.** Experiment 2: Mean exploration times (in seconds, -s; +SEM) for the correct and incorrect arms in the morning (AM) and afternoon (PM) sessions over the course of the 3-minute tests on Day 1 of testing.



**Figure 10:** Experiment 2: Mean exploration times (in seconds, -s; +SEM) for the correct and incorrect arms in the morning (AM) and afternoon (PM) sessions over the course of the 3-minute tests on Day 2 of testing.

Rats received a second day of testing, the results of which are shown in Figure 10. Inspection of this figure reveals that the levels of exploration were similar across each minute of the test, and that in the morning there was some tendency for rats to explore the incorrect arm in the AM session. ANOVA with arm (correct versus incorrect), test (morning or afternoon) and block (1-3) as factors revealed no effect of any variables (largest  $F_{(2,66)} = 1.45$ ,  $p > .05$ ). This second test was followed by a test of the effectiveness of the revaluation procedure. In this test, the rats were given a choice between objects A and B at midday in the open-field arena used for revaluation training. ANOVA with object and block (3 consecutive 1-min periods) as factors revealed no significant effect of object ( $F < 1$ ; A = 21.37s and B = 21.178 s), an effect of block ( $F_{(2,22)} = 3.56$ ,  $p > .05$ ; 1 = 19.23 s, 2 = 21.82 s and 3 = 22.78 s), and no significant interaction

between object and block ( $F < 1$ ). Post-hoc analysis revealed that exploration of objects A and B did not differ during each minute of training (largest  $t_{(11)} = 1.189, p > .05$ ).

## 2.8. Discussion

The results of Experiment 2 provide no support for the suggestion that rats form an integrated memory for which object (A or B) was presented where (left or right arm) and when (AM or PM). Of course, this failure should not be taken to undermine the utility of E-maze because the procedure differed considerably from that used successfully elsewhere (Eacott *et al.*, 2005; Eacott & Norman, 2004). However, other attempts to modify the current procedure to produce clearer results proved unsuccessful. Thus, further pilot studies (not reported here) using procedures that were more similar to those described by Eacott *et al.* (2005) were as unsuccessful as Experiment 2. In these studies, different times of day were again substituted for contexts, and the target object was revalued by pairing it with food, rather than by giving rats habituation to the object. However, the scoring procedure (i.e., first-turn choice) was more similar to that used by Eacott *et al.* (2005), as was the first stage of training in which the arms were no longer blocked: The removal of the blocks in the test stage of Experiment 2 might have resulted in generalization decrement - a disruption to what had been successfully learned during stage 1. However, these changes to the procedure did not result in an arm preference that depended upon the time of day that the arm was encountered. These pilot studies, in which the arms were not blocked during stage 1, did highlight another concern that was not evident in Experiment 2: observation of exploratory behaviour during training

sessions revealed that the rats did not spend an equivalent amount of time exploring each object and that could, in itself, contaminate subsequent arm preferences.

Finally, the test to assess whether the revaluation procedure had been successful revealed that pairing object A with Coco pops and B with no Coco pops resulted in no preference to approach object A during the final test. There are a variety of ways in which the procedure using the E-maze in Experiment 2 could be improved. However, the fact that the revaluation procedure did not produce a robust preference for object A motivated the development of a different procedure, which does produce a rapid and robust revaluation effect. The experimental designs used in subsequent chapters are formally equivalent to that of Experiment 2, but the procedures make use of more standard stimuli and apparatus.

## **2.9. General Discussion**

As previously stated in Chapter 1, the aim of this thesis is to provide an assay for the process of binding elements for a spatiotemporal context and use them to evaluate the hippocampal role in the processing of configural memories for stimuli that have episodic-like properties. Experiment 1 failed to provide clear-cut evidence that rats show a particularly marked preference for a familiar object that had undergone a spatiotemporal shift with respect to its training conditions. As has been discussed in previous sections, even had such a preference been visible, this would not have constituted evidence that the rats had formed an integrated configural memory for what had been presented where and when: Separate additive influences of a spatial and temporal shift would have been sufficient to explain such an effect (see Good *et al.*, 2007). In any case, the results of Experiment 1 did little to

encourage a more detailed analysis of the results (of the kind outlined in the Introduction) and prompted a move to a procedure which, it was hoped, would allow more control of the rats exploration to be induced. Experiment 2 used a novel experimental design and an E-maze (Eacott *et al.*, 2005) to investigate the possibility that rats could form integrated configural memories for which object was presented where and when. Rats first received training trials in which object A was presented in the left arm during the morning sessions, and in the right arm during the afternoon sessions, with object B occupying the opposite arm at both times of day (see Figure 7). After this training, object A was paired with food (Coco pops) at midday in a different apparatus. Finally, rats were allowed to explore the maze at during morning and afternoon test sessions. The prediction was that the revaluation training would result in the rats would showing a preference for the left arm in the morning and the right arm in the afternoon; and thereby provide evidence that they had encoded the combinations of where and when objects A and B had been presented. However, there was no significant evidence of such an effect.

There are reasons to be cautious before rejecting the general approach developed in Experiment 2. For example, the final revaluation test revealed that, at least after the conclusion of the tests in the E-maze, there was no preference for object A over object B. In addition, the modifications to the original E-maze procedure, although deemed necessary to fulfil the aims of the research, may have resulted in disrupting any significant effect. Repeating the experiment with a less-extensively modified E-maze procedure may yet prove successful. Although this option presented an approach for continuing the research, ongoing developments within the Behavioural Neuroscience Laboratory, aligned to the experiments discussed in this chapter, indicated that a new

procedure modelled on Experiment 2 produced results that were extremely promising. This procedure made use of contexts and stimuli that produce reliable sensory preconditioning effects in rats (e.g., Ward-Robinson & Honey, 2000) and are based on a revaluation procedure that is both rapid and highly effective, so it was decided to adopt this procedure for subsequent experiments. Chapter 3 describes the development of this procedure as both a behavioural effect and an assay to assess the role of the hippocampus in mnemonic integration.

## **Chapter 3**

### **A novel assay for configural integration and hippocampal function**

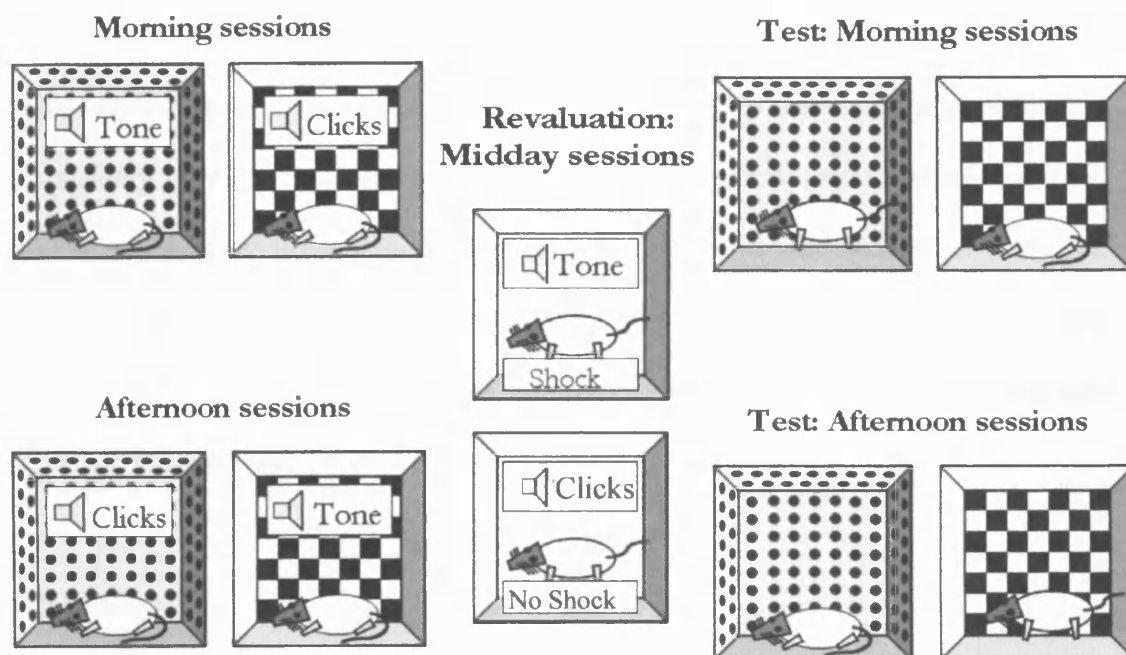
*Summary.* The results of the two experiments described in Chapter 2 (Experiments 1 and 2) prompted the development of an alternative procedure for demonstrating configural memory in rats (see Figure 1). The design of the first three experiments in Chapter 3 (Experiments 3, 4 and 5) was formally equivalent to that used in Experiment 2. However, in place of objects, auditory stimuli were used, and in place of arms of a maze, different experimental contexts were employed. In this procedure, like those discussed in Chapter 2, the information to be combined had an episodic nature and the task required a configural solution. In addition, the ineffective object revaluation procedure was replaced with an aversive conditioning procedure involving the two auditory stimuli. Experiments 3-5 examined how the effect of interest varies as a product of the amount of initial exposure to the various configurations. Experiment 6 examined whether the configural learning effect is reliant on the hippocampus. To this end, rats with excitotoxic lesions of the hippocampus made prior to behavioural training were run through the task.

### **3.0. Introduction**

The experiments discussed in Chapter 3 were based on the design of Experiment 2. As with the previous experiment, Experiment 3 uses a procedure in which rats were exposed to specific configurations of cues (what was presented where and when) and in which the anticipated pattern of test performance could not be based on binary what-where and what-when associations alone. The 'where' elements were provided by two

distinct skinner boxes with unique visual properties (spot or check patterns on the internal walls). The 'what' elements were provided by two distinct auditory stimuli (tone or click). As in Experiment 2, the 'when' element was provided by time of day (morning or afternoon). The design of the experiment is depicted in Figure 11. Initially, rats received trials in which whether the tone or click was presented was signalled by the combination of the time of day and context in which the rats were placed. For example, in the morning the tone was presented in the spot context and the click in the check context, whereas in the afternoon the tone was presented in the check context and the click in the spot context. After this training, rats received pairings of, for example, the tone with shock and the click with no shock in a third context at midday. Finally, the amount of fear (as evident in rats freezing behaviour) was assessed in both contexts, in the morning and afternoon. If the rats had learnt what stimulus was presented where and when, then they should show more fear to the context+time of day combinations that signalled the tone. In the previous example, they should be more fearful of the spot context in the morning and the check context in the afternoon than of the two remaining combinations. Experiments 3, 4 and 5 varied the amount of initial training. The principal reason for doing this was to pave the way for studying the effect of inactivating the hippocampus either during training or testing: If the behavioural effect can be demonstrated with little training then it would be possible to examine the effect of inactivating the hippocampus during training and avoid the possibility of repeated inactivation causing permanent damage to the hippocampus. As it transpired, the results of Experiments 3-5 meant that it was only possible, in Chapter 4, to examine the effect of hippocampal inactivation during testing.





**Figure 11.** Example of the treatments for rats in Experiments 3-5 in which the first stage of training involved 1 day (Experiment 3), 2 days (Experiment 4) or 4 days (Experiment 5). In the morning, a tone was presented in the spotted context and a series of clicks in the checked context; whereas in the afternoon the clicks were presented in the spotted context and the tone in the checked context. To assess if rats had formed configural memories of *where and when* the tone had been presented, fear was first established to the tone (but not the clicker) by pairing it with shock (in another context at midday on Days 5 and 6). Rats then received test configurations on Days 7 and 8 that should either result in the retrieval of the feared tone (i.e., morning with spotted context, and afternoon with checked context) or should not (i.e., the remaining combinations).

### 3.1. Experiments 3, 4 and 5: Integrating contexts, times of day and auditory stimuli

Experiments 3, 4 and 5 all used the experimental design summarized in Figure 11 and described above. The experiments matched the overall amount of presentations of the auditory stimuli within the four context+time of day combinations (check+morning, spot+morning, check+afternoon, spot+afternoon), but varied the number of days over which initial training took place (Experiment 3 = 1 day; Experiment 4 = 2 days; Experiment 5 = 4 days). Experiments 3-5 were conducted in parallel with those now published in Iordanova, Good and Honey (2008).

### 3.2. Method

*Subjects.* 48 naïve adult male hooded Lister rats (*Rattus norvegicus*; supplied by Harlan Olac Ltd., UK) were used in Experiments 3, 4 and 5 ( $n_s = 16$ ). The *ad libitum* weight ranges of the rats were: 320-600 g. Rats were maintained in the same way as rats in Experiment 1 and, like those rats, had *ad libitum* access to food and water in their home cages. As in previous experiments, all procedures were covered by Home Office Regulations (see Section 2.2).

*Apparatus.* Four chambers (L × W × H: 24cm × 24.5cm × 21cm; supplied by Camden Instruments Ltd. UK), arranged in a 2 × 2 array, were used during the exposure and test stages of the experiments. Each chamber was composed of three aluminium walls, an aluminium ceiling section and a clear, hinged plastic flap that served as door/wall to the chamber. The top two chambers in the array had their internal aluminium walls and ceiling covered with spotted laminated paper (black circles on white background). The lower two chambers had their internal aluminium walls and ceiling

covered with black and white and white checked laminated paper (black and white squares arranged in a grid pattern; see Honey & Watt, 1999). Each section of laminated paper was fixed behind cut sheets of clear plastic that lined the walls and ceilings of each chamber. The floor of each chamber consisted of stainless steel rods, 0.5 cm in diameter and placed approximately 1.5cm apart, centre to centre. Below the rods in each chamber was an aluminium tray containing a 24m x 24cm sheet of absorbent paper. Each chamber was illuminated by a single light source, a 15 V, 24-W jewel light housed in the centre of the ceiling section. The experimental room lights were turned off during the procedure. Each chamber was housed individually in a sound-attenuating cabinet which was left open during each trial to allow visual recording of rat behaviour during the test trials. Aversive conditioning took place in two further chambers, which were identical in structure to those used in the training and test trials but were undecorated, had plain aluminium walls and ceilings, and were not illuminated. These chambers were not contained within a sound attenuating cabinet, and were placed beneath the 2 x 2 array of chambers 1-4, at floor level. The floors of these two chambers were connected to a shock scrambler and generator (Camden instruments Ltd., Models 521C and 521S respectively) which enabled a 0.5 s, 0.5 mA electric shock to be delivered to the chamber floor. Each of the 6 chambers were equipped with a speaker mounted above the ceiling section, which was used to deliver either a 2 KHz tone or a 10 KHz click (depending on the configuration of stimuli required) at an intensity of approx 78 dB (A: Brüel & Kjaer, Type 2235). The auditory stimulus presented at each time point was the same in each chamber to avoid auditory contamination.

### *Procedure*

Experiments 3 (6 days), 4 (7 days), and 5 (9 days) were identical with the exception that the first stage of training occurred over 1 day, 2 days, and 4 days, respectively. During training trials, rats were placed in the two contexts during the morning (between 9.00 am and 11.30 am) and, 6-7 hours later, in the afternoon sessions (between 3.00 pm and 4 pm ). For a given rat, the order in which the contexts were presented, both within a day and between days, was the same. The context that was presented first and second was counterbalanced between subjects, and the auditory stimulus presented in the first and second context was also counterbalanced; with the designation in the morning dictating that the auditory stimuli would be presented in the opposite contexts in the afternoon session (see Figure 11). Each 10-s auditory stimulus was presented on 40 occasions in its designated context and successive presentations of a stimulus within a session were separated by 20 s. The first auditory stimulus was presented 20 s after the rat was placed in each context. Following the end of each session the rats were placed back in their holding cages and taken back to the colony room where they remained between sessions.

In Experiment 3, rats were placed in the two contexts for 20 min. in both the morning and afternoon. Rats received 40 presentations of the designated auditory stimulus in each context, and there was a 20-min interval between sessions at a given time of day. In Experiment 4, rats were placed in the two contexts in the morning and afternoon for 10 min, and there was a 10-min intervals between the two sessions at a given time of day. Rats received 20 presentations of the designated auditory stimuli in each context and training was repeated on two consecutive days. In Experiment 5, rats

were placed in the two contexts for 5 min. in the morning and the afternoon, with a 5-min interval between sessions at a given time of day. Rats received 10 presentations of the designated auditory stimulus in each context and training continued for 4 days. During the intervals between the pairs of morning sessions (and afternoon sessions) as well as the interval between the morning and afternoon sessions, rats were returned to their home cages.

On the two days following training, rats received an aversive revaluation procedure between 12.30 pm and 2.00 pm. On the first day, rats received 2 sessions where they were placed in the neutral, undecorated conditioning chamber for 90 s. In one session they received 3 presentations of an auditory stimulus (e.g., tone), separated by 20 s, that was followed by the delivery of shock. In the other session the second auditory stimulus (e.g., click) was presented, but no shocks were delivered. In the 20-min interval between sessions the rats were then returned to the holding cage. On the first day of aversive conditioning, half of the rats received the aversive conditioning session first and the other non-reinforced session second, and the remainder received the opposite sequence. On the second day, the sequence of sessions was reversed for each rat. The identities of the auditory stimuli that were paired with shock and no shock was fully counterbalanced with respect to the previous counterbalancing operations.

Following the aversive revaluation procedure, conditioned fear was assessed in both contexts in the morning and afternoon; with the sessions occurring at approximately the same time of day as the training sessions for each rat. Each test session was 3 min. and there was a 3-min interval between test sessions at a given time of day during which rats were returned to the home cage. The order in which the rats were placed in the

contexts was the same as during training; with the result that the context that should elicit the most fear was the first context in the morning and the second context in the afternoon for half of the rats and the second context in the morning and the first context in the afternoon for the remainder. This test procedure was repeated on day two.

On the final day of the procedure the level of fear elicited by the auditory stimuli was assessed at approximately midday in the contexts used for aversive conditioning. The test was conducted in the same way as revaluation training with the exception that no shocks were delivered. The sequence in which the auditory stimuli were presented in the two test sessions was counterbalanced.

*Behavioural measures and statistical analysis.* During testing sessions, for Experiments 3-5, the behaviour of rats was recorded by a stationary video camera (which was present but switched off during training and conditioning sessions). The freezing behaviour of rats was assessed from these recordings using a time-sampling procedure, where rats were observed every two seconds, and scored as either freezing or moving (inactive or active). Freezing was defined as the absence of movement, excluding that related to breathing or minor movements such as movement of the whiskers alone (Iordanova *et al.* 2008). The scoring was conducted by raters who were blind with respect to the specific treatment that each rats had received (Dean Burnett and Mihaela Iordanova). The percentages of observations on which freezing was observed in the tests was used to calculate separate ratio scores for the morning and afternoon sessions. These ratios were used in order to reduce individual variability and both ratios took the following form: Percentage of freezing in the context which predicted the now aversive stimulus in the morning, divided by the total amount of freezing during both sessions at a

given time of day. Using this measure, a morning ratio of above 0.50 indicates that rats are responding appropriately: showing more fear of the context+morning combination that had been paired with auditory stimulus that was later paired with shock than the other context+morning combination; and an afternoon ratio below 0.50 indicates that rats are showing more fear to the context+afternoon combination that had been paired with the same auditory stimulus than to the other context+afternoon combination.

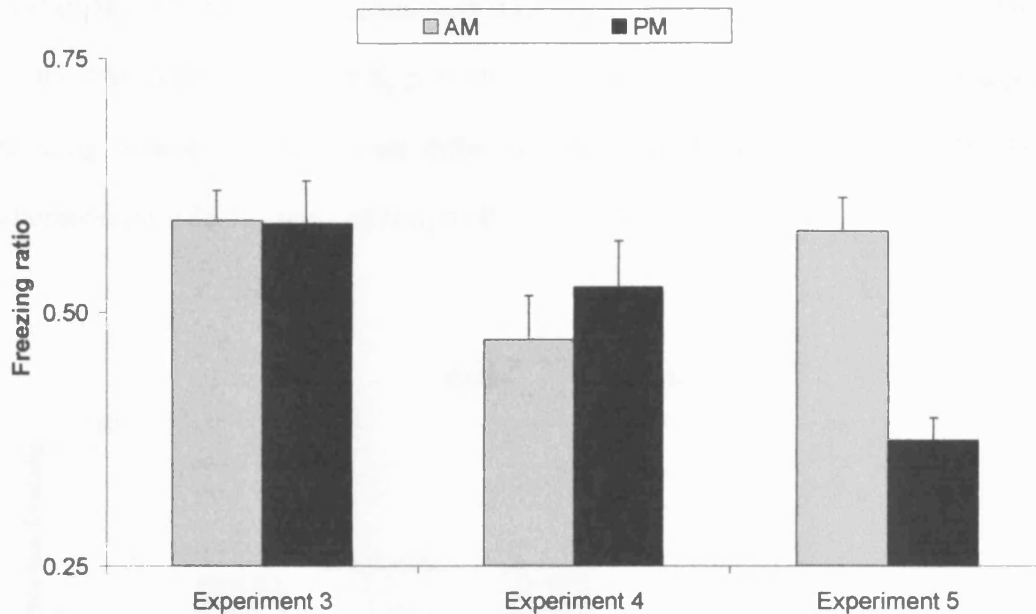
As previously stated, ANOVA and other parametric statistical analyses were used throughout the thesis. Parametric statistics (e.g., ANOVA) were used on the basis of the fact that the behavioural scores (e.g., freezing ratios) conformed to the conventional assumptions that underlie their use.

### **3.3. Results**

The results from the morning (AM) and afternoon (PM) tests for Experiments 3-5 are shown in Figure 12. Inspection of the left-hand pair of bars shows that the ratios for rats in Experiment 3 were the same in the morning and the afternoon. The fact that the ratios were both above 0.50 indicates that rats were consistently showing fear to the context associated with the revalued stimulus in the morning. That is, during the test a simple association was influencing their behaviour: namely the association resulting from the morning pairing of the context with the revalued stimulus. This description was supported by the fact that there was no difference between the morning and afternoon ratios ( $t_{(15)} = .049, p > .05$ ), and that both sets of ratios differed from chance (i.e., .50; AM :  $t_{(15)} = 3.240, p < .01$ , PM:  $t_{(15)} = 2.168, p < .05$ ). An analysis of the overall percentages of freezing in the AM ( $M = 38.13$ ) and PM ( $M = 30.48$ ) revealed a

significant difference between them ( $t_{(15)} = 2.743, p < .05$ ). This observation is consistent with the idea that extinction, during the AM session, influenced performance during the PM session: The AM sessions expose the rats to the contextual stimuli in the absence of the revalued auditory stimuli, causing a degree of extinction and resulting in a weaker context+auditory stimuli association in the PM sessions, which would logically result in reduced freezing levels. By contrast with the results of Experiment 3, the results from Experiment 4 (shown in the centre pair of bars in Figure 12) indicate that the scores in both morning and afternoon sessions are close to 0.50. This description of the results was supported by the fact that there was no difference between the morning and afternoon ratios ( $t_{(15)} = -.854, p > .05$ ), and that neither sets of ratios differed from 0.50 (AM:  $t_{(15)} = -.671, p > .05$ , PM:  $t_{(15)} = .566, p > .05$ ). An analysis of the overall percentages of freezing in the AM ( $M = 38.93$ ) and PM ( $M = 33.28$ ) revealed no significant difference between them ( $t_{(15)} = 1.125, p > .05$ ).

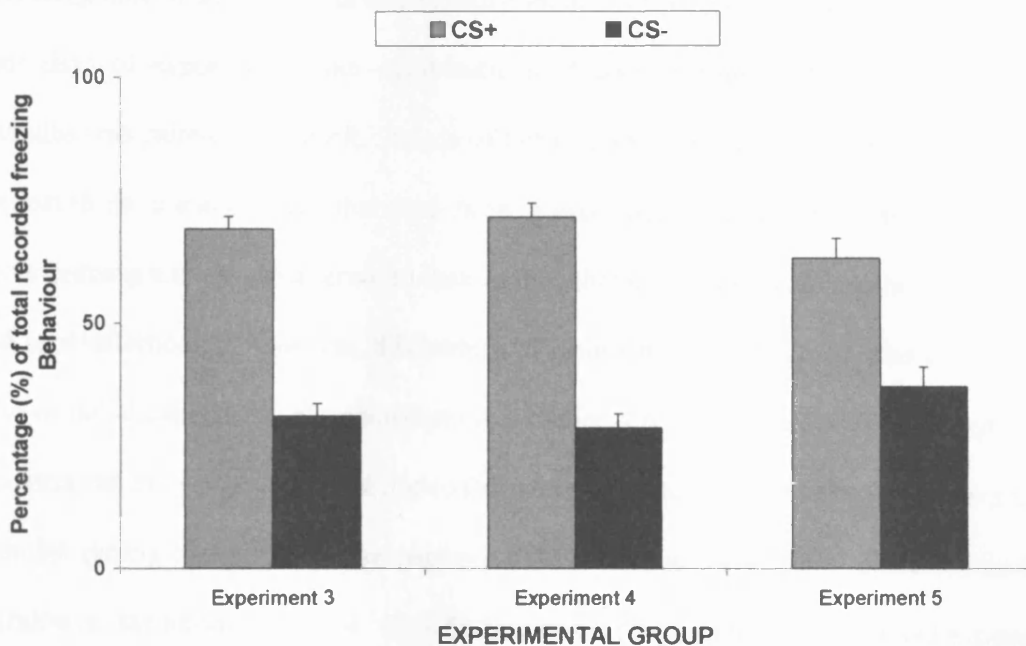




**Figure 12.** Experiments 3-5: Mean freezing ratios (+SEM) in Experiment 3 (1 day training procedure), Experiment 4 (2 day training procedure) and Experiment 5 (4 day training procedure).

Finally, inspection of the pattern of results in the right-hand pair of bars (Experiment 5) indicates that the time of day influenced which context provoked the most freezing. The fact that the ratio is above 0.50 in the morning signifies that the context+morning combination paired with the revalued auditory stimulus is eliciting more freezing than the other combination; similarly, the fact that the scores are below 0.50 in the afternoon indicates that the pattern of contextual freezing has reversed in the afternoon. Statistical analysis confirmed that AM and PM scores differed ( $t_{(15)} = 4.941, p < .05$ ) and that when the scores are combined in such a way as to maintain the direction of their differences from 0.50 the scores differ from chance (0.50;  $t_{(15)} = 4.485, p < .05$ ). This is also the case if the scores are analysed individually, with the mean score in the

AM (0.58) and PM (0.41) sessions both differing from chance (AM: 0.50;  $t_{(15)} = 2.535$ ,  $p < .05$ . PM: 0.50;  $t_{(15)} = -2.986$ ,  $p < .05$ ) Analysis of the overall percentage scores of freezing revealed no significant difference between the morning ( $M = 36.35$ ) and afternoon ( $M = 36.49$ ) sessions ( $t_{(31)} = -.042$ ,  $p > .05$ ).



**Figure 13.** Experiments 3-5: Mean percentages of observations freezing (+SEM) during presentations of the auditory stimulus paired with footshock (CS+) and the auditory stimulus that was not paired with footshock (CS-).

Figure 13 shows the results of the revaluation test in which the auditory stimuli were presented in the context in which aversive conditioning had taken place. The scores are pooled across the three presentations of each stimulus and indicate that freezing was, unsurprisingly, more evident during the stimulus paired with shock (CS+) than the other stimulus (CS-). Rats in each experiment showed this pattern of results (Experiment 3:  $t_{(15)}$

= 7.673,  $p < .005$ ; Experiment 4:  $t_{(15)} = 8.482$ ,  $p < .005$ ; Experiment 5:  $t_{(15)} = 3.381$ ,  $p < .005$ ).

### 3.4. Discussion

The results of Experiment 5 demonstrate that rats can form configural memories involving time of day, contexts and auditory stimuli. More specifically, rats first received four days of exposure to four combinations of context+time+stimulus, and then one stimulus was paired with shock. As a result of this training, rats showed more fear to the context+time combinations that had been paired with that auditory stimulus (e.g., spot+morning and check+afternoon) than to the other combinations (e.g., check+morning and spot+afternoon). However, this pattern of results was not observed after one or two days of initial exposure to the combinations. In fact, after a single day of initial exposure (Experiment 3) rats showed most fear to the context that was associated with the revalued stimulus during the morning, irrespective of whether this context was presented in the morning or the afternoon at test. This finding raises the possibility that massed exposure to the context+time+stimulus combinations in the morning session interfered with learning in the afternoon.

In Experiment 4 there level of fear to the contexts was the same (unlike in Experiment 3) and did not differ as a function of time of day (unlike in Experiment 5). Presented in isolation, the findings from Experiment 4 would suggest that no learning had taken place. However, taken together with the results of Experiments 3 and 5 this seems implausible. Instead it seems possible that the extra day of training has been sufficient to render the various binary associations (involving the contexts) equivalent, but insufficient

to result in appreciable configural learning involving what was presented where and when. The implications of this possibility for the role of the hippocampus in configural learning is discussed later in the chapter.

Although there is insufficient data to determine the exact mechanism(s) that mediate the outcomes of Experiments 3 and 4, the results of Experiment 5 suggest that rats can form configural memories involving episodic-like features, and provide a basis upon which to assess the role of the hippocampus in this form of learning. As such, the procedure used in Experiment 5 can be said to meet the aims of the thesis in part, as they provide a behavioural assay for the processing of configural memories for patterns of stimulation with episodic properties.

The procedure used in Experiment 5, in comparison to previous attempts to configural memories for what-where-when stimuli (discussed in Chapter 1), requires several days of training as opposed to minutes or hours, but has the distinct advantages of incorporating an effective revaluation method (shock) and not relying on spontaneous exploration or similar behavioural measurements, the outcomes of which risk being influenced by various motivational factors that are difficult to determine or recognise; the Experiment 5 procedure requires the subjects to take a more passive role, so this possibility is reduced.

It is also worth acknowledging that, due to the counterbalancing of the manner in which stimuli were presented, successful performance in the procedure requires the effective processing of configural memories, as the use of binary elemental associations alone (Figure 1) could not result in differentiation between patterns of stimuli based on

responses to any one or pair of stimulus elements. This property in particular was judged as potentially very useful in pursuing the aims of this thesis.

Specifically, the procedure used in Experiment 5 provide the opportunity, in Experiments 7-9, to investigate the effect of hippocampal inactivation on test performance (i.e., on the retrieval of configural information). In Experiment 6, however, the effect of excitotoxic lesions to the hippocampus made prior to behavioural training was examined.

### **3.5. Experiment 6: The role of the hippocampus in configural integration**

As discussed in Chapter 1, although many theorists have suggested that the hippocampus is involved in configural integration (Iordanova et al, 2008; O'Reilly & Rudy, 2001; Rudy & Sutherland, 1995), the evidence demonstrating that lesions to the hippocampus disrupt the formation of a reinforced configural discrimination is surprisingly sparse. Prompted by the finding that rewarded configural discriminations can be acquired by rats with hippocampal lesions, it has been suggested that the hippocampus might be especially important in forming and retrieving configural memories formed as the result of simple exposure to patterns of stimulations (e.g., O'Reilly & Rudy, 2001). Experiment 6 used the experimental design depicted in Figure 11, and used in Experiment 5, to examine the role of the hippocampus in configural integration that takes place during simple exposure to patterns of stimulation. There were two groups of rats that received different treatments prior to behavioural training and testing. One group received sham operations and the other group received excitotoxic lesions of the

hippocampus. The question of principal interest was whether the groups would differ in terms of their ability to show configural integration.

### **3.6. Method**

*Subjects.* 30 naïve adult male hooded Lister rats (*Rattus norvegicus*; supplied by Harlan Olac Ltd., UK) were used in Experiment 6, of which 3 were excluded from the experiment as a result of complications following surgery, resulting in a total of 27 subjects. The rats were approximately 10 months old (*ad libitum* weight range: 400-650 g) and were split into two groups: Rats in group Control received sham surgery ( $n = 10$ ) and those in group Hippocampal received bilateral lesions of the hippocampus ( $n = 17$  after the exclusion of 3 rats). Rats were maintained in the same way as Experiment 1.

*Surgery.* Before behavioural testing began, rats received either sham or hippocampal lesions. All rats were first placed in an induction chamber and anesthetized using an isoflurane-oxygen mix. When rats were observed to be sufficiently anaesthetised, the fur covering the area of the skull to be operated on was removed. Rats were then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). The skin was incised and the tissue covering the skull was retracted back and held in place with surgical clamps, thereby exposing the surface of the skull. For rats receiving sham surgery, this scalp incision was immediately sutured and the rats were then placed in an incubator at approximately 30 °C to facilitate recovery from anaesthesia. For rats receiving lesions, the bone covering the target regions of the cortex was removed and target regions were infused with ibotenic acid (Biosearch Technologies, San Rafael, CA; dissolved in phosphate-buffered saline [pH 7.4] to provide a solution with a concentration of 63 mM). The infusions were

administered with a 2  $\mu$ l Hamilton syringe, held in place and controlled by a microinjector (Kopf instruments, model 5000). There were a total of 28 infusions, 14 in each hemisphere. Each infusion occurred at a rate of 0.30  $\mu$ l/min. The precise location and infusion volumes used for complete bilateral hippocampal lesions are provided in Table 1.

AP (mm)	ML (mm)	DV (mm)	IBO ( $\mu$ l)
<b>-5.4</b>	-5.0	-6.1	0.08
		-5.3	0.08
		-4.5	0.09
	+5.0	-6.1	0.08
		-5.3	0.08
		-4.5	0.09
	-4.2	-3.9	0.10
	+4.2	-3.9	0.10
<b>-4.7</b>	-4.5	-6.5	0.05
	+4.5	-6.5	0.05
	-4.0	-7.2	0.10
		-3.5	0.05
	+4.0	-7.2	0.10
		-3.5	0.05
<b>-3.9</b>	-3.5	-2.7	0.10
	+3.5	-2.7	0.10
	-2.2	-3.0	0.10
		-1.8	0.10
	+2.2	-3.0	0.10
		-1.8	0.10
<b>-3.1</b>	-3.0	-2.7	0.10
	+3.0	-2.7	0.10
	-1.4	-3.0	0.10
		-2.1	0.10
	+1.4	-3.0	0.10
		-2.1	0.10
<b>-2.4</b>	-1.0	-3.0	0.05
	+1.0	-3.0	0.05

**Table 1.** The total number of infusions with associated coordinates and volumes of ibotenic acid administered. All coordinates are relative to bregma (bregma = 0.0). AP = Anterio-posterior; ML = Medio-lateral; DV = Dorso-ventral; IBO = Ibotenic acid (volume in microlitres,  $\mu$ l).

After infusion, the syringe was left in place for 1 min in order to allow the neurotoxin to diffuse out from the injection site and to minimise possible re-uptake



caused by the subsequent removal of the syringe from the cortex. Once all infusions were completed, the scalp tissue was replaced, sutured and cleaned, and rats were removed from stereotaxic frame and placed in an incubator at 30 °C to facilitate recovery from anaesthesia. When rats regained mobility they were returned to the holding room and placed individually in cages and allowed to recover fully. Soluble paracetamol was added to the rats' water bottles to aide recovery prior to and following surgery. Rats were returned to their home cage along with their cage mate when both showed normal levels of behaviour post-surgery. Rats were monitored for any signs of post-operative complications for 10 days post-surgery, and all rats were allowed at least 14 days of recovery time before undergoing behavioural testing.

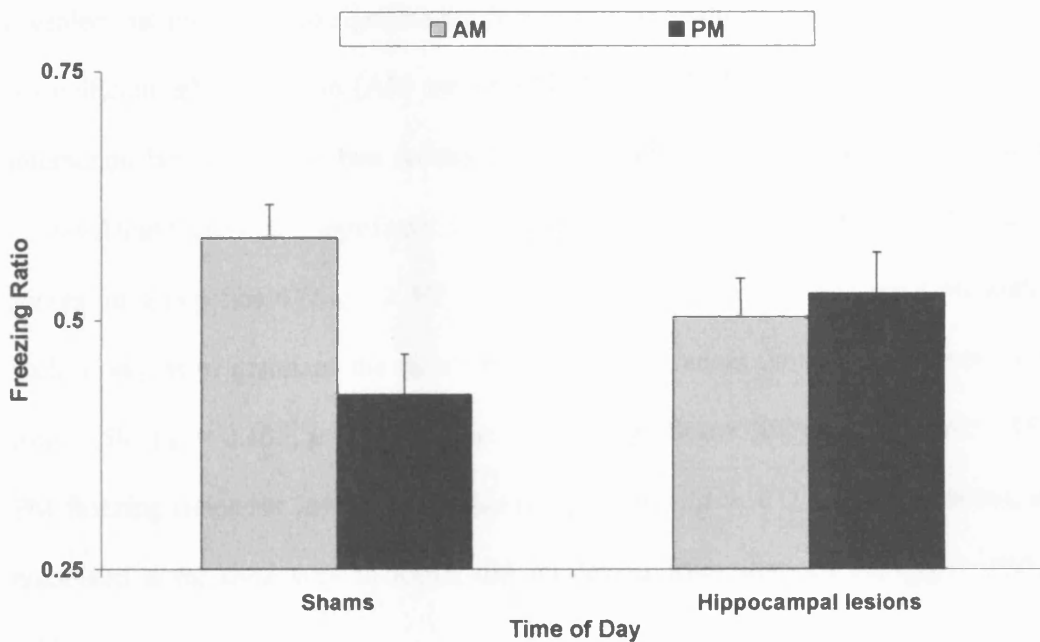
*Histology.* Following the completion of behavioural testing, rats received a lethal dose (approximately 1 ml) of sodium pentobarbitone (Euthatal) and were immediately transcardially perfused with a 0.9% saline solution followed by 10.0% formal-saline. The brains were then extracted and placed directly into 10.0% formal-saline and postfixed for 24 hours. The brains were then transferred to a 30.0% sucrose solution for approximately 48 hours, until the brains were observed to sink in the sucrose solution in order to cryo-protect the brains from the low temperatures encountered in the cryostat during sectioning. Brains were then removed from the solution and frozen in a cryostat at -20 °C and sliced coronally at 40 µm through the length of the hippocampus. Every fifth section was affixed to a glass slide, with 6 sections on every slide. Slides were then left to dry at room temperatures for a minimum of 24 hours before staining with cresyl violet. Slides were immersed in increasingly concentrations of alcohol which was then removed by immersion in xylene. Slides were then placed in cresyl violet to stain, before being fixed

in xylene and dehydrated again in alcohol of increasing concentration. A covering slide was then fixed in place and the slides were left in a fume cupboard to allow the xylene to evaporate.

*Apparatus and procedure.* The same chambers and procedures as described in Experiment 5 were employed in Experiment 6.

### 3.7. Results

#### *Behavioural Analysis*



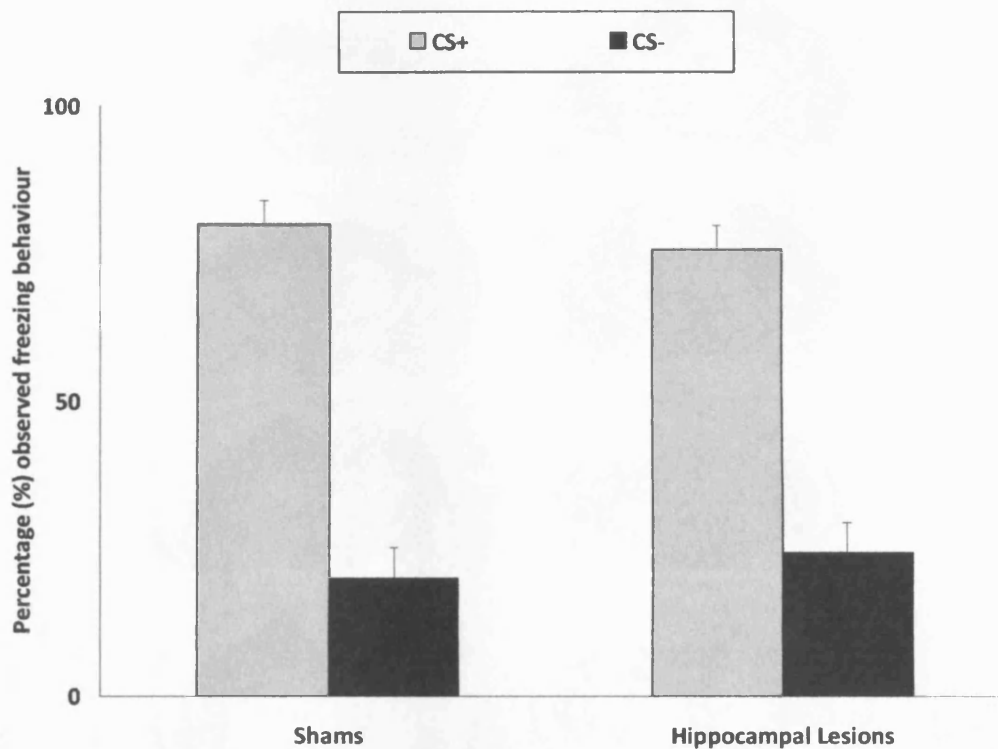
**Figure 14.** Experiment 6: Mean freezing ratios (+SEM) during the morning (AM) and afternoon (PM) tests for groups Sham and Hippocampal.

Figure 14 shows the mean freezing ratios for Experiment 6 for groups Sham and Hippocampal during the morning and afternoon test sessions. As previously stated, of the 20 rats that underwent hippocampal lesions, two did not recover from anaesthesia, and

one displayed no sign of hippocampal damage following histological analysis, resulting in a total of 17 rats in the hippocampal group. Inspection of the ratios for group Sham reveals the same pattern as in Experiment 5: The ratios were above 0.50 in the morning and below 0.50 in the afternoon. This pattern of results indicates that rats were exhibiting a configural integration effect, and replicated the pattern reported in Experiment 5. It is also evident from inspection of Figure 14 that the pattern of results in group Hippocampal is quite different from that of group Sham: The ratios for rats in group Hippocampal did not differ in the morning and afternoon, with both ratios being close to 0.50. ANOVA revealed that there was no significant effect of group (Sham versus Hippocampal;  $F < 1$ ), a significant effect of time (AM versus PM;  $F_{(1,25)} = 4.41, p < .05$ ), and a significant interaction between these two factors ( $F_{(1,25)} = 9.18, p < .05$ ). Pairwise comparisons revealed that there was a significant difference in the freezing ratios between AM and PM scores for group Sham ( $t_{(9)} = 2.490, p < .05$ ), and when these ratios were computed in such a way as to maintain the direction of their differences from chance, they differed from 0.50 ( $t_{(9)} = 2.467, p < .05$ ). There was no significant difference between AM and PM freezing ratios for hippocampal rats ( $t_{(16)} = -.974, p > .05$ ), and these ratios, when combined in the same way as above, did not deviate from chance (.50;  $t_{(16)} = -.955, p > .05$ ).

The overall percentages of freezing in the AM and PM sessions in group Sham (AM:  $M = 53.5$ ; PM:  $M = 43.9$ ) were somewhat higher than in group Hippocampal (AM:  $M = 32.6$ ; PM:  $M = 29.8$ ), and were slightly higher in the morning than in the afternoon. ANOVA revealed a significant effect of group ( $F_{(1,25)} = 4.976, p < .05$ ), an effect of time of day ( $F_{(1,25)} = 9.788, p < .05$ ) and a significant interaction between these factors ( $F_{(1,25)} =$

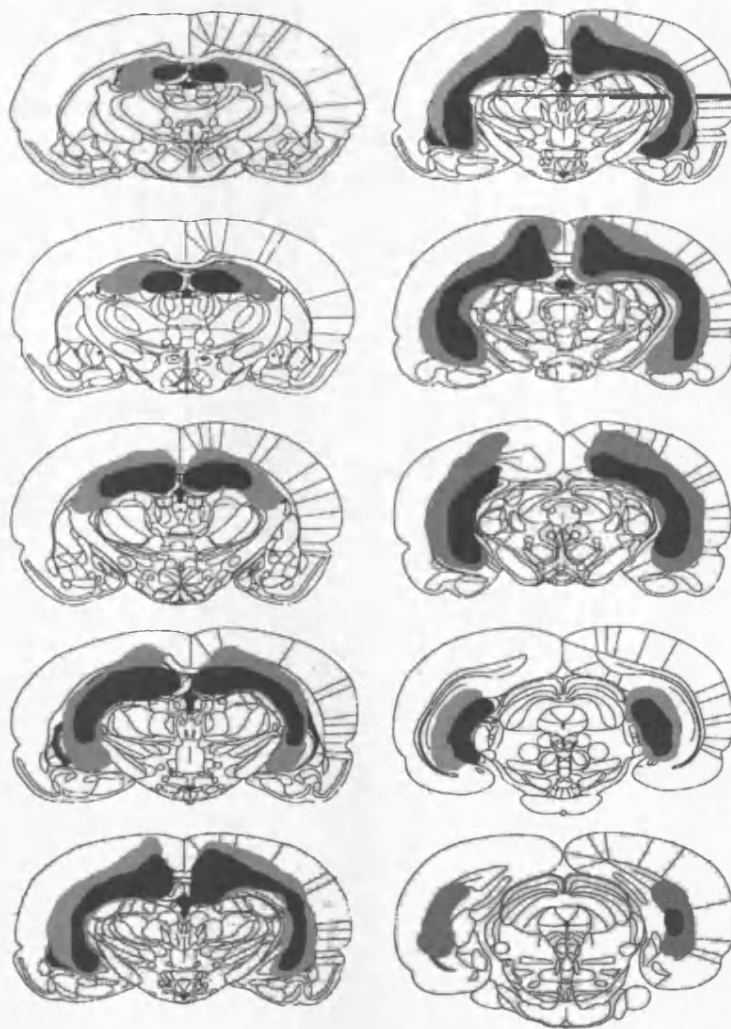
103.622,  $p < .005$ ). Pairwise comparisons revealed a significant difference between AM and PM scores for Sham rats ( $t_{(9)} = 3.082$ ,  $p < .05$ ), but not in hippocampal rats ( $t_{(16)} = 1.165$ ,  $p > .05$ ).



**Figure 15.** Experiment 6: Mean percentages of freezing behaviour (+ SEM) in groups Sham and Hippocampal to the auditory stimulus paired with shock (CS+) and the auditory stimulus that was not paired with shock (CS-).

Inspection of Figure 15 shows that rats in both groups Sham and Hippocampal showed more fear to the auditory stimulus that had been paired with shock (CS+) than the auditory stimulus that had not been paired with shock (CS-). ANOVA confirmed that there was a significant effect of stimulus ( $F_{(1, 25)} = 84.558$ ,  $p < .005$ ), but no effect of groups and no interaction between these factors ( $F_s < 1$ ).

#### *Histological analyses*



**Figure 16.** The maximum (dark grey) and minimum (black) extent of the lesion in Experiment 6. All sections are posterior to and occur at specific distances (in mm) from Bregma (top to bottom, then left to right: 2.12, 2.80, 3.30, 3.80, 4.30, 4.80, 5.30, 6.30, 6.80) according to the Paxinos and Watson (1998) stereotaxic atlas, from which diagrams were derived.

Figure 16 shows a series of coronal sections through the rat brain (adapted from Paxinos & Watson, 1998) with the maximum and minimum extent of cell loss for rats in group Hippocampal. Histological analysis revealed that there was a degree of sparing in the ventral area of the hippocampus (-6.80 mm from Bregma) in rats with the smallest

lesions, with the most extensive damage found in the dorsal regions of the hippocampus in all cases. All rats sustained damage to CA1 and CA2 regions, and to the dorsal subiculum. Sparing of areas of the dentate gyrus (granular, lacunosum and polymorph layers) in the left hemisphere (figure 3.8.3) that were close to the midline was present in ten of the rats. These rats were included in the experiment as the majority of the dentate gyrus and CA1 regions was missing in each case, and CA2 and CA3 lesions were effectively removed. As previously stated, analysis of one rat showed no visible damage to any areas of the hippocampus beyond that caused by insertion of the syringe, and as such this rat was excluded from the results. Two rats also showed extensive damage to the cortex and areas beyond the hippocampal formation in both dorsal and ventral lesions, and were therefore excluded from the hippocampal lesion group. Two rats did not recover from anaesthesia. The final number of rats in the hippocampal group was 17, with 10 in the sham group.

Although histological analysis revealed noticeable variation in the extent of hippocampal lesions, there was no evidence of significant variation in freezing behaviour between individuals in the hippocampal group that correlated with variations in the size of the lesions. Histological analysis also revealed consistent cortical damage in the areas situated between -4.30 and -5.30 mm from bregma. This was an unintended result of the extensive lesioning procedure used. It must be acknowledged, therefore, that the deficit demonstrated in lesioned rats in the current procedure may also be due, at least in part, to the effects of cortical damage. The role of cortical damage disrupting the formation of configural representations and associations (depicted in Figure 4; see also O'Reilly &

Rudy, 2001; Rudy & Sutherland, 1995) will be considered in more detail in the following sections.

### **3.8. Discussion**

The results from group Sham in Experiment 6 replicate those from Experiment 5 in showing that rats can form configural representations for patterns of stimulation involving contexts, times of day and auditory stimuli. There was no indication that rats with hippocampal lesions had formed (or could retrieve) such configural representations. There are a number of potential uninteresting explanations for the pattern of results in rats with hippocampal lesions, some of which can be discounted. One possibility is that they had formed configural representations during exposure to the patterns of stimulation, but that the revaluation procedure was ineffective. The results of the final test, in which the effectiveness of the aversive conditioning procedure was assessed, are inconsistent with this possibility. A more plausible possibility is that the deficit in configural learning is parasitic on a more basic deficit in learning about contexts or times of day (cf. Kim & Fanselow, 1992). There is nothing in the results of Experiment 6 that allow one to rule out these possibilities, and one of the aims of Chapter 4 is to assess its validity.

Another possibility which will be assessed in chapter 4 is that the deficit demonstrated by lesioned rats is actually caused by the incidental cortex damage that resulted from the lesioning procedure. Given the extent of the lesions and the fact that all lesions are administered prior to testing, there is no data from Experiment 6 that allows us to differentiate between a deficit caused by hippocampal or cortical lesions (cf. O'Reilly & Rudy, 2001; Rudy & Sutherland, 1989, 1995), or some combination of the two.

The possibility that cortical, rather than hippocampal, damage is responsible for the deficit observed in Experiment 6 is consistent with the view that the behavioural effect of interest emerges over several days; and that it has often been supposed that whereas the hippocampus is involved in the rapid learning of configurations (Kim & Fanselow, 1992; Gaffan, 1991; Good *et al*, 2007; O'Keefe & Nadel, 1978; Rudy & Sutherland, 1995) gradual learning is a feature of the cortex (O'Reilly & Rudy, 2001; Rudy & Sutherland, 1995). Although the rats with more extensive cortical damage were excluded from the results, the infusion procedure still resulted in less extensive but consistent damage to the cortex, and as such it is possible that the pattern of results is a result of this damage rather than damage to the hippocampus. The results presented in Chapter 4 will begin to address this concern by adopting an alternative method to manipulate hippocampal function that results in less cortical damage (i.e., temporary inactivation)

### **3.9. General Discussion**

The aims of the experiments described in Chapter 3 was to develop a behavioural assay to assess configural memory for patterns of sensory stimulation, to explore the boundary conditions of the assay, and to then assess the role of the hippocampus in configural memory. The results of Experiments 3-5 show that a configural integration effect is evident after four days of training, but not if training is massed and occurs over one or two days. Experiment 6 replicated the results from Experiment 5 and showed that the configural integration effect is abolished in rats who have received lesions of the hippocampus prior to behavioural training. This finding is consistent with view that the hippocampus supports configural memory under some circumstances (O'Reilly & Rudy,



2001). However, without contrasting the current assay with others involving the same content, but that do not require configural memory, this conclusion should be tempered with some caution. For example, rats with hippocampal lesions might be unable to learn about the contexts or times of day; such a deficit would almost inevitably influence performance in the procedures used in Experiment 6. Also, the results of Experiment 6 could not establish whether the involvement of the hippocampus (or cortex) is necessary during retrieval of configural memories. As previously stated, studies in both humans and nonhumans have described the hippocampus generally in terms of its role in the encoding of configural/episodic memories (Alvarado & Rudy, 1995; Bunsey & Eichenbaum, 1996; Morris, 2006; Nyberg, 1997; Wishaw & Tomie, 1991), but there is also evidence to suggest that it has a role in the retrieval process of the same memories process (Aggleton & Brown, 1999; Holt & Maren, 1999; O'Reilly & Rudy, 2001; Rudy & Sutherland, 1995). However, the nature of the lesioning procedure means that the hippocampus is absent during both encoding and retrieval stages of the experiment, so the effects of lesions on either process cannot be determined from the results provided in Experiment 6. These issues that will be addressed by the studies reported in Chapter 4. Before moving on to Chapter 4, however, some consideration should be given to why the behavioural procedures used in Experiments 5 and 6 were successful in generating a configural learning effect that involved time of day, when Experiments 2, 3 and 4 were not successful.

One obvious possibility is that the additional days of training in Experiment 5 and 6 allowed the animals to learn that both the context and the time of day were informative with respect to whether a tone or click would be delivered. That is, like many traditional

configural learning tasks the development of a configural association takes time. This could either reflect the fact that the links between the components of an "episode" (i.e., what, where and when) and a separate configural unit (see Figure 4) all form only slowly, or that any one of these components (e.g., when) requires many trials to become linked to the configural unit activated by the other components (what and where). One reason that learning involving morning and afternoon might be relatively slow is that, unlike the contexts and auditory cues, rats have experienced times of day on a regular basis before the experiment has started. It is well known that such exposure results in a retardation in that rate at which learning takes place - an effect known as latent inhibition (Lubow, 1973). Leaving this conjecture aside, the results of Experiment 6 provide a clear impetus for examining, in greater detail, the role of the hippocampus in the processing of patterns of stimulation. However, the results of Experiments 3-5 do place some constraints on how to proceed.

Taken together, the results of Experiments 3-5 suggest that investigation of the role of the hippocampus during encoding of the stimulus patterns (e.g., by temporary inactivation) might be technically difficult: the requirement to repeatedly inactivate the hippocampus over the course of the four days of training that are required to generate the effect of interest. However, there is still the possibility of examining the role of the hippocampus during testing using this technique. The final set of experiments have two aims: To assess the role of the hippocampus during the recollection of configural memory using the behavioural assay developed in Chapter 3; and to contrast the effect of hippocampal inactivation during the configural assay (developed in this chapter) with comparable assays based upon the same content but that do not require configural

processes. These assessments, coupled with a hippocampal inactivation technique, that presents a greatly reduced possibility of cortical disruption, will also help to address the concern that the deficit observed in Experiment 6 was a result of cortical damage rather than hippocampal damage. Once these aims have been achieved, it will be possible to offer a more definitive answer regarding the role of the hippocampus in mnemonic integration.



## **Chapter 4**

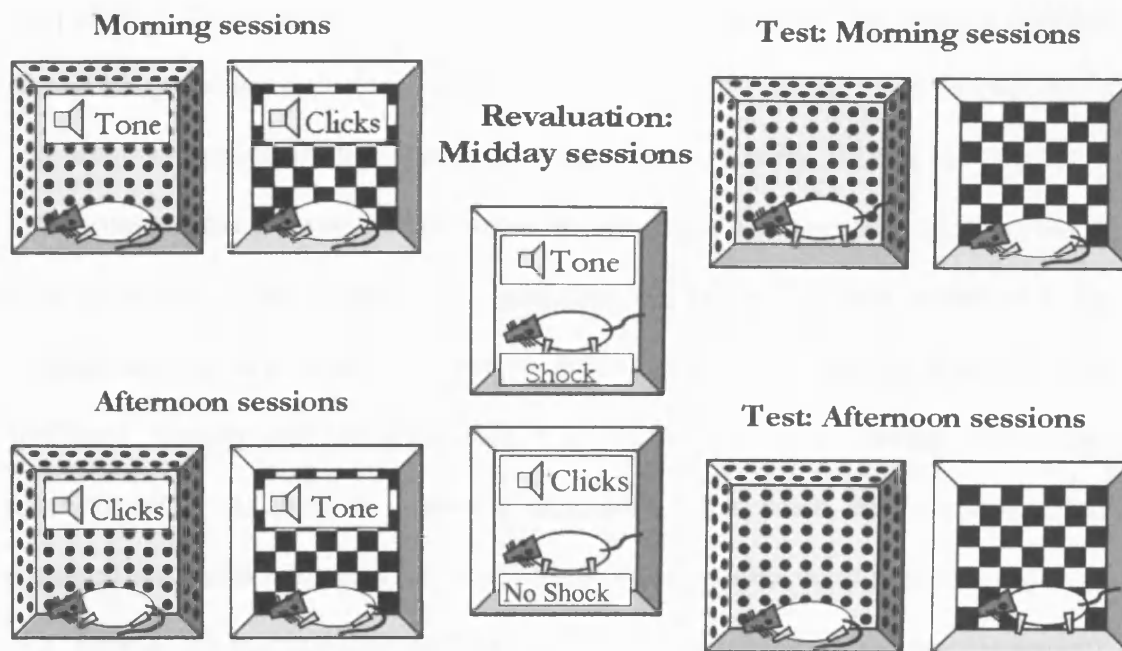
### **The role of the hippocampus in the retrieval of configural and elemental information**

*Summary.* The results from Chapter 3 demonstrate that rats acquire configural memories involving contexts, times of day, and auditory stimuli. This form of configural memory, based upon simple exposure to patterns of stimulation, was disrupted by excitotoxic lesions of the hippocampus. The three experiments reported in Chapter 4 investigated the role of the hippocampus during the retrieval of configural memories (Experiment 7) and the retrieval of those that could be based on simpler, binary associations (Experiments 8 and 9). The technique used to disrupt hippocampal function during retrieval was temporary inactivation induced by muscimol, which allowed the critical comparisons in each experiment to be conducted within subjects, and also greatly reduced cortical damage that resulted from the excitotoxic lesioning procedure.

#### **4.1. Experiment 7, 8 and 9: The role of the hippocampus in memory retrieval**

Experiment 7 used the same procedure as Experiment 6 where test performance requires the use of configural memory (see Figure 17) which is reproduced from Chapter 3 both to facilitate presentation and to contrast the procedure with those used in Experiments 8 and 9 that are presented on the succeeding pages. After the rats had received the exposure and revaluation procedure they received two tests days that were identical to the test day in Experiment 6. Briefly, in the morning and afternoon rats were placed in the two experimental contexts and freezing was assessed. On one test day, the hippocampus was inactivated by the administration of muscimol, a potent GABA agonist

that temporarily blocks synaptic transmission (Beaumont, Chilton, Yamamura. & Enna, 1978), immediately prior to the morning and afternoon tests. On the other test day, rats received administration of artificial cerebrospinal fluid (aCSF) immediately prior to both tests. Comparison of test performance on the two test days should allow to an assessment of the role of the hippocampus in the retrieval of configural memories. Two further experiments were conducted to examine the specificity of any effects of inactivating the hippocampus observed in Experiment 7. The same within-subject inactivation procedure was used, but the behavioural assays, were modified. The modifications were relatively minor, but theoretically important.

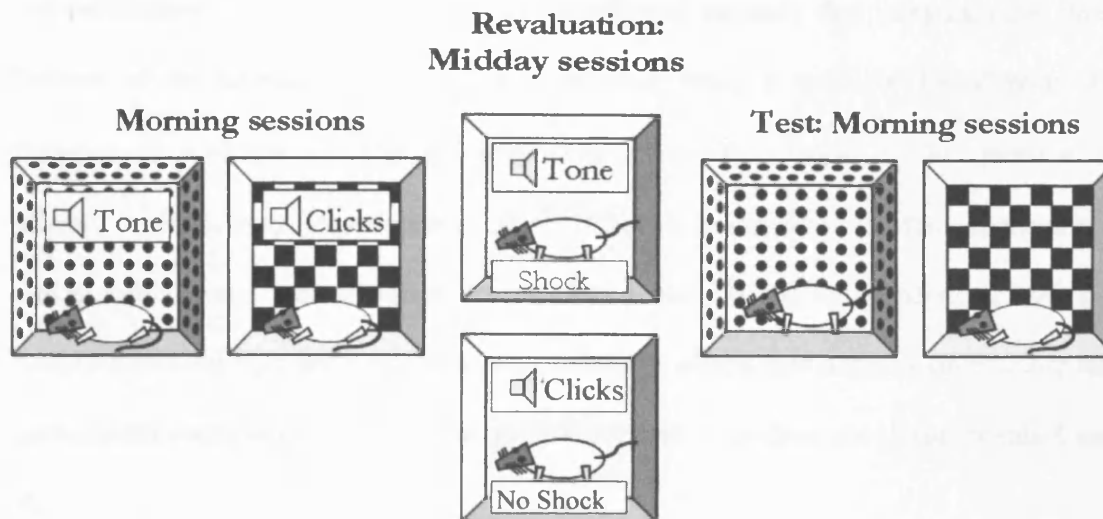


**Figure 17.** Example of the treatments given to rats in Experiment 7 (reproduced from Figure 11). In the morning sessions on Days 1-4 a tone was presented in the spotted context and a series of clicks in the checked context; whereas in the afternoon sessions on Days 1-4 the clicks were presented in the spotted context and the tone in the checked context. To assess if rats had formed configural memories of location and time of day in which the tone had been presented, fear was first established to the tone (but not the clicker) by pairing it with shock (in another context at midday on Days 5 and 6). Rats then received test configurations on Days 7 and 8 that should either result in the retrieval of the feared tone (i.e., morning with spotted context, and afternoon with checked context) or should not (i.e., the remaining combinations).

The design used in Experiments 8 is depicted in Figure 18, and is formally equivalent to the sensory preconditioning procedure described by Rescorla and Cunningham (1978) who used a flavour-aversion procedure. Rats were first placed in the two contexts (in the morning or afternoon) and received presentations of one auditory stimulus (e.g., tone) in one of the contexts (e.g., spot) and the other auditory stimulus

(e.g., click) in the second context (e.g., check). After this training, rats received the same revaluation procedure as in Experiment 6, where one of the auditory stimuli (e.g., tone) was paired with shock and the other (e.g., click) was not. Finally, the rats were placed in both contexts in the morning and freezing was assessed. It was anticipated that rats would show more fear to the context (e.g., spot) that had previously been paired with the revalued stimulus (e.g., tone). Although, such test performance could be mediated by a configural memory that integrates the three features of the training trials (e.g., spot+morning+tone) there is a simpler alternative: Rats could have formed direct elemental associations between the components of the patterns during the training stage (e.g., between the spot and tone) and between the tone and shock during the revaluations stage. The presence of a simple associative chain (spot→tone→shock) would be sufficient to provoke freezing behaviour (see Brogden, 1939; Rescorla & Cunningham, 1978).

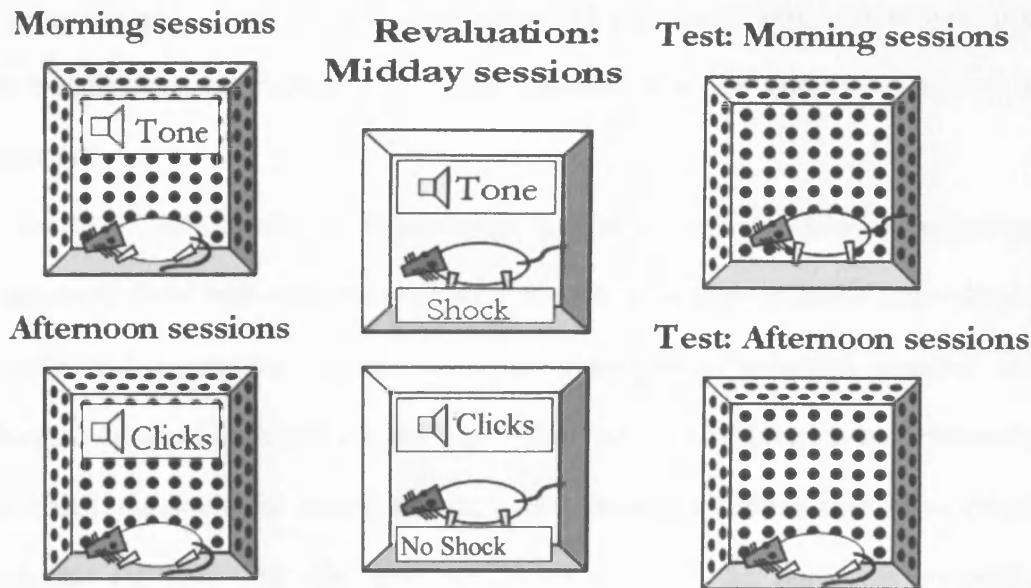




**Figure 18.** Example of the behavioural treatments given to rats in Experiment 8. On Days 1-4, a tone was presented in the spotted and a clicker in the checked context in the morning. Revaluation was identical to that described for Experiments 1 and 3: fear was established to the tone (but not the clicker) by pairing it with shock (in another context at midday on Days 5 and 6). Rats then received test configurations on Days 7 and 8 that should result in the retrieval of the feared tone in the appropriate context (i.e., in the spotted context, but not in the checked context). In order to counterbalance any effects of time of day, 50% of the rats were trained and tested in the morning only (as illustrated in Figure 18), whereas the remaining rats were trained and tested in the afternoon only. The stimulus-context pairings were also counterbalanced between subjects.

The design used in Experiment 9 is again similar to that employed in Experiment 7 and is depicted in Figure 19. The rats were placed in the same context (e.g., spot) in the morning and afternoon. In the morning, they received presentations of one stimulus (e.g., tone) and in the afternoon session they received another stimulus (e.g., click). All rats then received the revaluation procedure and then freezing was assessed in the morning and afternoon. It was anticipated that rats would show more fear at the time of day that had been paired with the revalued stimulus and, as in Experiments 7 and 8, although such

test performance could be mediated by a configural memory that integrates the three features of the training trials (e.g., spot+morning+tone) it could be based upon the presence of a simple associative chain (morning→tone→shock). The question of primary interest from Experiments 7-9, is whether inactivation of the hippocampus during testing has a general effect on retrieval at test, that is independent of how test performance could be mediated, or a more selective effect, that depends on whether test performance requires configural memory (Experiment 7) or does not (Experiments 8 and 9).



**Figure 19.** Example of the behavioural treatments given to rats in Experiment 9. On Days 1-4, in the morning a tone was presented in the spotted context whereas in the afternoon a clicker was presented in the spotted context. Revaluation was identical to that described for Experiments 1 and 3: fear was established to the tone (but not the clicker) by pairing it with shock (in another context at midday on Days 5 and 6). Rats then received test configurations on Days 7-10 that should result in the retrieval of the feared tone at the appropriate time of day (i.e., in the morning, but not in the afternoon). In order to counterbalance any effects of specific contexts, 50% of the rats were trained and tested in the spotted context only (as illustrated in Figure 18), whereas the remaining rats were trained and tested in the checked context only. The stimulus-time of day pairings were also counterbalanced between subjects

## 4.2. Method

*Subjects and apparatus.* 48 naïve adult male hooded Lister rats (*Rattus norvegicus*; supplied by Harlan Olac Ltd., UK) were used in Experiments 7, 8 and 9 ( $n_s = 16$ ). The *ad libitum* weight ranges of the rats were: 293-324 g (Experiment 7), 277-301 g (Experiment 8), and 288-329 g (Experiment 9). Rats were maintained in the same way as

rats in Experiment 1. As in previous experiments, all procedures were covered by Home Office Regulations (see Section 2.2). The apparatus was the same as that used in Experiment 5.

*Surgery.* All rats used in Experiments 7, 8 and 9, prior to behavioural testing, were surgically fitted with cannulae to directly administer substances to the hippocampus before the testing sessions. All rats were first placed in an induction chamber and anesthetized using an isoflurane-oxygen mix. When rats were observed to be sufficiently anaesthetised, the scalp was shaved and they were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). The skin and tissue covering the skull was surgically removed and retracted back, providing an exposed bone surface. Each rat was implanted with double 26-gauge guide cannulae (Blaney, UK), with the tip of guide cannulae aimed at the dorsal hippocampus by positioning it 2.0 mm below bregma through bilateral holes drilled 3.0 mm posterior to and 1.5 mm lateral to bregma. Guide cannulae were fixed in position with dental cement within a section of Perspex tubing (approx 5mm in length, 15 mm in diameter), four screws, and anchored in place with Super Glue. The guide cannulae contained dummy cannulae at all times outside of microinjection session, during which muscimol [1 µg/ml] (for a more detailed description of muscimol preparation and dosage, see Holt and Maren, 1999), or artificial cerebrospinal fluid (aCSF) was administered. Following surgery, the scalp was sutured around the cannulae housing, rats were removed from stereotaxic frame and placed in an incubator at 30 °C to facilitate recovery from anaesthesia. When rats were seen to regain mobility they were transferred back to the home cage and returned to the holding room. The rats were allowed to

recover until they re-established their pre-operative weights. During recovery and most days of experimentation the rats were handled and weighed daily.

*Histology.* Following behavioural testing, rats received a lethal dose of sodium pentobarbitone (Euthatal). The brains were then extracted (without fixing) and placed directly into 10.0% formal-saline and postfixed for 24 hours. Brains were then transferred to a 30.0% sucrose solution for approximately 48 hours, in order to act as a cryoprotectant before being removed from the solution and frozen in a cryostat at -20 °C. The brains were then sliced coronally at 40 µm through the hippocampus, mounted on slides, left to dry at room temperatures and then stained with cresyl violet using the same procedure as detailed in section 3.7.

*Infusion procedure.* For microinjection sessions, the dummy cannulae were replaced with a 33 gauge double microinjection cannula which projected a further 1mm ventral from the guide cannula. These microinjection cannula were connected to a 1ml glass syringe operated by an infusion pump (Harvard Apparatus, South Natick, MA). On control test days, rats received an infusion of 0.5 µl of aCSF immediately prior to the morning and afternoon test sessions. On inactivation test days, rats received an infusion of 0.5 µl of muscimol immediately prior to the morning and afternoon test sessions. The order in which these two treatments were given was counterbalanced.

*Behavioural procedures.* Experiment 7 was conducted over a period of 8 days. The procedure was identical to that described for Experiment 6 (the *what-where-when* procedure, see Figure 17) but without the final day of testing which assessed the efficacy of the revaluation procedure, as it was deemed that the revaluation method has been proven to be effective in subsequent tests. The stimuli and apparatus used in

Experiments 8 and 9 were identical to those used in Experiment 6. In the Experiment 8 (see Figure 18; the *what-where* procedure), during the first 4 days rats were placed in one context (e.g., spotted), where they received one auditory stimulus (e.g., tone), and a second context (e.g., checked), where they received the other auditory stimulus (e.g., clicks). This arrangement equates the number of pairings between a given context and auditory stimulus with the number of pairings between a given (context-time of day) configuration and auditory stimulus in the *what-where-when* procedure. Half of the rats received these sessions in the morning and the remainder received them in the afternoon. The sequence in which the rats received the two contexts was arranged in the same way as in Experiment 6. On Days 5 and 6, all rats received aversive conditioning trials in the undecorated chamber at midday, in the same way as Experiment 6. Finally, on Days 7 and 8 rats received test sessions in which their contextual fear in the two contexts was assessed. These test sessions were arranged in the same fashion as in Experiment 6 with the exception that rats only received a single pair of test sessions in the contexts at the same time as they had been presented during exposure. In Experiment 9 (see Figure 19; the *what-when* procedure), half of the rats received presentations of the tone in the morning and clicks in the afternoon during the first 4 days; for the remaining rats, this arrangement was reversed. This arrangement equates the number of pairings between a given time of day and a specific auditory stimulus with the number of pairings between a given (context-time of day) configuration and a specific auditory stimulus in the *what-where-when* procedure. For half of the rats in each of the sub-conditions created by the previous counterbalancing operation, exposure and test sessions occurred in a spotted chamber and for the rest they occurred in a checked chamber. The revaluation stage was

identical to that described in Experiment 6 and for Experiments 7 and 8: one of the auditory stimuli was paired with shock and the other was not. Rats in the *what-when* condition received testing over four days, Days 7-10. On each day, they received a single test either in the morning or the afternoon. For half of the rats the sequence of tests was morning (Day 7), afternoon (Day 8), afternoon (Day 9), morning (Day 10), and for the remainder the sequence of tests across the four days was afternoon, morning, morning, afternoon.

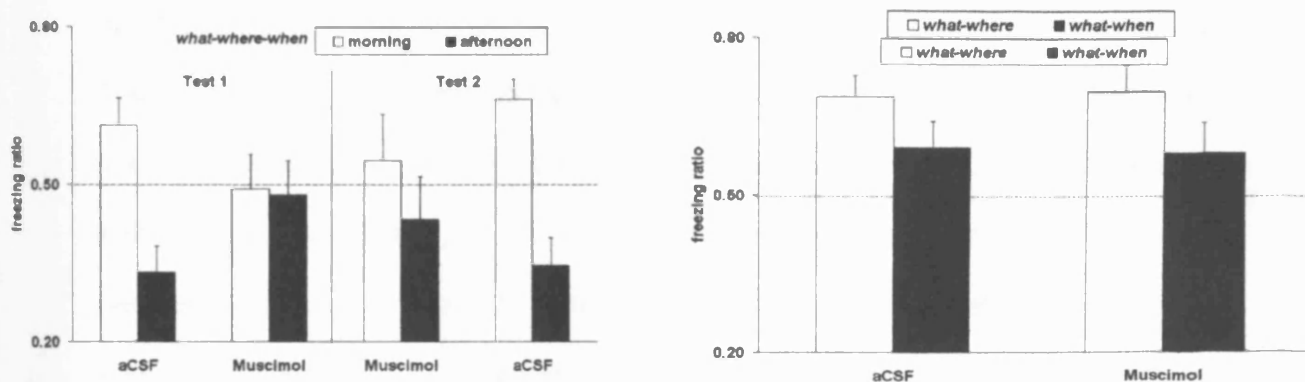
*Scoring and statistical analysis.* Experiment 7 used the same morning and afternoon ratio score as Experiment 6. For Experiment 8 the ratio took the following form: freezing in the context that had signaled the auditory stimulus that was later paired with shock, divided by freezing during both contexts. Using this measure, scores above 0.50 indicate that a rat is showing more freezing in the context that had signaled the now feared auditory stimulus (e.g., the tone in Figure 18) than the other context. Similarly, for Experiment 9 the ratio took the following form: Freezing at the time of day that was previously paired with the now aversive auditory stimulus, divided by freezing at both times of day. Again, scores above 0.50 indicate that a rat is showing greater freezing at the time of day at which the now feared auditory stimulus (e.g., the tone in Figure 19) had been presented during the first stage of training, than at the other time of day.

Scores were analysed using ANOVA and other parametric tests. In Experiment 7, the within-subject variables were aCSF/muscimol infusions and morning/afternoon freezing ratios. In Experiments 8 and 9 (analyzed in conjunction) the within-subject variable was aCSF/Muscimol infusions and the between-subject variable was contextual

(Experiment 8) and temporal (Experiment 9) procedure used. A variety of post-hoc t-tests was also used.

### 4.3. Results

#### *Behavioural analysis*



**Figure 20.** Experiments 7-9: Left panel – Mean contextual freezing ratios (+SEM) in the morning and afternoon for the aCSF and muscimol conditions in Experiment 7 on tests 1 and 2. Right panel – Mean contextual (Experiment 8) and temporal (Experiment 9) freezing ratios (+ SEM) for the aCSF and muscimol conditions.

*Experiment 7.* The mean freezing ratios for Experiment 7 are shown in the left panel of Figure 20. As in Experiment 6, scores above .50 in the morning and below .50 in the afternoon indicate that the test configurations are successfully retrieving the auditory stimuli that they had signaled during the first stage of training. Inspection of the left panel of Figure 20 reveals that when the rats received infusions of aCSF into the dorsal hippocampus (in either Test 1 or Test 2) they showed the same pattern of results as the sham rats from Experiment 6: the freezing ratios were above .50 in the morning and below .50 in the afternoon. However, there was no indication that the pattern of freezing



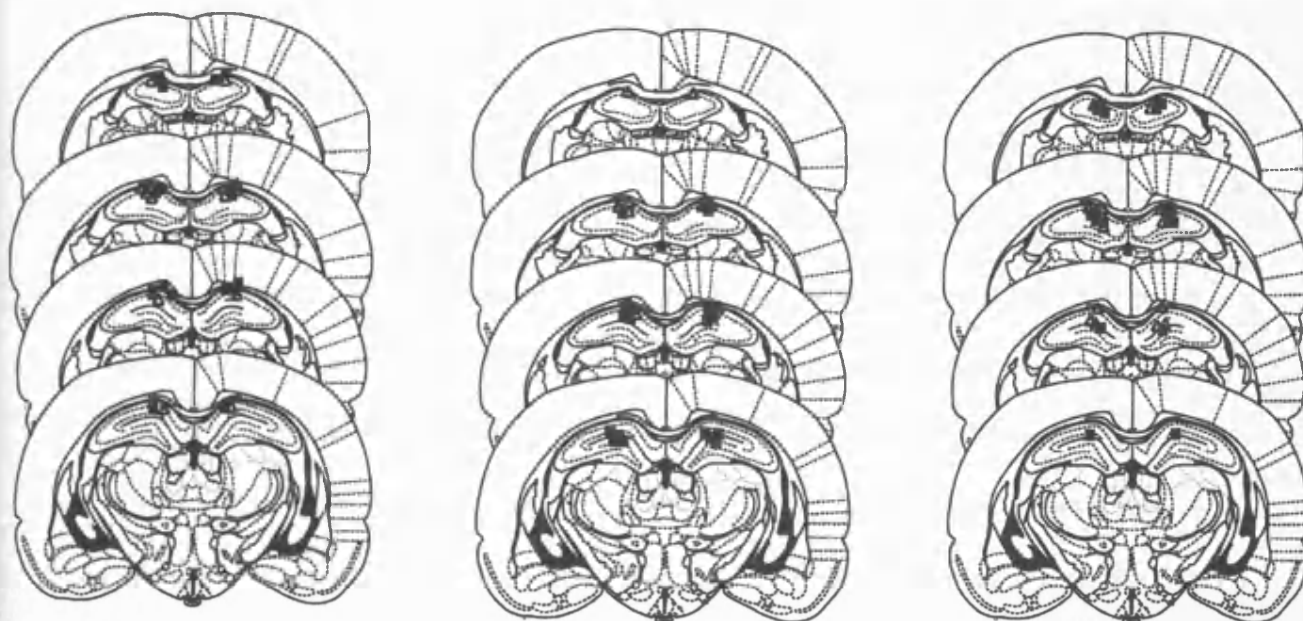
in the two contexts was modulated by the time of day when the same rats were given infusions of muscimol into the dorsal hippocampus; that is, the morning and afternoon ratios neither differed from one another nor from 0.50. A repeated measures ANOVA with inactivation treatment (aCSF versus muscimol) and time of day (morning versus afternoon) revealed that there was no effect of inactivation treatment,  $F_{(1,14)} < 1, p > .05$ , an effect for time of day,  $F_{(1,14)} = 28.15, p < .05$ , and a significant interaction between these factors,  $F_{(1,14)} = 8.53, p < .05$ . An analysis of simple main effects revealed that there was a difference in the freezing ratios between morning and afternoon for rats infused with aCSF,  $F_{(1,14)} = 34.81, p < .05$ ; and, when the morning and afternoon scores were averaged in the same way as in Experiment 6, these averaged ratios were different from 0.50,  $t_{(14)} = 5.90, p < .05$ . There was no difference between the morning and afternoon freezing ratios when the same rats were infused with muscimol,  $F_{1,14} = 1.07, p > .05$ ; and their ratios did not differ from chance,  $t_{(14)} = 1.03, p > .05$ .

*Freezing analyses.* An analysis of the overall contextual freezing levels when rats were given infusions into the dorsal hippocampus of aCSF versus muscimol revealed no differences between the two conditions (aCSF = 37%, Muscimol = 27%;  $t_{(14)} = 1.94, p > 0.05$ ).

*Experiments 8 and 9.* In Experiment 8, 1 rat did not recover from surgery and another rat irreparably damaged their guide cannulae during the post-surgery recovery period. Similarly, in Experiment 9, 2 rats irreparably damaged their guide cannulae during the post-surgery recovery period. All of these rats were excluded from the procedure, with the result that  $n = 14$  for Experiments 8 and 9 ( $n = 28$  for results collapsed across both experiments). The mean freezing ratios for Experiments 8 and 9 are

shown in the right panel of Figure 20. Inspection of this figure reveals that rats showed greater freezing in the context and at the time of day previously paired with the revalued auditory stimulus than in the context and at the time of day associated with other auditory stimulus; that is, the ratios were above .50. Inspection of this figure also reveals that this pattern of results was not influenced by whether the rats had received infusions of aCSF or muscimol prior to the test sessions. ANOVA with drug and Experiment (8 or 9) confirmed that there was no effect of inactivation treatment,  $F < 1$ ,  $p > .05$ , no effect of Experiment,  $F_{(1,26)} = 3.26$ ,  $p > .05$ , and no interaction between these factors,  $F < 1$ ,  $p > .05$ . The freezing ratios differed from chance both when rats were infused with aCSF,  $t_{(27)} = 4.58$ ,  $p < 0.05$ , and when the same rats were infused with muscimol,  $t_{(27)} = 3.60$ ,  $p < 0.05$ . Despite freezing ratios in Experiment 8 (context, *what-where* test) being noticeably higher than those in Experiment 9 (time of day, *what-when* test), analysis of the combined aCSF and muscimol scores for both experiments revealed that freezing ratios were significantly higher than chance (context:  $t_{(27)} = 6.206$ ,  $p < .05$ ; time of day:  $t_{(27)} = 2.314$ ,  $p < .05$ ). When the scores were averaged across aCSF and muscimol conditions, both the *what-where* and *what-when* test scores differed from chance (context:  $t_{(13)} = 9.277$ ,  $p < .05$ ; Time of day:  $t_{(13)} = 9.277$ ,  $p < .05$ )

*Freezing analyses.* Analyses of the overall contextual freezing levels revealed no differences between aCSF and Muscimol infusion conditions (context: aCSF = 38%, Muscimol = 27%;  $t_{(13)} = 1.64$ ,  $p > 0.05$ ; time of day: aCSF = 36%, Muscimol = 26%;  $t_{(13)} = 1.55$ ,  $p > 0.05$ ).



**Figure 21.** Coronal sections taken throughout the dorsoventral extent of the brain identifying the placement of guide cannulae into the dorsal hippocampus for Experiment 7 (left), Experiment 8 (centre), and Experiment 9 (right); the open triangles denote rats infused with muscimol during Test 1 and the closed squares denote rats infused with muscimol during Test 2. The sections are posterior to and at specific distances (in mm) from Bregma (top to bottom, 2.12, 2.80, 3.30, 3.80, 4.30) taken from the Paxinos and Watson (1996) stereotaxic atlas.

Figure 21 shows the location of the ends of the guide cannulae, and inspection of this figure confirms that they were within area CA1 of the dorsal hippocampus. There was a small amount of damage associated with the guide cannulae tract in the overlying cortex in all rats. There was no evidence that the infusions of either muscimol and aCSF had caused any long-lasting damage to the hippocampus. The data from one rat were excluded from the statistical analyses because the guide cannulae became displaced prior to testing thereby precluding either infusing muscimol or aCSF.

#### 4.4. General Discussion

Behavioural, computational and neural levels of analysis draw a fundamental distinction between two ways in which the components of a pattern of stimulation can be bound together in memory: Elemental accounts assume that exposure to a pattern allows links to form between its components (e.g., Hebb, 1949; Rescorla & Cunningham, 1978), whereas configural accounts hold that the components of a pattern of stimulation become bound together through becoming linked to a shared configural memory (e.g., Pearce, 1994; Sutherland & Rudy, 1989, 1995; see Figure 1). The experiments reported in Chapters 3 and 4 examined the role of the hippocampus in mediating these processes. In particular, they assessed the effects of disrupting the operation of these structures (using lesions in Chapter 3 and temporary inactivation in Chapter 4) on behavioural assays where successful performance could reflect the operation of elemental or configural processes (Experiments 8 and 9), and on a novel assay where successful performance requires the operation of configural processes (Experiments 6 and 7). The use of this novel combination of assays (involving the same content and procedures) allowed me to provide converging evidence that both elemental and configural processes are involved in pattern memory and that disrupting hippocampal function leaves rats reliant on elemental processes. Experiments 7 confirmed that rats form integrated configural memories in which the combination of a specific time of day and context indicates which auditory stimulus will be presented (cf. Experiments 5 and 6). The results of my assessment of the effect of inactivating the hippocampus during retrieval were clear-cut: Inactivating the hippocampus at test disrupted the performance in the task that required configural memory (Experiment 7), while leaving intact performance in the tasks that only required

elemental associations (Experiments 8 and 9). These results provide a firm basis for the suggestion that the hippocampus is necessary for the acquisition and/or retrieval of configural memories, but not elementary memories, involving the same content (the uncertainty regarding the acquisition or retrieval stems from the fact that experiments have yet to be performed where the hippocampus is deactivated during the acquisition phase of the experiment only, as the lesion studies described in Chapter 3 introduced hippocampal disruption to both acquisition and retrieval phases, so the effect of lesions cannot be specifically attributed to disruption of a specific stage). This precise pattern of dissociations is also evident when the hippocampus is lesioned prior to behavioural training and testing (see Iordanova, Burnett, Aggleton, Good & Honey, in press). In the discussion of Experiment 6, it was suggested that the reported deficit may have been due to the cortical damage caused by the extensive lesioning procedure (see Chapter 3). Although there remains insufficient data to completely rule out this possibility, the fact that Experiment 7 demonstrated the same deficit when rats underwent temporary hippocampal deactivation by infusion of muscimol (a procedure that greatly reduced the amount of cortical damage visible) strongly suggests that hippocampal disruption in both experiments is responsible for the recorded deficit.

This type of configural/elemental dissociation was foreshadowed by Rudy and Sutherland (1989; Rudy, Huff & Matus-Amat, 2004; see also, O'Keefe & Nadel, 1978) and is consistent with earlier evidence implicating the hippocampus in the process of forming and/or retrieving memories of complex patterns of contextual stimulation, but not simple stimuli (e.g., auditory cues; see Fanselow, 1990; Kim & Fanselow, 1992; Phillips & LeDoux, 1992). The results of Experiments 7-9 are informative because they suggest

that the results upon which these analyses were based (e.g., deficits in contextual fear conditioning, but not discrete cue conditioning) might well have reflected a disruption to configural integration rather than elemental integration (see also, Good & Honey, 1997). An equivalent argument applies to more recent results reported by Holt and Maren (1999). They showed that inactivation of the hippocampus disrupts the contextual dependence of latent inhibition during testing (see also, Honey & Good, 1993). The contextual dependence of latent inhibition might reflect either the operation of elementary associative processes (see Wagner, 1981; Honey, Iordanova & Good, 2009) or configural/hierarchical processes: where latent inhibition accrues to the configuration of the stimulus and the context in which it was presented. Our results are consistent with the suggestion that hippocampal inactivation disrupted the latter, configural process in the study reported by Holt and Maren (1999). The conclusion that the hippocampus has an important role in the effective binding of elements of complex patterns is in-keeping with similar observations reported in the literature (e.g. Eacott & Gaffan, 2005; Iordanova *et al.* 2008; O'Reilly & Rudy, 2001). One important feature of the two types of behavioural task used in Chapter 4 (i.e., the *what-where-when* versus *what-where* and *what-when* tasks) is that they are very similar. For example, all of the training patterns involve three components (an auditory stimulus presented in a context at a given time of day), the aversive conditioning stage is identical, and each of the test patterns involved a context presented at a given time of day. In fact, the first pair of test trials were identical in the *what-where-when* and *what-where* tasks (i.e., the two contexts were presented in the morning; compare Figures 17 and 18). Also, the tests in the *what-when* task matched the first tests in the *what-where-when* task (i.e., the same context was presented in the

morning and afternoon). Moreover, as we have noted in passing, the training stages equate the number of pairings between a given pattern (context and time of day) and auditory stimulus. Not only are the two types of behavioural task very similar at a procedural level, they also resulted in very similar levels of test performance (see Figure 20, left and right panels): the idea that the dissociations that we have observed between the tasks reflect different levels of baseline performance or indeed task difficulty, therefore, seems to be implausible.

The findings from Experiments 8 and 9 provide a relatively direct means of assessing the suggestion, from the discussion of the results of Experiments 3-5, that rats might have a particular problem in learning about time of day, relative to other stimuli. Now, although the ratios for the *what-when* task were numerically lower than those for the *what-where* task, these differences were not statistically significant and, what is more, both differed from chance. Admittedly, this is a null result and one where the levels of performance are rather low. However, keeping these caveats in mind, the results of Experiments 8 and 9 do begin to inform our understanding of why the configural learning effect takes several days to emerge (see Chapter 3). The results of these experiments are more consistent with the suggestions that configural learning occurs slowly than with the view that rats have a specific problem with learning about time of day.

In summary, the results of the Experiments detailed in this chapter clearly show that rats can acquire integrated long-term configural memories (involving what occurs where and when) and that this form of long-term memory, unlike elementary associative memory involving the same content, requires the integrity of the hippocampus. In

Chapter 5 I will draw out the general implications of the experimental work reported in this thesis particularly with regards to the literature available of episodic-like memory and previous studies in this area, and suggest further ways in which to pursue the issues of central interest.



## **Chapter 5**

### **Configural memory and the hippocampus**

*Summary.* In this chapter, a summary and evaluation of the new experimental results presented in this thesis is presented. The overarching aim of this thesis was to investigate the role of the rat hippocampus in processing and retrieving memories for patterns of stimulation and whether these memories are based on elemental or configural mnemonic structures, with the secondary aim of assessing memory for stimuli with episodic content. More specifically, the new experimental work assessed the idea that the rat hippocampus contributes to the formation and retrieval of configural, as contrasted with elemental, memories of what happened, where and when (cf. Aggleton & Brown, 1999; Tulving, 1978; 2002). The experimental work focussed on the case in which the information to be bound together involved stimulus dimensions that are aligned to the critical components of episodic memory (i.e., what: object or auditory stimulus; where: which location or experimental context; and when: relative recency or time of day). After a summary of the new results presented in this thesis, and an evaluation of their theoretical significance, there follows a discussion of a series of further experiments that would serve to further refine our understanding of hippocampal function.

#### **5.1. Summary of new results**

##### **5.1.1. Spontaneous exploration as a measure of mnemonic integration**

Experiment 1 attempted to replicate the results reported by Good *et al* (2007; see also, Kart-Teke *et al*, 2006) as a prelude to examining the nature of their results. Unfortunately, the pattern of results, although similar to that reported by Good *et al*

(2007), was not entirely consistent with their results. In particular, the rats were no more likely to explore an object that had undergone a spatiotemporal change than objects that had either undergone either a spatial or temporal change. Rather than directly examine the basis for this discrepancy, research was conducted using an alternative behavioural assessment of mnemonic integration. Experiment 2 employed a modified E-maze procedure that Eacott *et al.* (2005) had successfully used to examine ‘what-where-which’ memory. Briefly, this modification involved exposing rats to patterns of stimulation (e.g., morning+left arm+object X; morning+right arm+object Y; afternoon+left arm+object Y; and afternoon+right arm+object X). After this training, X was paired with food, and an assessment was made of whether rats explored the configurations associated with X (i.e., morning+left arm, and afternoon+right arm) significantly more than the remaining configurations. Unfortunately, there was no sign of any such effect, but there was also no sign that the revaluation procedure involving X had been successful.

An extensive consideration of the potential reasons for the failure of Experiments 1 and 2 is presented in the discussion sections of Chapter 2. No doubt, with further refinement of the procedures used in Experiments 1 and 2 it would prove possible to replicate and extend the findings upon which these experiments were based (i.e., Eacott *et al.*, 2005; Good *et al.*, 2007). Moreover, there is other evidence, some from studies using spontaneous exploration, that encourage the view that animals can form integrated memories for what happened, where and when (e.g., Babb & Crystal, 2006a; Clayton & Dickinson, 1998; Clayton *et al.*, 2001; Eacott & Norman, 2004; Ergorul & Eichenbaum, 2004; Good *et al.*, 2007). However, as has been discussed in Chapter 1, many of these results might be based upon either elementary or configural processes; and this thesis was

particularly concerned with the contrast between these two types of process in the context of integrating information with an obvious episodic content. For example, scrub jays may be exploring caches based on differences in *what-where* memory trace strength, rather than memory for specific *what-where-when* configurations (Clayton & Dickinson, 1998; Clayton *et al.*, 2001; Eichenbaum & Fortin, 2003), whereas the finding that rats preferentially explore an object that has undergone a spatiotemporal context change might be based on the combined influence of both *what-where* and *what-when* elemental memories (Ergorul & Eichenbaum, 2004; Good *et al.*, 2007).

Having spent a considerable amount of time using spontaneous exploration to study mnemonic integration in rats (see Chapter 2) it is perhaps worth making it clear that the failure of this enterprise to generate useful assays should not be taken to undermine the use of these types of procedures more generally. It is worth noting, however, that the use of explicit reinforcement often produced more robust behavioural effects. For example, sensory preconditioning procedures often produce behavioural effects that are marked (e.g., Rescorla & Cunningham, 1978). So, why is it that effects that are based on spontaneous exploration are, or appear to be, less marked? For example, even though Eacott and Norman (2004) reported evidence showing *what-where-which* memory in a configural task, the behavioural effect itself was relatively small. What factors might limit the size of effects produced in experiments that solely use spontaneous exploration as a measure? One observation is that in such procedures, the experimenter relinquishes some control of how the animal interacts with the critical aspects of the stimulation. For example, the rats might spend a good deal of time approaching an (asymmetric) object from one angle, and not experience its other feature, which will thereby remain

unfamiliar. If these elements are encountered during the test, then they will generate approach to an object that is operationally familiar. Also, at a more theoretical level, spontaneous exploration tasks rely on exposure to a stimulus or pattern of stimulation resulting in habituation. There have been many attempts to explain habituation and few have considered the possibility that habituation occurs to configurations of stimuli (for a recent review, see Honey *et al.*, 2010). Indeed, there is evidence to show that simple habituation might be multiply determined: under these circumstances, perhaps the apparent simplicity of the procedures might belie the fact that the target behaviour (e.g., approach and contact) is itself under the control of a range of theoretical and neural processes (Honey, Watt, & Good, 1998).

Leaving to one side the discussion related to Experiments 1 and 2, it is now appropriate to consider the use that was made, in subsequent chapters, of a different procedure to investigate mnemonic integration. This procedure was based on the type of sensory preconditioning procedure mentioned, in passing, above.

### **5.1.2. Configural memory in a sensory preconditioning procedure**

The design of Experiments 3-5 was based on that used in Experiment 2. They again used times of day as *when*, but used the visual contexts as *where*, and auditory stimuli as *what*. Rats were exposed to four configurations. For example, morning+spotted context+tone, morning+checked context+click, afternoon+spotted context+click, and afternoon+checked context+tone. In Experiments 3-5, the duration of training was increased from 1 day to 2 days, and finally to 4 days, respectively. After this period of exposure, rats then received pairings of, for example, the tone with shock and click with

no shock and their tendency to show freezing to the remaining components of the configurations was assessed (i.e., morning+spotted context, morning+checked context, afternoon+spotted context, and afternoon+checked context). Importantly, in Experiment 5 (after 4 days of initial training), rats showed more fear to the configurations that had been paired with the tone (in the current example, morning+spotted context and afternoon+checked context) than the remaining configurations. The fact that 4 days of training was required to generate the configural learning effect was taken to reflect the fact that after 1 or 2 days might have been insufficient for the rats to encode the time of day at which the other stimuli were occurring (see Chapter 3 for further discussion of this issue).

Experiment 5 demonstrated that rats show significant differences in freezing behaviour when presented with a combination of *where-when* stimuli that was previously presented with an audio '*what*' stimulus which was subsequently revalued with shock. This result is important, as it demonstrates that rats are capable of forming integrated memories for different configurations of *what-where-when*. Although evidence consistent with possibility has been presented before, Experiment 5 uses a testing procedure which rules out the possibility that the test performance of rats is based upon simple elemental associations: these are equated in Experiment 5 and so could not provide a basis for the test results (see Figure 4). The procedure pioneered by Eacott and Norman (2004) is another example of a test where elemental associations or links cannot explain successful test performance, but Experiment 5 incorporates a '*when*' element (time of day) making the results more consistent with the traditional definition of episodic-like memory (e.g., Clayton & Dickinson, 1998). The procedure developed in Experiment 5 therefore

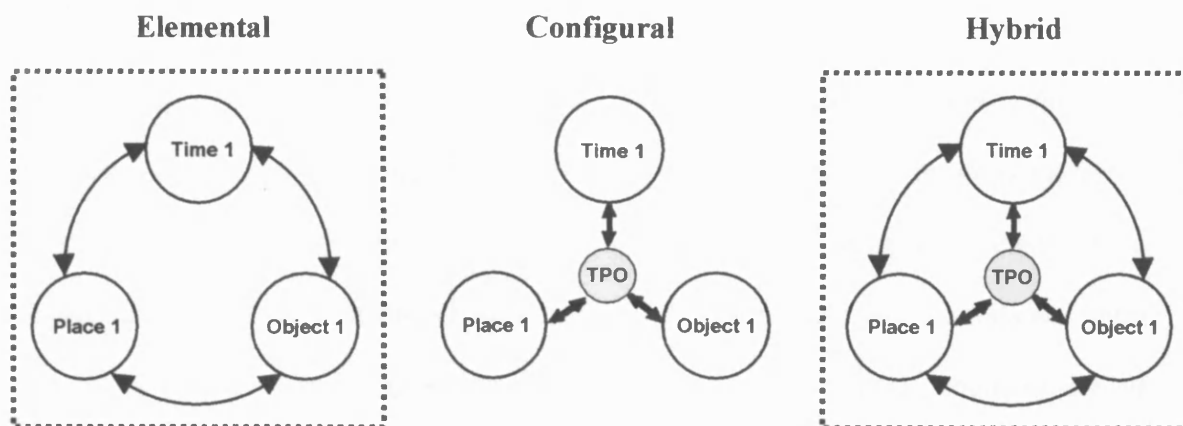
allowed subsequent investigation of the role of the hippocampus in the formation and retrieval of such configural memories, and contrasting such memories with other, elemental memories based upon the same content.

### **5.1.3. Assessing the contribution of the hippocampus to configural memory**

Experiments 6-9 examined the role of the hippocampus in mediating configural memory. In particular, these experiments assessed the effects of disrupting the function of the hippocampus using lesions (Experiment 6) and temporary inactivation (Experiments 7-9) on behavioural assays where successful performance could reflect the operation of either elemental processes (Experiments 8 and 9) or required configural processes (Experiments 6 and 7). The use of this novel combination of assays (involving the same content and procedures) allowed the provision of converging evidence that both elemental and configural processes are involved in pattern memory and that disrupting hippocampal function leaves rats reliant on elemental processes. Experiment 6 demonstrated that hippocampal lesions disrupt configural memory using a novel behavioural procedure (i.e., that of Experiment 5). Experiment 7 demonstrated that hippocampal inactivation during test disrupted successful performance in the configural memory procedure used in Experiments 5 and 6. Experiments 8 and 9 showed that hippocampal inactivation did not disrupt more standard sensory preconditioning effects where performance could be mediated by elementary, associative chains.

## **5.2. Theoretical implications of experimental results**

Behavioural, computational and neural levels of analysis draw a fundamental distinction between two ways in which the components of a pattern of stimulation can be bound together in memory: Elemental accounts assume that exposure to a pattern allows links to form between its components (see Figure 20; e.g., Hebb, 1949; Rescorla & Cunningham, 1978), whereas configural accounts hold that the components of a pattern of stimulation become bound together through links to a shared configural memory (e.g., Pearce, 1994; Sutherland & Rudy, 1989, 1995). After establishing that rats could form configural memories for patterns of stimulation with episodic content, Chapters 3 and 4 examined the role of the hippocampus in mediating these processes. The research focussed specifically on the effects of disrupting the operation of these structures (using lesions in Chapter 3 and temporary inactivation in Chapter 4) on behavioural assays where successful performance could reflect the operation of elemental or configural processes (Experiments 8 and 9), and on a novel assay where successful performance requires the operation of configural processes (Experiments 6 and 7). As previously stated, the use of this novel combination of assays (involving the same content and procedures) allowed converging evidence to be provided for the proposition that both elemental and configural processes are involved in pattern memory and that disrupting hippocampal function leaves rats reliant on elemental processes. This theoretical analysis is presented characterized in Figure 22.



**Figure 22.** Alternative patterns of connections (denoted by arrows) created within elemental (left), configural (centre) and hybrid (right) associative networks after exposure to a pattern consisting of neutral episodic-like stimuli (Time 1, Place 1, Object 1; note: TPO is a configural unit activated by a specific pattern of stimulation). The right-hand, hybrid model is assumed to underlie performance in control rats, and the left-hand, elemental model is assumed to underlie the residual ability of rats with hippocampal damage (or inactivation) to form/retrieve memories of patterns of stimulation.

If we accept that ordinarily rats are hybrid animals, using both elemental and configural processes to combine information about what has happened where and when, whereas hippocampal animals are forced to rely on elementary associations there is at least one important theoretical question that remains: Why is the hippocampus required for configural memory in the behavioural assays discussed in Chapters 3 and 4, but not elementary associations involving the same content? One possible answer to this question is that the hippocampus is required to co-ordinate, or to otherwise maintain, the pattern of activation provoked by a test pattern and thereby allow the corresponding configural memory to become activated (cf. Olton *et al*, 1979). Without a co-ordination/maintenance process like that postulated, ensuring that the neural correlates



role for the hippocampus in maintaining the relevant pattern of activation might be limited until the more gradual cortical system can encode the pattern as part of long-term memory. This possibility is supported by the fact that hippocampal lesions do not affect at least simple contextual conditioning if these lesions are made once a sufficiently long period has elapsed after training (Kim & Fanselow, 1992). Rudy and Sutherland (1995) propose a related pattern-enhancing role for the hippocampus in their model of hippocampal function.

One feature of this analysis is that it does not assume that the formation and retrieval of all configural memories will be equally reliant on the hippocampus: the involvement of the hippocampus should be restricted to cases where the information to be combined comes from disparate sources (either spatially or temporally). Indeed the analysis might well provide grounds for supposing that the binding of certain kinds of (episodic) information will be particularly dependent upon the hippocampus (see Aggleton & Brown, 1999; Day *et al*, 2003; Eichenbaum *et al*, 1994; Ergorul & Eichenbaum, 2004; Good *et al*, 2007; Morris, 2006). This idea is consistent with the view that there are other regions of the brain (e.g., the perirhinal cortex) that might also be involved in representing configurations of certain classes of stimulation (e.g., the combination of visual features; e.g., Bussey, Saksida & Murray, 2002; Eacott & Gaffan, 2005; Iordanova *et al*, 2009).

Another feature of the analysis of hippocampal function that is described above is that it predicts that other forms of memory involving the same content (e.g., context, time of day and auditory stimulus) need not require the involvement of the hippocampus. For example, provided it is the case that simple links have formed between the components of

a pattern of stimulation, then presenting one of its components should be sufficient to allow propagation of activity to other components of the pattern (cf. Hebb, 1949; Rudy *et al.*, 2004); such associative chains could not, of course, support configural memory. Of course, the results of Experiments 8 and 9 provide direct support for this suggestion, as hippocampal inactivation does not have any discernable effect on recall of what-where and what-when information. These findings are themselves not without interest: previous studies suggest that the hippocampus is involved in spatial memory (Ergorul & Eichenbaum, 2004; O'Keefe, 1991); but more pertinent is the fact that hippocampal lesions disrupt context fear conditioning (e.g., Kim & Fanselow, 1992). In Experiment 8, in order to show selective fear in one of the test contexts, they must have learnt the location/context in which the revalued auditory stimulus was presented. Clearly, hippocampal lesions do not influence the process of associating a context with an auditory stimulus in Experiment 8, and this places further constraints on the role of the hippocampus in learning about contextual cues.

The theoretical analysis presented above and summarized in Figure 22 can also provide one account for extant evidence suggesting that non-humans animals might possess the rudiments of episodic memory. For example, the results of the scrub jay experiments performed by Clayton and Dickinson (1998; see also Clayton *et al.*, 2001b) could be based upon the scrub jays using configural memories for the location+interval+food stored. Alternatively, as suggested in Chapter 1, they could use a sequence of elemental associations, interval→food stored→location (Clayton *et al.*, 2001b).

Finally, the procedures used in Experiments 5, 6, 7 and 9 demonstrate that time of

day can be used as a cue by rats. Although the manner in which time of day is encoded in these experiments remains unknown, Experiment 9 reveals that successful recall of time of day is not based on the within-day order of sessions (the AM and PM tests were conducted across days). However, there was some evidence that time of day might be less salient than other cues (e.g., the contexts). In Experiment 3, there was evidence that rats had acquired a context-stimulus association, before they had acquired a time-of-day-stimulus association.

### **5.3. Some future directions**

There are a variety of issues that remain unresolved in the context of the empirical work presented in this thesis, and the theoretical interpretation of the patterns of dissociations that have been observed (especially in Chapter 4). For example, although the configural assay was well matched to the assays where test performance could be supported by elemental associations, there remain some differences. The training procedure for the configural (*what-where-when*) task involved twice the number of training patterns as the training procedure for the *what-where* and *what-when* tasks (compare Figure 16 with Figures 17 and 18). This difference between the tasks might have contributed, in some way, to the patterns of dissociations that were observed in Chapter 4. In a series of follow-up experiments, Iordanova, Burnett, Good and Honey (in preparation) doubled the number of training patterns in the *what-where* task (Experiment 8): by adding afternoon sessions in which the patterns presented in the morning were repeated; and in the *what-when* tasks (Experiment 9): by adding a second session in the morning and afternoon in which the same auditory stimuli were presented, but in a

second context. These modifications to the procedures in the *what-where* and *what-when* tasks equate the number of training patterns with the *what-where-when* task. We found that lesions made prior to behavioural training were again without effect on tasks where test performance required only elemental processing (i.e., the *what-where* and *what-when* tasks). Therefore, the number of training trials does not appear to be the critical factor in determining whether or not lesioning the hippocampus prior to behavioural training influences test performance (cf. Iordanova *et al*, 2009). Clearly, it would be worthwhile confirming that this pattern of results is also observed when hippocampal function is temporarily disrupted during testing (cf. Experiments 7-9).

The inactivation studies in Chapter 4 reveal that the hippocampus has a role in retrieval of configural memory. It would be of interest to examine whether the hippocampus has a role in the encoding of configural memories; certainly the theoretical analysis described in Section 5.2 would predict that inactivating the hippocampus during training should also disrupt the formation of configural memories. Unfortunately, the use of temporary inactivation procedures is counter-indicated by the results of Experiments 3-5 - where 4 days of training proved to be necessary in order to observe the effect of interest. It might still be possible, however, to temporarily inactivate the hippocampus on two of the training days and still be sensitive to observing a disruption of configural learning. Of course, a null result under such conditions would not be readily interpretable. An alternative approach would be to examine whether or not temporary inactivation of the hippocampus during the revaluation stage is effective. There is no requirement that the configural memories involving the auditory stimuli are re-activated during revaluation in order for the test effect to be observed: for example, the various

context+time of day configurations would remain able to activate the auditory stimuli with which they were paired. Accordingly, inactivating the hippocampus during revaluation might be without effect.

Another question involves whether the effects observed in Chapter 4 are restricted to the stimuli with the episodic-like properties, or might the effects also be observed when other stimuli with different properties are used. It is well established that exposure to flavour compounds produce robust sensory preconditioning effects (e.g., Rescorla & Cunningham, 1978) and that such effects are not influenced by lesions of the hippocampus made prior to behavioural training (Ward-Robinson *et al*, 2001). The latter sensory preconditioning procedures are formally equivalent to the *what-where* and *what-when* assays used in Chapter 4, which were not influenced by hippocampal inactivation at test. It would be interesting to examine whether an assay that was formally equivalent to the configural what-where-when procedure developed here, would be sensitive to the effects of hippocampal lesions and inactivation. As indicated in Section 5.2, it seems possible that the role of the hippocampus in configural memory might be restricted to certain types of stimulus materials; namely, phasic stimuli that require short-term maintenance or co-ordination.

Finally, the test procedures used in Experiments 6-9 occurred relatively shortly after training had taken place. It has been argued that the role of the hippocampus in some forms of memory might be time dependent: with lesions placed shortly after training having a greater impact on later memory than lesions placed some time later (e.g., Kim & Fanselow, 1992). If the behavioural effects observed in Experiments 6-9 proved to be relatively robust (lasting for many weeks) then it would be interesting to

examine whether temporary inactivation of the hippocampus at test still produces the pattern of dissociations observed in Chapter 4. Certainly, the theoretical analysis presented in Section 5.2 predicts, other things being equal, temporary inactivation should disrupt the retrieval of configural memories.

#### **5.4. Summary statement**

The results of primary theoretical significance from this thesis are those pertaining to the dissociation between different ways in which patterns of stimulation can be represented. The finding that at least some forms of configural integration are reliant on the hippocampus, whereas other forms of elementary integration are not, is entirely consistent with some psychobiological models of memory that rely on a hybrid of elemental and configural processes (see right-hand panels of Figure 20; e.g., Sutherland & Rudy, 1989, 1995). The same results are inconsistent with the more parsimonious accounts that rely on either elemental (see left-hand panels of Figure 20; e.g., Rescorla & Wagner, 1972) or configural processes (see centre panels of Figure 20; e.g., Pearce, 1994). However, I have argued that the dissociation between elemental and configural memory might be a secondary consequence of a disruption to the short-term maintenance of stimulus traces (cf. Olton *et al*, 1979) that has a greater impact when there is an obvious requirement to co-ordinate or maintain disparate stimulus traces than when there is no such requirement.



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