The role of the hippocampus in forming integrated memories for patterns of stimulation

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This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

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Contents

Summary

The overarching aim of this thesis was to gain a better understanding of the involvement of the rodent hippocampus in configural learning and memory. To do so, I developed novel behavioural procedures to assess (i) configural integration of where and when reinforcers are delivered during conventional conditioning procedures, and (ii) configural processes involving standard stimuli during sensory preconditioning procedures. Firstly, it was important to establish that rats with hippocampal lesions are able to learn about where or when a reinforcer is presented (Experiments 1-2). I then developed appetitive and aversive conditioning procedures that enable the formation of configural memories involving what happened where and when to be studied (Experiments 3-4), and assessed the performance of rats with hippocampal lesions in these procedures (Experiments 5-7). These experiments revealed that rats with hippocampal lesions are not impaired at acquiring configural memories for patterns of stimulation requiring the integration of contextual and temporal cues. In order to further investigate the role of the hippocampus in configural learning and memory novel sensory preconditioning procedures were developed using more standard stimuli (Experiment 8). In this case, hippocampal lesions abolished a sensory preconditioning effect that was based on mediated configural learning (Experiment 9). The findings presented in this thesis suggest that the hippocampus is not involved in the acquisition of configural memories generally, or in the integration of the components of episodic-like memory. However, the results add to evidence suggesting that the hippocampus does play a general role in retrieval-mediated learning about configurations.

Publications

Chapter 3 of this thesis contains the research presented in the following publication:

Dumigan, N. M., Lin, T. E., Good, M., & Honey, R. C. (2015). Configural integration of temporal and contextual information in rats: Automated measurement in appetitive and aversive preparations. *Learning & Behavior, 43,* 179-187.

The results from Experiment 9 are included in the following publication:

Lin, T. E., Dumigan, N. M., Good, M., & Honey, R. C. (2016). Novel sensory preconditioning procedures identify a specific role for the hippocampus in pattern completion. *Neurobiology of Learning & Memory*, *130*, 142-148.

Chapter 1: General Introduction

1.1. Introduction

The main aim of this thesis was to investigate the role of the rodent hippocampus in forming integrated memories for patterns of stimulation. This overarching aim required the development of novel behavioural procedures that allowed assessments of whether or not rats could learn about where or when a motivationally significant event would occur (Chapter 2) and whether or not rats could learn about configural discriminations involving these elements (Chapter 3). It also required the development of novel procedures to assess the content of the memories for more prosaic patterns of stimulation (e.g. a tone with a light; Chapter 5). Chapter 1 begins with a brief overview of models from the field of associative learning theory that are relevant to this enterprise. I then consider the possibility that the hippocampus might play a specific role in certain classes of stimulation; namely, stimulation that might be regarded as having episodic content (what, where and when); or that it plays a selective role in learning about retrieved configural memories that play a role in some forms of sensory preconditioning.

1.2. Associative Learning

The capacity to learn and form memories has clear adaptive significance. For example, learning the route to a water source, or remembering that eating a particular food makes you ill, is clearly beneficial to health. Exactly how animals learn about and represent their environments is of fundamental interest in its own right, but also because it provides a means of studying associative processes of learning “untrammeled by other complexities” (p.11, Mackintosh, 1994). Moreover, the study of these processes in rodents enables the precise manipulation of their experiences and for these experiences to be understood at both the computational level and in terms of the brain systems involved. The dominant

theories that have attempted to describe and explain learning and memory in animals involve the idea that connections or links form between internal representations of stimuli in the world (see, for example, Hall, 1991). Theories of associative learning posit that stimuli are represented in the brain through particular patterns of activity and when two stimuli are experienced together their representations become linked. This will allow activity in one of the stimulus representations to engender activity in the other. An obvious example of the operation of this process is Pavlovian conditioning in rats. For instance, when a conditioned stimulus (CS; e.g., a tone) is presented to rats and paired with an unconditioned stimulus (US; e.g., food) rats come to show a conditioned response (CR; approaching the site of food delivery) upon presentation of the tone (Pavlov & Anrep, 1927).

The precise nature of the associative structures involved in learning is a contentious issue. For many years, the presumed associative structure was between the processes activated by the CS and those motor programs directly responsible for the generation of the response (e.g., Hull, 1943). The formation of such stimulus-response connections was thought to depend on close temporal contiguity between the processes activated by the stimulus and the processes activated by the response. Although stimulus-response associations are still considered to play a role in learning and behaviour, over the years, focus has shifted away from stimulus-response associations to consideration of the role of stimulus-stimulus associations. This change in emphasis reflects the existence of reliable demonstrations of learning in animals that occurs in the absence of a reinforcer, such that there is no immediately expressed overt response, and no obvious way for stimulus-response associations to mediate learning. The most obvious instance of this form of learning is sensory preconditioning.

Sensory preconditioning refers to the observation that after two neutral stimuli (e.g., a tone and a light) have been paired, establishing a conditioned response to the light is

reflected in performance to the tone (e.g., Brogden, 1939; Rescorla & Cunningham, 1978). Observations of this type suggest that an integrated memory of the tone and light has been formed that can mediate the generalisation of the conditioned response between them. The nature of this integrated memory and how its activation mediates generalisation remains a matter of considerable debate and interest. There are two principle types of representation that have been implicated in sensory preconditioning: configural and elemental (see Figure 1). These accounts of sensory preconditioning have important counterparts in models of Pavlovian conditioning itself. These general models of conditioning will be briefly described before specific accounts of sensory preconditioning are presented in more detail.

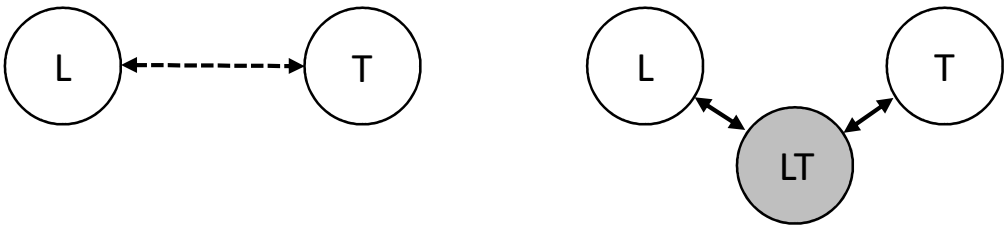

Figure 1. Elemental (left-hand side) and configural (right-hand side) associative structures that could provide the basis for demonstrations that two stimuli, L (e.g., light) and T (e.g., tone) co-occur (e.g., in sensory preconditioning). Dashed arrows represent "elemental" associations directly linking the two stimuli. Solid arrows represent associations between each of these stimuli and a separate configural representation, LT, which represents the compound pattern (e.g., light+tone).

One of the most influential elemental theories was proposed by Rescorla and Wagner (1972; Wagner and Rescorla, 1972). The Rescorla-Wagner model attempts to explain the conditions under which classical conditioning occurs and is expressed within an elemental framework that has been adopted by other influential theories (e.g., Pearce & Hall, 1980; Wagner, 1981). Elemental theories share the assumption that each element in a stimulus pattern (e.g., a light and a tone) forms an associative link with the reinforcer with which it is paired and that responding in the presence of the stimulus pattern is determined by the sum of the associative strengths of the constituent elemental links. It is worth noting

that in addition to these elementary associations it has also been recognised that elementary within-compound associations (i.e., between the light and tone) also contribute to performance (see Durlach & Rescorla, 1980).

While the range of phenomena that elemental accounts can address is impressive, there are behavioural phenomena for which elemental accounts provide a less satisfactory explanation. There is abundant evidence that animals can solve discriminations that would be impossible if animals could only rely on elemental associations. For example, in a negative patterning discrimination the presentation of a compound stimulus (e.g. tone + light) results in one outcome (e.g. no food) whereas the presentation of either component stimulus alone results in a different outcome (e.g. food; see Figure 2). According to elemental models, such as the Rescorla-Wagner (1972) model, the presentation of the compound should cause summation of the associative strengths of the individual components and result in an increased conditioned response to the compound compared to the individual components. However, animals are able to learn to withold responding to the compound and increase responding to the individual components (e.g., Woodbury, 1943; Grand & Honey, 2008).

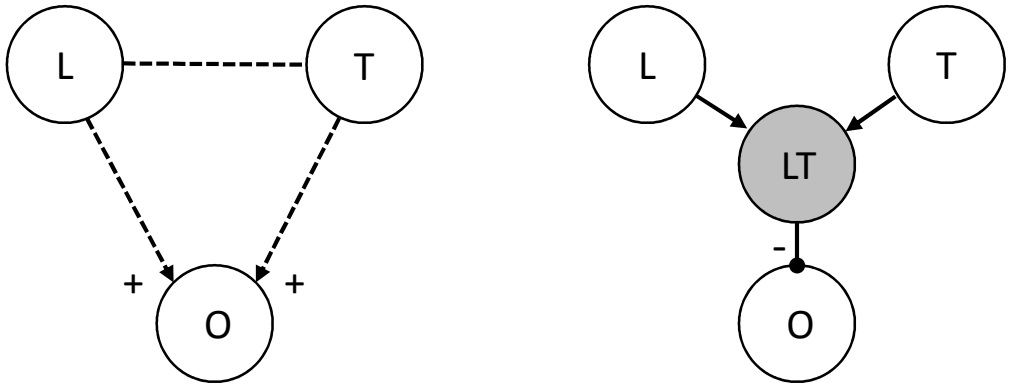

Figure 2. Associative structures thought to underlie negative patterning. Dashed arrows represent (excitatory) "elemental" associations directly linking individual stimuli, L (e.g., light) and T (e.g., tone), with a particular outcome, O (e.g., food). Solid arrows represent associations between each of these stimuli and a separate configural representation, LT, which represents the compound pattern (e.g., light+tone). This configural representation (LT) is linked to the same outcome (O), but via an inhibitory association.

The basic fact that animals can learn negative patterning discriminations can be explained by configural theories. For example, the configural models developed by Pearce (1987, 1994) propose that every pattern of stimulation recruits a separate configural representation or unit that enters into association with the outcome that it precedes (see Figure 2). In the case of negative patterning, while the configural units separately activated by the light (L) and tone (T) are associated with food, the configural unit activated by their combination (LT) develops an inhibitory link with food or, in principle, could be linked to a representation of no food.

The view that Pavlovian conditioning could be construed as an elemental or configural learning process, with both types of model being subject to modifications that enable them to explain phenomena that appeared to favour one or the other (e.g., Wagner, 2003), is complemented by other models that have supposed that both elemental and configural processes contribute (see Sutherland & Rudy, 1989; Kehoe, 1988; see also, Honey, Iordanova & Good, 2014). These models are of direct relevance to understanding the role of the hippocampus in learning and memory, and will be considered in the sections relating to hippocampal function (Section 1.5.).

1.3. Associative Structures in Sensory Preconditioning

As I have already noted, there are many instances of learning where it is not straightforward to assign an elemental or configural associative structure. For example, while a configural explanation for negative patterning might seem to be the most natural, elementary analyses have been developed in which a hypothetical unique element is produced by the juxtaposition of two stimuli (e.g., a light and a tone; Rescorla, 1972). This unique cue will acquire inhibitory properties on trials on which the compound (LT) is followed by no food during a negative patterning procedure. The issues that arise in the context of sensory preconditioning are similar: There are several potential mechanisms that could underlie this

simple behavioural phenomenon. As will become clear, there is no simple way to establish which mechanism is at play, and another aim of this thesis is to develop procedures to enable elucidation of the mechanism(s) in operation.

A typical sensory preconditioning procedure consists of three phases: exposure, conditioning and test. During the exposure stage, rats might receive two audio-visual compounds (AX and BY), a conditioning procedure is then used to establish a conditioned response to X and not Y, and at test responding during A and B is assessed. The fact that A provokes more responding than B is taken to reflect the formation of memories for the exposed compounds (Brogden, 1939; Rescorla & Cunningham, 1978). How these memories develop and the ways in which they mediate sensory preconditioning are not completely understood.

One simple explanation for sensory preconditioning is based on an elemental associative chain (e.g., Jones, Esber, McDannald, Gruber, Hernandez, Mirenzi & Schoenbaum, 2012; see also, Wimmer & Shohamy, 2012). During the exposure stage links form between the components of the compound (e.g., an A-X link) and conditioning results in a link between X and the unconditioned stimulus (US; e.g., shock). According to this explanation, test performance reflects the operation of the resulting A-X-shock chain. It is important to note that this analysis only requires that the animal is capable of representing the relationships that have actually been presented (Dickinson, 1980). However, it has been argued that sensory preconditioning might instead rely on a process of retrieval-mediated conditioning, in which associations are formed between the representations of stimuli that have been associatively evoked rather than directly activated by their corresponding stimuli (e.g., Hall, 1996; Ward-Robinson & Hall, 1996). According to this analysis, when X is presented during the conditioning stage it will evoke a representation of A, through the

elemental A-X link, and the representation of A will become directly linked to the US (e.g., footshock; see also, Lin, Dumigan, Dwyer, Good, & Honey, 2013).

The two elemental accounts outlined above have counterparts that rely on the idea that configural memories (e.g., of AX) form during the exposure stage. According to one such account, after the exposure stage conditioning with X results in an association between X and the US, and when A is presented at test it evokes the configural memory of AX, which in turn activates X and thereby the memory of the US (see Pearce, 2002). In an analogous fashion to the elemental associative chain, each stage of training results in learning involving stimuli that were physically presented: AX during exposure, X-shock during conditioning, and when A is presented at test it activates a memory of AX which activates one of X and thereby shock. An alternative analysis relies on a process of mediated learning in which the presentation of X during conditioning directly evokes the configural memory of AX, which becomes linked to the US. According to this account, test performance depends on the capacity of the test stimulus (e.g., A) to activate the memory of AX (e.g., Rescorla & Durlach, 1981).

These types of processes outlined above, where the elements of a compound get linked together, are assumed to be general: they are assumed to provide a basis for forming integrated memories for any types of information. Recent studies of sensory preconditioning have confirmed that configural processes provide one basis for forming an integrated memory for the components of episodic memory: what happened, where and when (Iordanova, Good & Honey, 2008; Iordanova, Burnett, Aggleton, Good & Honey, 2009; Iordanova, Burnett, Good & Honey, 2011a; Iordanova, Good & Honey, 2011b). In these studies, rats received presentations of a tone in a spotted context and a clicker in a checked context in the morning and presentations of the tone in the checked context and clicker in the spotted context in the afternoon (see upper panel of Figure 3). Presentations of the tone

were then paired with shock in a third context (an undecorated test chamber) at midday while those of the clicker were not. On the next day, the levels of freezing were assessed in both contexts in the morning and afternoon. Rats showed more freezing in the context+time of day configurations in which the tone had been presented: the spotted context in the morning and the checked context in the afternoon. These results implicate configural processes in this specific case of sensory preconditioning because the tone had been presented in both contexts and at both times of day, and as such, knowledge of where the auditory stimuli had been presented must be based on some form of configural information.

Iordanova *et al*. (2009, 2011ab) went on to demonstrate that the hippocampus was critical for this form of configural memory. Thus, lesions of the hippocampus made before preexposure to the four patterns resulted in rats showing equivalent levels in fear in each of the test configurations (Iordanova *et al*., 2009, 2011a). Moreover, if synaptic transmission in the hippocampus is temporarily disrupted by the infusion of muscimol during fear conditioning with the auditory stimuli (Iordanova *et al*., 2011b) or during the test (Iordanova *et al.,* 2011a) then rats show equivalent levels in fear in each of the test configurations. Finally, while infusion of AP5 into the hippocampus during fear conditioning with the auditory stimuli disrupted test performance, such infusions during the test itself did not (Iordanova *et al.,* 2011b). Infusions of AP5 disrupt NMDA-dependent synaptic plasticity. Notably, none of these manipulations had any effect on test performance that could be supported by elementary (*what-where* or *what-when*) associations (see centre and lower panels of Figure 3). Taken together these results suggest that the hippocampus is involved in mediated learning involving configural representations (Iordanova *et al*., 2011b) and in the expression of such learning at test (Iordanova *et al*., 2011a).

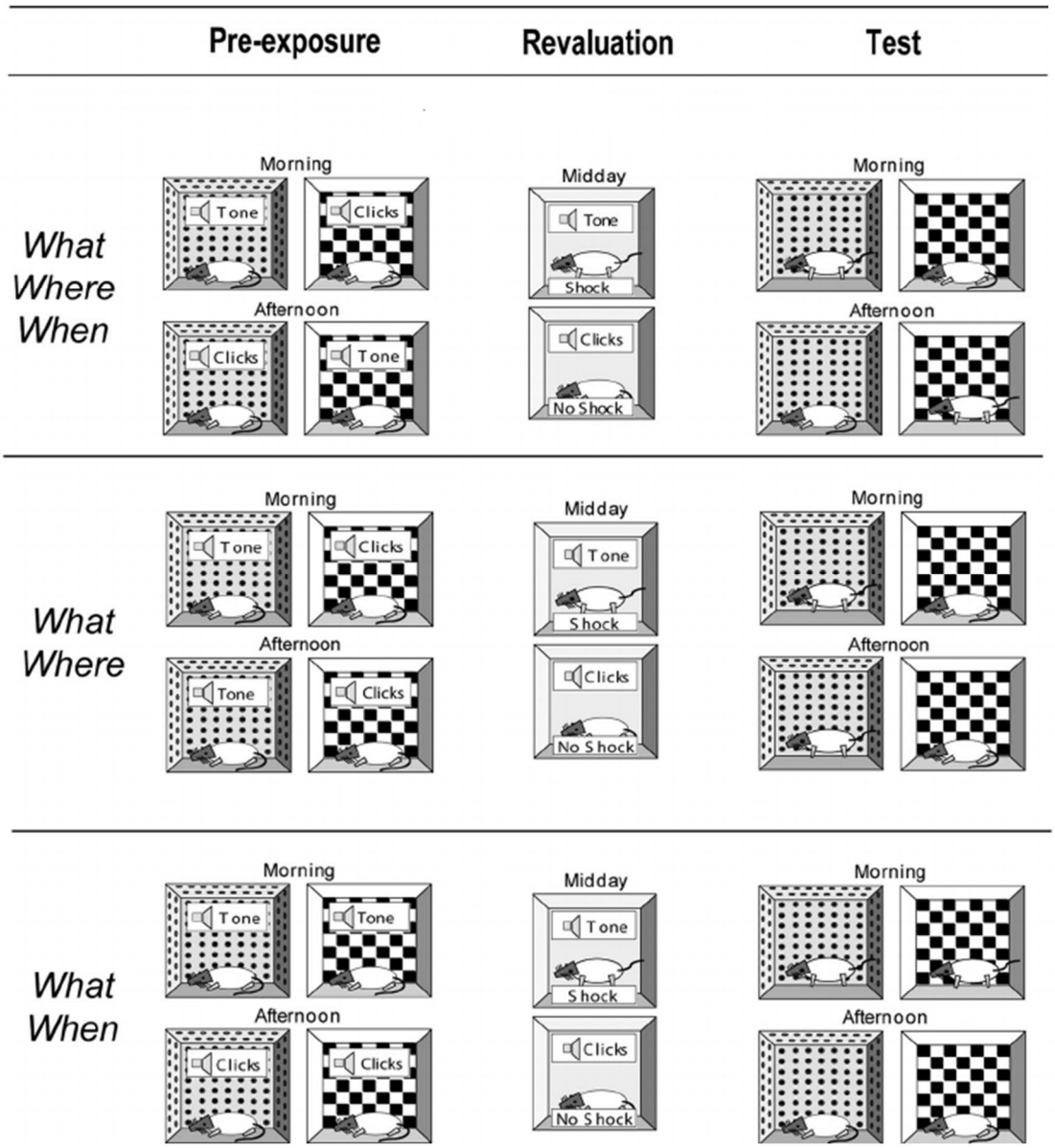

Figure 3. Design of *what-where-when* (upper panel), *what-where* (centre panel), and *what-when* (lower panel) experimental procedures used by Iordanova and colleagues (2008, 2009, 2011a, 2011b). Each experiment involved three stages (left to right). In the first stage, rats were placed in two visually distinct contexts in the morning and afternoon, where they received auditory stimuli. In the second stage, rats received presentations of both auditory stimuli, one of which was paired with shock. Finally, freezing was assessed in both contexts in the morning and afternoon.

In the context of the studies by Iordanova and colleagues, it is interesting to note that there is good evidence that the hippocampus codes temporal and spatial information and this will be discussed in Section 1.5. The extent to which configural processes play a role in sensory preconditioning procedures involving more conventional stimuli (e.g., a light and

a tone) has not been investigated in great detail. The development of procedures that would allow this issue to be interrogated would be of theoretical importance in its own right, but it would also enable me to investigate whether such configural processes rely on the hippocampus. These two aims were pursued in Chapter 5. To date, examination of the role of the hippocampus in standard sensory preconditioning procedures has revealed inconsistent results. Thus, while there are some reports that hippocampal lesions disrupt sensory preconditioning (e.g., Port, Beggs & Patterson, 1987; Talk, Ghandi & Matzel, 2002), there are others that have reported no effect of hippocampal lesions on sensory preconditioning (e.g., Ward-Robinson, Coutureau, Good, Honey, Killcross & Oswald, 2001). This inconsistency is easy to understand given the many differences (in species and procedures) that have been used together with the lack of selective assays for the contribution of elemental or configural processes (cf. Iordanova *et al*., 2011ab). These issues were assessed in Chapter 5. However, the first empirical chapters (Chapters 2, 3 and 4) were concerned with assessing whether or not the hippocampus plays a general role in configural learning involving what happened where and when.

1.4. Episodic and episodic-like memory

Tulving (1972, 1983) used the term episodic memory to describe memory for experienced events in particular places at particular times, and distinguished this type of memory from semantic memory, which is memory for general factual information. He proceeded to argue that for an animal to possess true episodic memory it must have a sense of subjective time and a capacity for mental time travel. Humans are able to mentally go back in time and re-live past experiences without confusing them with present experiences. In contrast to humans, it has been argued that non-human animals do not have this capacity and are "bound to a present that is defined by their current motivational states" (Suddendorf & Corballis, 1997, p. 143). Tulving (2002) acknowledged the difficulty of confirming the

existence of autonoetic consciousness, awareness of one's own existence as an entity in time, in non-verbal animals and suggested that humans are unique in their capacity to experience true episodic memory.

This restrictive definition of episodic memory has not discouraged researchers from attempting to demonstrate episodic-like memory in non-human animals. I have already described a series of experiments by Iordanova and colleagues that investigated various ways in which information about what happened where and when could be integrated. These results do not stand alone in showing that some animals exhibit episodic-like memory.

The first convincing demonstration of this type of memory in non-human animals were recorded by Clayton and Dickinson (1998) in scrub jays. They found that food-caching scrub jays were able to remember what foods they hid in which locations at particular points in time. While Bird, Roberts, Abroms, Kit and Crupi (2003) could not demonstrate an analogous effect in rats, others have enjoyed greater success in showing that rats could integrate temporal and spatial information in order to find food. Thus, Babb and Crystal (2006) found that rats were able to determine which arm of a radial arm maze would contain food after a given interval. Also, there is evidence from studies using variants of the spontaneous recognition task that rats can encode the spatio-temporal context in which objects have been presented (Good, Barnes, Staal, McGregor & Honey, 2007; see also Eacott & Norman, 2004; Eacott, Easton & Zinkivskay, 2005; Fortin, Agster & Eichenbaum, 2002).

Showing that "when" has been encoded has proved more difficult than showing that "where" has been encoded, and many attempts to demonstrate episodic-like memory have been criticised because they might instead be based on relative familiarity, sequence learning or interval timing judgements (Clayton, Bussey & Dickinson, 2003), which do not necessarily require learning about specific events or episodes in time (Tulving, 2002). It has been suggested that "when" serves only as an occasion setter to distinguish one experience

from another similar experience, and that "what-where-which occasion" might be a more appropriate non-human analogue of human episodic memory than is "what-where-when" (e.g., Eacott & Norman, 2004; Eacott, Easton & Zinkivskay, 2005). It is widely known that rats have a spontaneous tendency to explore novel aspects of their environment, and Eacott and Norman (2004; see also Eacott, Easton & Zinkivskay, 2005) used this behaviour to assess rats' integrated memory for an object, its spatial location and the context in which it appeared. They showed that rats preferentially explored an object that appeared in a novel configuration of location and context over an object that was in a familiar location and context. This was interpreted as evidence for episodic-like memory using a broader definition of "what-where-which" memory, where "which" is any occasion-setter that defines the experience as unique.

Alternatively, using absolute time of day as the "when" component is one way that may help to alleviate some of the concerns about accessing an appropriate temporal aspect of episodic-like memory (Clayton, Bussey & Dickinson, 2003). As previously mentioned, Iordanova and colleagues demonstrated that rats can learn to associate a particular auditory stimulus with a particular configuration of visual context and time of day (Iordanova *et al*., 2008; 2009; 2011ab). O'Brien and Sutherland (2007) also showed that rats can acquire memories that include integrated information about the time of day that an event occurred. They exposed rats to two distinctive contexts, one in the morning and one in the afternoon and then paired one of the times of day (either morning or afternoon) with footshock in a third context. Subsequently, when they measured conditioned freezing at an intermediate time of day, rats showed significantly more fear in the context congruent to that in which the footshock was presented. Also, Fellini and Morellini (2013) found that male mice are capable of learning and remembering the spatial location and time of day that a female mouse has been present (and that this requires the hippocampus). Finally, it has been shown in a T-maze that rats can learn to choose the left choice arm of the T-maze rather than the

right arm to gain food in the morning, and to do the reverse to gain food in the afternoon (e.g., Means, Arolfo, Ginn, Pence & Watson, 2000a; see also, Means, Ginn, Arolfo & Pence, 2000b; Thorpe, Bates & Wilkie, 2003).

It remains the case that there is a dearth of experimental procedures that enable the acquisition of integrated memories for what happened where and when to be monitored. The aim of Chapter 3 was to use more conventional configural conditioning procedures to assess the development of integrated *what-where-when* memories. The successful demonstration of configural learning involving where and when a reinforcer (food or shock) was delivered would enable me to investigate (in Chapter 4) the role of the hippocampus in such instances of configural learning. Previous research has failed to demonstrate a consistent or convincing role for the rodent hippocampus in configural learning *per se* (see next section). However, recent research conducted by Iordanova and colleagues provides just such demonstrations in sensory preconditioning procedures. Hence, Chapter 4 examined the role of the hippocampus in conventional configural discriminations involving what happened (e.g., food or no food; shock or no shock), where (one context or another) and when (in the morning or afternoon).

1.5. The hippocampus and configural learning

The disruption to configural processing observed after various manipulations reported by Iordanova and colleagues can be interpreted in several ways. The first interpretation is that their results reflect a more general disruption to configural learning. This possibility is consistent with the theoretical analysis developed by Rudy and colleagues (O'Reilly & Rudy, 2001; Rudy & Sutherland, 1989, 1995). According to this analysis, the hippocampus plays an important role in configural learning, but not learning that can be subserved by elemental associations. This analysis predicts that the damage to the hippocampus will disrupt any task provided it is the case that configural processes are required (e.g., Alvarado & Rudy, 1995;

Rudy & Sutherland, 1989). Consistent with this is evidence of hippocampal involvement in configural learning tasks when so-called elemental learning about the same components is unimpaired (Eacott & Gaffan, 2005; Iordanova *et al*., 2009; Langston & Wood, 2010; Li & Chao, 2008; Save, Poucet, Foreman & Buhot, 1992). However, this configural learning analysis of hippocampal function is not well placed to explain the occasions where hippocampal lesions do not influence the acquisition of configural discriminations (e.g., Coutureau, Killcross, Good, Marshall, Ward-Robinson & Honey, 2002; Davidson, McKernan & Jarrard, 1993; Gallagher & Holland, 1992).

The alternative, but related, interpretation is that the hippocampus is involved in configural processing of information that has episodic content. This analysis predicts that configural discriminations will involve the hippocampus to the extent that they are based on the integration of certain types of information; notably where and when something happens.

The "where" aspect of an event memory is provided by some form of spatial information. The discovery of "place cells" in areas CA1 and CA3 of the hippocampus (O'Keefe & Dostrovsky, 1971) led to a theory that the hippocampus acts as a cognitive map and plays a key role in processing spatial memory (O'Keefe & Nadel, 1978). It is well established that spatial learning that involves a navigational component is disrupted in rats with damage to the hippocampus (e.g., Jarrard, 1978; Olton, Becker & Handelmann, 1979; Steele & Morris, 1999; Clark, Boradbent & Squire, 2005). Additionally, MRI studies in humans have implicated the hippocampus in the processing of spatial information used for navigation (e.g., Maguire, Frackowiak & Frith, 1997; Maguire, Burgess, Donnett, Frackowiak, Frith & O'Keefe, 1998; Maguire, Mummery & Büchel, 2000). In support of the theory that the hippocampus is only necessary for configural tasks that involve integrating spatial information, Sanderson, Pearce, Kyd and Aggleton (2006) found that rats with hippocampal damage were able to acquire two non-spatial configural discriminations (transverse

patterning and biconditional discrimination) but not a “structural” configural discrimination, where spatial information was key. Also, Albasser and colleagues found that rats with hippocampal lesions were impaired at a spatial biconditional task in which distal location cues determined in which of two media to dig for food (Albasser, Dumont, Amin, Holmes, Horne, Pearce & Aggleton, 2013). However, the same rats could learn about the digging media and the location cues separately. Moreover, they could also learn a biconditional task when contextual floor and wall cues signalled the correct media in which to dig for food. These results lend further support to the idea that the hippocampus is not required for all configural learning, but is required for integrating specific types of information, noteably involving spatial cues (but see Kumaran, Hassabis, Spiers, Vann, Vargha-Khadem & Maguire, 2007).

The hippocampus has also been implicated in the processing of temporal information. The severe memory loss of the patient, H. M., that resulted from bilateral surgical removal of major parts of the medial temporal lobe including the hippocampus (Scoville & Milner, 1957), was accompanied by an impairment in the ability to accurately estimate time intervals greater than 20 seconds (Richards, 1973). This suggests that within the medial temporal lobe there is a system for evaluating the pasasge of time.

Studies in non-human animals have specifically identified the hippocampus as having a role in processing temporal information. For example, MacDonald, Lepage, Eden and Eichenbaum (2011) found evidence for hippocampal “time cells” that are active at particular points in time in a similar way to how “place cells” in the hippocampus are active at particular locations in space (see O’Keefe & Dostrovsky, 1971). Coding of time over longer intervals has been investigated by Mankin and colleagues, who described a hippocampal neuronal code for when, or how long ago, events occurred (Mankin, Sparks, Slayyeh, Sutherland, Leutgeb & Leutgeb, 2012). They repeatedly measured the firing patterns of the same hippocampal

cell populations over extended time intervals as the rats experienced repeated events in the same, highly familiar environment. They found that the activity of cells in area CA1 changed with time, and that cells in area CA3 generated nearly identical firing patterns during the repeated events. This changing activity in CA1 is consistent with the idea that this area codes for the passage of time (see also Munn & Bilkey, 2012) and that rats are able to use information about "how long ago" an event occurred (e.g., Roberts, Feeney, Macpherson, Petter, McMillan & Musolino, 2008; Jacobs, Allen, Nguyen & Fortin, 2013). The highly reproducible firing patterns in CA3 suggests that this area might represent more stable aspects of event context. Consistent with temporal coding in CA1 neurons, Rubin, Geva, Sheintuch and Ziv (2015) found hippocampal activity patterns unique to specific points in time. These results suggest that the hippocampus could be involved in representing and associating events based on their temporal distance. Behavioural evidence for this comes from a study by Jacobs and colleagues (Jacobs *et al*., 2013). They found that inactivating the hippocampus produced a deficit in rats' ability to measure elapsed time, particularly when performing high resolution temporal discriminations. Hippocampal damage-related impairment in assessing "how long ago" has also been demonstrated by Good *et al*. (2007), who found that lesions of the hippocampus disrupted the tendency shown by control rats to explore objects that were presented to the rat least recently (see also Albasser *et al*., 2012). Additionally, the hippocampus has generally been found to be required for tasks involving memory for the order in which stimuli are presented, for example, sequential spatial locations on a radial-arm maze (Chiba, Kesner & Reynolds, 2004) or runway box (Hunsaker, Lee & Kesner, 2008), or sequentially presented odours (e.g., Fortin *et al*., 2002; Kesner, Gilbert & Barua, 2002). Thus, there are many instances where the processing of temporal information, something that is crucial for episodic memory, has been shown to be reliant on the hippocampus.

Moreover, there is direct evidence that the hippocampus is involved in the configural integration of temporal and spatial information. In humans, episodic memory has been frequently associated with the medial temporal lobe, particularly the hippocampus (Burgess, Maguire & O'Keefe, 2002; Eichenbaum & Cohen, 2001; Tulving & Markowitsch, 1998; Vargha-Khadem, Gadian, Watkins, Connelly, Paesschen & Mishkin, 1997). In an episodic-like memory paradigm in mice, Fellini and Morellini (2013) demonstrated that hippocampal damage impaired the ability of males to remember the time and place that a female was previously located. Deficits in spatio-temporal integration have also been demonstrated in tasks involving spontaneous exploration. The spontaneous exploratory behaviour of rats is such that, when given a choice between two objects that have both been presented to rats relatively recently, rats will choose the object that has been presented in a novel location. When given a choice between two familiar, older (presented relatively less recently) objects, rats will choose the object presented to them least recently. Li and Chao (2008) explored the effects of electrolytic lesions of dorsal CA3 in a version of an object exploration task which is thought to require the association of spatial, temporal and object information. During the test phase of this task, rats were exposed to four familiar objects, two recently presented, and two less recently presented. From each set, one was presented in the same location as during the initial exposure, and the other in a different spatial location. For the recent objects, control animals explored the displaced object significantly more than the un-displaced object, whereas for the less recently presented objects, they explored the non-displaced object more than the displaced one. This was taken as evidence for formation of an integrated representation of object, location and temporal order. In contrast, animals with CA3 lesions displayed significant preference for the old displaced object than for the old stationary object, and for the recent displaced object than for the recent stationary object, i.e., these rats did not demonstrate learning about integrated spatio-temporal information (see also Save, Poucet, Foreman & Buhot, 2002; Barker & Warburton, 2011). However, these

same animals showed no impairments in the temporal or spatial aspects of the task when tested in isolation. Further studies provide evidence for a role of the hippocampus in integrating spatial/contextual and temporal information (Aggleton & Brown, 1999; Eichenbaum & Fortin, 2003; Ergorul & Eichenbaum, 2004; Cassel *et al*., 1998; Jarrard, 1993; Olton, Becker & Handelmann, 1979). These results suggest that the hippocampus is well positioned to provide a basis for integrating information about where and when something happened; and provides an obvious substrate for the configural processes of the kind studied by Iordanova and colleagues. This prediction was tested in Chapter 4 using the novel configural discriminations developed in Chapter 3.

The final possibility for explaining the configural learning deficits obtained by Iordanova and colleagues is that the hippocampus is involved in learning about configural representations that are retrieved as opposed to being directly activated. This more circumscribed claim receives support from the observation that infusing AP5 into the hippocampus during conditioning in the procedure used by Iordanova and colleages abolishes evidence of configural learning during the test (Iordanova *et al*., 2011b). This finding suggests that conditioning with the auditory stimuli reactivates the configural memories formed during the exposure stage and these memories ordinarily become linked to shock. This analysis allows that directly activated configural memories might be distinct from retrieved configural memories, and that learning involving these two types of representation might be dissociated when hippocampal function is disrupted.

1.6. Aims of the thesis: A summary

The overarching aim of this thesis was to gain a better understanding of the involvement of the rodent hippocampus in configural learning and memory. To do so, I needed to develop novel behavioural procedures to assess (i) configural integration of where and when reinforcers are delivered during conventional conditioning procedures, and (ii) configural

processes involving standard stimuli during sensory preconditioning procedures. Thus, the experiments described in Chapter 2 investigated whether or not rats with selective hippocampal lesions could learn where or when reinforcers are delivered (Experiments 1 and 2). That is, Chapter 2 investigated whether rats with hippocampal lesions could learn where a reinforcer was presented and where it was not presented (a *what-where* discrimination; e.g., food is presented in the spotted context, but not in the checked context); and it investigated whether rats with hippocampal lesions could learn at which time of day a reinforcer would be presented and at which time of day it would not be presented (a *what-when* discrimination; e.g., food is presented in the morning, but not in the afternoon). These are *what-where* and *what-when* discriminations in the sense that in order to learn the discrimination rats need to be sensitive to the contingencies between the relevant dimensions (where or when) and the presence or absence of food (cf. Iordanova *et al*., 2011ab). Chapter 3 reports the results of appetitive and aversive conditioning procedures that enable the formation of configural memories involving what happened where and when to be studied (Experiments 3 and 4). That is, Chapter 3 used appetitive and aversive procedures to investigate whether rats could learn that the presence or absence of a reinforcer was signalled by specific configurations of spatial and temporal information (a *what-where-when* discrimination; e.g., food is presented in a spotted context in the morning and a checked context in the afternoon; but not in a checked context in the morning and a spotted context in the afternoon). This is a *what-where-when* discrimination in the sense that in order to learn the discrimination rats need to be sensitive to the contingencies between configurations of the relevant dimensions (where and when) and the presence or absence of food (cf. Iordanova et al., 2011ab). The research experiments described in Chapter 4 evaluated the effect of selective hippocampal lesions on configural learning using the procedures developed in Chapter 3 (Experiments 5-7). In Chapter 5, novel sensory

preconditioning procedures, involving audio-visual compounds, were used to assess the involvement of the hippocampus in configural learning and memory (Experiments 8 and 9).

Chapter 2: Learning about *where* or *when*

2.1. Summary

One of the primary objectives of this thesis, identified in Chapter 1, was to assess the role of the hippocampus in forming configural memories involving where and when a stimulus (e.g., food) will be delivered. The first step towards meeting this objective was to assess whether the hippocampus plays a role in learning about where a given reinforcer is delivered (Experiment 1) or when it is delivered (Experiment 2). If either of these elemental learning processes were disrupted in rats with lesions to the hippocampus then this would require further investigation, but the development of tasks to assess the role of the hippocampus in configural integration would be of less theoretical interest.

2.2. Introduction

There is evidence suggesting that learning where something is presented or when it is presented can be disrupted in rats with lesions to the hippocampus (see Chapter 1). Thus, it is well established that spatial learning that involves a navigational component is disrupted in rats with damage to the hippocampus (e.g., Jarrard, 1978; Olton *et al*., 1979; Steele & Morris, 1999). Also, hippocampal involvement in processing spatial relationships is evident from the results of spontaneous exploration tasks in rats with hippocampal damage. Normal rats display a preference for, and spend more time exploring, novel objects, and objects that have been positioned in a different spatial location from where the rats previously experienced them. It has been shown that rats with hippocampal lesions do not show increased exploration of objects that have been re-positioned between exposure and test (e.g., Save *et al*., 2002; Barker & Warburton, 2011), indicating a disruption in processes of representing the spatial environment. Similarly, Gilbert and Kesner (2002) trained rats on two paired-associate tasks: object-place and odour-place. Over a series of multiple training

trials, Object A (or Odour A) was rewarded in Location 1 but not in Location 2, and Object B (or Odour B) was rewarded in Location 2 but not in Location 1. Control rats learned which object (or odour) was presented in which location, but rats with hippocampal lesions showed no evidence of this learning (see also, Day, Langston & Morris, 2003; Tse, Langston, Kakeyama, Bethus, Spooner, Wood, Witter & Morris, 2007; Yoon, Seo, Kim & Lee, 2012).

The temporal component of episodic memory has proved more difficult to investigate in rats. "When" is sometimes investigated as temporal order, relative recency or as absolute time of day. The hippocampus has generally been found to be required for tasks involving memory for the order in which stimuli are presented, for example, sequential spatial locations on a radial-arm maze (Chiba, Kesner & Reynolds, 2004) or runway box (Hunsaker, Lee & Kesner, 2008), or sequentially presented odours (e.g., Fortin *et al*., 2002; Kesner, Gilbert & Barua, 2002). Additionally, Good *et al*. (2007) found that lesions of the hippocampus disrupted the tendency shown by control rats to explore objects that were presented to the rat least recently. Similarly, Albasser *et al*. (2012) found that rats with hippocampal lesions were impaired in performing an object recency task (requiring processing of temporal order information) but were not impaired on tasks that required object recognition memory. Other research has implicated the hippocampus in learning about temporal cues across longer time scales. Mankin *et al.* (2012) showed that there are populations of neurons in the hippocampus that show different patterns of activity for events that are repeated, i.e. events that are identical in every way except the time at which they occur. Other studies have also found evidence for neuronal activity in the hippocampus signalling the passage of time (e.g., Rubin *et al*., 2015; Munn & Bilkey, 2012; Jacobs *et al*., 2013).

Langston and Wood (2010) showed that hippocampal lesions did not impair object-place or object-context memory. In this study a deficit was seen only on object-context-place

tasks, when rats were required to integrate contextual and spatial information. This suggests that there might be something about the integration of these types of information that is dependent on the hippocampus, but not necessarily acquiring spatial or contextual discriminations independently. Similarly, Iordanova *et al*. (2009) found that rats with hippocampal lesions were able to associate a particular contextual cue (wallpaper of an operant chamber) with a particular auditory stimulus, and similarly, a particular time of day with an auditory stimulus.

Given the fact that the conditions under which the hippocampus contributes to learning about where or when are not established, the aim of the two experiments reported in Chapter 2 was to investigate the impact of hippocampal lesions on learning about where (Experiment 1) or when (Experiment 2) a reinforcer (food) would be delivered. The two experiments used the contexts (where) or times of day (when) that were employed by Iordanova *et al*. (2009). Importantly, the procedures used in the current study provided a method of continual assessment throughout the acquisition of *what-where* and *what-when* discriminations.

2.3. Design of Experiments 1 and 2

The experimental designs used in Experiments 1 and 2 are presented in Figure 4. Rats were exposed daily to four patterns that each consisted of a context (spotted or checked wallpaper) and time of day (morning or afternoon). In Experiment 1 (*what-where*), food pellets were presented in one context and not in the other irrespective of the time of day when rats were placed in the contexts, and in Experiment 2 (*what-when*), food pellets were presented at one time of day and not at the other, irrespective of the context in which the rats were placed. Rats' ability to learn the two discriminations was assessed by recording the number of entries to a foodwell in the first 30s of a trial, when no food pellets were delivered in any of the four patterns.

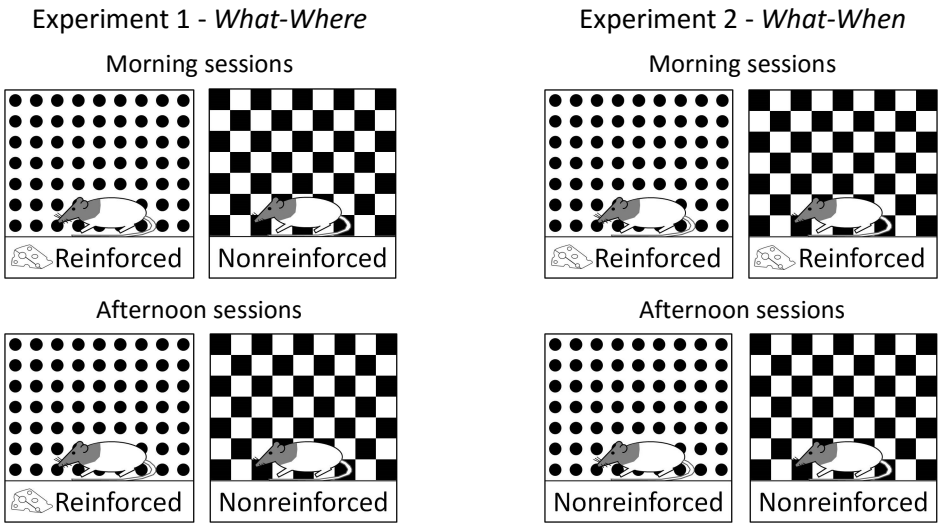

Figure 4. Design of Experiment 1 (left) and Experiment 2 (right): Rats received exposure to four context+time of day patterns. In Experiment 1, food pellets were delivered in one of the contexts (e.g. spotted) but not the other (e.g. checked). In Experiment 2, food pellets were delivered at one time of day (e.g. morning) but not the other (e.g. afternoon) in both contexts. Which context (Experiment 1) or time of day (Experiment 2) was reinforced was fully counterbalanced.

2.4. Method

2.4.1. Subjects

Sixteen male naïve Lister hooded rats (*Rattus norvegicus*; supplied by Harlan Olac Ltd, UK) were used in each experiment. The rats in Experiment 1 were ≈ 3.5 months old at the start of the experiment (mean weight = 306g; range: 285 – 341g) while those in Experiment 2 were ≈ 3.5 months old (mean weight = 317g; range 275 - 357g). They were maintained at 85% of their free-feeding weights by giving them a restricted amount of food at the end of the day (≈ 18:30), and they were housed in pairs in a colony room that was illuminated between the hours of 08:00 and 20:00. Rats were weighed on each day and separated for feeding if this was required to maintain their weights. Behavioural training began at, approximately, 09:30 each day.

2.4.2. Surgery

There were two groups in Experiment 1: Group Sham (n = 8) and Group Hippocampal (n = 8), and two groups in Experiment 2: Group Sham (n = 8) and Group Hippocampal (n = 8). All rats were first anaesthetised using an isofluorane-oxygen mix and then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) with the nose bar set at +3.0. The bone above the region to be lesioned was removed, and rats in group Hippocampal (for both Experiments 1 and 2) were infused with ibotenic acid (Biosearch Technologies, San Rafael, CA; dissolved in phosophate-buffered saline [pH 7.4] to provide a solution with a concentration of 63 mM) through a 2-µl Hamilton syringe held with a microinjector (Kopf Instruments, Model 5000). Table 1 shows the coordinates where the microinjector was positioned and associated volumes that were injected. A total of 15 infusions per hemisphere were made with an infusion rate of 0.05 µl/min and diffusion time of 2 min. After each injection, the needle was left in position for 2 min to allow diffusion of the ibotenic acid and to limit the spread of the drug into overlying cortical areas. Rats in group Sham received the same surgical preparation with the exception that the dura was perforated with a 25-gauge Microlance3 needle (Becton Dickinson, Drogheda, Ireland), but no fluid was infused into the brain. Following surgery, the scalp was sutured and antibiotic powder (Dalacin C, Pharmacia) was applied topically to the wound. Rats were given a subcutaneous injection of 0.06ml Metacam (Boehringer Ingelheim, Alkmaar, NL) to reduce post-operative pain, and a subcutaneous injection of 5ml glucose-saline to replace lost fluids. Rats were left to recover in a warm, quiet area before being returned to their home cage. During recovery, the rats were handled and weighed daily and food restriction and behavioural testing did not commence for a minimum of 14 days post-surgery when rats had established their preoperative weights.

Table 1. Stereotaxic coordinates and volume of ibotenic acid for lesions of the hippocampus

AP	ML	DV	Volume (µl)
-5.5	±4.2	-7.6	0.10
		-3.9	0.10
	±5.5	-6.8	0.10
		-5.8	0.10
		-5.0	0.10
-4.7	±4.0	-7.5	0.10
		-3.5	0.05
	±4.5	-8.0	0.10
-3.9	±2.2	-3.7	0.10
		-3.0	0.10
	±3.5	-2.7	0.10
-3.1	±1.4	-4.0	0.10
		-3.0	0.10
	±3.0	-2.7	0.10
-2.4	±1.0	-3.8	0.05

Note: AP, ML and DV indicate the coordinates in relation to bregma from anterior to posterior (AP), from medial to lateral (ML) and from dorsal to ventral (DV).

2.4.3. Apparatus

Experiment 1 employed a set of four chambers (23.0cm×24.5cm×21.0cm, L×W×H; supplied by Camden Instruments Ltd., UK) arranged in a 2×2 array. The chambers were constructed from three aluminium walls, an aluminium ceiling, and a plastic wall that served as the door to the chamber. The ceilings and walls of the top pair of boxes in the array were decorated with spotted laminated paper (black circles on a white background), whereas the walls and ceiling of the lower two chambers were decorated with black and white checked laminated paper (for further details, see Honey & Watt, 1999). There was a foodwell in the left hand aluminium wall into which 45mg of food pellets (supplied by P. J. Noyes, Lancaster, NH) could be delivered. A top-hinged transparent plastic flap guarded access to this food cup. Food-well entries were automatically recorded when the top-hinged magazine flap was pushed approximately 3 mm. A series of stainless steel rods, 0.50 cm in diameter and 1.5 cm apart (centre-to-centre), served as the chamber floor, below which was a tray containing a 24cm x 23cm absorbent sheet. The chamber received local illumination from a single 15-V, 24-W jewel light positioned in the centre of the ceiling, and ambient illumination from the striplight

in the experimental room. The doors of the sound-attenuating shells in which the chambers were housed were left open throughout training.

2.4.4. Procedure

Following a minimum of 14 days recovery from surgery, rats were food restricted to 85% of their free-feeding weights by giving them a restricted amount of food. Once rats had been food restricted to this level, behavioural training began.

On the first day, in two 10-min sessions, conducted between 13:30 and 15:30, rats were trained to retrieve food pellets from a foodwell in undecorated chambers. In the first session, the flaps in front of the foodwells were fixed in a raised position to allow rats ready access to the food pellets; and in the second session these flaps were lowered and rats had to move them to gain access to the food pellets. During both sessions, 20 food pellets were delivered two at a time on a variable time 60-s schedule. Successful foodwell training was followed by 20 days of discrimination training. On each day, rats were placed in the two contexts (spotted and checked) in the morning and the same two visual contexts in the same sequence in the afternoon (spotted and then checked for half of the rats, and checked and then spotted for the remainder). For a given training day, the order in which the contexts were presented for a given rat was consistent, but across days the order pseudo-randomised with the constraint that there were no more than two consecutive days with the same order. Morning sessions took place between 09:30 and 11:30, and afternoon sessions took place between 16:30 and 18:30. Each context placement lasted 5 min, and there was a 5 min interval between the two morning and afternoon sessions during which rats were placed in a holding cage outside the testing room.

In Experiment 1 (*what-where*), in the morning, during placement in one context (e.g., spotted) two food pellets were delivered to the foodwell every 30s (resulting in a total of 20 pellets delivered per session), and during placement in the other context (e.g., checked) no

food pellets were delivered. This was repeated in the afternoon so that food pellets were delivered on the same schedule in the same context that they had been delivered in the morning (e.g., spotted) and not in the other context (e.g., checked). Which of the contexts was reinforced was fully counterbalanced in each group of rats (Sham and Hippocampal). In Experiment 2 (*what-when*), food pellets were delivered to the foodwell in both contexts at one time of day (e.g., morning) and neither context at the other time of day (e.g. afternoon). Which time of day was reinforced was also fully counterbalanced. At the end of each pair of sessions, the rats were taken back to the colony room and replaced in their holding cages.

To reduce the likelihood of rats learning that food would not be delivered for the first 30-s of any session, each cycle of 4 training days had the following structure. On the first two days of the cycle, on placement in the reinforced contexts, an additional 2 pellets were present in the foodwell. For the following two days of the cycle, no food pellets were present in the foodwell for the first 30-sec periods. This method was used in an effort to increase the rate at which the discrimination was acquired, compared to a pilot study, in which food pellets were not present in the foodwell at the start of any trials. The number of foodwell entries made by a rat during this first 30s reinforcer-free periods during the second pair of training days in each cycle was used to assess the acquisition of the discrimination. To do so, a discrimination ratio (DR) was used: foodwell entries per minute during reinforced sessions (e.g. spotted + morning) divided by the combined number of entries per minute during reinforced and nonreinforced sessions (e.g. for Experiment 1: spotted + morning, and spotted + afternoon; for Experiment 2: spotted + morning, and checked + morning). For Experiment 1, one DR was calculated for the morning and one for the afternoon. For Experiment 2, one DR was calculated for the spotted context and one for the checked context. In both experiments, a mean of the two DRs for each day was calculated to provide one DR value for each rat per day. A DR of 0.50 indicates that the number of foodwell entries was the same during the reinforced and nonreinforced sessions, whereas scores greater than

0.50 indicate that responding is greater during reinforced than nonreinforced sessions. The raw rates of responding on the reinforced and nonreinforced trials were also reported.

2.4.5. Histology

Following behavioural testing, rats received a lethal overdose of sodium pentobarbitone (Euthatal) and were then transcardially perfused, first with 0.9% saline followed by 10.0% formal-saline. The brains were then removed and placed in 10.0% formal-saline for 24 hours, and transferred to phosphate-buffered (0.1 M) 25.0% sucrose solution for 24 hours. Subsequently, each brain was frozen, sectioned coronally using a sliding microtome, and the 40µm sections were collected on gelatine-coated slides. These slides were left to dry for 24 hours and the sections were stained with cresyl violet. To estimate the extent of hippocampal damage all lesions were plotted onto six equally spaced, coronal sections (Bregma -2.28, -3.12, -3.96, -4.80, -5.64, -6.48; adapted from Paxinos & Watson, 2005) and the percentage cell loss was estimated following examination under a microscope. Rats were excluded from the analysis if the total cell loss in the hippocampus was found to be less than 50%. The tissue loss in the dorsal (septal) hippocampus was also estimated and recorded, as there is evidence that this region is particularly important for learning and memory, including spatial learning (e.g., Bannerman *et al*., 2004; Barkus *et al*., 2010). For this, the border between dorsal and ventral hippocampus was arbitrarily placed at -5.5 below bregma (Paxinos & Watson, 2005).

2.4.6. Statistical Methods

Results were analysed using parametric statistics. The software package, SPSS 16.0 (SPSS Inc., Chicago), was used, which enabled the underlying assumption of sphericity of the data to be confirmed for ANOVA. The rejection level that was adopted for analyses was $p \leq .05$. When ANOVA had established already that there was a significant effect of training block (or

an interaction involving block), *t*-tests were used to establish the blocks on which the scores differed from one another (or from chance level; i.e., 0.50 for discrimination ratios).

2.5. Results

2.5.1. Histology

Inspection of the cell loss in the rats with hippocampal lesions across both experiments revealed that 13 of the rats had a minimum of 50% total cell loss in the hippocampus (mean 73%), with a minimum of 70% cell loss in the septal region (mean 92%). The other 3 rats had less than 32% total cell loss in the hippocampus and so were excluded from the analysis. The areas of largest and smallest lesions can be seen in figure 5. As in previous experiments using this lesion method (e.g., Iordanova *et al*., 2011a), there was limited cortical damage caused by the insertion of the needle and some diffusion of the neurotoxin around the needle, but in all cases this damage was minor. Following histological analysis there remained 6 rats in group Hippocampal in Experiment 1, and 7 rats in group Hippocampal for Experiment 2.

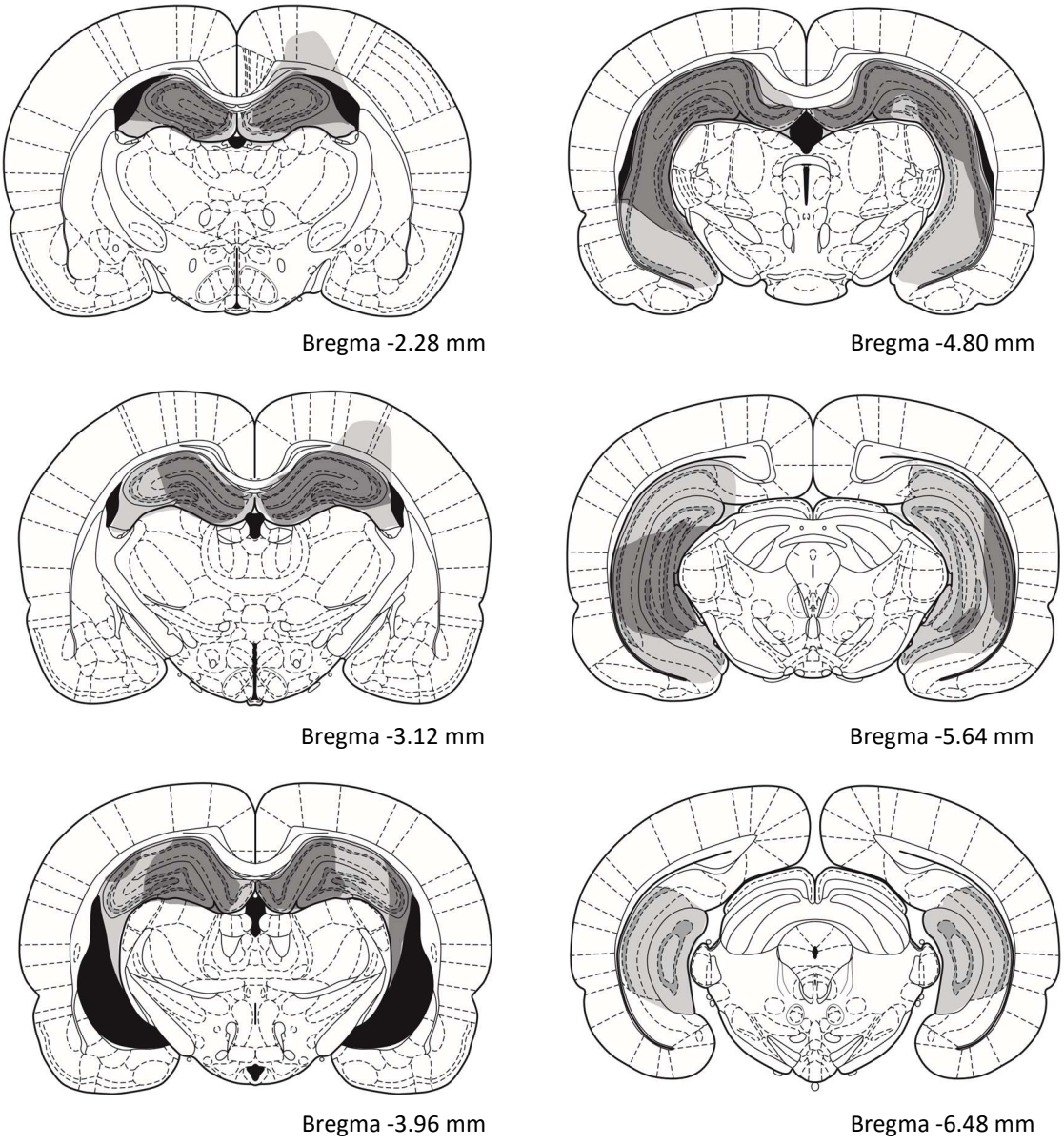

Figure 5. Experiments 1 and 2 Histology combined. Dark grey indicates the area of the smallest lesion and light grey indicates the area of the largest lesion.

2.5.2. Experiment 1: Acquisition of a *what-where* discrimination

As shown in the upper left panel of Figure 6, the discrimination ratio increased across training blocks in both groups Sham and group Hippocampal. ANOVA confirmed that there was a significant main effect of block ($F(4, 48) = 6.05$, $p < .001$), no effect of group ($F(1, 12) < 1$) and no interaction between block and group ($F(4, 48) = 1.913$, $p = 0.124$). A pooled error term was used here and elsewhere in the thesis, and analysis confirmed that the assumptions of the statistical tests had not been violated. Further analysis of the main effect of block, using two-tailed one-sample *t*-tests, confirmed that the DR was significantly different from 0.5 for group Sham in all blocks (smallest $t(7) = 4.09$, $p < .05$), and for group Hippocampal in blocks 2, 3, 4 and 5 (smallest $t(5) = 3.74$, $p < .05$). The use of a two-tailed test is conservative given the facts that there is a main effect of block and discrimination training (here and elsewhere in the thesis) is predicted to result in DR scores being above 0.5.

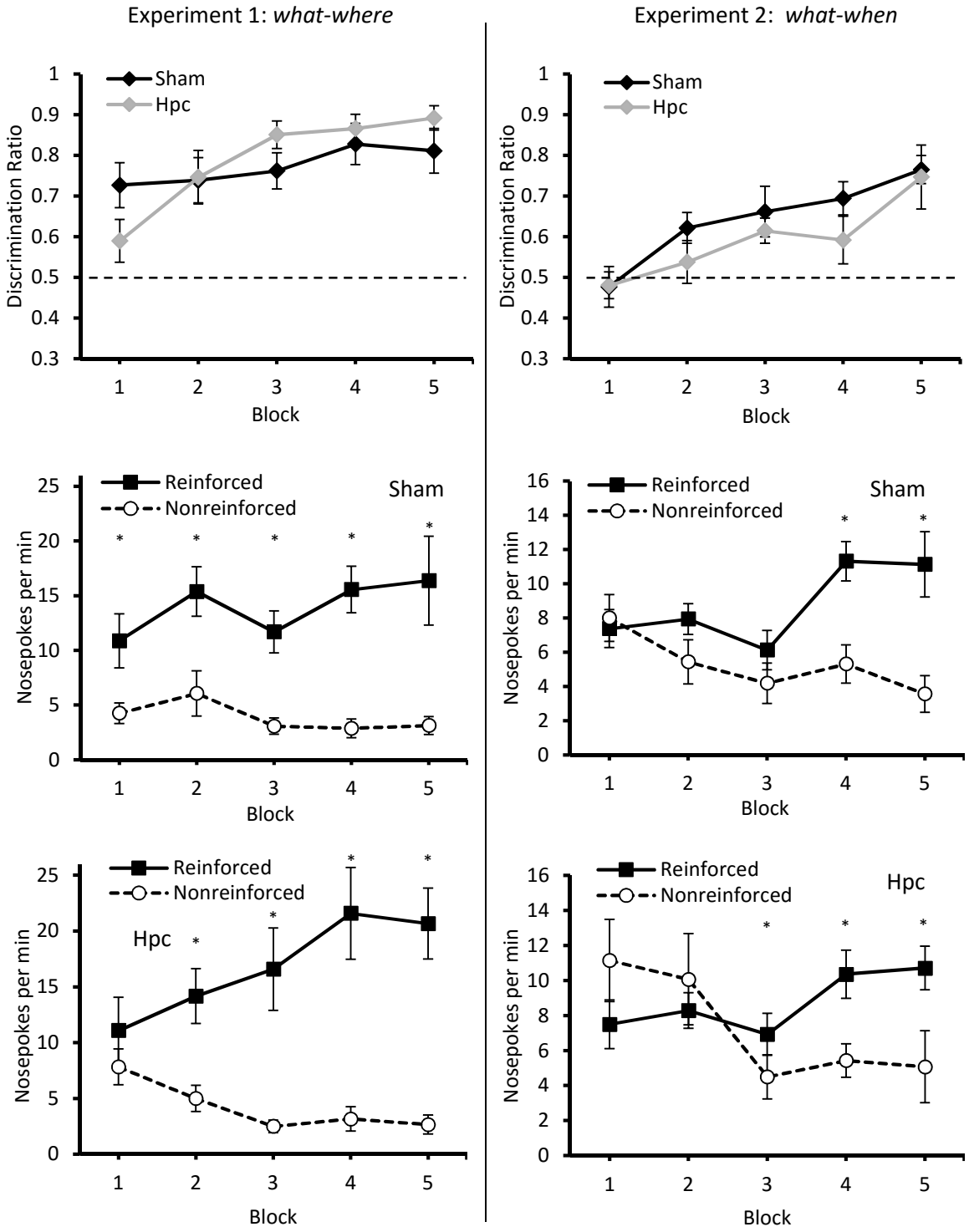

Figure 6. Experiment 1 (left-hand panels) and Experiment 2 (right-hand panels): Mean discrimination ratio (±SEM) over training blocks for Groups Sham and Hippocampal (top panels). Each block represents data from the last two days of a four-day training cycle, during the food-free 30-sec periods at the start of the sessions. Mean number of foodwell entries ("nosepokes") per minute in reinforced and nonreinforced sessions over two-day blocks for Group Sham (centre panels) and Group Hippocampal (bottom panels). Asterisks represent *p* values < .05.

The middle and lower left-hand panels of Figure 6 show the corresponding raw rates of nosepokes on reinfroced and nonreinforced trials. Inspection of these panels shows that the rates of responding were similar in the two groups, and the rates of responding on the reinforced and nonreinforced trials diverged similarly over the course of training. ANOVA on the number of nosepokes per minute in the first 30s of reinforced and nonreinforced sessions revealed a significant main effect of reinforcement ($F(1, 12) = 59.72$, $p < .001$), no main effect of block ($F(4, 48) = 1.7$, $p > .16$) and no main effect of group ($F(1, 12) < 1$). There was a significant interaction between reinforcement and block ($F(4, 48) = 5.49$, $p < .01$), no interaction between block and group ($F < 1$) and no three-way interaction ($F(4, 48) = 1.108$, $p > .36$). Paired *t*-tests confirmed that there was a significant difference between the number of nosepokes in the first 30s of reinforced trials compared to nonreinforced trials for group Sham in all blocks (smallest $t(7) = 2.84$, $p < .05$) and for group Hippocampal in blocks 2, 3, 4 and 5 (smallest $t(5) = 3.43$, $p < .05$).

2.5.3. Experiment 2: Acquisition of a *what-when* discrimination

The upper right panel of Figure 6 shows that the discrimination ratios increased across training blocks in both groups Sham and Hippocampal. ANOVA revealed a significant main effect of block ($F(4, 52) = 9.71$, $p < .001$), no effect of group ($F(1, 13) = 1.54$, $p > .23$) and no interaction between block and group ($F < 1$). Subsequent analysis confirmed that the discrimination ratios were significantly different from 0.5 for group Sham in blocks 2, 3, 4 and 5 (smallest $t(7) = 2.59$, $p < .05$), and for group Hippocampal in block 3 ($t(6) = 3.67$, $p < .05$) and block 5 ($t(6) = 3.14$, $p < .05$).

The middle and lower right-hand panels of Figure 6 show the corresponding raw rates of nosepokes on reinforced and nonreinforced trials. Inspection of these panels shows that the rates of responding were similar in the two groups, and the rates of responding on the reinforced and nonreinforced trials diverged similarly over the course of training. ANOVA

on the number of nosepokes per minute in the first 30s of reinforced and nonreinforced sessions revealed a significant main effect of reinforcement ($F(1, 13) = 20.05$, $p < .01$), a significant main effect of block ($F(4, 52) = 5.00$, $p < .005$) and no main effect of group ($F < 1$). There was a significant interaction between reinforcement and block ($F(4, 52) = 10.24$, $p < .001$), no interaction between block and group ($F(4, 52) = 1.07$, $p > .37$) and no three-way interaction ($F < 1$). Paired *t*-tests confirmed that there was a significant difference between the number of nosepokes in the first 30s of reinforced trials compared to nonreinforced trials for group Sham in block 4 ($t(7) = 4.65$, $p < .005$) and block 5 ($t(7) = 4.61$, $p < .005$), and for group Hippocampal in blocks 3, 4 and 5 (smallest $t(6) = 2.53$, $p < .05$).

2.6. Discussion

Two experiments assessed the capacity of rats to acquire discriminations involving contextual cues or cues associated with time-of-day, using a procedure that enabled repeated comparison of groups Sham and Hippocampal across training. The results of Experiment 1 showed that rats with hippocampal lesions, like rats with sham lesions, were able to learn that food pellets would be delivered in one context and not another. These results are clearly consistent with those reported by Langston and Wood (2010) and Iordanova *et al*. (2009; see also Good & Honey, 1991). The results of Experiment 2 showed that hippocampal lesions did not affect the acquisition of a discrimination in which food pellets were delivered at one time of day but not another. These results are also consistent with Iordanova *et al*. (2009).

There are several important issues that are raised by the results presented in Chapter 2, one of which we will return to in the general discussion of Chapter 3 (section 3.6.). Namely, what is the nature of the cues that rats use in the temporal discrimination described in Experiment 2? Other questions pertaining to why some discriminations involving contexts (or times of day) are disrupted by hippocampal lesions while others are not, will be reserved

until Chapter 4, where the effects of lesions to the hippocampus on novel *what-where-when* discriminations are presented. I now proceed to Chapter 3, where the development of these new behavioural procedures is described.

Chapter 3: Configural *what-where-when* learning

3.1. Summary

The results from Chapter 2 demonstrated that rats can acquire simple, elemental discriminations involving contextual or temporal (time-of-day) cues, and that the acquisition of these discriminations was unaffected by lesions to the hippocampus. The purpose of the current chapter was to develop configural learning procedures involving the integration of these contextual (where) and temporal (when) cues that could then be used to assess the role of the hippocampus in such configural learning. Experiments 3 and 4 required rats to use information associated with the times of day (morning or afternoon) when they were placed in two contexts (A, spotted, or B, checked). Experiment 3 employed an appetitive reinforcer (food) and Experiment 4 an aversive reinforcer (footshock). The aversive procedure allowed for the assessment of the acquisition of the configural discrimination both through measurement of the conditioned response (i.e., inactivity or freezing) as well as modulation of the unconditioned response (i.e., post-shock activity bursts).

3.2. Introduction

As noted in Chapter 1, the ability of an animal to combine information from target stimuli with other non-target information is advantageous for survival: the deep purple berries on one variety of shrub might be nutritious while those on another might be poisonous, and drinking from a specific watering hole might be safe at daybreak but dangerous at dusk. One can demonstrate such integration through the ability of rats to acquire configural discriminations involving contexts. For example, rats can learn that when they are placed in one context (A; e.g., a chamber decorated with spotted wallpaper) presentations of a tone (X) are followed by food and those of a clicker (Y) are nonreinforced, whereas when they are placed in a different context (B; e.g., a chamber decorated with checked wallpaper)

presentations of Y are reinforced and those of X are not (e.g., Honey & Watt, 1999; Preston *et al*., 1986).

Most often configural discriminations involve stimuli that are present in an animal's immediate (external) environment. However, an animal that can only learn about such configurations is unlikely to survive as long as one that can make use of information that is (i) temporally or spatially remote from stimuli that are currently impinging on them (e.g., when fallen berries have scattered some distance from where they originated), or (ii) internally generated (e.g., when cloud cover means that there are no obvious visual cues about time of day). There is evidence that rats can form integrated configural memories involving stimulus traces (see Lin & Honey, 2010), and some evidence that is consistent with the possibility that rodents can learn configural discriminations involving time of day (e.g., O'Brien & Sutherland, 2007; Fellini & Morellini, 2013).

Rats can learn to choose the left choice arm in a T-maze rather than the right arm to gain food in the morning, and to do the reverse to gain food in the afternoon (e.g., Means *et al*., 2000a; see also, Means *et al*., 2000b; Thorpe *et al*., 2003). However, such observations simply require the cues that are correlated with different times of day to become linked to specific responses (e.g., turn left or right). Evidence that such (time of day) cues become configured with information from other sources comes from studies that have investigated whether rats can learn what happened where and when (Iordanova *et al*., 2008; see also, Iordanova *et al*., 2009, 2011ab; for a review, see Honey *et al*., 2014). In these studies, in the morning, placement in context A is associated with presentations of one auditory stimulus (X) and placement in context B is associated with a second auditory stimulus (Y); and in the afternoon the contexts in which the auditory stimuli are presented are transposed. The fact that these configurations have been encoded can be revealed by showing that pairing X with shock (at midday in a third context) results in more fear in the time-of-day+context

configurations in which X had been presented (morning+A and afternoon+B) than in the other configurations (morning+B and afternoon+A; see also, Cain, Ko, Chalmers & Ralph, 2004; but see, McDonald, Hong, Ray & Ralph, 2002).

A main aim of this chapter was to obtain rather more direct evidence that temporal information can be integrated with contextual information using standard configural training procedures (cf. Means *et al*., 2000ab) that allowed both an automated and relatively continuous measurement of the process of integration (cf. Iordanova *et al*., 2008). Assays that employ automated measures are useful insofar as they obviate the needs for time consuming video scoring or the experimenter being in the room when the rat is being tested; and continuous measures allow assessment of the rate at which integrated configural memories are formed in rats (cf. Clayton & Dickinson, 1998). To do this, two procedures were developed that investigated the capacity of rats to acquire configural discriminations in which critical components of the configurations were cues associated with the times of day (morning or afternoon) when animals were placed in two contexts, A and B. Experiment 3 employed an appetitive reinforcer (food) and Experiment 4 an aversive reinforcer (footshock). Each day, rats were placed in two contexts (A and B) in the morning and the same two contexts in the afternoon. In the morning, food (or shock) was presented in context A (but not in B) and in the afternoon food (or shock) was presented in context B (but not in A). The development of the appetitive configural discrimination (in Experiment 3) was assessed by the tendency of the rats to enter the foodwell when presented with the four configurations, and that of the aversive configural discrimination (Experiment 4) by the tendency of rats to suppress ongoing behaviour when presented with the same configurations. Experiment 4 also allowed an assessment of whether or not configural learning involving where and when footshock is delivered has the same behavioural sequelae as more standard forms of Pavlovian conditioning: where the immediate unconditioned response to shock (an activity burst; e.g., Fanselow, 1982) contrasts with the conditioned

response (freezing or inactivity), and the unconditioned response is modulated by the presence of an effective conditioned stimulus (cf. Wagner, 1981). Briefly, Wagner's (1981) model assumes that the formation of an association between the memory of a conditioned stimulus and an unconditioned stimulus allows the future presentation of the conditioned stimulus to place the unconditioned stimulus into a refractory state in which it is less likely to provoke its unconditioned response (e.g., an activity burst). We assessed such modulation by presenting shock in where+when configurations in which shock had either been delivered or not. A so-called conditioned diminution of the unconditioned response would be evident if the post-shock activity burst was less marked in the configuration in which shock had been delivered than in the configuration in which no shock had been delivered.

3.3. Design of Experiments 3 and 4

The design of Experiments 3 and 4 is shown in Figure 7. In Experiment 3, rats were placed in two contexts (A and B; a spotted and a checked chamber) in both the morning and afternoon. During the morning sessions, food pellets were delivered in A but not B, whereas in the afternoon sessions, food pellets were delivered in B but not A. Acquisition of this appetitive discrimination was assessed by recording the tendency of rats to approach the foodwell during the food-free periods at the outset of each of the four types of trial.

Experiment 4 used the same design but footshock was delivered in the morning in context A but not B, and in the afternoon footshock was delivered in context B but not A. Acquisition of this aversive discrimination was measured using an automated system that recorded the levels of general activity in footshock-free periods at the start of each of the sessions (cf. Lin *et al*., 2013). It was anticipated that the level of activity would be lower in the configurations paired with footshock (i.e., morning+A and afternoon+B) than those that were not (i.e., morning+B and afternoon+A). Using the same system, the levels of post-shock activity were measured. It is well established that the delivery of footshock can result in a

post-shock activity burst (e.g., Fanselow, 1982) and we assessed whether this burst changed over the course of training. Finally, footshock was presented in previously reinforced and nonreinforced configurations to assess whether the response to shock was modulated by whether the configuration had been paired with shock or not (cf. Wagner, 1981; see also, for example, Honey, Good & Manser, 1998a; Honey, Watt & Good, 1998b; Honey & Good, 2000).

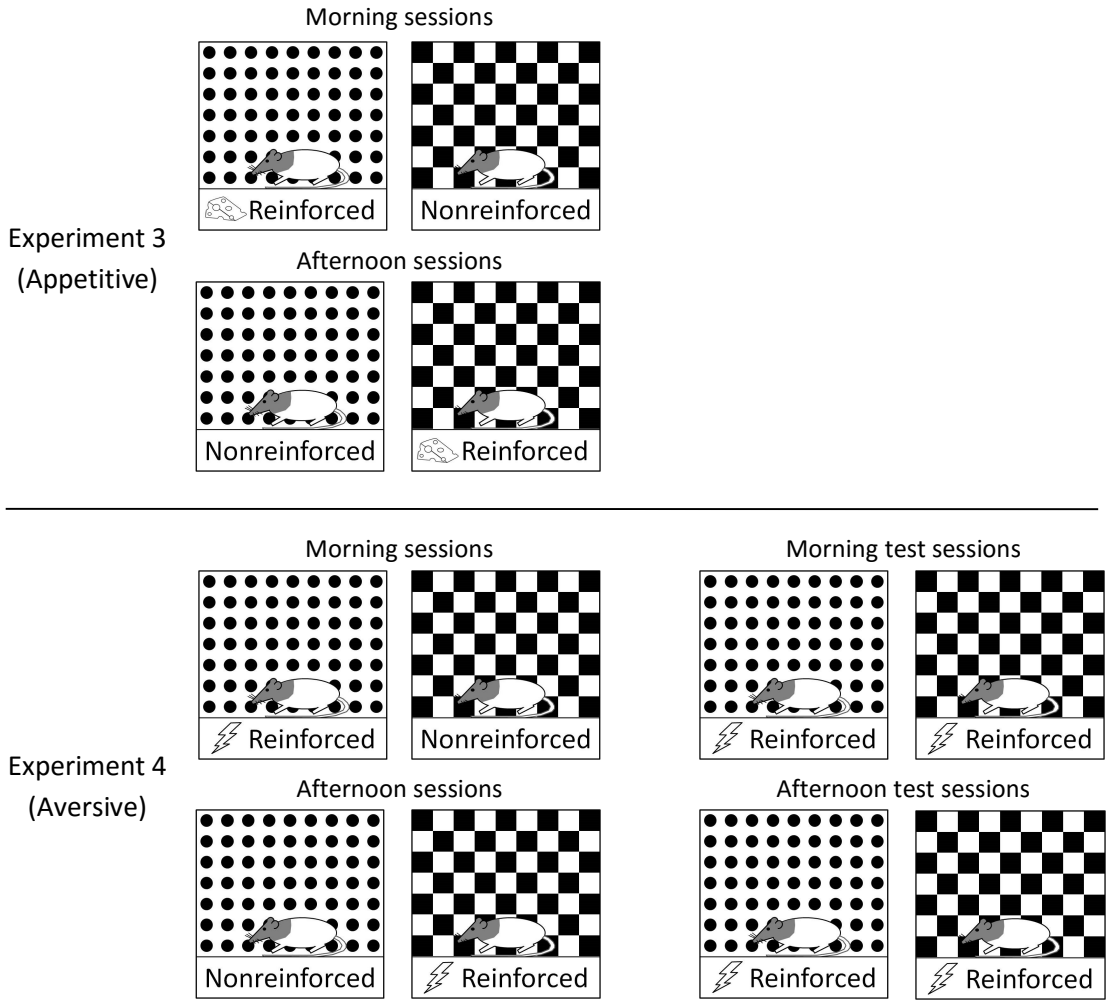

Figure 7. Design of Experiment 3 (upper panel) and Experiment 4 (lower panels): Rats received exposure to four context+time of day patterns daily. During morning sessions, reinforcement in the form of food pellets (Experiment 3) or footshock (Experiment 4) was delivered in one of the contexts (e.g. spotted+morning) and not in the other (e.g. checked+morning). This arrangement was reversed in the afternoon. Which configurations were reinforced was fully counterbalanced. In Experiment 4, on following test days, rats received test sessions in which footshock was presented in both contexts at both times of day.

3.4. Method

3.4.1. Subjects

Sixteen naïve male Lister hooded rats (*Rattus norvegicus*; supplied by Harlan Olac Ltd, UK) were used in each experiment. The rats were housed in the same way as in Experiments 1 and 2 and they were maintained at 85% of their *ad libitum* weight (Experiment 3: *M* = 298 g, range 278 - 314 g; Experiment 4: *M* = 287 g, range 275 - 311 g). The rats in both experiments were ≈ 3 months old at the start of the experiments. Behavioural training began at, approximately, 09:30 each day.

3.4.2. Apparatus

Experiment 3 employed the same apparatus and materials as Experiments 1 and 2, described in detail in Chapter 2 (Section 2.4.3.). Four operant chambers arranged in a 2x2 array were decorated with spotted (upper two chambers) or checked (lower two chambers) laminated paper. There was a foodwell in the left hand wall of each chamber into which food pellets could be delivered. A computer controlled the apparatus and recorded foodwell entries.

Experiment 4 used a set of eight operant chambers (30.5 cm×26 cm×20 cm; Test chamber 80004-D001; supplied by Campden Instruments Ltd., Loughborough, England) arranged in a 4×2 array that were positioned within sound-attenuating boxes. Each chamber had two aluminium sidewalls, a transparent Perspex back wall and a transparent Perspex ceiling. The front walls were also constructed from transparent Perspex and served as the doors to the chamber. The walls of the upper row of boxes were decorated with spotted wallpaper and those of the lower row were decorated with checked wallpaper. The chambers were illuminated by a 3-W light bulb, with a white plastic cover, positioned centrally and at 13.5 cm above the floor of the left aluminium wall. There was a stainless steel grid floor, constructed from 19 bars (diameter 0.47 cm, spacing from bar centre to bar centre 1.07 cm) to which a 0.5-s. 0.64 mA electric shock could be delivered using a scrambled

shocker (Campden Instruments Ltd. Model HSCK1000). Beneath the floor was a tray that was lined with absorbent paper. The level of rat activity in the chambers was measured using an ambulatory monitor (Campden Instruments Ltd. Model 80004 AM) that consisted of a horizontal strip attached to the back wall of the chamber and another to the front wall positioned 3.0 cm above the grid floor. These strips contained three infrared light sources and photo beam detectors that were located 3.0 cm from the left hand wall, in the centre of the chamber, and 3.0 cm from the right hand wall. Detection of the presence of the rat in the area covered by a photobeam followed by detection of the absence of the rat in this area, as the rat moved, was recorded as a value of 1. The number of times this occurred for each of the three beams gave a single value for the total movement made by the rat in the chamber. A computer (Mark II Control Unit) controlled the apparatus, operated the programs (using Behavioural Net Controller Control 1.0), and recorded ambulatory movement data (all equipment was supplied by Campden Instruments Ltd.).

3.4.3. Procedure

Experiment 3: Appetitive configuration discrimination

Behavioural training began once rats had been restricted to 85% of their free-feeding weights. In the same way as in Experiments 1 and 2, in two 10-min sessions conducted between 13:30 and 15:30, rats were first trained to retrieve food pellets from a foodwell in undecorated chambers. In the first session, the flaps were fixed in a raised position to allow rats ready access to the food pellets; and in the second session they were lowered and rats had to move them to gain access to the food pellets. During both sessions, the 20 food pellets were delivered in pairs on a variable time 60-s schedule.

Successful foodwell training was followed by 20 days of discrimination training. On each day, rats were placed in the two contexts (spotted and checked) in the morning and the same two visual contexts in the same sequence in the afternoon (spotted and then checked

for half of the rats, and checked and then spotted for the remainder). For a given training day, the order in which the contexts were presented for a given rat was consistent, but across days the order was pseudo-randomised with the constraint that there were no more than two consecutive days with the same order. Morning sessions took place between 09:30 and 11:30, and afternoon sessions took place between 16:30 and 18:30. Each context placement lasted 5 min, and there was a 5 min interval between the two morning and afternoon sessions during which rats were placed in a holding cage outside the testing room. In the morning, during placement in one context (e.g., spotted) two food pellets were delivered to the foodwell every 30s (resulting in a total of 20 pellets delivered per session), and during placement in the other context (e.g., checked) no food pellets were delivered. In the afternoon this arrangement was reversed. Which of the contexts was reinforced in the morning or the afternoon was fully counterbalanced. Following the end of each pair of sessions the rats were taken back to the colony room and replaced in their holding cages.

As in Experiments 1 and 2, to reduce the likelihood of rats learning that food would not be delivered for the first 30-s of any session, each cycle of 4 training days had the following structure. On the first two days of the cycle, on placement in the reinforced contexts, an additional 2 pellets were present in the foodwell. For the following two days of the cycle, no food pellets were present in the foodwell for the first 30-sec periods. The number of foodwell entries made by a rat during this first 30s reinforcer-free periods during the second pair of training days in each cycle was used to assess the acquisition of the discrimination. Two measures of learning were employed. A discrimination ratio (DR) in which the rate of foodwell entries per minute during reinforced sessions (e.g. spotted+morning) was divided by the combined number of entries per minute during reinforced and nonreinforced sessions (e.g. spotted+morning, and checked+morning), for each morning and afternoon session. Using this ratio, a score of 0.50 indicates that the number of foodwell entries was the same during the reinforced and nonreinforced sessions,

whereas scores greater than 0.50 indicate that responding was greater during reinforced than nonreinforced sessions. The second measure was the raw rates of responding on the reinforced and nonreinforced trials.

Experiment 4: Aversive configuration discrimination

To acclimate rats to the procedure, on both of the first two days each rat was placed into an undecorated operant chamber for ≈ 20 min. The lights in the testing room were turned off and the house light in each chamber was illuminated for 20-min once each squad of 8 rats had been placed in the chambers. These sessions, conducted between 12:00 and 14:00, were followed by 12 days of training. On each day, rats were placed in the two contexts (spotted and checked) in the morning and afternoon in the same sequence (spotted and then checked for half of the rats, and checked and then spotted for the remainder). As in Experiment 3, across days the sequence in which rats were placed in the two contexts varied according to a pseudo-randomised sequence. Each exposure to a context lasted for 3 min and there was an interval of 5 min between the pairs of exposures (to the spotted and checked contexts) in the morning and afternoon. In the morning, during exposure to one of the contexts (e.g. spotted) two mild electric shocks were delivered through the grid floor of the chamber, one after the first minute and another after the second minute of the 3- minute session. During exposure to the other visual context (e.g. checked) no shocks were delivered. In the afternoon this arrangement was reversed (e.g. shocks were delivered in the checked but not in the spotted context). Note that in Experiment 4 there were no sessions on which shock was presented immediately after the rats entered the chamber because such presentations disrupt fear conditioning (Fanselow, 1986). Details of the procedure that have not been mentioned were the same as for Experiment 3.

To assess acquisition of the configural discriminations, a discrimination ratio (DR) was used: activity during first 30s of session without shocks (e.g., checked+morning) divided

by the combined activity during the first 30s of both sessions at that time of day (e.g., spotted+morning, and checked+morning). Using this measure, a score of 0.50 indicates that rats were equally active in the context in which shock is delivered and the context where no shocks were delivered, and scores above 0.50 indicate rats are more active in sessions without shocks than with shocks. As in Experiment 3, the raw rates of responding on the reinforced and nonreinforced trials were also reported. In addition, we assessed the impact of the shocks themselves by measuring activity in the 30-s period that immediately followed shock presentation, and during the same time periods in the corresponding sessions where no shocks were delivered.

On the thirteenth day, rats were again placed in the two contexts at both times of day, but in every session two shocks were delivered to the grid floor (one after 1 min and the other after 2 min). Activity was measured during the 30s immediately following each shock presentation. Our analysis focused on the impact of the first presentations of shock in the two morning sessions, because the rats became inactive in both contexts in the afternoon.

3.5. Results

3.5.1. Experiment 3: Appetitive configuration discrimination

The upper panel of Figure 8 depicts the mean of the morning and afternoon discrimination ratios for the ten days of training on which food was not presented in the first 30-sec periods (i.e., days 3, 4, 7, 8, 11, 12, 15, 16, 19, 20). Inspection of this figure indicates that the discrimination ratios increased across training. ANOVA confirmed that there was a main effect of day ($F(9, 135) = 2.10$, $p < 0.05$), and subsequent one-sample t tests revealed that the discrimination ratios were significantly different from 0.50 on days 8, 16, 19 and 20 (smallest $t(15) = 2.21$, $p < 0.05$). The lower panel depicts the rates of responding on reinforced and nonreinforced trials that were used to calculate the ratios. It is evident that there was a marked difference in the rates of responding on the reinforced and nonreinforced trials by the final stages of training. ANOVA revealed a main effect of day, $F(9, 135) = 4.02$, $p < 0.01$, a main effect of trial type, $F(1, 15) = 10.88$, $p < 0.05$, and no interaction between these factors, $F(9, 135) = 1.85$, $p = .065$. There was a significant difference between the rates of responding on reinforced and nonreinforced trials on days 8, 15, 16, 19 and 20 (smallest $t(15) = 2.39$, $p < 0.05$). These results demonstrate that rats can learn an appetitive configural discrimination where the configuration of the context (spotted or checked) and time of day (morning or afternoon) indicates whether or not food will be delivered.

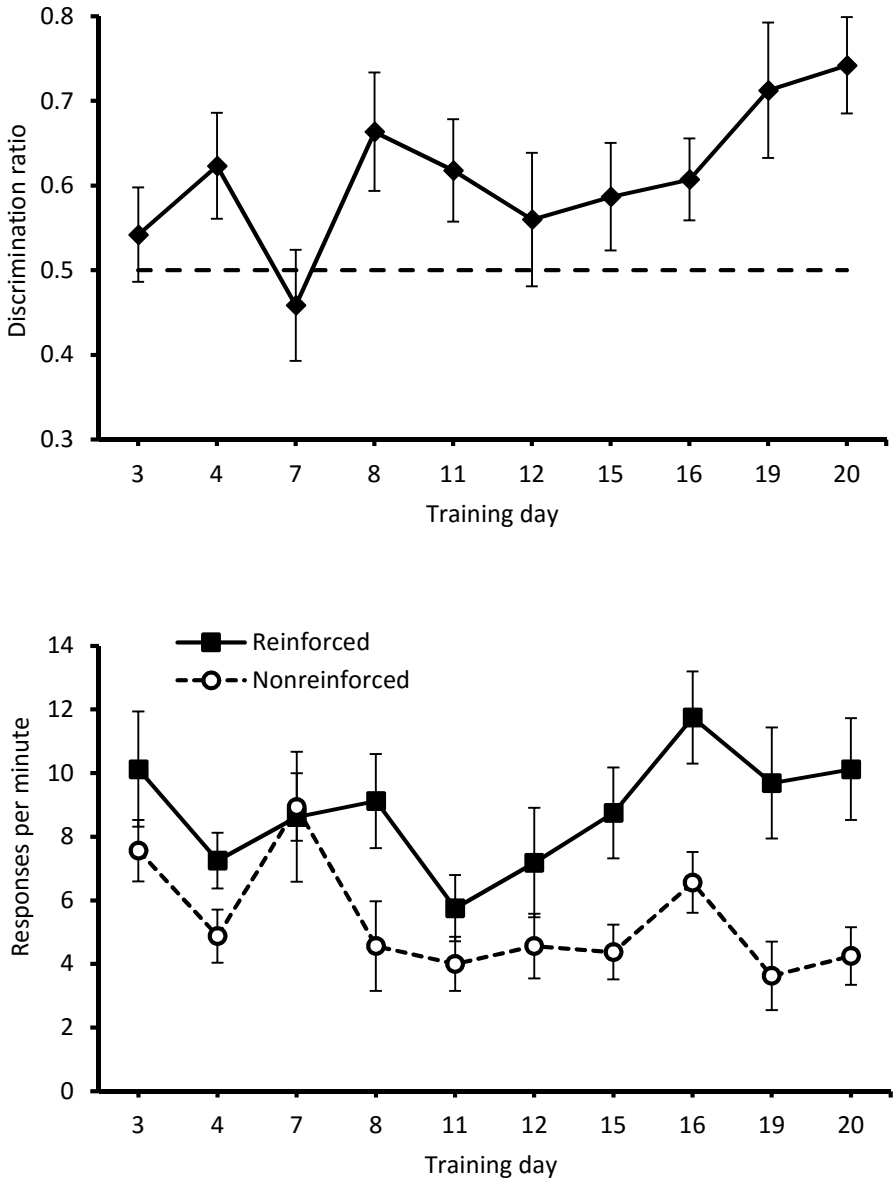

Figure 8. Experiment 3: The upper panel shows the mean discrimination ratios (±SEM) across the training days on which no food was presented for the first 30 s in any of the configurations (i.e., days 3, 4, 7, 8, 11, 12, 15, 16, 19 and 20). These ratios are derived from the food-free 30-sec periods at the start of the sessions. The lower panel shows the mean rates of responding (±SEM) on reinforced and nonreinforced trials that were used to calculate the ratios.

3.5.2. Experiment 4: Aversive configuration discrimination

The upper panel of Figure 9 shows the discrimination ratios for the six, two-session blocks of training. ANOVA revealed a significant main effect of block ($F(5, 75) = 4.04$, $p < .005$), with the discrimination ratios being significantly higher than 0.50 on blocks 3 and 5 (smallest $t(15) = 2.29$, $p < .05$). The fact that the discrimination ratios are below 0.50 on the first block ($t(15) = 4.27$, $p < 0.005$, suggests that the recent presentation of shock reduces activity in an immediately succeeding session, or the recent presentation of no shock increases activity in a succeeding session. These effects of trial sequencing oppose the pattern of behaviour that would be expected on the basis of rats learning the configural discrimination. The lower panel of Figure 9 depicts the rates of responding on reinforced and nonreinforced trials that were used to calculate the ratios. It is evident over the course of training there was a gradual increase in activity, perhaps reflecting the conditioned modulation in responding to footshock (see below), and that after the first block of training there was less activity on the reinforced than the nonreinforced trials. ANOVA revealed no significant effect of block, $F(5,75) = 2.14$, $p = 0.07$, a main effect of trial type, $F(1, 15) = 12.04$, $p < .005$, and an interaction between these factors, $F(5, 75) = 4.49$, $p < .005$. There were differences in responding between the trial types on blocks 1, 3 and 4 (smallest $t(15) = 3.21$, $p < .05$). These results complement those from Experiment 3 in confirming that rats can learn a configural discrimination where the configuration of the context (spotted or checked) and time of day (morning or afternoon) indicates whether or not shock will be delivered.

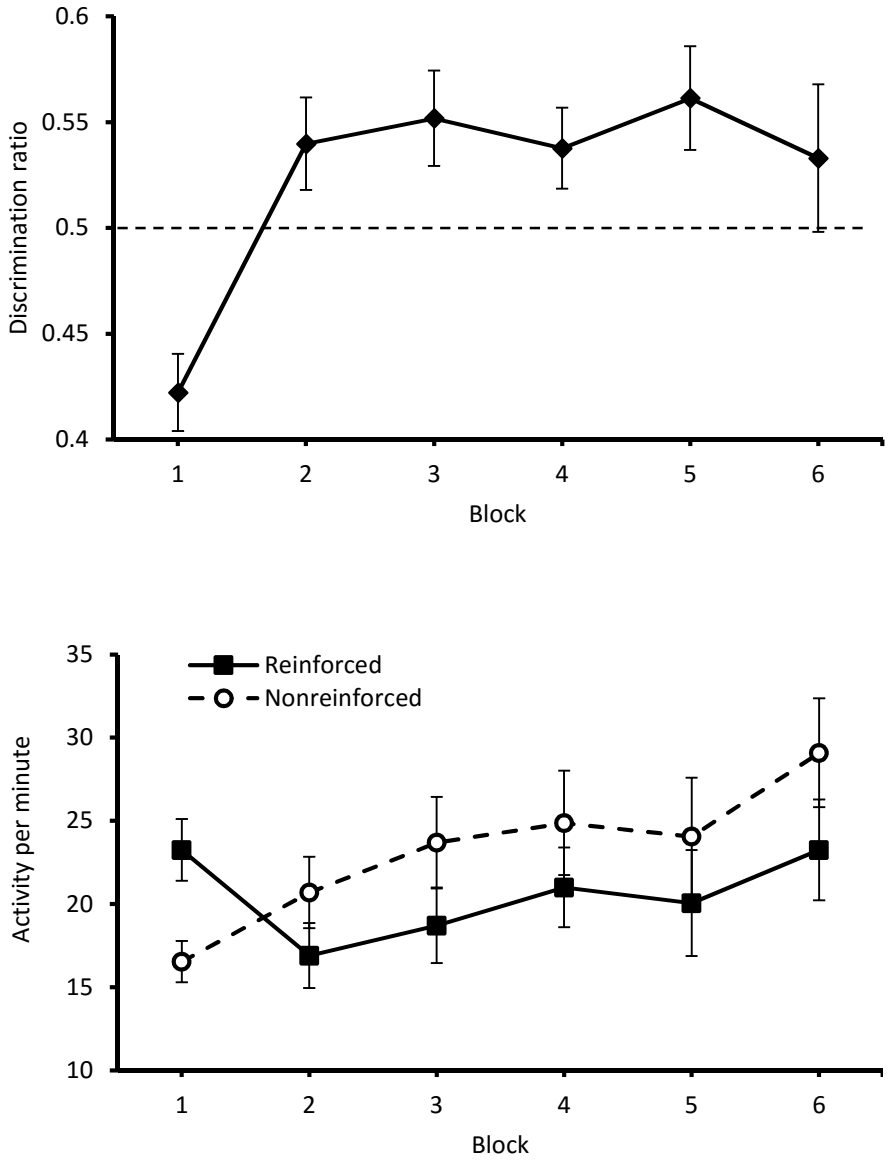

Figure 9. Experiment 4: Mean discrimination ratios (±SEM) across the six, two-day blocks of training. These ratios are derived from the footshock-free 30-sec periods at the start of the sessions. The lower panel shows the mean rates of activity (±SEM) during the reinforced and nonreinforced trials that were used to calculate the ratios.

3.5.3. Experiment 4: Post-shock activity during training

The left-hand panel of Figure 10 shows the levels of post-shock activity in the 30-sec periods after the presentation of the two footshocks in each of the six, two-session blocks of training; and the activity levels in the corresponding periods when shock was not delivered. Inspection of this figure suggests that there was a general increase in activity across training, and that the presence of shocks increased activity in the post-shock period (relative to the equivalent period in the context+time of day configurations where no shock was present). There was also some indication that the second shock increased activity more than the first shock, and that in the nonreinforced configurations there was a reduction in activity between the corresponding first and second periods within a session. These impressions were confirmed by an ANOVA, which revealed a significant main effect of shock ($F(1, 15) = 151.49$, $p < .001$), no effect of shock number ($F(1, 15) = 1.96$, $p > .05$), and an effect of block ($F(5, 75) = 17.31$, $p < .001$). There was an interaction between shock and shock number ($F(1, 15) = 22.04$, $p < .001$), an interaction between shock and block ($F(5, 75) = 4.55$, $p < .005$), no interaction between block and shock number, and no three-way interaction (largest $F(5, 75) = 1.43$, $p > .05$). A secondary ANOVA on the reinforced trials revealed an effect of shock number ($F(1, 15) = 11.12$, $p < .01$), an effect of block ($F(5, 75) = 8.08$, $p < .001$), and no interaction between these factors ($F < 1$). A parallel ANOVA for the nonreinforced trials revealed an effect of block ($F(5, 75)= 20.83$, $p<.001$), period within session ($F(1, 15)=6.43$, $p<.05$), and no interaction ($F(5, 75)=1.41$, $p>.05$).

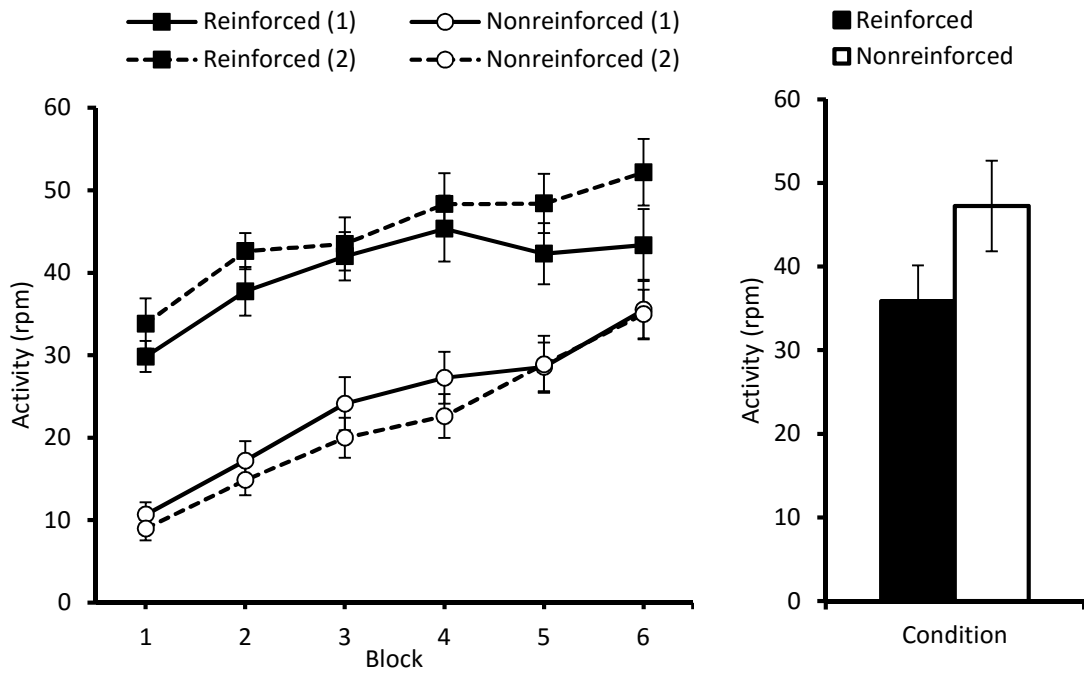

Figure 10. Experiment 4: Mean rates of activity (in responses per minute; ±SEM) during the two periods (1 and 2) that immediately followed the two footshocks on reinforced trials, and during equivalent periods (1 and 2) on nonreinforced trials (left panel); and mean rates of post-shock activity during a morning test where footshocks were presented in the previously reinforced and nonreinforced configurations (right panel).

3.5.4. Experiment 4: Post-shock activity during the test

The mean levels of activity during the 30s following the first footshock in each of the morning sessions of the test day are shown in the right-hand panel of Figure 10. The levels of activity were significantly greater when the footshock was presented in the previously nonreinforced context+time of day configuration than in the reinforced configuration, $t(15) = 2.22$, $p < 0.05$. These results, involving the modulation of the unconditioned response provoked by shock, provide a second assay for the acquisition of the configural discrimination, and suggest one explanation for the loss of this discrimination in the later blocks of training: a reduction in the effectiveness of shock as training progressed (cf. Wagner, 1981).

3.6. Discussion

Two behavioural procedures are described that provide automated measurements of the acquisition of configural discrimination involving time of day and contextual cues. One procedure used an appetitive reinforcer and approach of the foodwell as the conditioned response in conventional operant chambers; and the second procedure employed an aversive reinforcer and activity as the conditioned response in chambers equipped with an ambulatory monitor. Both procedures produced robust changes in behaviour (cf. Means *et al*. 2000ab; Thorpe *et al*., 2003) similar to those seen when rats are given discriminations where the configurations of contextual with auditory cues signalled whether or not food would be delivered (e.g., Honey & Watt, 1999).

There are two specific aspects of the results that are worth dwelling on: The difficulty of the two configural discriminations and the nature of the cues correlated with times of day that the rats might have used. An animal that can rapidly form episodic-like (*what-where-when*) memories (cf. Clayton & Dickinson, 1998) should be well placed to acquire configural discriminations that require them to learn where and when food or shock are delivered. However, the appetitive and aversive discriminations in Experiments 3 and 4 took many days to emerge. This observation is consistent with results reported by Iordanova *et al*. (2008, 2009, 2011ab), in which rats were exposed to four configurations (morning+contextA+tone, morning+contextB+clicker, afternoon+contextA+clicker, afternoon+contextB+tone) on each of four days before the critical test. That is, in neither type of procedure was there evidence that the configural memories developed particularly rapidly (cf. Honey & Watt, 1999). One could argue that the procedures were not sensitive to capturing the rapid acquisition of configural memories. This argument seems reasonable in the context of Experiment 3, but the procedure used in Experiment 4 was sensitive to observing some differences early in training. The most economical interpretation of these differences was that they reflected the

fact that early in training the presentation of shock (or no shock) in the first of a pair of sessions (at a given time of day) directly affected performance in the second session (at that time of day). However, the observation that rats can learn configural discriminations involving cues associated with different times of day raises the issue of which cues provide a basis for this capacity (see Means *et al*., 2000a). The following supplementary analyses bear on some of them.

3.6.1. Supplementary analyses

The facts that the procedures were conducted in a quiet experimental room, within a quiet laboratory, with weekly (not daily) changes to bedding means that it is unlikely that there were auditory cues that distinguished between morning and afternoon sessions. However, a related possibility is that there were odour cues that distinguished between these sessions. For example, for the first two squads of rats in the morning the chambers might have a different odour than when they re-enter the same chambers in the afternoon, after other squads of rats have been in the chambers. This form of account seems less plausible for later squads of rats. For these rats the odours in the chambers in the afternoon are likely to be similar to those present in the morning, having been previously occupied by the same squads of rats in both sessions. In any case, the prediction that the discrimination ratios for rats tested earlier in the day should be better than those tested later in the day was not borne out in the data. For the final four test days of Experiment 3 (15, 16, 19, 20) the discrimination ratios for the first two (n = 8) and last two squads (n = 8) were 0.66 and 0.66, respectively; and for the final three blocks of training in Experiment 4 the corresponding scores were: Squads 1 and 2 (n = 8) = 0.55, and Squads 3 and 4 (n = 8) = 0.54. ANOVA with squad and experiment as factors revealed an effect of experiment ($F(1, 28) = 9.00$, $p < .01$), no effect of squad ($F < 1$) and no interaction between the two factors ($F < 1$). These four discrimination ratios were each different from 0.50 (smallest $t(7) = 2.43$, $p < .05$), and they provide little

support for the contention that differences in odours (generated by the rats) between the morning and afternoon were used to discriminate between the different times of day.

A different possibility is that the rats' behaviour was based on some form of alternation strategy. For example, they might have learned that if food (or shock) was presented in the spotted box on the immediately preceding trial then it would be presented in the checked box on the next trial. If this was the case, then one might predict that discriminative performance would be superior when the immediately preceding reinforced trial occurred within a day (i.e., in the morning) than across days (i.e., in the afternoon of the previous day). However, in Experiment 3 a paired *t*-test conducted on the pooled discrimination scores from the final 4 test days (15, 16, 19, and 20) revealed no significant difference between the morning sessions (.67), when the preceding reinforced trial was on the previous afternoon, and afternoon sessions (.66), for which the preceding reinforced trial was in the morning ($t(15) = 0.16$, $p > .87$). Similarly, in Experiment 4 a paired *t*-test conducted on the pooled scores for the final 3 blocks of training revealed no significant difference between the discrimination ratios in the morning (.53) and afternoon (.55) sessions ($t(15) = 0.77$, $p > .45$).

There are other potential mediators of discriminative performance in Experiments 3 and 4. Thus morning and afternoon sessions might be correlated with differences in either the rats' state of food deprivation or arousal, and they might encode cues correlated with these differences. In keeping with this possibility, in Experiment 3 the overall levels of magazine entries during the reinforcer-free periods at the start of the sessions was higher in the afternoon (7.9 rpm) than in the morning (6.2 rpm; $F(1,15) = 15.48$, $p < .05$); and this difference was also evident during the final four test sessions (15, 16, 19, 20) when the discrimination was most evident (afternoon = 8.4 rpm and morning = 6.4 rpm; $F(1,15) = 5.07$, $p < .05$). However, time of day ratios (rate of foodwell entries in the afternoon divided by the

rate of entries at both times of day) did not correlate with configural discrimination ratios when both were pooled across the entire course of training (r = −.01, p = .98), and there was a negative correlation between time of day ratios and discrimination ratios during the final four test sessions (r = −.531, p < .05): Rats that were more adept at responding to the reinforced than the nonreinforced configurations were less likely to show different overall levels of responding in the morning and afternoon (see Figure 11). The rates of activity during the corresponding periods in Experiment 4 were similar in the morning and afternoon when pooled across all sessions of training (AM = 21.2 rpm and PM = 22.5 rpm, F(1, 15) = 1.28, p > .05), but these rates were higher in the afternoon (26.2 rpm) than the morning (21.3 rpm) in the final 3 blocks of training (F(1, 15) = 10.85, p < .05). Again, however, time of day ratios for all sessions did not correlate with the corresponding configural discrimination ratios, that were not statistically different from chance as a group (r = .45, p = .079); and there was no correlation between these two types of ratios for the final three blocks of training (r = .22, p = .41; see Figure 11). These results provide little support for the idea that rats encoded time of day in terms of their relative deprivation state or level of arousal, at least insofar as the behavioural measures provided a sensitive assay of such processes.

Another possibility is that internal biological rhythms entrained to cues associated with the light cycle in the laboratory provided the basis for differentiating the morning and afternoon sessions. To evaluate this possibility, as opposed to accepting it by default, would require further experimental work to be conducted in which the light cycles were reversed or shifted between training and testing. For example, one could delay the onset of the vivarium lights by several hours so that rats are placed in the chambers at the same time as the afternoon session, but only one hour after the onset of the vivarium lights. If the critical (internal) cue for time of day is related to the light cycle in the vivarium, then this manipulation should result in behaviour indicative of the morning sessions, but in the

afternoon. However, the light cycle is only one of the potential zeitgebers in a laboratory (e.g., Aschoff, 1984).

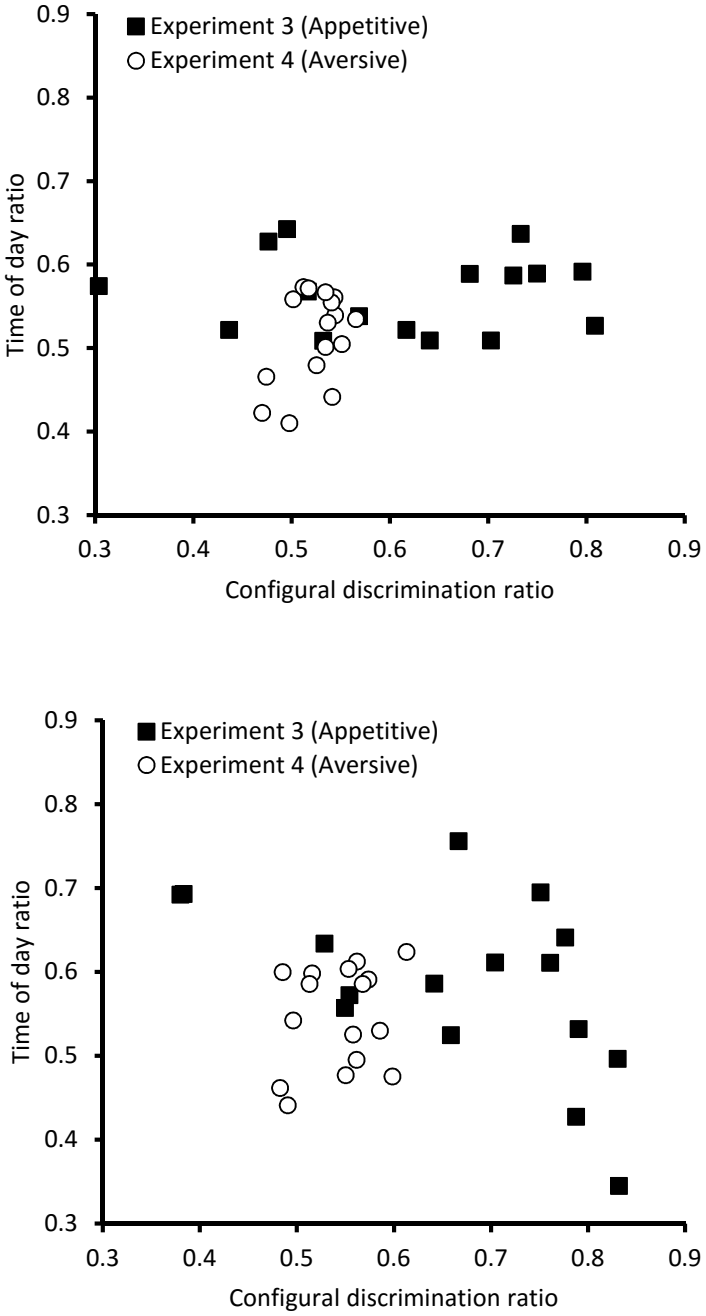

***Figure 11*.** Experiments 3 and 4: Relationship between time of day discrimination ratios and configural discrimination ratios over the entire course of training (upper panel) and for the final sessions of training (lower panel; see text for further details).

3.6.2. Summary

The novel procedures described in this chapter provide automated measures of the acquisition of configural discriminations involving where and when appetitive and aversive events were presented. The results demonstrate that, as with other forms of simple conditioning, this learning takes the form of changes in conditioned responding (i.e., inactivity) that do not necessarily match the immediate unconditioned response to the unconditioned stimulus (i.e., hyperactivity; Experiment 4). Also, learning is not only evident as conditioned changes in behaviour to the configural stimuli, but also the capacity of these stimuli to modulate unconditioned responding. I proceed now to chapter 4, in which I present experiments investigating the performance of rats with hippocampal lesions in these configural episodic-like tasks.

Chapter 4: The role of the hippocampus in configural *what-where-when* learning

4.1. Summary

In Chapter 3, two configural learning procedures were developed. Both procedures showed that rats are capable of acquiring configural discriminations involving where and when food (or shock) will be delivered. It has been suggested that the hippocampus is critical for configural learning, with recent evidence suggesting that this involvement might be particularly apparent when the configurations involve the components of episodic memory. The experiments in the current chapter investigated the performance of rats with lesions of the hippocampus in the configural learning procedures developed in Chapter 3.

4.2. Introduction

Many theorists have argued that the hippocampus is involved in associating or binding together the different elements that make up patterns of stimulation (e.g., Ergorul & Eichenbaum, 2004; O'Reilly & Rudy, 2001; Rudy & Sutherland, 1989, 1995). For example, configural learning theory (Rudy and Sutherland, 1989, 1995) posits a critical role for the hippocampus in the formation of distinct configural units that represent specific patterns. Original support for this idea comes from the finding that rats with hippocampal lesions could not solve a negative patterning discrimination (Rudy & Sutherland, 1989). In such discriminations the presentation of either a light (L) or a tone (T) is reinforced but the light-tone compound stimulus (LT) is nonreinforced; and rats come to show less conditioned responding to the compound than the elements. The acquisition of this discrimination cannot be solved on the basis of learning about the individual elements (L and T) and requires the animals to encode the compound (LT) as different to the sum of its parts; otherwise, responding to the LT compound should be greater than to L and T (see Rescorla & Wagner,

1972). As mentioned in Chapter 1, however, configural learning is not always disrupted by lesions to the hippocampus (e.g., Coutureau *et al*., 2002; Davidson, McKernan & Jarrard, 1993; Gallagher & Holland, 1992).

Episodic memory can be thought of as a special kind of configural memory, involving learning about "what" happened "where" and "when" (Tulving, 2002). There is abundant evidence linking the human medial temporal lobe, and particularly the hippocampus, to episodic memory (Burgess, Maguire & O'Keefe, 2002; Eichenbaum & Cohen, 2001; Tulving & Markowitsch, 1998; Vargha-Khadem, Gadian, Watkins, Connelly, Paesschen & Mishkin, 1997). Consistent with these findings, the results from animal studies have implicated a crucial role for the non-human hippocampus in the integration of spatial/contextual and temporal information (Aggleton & Brown, 1999; Eichenbaum & Fortin, 2003; Ergorul & Eichenbaum, 2004; Iordanova *et al.,* 2011). For example, rats with hippocampal lesions make more errors than normal rats in a radial arm maze task, repeatedly reentering previously visited arms (Cassel *et al*., 1998; Jarrard, 1993; Olton, Becker & Handelmann, 1979). Also, in a spontaneous exploration task, Li and Chao (2008) found that rats with electrolytic lesions in the dorsal CA3 did not demonstrate learning about integrated spatio-temporal information. However, these same animals showed no impairments in the temporal or spatial aspects of the task when tested in isolation (Li & Chao, 2008). Other studies have also highlighted hippocampal involvement in configural learning tasks when so-called elemental learning about the same components has been unimpaired (Eacott & Gaffan, 2005; Iordanova *et al*., 2009; Langston & Wood, 2010; Li & Chao, 2008; Save, Poucet, Foreman & Buhot, 1992).

Evidence that is directly relevant to the claim that the hippocampus is involved in configural *what-where-when* memory comes from studies that have used variants of sensory preconditioning procedures. Iordanova *et al*., (2009, 2011) found that rats with hippocampal

damage were unimpaired in learning what happened either where or when (see also Chapter 2), but were impaired in configural learning involving where and when. In these studies, rats received four training trials daily in which they were placed in two contexts (A and B) at two times of day (morning and afternoon) and were presented with two auditory stimuli (tone and click). In one elemental procedure, one auditory stimulus (e.g. the tone) was presented in context A and the other auditory stimulus (e.g. the click) was presented in context B, irrespective of the time of day. In the second elemental procedure, one auditory stimulus (e.g. the tone) was always presented in the morning and the other auditory stimulus (e.g. the click) was presented in the afternoon, irrespective of whether they were in context A or B. Following four days of training, rats were placed in a third context (C) at midday where one of the auditory stimuli (e.g. the tone) was paired with footshock. On subsequent test days, rats were placed back into contexts A and B at both times of day (morning and afternoon) but no auditory stimuli were presented. In the first elemental procedure there was more freezing in context A than in context B irrespective of the time of day, whereas in the second elemental procedure there was more fear in the morning than in the afternoon irrespective of the context in which they were placed. This pattern of results was seen in both normal rats and rats with hippocampal lesions. These results suggest that both groups of rats could acquire context-auditory stimulus associations and time of day-auditory stimulus associations, and were able to integrate this knowledge with auditory stimulus-shock associations. These results are mirrored in the results from Chapter 2, which used more conventional conditioning procedures to assess the development of associations involving contexts and times of day.

In marked contrast to the results described in the immediately preceding paragraph, Iordanova *et al*. (2009, 2011) demonstrated that when test performance required that configural processes were involved, rats with lesions to the hippocampus were impaired. In the training trials for the configural versions of the task, in the morning, one of the auditory

stimuli (e.g. tone) was presented in context A but not in B, and the other auditory stimulus (e.g. click) was presented in context B but not in A; and these arrangements were reversed in the afternoon. Following tone-shock pairings in a third context at midday, the rats were exposed to test trials in both contexts at both times of day without presentation of either of the auditory cues. Normal rats showed greater levels of freezing in the configurations in which the tone had been played in training trials (e.g. context A+morning and context B+afternoon) than in the configurations in which the click had been played in training trials (e.g. context B+morning and context A+afternoon), and in doing so, these rats demonstrated learning about the four training configurations. Rats with hippocampal lesions were equally likely to show freezing in each of the configurations.

In the Iordanova *et al*. (2009, 2011ab) experiments there are a number of different learning processes that the hippocampus could be influencing. For example, to exhibit evidence of configural learning at test rats not only had to encode the configural representations in the first stage, but also to link one of the constituent components with footshock in the second stage, and integrate or update the previously acquired configural representations with this new information. The disruption to any of these processes might affect test performance. While it might seem appealing to suggest that the original encoding of the configurations was disrupted (e.g. Rudy & Sutherland, 1989) there are reasons to be cautious before accepting this suggestion. First, some types of configural learning are not disrupted in rats with hippocampal lesions (e.g. Coutureau *et al*., 2002; Davidson, McKernan & Jarrard, 1993; Gallagher & Holland, 1992). Second, Iordanova *et al*. (2011b) showed that temporarily disrupting hippocampal function during the second, aversive conditioning stage was sufficient to produce a deficit in test performance. The implication of these two observations is that it would be premature to assume that the original encoding of what happened where and when was disrupted during the first stage of training in the studies reported by Iordanova *et al*. (2009, 2011). In this chapter I sought to assess the role of the

hippocampus in the acquisition of *what-where-when* configural representations by making use of the two procedures developed in Chapter 3. In Experiments 5 and 6 I assessed the performance of rats with hippocampal lesions in a task requiring the acquisition of an appetitive configural discrimination involving the time of day (morning or afternoon) they were placed in one of two contexts (A or B), and in Experiment 7 I employed the formally equivalent aversive conditioning procedure. If the hippocampus plays a (general) role in the encoding of spatio-temporal configurations then the acquisition of these two discriminations should be disrupted in rats with lesions of the hippocampus.

4.3. Design of Experiments 5, 6 and 7

The design of Experiments 5 and 6 was the same as Experiment 3 and the design of Experiment 7 was the same as Experiment 4 (see Figure 7, Chapter 3). There were two groups in each experiment: group Hippocampal comprised rats with lesions of the hippocampus and group Sham comprised rats with sham control lesions. In Experiments 5 and 6, rats were placed in two contexts (A and B; a spotted and a checked chamber) in both the morning and the afternoon. During the morning sessions, food pellets were delivered in A but not B, whereas in the afternoon sessions, food pellets were delivered in B but not A. Acquisition of this appetitive discrimination was assessed by recording the tendency of rats to approach the foodwell during the food-free periods at the outset of each of the four types of trial. Experiment 7 used the same design but footshock was delivered in the morning in context A but not B, and in the afternoon footshock was delivered in context B but not A. Acquisition of this aversive discrimination was measured using an automated system that recorded the levels of general activity in footshock-free periods at the start of each of the sessions.

4.4. Method

4.4.1. Subjects

Twenty-seven naïve male Lister Hooded rats (*Rattus norvegicus*; supplied by Charles River, UK) were used in Experiment 5. These rats were approximately 8 months old at the start of the experiment (mean weight = 454g). Thirty-two naïve male Lister Hooded rats (*Rattus norvegicus*; supplied by Harlan, UK) were used in Experiment 6 (mean weight = 321g), and thirty-two were used in Experiment 7 (mean weight = 370g). These rats were approximately 3.5 months old at the start of the experiments. All rats were housed in pairs in the same way as in Experiments 1-4 and they were maintained at 85% of their *ad libitum* weights.

4.4.2. Surgery and histology

In Experiments 5, 6 and 7, surgery was conducted identically to surgery for Experiments 1 and 2 (Chapter 2, Section 2.4.2.). Briefly, rats were anaesthetised, placed in a stereotaxic frame and the bone above the area to be lesioned was removed. Rats in group Hippocampal (Experiment 5: n = 14; Experiments 6 and 7: n = 16) received infusions of ibotenic acid. Table 1 (Chapter 2) shows the coordinates and volumes used. Rats in group Sham received identical treatment to rats in group Hippocampal except that no infusions were made and, instead, a needle was used to perforate the dura. Rats recovered for a minimum of 14 days and their preoperative weights were restored before behavioural training began.

Histological procedures were identical to those described for Experiments 1 and 2 (Chapter 2, Section 2.4.5.).

4.4.3. Apparatus

Experiments 5 and 6 were carried out with the same apparatus as that used for Experiments 1, 2 and 3. Four operant chambers were decorated with spotted or checked laminated paper.

There was a foodwell in one side of each chamber into which food pellets could be delivered. A computer controlled the apparatus and recorded foodwell entries.

Experiment 7 was carried out with the same apparatus as that used for Experiment 4. Eight operant chambers were decorated with spotted or checked laminated paper. The floor of each chamber was a stainless steel grid through which a scrambled shocker could deliver a mild electric shock. Infrared light sources and photobeam detectors connected to a computer were used to detect and record ambulatory movement data.

4.4.4. Procedure

Experiments 5 and 6: Appetitive configuration discrimination

In Experiment 5, both group Hippocampal (n = 14) and group Sham (n = 13) underwent an identical procedure to the rats in Experiment 3 (Chapter 3, Section 3.4.3.), except that training continued for 24 days. Following two days of successful magazine training, behavioural training commenced. On each day, rats were placed in two contexts (spotted and checked) in the morning and the same two contexts in the afternoon. In the morning sessions, food pellets were delivered to the foodwell in one of the contexts (e.g. spotted) and not in the other (e.g. checked), and in the afternoon sessions this arrangement was reversed. The ability of rats to discriminate between the four configurations was assessed by recording the number of entries to the foodwell in the 30-s reinforcer-free period at the start of each reinforced and nonreinforced sessions. Data were not recorded from the first two days in every cycle of four training days because on these days two additional food pellets were present in the foodwell at the start of each reinforced session. As in Experiments 1-3, these four-day cycles were used to reduce the likelihood that rats would learn that no food pellets were presented in the first 30s of any trial. To assess the acquisition of the discriminations a discrimination ratio (DR) was used: foodwell entries per minute during reinforced sessions (e.g. spotted+morning) divided by the combined number of entries per

minute during reinforced and nonreinforced sessions (e.g. spotted+morning, and checked+morning). Rats in Experiment 6 (group Hippocampal, n = 16; group Sham, n = 16) also underwent this procedure and had 20 days of training.

Experiment 7: Aversive configuration discrimination and response to footshock

In Experiment 7, both group Hippocampal (n = 16) and group Sham (n = 16) underwent an identical procedure to the rats in Experiment 4 (Chapter 3, Section 3.4.3.), except training continued for 24 days and test sessions were conducted on days 25 and 26. Before training began there were two days on which rats were placed in an undecorated operant chamber for 20 min, in order to acclimate the rats to the procedure. On each of the 24 days of training, rats were placed in two contexts (spotted and checked) in the morning and the same two contexts in the afternoon. In morning sessions, during exposure to one of the contexts (e.g. spotted), two mild electric shocks were delivered through the grid floor of the chamber, one after the first minute and another after the second minute of the 3-minute session. During exposure to the other context (e.g. checked), no shocks were delivered. In afternoon sessions this arrangement was reversed. To assess acquisition of the configural discriminations during training, a discrimination ratio (DR) was used: activity during first 30s of session without shocks (e.g., checked+morning) divided by the combined activity during the first 30s of both sessions at that time of day (e.g., spotted+morning, and checked+morning). Activity levels immediately following each shock presentation in reinforced sessions, and the corresponding period in nonreinforced sessions were also recorded. This enabled assessment of the unconditioned response to footshock over the course of training. On days 25 and 26, footshock was presented in both "reinforced" and "nonreinforced" configurations. These "test" presentations of footshock allowed assessment of the response when footshock presentation conflicted with any retrieved configural memories acquired during training.

4.5. Results

4.5.1. Histology

Inspection of the cell loss in the rats with hippocampal lesions in Experiment 5 revealed that 12 of the rats had a minimum of 50% total cell loss in the hippocampus (mean 87%), with a mean of 98% cell loss in the septal region. The other two rats had large lesions such that sufficient transfer of brain slices onto slides was unsuccessful, meaning that precise measurement of lesion size was not possible, and behavioural data from these rats were excluded from the analysis. In addition to the intended damage to the hippocampus, all rats had some limited damage to the overlying cortical regions, as seen in previous studies using this lesion method (e.g. Iordanova *et al*., 2011a). In all rats (other than the two already excluded from Experiment 5) this damage was considered to be within a satisfactory limit. Inspection of the cell loss in the rats with hippocampal lesions in Experiment 6 revealed that 13 of the rats had a minimum of 50% total cell loss in the hippocampus (mean 69%), with a mean of 92% cell loss in the septal region. The other 3 rats had less than 42% total cell loss in the hippocampus and so were excluded from the analysis, leaving 13 rats in group Hippocampal for Experiment 6. Inspection of the cell loss in the rats with hippocampal lesions in Experiment 7 revealed that 11 of the rats had a minimum of 50% total cell loss in the hippocampus (mean 67%), with a minimum of 65% cell loss (mean 87%) in the septal region. The other 5 rats had less than 46% total cell loss in the hippocampus and so were excluded from the analysis, leaving 11 rats in group Hippocampal for Experiment 7.

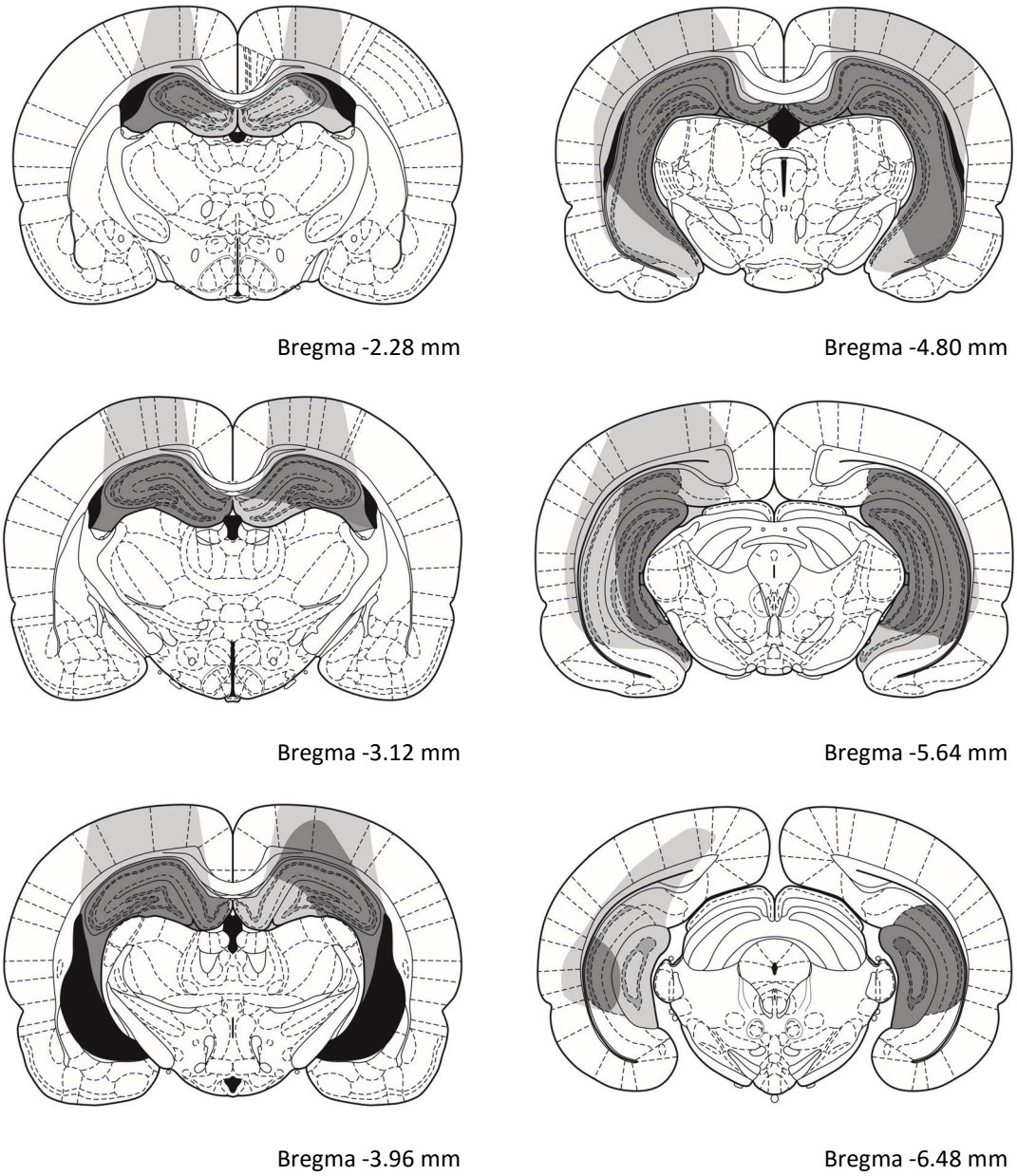

Figure 12. Experiment 5 Histology. Dark grey indicates the area of the smallest lesion and light grey indicates the area of the largest lesion.

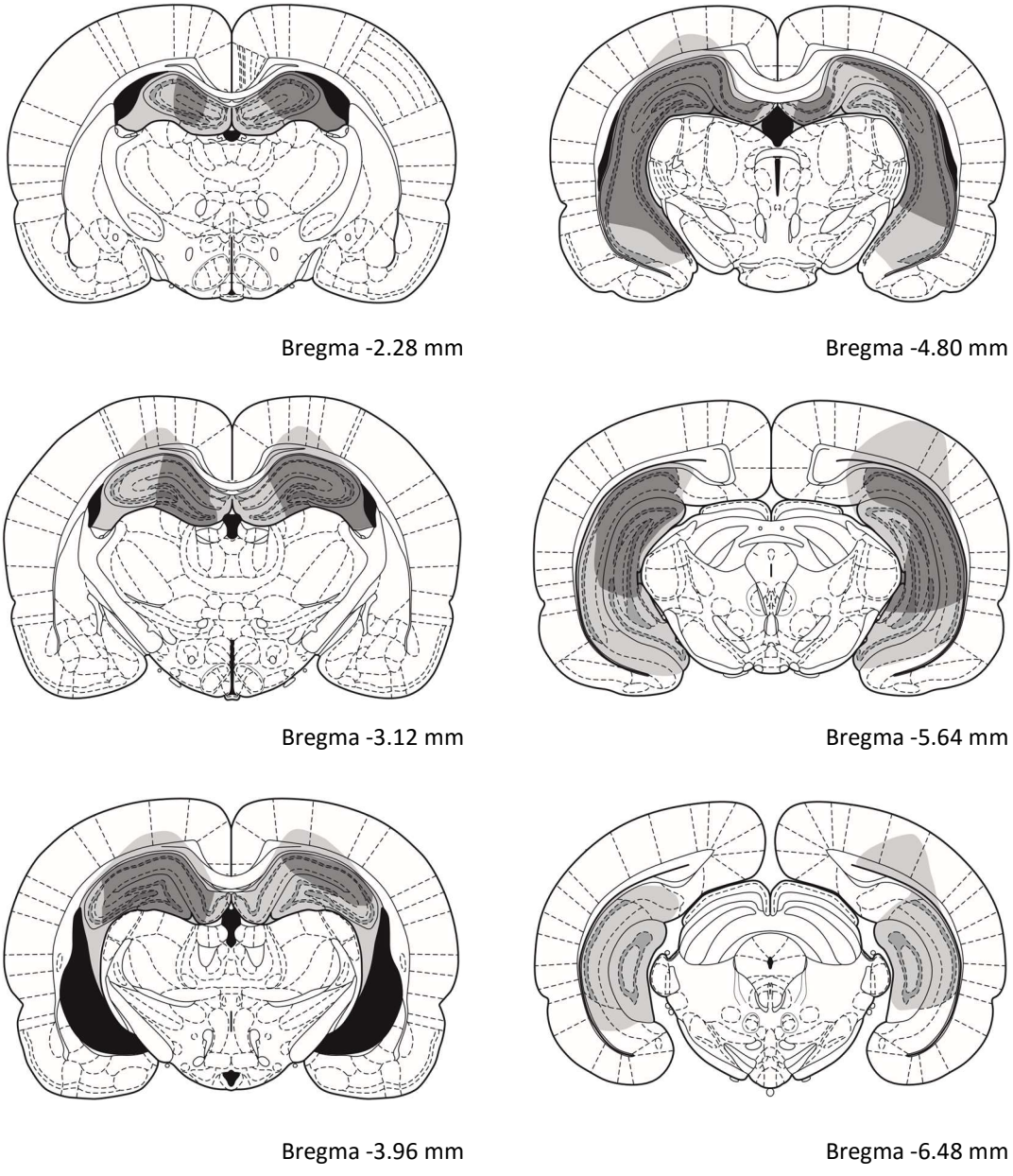

Figure 13. Experiment 6 Histology. Dark grey indicates the area of the smallest lesion and light grey indicates the area of the largest lesion.

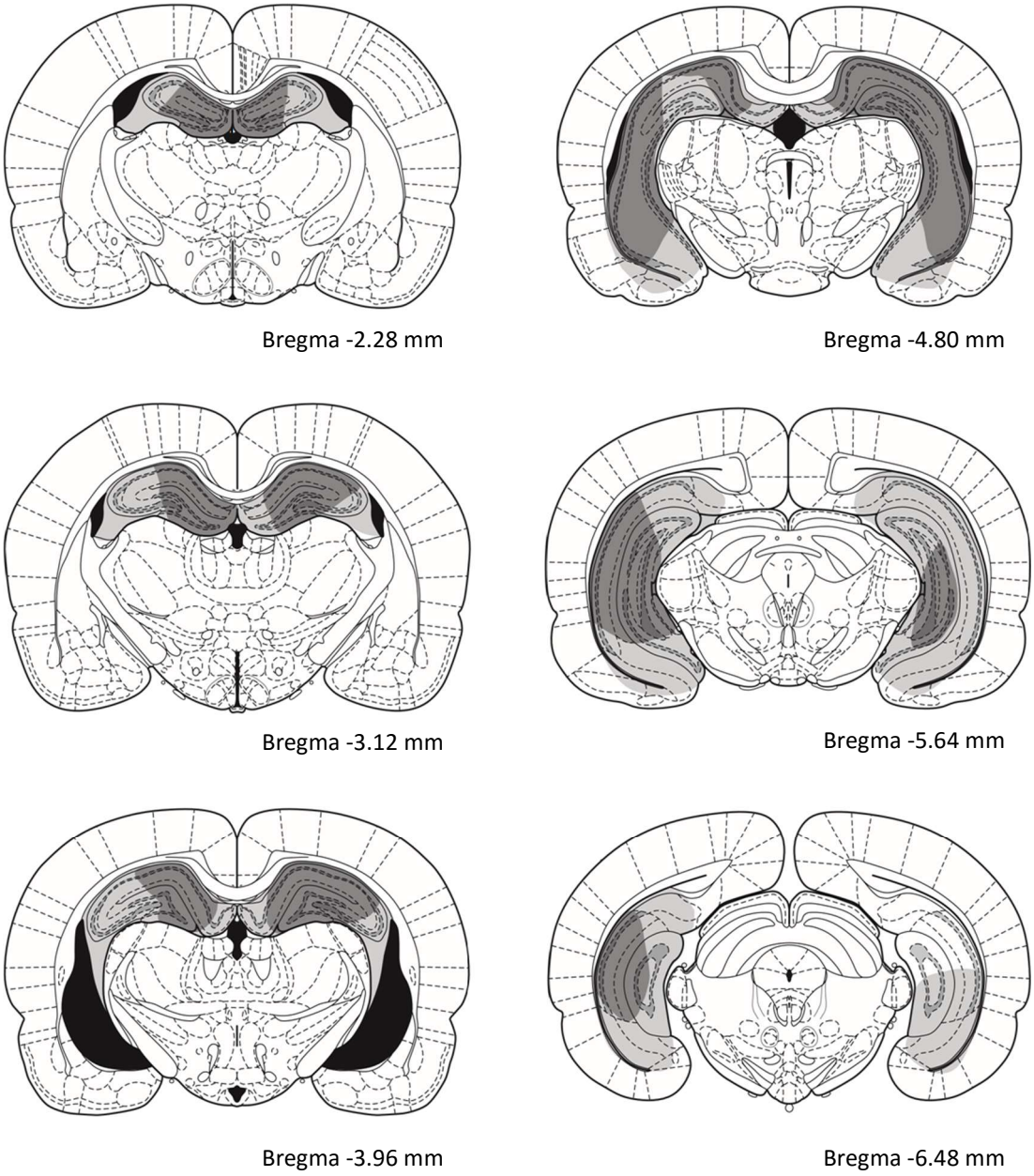

Figure 14. Experiment 7 Histology. Dark grey indicates the area of the smallest lesion and light grey indicates the area of the largest lesion.

4.5.2. Experiment 5: Appetitive configuration discrimination

The mean discrimination ratios and numbers of food well entries for groups Hippocampal and Sham in Experiment 5 are shown in Figure 15. The discrimination ratios for both groups show increase over the course of training. ANOVA revealed a significant effect of block ($F(5,115) = 9.10$, $p < .001$), no effect of group ($F(1,23) = 1.60$, $p = .218$) and no interaction between these factors ($F < 1$). The discrimination ratios were significantly different from 0.50 in group Sham in block 5 ($t(12) = 3.65$, $p < .01$) and block 6 ($t(12) = 2.30$, $p < .05$) and in group Hippocampal in blocks 4, 5 and 6 (smallest $t(11) = 3.25$, $p < .01$). The number of nosepokes in reinforced sessions was greater than in nonreinforced sessions for both groups, and there was some indication that the level of responding was lower in group Sham than in group Hippocampal. ANOVA confirmed a significant main effect of reinforcement ($F(1,25) = 12.11$, $p < .01$), a significant main effect of block ($F(5,115) = 5.34$, $p < .001$) and a significant interaction between these two factors ($F(5,115) = 6.72$, $p < .001$). There was also a significant effect of group ($F(1,23) = 10.20$, $p < .01$) and a significant interaction between group and reinforcement ($F(1,23) = 5.58$, $p < .05$). There was no significant interaction between group and block ($F(5,115) = 2.17$, $p > .05$) and no significant three-way interaction between reinforcement, block and group ($F = 1.73$, $p > .05$). Paired *t*-tests revealed a significant difference between the number of nosepokes in the reinforced and the nonreinforced sessions in group Sham on blocks 5 ($t(12) = -3.07$, $p < .05$) and 6 ($t(12) = -2.26$, $p < .05$) and in group Hippocampal on blocks 3, 4, 5 and 6 (smallest $t(11) = -2.34$, $p < .05$). This pattern of results indicates that both groups acquired the configural discriminations.

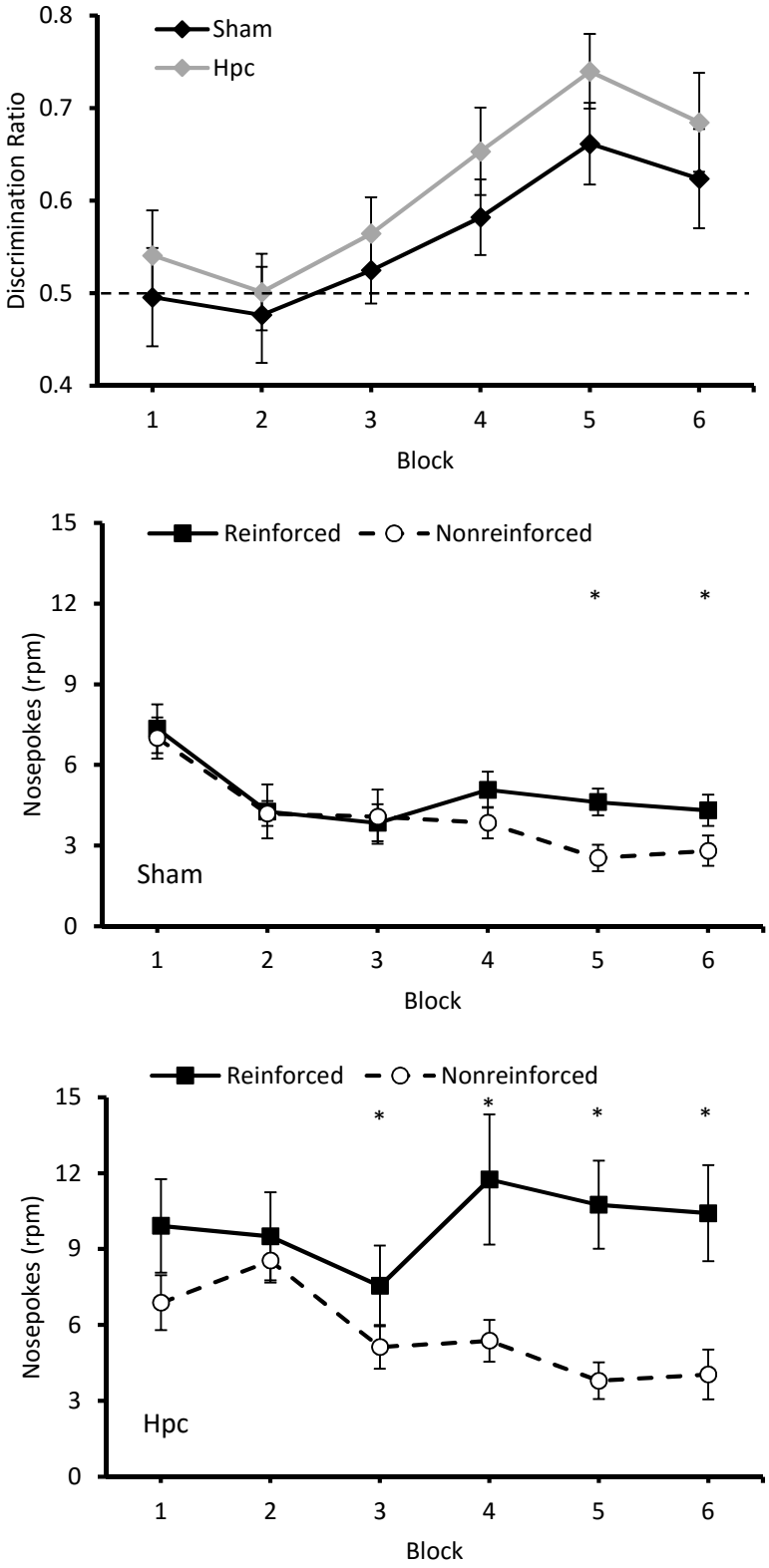

Figure 15. Experiment 5: The upper panel shows the mean discrimination ratios (±SEM) for groups Sham and Hippocampal across training blocks. Each block represents data from the last two days of a four-day training cycle, on which no food was presented for the first 30 s in any of the configurations. The ratios are derived from the food-free 30-sec periods at the start of the sessions. The middle and lower panels show the mean rates of responding (±SEM) on reinforced and nonreinforced trials that were used to calculate the ratios in group Sham (middle panel) and group Hippocampal (lower panel). Asterisks represent *p* values < .05.

4.5.3. Experiment 6: Appetitive configuration discrimination

The results from Experiment 6 are similar to those from Experiment 5. As shown in Figure 16, the DR increased across training in both groups. ANOVA confirmed there was a main effect of block ($F(4, 108) = 5.03$, $p < .005$), no effect of group ($F < 1$) and no interaction ($F < 1$). One sample *t*-tests revealed that the discrimination ratios were significantly different from 0.50 for group Sham in blocks 2 - 5 (smallest $t(15) = 2.31$, $p < .05$) and for group Hippocampal in block 2 ($t(12) = 2.40$, $p < .05$) and block 5 ($t(12) = 4.96$, $p < .001$). Also, both groups showed a consistent difference in responding between reinforced and nonreinforced trials. ANOVA revealed a main effect of block ($F(4, 108) = 2.72$, $p < .05$), a main effect of reinforcement ($F(1, 27) = 26.87$, $p < .001$) and no effect of group ($F < 1$). There were no interactions between these factors (largest $F(4, 108) = 2.34$, $p > .05$). There was a significant difference between the rates of responding on reinforced and nonreinforced trials in group Sham in blocks 2 - 5 (smallest $t(15) = 2.40$, $p < .05$) and in group Hippocampal in blocks 1, 3 and 5 (smallest $t(12) = 2.25$, $p < .05$).

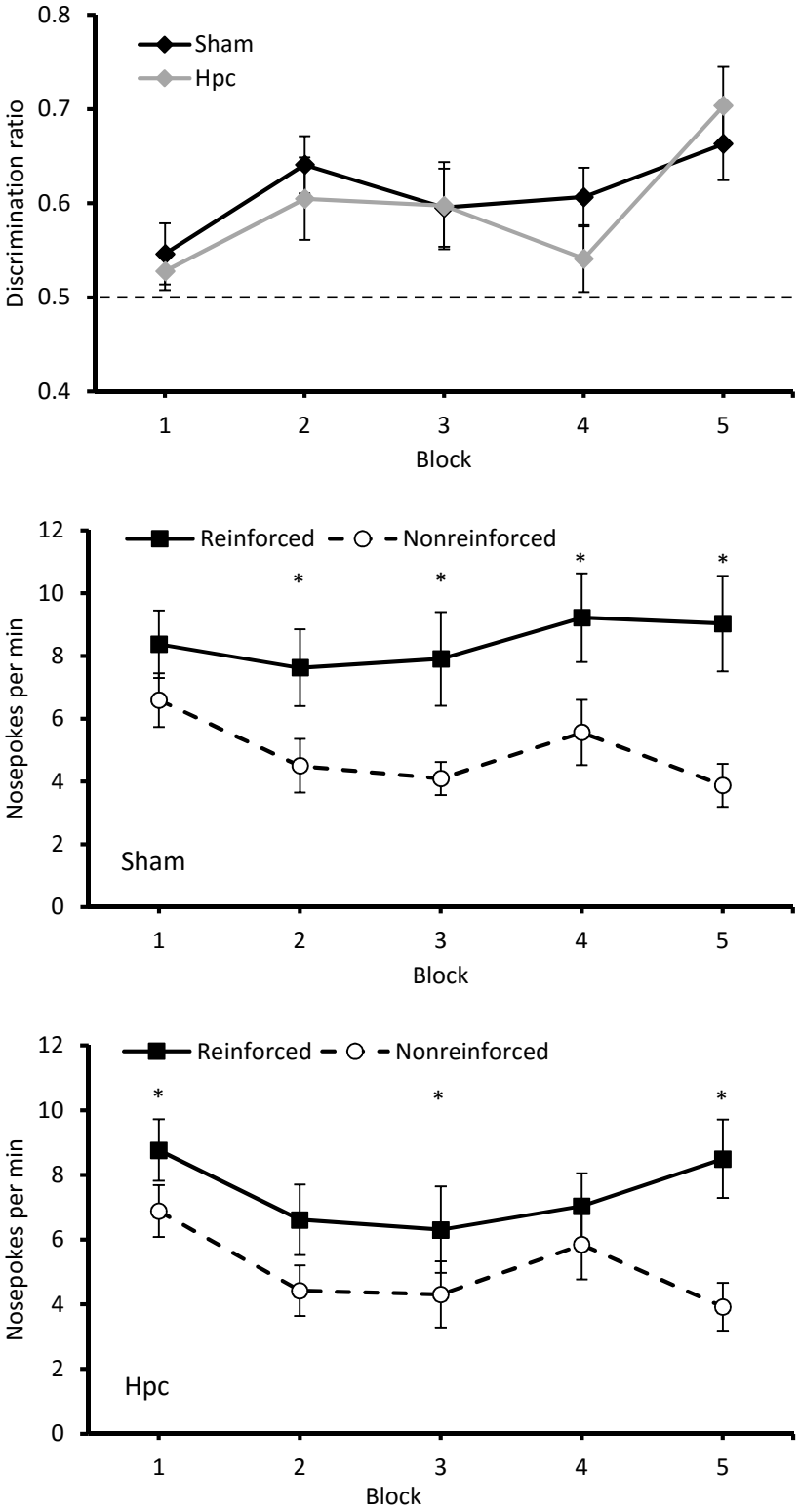

Figure 16. Experiment 6: The top panel shows the mean discrimination ratios (±SEM) for groups Sham and Hpc across training blocks. Each block represents data from the last two days of a four-day training cycle, on which no food was presented for the first 30 s in any of the configurations. The ratios are derived from the food-free 30-sec periods at the start of the sessions. The middle and bottom panels show the mean rates of responding (±SEM) on reinforced and nonreinforced trials that were used to calculate the ratios in group Sham (middle panel) and group Hpc (bottom panel). Asterisks represent *p* values < .05.

4.5.4. Experiment 7: Aversive configuration discrimination

As shown in Figure 17, the discrimination ratios increased across training in both groups. ANOVA confirmed there was a main effect of block ($F(7, 175) = 4.26$, $p < .001$), no effect of group ($F < 1$) and no interaction ($F(7, 175) = 1.35$, $p > .23$). One sample *t*-tests revealed that the discrimination ratios were significantly different from 0.50 for group Sham in block 7 ($t(15) = 2.44$, $p < .05$) and for group Hippocampal in blocks 1, 4 and 7 (smallest $t(10) = 2.58$, $p < .05$). Overall activity levels were lower in group Hippocampal compared to group Sham, but both groups showed a consistent difference in responding between reinforced and nonreinforced trials. ANOVA revealed a main effect of block ($F(7, 175) = 4.02$, $p < .001$), a main effect of reinforcement ($F(1, 25) = 5.50$, $p < .05$) and a main effect of group ($F(1, 25) = 9.56$, $p < .01$). There was a significant interaction between block and reinforcement ($F(7, 175) = 2.57$, $p < .05$) and no other interactions (largest $F(7, 175) = 1.70$, $p > .11$).

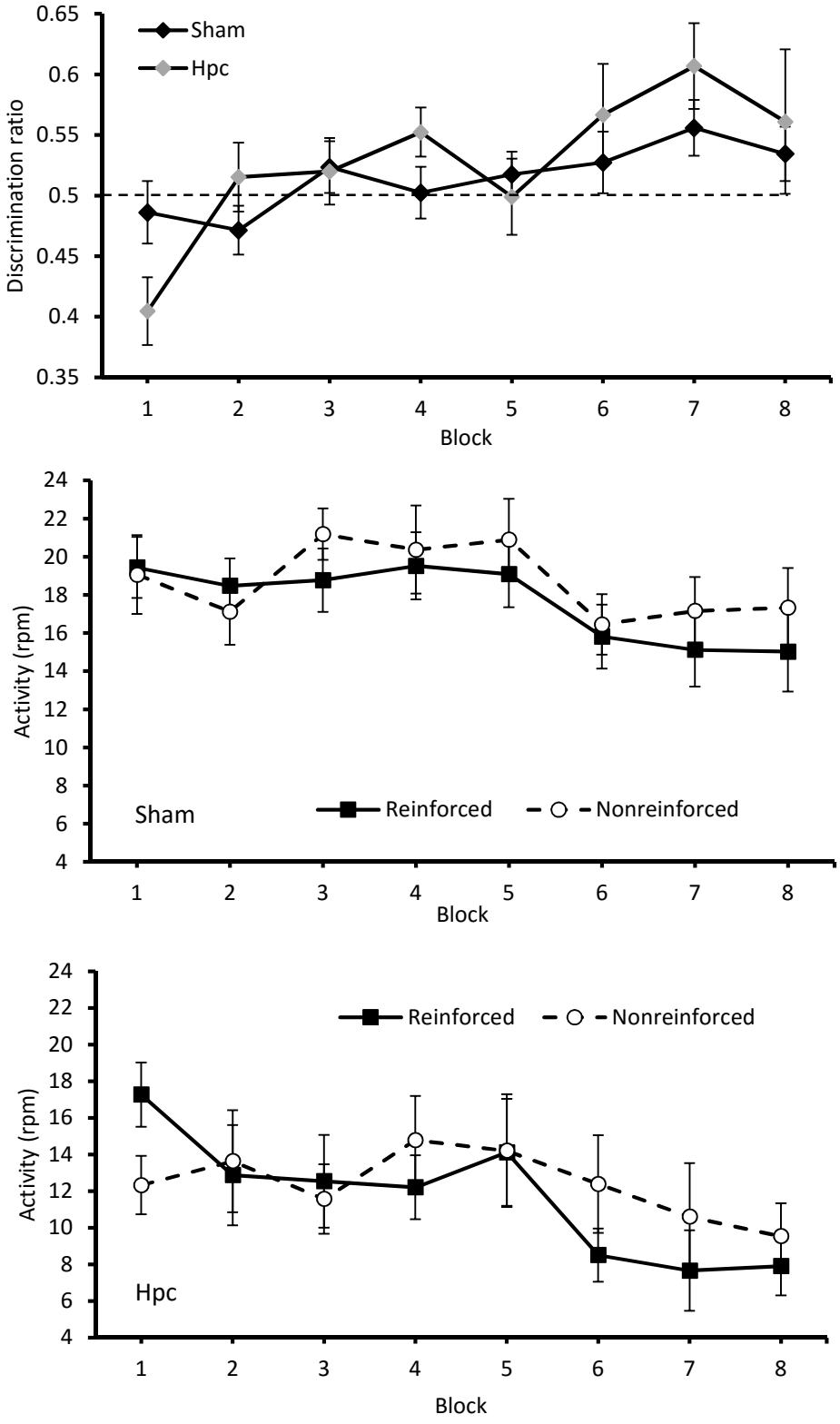

Figure 17. Experiment 7: Mean discrimination ratios (±SEM) across the eight, three-day blocks of training in group Sham and group Hippocampal (upper panel). These ratios are derived from the footshock-free 30-sec periods at the start of the sessions. The middle and lower panels show the mean rates of activity (±SEM) during the reinforced and nonreinforced trials that were used to calculate the ratios in groups Sham and Hippocampal.

4.5.5. Experiment 7: Post-shock activity

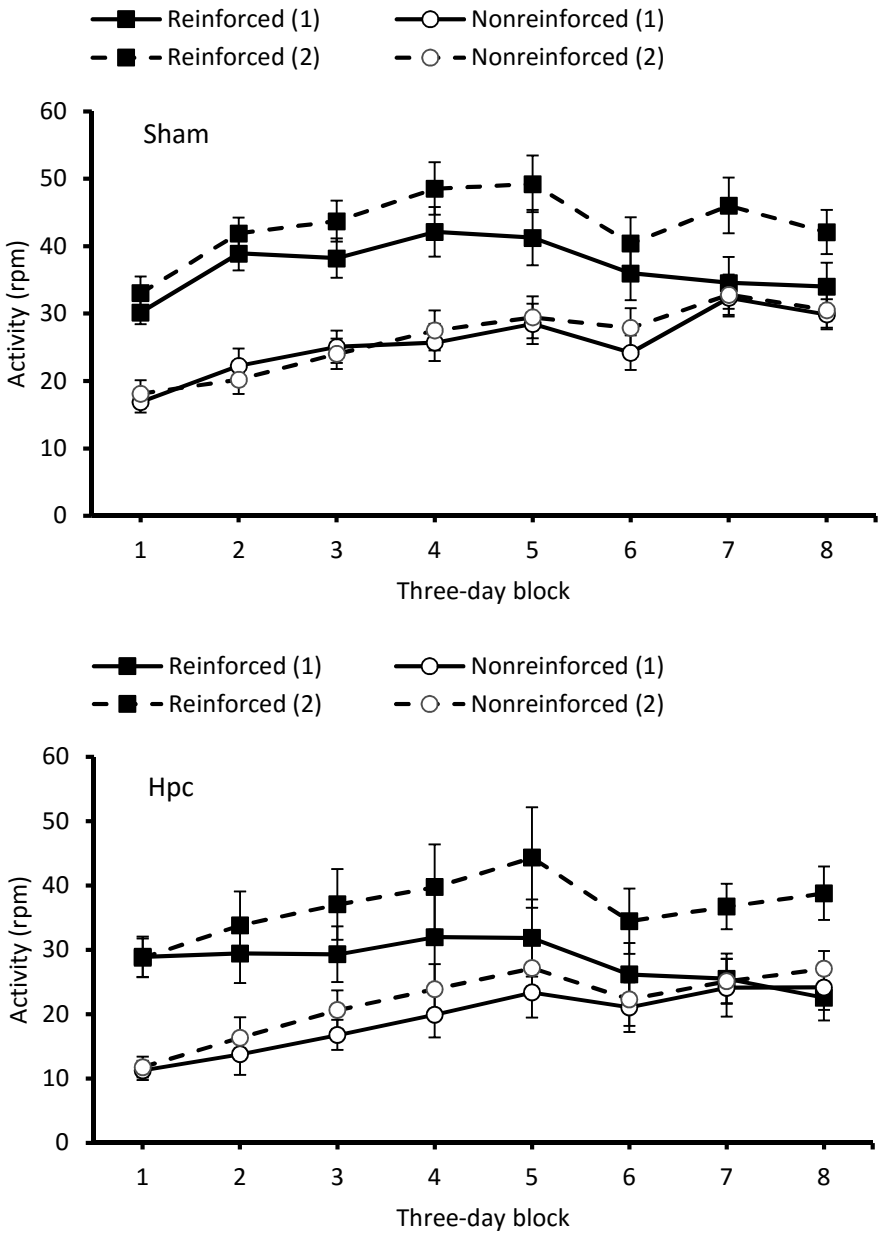

Figure 18. Experiment 7: Mean rates of activity (in responses per minute; ±SEM) during the two 30-s periods (1 and 2) that immediately followed the two footshocks on reinforced trials, and during equivalent periods (1 and 2) on nonreinforced trials for group Sham (upper panel) and group Hippocampal (lower panel).

As shown in Figure 18, in both groups, there was a general increase in activity across blocks, and activity in the 30-s following the delivery of shock was greater relative to activity in corresponding periods when shock was not delivered. It is also evident that the second

shock appeared to increase activity more than the first. ANOVA confirmed a significant main effect of the presence of shock ($F(1, 25) = 92.24$, $p < .001$), an effect of shock number ($F(1, 25) = 68.84$, $p < .001$), an effect of block ($F(7, 175) = 10.24$, $p < .001$) and no effect of group ($F(1, 25) = 2.61$, $p > .12$). There was an interaction between shock and shock number ($F(1, 25) = 80.41$, $p < .001$), an interaction between shock and block ($F(7, 175) = 9.58$, $p < .001$) and an interaction between shock number and block ($F(7, 175) = 4.65$, $p < .001$). There was a three-way interaction between shock, shock number and block ($F(7, 175) = 4.10$, $p < .001$). There were no significant two- or- three-way interactions where group was a factor (largest $F(7, 175) = 1.43$, $p > .19$) and there was no four-way interaction ($F(7, 175) = 1.14$, $p > .34$).

4.5.6. Experiment 7: Post-shock activity during the test

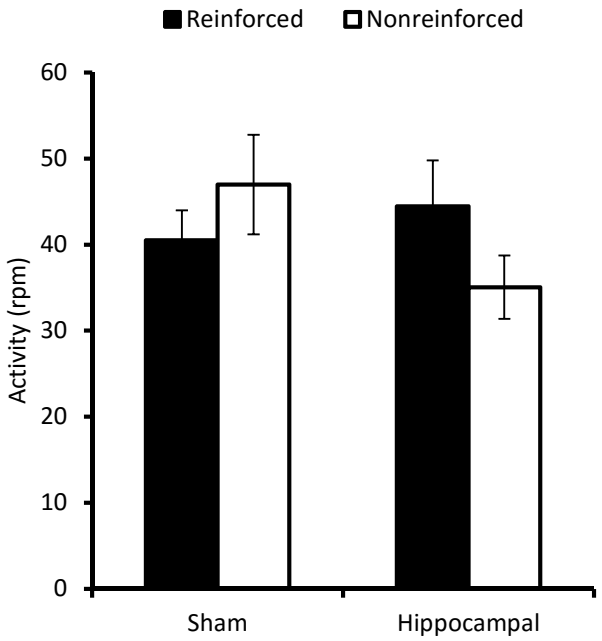

Figure 19. Experiment 7: Mean rates of post-shock activity (in responses per minute; ±SEM) during the 10s following presentation of the first footshock on two test days where footshocks were presented in the previously reinforced and nonreinforced configurations.

The mean levels of activity during the 10s following the first footshock in each of the morning sessions of the test day are shown in Figure 19. ANOVA revealed no significant effect of whether the shock was presented in the previously reinforced context+time of day configuration or the previously nonreinforced configuration ($F < 1$) and no effect of group ($F < 1$). There was a significant interaction between these factors ($F(1, 25) = 5.79$, $p < .05$). Paired *t*-tests showed no significant difference between the post-shock activity in the previously reinforced and nonreinforced configurations for group Sham ($t(15) = -1.46$, $p = .165$) or for group Hippocampal ($t(10) = 2.02$, $p = .071$).

4.6. Discussion

Two experimental procedures assessed the ability of rats with hippocampal lesions to learn a configural discrimination involving four *what-where-when* patterns of stimulation. The results from Experiments 5 and 6 provided clear evidence that rats with hippocampal lesions learned an appetitive configural *what-where-when* discrimination. The results of Experiment 7 suggest that rats with hippocampal lesions can also learn an aversive configural *what-where-when* discrimination. However, it should be acknowledged that the results of Experiment 7 are less compelling than those of Experiments 5 and 6. Taken in the round, however, these results are inconsistent with the general view that the hippocampus is required for configural learning (Rudy & Sutherland, 1989; O'Reilly & Rudy, 2000) or the more restricted view that it is required for some forms of configural learning that involve the components of episodic memory (i.e., what, where and when; cf. Iordanova *et al*., 2009, 2011; Save *et al*., 1992; see also Aggleton & Brown, 1999; Allen & Fortin, 2013; Ergorul & Eichenbaum, 2004; Tulving, 2002). However, these results are consistent with studies where damage to the hippocampus did not interfere with the ability of rats to learn reinforced discriminations where the configuration of a context and auditory stimulus signalled whether or not food would be delivered (e.g., Coutureau *et al*., 2002).

The results of the final test in Experiment 7, in which footshocks were presented in both contexts at both times of day (c.f. Experiment 4), were not as clear as was hoped. In the sham rats there was more post-shock activity when the shock was presented in the configurations that had not previously been paired with shock, compared to the configurations that had been paired with shock, but this difference was not statistically significant. That this result is different from Experiment 4 may reflect the greater number of training days and poorer acquisition of the discrimination in Experiment 7 compared to Experiment 4. There was no sign of this result in rats with hippocampal lesions, suggesting that there may be a difference in the way in which these rats processed the acquired information about the configurations. The associative mismatch effect (see Honey & Good, 2000; Honey *et al*., 1998; Kumaran & Maguire, 2007) may be compromised in rats with hippocampal lesions, but further work is needed to reveal this. The results from Experiment 7 need to be interpreted cautiously, especially because the sham rats did not acquire the discrimination as quickly as was predicted.

The immediate question that the results of Experiment 5, 6 and 7 prompt is: Why is the hippocampus necessary for configural *what-where-when* processes in some procedures but not others? Perhaps the key difference between the experiments reported by Iordanova *et al*. (2009, 2011a) and the results presented here is the requirement for retrieval-mediated learning. In the Iordanova *et al*. studies, rats not only had to encode the training configurations, but then to update these configurations with new information about one of the components, and then retrieve this information at test. In contrast, the procedures in Experiments 5, 6 and 7 required rats only to directly link specific configurations with specific outcomes. Indeed, as already noted in the introduction to Chapter 4, Iordanova *et al*. (2011b) showed that temporarily disrupting hippocampal function during the aversive conditioning stage was sufficient to produce a deficit at test. One prediction that follows from the view that the hippocampus plays a role in retrieval-mediated learning involving configurations is

that test performance in other procedures in which this process operates will be disrupted by hippocampal lesions. This prediction was tested in Chapter 5.

Chapter 5: Configural processes in sensory preconditioning

5.1. Summary

One hypothesis based on the results of Chapter 4 is that the hippocampus has a general role in retrieval-mediated learning about configurations. The two experiments reported in Chapter 5 used sensory preconditioning procedures to assess this hypothesis. Experiment 8 sought evidence that configural processes play a part in sensory preconditioning procedures that do not involve the components of episodic memory. Experiment 9 used a variant of this procedure to assess the contribution of the hippocampus to these configural processes.

5.2. Introduction

Models of associative learning assume that in order for an association to form between two representations their corresponding stimuli need to be present in the environment in close temporal contiguity (e.g., Rescorla & Wagner, 1972). This assumption is violated by instances of *representation-mediated learning* where associations are formed between the representations of stimuli that have been associatively evoked rather than directly activated by their corresponding stimuli. For example, rats given trials where a tone is first paired with the delivery of food pellets and is later paired with illness, show an aversion to food pellets in spite of the fact that food pellets have never been directly paired with illness (e.g., Holland, 1981). Here, the first stage of training is held to result in the tone coming to activate a representation of food, and when the tone is later paired with illness both the directly activated representation of the tone and the associatively evoked representation of food pellets become linked to illness (for a review, see Hall, 1996). As described in Chapter 1, this instance of representation-mediated learning has often been cast in elemental terms, in the sense that the representations that are being (directly and associatively) activated during

pairings with illness are held to form separate associations with illness during the critical conditioning stage. However, as I have already noted elsewhere, there is also some evidence suggesting that representation-mediated learning can involve evoked configural representations.

Briefly, Iordanova, Good and Honey (2008) reported a study in which rats received presentations of different configurations of the same contexts and auditory stimuli in morning and afternoon sessions. For example, rats might receive morning sessions where a tone is presented in a spotted context and a clicker in a checked context, and afternoon sessions where the tone is presented in the checked context and clicker in the spotted context. After this training, presentations of the tone were paired with shock in a third context (an undecorated test chamber) at midday while those of the clicker were not. On the next day, the levels of freezing were assessed in both contexts at both times of day. Rats showed more fear in the context+time of day configurations in which the tone had been presented: the spotted context in the morning and the checked context in the afternoon. These results implicate configural processes because the tone had been presented in both contexts and at both times of day.

However, they do not require that mediated configural learning played a role: the tested configurations might have provoked fear to the extent that they activated the memory of the tone at test rather than because of their similarity to the configural representations linked to shock during fear conditioning (i.e., spotted+morning+tone and checked+afternoon+tone). Direct evidence that mediated configural learning was the source of the critical test effect came from a further study that attempted to disrupt mediated learning during the conditioning stage. Iordanova *et al*. (2011b) demonstrated that the critical difference in fear to the test configurations was abolished if AP5 (an NMDA receptor antagonist) was infused into the hippocampus during fear conditioning; with the same

infusion being without effect when administered during the test itself. These results, together with various control experiments, implicate a process of mediated configural learning in generating the test results (for a review, see Honey, Iordanova & Good, 2014).

The experiments in this chapter were designed to assess the contribution of configural processes to sensory preconditioning effects when conventional stimuli are used in place of configurations involving episodic information (i.e., what happened where and when). There is some evidence that is consistent with a role for configural processes in this type of procedure. For example, Lin, Dumigan, Dwyer, Good and Honey (Experiment 1a, 2013) exposed rats to two audio-visual compounds, AX and BY, prior to presentations of X that were followed by shock and Y that were not. After these treatments, rats showed more fear (less activity) during AX than during BX. This finding is consistent with the view that during conditioning with X the configural representation AX was linked to shock. The finding is inconsistent with an account of sensory preconditioning in terms of an associative chain (i.e., A-X-shock), because the presentation of B with X should mean that both compounds will be equally able to activate a memory of shock. The second experiment in this chapter, Experiment 9, used a similar procedure to that developed by Lin and colleagues to investigate the involvement of the hippocampus in learning about retrieved configural memories. There is evidence from sensory preconditioning procedures using neutral stimuli that animals with damage to the hippocampus are impaired at sensory preconditioning (e.g., Port, Beggs & Patterson, 1987; Talk, Ghandi & Matzel, 2002). However, other researchers have found no effect of hippocampal manipulation on the sensory preconditioning effect (e.g., Ward-Robinson *et al*., 2001). The basis for these differences is unclear; but once one acknowledges that there are several mechanisms that could underpin sensory preconditioning effects (e.g., associative chains, retrieval mediated configural learning) then it is difficult to establish whether a given manipulation (e.g., a lesion to the hippocampus) is effective because it

affects one mechanism or another. Experiment 9 was designed to investigate the contribution of the hippocampus to configural processes in sensory preconditioning.

5.3. Experiments 8 and 9

Table 2. Design of Experiments 8 and 9

Experiment 8		
Pre-exposure	Conditioning	Test
AX	X→40s→shock	AX, BX
BY	Y→40s→shock	AY, BY
Experiment 9		
Pre-exposure	Conditioning	Test
AX	X→40s→shock	AX, BX
BY	Y-	AY, BY

Note: A and B were localized visual stimuli (left and right jewel lights); X and Y were auditory stimuli (tone and clicker).

The experimental design employed in Experiment 8 is shown in the upper panel of Table 2. After exposure to two stimulus compounds, AX and BY, rats were given separate presentations of X and Y that were followed by shock. During the test, the levels of fear (as indexed by inactivity) during compounds AX, BX, AY and BY were assessed. Whereas elemental analyses of sensory preconditioning predict less fear to AX and BY than to AY and BX, an account based on configural learning predicts more fear to AX and BY than to AY and

BX. First consider the contribution of elementary associative chains. The presence of A or B in each test compound will mean that each should be capable of evoking a memory of a stimulus (X and Y, respectively) that has been directly paired with shock. However, while on AX and BY trials these evoked memories will also be physically present, on AY and BX trials the evoked memory will differ from the stimulus that is present. On this basis, there should be greater fear during AY and BX than during AX and BY. Now consider the contribution of elementary mediated conditioning. A memory of A will be evoked on conditioning trials with X and a memory of B will be evoked on conditioning trials with Y, and the contribution of any resulting mediated conditioning to test performance should be equally apparent across the four compounds: each contains either A or B. However, on an AX test trial X will only be capable of activating a memory of a stimulus that is physically present (i.e., A), whereas on an AY trial Y can activate a memory of B that could provide an additional basis upon which the memory of shock becomes active. On this basis, there should be more fear during AY and BX than during AX and BY. In contrast, an account based upon configural processes makes a different prediction: If during conditioning X evokes AX and Y evokes BY and these configurations become linked to shock, then there should be greater fear (less activity) to both AX and BY than to AY and BX. Similarly, if A elicits fear because it has the capacity to activate the AX configural memory and thereby the memory of X (Pearce, 2002), then the presentation of AX should be a more effective means of doing this than the presentation of AY or BX.

The experimental design of Experiment 9 is shown in the lower panel of Table 2. A similar procedure to that used by Lin and colleagues (2013), the procedure involves differential conditioning to X and Y. This allows investigation not only into differences in responding to AX and BX but also differences in responding to X and Y. As in Experiment 8, elemental analyses predict more fear to BX than to AX, whereas configural analyses predict

the opposite. Rats with hippocampal lesions were used to investigate hippocampal involvement in these sensory preconditioning processes.

5.4. Method

5.4.1. Subjects

Sixteen male Lister hooded rats (*Rattus norvegicus*; supplied by Harlan Olac Ltd, UK), with mean *ad libitum* weight of 428 g (range = 367-478 g) were used in Experiment 8. An additional sixteen male Lister hooded rats (*Rattus norvegicus*; supplied by Harlan Olac Ltd, UK) were used in Experiment 9 (group Sham: *n* = 8; group Hippocampal: *n* = 8; mean *ad libitum* weight: 421 g, range: 394 - 492 g). In both experiments the rats were ≈ 4 months old at the start of the experiment. The rats were housed in pairs in the same way as in Experiments 1-7 except that food and water were available *ad libitum* in the home cage throughout the experiment. In Experiment 8, behavioural training began at ≈ 10:00 on each day. In Experiment 9, rats had a minimum of 2 weeks of postoperative recovery before receiving behavioural training that began at ≈ 09:30 on each day.

5.4.2. Experiment 9: Surgery and histology

The surgical procedure and the coordinates of injection sites were identical to those for Experiments 1, 2, 5, 6 and 7. Briefly, rats were anaesthetised, placed in a stereotaxic frame and the bone above the area to be lesioned was removed. Rats in group Hippocampal (n = 8) received infusions of ibotenic acid. Table 1 (Chapter 2, Section 2.4.2.) shows the coordinates and volumes used. Rats in group Sham (n = 8) received identical treatment to rats in group Hippocampal except that no infusions were made and, instead, a needle was used to perforate the dura. Rats recovered for a minimum of 14 days and their preoperative weights were restored before behavioural training began.

Following behavioural testing, histological procedures were performed identically to the procedures described for Experiments 1 and 2 (Chapter 2, Section 2.4.5.) except that the brains were frozen and sectioned using a -20°C cryostat rather than a sliding microtome. The 40µm sections were collected on gelatine-coated slides, left to dry at room temperature for 24 hours, and then stained with cresyl violet before being examined under a microscope.

5.4.3. Apparatus

Experiments 8 and 9 were carried out with the same eight operant chambers that were used for Experiments 4 and 7, however the walls of the operant chambers were undecorated. As such, each operant chamber had two aluminium side walls, a transparent Perspex back wall and ceiling. The front wall was also Perspex, and served as the door to the chamber. The chambers were housed within sound-attenuating shells and were lit by a 3-W light bulb, with a white plastic cover, positioned centrally and 13.5 cm above the floor. Two 30-sec visual stimuli served as A and B: illumination of covered 3-W jewel lights that were located on the left- and right-hand sides of the left aluminium wall that contained the foodwell. These lights, each constantly illuminated during the 30-sec, were mounted 13.5 cm above the floor and were positioned 9.2 cm to the left and right of an unused central wall light mounted at the same height above the floor but immediately above the foodwell. Two 30-s auditory stimuli served as X and Y: a 2-kHz tone and a 2-Hz clicker. These stimuli were presented at an intensity of ≈ 75 dB through a speaker located centrally and at 14.5 cm above the floor on the left aluminium wall; and were produced by an internal audio generator. A 0.5-s 0.64 mA electric shock could be delivered through the grid floor (19 stainless steel bars; diameter 0.47 cm, spacing from bar centre to bar centre, 1.07 cm).

The activity levels of the rats in the chambers were measured in the same way as in Experiments 4 and 7. Infrared light sources and photobeam detectors connected to a

computer were used to detect and record ambulatory movement data. It was assumed that lower levels of activity were indicative of greater fear during the final test.

5.4.4. Experiment 8: Procedure

Rats received one preexposure session per day for six days (days 1-6). In each session they received two types of 30-s simultaneous compound: AX (e.g., the left light presented with the tone) and BY (e.g., the right light presented with the clickers). The identity of the visual stimulus that served as A or B, and of the auditory stimulus that served as X or Y, was fully counterbalanced: For half of the rats, the left light served as A and the right light served as B and for the remainder the reverse was the case; and within these subgroups half of the rats received the tone as X and the clicker as Y, and for the rest this arrangement was reversed. Each trial type was presented 10 times per session and the order of presenting these trials was pseudorandom, with the constraint that there were no more than two trials of the same type in each session. The intertrial interval (ITI) was 2.5 min.

In the conditioning stage, rats received one session per day for four days (days 7-10). Rats were given three presentations of X (e.g., the tone) followed by shock after a 40-s trace interval in one session, and three presentations of Y (e.g., the clicker) that were followed by shock after a 40-s trace interval in another session (i.e., X→40s→footshock, Y→40s→footshock). Half of the rats received X→40s→shock trials on days 7 and 9 and Y→40s→shock trials on days 8 and 10, and for the remainder, this arrangement was reversed. The ITI was 8 min. This trace conditioning procedure was the same as that used by Lin *et al*. (2013), and it has been argued that such procedures are more likely to result in mediated learning than those in which the reinforcer is delivered immediately after the conditioned stimulus (see Ward-Robinson & Hall, 1998). When using a trace period, it is less likely that the associatively evoked representation of AX will be overshadowed by a directly

activated representation of X; using a trace conditioning procedure makes it more likely that the associatively evoked representation of AX will be paired with shock.

During the following two test days (days 11 and 12), rats received four types of 30-s simultaneous compounds: AX, BX, AY and BY. For half of the rats, Test 1 (involving a comparison of AX versus BX) was on day 1 and Test 2 (involving AY versus BY) was on day 2; and for the remainder the assignment of stimulus compounds to Test 1 and Test 2 was reversed. For half of the rats on each day, the order of trials was AX, BX, BX, AX, BX, and AX and for the remainder it was BX, AX, AX, BX, AX and BX. Similarly, for half of the rats on each day, the order of trials was AY, BY, BY, AY, BY and AY, and for the remainder it was BY, AY, AY, BY, AY and BY. The ITI was again 8 min.

The conditioning ratio used to provide an assessment of the change in activity to X and Y during the conditioning stage was calculated in the following way: activity during the final trial of conditioning divided by activity during the first and final trial. Using this ratio, scores approaching zero indicate that activity declined over the course of conditioning. Test performance was also assessed by means of a ratio that took the following form: activity during the familiar compounds (i.e., AX and BY) divided by activity during both familiar and novel compounds (i.e., AX, BY, AY and BX). When this measure is used, a score below .50 indicates that rats are less active during the familiar compounds than during the novel compounds.

5.4.5. Experiment 9: Procedure

The procedure was similar to that of Experiment 8 and was modelled on the procedure described in Lin *et al*. (2013). Rats in Experiment 9 received a sensory preconditioning procedure that each involved 3 stages: preexposure, conditioning and test (see Table 2). As in Experiment 8, the preexposure stage consisted of one session per day for 6 days (days 1-6). In each session, there were two types of 30-s simultaneous compound: AX (e.g., the left

light presented with the tone) and BY (e.g., the right light presented with the clicker). For half of the rats in each group (Sham and Hippocampal), the left light served as A and the right light served as B, and for the remainder the reverse was the case. In the subgroups created by the previous counterbalancing operation, for half of the rats the tone served as X and the clicker served as Y and for the remainder the reverse was the case. There were 10 presentations of each compound per session that were presented in a pseudorandom order with the constraint that there were no more than two trials of the same type in succession. The intertrial interval (ITI) was 2.5 min.

A trace conditioning procedure was used because this procedure results in the theoretically important difference between AX and BX during the test (see Lin *et al*., 2013). It has been suggested that when using a trace period before the shock, overshadowing by the direct presence of X is less likely and the associatively evoked representation of AX has more of a chance to be paired with shock (Ward-Robinson & Hall, 1998). Moreover, it has been established that the trace interval used here has equivalent effects on rats with lesions of the hippocampus as it does on rats with sham lesions (see Lin & Honey, 2011). Rats received 2 conditioning sessions (on days 7 and 8), one session per day. In each session, rats received 3 presentations of X (e.g., tone) followed by footshock after a 40-s trace interval and 3 presentations of Y that were not followed by footshock (i.e., X-trace-footshock, Y-no footshock). For half of the rats, the sequence was XYYXYX, and the rest received YXXYXY with an ITI of 8 min. The levels of activity during X and Y in this experiment were similar in the two groups, but also similar during both stimuli. I therefore focus on activity during the trace periods that followed X and Y in Experiment 9.

During the critical two test days, rats received tests with four configurations: AX, BX, AY and BY. For half of the rats, Test 1 (involving a comparison of AX versus BX) was on day 1 and Test 2 (involving AY versus BY) was on day 2; and for the remainder the order of Test 1

and Test 2 was reversed. For half of the rats on each day, the order of trials in Test 1 was AX, BX, BX and AX, and for the remainder it was BX, AX, AX and BX. Similarly, for half of the rats on each day, the order of trials was AY, BY, BY and AY, and for the remainder it was BY, AY, AY and BY. These tests allow retrieval-mediated learning involving a configural memory of AX to be assessed (e.g., by comparing AX and BX). Each test compound was presented for 60s and the ITI during the tests was 8 min.

5.5. Results

5.5.1. Experiment 8: Conditioning and test

Over the course of conditioning rats became less active (mean conditioning ratio = 0.40; SEM = 0.035). A one-sample *t* test confirmed that the scores were significantly below 0.50 ($t(15) = -2.87$, $p < .05$, $d = -0.72$). The mean level of activity (in responses per minute, rpm) on the first X and Y trial (pooled) was 26.94 (SEM = 2.98).

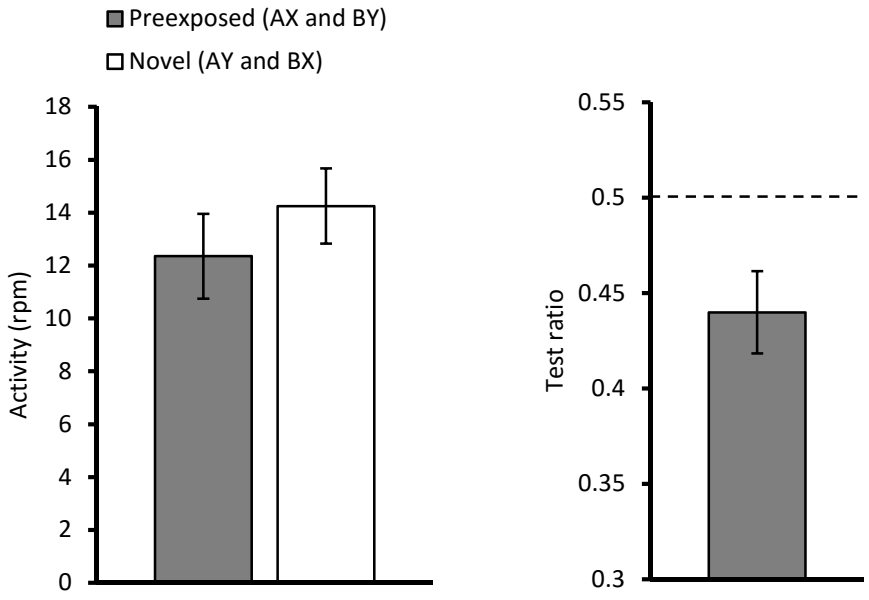

Figure 20. Experiment 8. Left-hand panel: Mean activity (responses per minute; ±SEM) during the pre-exposed compounds (AX and BY) and re-configured (AY and BX) test compounds. Right-hand panel: Test ratio, calculated by dividing the level of activity during the pre-exposed compounds (AX and BY) by the total amount of activity during all test compounds (AX, BX, AY and BY).

The test results from Experiment 8 are shown in Figure 20. Inspection of this figure suggests that responding to the pre-exposed compounds, AX and BY, was lower than to the novel compounds, BX and AY, and that the test ratio was below 0.50. A paired *t*-test confirmed that the activity during the pre-exposed compounds was lower than during their reconfigured counterparts ($t(15) = -2.12$, $p = .050$; although note that this is on the borderline for significance with an alpha level of 0.05). A one-sample *t*-test confirmed that the test ratios were below 0.50 ($t(15) = -2.79$, $p < .05$).

5.5.2. Experiment 9: Histology

Figure 21 depicts a series of coronal sections through the rat brain (adapted from Paxinos & Watson, 2005), with the largest overall lesion (in light grey) and the smallest lesion (in dark grey). All eight rats in group Hippocampal had a total hippocampal tissue damage of greater than 60%, and greater than 75% damage in the dorsal (septal) region, therefore data from all eight rats were included in the analysis. As in Experiments 1, 2 and 5-7, there was some cortical damage and limited diffusion of the neurotoxin but in all rats this damage was minor.

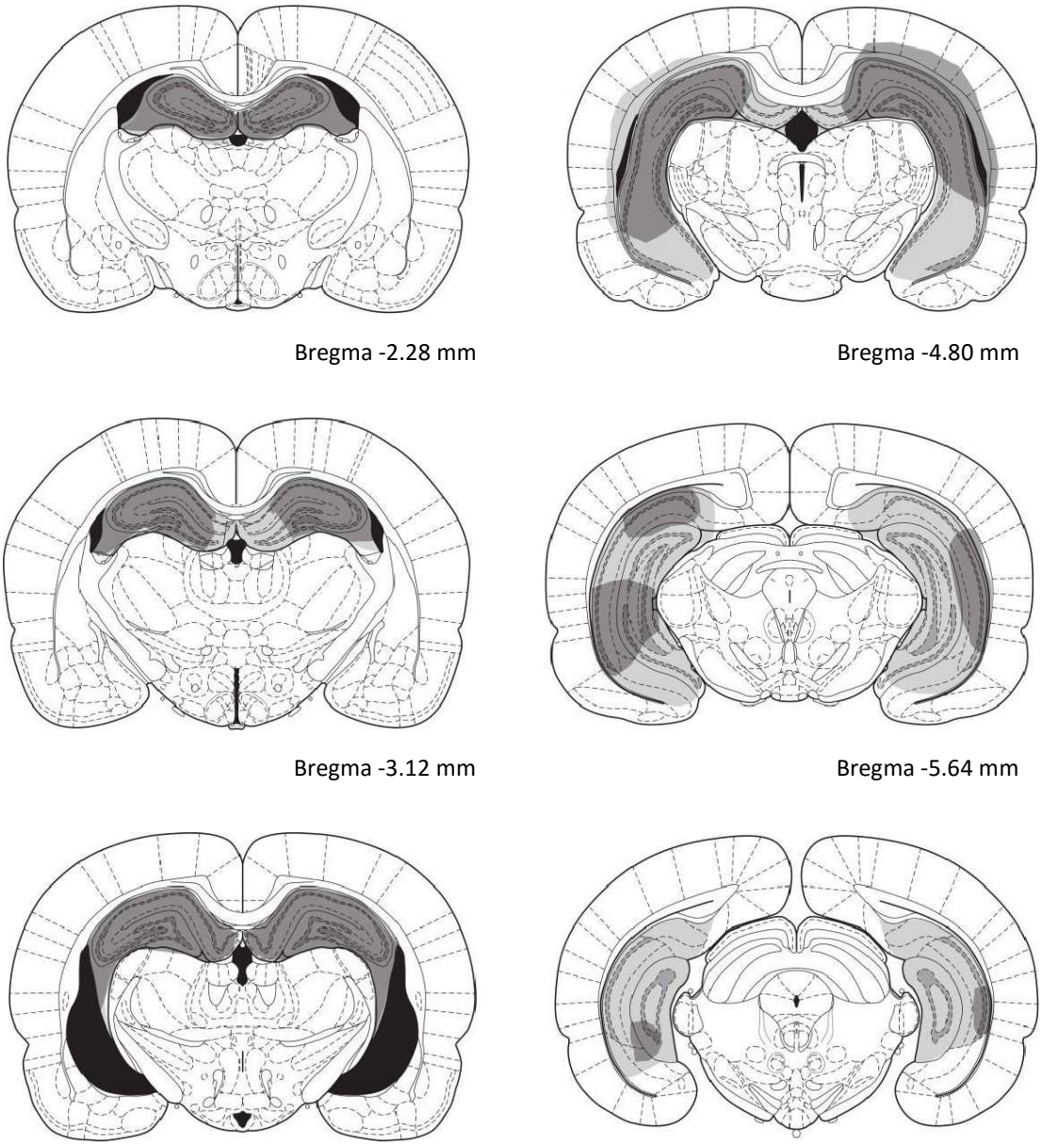

Figure 21. Experiment 9 histology. Dark grey indicates the area of the smallest lesion and light grey indicates the area of the largest lesion.

5.5.3. Experiment 9: Conditioning

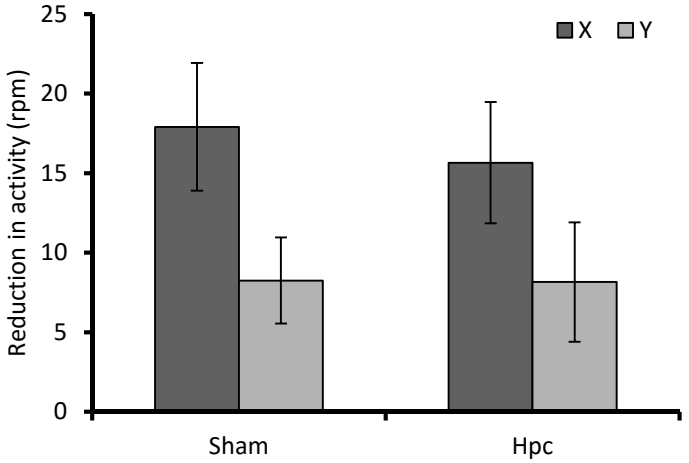

Figure 22. Experiment 9: Mean reduction in activity (responses per minute; ±SEM) between the first and final conditioning sessions, during the trace periods that followed presentation of X and Y, in group Sham and group Hippocampal. The scores are pooled over the two conditioning sessions.

Figure 22 shows the reduction in the activity levels between the first and final conditioning sessions during the trace periods that followed presentations of X and Y. Inspection of this figure suggests that the reduction in activity was greater during the trace of X than during the trace of Y; and that this difference was similar in groups Sham and Hippocampal. ANOVA with group and stimulus as factors confirmed that there was a main effect of stimulus ($F(1, 14) = 6.805$, $p < 0.05$), but there was no effect of group and no interaction between these two factors (largest $F(1,14) = 2.31$, $p > 0.15$).

5.5.4. Experiment 9: Test

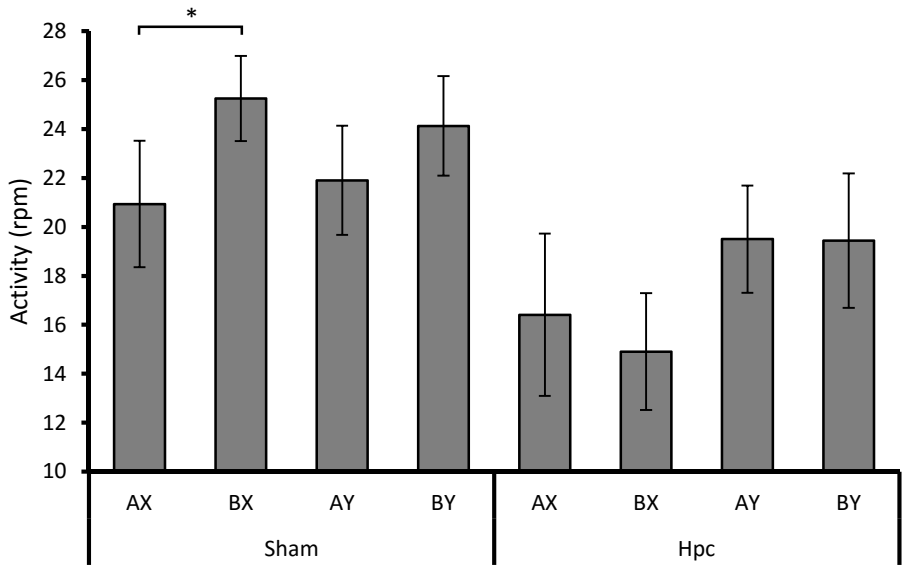

Figure 23. Experiment 9: Mean activity levels (in responses per minute; ± SEM) during presentation of test compounds AX and BX (and AY and BY) in group Sham and group Hippocampal.

The mean activity levels during the tests in Experiment 9 are shown in Figure 23. The test patterns (AX, BX, AY and BY) clearly differ in their similarity to the configuration (AX) that should have been stored during preexposure and activated on the trace conditioning trials with X: AX most closely matches this configural trace, whereas BX is less similar to it. However, it should be noted that the comparison involving compounds that differ from AX in terms of the absence of either A or X (i.e., comparing BX with AY) is complicated by the fact that A and B were visual stimuli and X and Y were auditory (and the similarity of A to B and X to Y not known). In any case, rats in group Sham showed less activity (i.e., more fear) during AX than BX and, to a lesser degree, less activity during AY than BY; and these rats showed little difference in activity between compounds containing X and compounds containing Y. This pattern of results was not evident in rats in group Hippocampal, who showed no difference between activity to AX and BX (or AY and BY). ANOVA with group (Sham and Hippocampal), presence of directly conditioned stimulus (X or Y) and nature of configuration (containing A, AX and AY, or containing B, BX and BY), revealed no effect of

group ($F(1, 14) = 3.53$, $p > .08$), conditioned stimulus ($F(1, 14) = 1.93$, $p > .18$), or configuration ($F(1, 14) = 1.78$, $p > .20$); but there was an interaction between group and configuration ($F(1, 14) = 4.72$, $p < .05$), and no other interactions (largest $F(1, 14) = 2.09$, $p > .17$). Analysis of simple main effects confirmed an effect of configuration in group Sham ($F(1, 14) = 6.15$, $p < .05$) but not group Hippocampal ($F < 1$).

In order to further understand the results from the two types of test (involving AX and BX or AY and BY), supplementary analyses were conducted on the two types of test. Analysis of the results from the AX versus BX test showed that there was an effect of group ($F(1, 14) = 4.72$, $p < .05$), no effect of configuration ($F(1, 14) = 1.32$, $p > .27$), and an interaction between these factors ($F(1, 14) = 5.64$, $p < .05$). Analysis of simple main effects revealed a difference between AX and BX in group Sham ($F(1, 14) = 6.21$, $p < .05$), but not in group Hippocampal ($F < 1$). A parallel analysis of the comparison between AY and BY revealed no effect of group or configuration and no interaction between the two (largest $F(1, 14) = 1.39$, $p > .25$). The mean rates of responding during the first minute of both tests did not differ significantly (Sham: $M = 19.00$ rpm, $SEM = 3.17$; and Hippocampal: $M = 14.12$ rpm, $SEM = 2.28$; $F(1, 14) = 1.52$, $p > .23$).

5.6. Discussion

The finding that exposure to AX allows responding directly conditioned to X (e.g., by pairing it with shock) to be exhibited during A is most often explained in terms of the formation of an A-X-shock associative chain, the components of which were forged during exposure and conditioning, and then put together "on the fly" during the test (e.g., Jones *et al*., 2012). An alternative elemental analysis relies on the idea that when X is presented for conditioning it will evoke a memory of A and this memory will become linked to shock (e.g., Hall, 1996; Ward-Robinson & Hall, 1996). In Experiment 8, after exposure to AX and BY, and conditioning trials with X and Y, rats showed less activity (more fear) during the exposed, familiar

compounds (AX and BY) than novel compounds constructed from familiar components (AY and BX). This pattern of results is inconsistent with elemental analyses of sensory preconditioning, which predict less activity (more fear) to AY and BX than to AX and BY. Instead, the results of Experiment 8 provide support for the suggestion that exposure to AX and BY results in the formation of configural representations of these compounds, that can be (1) activated by X and Y during conditioning and enter into an excitatory association with shock, or (2) activated during the test and thereby activate the X-shock association.

The results of group Sham in Experiment 9 support the view that conditioning with X allowed a reactivated configural AX representation to become linked with shock. In Experiment 9, after the same form of exposure as in Experiment 8, conditioning with X but not Y resulted in significantly more fear to AX than to BX, but no difference in fear between BY and AY. Analyses that rely on an associative chain do not predict this outcome because A is only held to provoke more fear than B by dint of its capacity to activate X, and the presence of X with A and B at test will mean that both compounds should have this capacity. Instead an analysis that relies on mediated conditioning, involving either A or the configural memory AX, provides a straightforward account of the fact that the presence of A is critical.

However, there are alternative interpretations. Thus, the tendency for the AX compound to provoke unconditioned activity might have habituated during the exposure stage through the operation of conventional associative processes; for example, as a result of the formation of within-compound elementary associations (see Honey, Manser & Good, 1998; Honey, Watt & Good, 1998; Honey & Good, 2000). This reduction in unconditioned activity during AX relative to BX could then masquerade as greater fear (also measured as less activity) during AX than BX. However, the latter possibility is rendered implausible by a secondary observation from the test stage of the same experiment. During the test, the novel compound AY was, if anything, more likely to provoke fear than the familiar compound BY.

This observation is inconsistent with an account of test performance in terms of differences in unconditioned activity, but it immediately suggests another explanation for the finding that AX provokes more fear than BX: The representation of A might have been linked to shock during the conditioning trials with X and thereby resulted in more fear to AX than BX. In fact, however, the latter possibilities are rendered moot to the extent that they rely, at least in part, on the absence of a statistical interaction between the effects at test of the presence of A (versus B) and X (versus Y) in the four compounds. Thus, the pattern of results observed in group Sham in Experiment 9 is consistent with the suggestion that during conditioning with X the configural memory of AX was linked to shock; or with the more general possibility that the memory activated during conditioning more closely matches AX than BX (cf. Lin *et al*., 2013).

The results of Experiment 8, and the results from group Sham in Experiment 9 implicate configural mediated learning in these cases of sensory preconditioning. In group Hippocampal in Experiment 9 there was no evidence of a difference in fear to AX and BX, suggesting that these configural mediated learning processes are underpinned by the hippocampus. This is consistent with the results from Iordanova and colleagues (2009, 2011b) who found that their critical test effect was abolished in rats with lesions of the hippocampus and by infusing AP5 (an NMDA receptor antagonist) into the hippocampus during conditioning. In this context, it is interesting to note that in a recent fMRI study using a sensory preconditioning procedure with visual stimuli in humans, Wimmer and Shohamy (2012) presented evidence suggesting that their sensory preconditioning effect (at test) was correlated with hippocampal activity during the equivalent of the conditioning stage of the study, but not the exposure or test stage. These results provide evidence for the suggestion that the involvement of the hippocampus in retrieval-mediated learning is not restricted to configurations involving episodic content. This suggestion will be discussed in the following, final chapter.

Chapter 6: General Discussion

6.1. Overall summary

The principal aim of this thesis was to investigate the contribution of the hippocampus to forming integrated memories for patterns of stimulation. To do so required the development of novel behavioural procedues that provided assays of different forms of mnemonic integration (i.e., elemental and configural) and involved the use of different kinds of patterns (i.e., involving the components of episodic memory or audio-visual compounds). In this chapter the findings from Chapters 2-5 will be summarised briefly and the theoretical implications of the results will be considered. Finally, I will focus on the broader importance of these findings and explore possible future research directions.

6.2. Summary of main results

6.2.1. Assessing the role of the hippocampus in learning about contextual and temporal cues

It has been argued that the hippocampus has a critical role in the processing of spatial/contextual and temporal information. Before investigating the contribution of the hippocampus to configural learning involving the integration of the components of episodic memory (i.e., what happened, where and when), it was important that I establish a procedure in which I could be confident that rats with lesions to the hippocampus were not impaired in learning about these components individually. To do this, rats received a discrimination in which the availability of food was predicted by either the context in which they were placed or the time of day (morning or afternoon) at which they were placed in the experimental apparatus. The results from the experimental work described in Chapter 2 indicate that rats with lesions to the hippocampus were not impaired in learning simple, elemental discriminations involving context (Experiment 1) or times of day (Experiment 2).

These results provided the basis for me to go on to develop configural learning procedures using the same contextual and temporal cues (Chapter 3), and subsequently investigate the role of the hippocampus in these procedures (Chapter 4).

6.2.2. Developing configural learning procedures involving contextual and temporal cues

The aim of the experiments presented in Chapter 3 was to obtain direct evidence that temporal information could be integrated with contextual information, using standard configural training procedures. Two procedures were developed, one appetitive (Experiment 3) and one aversive (Experiment 4), that required rats to learn which specific context+time of day configurations signalled delivery of reinforcement. For example, in the morning food might be presented in the spotted context, but not in the checked context, and in the afternoon these contingencies were reversed. In Experiment 3, rats came to approach the site of food delivery during the configurations that signalled the delivery of food and were less likely to do so during the configurations that did not. Similarly, in Experiment 4, rats became less active during the configurations that signalled shock than those that signalled no shock. These procedures allowed both an automated and relatively continuous measurement of configural learning in contrast to those that had been available previously (e.g., Iordanova *et al.*, 2008).

6.2.3. Investigating the role of the hippocampus in configural learning involving the components of episodic memory

The configural learning procedures described in Chapter 3 were then used to assess the role of the hippocampus in standard configural discrimination procedures, but that involved the components of episodic memory. The results of the experiments described in Chapter 4 provided evidence that rats with hippocampal lesions, like control rats, can acquire configural discriminations requiring the integration of contextual and temporal cues. That is, in both an appetitive procedure (Experiments 5 and 6) and an aversive procedure

(Experiment 7), rats with hippocampal lesions did not show any deficit in acquiring the configural discriminations. These results stand in maked contrast to predictions made by several influential accounts of hippocampal function that were outlined in Chapter 1 (Sutherland & Rudy, 1989; O'Reilly & Rudy, 2001). They also appear to be inconsistent with the results reported by Iordanova and Collegues (Iordanova *et al*., 2009, 2011ab). The fact that lesions to the hippocampus did not appear to affect the direct encoding of *what-where-when* configurations suggested that the hippocampus might instead be involved in retrieval-mediated learning. It will be remembered that Iordanova *et al.* (2011b) showed that disrupting hippocampal function during the conditioning phase (involving the two auditory stimuli) was sufficient to abolish the difference in test performance between the configurations that had accompanied the two auditory stimuli. Taken together, these results suggest that investigating the role of the hippocampus in sensory preconditioning, where mediated learning has been implicated, should yield potentially important information. The aim of the experiments reported in Chapter 5 was to assess the hypothesis that the hippocampus might play a general role in retrieval mediated learning involving configural memories. In order to do so, I first needed to establish a *prima facie* case for the involvement of mediated configural learning in sensory preconditioning.

6.2.4. Assessing the contribution of configural processes to sensory preconditioning and the role of the hippocampus

To establish whether the hippocampus plays a general role in mediated (configural) learning I clearly needed to move to another procedure in which this process had been implicated. One obvious candidate was sensory preconditioning (see Ward-Robinson & Hall, 1996). However, the results reported by Iordanova *et al*. (2011a) suggested that the hippocampus was involved in mediated configural learning. Therefore, I conducted a behavioural experiment to assess the role of configural processes in an extant sensory preconditioning procedure (Lin *et al*., 2013). The results of Experiment 8, when taken together with Lin *et al*.

(2013), implicated mediated learning involving configurations in sensory preconditioning effects. Experiment 9 used a variant of this procedure to show that the hippocampus is involved in mediated learning involving more conventional audio-visual compounds. Thus, in control rats, after exposure to two compounds (AX and BY), conditioning with X resulted in greater fear to AX than to BX; and this effect was abolished in rats who had received lesions to the hippocampus prior to the exposure stage.

6.3. Theoretical implications

6.3.1. Episodic memory in animals

As described in Chapter 1, there are a number of studies that have attempted to document instances of episodic, or episodic-like, memory in non-human animals. Traditional interpretations of an animal's capacity for episodic memory have proposed the requirement to demonstrate mental time travel to single events or episodes that occurred in the past (Tulving, 2002). Other researchers have coined the term "episodic-like" memory to describe instances of animal learning about the components of episodic memory, and highlighted the formation of *what-where-when* integrated memories (Clayton & Dickinson, 1998).

The experimental work described in Chapter 3 was not intended to provide conclusive evidence in rats of true episodic memory as defined by Tulving (1972; 2002), particularly as the appetitive and aversive discriminations observed in Experiments 3 and 4 took many days to emerge. Nevertheless, successful discrimination performance in both procedures required configural integration of information about what reinforcer was presented (Experiment 3, food; Experiment 4, shock), where the reinforcer was presented (spotted or checked context) and when it was presented (morning or afternoon). This integration of contextual and temporal information is of central importance to episodic or episodic-like memory and the procedures developed in this thesis allowed for automated

measurement of how learning progressed over training. This contrasts with similar procedures developed by Iordanova *et al*. (2008), where successful learning about the configurations could only be revealed following modification of the information contained within each of the configurations involved in the discrimination.

There was some evidence from the results of the experiments in Chapter 2 that learning about time of day is more difficult than learning about visual context. This is consistent with other studies in animals that have suggested that learning about the *when* component of episodic-like memory tasks is more difficult than learning about the what or where components (Marshall, Hurly, Sturgeon, Shuker & Healy, 2013; see also Cain *et al*., 2004; Bird *et al*., 2003). One hypothesis is that this is due to the *what* and *where* components being made up of information from a single sensory stream, i.e., the nutritional benefit of food, or the visual input from a particular pattern, whereas information about the *when* component could be coming from a variety of different modalities, for example, the environmental light or sound levels, current states of arousal or hunger, or internal circadian cycles, and all of these may need to be integrated before they can provide information about the time of day. It was not possible to determine exactly which cues rats were using to signal the time of day in these procedures and such an issue was beyond the scope of this thesis. A discussion of the possible ways in which rats were using temporal cues is presented in Chapter 3 (Section 3.6.1.).

6.3.2. The role of the hippocampus in configural/episodic memory

The finding that rats with hippocampal lesions are not impaired in acquiring the configural discriminations in Chapter 4 is interesting on two counts. First, configural theories of hippocampal function propose a critical role for the hippocampus in configural learning, but not learning that can be subserved by elemental associations (see e.g., Rudy & Sutherland, 1989; O'Reilly & Rudy, 2001). Second, the hippocampus is often thought to influence learning

about spatial/contextual and temporal information, and is thought to be particularly important for the integration of these episodic memory components (e.g., Aggleton & Brown, 1999; Allen & Fortin, 2013; Ergorul & Eichenbaum, 2004; Tulving, 2002). The results from Experiments 1 and 2 indicate that the hippocampus is not critically involved in learning to associate cues from the visual environment with the presence of food, or in using some form of temporal cue to predict the presence of food. This finding is consistent with the idea that the hippocampus is not involved in the formation of simple associations and is consistent with results from Iordanova *et al*. (2009, 2011) who found no deficit in rats with hippocampal damage in forming elemental *what-where* and *what-when* associations.

However, the results from Experiments 5-7 show that the hippocampus may also not be involved in learning to integrate these types of information in a configural way. As mentioned previously, these results are not predicted by configural theories, which posit a critical role for the hippcoampus in configural learning. There are, however, other studies that have found that damage to the hippocampus does not affect the ability of rats to learn a configural discrimination. For example, Coutureau *et al.* (2004) reported that hippocampal rats readily acquired a conditional discrimination in which when exposed to contexts A and B, presentations of X were followed by food and those of Y were not, whereas when exposed to contexts C and D, presentations of Y were paired with food and those of X were not (Coutureau *et al.,* 2004; see also Saksida *et al*., 2007; McKernan & Jarrard, 1993; Gallagher & Holland, 1992).

It has been suggested that the hippocampus is primarily involved in *spatial* configural learning (Aggleton & Pearce, 2001; Sanderson, Pearce, Kyd & Aggleton, 2006), which is consistent with the role of the hippocampus in the processing of spatial cues (e.g., Olton *et al*., 1979). However, this does not account for studies where deficits in learning about non-spatial configurations have been found (e.g., Alvarado & Rudy, 1995; Rudy & Sutherland,

1989) and this does not explain the deficit seen in hippocampal-lesioned rats in the non-spatial configural learning tasks carried out by Iordanova and colleagues (Iordanova *et al*., 2009; 2011ab). Alternatively, it has been suggested that the hippocampus is only required for tasks that require the rapid formation of conjunctive representations (O'Reilly & Rudy, 2001). The procedures used in Experiments 5, 6 and 7 involved a relatively large number of days of training (24, 20 and 24 training days, respectively) and this could be the reason for the fact that lesions to the hippocampus had no effect on the acquisition of the configural discriminations. Nevertheless, the results presented in Chapter 4 add to evidence that an intact hippocampus is not a requirement for the encoding of configural memories, in this case involving the integration of contextual and temporal cues.

6.3.3. The role of the hippocampus in mediated configural learning

The finding that the hippocampus is not involved in acquiring configural memories in standard discrimination learning procedures leads to consideration of the basis for the deficit observed in rats with lesions of the hippocampus in the studies by Iordanova and colleagues (Iordanova *et al*., 2009; see also Iordanova *et al*., 2011ab). One possibility is that those memories were acquired relatively rapidly (involving four days of training; cf. O'Reilly & Rudy, 2001). A second possibility, however, is that the direct acquisition of configural memories is not dependent on the hippocampus and it is only in procedures involving mediated configural learning where a deficit is seen. In the studies by Iordanova *et al*., rats not only had to encode the training configurations, but then to update these configurations with new information about one of the components, and then retrieve this information at test. In contrast, the procedures in Experiments 5, 6 and 7 required rats only to directly link specific configurations with specific outcomes. Thus, one key difference between the procedures used in Experiments 5-7 and the procedures used by Iordanova and colleagues is the potential involvement of learning about associatively evoked configurations. Consistent with this view, Iordanova *et al*. (2011b) showed that temporarily disrupting

hippocampal function during the aversive conditioning stage was sufficient to produce a deficit at test. The suggestion that retrieval-mediated learning about the configurations was impaired is supported by the results from Experiment 8 and 9, which showed that mediated configural learning processes are evident in sensory preconditioning procedures and that hippocampal lesions disrupt performance in these procedures.

6.4. Future directions

Almost inevitably, there have been issues that I should have liked to pursue further or have been pursued without clear results. There are three examples where the results of research that I had conducted were inconclusive, or not sufficiently clear-cut to be presented in the main body of the thesis, which represent potential avenues for future research. I will briefly describe these below.

In Experiment 7, the results of a final test in which footshocks were presented in both contexts at both times of day (cf. Experiment 4) were inconclusive: While there tended to be more post-shock activity when the shock was presented after the configurations that had not previously been paired with shock than after the configurations that had been paired with shock, there was no sign of this effect in rats with hippocampal lesions. However, perhaps because of the differing amounts of training given in Experiments 4 and 7, the difference in the control group was not statistically significant. This test had the potential to reveal that the nature of learning differed in control rats and rats with hippocampal lesions. Thus, the kind of associative mismatch effect (cf. Honey & Good, 2000; Honey *et al*., 1998; see also Kumaran & Maguire, 2007) that this probe test reveals might be compromised in rats with hippocampal lesions. That is, this test had the potential to reveal whether the basis for successful discrimination learning was the same in the two groups. A related possibility is that for both groups configuration-shock associations formed, but the way in which the associatively provoked memory of shock interacted with the presentation of shock in the

world differed in the two groups. Of course, this analysis is moot given the fact that the results of the test were inconclusive.

In the analogous studies by Honey and Good (2000; Honey *et al*., 1998), rats received training in which the presentation of one of two distinct auditory stimuli (a tone or a series of clicks) was immediately followed by the presentation of a particular visual stimulus (constant light or flashing light). During mismatch trials, in which one of the auditory cues preceded the simultaneous presentation of both visual stimuli, control animals oriented more to the visual stimulus that was not predicted by the auditory cue rather than to the visual stimulus that was predicted by the auditory cue. In contrast, hippocampal rats showed greater orienting to the visual stimulus that was predicted by the auditory cue. Hippocampal involvement in detecting associative mismatches has also been suggested from the results of a human neuroimaging study in which participants viewed familiar items being presented in a novel temporal order and/or in a novel location (Kumaran & Maguire, 2007). It was found that hippocampal activation occurred whenever novel input patterns consisted of both associative match and mismatch components at the same time and in separate domains (temporal and spatial). These results suggest that the hippocampus is involved in representing the difference between associatively activated memories and those that are directly activated by events in the world. In the context of this thesis, this would have been to investigate in rats, mismatches between the stimulus that different configurations predict (i.e., one light or another) and what actually happens. In fact, I conducted a series of experiments where this idea was pursued and the orienting response to lights was measured as a function of whether or not they were predicted by a configuration of context and time of day. Unfortunately, the results were not significantly clear-cut to be presented in detail here.

Finally, I also attempted to "re-value" food after the appetitive configural discriminations reported in Chapter 4. Thus once the configural discrimination had been acquired food was presented in a blank chamber at midday and paired with shock. It was hoped that this procedure would yield the effect seen by Iordanova and colleagues in the control condition: more fear to the test configurations that had predicted food than the configurations that had predicted no food; and no effect of this manipulation in rats with hipocampal lesions. However, this manipulation proved to be ineffective, perhaps because there were too few aversive conditioning trials or because the response established to the configurations during appetitive training (movement towards the foodwell) interfered with the expected response during the test after aversive conditioning (freezing in the previously rewarded configurations).

In addition to these avenues for future research that were left incomplete, there are two others that remain to be explored and are prompted by the theoretical analysis developed in this thesis: That the hippocampus plays a role in learning about evoked configural memories, but not in direct learning about the same configural memories. First, one obvious prediction that follows from this analysis is that disrupting hippocampal function during the conditioning stage of the sensory preconditioning procedures described in Chapter 5 should abolish sensory preconditioning in those procedures. The use of sensory preconditioning effects that are not based on retrieval-mediated learning (i.e., are based on elemental chains) would provide an effective control for this type of study. Second, the hippocampus is most often associated with spatial processing and it would be interesting to examine the role of retrieval-mediated learning during various spatial tasks. However, little is known about how the process of forming the representation/s necessary to learn to escape from the watermaze interacts with connecting this representation to the goal (i.e., escaping from the watermaze). It certainly seems possible that the evoked memory of the specific location, where the platform has been located in the past, might need to be discriminated

from the directly activated memory of the location in order for the search for a safe haven to come to a successful conclusion.

6.5. Conclusions

My findings suggest that the hippocampus is not involved in the acquisition of configural memories generally, or in the integration of the components of episodic-like memory. However, on the basis of the research that I have conducted, it appears to be the case that the hippocampus does play a general role in retrieval-mediated learning about configurations. The way in which this role might be related to other functions that have been ascribed to the hippocampus is an intriguing issue that requires further investigation.

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