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The effects of hippocampal lesions on acquisition and memory for context.

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Declaration

I hereby declare that this submission is my own work, and that to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning., except where due acknowledgement has been made in the text.

Signature:

Name:

Date

To Andrew

Thank you

You have a God who hears you,
the power of love behind you,
the Holy Spirit within you,
and all of heaven ahead of you.

(The words of Max Lucado, given to me on a card by St Columba's Sunday School when I left Aberdeen. This card was pinned to the notice-board above my desk throughout my PhD.)

Abstract

The effects of hippocampal lesions on acquisition and memory for context

Hippocampal lesions impair memory for context in some tasks but not others. Factors that may contribute include:

a) whether context is encoded in configurally or elementally;

b) whether lesions are performed before or after acquisition of contextual information (Jeffery et al 2004 P 201-218[1]).

c) the size of the lesion.

This study compared the effects of pre- vs post-acquisition hippocampal lesions on performance of a novel context-dependent odour discrimination task that required explicit processing of the contextual features. As the task required a configuration to be formed between context, odour and reward, it was hypothesised that the hippocampus would be essential for the acquisition and performance of this task (Sutherland and Rudy 1989[2]).

Pre-surgery training consisted of simultaneous presentations of a context-dependent and a context-independent odour discrimination task. In the context dependent task, odour A but not odour B was rewarded in context 1, whereas odour B but not odour A was rewarded in context 2. In the context independent task, odour C was rewarded in both contexts, whereas odour D was rewarded in neither. Rats took around 60 days to reach criterion level (2 days >80% correct on both tasks). Subsequently, they received either bilateral ibotenic acid lesions of the hippocampus or sham surgery.

After a 14 day recovery period, post-surgery testing began. On the first 2 days of post-operative testing, lesioned animals were significantly impaired on the CD task,

but not on the CI task. Thereafter they performed as well as controls. Thus, the data demonstrate that although the hippocampus normally contributes to the retention of contextual information, it is not necessary for the performance of this context dependent odour discrimination task. Other areas can take over these functional demands in its absence. However, the involvement of the hippocampus cannot be completely disregarded due to the high degree of correlation between spared hippocampal tissue and the immediate post-surgery performance level of the animals (i.e. larger the volume of tissue spared the higher the initial degree of accuracy on the CD task). These findings were shown to be highly replicable, regardless of whether the odorous stimuli were presented simultaneously or successively.

Furthermore, the hippocampal and extra-hippocampal methods of task resolution were not identical. When a cue conflict situation arose between intra-maze and self-motion cues, it affected the two groups in a differentially. The ambiguity between cues had a highly detrimental affect on the performance of the intact animals; yet the hippocampal lesioned animals appeared oblivious to the inconsistency. They continued to perform the context dependent odour discrimination task as normal. Thus although apparently able to process the major contextual cues, the hippocampal lesioned animals had a deficit in detecting and responding to more subtle distinctions that were not integral to normal success on the task.

In the final aspect of this thesis, hippocampal lesioned animals were found to demonstrate no deficits in the acquisition of new variants of the context dependent odour discrimination task (new odours / contexts), thus the hippocampus is not essential for learning contextual discriminations.

Overall, the hypothesis that the hippocampus would be necessary for contextual representations, is unsupported by this thesis. Nevertheless, if present during training, the hippocampus will contribute to the retention of contextual stimuli and provides a more all encompassing view of ‘context’ than other areas can achieve alone.

1. *Jeffery, K.J., et al., A proposed architecture for the neural representation of spatial context. Neuroscience & Biobehavioral Reviews, 2004.* ***28****(2): p. 201-218.*

2. *Sutherland, R.J.R., J.W., Configural association theory: The role of the hippocampal formation in learning, memory and amnesia;. Psychobiology, 1989.* ***17****: p. 129-144.*

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1. Introduction

In order to interact successfully with our environment, our memory systems must assemble the disjointed set of pictures, actions, sensory inputs and emotions we experience, into one fluid representation, which can be modified by new occurrences. But where and how is this done?

"Memories light the corners of my mind" (Bergman 1973) sang Barbara Streisand, but was she right?

Despite being studied by philosophers and researchers for centuries, the exact anatomical basis for memory is still controversial. However, the association of the medial temporal lobe with learning and memory has long been established. Deep within this structure is a region called the hippocampus that is understood to play a critical role in at least some types of memory – but the question this thesis aims to address is whether contextual memory is one of them?

But what is a 'context'?

Context has been defined to mean many different things:

- **Spatial context** – a space you can move around in (O'Keefe and Nadel 1978)
- **Event context** – a discrete event has a time and /or place context (Nadel and Willner 1980)
- **The non-geometric features of an environment** – i.e. colour or smell (Anderson et al 2003)
- **Temporal context** – the timing of 2 events (Fortin et al 2002)
- **Motivational State** – i.e..g. hunger or thirst (Kennedy and Shapiro 2004)
- **Drug state** – and the feeling associated with it (Overton 1964)
- **Cognitive context** – a mental framework or schema (Morris 2006, Amaral and Levenex 2007)

Context as defined in this thesis will mean the **non-geometric features of the environment** (colour, texture etc). But why should these factors matter? Why should the memory systems encode non-geometric context at all?

In real life, items do not appear in isolation. They always have a time, space and context - and indeed many events would be nonsensical without this. Interpreting environmental cues means situations can be predicted and potentially avoided. For example if a predator appears, it may be best to freeze in the dark, whereas in the daylight, running may be a better option. Similarly, a stimulus may be dangerous in one situation, but not in another. Learning which environments are safe and which are aversive could be critical to an animal's reproductive success (Sanders and Wiltgen et al. 2003). Hence context associates are crucial to the very survival of the animal.

This chapter will begin by discussing how the hippocampus came to be associated with memory, and how its anatomy and connectivity make it ideally suited for the encoding of context. It will then move onto discussing some of the various theories of hippocampal function and why the configural theory is of particular interest when thinking about context encoding. Finally, I will describe some of the tasks that have been used previously by other experimenters in an attempt to clarify the role of the hippocampus in the encoding of context, in particular within the field of contextual fear conditioning. In the last section, I will set out the major questions that still remain regarding the role of the hippocampus in learning and memory for context, and the hypotheses that will be addressed specifically in the experimental chapters of this dissertation.

1.1. Hippocampus and Memory

The term hippocampus was first adopted by the anatomist Giulio Cesare Aranzi in 1564 (Thammaroj and Santosh et al. 2005) because of the structures seahorse shape. However, it was originally thought to be involved with smell, or alternatively (because of its position), emotions (Anderson 2007). The psychiatrist and neurophysiologist Vladimir Bekhterev was the first to make the link to memory based on observations of his patients. However, the paper that brought the focus for memory strongly onto the hippocampus was one describing a patient called H.M. by Scoville and Milner (1957). H.M. received neurosurgery to treat severe epilepsy (Gleitman et al 1999). During the surgery several parts of his medial temporal lobe were removed bilaterally, including most of the hippocampus. As a result H.M. suffered from severe anterograde amnesia. He completely lost the capacity to make new memories; he could not remember any new people, facts or events that occurred after the point of surgery (Gleitman et al 1999). In addition, H.M. had moderate retrograde amnesia – he could not remember events and facts that occurred in the 1-3 years before surgery. Conversely, his memory for events prior to this (3yrs + before surgery) was largely intact. As was his ability to perform broad intellectual functions (e.g. reading, writing and conversational skills; Scoville and Milner 1957).

1.2. Classes of memory

Memory has been classified into several different types. The first segregation was described by Waugh and Norman (1965) who defined 2 types of memory dependent on their timescale a) short term memory and b) long term memory – he called these primary and secondary, respectively. Long term memory is a large data storage system (like a library). The capacity is vast, but the access to it is difficult. Short term memory however, is more in limited size, but is immediately accessible (like the

limited number of books that you would actively work from on a desk (Gleitman 1999).

Long-term memory has been divided into many subtypes (see Figure 1-1), all of which are thought to be processed in different ways by the brain. Procedural memory is defined as memory for skills, such as riding a bike (i.e. knowing how). Declarative memory by contrast is defined as "remembering that" and includes memory for facts and events (i.e. episodic memory, Tulving 1972). These are generally things that humans can describe with language e.g. facts about the world, as well as our own personal experiences. Declarative memory is further broken down into semantic and episodic memory. Semantic memory is known as general knowledge (e.g. cars run on petrol). There is no recollection of when or where we learnt this piece of information, just the knowledge itself. Episodic memory on the other hand is memory for personal events that each have a 'context' – a time and a place in which they occurred (e.g. 'I had dinner at my friend's house last night').

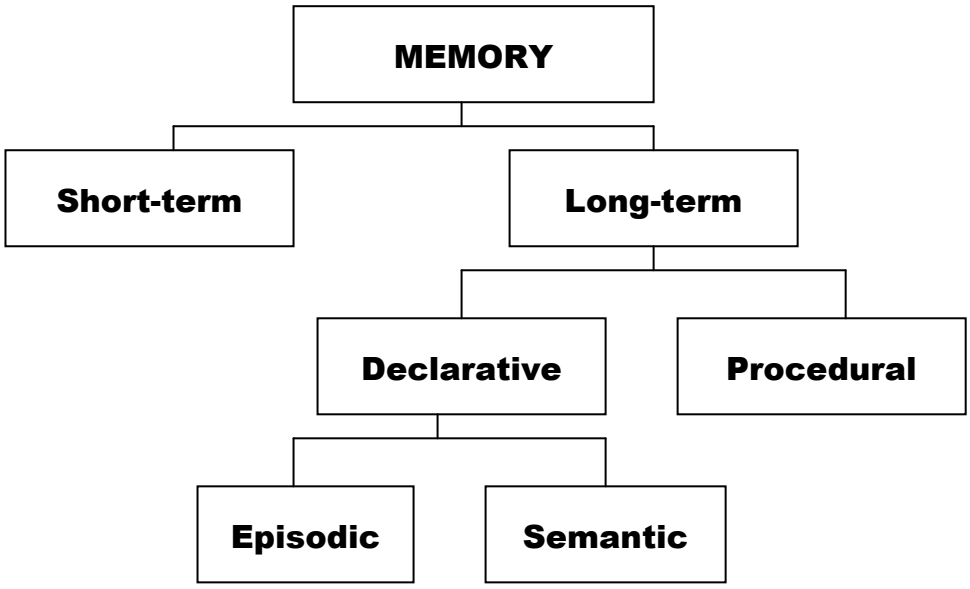

Figure 1-1: Types of memory and how they are related

H.M. was found to have impaired declarative memory but intact non-declarative memory. Therefore, MTL structures, comprising of the hippocampus, perirhinal cortex and parahippocampal cortex were thought to play a role in declarative but not procedural memory. This has been substantiated, both in studies of other patients (Stefanacci et al 2000, Bayley et al 2005,) as well as in animal studies testing the effects of large medial temporal lobe lesions in primates (Zola-Morgan and Squire 1984, 1985). But what about the hippocampus specifically? Is it required for context memory? The specifics of this are still being debated and many theories have arisen as to exactly how the hippocampus works and what information it processes. However, before moving into descriptions of the more physiological role for the hippocampus, it seems necessary to anatomically place the hippocampus with respect to the rest of the brain.

1.3. Anatomy of the hippocampal regions[a]

The hippocampus is an evolutionally old part of the brain that is highly similar across mammalian species (including rats and humans). It sits prominently as an elongated C-shape in the medial temporal lobe of the brain (see Figure 1-2). The hippocampus has a very precise and organised structure, which is made up of distinct regions called the dentate gyrus and the cornus ammonis (CA); the latter of which is further subdivided into the CA1, CA2 and CA3 areas. The anatomical distribution of the intra-hippocampal connections produces a primarily uni-directional internal circuitry. This pathway runs through the dentate gyrus, to the CA3 region, then CA1 region and then finally outputs outside the hippocampus to the subiculum and entorhinal cortex (The CA2 region does not appear to be a critical component of the internal circuitry. Indeed its connectivity is still somewhat unknown).

In addition to the unidirectional pathway, there are supplementary inputs, outputs and associational fibres which are thought to aid the hippocampus in its more integrative role. These include many associational (to self) and commissural (to contralateral hemisphere) fibres in the DG and CA3. The recurrent collaterals between neighbouring CA3 neurons are abundant (each CA3 pyramidal cell receives ~12000 collateral inputs) but highly distributed (~2% connectivity across hippocampus). They are thought to be important for making arbitrary associations between cues in different modalities, and for the subsequent reactivation of an entire representation from one or a small number of cues (Rolls and Kesner 2006). This would make them ideal for the encoding of context.

Furthermore, inputs from the entorhinal cortex and subcortical structures (e.g. perirhinal and postrhinal cortices) arise later in the process and input directly to the CA3 and CA1 regions. For example, the CA1 region receives inputs both via the tri-synaptic loop from CA3 and also directly from the entorhinal cortex, making it a

[a] The information in this anatomy section came from {Alheid et al, 2004 #143}

potential comparator or pattern separator. This would make it ideal for distinguishing between contexts.

All regions within the hippocampus maintain a distinct 3 layer structure that is the hallmark of hippocampal tissue. This gives the region anatomical potential for both making associations as well as maintaining segregation of inputs. This dual property makes the hippocampus an ideal candidate for learning and memory processes. The ability of the CA3 region to associate diverse inputs (Kesner 2007) may be especially useful for the encoding of context, which requires the association of many different sensory modalities (e.g. temperature, light levels, texture).

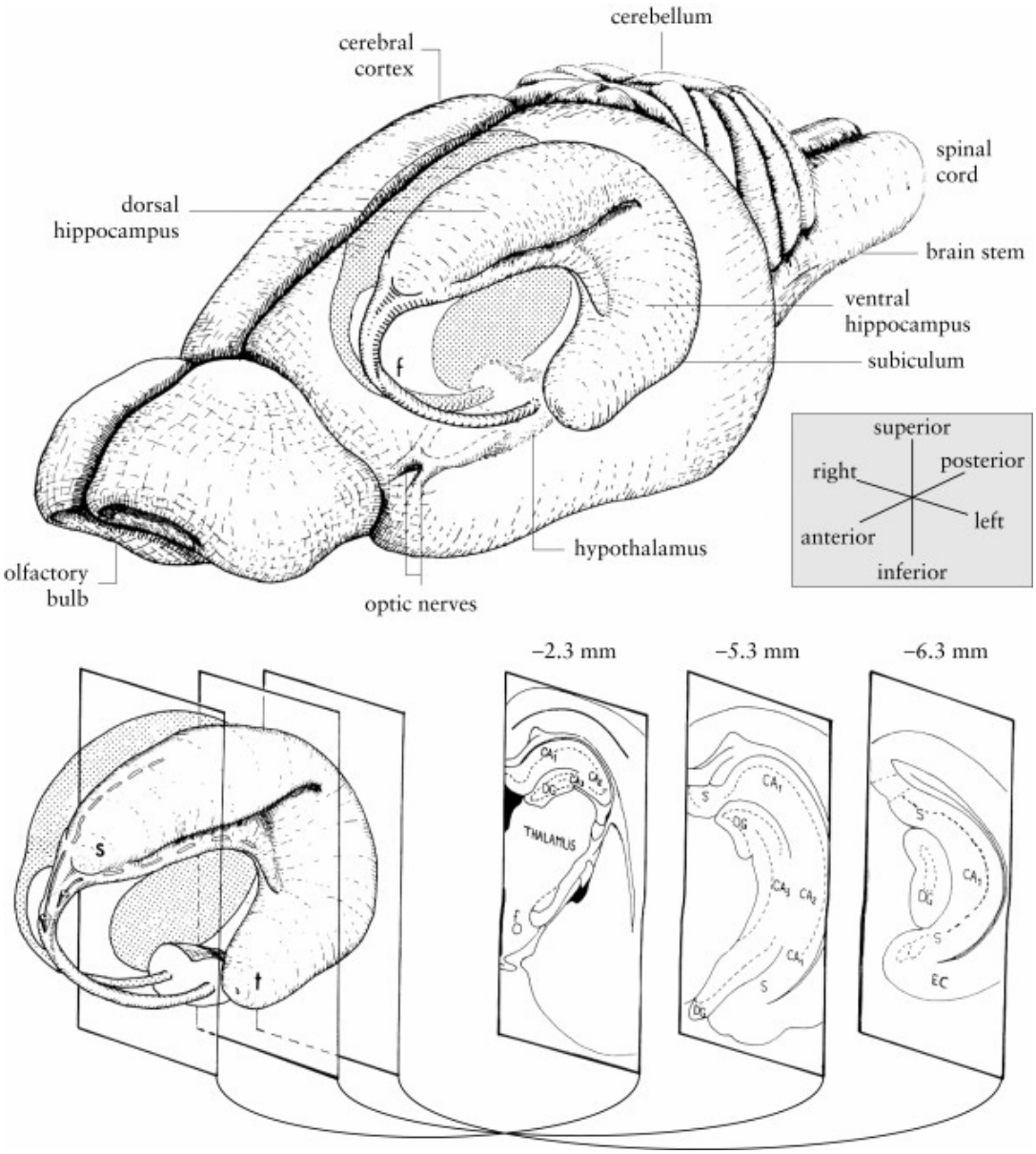

Figure 1-2: a) Schematic Illustration showing the 3D location of the hippocampus in relation to other brain structures. b) Three coronal sections through the left hippocampus showing the different fields that make up each part of the hippocampus. CA1, CA2, CA3 = cornus ammonis fields, DG = dentate gyrus, EC = entorhinal cortex, f = fornix, s = septal pole of hippocampus, S = subiculum, t = temporal pole of hippocampus (From Amaral and Witter 2000 as found in the first edition of the book 'The Rat Nervous System' (Alheid et al, 2004}

1.4. Theories of hippocampal function

When describing the anatomy of the hippocampus, it has become obvious that the hippocampus can both segregate and integrate its inputs. However, despite being the subject of extensive research, the controversy over the exact function of the hippocampus still continues. The following section gives an overview of the differences between cortical and hippocampal learning, and then moves onto describing four of the main theories of hippocampal function and the experiments which have been used to test them.

Cortical learning is slow and methodical; Hippocampal learning is fast and flexible.

One hypothesis is that to get maximum use out a memory system, it must be both stable and flexible. This combination is difficult for just one area system alone to achieve. So instead it has been proposed that the brain uses a combination of two interacting systems, a slow methodical one in the cortex and a fast and flexible one in the hippocampus.

The cortical system is the solid, regular and dependable site that forms the basis of brain function. It is not set up to rapidly encode information. Indeed if it did, all concepts of regularity could be lost and the memory system could collapse. Instead the cortical system normally learns in a slow error driven way (Rumelhart et al 1986), adjusting its neuronal weights to minimize errors in the system. It produces one particular output when given a certain input (i.e. stimulus-response) so its coding can be rather inflexible. The primary role of the cortex therefore is to extract generalities about the environment (Morris et al 2007).

The hippocampus on the other hand primarily uses a fast hebbian learning mechanisms; linking co-active neurons and subduing links to inactive ones (O'Reilly et al 2000). In this way the hippocampus can efficiently and rapidly encode the

specifics of a situation. It is thought to be useful for binding together co-occurring features (conjunctive representations), learning about relationships between stimuli (pattern completion), and for keeping opposing features separate (pattern separation; O'Reilly et al 2000). In this way the hippocampus is thought to be ideal for both forming representations of contexts (with all their co-occurring stimuli), and for keeping the representations of each context separate and independent (Leutgeb and Moser 2007, Jeffrey et al 2004, Goodrich-Hunsanker et al 2005).

With the cortical and the hippocampal systems working together in this way, a flexible but stable system memory system is obtained. However, there is some debate as to whether all incoming information is processed in this way. Do the hippocampal or cortical memory systems actually work alone to processes certain types of information? What does it take to activate them both? These questions have formed the basis of several theories of hippocampal function – four of which are described below.

1.4.1. Cognitive Map Theory

O'Keefe and Nadel (1978) propose that the hippocampus holds a distinct map of space, that is built up during exploration of an environment. This map is allocentric (world view), and can be used for place recognition and navigation (Amaral et al 2007). This theory focuses predominantly on space. Supporters of this view are resolute that a representation of space can only exist after substantial mental computations of geometry. Changing simple sensory inputs is thought to have little effect on spatial representations.

In general this theory is widely accepted. However, it is considered too narrow a concept. Couldn't the cognitive map be expanded to include all spatial, non-spatial, temporal and contextual aspects and thus become a more inclusive theory of memory?

1.4.2. Declarative Memory Theory

Squire (1983) proposed that memory is either declarative or non-declarative. Declarative memory is described as the conscious recollection of facts and events. It is dependent upon the medial temporal lobe (including the hippocampus). Non-declarative memory by contrast describes the unconscious memory for skills, habits and non-associative learning. Furthermore, this theory suggests that the role of the medial temporal lobe is time-limited. Over time, memories are reorganised / consolidated to long term storage sites in the cortex (Squire 1992). Thus, according to this theory, any task that requires an association between context and another factor should depend upon the hippocampus, at least temporarily, unless the response becomes habitual.

Nevertheless, several objections have been raised to this theory. The first is that it suggests the entire medial temporal lobe acts as one unit. Although it is likely that the rest of the medial temporal lobe plays some role in supporting the hippocampus, this level of involvement is debatable (Amaral et al 2007). Furthermore the declarative memory theory combines both semantic (facts) and episodic memory (events) into one category – an idea that is seen as an oversimplification. Thus, the declarative memory theory appears provide only a very broad view over a very complex process (Amaral et al 2007).

1.4.3. Explicit and Incidental memories

Most tests of memory challenge explicit memories - memories that were deliberately or consciously formed. Explicit memories normally encompass the key features of the task. The opposite of this is incidental memory. This term is used to describe

memories that are not deliberately encoded, (for example the background when you look at an object).

In rats, the hippocampus has been shown to be necessary for incidental encoding, but not always for explicit encoding of context. For example, Philips and LeDoux (1994) demonstrated that dorsal hippocampal lesions impaired context fear when a tone was present during conditioning (i.e. context was a background cue and encoded incidentally). On the other hand, when context became the foreground explicitly encoded stimuli after the removal of the salient tone, (i.e. the only thing the animals could associate the shock to was the context) hippocampal lesions caused no impairment in contextual fear. Similarly, Good et al (1998) demonstrated that rats could perform an explicit biconditional task as well as sham animals, but yet failed to show contextual control (decrease in responding) when the stimuli were presented in a new context for the first time. Thus, for context associations at least, it is incidental stimuli that are dependent on the hippocampus, whereas explicit learning can proceed without it.

1.4.4. Configural Association Theory

The configural association theory was proposed by Rudy and Sutherland in 1989 (and revised by Rudy and Sutherland 1995 and Rudy and O'Reilly 2001). A configuration is:

" an association comprising of more than one distinguishable cue. The association includes a description of each cue and of its relationship to at least one other cue"

(McDonald et al 1997, Hippocampus 7(4) Page 371)

This theory is principally concerned with situations where ambiguity occurs (i.e. the same stimulus signifies different events in different contexts). These are called 'non-linear' associative problems. For example a tone could predict food in one situation, but not in another (Amaral et al 2007).

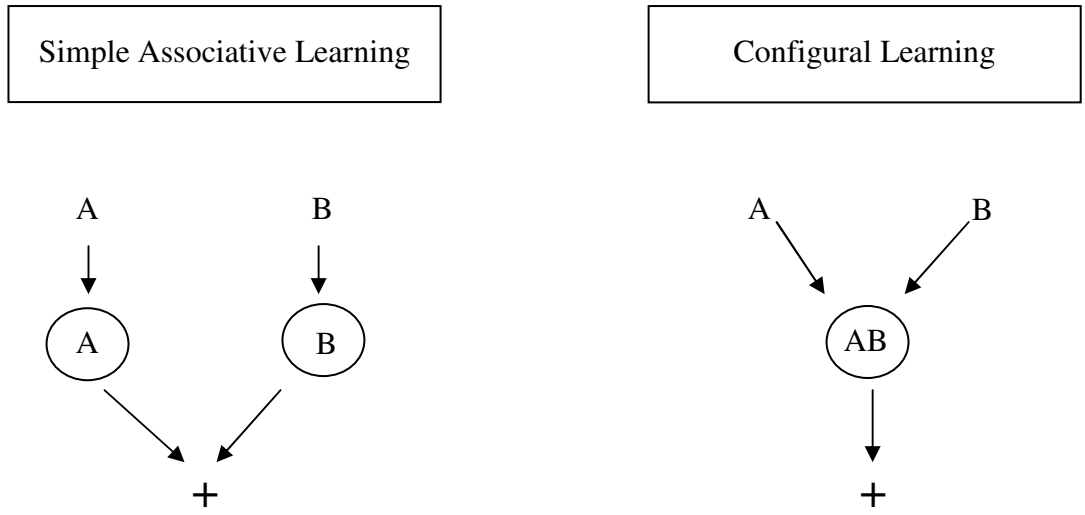

Figure 1-6: Illustration of the encoding of a compound stimulus AB by both a simple and a configural association system. For the simple association, the associative strength of the compound is equal to the sum of its elements and individual presentation of an element would lead to reduced responsiveness. By representing the compound in a configural way, presentation of each element individually would only partially activate the representation and hence the reward contingencies of a compound and its separate elements can be kept apart.

The theory proposes that the hippocampus binds and stores the configural associations between elemental (individual) stimuli to form, for example, spatial relationships and logical associations (Rudy and Sutherland 1989). In this sense, two stimuli can be associated together and hence become 'one' combined cue. In the above example, the tone and situation 1 would be bound together as 'cue 1' (predicting reward), whereas the tone and situation 2 would be bound together as 'cue 2' (no reward). The tone is therefore no longer a conflicting or 'ambiguous' stimulus. In this way normal associative learning would occur, (i.e. an increase in

synaptic strength with exposure to that specific combination) and the animal would learn the solution to the problem. Rudy and Sutherland (1989) proposed that this associative learning process could be used to bind together any combination of stimuli – even that coming from different sensory modalities or arriving separated in time or space. Hence, context learning may be an example of configural learning because it is dependent upon the integration of multiple stimuli into one representation. Examples of studies that have been used to test the configural theory (see Table 1-1) include negative patterning (Impaired - Rudy and Sutherland 1989, Alvarado and Rudy 1995a, McDonald and Murphy et al 1997, unimpaired – Davidson, McKernan and Jarrard 1993), transverse patterning (Alvarado and Rudy, 1995a, 1995b, Dusek and Eichenbaum 1998) biconditional discrimination (unimpaired – Good, de Hoz and Morris 1998, Coutereau and Kilcross et al 2002; slow acquisition – Wishaw and Tomie 1991, McDonald et al 1997), and feature neutral discrimination tasks (Gallagher and Holland 1992, Alvarado and Rudy 1995a).

Table 1-1: Range of Configural tasks. The letters represent individual stimuli. + means rewarded, - means non-rewarded.

Task	Effect of Hippocampal Lesions
Negative Patterning A+, B+, AB-	Most studies find impairments
Transverse patterning A+B-, B+C-, C+A- (rock, paper, scissors)	Significantly impaired performance
Biconditional discrimination AB+, CD+, AC-, BD-	Slow learning, but can learn
Feature neutral discrimination AC+, B+, AB- C-	No impairment

However, despite predictions that performance on all configural tasks would be impaired by hippocampal lesions, this was not the case. Although the overlapping negative patterning and transverse patterning paradigms, were indeed impaired by hippocampal lesions (e.g. Spence 1952; Rudy and Sutherland 1989), other supposedly configural tasks were not (e.g. Whishaw and Tomie 1991; Gallagher and Holland 1992; Good et al 1998; Honey and Ward-Robinson 2002). Thus it was shown that the hippocampus is only required for tasks where an elemental solution is impossible. If compound elements can be permanently bound together, such that they reliably predict the outcome, the hippocampus is not required (Whishaw and Tomie 1991; Gallagher and Holland 1992; Good et al 1998; Honey and Ward-Robinson 2002).

In the face of this mounting evidence, Rudy and Sutherland revised their configural theory in 1995. Configural units were now postulated to exist outside (instead of inside) the hippocampus. Instead of being the origin for configural units, the hippocampus was instead postulated to contribute to configural processing by enhancing the activation or salience of configural / conjunctive representations formed in other parts of the cortex. This increases the difference between elements and their compounds and increases the rate at which configurations acquire associative strength (Rudy and Sutherland 1995). Thus, although the hippocampus would aid the formation of a configural representation of all the elements of a context, this representation would not lie in the hippocampus itself.

1.4.5. Summary of theories

Overall, the main theories of hippocampal function focus strongly on the encoding of space, spatial context and the relationships between stimuli. They do not preclude however a more general role for the hippocampus in more varied memory processes such as non-spatial encoding and temporal, logical and abstract associations.(Morris

2007). Indeed, due to its integrative capacity, the hippocampus would be ideally suited for the purpose of linking together multi-modal contextual information. Thus the question arises, is the hippocampus involved in memory for context, and if so under what conditions?

1.5. Memory for Context

Context as defined in this thesis as the combination of non-geometric features in the environment (colour, texture etc). Thus a major factor in contextual encoding is the association of 2 or more factors or stimuli. This can be done is two ways, either:

- an elemental way, where each independent feature (or element) is encoded in isolation and can enter into a separate association with the event;

or

- a configural way, where the separate features are bound together into one unit, which is then associated to the event. The latter method emphasises relationships and co-occurrences.

As is consistent with the cognitive mapping theory of O'Keefe and Nadel (1978) it seems likely that the cortex is responsible for encoding the individual elements of a context, and simple stimulus-stimulus associations, but that it must interact with the hippocampus to perform more complex functions such as binding these into a unitary representation (Rudy and O'Reilly 2001). One of the key questions however (as stated by Jeffrey and Anderson et al 2004) was whether behavioural studies could give insights into this contextual memory process.

1.5.1. Contextual Fear Conditioning

Contextual fear conditioning is a well studied behavioural paradigm that forms the basis of much current knowledge regarding contextual processing. This next section will discuss the role of the hippocampus in contextual fear conditioning in terms of encoding and retention.

Fear conditioning involves making an association between a tone and/or a context (conditioned stimulus – CS) and a foot-shock (unconditioned stimulus or US). The general protocol involves placing an animal into a distinct conditioning chamber (context) for a training phase. After a period of exploration, an auditory tone is paired with an electric shock to the animal's feet. The instant response of the animal involves vigorous locomotor activity (unconditional reaction – UR) that gradually gives way to freezing (conditioned response – CR). Freezing is defined as complete immobility, with the exception of that normally associated with breathing (Fanselow 2000). At this point the rat is removed from the apparatus and returned to its home cage for a period of time (often 24hrs). The rat is then replaced into the conditioning chamber for a testing phase where the amount of time spent freezing is noted.

To measure context fear only the environmental cues are present (no tone). Conditioning to context is thought to be highly dependent upon the hippocampus, but to the tone hippocampal independent. This would be consistent with the view that the hippocampus is necessary for supporting conjunctive representations (Nadel and Wilner 1980), but is not necessary for the encoding of single unitary cues.

What the hippocampus does not do!

Before moving on to the exact role of the hippocampus, it is important to establish what the hippocampus does not do and the characteristics of other brain regions involved in contextual fear conditioning. The amygdala is a heterogeneous group of nuclei buried deep within the temporal lobe that is involved in the processing of

emotional stimuli (Alheid et al 2004). It receives sensory input from the thalamus and cortex, as well as complex conjunctive stimuli (e.g. spatial representations) from the hippocampus and subiculum (the major output region of the hippocampus). Due to its complementary emotional and sensory role, the amygdala is thought to be responsible for associating the CS with the US during fear conditioning studies (Alheid et al 2004). For this reason the amygdala has been implicated in almost all forms of fear conditioning including conditional freezing to simple stimuli such as tone, or polymodal stimuli like context (Alheid et al 2004). In addition to the amygdala, a critical component involved in contextual fear conditioning is the nucleus accumbens, which regulates exploration of the environment, ensuring that sufficient sampling of the cues can occur (Mogensen et al 1980). When a rat is placed into a novel environment, it will explore until it has had adequate exposure to form an integrated memory. At this point exploration will decrease and associations can be made.

The hippocampus is necessary for retention of contextual fear

Post-training lesions have been used to test the involvement of the hippocampus in the retention of contextual memories. If the hippocampus was used to form associations, then its removal should result in a deficit. And indeed this is exactly what was observed for contextual fear conditioning – removal of the hippocampus post-training causes an impairment in contextual freezing upon testing (Anagnostaras and Maren et al. 1999; Lehmann and Lacanilao et al. 2007)

The hippocampus is not absolutely necessary for the acquisition of contextual fear

Experiments using pre-training excitotoxic lesions often find no impairment on contextual fear conditioning (Maren and Aharonov et al. 1997; Gisquet-Verrier, Dutrieux et al. 1999; Anagnostaras et al 2001). It is hypothesised that a lesioned rat simply finds an elemental solution to the problem, instead of a configural one, when

the hippocampus is absent during training. Hence it appears that the hippocampus is not required for learning a contextual fear association.

Nevertheless, some confounding evidence has been found. Pre-training electrolytic lesions **do** produce impairments in acquisition of contextual fear (Maren and Fanselow 1997, Fanselow 2000). However, this effect has been discredited as being a hyperactivity artefact (as is characteristic of this type of lesion).

Hence, it appears that the hippocampus is not required for learning a contextual fear association if simple associations can be used instead.

The hippocampus is more involved when contextual complexity is high.

A recent paper by Moses et al (2007) showed that even the complexity of the environment can have an effect on the ability of a hippocampal lesioned animal to perform a contextual fear conditioning task. In normal rats, less freezing was observed during the test phase if rats were conditioned within a complex, multi-stimulus environment, than if conditioning took place in a more simple, homogenous (mainly white) environment. Hippocampal lesions led to reduced freezing in both contexts, but the effect was significantly greater in the complex one. Furthermore, lesioning the hippocampus had an interesting effect on generalisation. If the conditioning occurred in the simple context, the lesioned rats did not display the freezing response (generalisation) to the complex context. Conversely, if conditioning occurred in the complex context – a large level of generalisation was observed, (i.e. the rats froze as often in the simple context, as they did if exposed to the complex one). This provides further evidence that substantial and effective conditioning to simple environments is possible without the hippocampus. However, the formation of a conjunctive representation of a complex environment is necessary to prevent the application of a behavioural outcome in an inappropriate context

Configural representations of context are required for context fear conditioning - insights from Immediate Shock Deficit experiments

The immediate shock deficit is observed when the delay between entering the context and the onset of shock is very short. The result is a proportional reduction in freezing during the testing phase (Fanselow et al 1990). The short pre-shock period is thought to be insufficient for the formation of a configural relationship between the elements of the context. Thus, the association between shock and context does not occur (Fanselow et al 1990). Nevertheless, a short period of pre-exposure can ensure the context representation is made in advance of conditioning and thus rescue performance (context pre-exposure facilitation effect, CPFE). Upon re-entry, the former representation can be recalled and associated with the aversive stimulus (Fanselow 1990). However, pre-exposure itself is not sufficient. Rudy and O'Reilly (1999) demonstrated that pre-exposure to the elements of a context individually was not sufficient to result in a facilitation of contextual freezing (No CPFE). Only the formation of a configural representation will result in the CPFE. Therefore, for context conditioning to occur, there must be sufficient pre-exposure to the context (for a configural representation to be formed), and a small amount of exposure that is contiguous with the shock (to re-activate the representation and produce the association).

It is hypothesised that the hippocampus is required for the formation of these configural representations of context. Indeed, inactivation of the hippocampus that prevents either the formation of the configuration or its retrieval during conditioning or testing impairs context fear conditioning (Matus-Amat 2004). Hence the role of the hippocampus in pattern completion and the formation of a configural representation of context is well supported by contextual fear conditioning data.

Summary of fear conditioning studies

Contextual fear conditioning has demonstrated that the hippocampus is required for acquisition, retention and retrieval of contextual information when a configural representation is required. Configural representations also prevent inappropriate generalisation and allow memories to be used more flexibly. However, if behaviour can be supported by elemental associations, normal behavioural performance may still be achieved even in the absence of the hippocampus.

1.5.2. Alternative methods of assessing contextual fear

The standard assessment of context fear measures freezing. Although this is easily measured and is highly sensitive to hippocampal lesions, it is potentially confounded by the hyperactivity side effects that are often seen after electrolytic lesions. Furthermore, hippocampal lesions have been shown to disrupt freezing to unconditioned stimuli (e.g. freezing in response to the appearance of a cat; Blanchard and Blanchard 1972). To control for this, contextual freezing experiments usually demonstrate that conditioning to a tone remains intact. Nevertheless, there is now evidence that even tone fear can be disrupted by hippocampal lesions (Maren 1999; Bast 2003).

In addition, there is a growing body of evidence suggesting that the hippocampal dependence observed with contextual freezing may not be applicable to other context fear paradigms. For example, McNish and Gewirtz et al (1997) demonstrated that rats with dorsal hippocampal lesions showed no deficit in fear potentiated startle, despite the same animals showing impairments in contextual freezing. Similarly, in 2000, McNish and Gewirtz demonstrated that rats with lesions to their dorsal hippocampus displayed no impairment in a contextual blocking paradigm. This is an

indirect method of measuring contextual fear, where the animals are exposed to the conditioned stimulus in both the conditioning chamber and a neutral chamber. Normal animals display a decrease in fear potentiated startle to the conditioned stimulus. In this experiment, the lesioned animals still displayed this decrease (McNish and Gewirtz 2000), indicating that they had indeed picked up the context ambiguity. Similarly, Winocur et al (1987) demonstrated that rats with dorsal hippocampal lesions displayed no impairment in active avoidance of a chamber in which the shock had been previously delivered.

Thus it seems that the expression of context fear after hippocampal lesions may depend upon experimental conditions. Perhaps freezing is not the best measurement. Perhaps instead using non-fearful paradigm would avoid these points of debate.

1.5.3. Other contextual tasks

Context has been studied in other paradigms aside from fear conditioning.

Hippocampal lesions impair acquisition of object-context recognition

One principle method has involved object recognition. Rats have a natural tendency to explore things that appear novel more than those that are familiar (Ennaceur 1988). In 1999 Dix and Aggleton designed a context version of this paradigm. For this rats are familiarized with two different objects, each in a unique context (i.e. two copies of object A in context 1 (striped walls, sawdust floor), two copies of object B in context 2 (grey walls, aluminium floor). During the test phase, one copy of each object is presented in one of the two contexts, hence one is familiar in that context and the other is novel in that context. Rats prefer novelty, so will spend a large proportion of their time investigating the novel combination. Mumby et al (2002) showed that rats with hippocampal lesions were impaired at this task. Thus again the

hippocampus and its role in encoding configurations is shown to be important for contextual memory. The researchers suggested that (as was seen in contextual fear conditioning), the lack of a configural representation prevented the association of the object with its context, and hence both objects had equal familiarity, there would be no reason to explore them differently. In contrast, an animal with an intact hippocampus would form configural representations of each object with a particular context, so when faced with two objects, one in a familiar object-context configuration and one in a novel configuration, it would explore the novel object-context configuration more.

Conflicting studies suggesting the hippocampus is <u>not</u> necessary for object-context recognition memory

In contrast to Mumby et al (2002), who used different rooms for their 2 contexts, Langston et al (2006) found that the hippocampus was not necessary for object-context encoding if the two contexts are presented within the same room. Similarly, Norman and Eacott (2005) demonstrated that fornix lesions caused only a mild impairment in object-context recognition. However, the retention delays were shorter in these studies than in the Mumby et al (2002) study, which could also contribute to the differential findings. Thus the data within the field is not entirely consistent and requires some further investigation.

The hippocampus is required for contextual control

Good and Honey (1991) demonstrated that the hippocampus is required for contextual control of responding to a cue. Rats were trained to approach a food tray after presentation of a visual cue (X) in context A or an auditory cue (Y) in context B. They were then tested for generalisation, by presenting the cues in the opposite contexts. Control rats showed a much lower level of responding when the cue was presented within an 'inappropriate' context. Rats with pre-training dorsal hippocampal lesions did not display this reduction in responding. This suggests that although the lesioned rats encoded the explicit cue reward association between the

tone/clicker and the contexts (light /dark), the implicit encoding of background cues was absent. Furthermore, McDonald et al (1997) demonstrated that it took extensive training (with rewarded and non-rewarded trials) for lesioned rats to gain this kind of contextual control. Hence, the hippocampus provides a rapid way to encode relationships between stimuli. Without it generalisation will tend to occur.

The hippocampus is required for appropriate generalisation – evidence from the acquired equivalence effect

Further proof of this appropriate generalisation effect was found by Honey and Watt (1999) who showed that normal rats will generalise an aversive stimuli between contexts that had been similarly paired during a biconditional task (auditory and context). Initially in context A and B, sound X was food rewarded, but Y was not. In contexts C and D, the opposite was true (i.e. Y rewarded, X not – see Table 2). Rats then received evaluation training whereby context A became associated with shock and context C with absence of shock. Generalisation of the freezing fear response was seen to transfer to context B which had shared reward properties with A during the biconditional training, but not D which had been paired with the unshocked context C. This is described as the "acquired equivalence effect" (Good and Honey 1991) and occurs between the context and a feature that have come to represent the same associations. If a simple elemental strategy was employed, this generalisation would not have occurred because context B and the shock stimulus never occurred together, and context A and B were visually distinct and so would activate different representations. Only a configural representation where the similarities between the reward properties of contexts A and B are recognised could result in the representational flexibility and generalisation observed.

Table 2: Design of Acquired Relational Equivalence Task

(Adapted from Honey and Watt 1999)

Bi-conditional Training		Revaluation	Test
A-X → food	A-Y → no food		
B-X → food	B-Y → no food	A → shock	B & D
C-X → no food	C-Y → food	C → no shock	
D-X → no food	D-Y → food		

Therefore, just like the fear conditioning studies, other contextual studies have emphasised the need for the hippocampus in encoding configural representations.

1.5.4. The role of the hippocampus in the encoding and retention of context

The role of the hippocampus in the encoding and retention of context is not clear in the literature. Hippocampal lesion studies have been shown to both impair memory for context and to leave it intact, depending upon the task employed. For example, the hippocampus is essential for the retention of contextual fear, but yet is not required for the retention of fear potentiated startle to context (McNish et al 1997). Similarly, object context recognition has been found to be both impaired by hippocampal lesions (Mumby et al 2002) and to be left intact (Langston et al 2006). Some of the factors thought to determine the level of hippocampal involvement in a task may include whether context is encoded implicitly or explicitly, configurally or elementally and the timing of the lesion (pre- or post acquisition). This thesis aims to explore some of these facets in more detail.

At the end of their 2004 paper, Jeffery and Anderson et al identified several specific questions that they felt had yet to be answered regarding contextual encoding. These included:

- "Where and how does the brain represent context, and can study of this representation find parallels between behavioral and neural phenomena?
- Can/does an animal use just one particular contextual stimulus element to solve a problem, or must it know about how they are combined as compounds (configurations)?"

The experiments in this thesis were designed to address these questions. Therefore, it is proposed that the hippocampus is responsible for encoding representations of context, and that behavioural tasks can be used to prove this. Furthermore, lesions to the hippocampus will result in impairments in the acquisition and retention of contextual information.

Contextual fear conditioning studies provide the most comprehensive outline of what it takes to induce hippocampal involvement in a task. These studies have illustrated that it is important both to emphasise the use of a configural representation and to examine features like retention as well as acquisition in order to fully understand the complexity of hippocampal involvement. Nevertheless, an important feature of the task used in this thesis is it does *not* use fear. Instead it deploys positive reinforcement that enables the study of hippocampal memory mechanisms separately from basic mechanisms of aversively motivated behaviour in which the hippocampus has been implicated (e.g. Gray and McNaughton, 2000; Bannerman et al. 2004)

The task used in this thesis is biconditional (AX^+, AY^-, BX^-, BY^+). It cannot therefore be solved using summation of the associative strengths of its elements – it requires a configural solution. Furthermore, in order to emphasise associations, the contexts (A and B) will be composed from several different elements. As odour discrimination is a natural behaviour for rats and is not dependent on the

hippocampus (Eichenbaum et al 1986, 1988), it was decided that the task be a biconditional context dependent odour discrimination. Thus, in order to solve the task, the rats had to make a configural association between the contextual stimuli and the reward association of the odour. The emphasis on a configural association prevents simple stimulus-response encoding and thus should induce the use of the hippocampus.

1.6. Hypothesis

This thesis aims to test the hypotheses that:

a) Rats can learn a novel context dependent odour discrimination task

b) Lesioning the hippocampus after training will result in a performance deficit on the context dependent odour discrimination task. Performance on a concurrently run context independent task will be unaffected.

c) Post-surgery acquisition of the context-dependent odour discrimination task will be impaired in animals with hippocampal lesions. No deficit in acquisition will be observed for the context independent task.

d) Rats with hippocampal lesions will have a more simplistic representation of the task and their environment than sham animals.

e) Hippocampal lesions will prevent the implicit encoding of background stimuli.

2. Task development – Designing the context dependent odour discrimination task and establishing a protocol

2.1. Introduction

The aim of this study was to design and develop an appetitive context dependent task and to do initial tests on its hippocampal dependence.

Many contextual fear conditioning studies have suggested that the hippocampus is involved in context processing (e.g. Anagnostaras and Maren et al 1999, Lehmann and Lacanilao et al 2007). However other fear related (e.g. Winocur et al 1987, Maren and Aharonov et al 1997, McNish et al 1997, 2000, Gisquet-Verrier and Dutrieux et al 2001), and non-fear tasks (e.g. Langston et al 2006), have demonstrated opposing results. Thus perhaps fear motivated learning is not the best way to address hippocampal encoding (Gray and McNaughton, 2000; Bannerman et al., 2004). Nevertheless, aside from the presence or absence of a fear inducing stimulus, other factors may also determine whether the hippocampus is required for a context dependent task. These include whether the context is encoded incidentally or explicitly, configurally or elementally and the timing of the lesion (pre- or post acquisition). The task design for this thesis aimed to address these facets in more detail with reference to contextual encoding.

As the hippocampus is known to be highly involved in spatial encoding, to avoid confounding factors it was important that this task was non-geometric. However, to keep the level of complexity high enough to require configural representations and not simple stimulus-responses, a secondary associative cue was necessary. As rats have a highly developed sense of smell, odour was selected for this purpose. In a natural environment, remembering which odours are associated with food in a particular context could be crucial to survival. Scientists have taken advantage of this

and developed many tasks based on odour discrimination (Eichenbaum, Fagan et al. 1988; Wood, Dudchenko et al. 1999; Wood et al. 2004).

Combining these principles gave the idea for a protocol that was based around context dependent odour discrimination. The basic premise was that the task should be suitable both for a lesion study (as described in this thesis) and for future adaptation into a single-unit recording study. The task design was inspired by the double Y-maze apparatus that Bussey et al used in their 2001 paper (see Figure 2-1). For this task the rats learnt the conditional rule, in place X choose object A not B (A^+B^-), in Place Y choose object B not A (A^-B^+). The order of presentations and the left / right position of objects were pseudo-randomised. They found that perirhinal cortex lesions cause impairment in acquisition of this task, but hippocampal lesions were not tested.

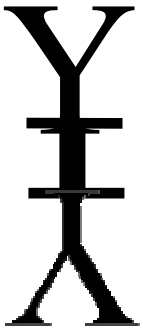

Figure 2-1: Diagrammatic representation of double ended Y-maze apparatus used by Bussey et al 2001. Barriers at centre contain rat before each trial begins.

For this thesis, the task rules were retained, but the apparatus was reconfigured so as to examine contextual processing instead of spatial or object processing (see Figure 2-2). The two arms found at each end of the double Y-maze were replaced with two

distinct contexts at either end of a central stem and the objects replaced with pots of scented sand. Thus the task became a context dependent odour discrimination.

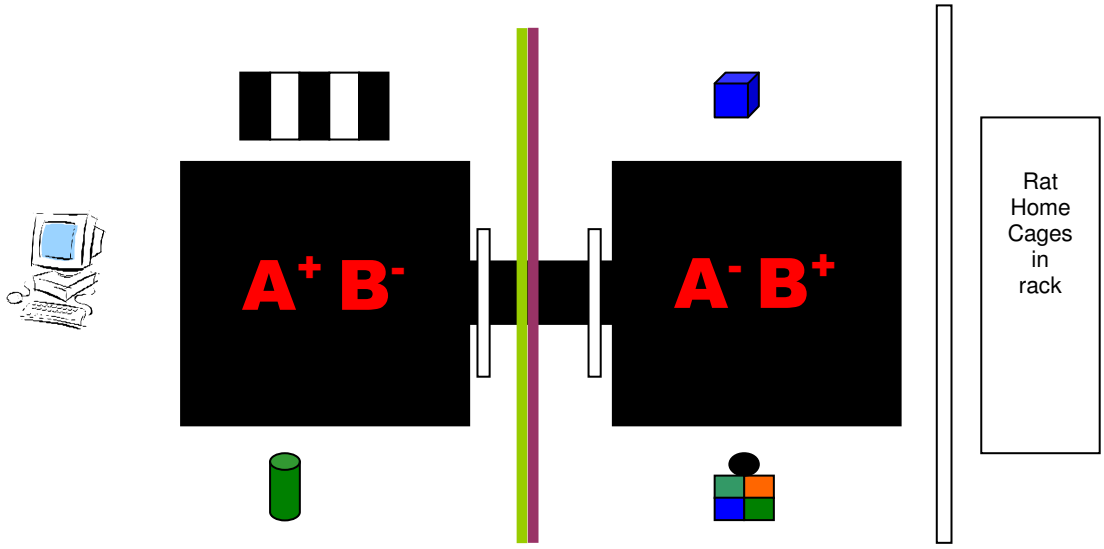

Figure 2-2: Schematic diagram of apparatus used for pilot study

With the task and apparatus in place, a protocol for training and testing rats on this context dependent odour discrimination task was required. The following discussion outlines the initial training schedule, as well as some of the problems that were encountered and how these were overcome. In addition, some preliminary hippocampal lesion results are outlined, alongside a description of how the final protocol was modified in light of them.

2.2. Methods

2.2.1. Subjects and Housing

The subjects were 6 Male Lister-hooded rats (Charles River, UK), weighing 250-300g at the start of pre-surgery training. The rats were individually housed and kept on a 12h light/dark cycle. Behavioural testing was carried out during the light phase. Rats were maintained at 90% of their free-feeding body weight by feeding them a restricted amount (20-30g) of standard laboratory chow (SDS, UK) at the end of each test session. Individual bodyweights were recorded at least once per week. All rats had *ad libitum* access to water for the duration of the experiment. The bedding consisted of dust free shavings. All procedures were carried out in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986 and European Communities Council Directive of 24 November 1986 [86/609/EEC], and all efforts were made to minimise suffering.

2.2.2. Apparatus

Contexts

The experimental apparatus consisted of two structurally identical platforms that were defined as separate contexts according to their distal cues. Diagrams of the apparatus are shown below and a moving representation can also be observed within the PowerPoint file on the CD in the appendix of this thesis.

The platforms were constructed from two 1m x 1m squares of 18mm thick exterior plywood. 2cm high walls were affixed to each side. The platforms rested approx 75 cm above floor level on tables, and were connected in the centre by a bridge, (10 cm wide by 92cm long) constructed from 18mm exterior plywood. The bridge also had 4

cm high walls. Two removable blockades were placed either end of the bridge to constrain the rat on the bridge during the inter-trial period. These barriers were 45 cm wide by 40 cm high. The entire apparatus was painted black with floor paint.

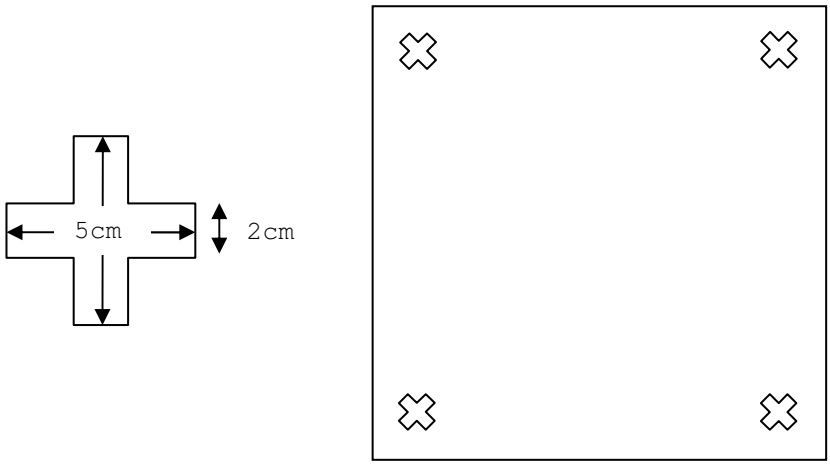

Figure 2-3: Schematic of the attachment points for odour pots

Crosses of Velcro (see Figure 2-3) were placed in the corners of the platforms to allow for the attachment of odour pots. There were 8 potential positions an odour pot could be located (4 on each platform).

The apparatus was located in a small testing room amid a rich array of constant distal spatial cues, which served to define the two platforms as separate 'contexts' (see Figure 2-2 above). Two coloured curtains across the centre of the bridge prevented the animals from observing one context while located in the other, whilst not obstructing their passage along the bridge. A white curtain separated the testing arena from the rat home cages.

Odour Pots

The reward system used here is modelled on that used by Wood et al (1999). Clear Nalgene cups (6.5 cm diameter, 6.5 cm high) were prepared by affixing crosses of Velcro to the base. They were filled with 0.8g of household herbs and spices (mint, basil, cumin, cinnamon, rosemary, tarragon – Swartz herbs & spices) and 150g of dry childrens' play sand thoroughly mixed together. Rewards were ½ chocolate wheetos (Nestle) submerged 2.5-3 cm deep into the sand.

2.2.3. Behavioural Protocol

Handling

Rats were habituated to handling for 5 days (5mins/day) before training. Furthermore, during this time the rats were taught to dig by giving them pots of unscented sand in their homecages within the animal housing room. These pots contained chocolate wheeto cereal rewards and the animal's food ration buried within approx 150g of plain sand. Each pot was affixed with Velcro to a wooden base for stability.

General Protocol

Testing took place on weekdays (Mon - Fri) between 8.30am and 8.30pm (during the light phase of their diurnal cycle). Effort was taken to ensure individual rats were tested at approximately the same time of day, every time. The rat was carried from the animal housing room into the testing room in its home cage and placed in the rack behind the white curtain. All 6 animals were brought in at the start of the session and removed at the end.

Immediately before testing, an individual rat was placed into a transferral container (blue bucket, 33 cm in diameter, 35 cm high with ventilation holes drilled in lid and sawdust in the bottom) and carried past the curtain to the apparatus. It was then placed on the centre of the bridge.

The experimenter remained visible at all times during the task. To minimise their effects, the experimenter's exact location was unpredictable and bore no relevance to the site of the reward or to the behaviour expected.

At the end of their trials, the rat was lifted off the bridge and carried in the transferral container back to their home-cage within the room.

Habituation and shaping

Prior to actual behavioural training, the rats were habituated to the plain empty platform for 15 minutes (pre-training session 1). Over the following 6 sessions (pre-training days 2-7) the rats were shaped to dig for single chocolate reward in plain (non-odourous) pots of sand (x 8) which they encountered in random positions on the platform. Having previously been shaped to dig in similar pots in their cages, the digging response was familiar to the animals. Although the rats were given freedom to move around the entire apparatus during the first 3 sessions of pre-training, from that point on barriers were introduced to restrict the movement of the animal so that it had access to only one platform at a time. Hence all trials then began and ended with the rat on the bridge between the two blockades. On each trial, **one** blockade would be removed revealing **one** platform with **one** pot in a corner. The rat would enter the context, dig for and consume the reward and return back to the bridge, wherein the experimenter would replace the blockade. During the inter-trial interval of 20-30sec the rat would remain on the bridge and the experimenter would remove any spilt sand, before setting up for the next trial. (For a moving representation of this see the PowerPoint file in the CD in the appendix of this thesis).On pre-training

session 8, odours were introduced to the sand (mixed as described above). The trials were configured such that each odour was only encountered on one platform (e.g. basil only on left platform, mint only on right platform) to conform to task rules that would be introduced later. Rats were given 12 trials per session – 6 on each platform (For overview see Table 2-1).

Training Phase

The aim of training phase was to teach the animals that not all trials would be rewarded, but that the presence or absence of reward could be predicted from the odour of the sand. Thus for this stage, two completely unrewarded foils were introduced (odours X and Y; rosemary and tarragon). The rats received 16 pseudo-randomised trials per session of A^+ and X^- on one platform; B^+ and Y^- on the other (see Figure 2-4).

The correct response was:

a) On a rewarded trial – to sniff the sand, dig for reward, eat it, return to bridge;

or

b) On an unrewarded trial – to sniff not touch sand, turn away and return to bridge.

Rats were trained for 16 trials per session (5 A^+, 5 B^-, 3 X^-, 3 Y^-) until they reached a criterion of 15/16 trials correct for 2 consecutive sessions.

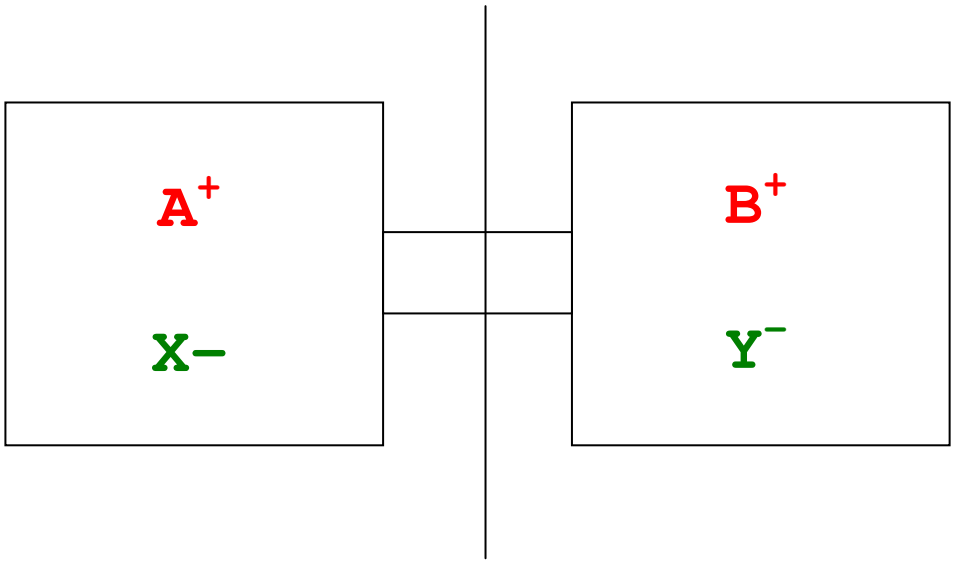

Figure 2-4: Rules of training task. Letters refer to particular odours. + is rewarded. – is unrewarded.

Table 2-1: Schedule for pilot study. Varying numbers of sessions represent variation in time spent on that part of task

	Pre 1	Pre 2-3	Pre 4-7	Pre 8	Training	
	1 Session	2 Sessions	4 Sessions	1 Session	3-5 sessions	CRITERION
	Habituation to platform	Habituation 8 plain pots All Rewarded	Habituation 8 plain pots (All Rewarded) with barriers	12 dig trials Random order A^+, B^+	16 trials 5 x A^+, 5 x B^+ 3 x X^-, 3 x Y^-	15/16 Correct for 2 Consecutive Sessions
ACQUISITION	11-13 Sessions	8 Sessions	2 Sessions	3 Sessions	5 Sessions	0-22 Sessions
	Context Dependent Task 16 Trials 4 each of A^+, B^-, A^-, B^+	Context Dependent Task 20 Trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^-	Blocks of 8 trials Stopped X/Y trials Introduced Correction Trials 4 each of A^+, B^-, A^-, B^+	No Correction Trials Blocks of 8 trials 4 each of A^+, B^-, A^-, B^+	Blocks of 8 trials 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^-	Blocks of 8 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- up to 2 Correction trials
	CRITERION	2-14 Sessions	CRITERION	2-7 Sessions		
	5/8 correct Non Rewarded Trials for 2 consecutive sessions (for 3 rats) Others were moved on Even though no apparent learning	Blocks of 4 trials 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- up to 2 Correction trials	5/8 correct Non Rewarded Trials For 2 consecutive sessions (for 3 rats) 6/8 criterion for other 3 (improved measure)	Random Order 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- Up to 2 correction trials		
TESTING	CRITERION	4 Sessions	2 Sessions	4 Sessions	4 Sessions	
	6/8 correct Non-rewarded Trials For 2 consecutive Sessions	21 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- & 1 randomly placed A/B probe trial per session up to 2 Correction trials	Get back to Criterion after Probe Trials 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- up to 2 Correction trials	FULL TASK Random Order 24 Trials 4 each of A^+, B^-, A^-, B^+ 4 x E^+, 4 x F^- up to 2 Correction trials	FULL TASK Random Order 26 Trials 4 each of A^+, B^-, A^-, B^+ 4 x E^+, 4 x F^- & 1 A/B and 1 E/F probe trials up to 2 Correction trials	

2.2.4. Acquisition Phase - Context dependent task

Once the animals had reached criterion performance on the training stage, they were moved onto the acquisition phase, where the context dependent odour discrimination task that forms the basis of this thesis was introduced. To solve this task, the animals had to pay attention not only to the odour of the sand, but to the spatial context, as both odour A and odour B were presented on both platforms. Thus the rules of the task were $A^{+}B^{-}$ on one platform, $A^{-}B^{+}$ on the other (see Figure 2-5).

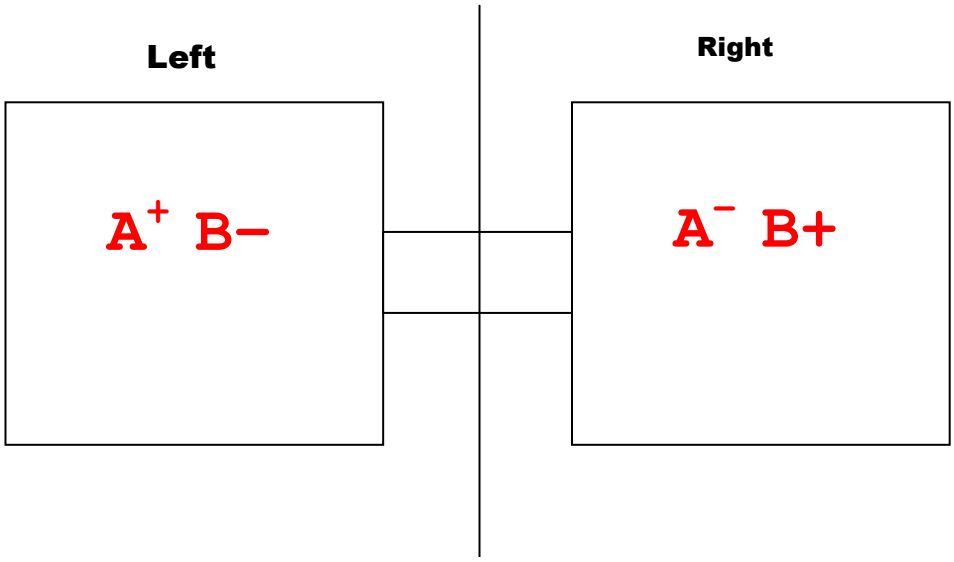

Figure 2-5: Rules of context dependent task

Rats were initially trained for 16 trials per session ($4A^{+}$, $4B^{-}$, $4A^{-}$, $4B^{+}$) presented in pseudo-random order and counterbalanced across the 8 positions. However, the rats failed to learn the task under these conditions.

Many combinations of training protocol were introduced in an effort to get the rats to learn this task (see Table 2-1) including the introduction of:

a)	Correction trials	Any mark on the top of the sand counts as 'dug in'. If a rat 'digs' during an unrewarded trial, it receives that exact trial again.
b)	Reintroduction of X/Y	To remind rats that not all odours are rewarded.
c)	Blocks of 8 trials	By running 8 odour A trials, followed by 8 odour B trials (or vice versa, randomised each session) the rats could learn that one platform is rewarded, and the other is not for that particular odour.

A combination of the above three was the most successful (i.e. 20 trials per session, 4 each of A^+, B^-, A^-, B^+ in blocks of 8, with 4 X^- / Y^- non-rewarded trials randomly interspersed and up to 2 correction attempts per trial). Hence the rats were trained in this way and rewarded according to the rules shown in Figure 2-6 until they reached a criterion of 5/8 non-rewarded trials for 2 consecutive sessions.

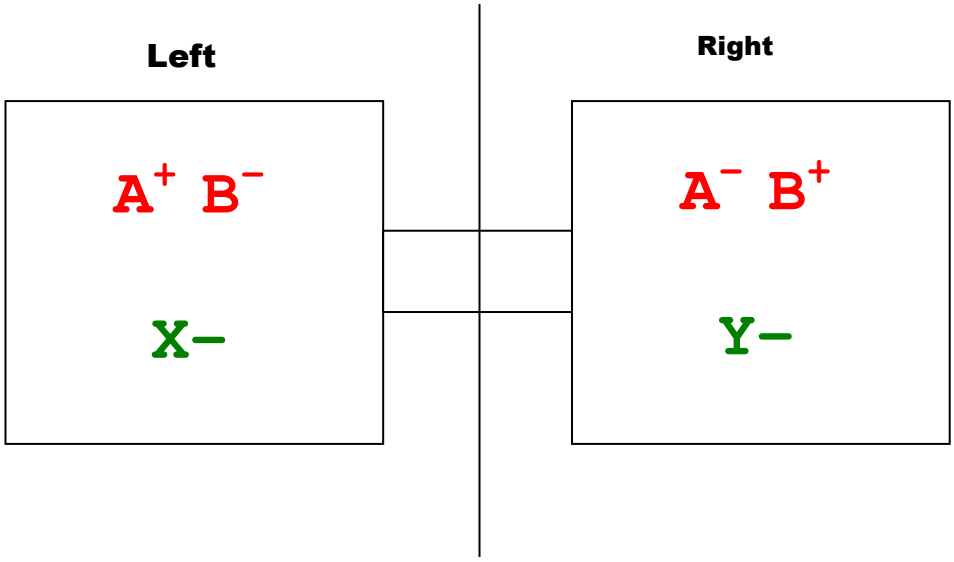

Figure 2-6: **Rules of task during acquisition phase**. Letters represent odours – red for context dependent task and green for simple discrimination. + is rewarded, - is unrewarded

As the natural tendency of the rats was to dig in every pot they encountered, criterion levels were set according to the number of non-rewarded trials the rats correctly identified – hence criterion initially became 5/8 non-rewarded trials correct for 2 consecutive sessions. Later this was adjusted to 6/8 correct in order to ensure the rats had fully learnt the task and to reduce the probability of the rats achieving criterion by chance. Other difficulties experienced during training and their solutions are summarised in Table 2-2.

Table 2-2: Problems Overcome

Problem	Solution
Rats climbed over / knocked over barriers	Redesigned blockades to make them bigger and more stable.
Rats dug in every pot presented to them	Increased food intake; Introduced correction trials; Reintroduced E /F non-rewarded trials;
Rats did not dig sufficiently to get wheeto on rewarded trials.	Gave rat 2 further chances to find the reward (i.e. let rat return to bridge twice). On third approach to pot, the rat was encouraged to dig by a) tapping the side of the pot, b) flicking top of sand (eventually) c) using a pen to lift reward nearer surface. If rat started to become too dependent on experimenter's help, they were given 3 chances to dig, before being moved onto next trial (i.e. no reward received).
Rats did up to 10 correction trials and still dug in a 'non-rewarded' pot	Correction trials were limited to 2 per non-rewarded trial.
Rats refused to leave bridge / dig.	Remedial digging pots placed in cage(as described in 'handling' section above); Moved onto next stage of task without reaching criterion (see Table 2-1)

Upon successful completion of the 'blocks of 8' phase, the rats were moved onto blocks of 4 trials (i.e. 4 odour A trials followed by 4 odour B trials). This pattern was repeated twice per session alongside 4 randomly interspersed X^- / Y^- trials (total of 20 trials per session; 16 context dependent, 4 foils). In each session the trials were balanced but pseudo-randomised for position and reward properties, and up to two corrections were allowed per trial.

(2 rats who failed to reach criterion on the 'blocks of 8' phase despite 20+ sessions of training were moved onto the 'blocks of 4' stage anyway. Where this variation was implemented, it was a successful strategy and quickly resulted in signs of behavioural improvement)

Training continued in blocks of 4 until the rats reached criterion (5/8 or 6/8 trials correct non-rewarded trials for 2 consecutive sessions, as discussed above). The rats rapidly progressed though blocks of 4 and thus moved onto 20 context dependent trials presented in completely pseudo-randomised order (4 each of A^+, B^-, A^-, B^+ and 2 each of X^- and Y^- with up to 2 corrections per trial). Again criterion was set at 6 out 8 correct non-rewarded trails for 2 consecutive sessions.

Testing Phase – Context Dependent Task

Having reached criterion on the context dependent task when it was presented in a pseudo-randomised manner, the rats underwent a period of testing. Testing days were exactly like the last stage of training (4 trials each of A^+, B^-, A^-, B^- and 2 each of X^- and Y^- with up to 2 corrections per trial), except for the addition of 1 randomly placed odour A or B probe trial. These were used to verify that the animals were using the scent of the sand to solve the task and not detecting the scent of the cereal reward itself. Probe trials were presented as rewarded trials (i.e. A or B in their appropriate context), however no cereal reward was available within the pot. The reward was only dropped into the pot after the animal dug for 5 sec. (For a

demonstration of a probe trial performed in the revised task apparatus, see Probe Trial video on the CD in the appendix of this thesis). Following probe trial sessions, the rats were given two more standard acquisition sessions (4 each of A^+, B^-, A^-, B^+ and 2 each of X^- and Y^- with up to 2 corrections per trial), to ensure they were performing at criterion level before adding the next task.

Context Independent Task

The end training stage (Full task) involved the rats performing both the context dependent task and context independent tasks at the same time (see Figure 2-7). For the context independent task, odour E was always rewarded and odour F was never rewarded, regardless of the context they were presented in (odours E /F were cinnamon and cumin; which was rewarded/unrewarded was balanced across the group). At this stage the X/Y non-reward 'reminder' trials were stopped, and the E^+ / F^- trials were randomly interspersed with the context dependent trials. The rats were performing 24 trials per session – 16 context dependent trials (4 each of each of A^+, B^-, A^-, B^+) and 8 context independent trials (each of E^+, F^-) with up to 2 corrections per trials (as described above).

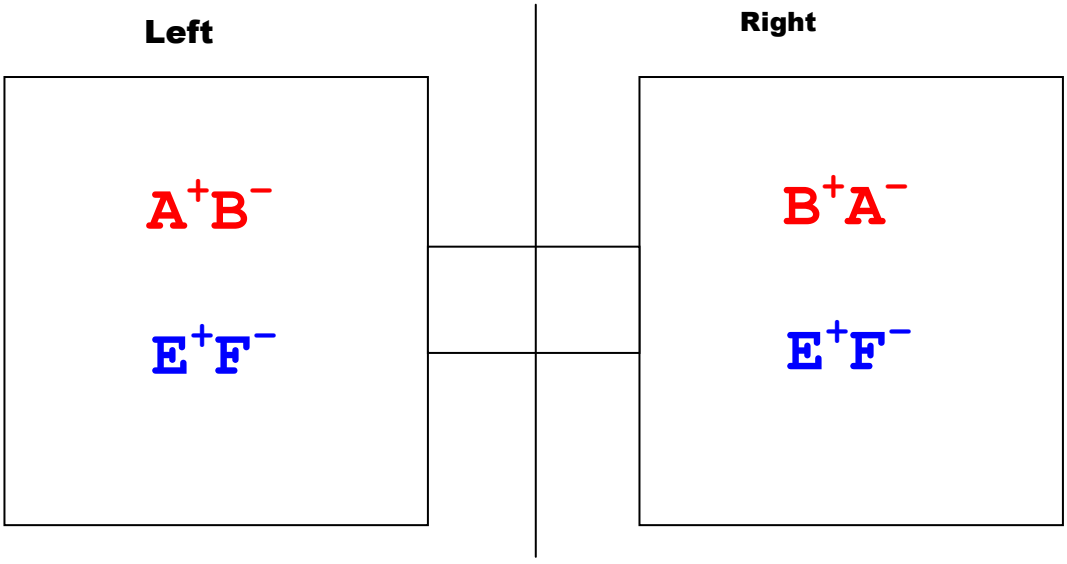

Figure 2-7: Rules of full task; Letters represent odours; red for context dependent and blue for context independent tasks. + is rewarded, - is unrewarded

The rats were so competent at learning the context independent task, that a fixed training period of 4 sessions was used instead of a criterion. Thus, after 4 sessions of training on the full task the rats underwent a further period of testing.

Testing Phase – Full Task (Context Dependent and Context Independent)

The four testing sessions followed the same protocol as the full task described above. The rats received 24 trials per session, 16 context dependent trials (4 each of each of A^+, B^-, A^-, B^+) and 8 context independent trials (4 each of E^+, F^-) with up to 2 corrections per trials. In addition they also received 1 context dependent probe trial and 1 context independent probe trial per session (i.e. rats performed 26 trials/testing session). The probe trials were set-up to look like rewarded trials, however the reward was not dropped into the pot until after the animal had dug for 5 sec. This was to check that the animals were indeed using the odour of the sand to dictate behaviour and not sensing the reward itself.

This marked the end of pre-surgery Training.

2.2.5. Hippocampal Lesion Surgery

Upon finishing their final pre-surgery session, the rats were returned to their home-cage in the animal housing room and given *ad libitum* access to food and water. Pre-surgery analgesia was administered in their water (Large Animal Rimadyl, Pfizer, UK).

The day after completing pre-surgery training, the animals underwent either bilateral hippocampal lesion surgery (n=3) or sham surgery (n=2). Lesions were made using

ibotenic acid (Biotechnology, CA) dissolved in phosphate buffered saline (pH 7.4) at 10mg/ml following the protocol of Jarrard (1989).

Anaesthesia was induced and maintained using halothane (Merial Animal Health, UK) and rats were positioned in a stereotaxic frame (Kopf, USA). The head was shaved and swabbed with alcohol. A heat blanket with rectal probe was used to maintain the rat's temperature throughout the procedure.

A longitudinal incision was made midway between the ears, to expose the top of the skull. After ensuring the head was flat, measurements of bregma were taken and used as a reference point to calculate injection sites. An oval bilateral craniotomy was carried out using a dental drill. Care was taken not to damage the dura during drilling, and the area irrigated with saline. Attention was paid to removing all rough edges and to ensuring the drilling was taken fully out to the bone ridge at the most lateral aspect of the hole.

A 1µl bevelled syringe (SGE, UK) was filled with sterile saline and used to obtain a dura depth (dorsoventral origin) measurement at a predetermined point (AP -4.8, ML + or – 4.1). 0.4mm was subtracted from this measurement to account for the distance between dura and the top of the cortex. The result gave a baseline depth, from which all injection co-ordinates could be calculated.

From this point on the brain surface was kept moist with either sterile saline on the side where injections were taking place, or sterile absorbable gelatin sponge (Johnson & Johnson) soaked in saline on the contralateral side. The syringe was emptied of saline and refilled with ibotenic acid. The outside of the needle was swabbed using saline soaked cotton buds to remove any extra ibotenic acid and prevent contamination of the cortex.

Thirteen injections of 0.05 or 0.1µl ibotenic acid were made into each hippocampus at different rostrocaudal and dorsoventral levels. Co-ordinates were calculated from bregma and are shown in Table 2-3. A total of 0.91ul per hemisphere was injected.

Table 2-3: Stereotaxic co-ordinates calculated from bregma.

Right Side				**Left Side**			
AP	**ML**	**DV**	**µl**	**AP**	**ML**	**DV**	**µl**
-2.4	-1.0	-3.0	(0.05)	**-2.4**	1.0	-3.0	(0.05)
-3.0	-3.0	-2.7	(0.10)	**-3.0**	3.0	-2.7	(0.10)
	-1.4	-2.1	(0.05)		1.4	-2.1	(0.05)
		-2.9	(0.05)			-2.9	(0.05)
-4.0	-3.7	-2.7	(0.10)	**-4.0**	3.7	-2.7	(0.10)
	-2.6	-1.8	(0.05)		2.6	-1.8	(0.05)
		-2.8	(0.05)			-2.8	(0.05)
-4.3	-4.0	-7.0	(0.05)	**-4.3**	4.0	-7.0	(0.05)
-5.0	-3.0	-3.0	(0.10)	**-5.0**	3.0	-3.0	(0.10)
	-3.9	-7.0	(0.10)		3.9	-7.0	(0.10)
	-5.4	-5.1	(0.10)		5.4	-5.1	(0.10)

AP: anterior-posterior (mm)
ML: medial-lateral (mm)
V: ventral from dorsoventral origin (mm)
µl: volume of ibotenic acid injected
The co-ordinates were modified from Jarrad (1989) by de Hoz et al (2003) to suit the brain size of male Lister Hooded Rats.

A careful injection protocol was followed to allow complete absorption of the toxin solution by the brain tissue. Before each infusion, the needle was lowered to 0.03mm lower than the injection depth to make a space for the toxin to fill and a delay of 30sec imposed to let the tissue recover back to its original position. The appropriate volume of ibotenic acid was then injected at a rate of 0.1µl/min. The needle was brought up by approx 0.02mm and a further delay of 1 min imposed to allow the dispersal of the toxin into the tissue. The needle was removed very slowly (over approx 1 min) to prevent the extraction of the toxin. The outside of the needle was cleaned with cotton buds soaked in sterile saline before proceeding with the next injection, to prevent any external ibotenic acid damaging the cortex. Some injections are double (i.e. two injections occur at the same anterior-posterior and medial-lateral positions). In these circumstances, the less ventral injection was always done first. Once all the injections had been performed on one hemisphere, a needle check was carried out to ensure that it was not blocked, and the syringe refilled with ibotenic acid. Before proceeding to the other side, the exposed area was thoroughly irrigated with sterile saline and covered in gelatin sponge.

Sham lesions were carried out in the same way, but the needle injections were substituted instead with several pierces though dura with a 23G needle. This was to simulate the mechanical damage to the cortex caused by needle entry.

After completion of the ibotenic acid injections (or piercing of dura for sham rats), the area was thoroughly irrigated and cleaned. Gelatin sponge was placed over the two areas where bone had been removed and the skin sutured closed over the top. The wound was covered in aureomycin power. Rats were given 5ml of subcutaneous saline for rehydration. Rats were returned to a clean homecage on a heated platform for approx 2hrs post-surgery, and then returned to the animal housing room before the end of the light phase.

Post-surgery the rats had *ad libitum* access to food and water. Rimadyl analgesia was present in their water for the first 48hrs. Rats were given 14 days to recover and regain pre-surgery weights before post-surgery testing began. 48hrs before post-surgery testing, the rats were returned to their restricted diet.

Post-surgery Behavioural Testing

Post-surgery Testing was exactly the same as the full-task stage of pre-surgery training and followed the same rules (see Table 2-4). Thus the rats received 24 counterbalanced but pseudo-randomised trials per session; 16 context dependent trials (4 each of each of A^+, B^-, A^-, B^+) and 8 context independent trials (each of E^+, F^-), with up to 2 corrections per trials. Once the rats were again performing at criterion level (6 out of 8 non-rewarded trials correct for 2 consecutive sessions), 4 testing sessions were given. These were exactly the same as those given immediately before surgery (26 trials, 16 context dependent (4 each of each of A^+, B^-, A^-, B^+) and 8 context independent trials (4 each of E^+, F^-), and 1 A/B and 1 E/F probe trial per session.)

Table 2-4: **Post-surgery testing schedule for pilot study**

4 Sessions	CRITERION	4 Sessions
FULL TASK Random Order 24 Trials 4 each of A^+, B^-, A^-, B^+ 4 each of E^+, F^- Up to 2 Correction trials	6 /8 non-rewarded trials correct For 2 consecutive sessions	FULL TASK Random Order 26 Trials 4 each of A^+, B^-, A^-, B^+ 4 each of E^+, F^- & 1 M/B and 1 E/F rewarded position probe trial Up to 2 Correction trials

New odours

The same 5 rats that had completed the above protocol, continued on to learn this new task. It followed the same rules as the original context dependent task and training/testing took place in the **same** room and utilised the **same** apparatus (see Figure 2-2). The only differences were that:

- two new odours were used (fennel and all-spice (G and H) counterbalanced across groups)
- the training protocol jumped straight into random order (i.e. 16 context dependent trials; 8 rewarded, 8 non-rewarded randomized for order and position on platform).

No context independent task was run at this stage.

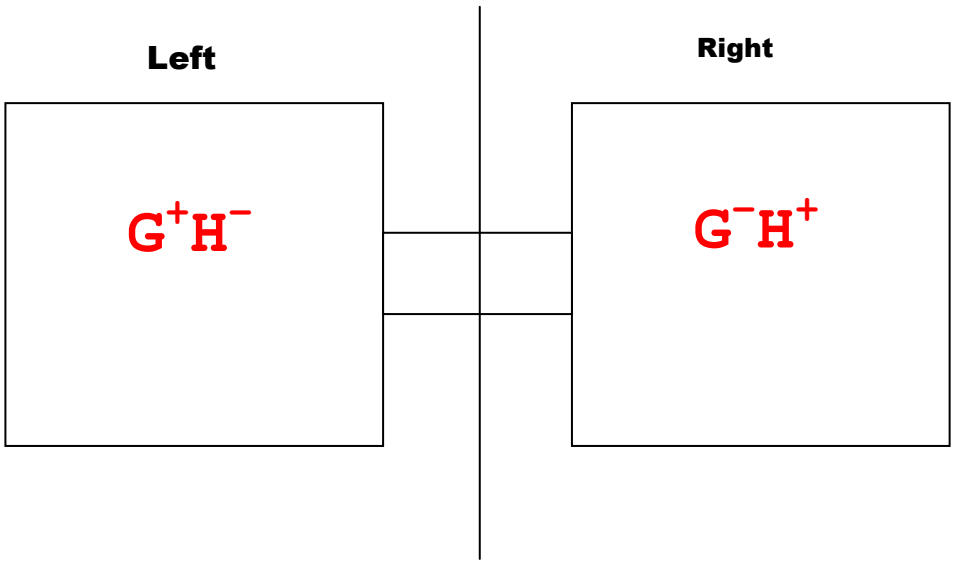

Figure 2-8: Rules of new odours task

Thus the animals were trained on 16 counterbalanced, pseudo-randomised trials per session (4 each of each of G^+, H^-, G^-, H^+) with up to two corrections per trial. Training continued until the rats reached criterion (14/16 trials correct for 2 consecutive sessions). At this point 4 testing sessions were given (as above). Within each testing session the rat received a standard set of trials (16 trials: 4 each of each of G^+, H^-, G^-, H^+) with a probe trial added in at a randomised point (17 trials in total). Probe trials were used to ensure the animal was using the odour of the sand to determine the reward properties of a pot, and not directly sensing the reward itself.

New Contexts

The same rats that had completed the previous tasks went on to be trained on one further protocol. It again followed the same rules as the original context dependent task, except that this time training and testing took place in a **new** room, with a **new** set of contexts and odours (sage and parsley; (J and K) counterbalanced across groups).

The two new contexts were of the same general construction as described in section 2.2.2). The main differences were:

a) The platforms were round (1m diameter, with 2cm high walls) instead of square;

b) The distal cues were different;

c) Each platform was surrounded by thick black curtains, upon which the cues were hung.

An illustration of the new contexts is given in Figure 2-9.

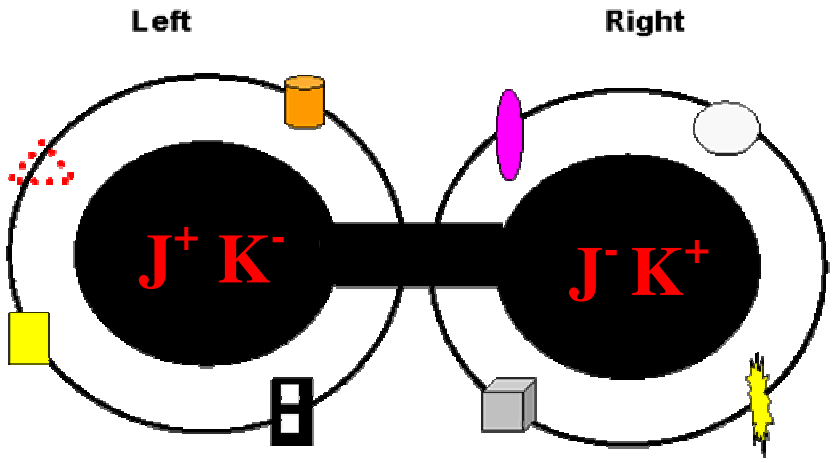

Figure 2-9: Schematic illustration of the apparatus used for the new context protocol

The protocol proceeded in exactly the same manner as for the new odour task above. Rats were trained on only the context dependent task for 16 randomised but counterbalanced trials per session (4 each of each of J^+, K^-, J^-, K^+) with up to two corrections per trial (for rules see Figure 2-9). Training continued until the rats reached criterion (14/16 trials correct for 2 consecutive sessions). At this point 4 testing sessions were given (as above). Within each testing session the rat received a standard set of trials (16 trials: 4 each of each of J^+, K^-, J^-, K^+) and probe trial added in at a randomised point (17 trials in total).

2.2.6. Histology

Perfusion

At the end of behavioural testing the rats were given an injection of Euthatal (Sodium Pentobarbitol, Merial Animal Health, UK) while under the effect of halothane gas. They were then perfused intracardially, first with 0.9% saline and then

with 4% formalin. The brain was removed and placed in a jar containing 4% formalin for at least 24hrs to ensure full fixation.

Embedding

To stabilise the tissue before slicing and to maintain their shape, the brains were embedded in egg yolk. After trimming away unwanted tissue (around the hippocampus to approx 0.7mm and -0.75 mm from bregma), the brains were placed in ice cube trays and surrounded with fresh egg yolk. Incubation in formalin vapours for 24hrs solidifies the egg yolk around the brain. This was done by placing the mould in a shallow formalin bath in an incubator at 37^0C. The egg embedded brains were then removed from the mould and transferred back into jars of 4% formalin, to fix for a further 48hrs at room temperature. I embedded only a subset. The rest were done by Jane Tulloch.

Sectioning and Staining

The brains were cut from posterior to anterior to maintain orientation once mounted on the slides (i.e. right side of brain on right side of slice). 30µm coronal sections were cut using a cryostat, with every fifth section mounted onto gelatine coated slides and stored for histological analysis. The slides were stained with cresyl violet acetate and mounted with DPX. (The protocol for this is included in the appendix of this thesis)

The majority of the sectioning and staining was done by Jane Tulloch, with only a subset done by myself.

Volumetric Measurements

The extent of the lesions was assessed by transferring an image of each slice to the computer using a camera (Leica), mounted on a macroscope (Wild M420,

Switzerland) and the Lecia Qwin Program. The image was then opened using Image J and the area measurement tool used to outline any spared hippocampal tissue and calculate its area. When repeated for each slice, this gave a measurement of spared tissue in mm^2 for each animal. The area present in sham animals was taken as a value for 100% sparing of hippocampus, and an average of this used to calculate percentage sparing for the lesioned rats. As the distance between sections is constant for all animals, a calculation of actual 'volume' is un-necessary.

2.2.7. Statistical Analysis

Data analysis was done using SPSS for windows version 12. All numerical values and graphs are reported as $\pm$ SEM. Criterion was 80% and is marked with a black horizontal line on the graphs (where appropriate).

Independent samples t-tests were used to compare the performance of the groups (hippocampal lesioned and sham operated) throughout.

A Repeated measures ANOVA followed by simple effects analysis (with bonferonni corrections) was used to compare performance across the first 5 sessions of retesting and the new tasks variants. Homogeneity of covariance was tested for using a Mauchly sphericity test. If the data failed this test, the Greenhouse-Geisser correction was used.

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2.3. Results

2.3.1. Acquisition of Task

Initially, the rats were trained for around 20 sessions (range = 19-21 sessions) on various protocols (see Table 2-1) without showing any of noteworthy signs of improvement (see Figure 2-10 and Figure 2-11). Around session 25 (range: Session 24-26) all rats were moved onto blocks of 8 trials (see methods section 2.2.3).From this point forward they took an average of 30 $\pm$ 4 sessions to acquire the context dependent part of the task (i.e. to complete blocks of 8 and blocks of 4, and to ultimately reach criterion on the random ordered context dependent part of the task.) Finally, the context independent part of the task was added. This task proved much simpler, and was acquired within 4-5 sessions (see Figure 2-10 and Figure 2-11).

The instinctive response of the rats was to dig in all pots that they were presented with. As this is a 'go / no-go' task, which was counterbalanced for rewarded and non-rewarded trials, performance never dipped below 50% (i.e. rats got all rewarded trials correct and all non-rewarded trials incorrect) The origin of the y-axis on the graphs below is therefore set at 50%.

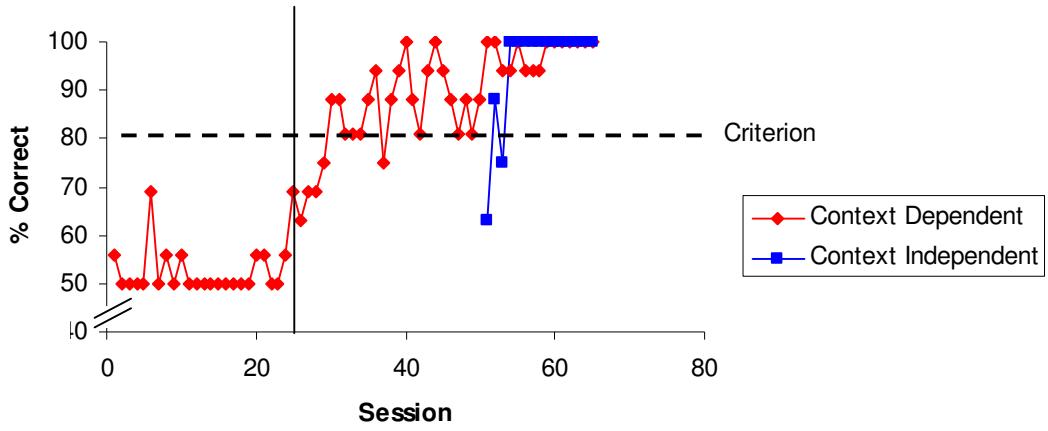


Figure 2-10: Performance of first rat to complete pre-surgery training, expressed in terms of % correct. The vertical black line shows where blocks of 8 trials were introduced. Performance rapidly improves after this point.

The performance of *first* rat to complete pre-surgery training is shown in Figure 2-10. This is representative of the performance of the majority of the pilot study rats. Performance improved rapidly after the introduction of blocks of 8 trials. A drop in performance was seen on session 37 when the rat was first moved onto randomised order of context dependent trials. Once the tasks are learnt, performance remains consistently above criterion.

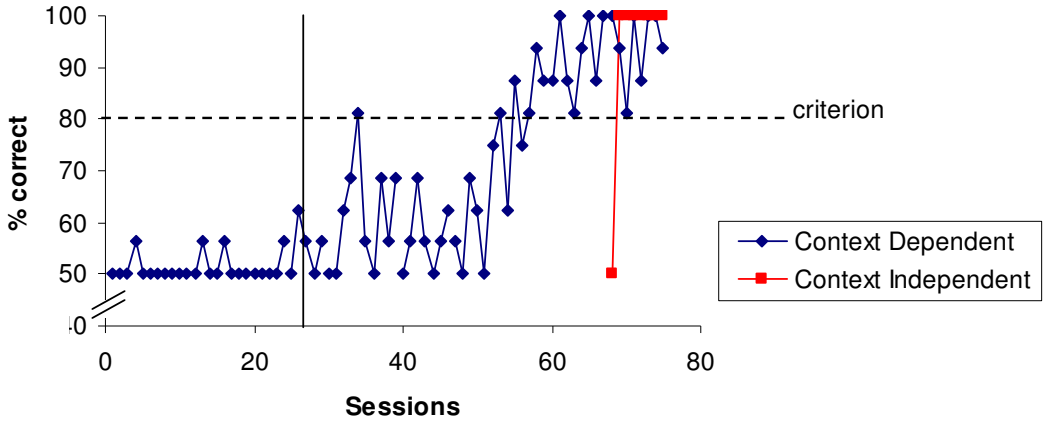


Figure 2-11: Performance of last rat to reach full task expressed as % correct. The vertical black line shows where blocks of 8 trials were introduced. Performance varied rapidly from this point onwards, but never reached criterion. The green line shows where the rat was moved onto blocks of 4. After this, performance improved dramatically.

The performance of the *last* rat to complete training is shown in Figure 2-11. After the introduction of blocks of 8 trials, performance fluctuated widely, but never reached criterion. However, a move onto blocks of 4 trials on session 48 prompted an improvement in performance that led to successful completion of pre-surgery training.

2.3.2. Pre-surgery Performance on task

No difference in performance of groups before surgery

6 rats completed pre-surgery training. Unfortunately, 1 rat died during surgery, so the data given below represents 5 rats (sham n = 2, lesion n = 3). The pre-surgery data is divided into sham and lesion groups according to the surgery the rats subsequently received (i.e. all data within section 2.3.2 is based on intact rats). The data are here illustrates that there was no difference in the performance of the two groups before surgery.

The average number of sessions taken to reach criterion after the introduction of blocks of 8 trials was 36±5 sessions. As is shown in Figure 2-12, there was no pre-surgery difference in learning rate between groups ($t < 1$).

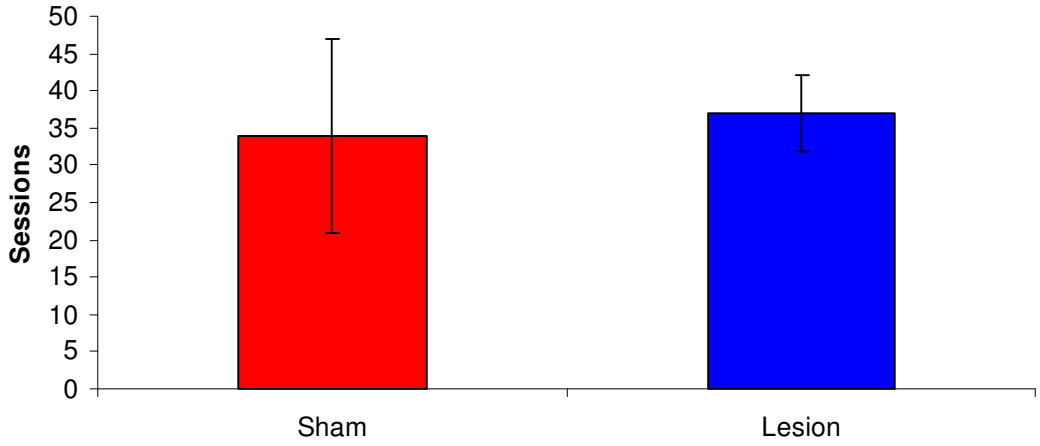


Figure 2-12: Average number of sessions taken to reach criterion on original task before surgery (counted from start of blocks of 8). Animals are split according to the surgery they would receive. (sham n= 2, lesion n = 3)

Performance on the four pre-surgery testing days was consistently high across all animals (see Figure 2-13). An independent t-test on the CD task showed no difference between the groups ($t < 1$).

All probe trials were correct, indicating the rats were not detecting the odour of the reward, but were indeed using the odour of the sand to determine the presence or absence of reward.

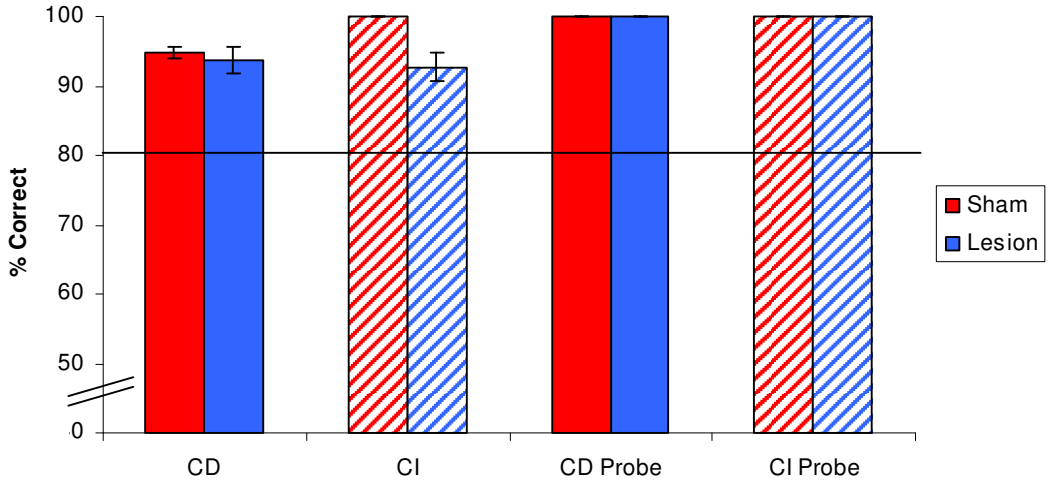


Figure 2-13: Average performance on the four pre-surgery Testing Sessions expressed as % correct. CD = context dependent task, CI – context independent task. The rats received 12 context dependent and 12 context independent trials per session, as well as 1 context dependent probe and 1 context independent probe per session. (sham n = 2, lesion n = 3)

2.3.3. Histology

Volumetric Measurements of the lesions showed that the 3 rats had sparing of 4.75%, 8.47% and 32.17%. The sparing was in the deepest (most ventral) regions of the hippocampus.

(The variance in hippocampal lesion size and its implications are discussed in chapter 3)

The entorhinal cortex showed minimal damange. However both the subiculum and post-subiculum showed extensive bilateral damage, that was consistent in position across the lesioned group. Furthermore, significant cortical damage to the regions directly above the hippocampus was sustained. This was notable as a hole in the cortex upon removal of the fixed brain from the skull.

2.3.4. Post-surgery Performance

Initial deficit in performance of hippocampal lesion group on CD task post-surgery

Within 48 hours of completing pre-surgery training, the rats received either bilateral hippocampal lesions (n = 3) or sham surgery (n = 2) and were given 14 days to recover. Figure 2-14 shows the average performance of the groups on the first 5 sessions of testing after post-surgery recovery.

All animals immediately performed above criterion on the CI task and from session 2 onwards scored 100%. This shows that the rats are not impaired in their ability to perform the basic task (i.e. they can still see, run, smell, discriminate, dig etc). An analysis of variance was performed on the CD task. A significant effect of session ($F_{(4,12)} = 4.72$, $p = 0.016$) reflected the improvement in performance observed across the 5 days. The significant effect of group ($F_{(1,3)} = 19.78$, $p = 0.021$) and a session by group interaction ($F_{(4,12)} = 3.75$, $p = 0.033$) indicates that the two groups are performing differently on the CD task. Post-hoc simple effects showed that the groups performed significantly differently on days 1 and 2 (Day 1: $p = 0.007$, Day 2: $p = 0.019$) but not on days 3 to 5 (Day 3: $p = 0.081$, Day 4: $p = 0.720$, Day 5: $p = 0.532$). This reflects the fact that although the sham animals can immediately perform the CD task at above criterion level, the lesioned animals are initially impaired (see Figure 2-14).

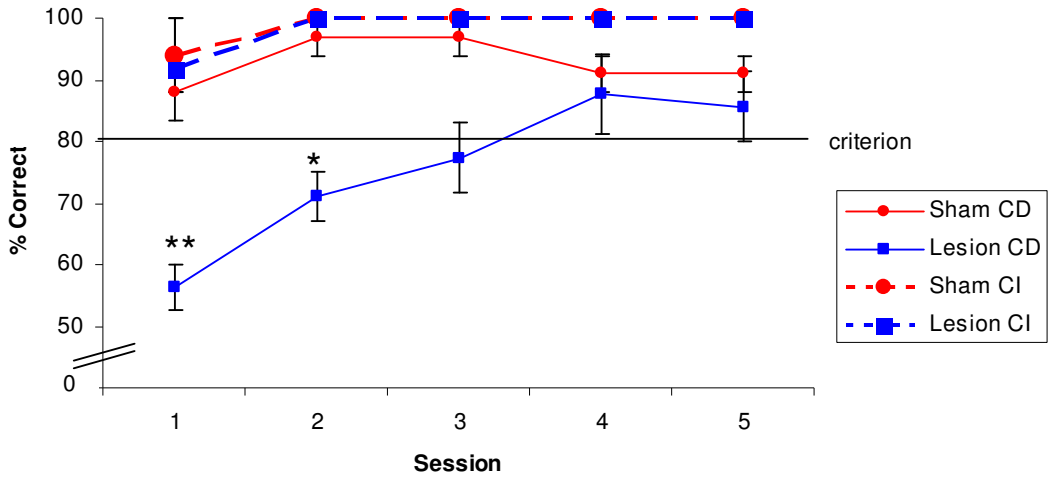


Figure 2-14: Average performance on sessions 1-5 of retest expressed in terms of % correct. (Sham n = 2, Lesion n = 3). All animals can immediately perform the CI task well, however the lesion animals takes 2 sessions for the average lesion performance to reach criterion level on the context dependent task, whereas the average sham performance is above criterion from the start.

No difference between groups in sessions to regain criterion post-surgery

Although the performance of the lesion group was initially impaired, the average number of sessions taken to regain criterion post-surgery did not differ significantly between groups (average sham = 4±2, lesion = 6±1; $t_{(3)} = -1.53$, $p = 0.222$; see Figure 2-15). All animals took less time to *regain* accurate performance, than they did to learn the tasks pre-surgery (average sessions to criterion pre-surgery = 34 ± 4, re-test = 5 ± 1).

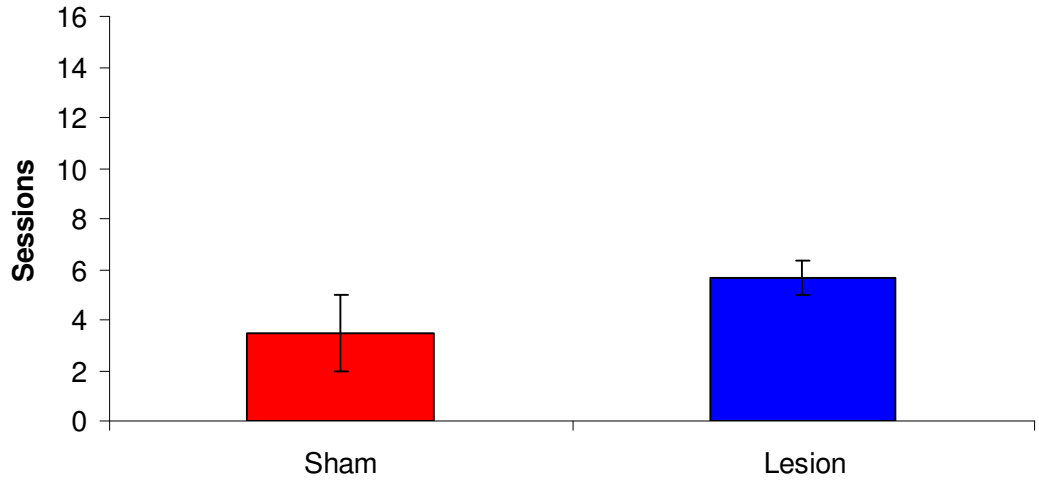


Figure 2-15: Average number of sessions taken to regain criterion on the original task after surgery. Sham n = 2, Lesion n = 3

Consistently high performance in both groups after criterion regained

During the four post-surgery testing sessions, performance was consistently high for all animals (see Figure 2-16). An independent samples t-test showed no difference between the groups ($t < 1$). All animals got their probe trials correct, indicating they were not using the odour of the reward itself to guide their behaviour.

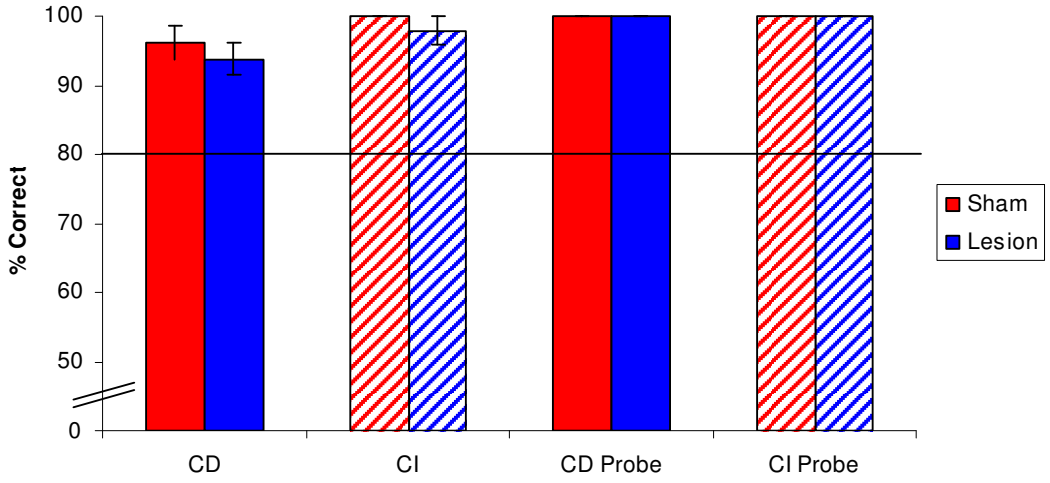


Figure 2-16: Average performance on the four post-surgery testing sessions after reaching criterion (expressed as % correct). CD = context dependent task. CI = context independent task. The rats received 12 context dependent and 12 context independent trials per session as well as 1 context dependent probe and 1 context independent probe. (sham n = 2, lesion n = 3))

2.4. Performance on 'new odours' task

Although hippocampal lesioned animals were initially impaired on the Context dependent part of the task, they did (with time) regain a consistently high performance level. This suggests that an intact hippocampus is not necessary for performance on this task. The next question that arose was whether the hippocampus would be necessary to learn a new version of the task (i.e. one in which a new set of context dependent odour discriminations had to be learnt).

No difference between groups on acquisition and performance of 'new odours' task.

A repeated measures ANOVA on the performance data for the first 5 sessions of this new task showed a significant effect of Session ($F_{(4,12)} = 9.84$, $p = 0.001$) reflecting gradual learning of the task, but no effect of group ($F_{(1,3)} = 0.17$, $p = 0.704$) and no

session by group interaction ($F_{(4,12)} = 1.70$, $p = 0.215$). Over the first 5 sessions, both groups were acquiring the task at the same rate.

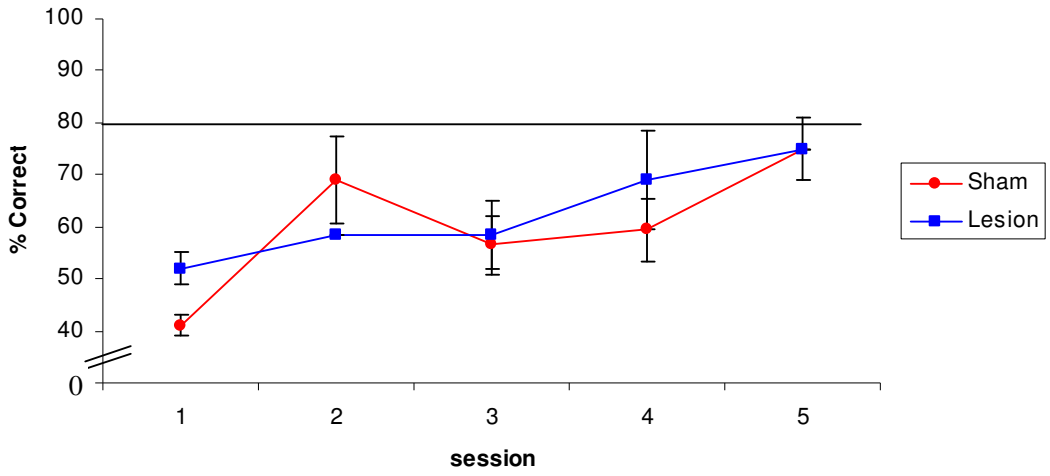


Figure 2-17: Average performance on sessions 1-5 of the new odours task, expressed in terms of % correct. (Sham n = 2, Lesion n = 3). Only the CD task is performed at this stage.

The average number of sessions taken to achieve criterion level on this new task did not differ significantly between groups (sphericity test failed so equal variance not assumed; $t < 1$; see Figure 2-18). Thus hippocampal lesions did not prevent the rats from learning a new version of the context dependent odour discrimination task.

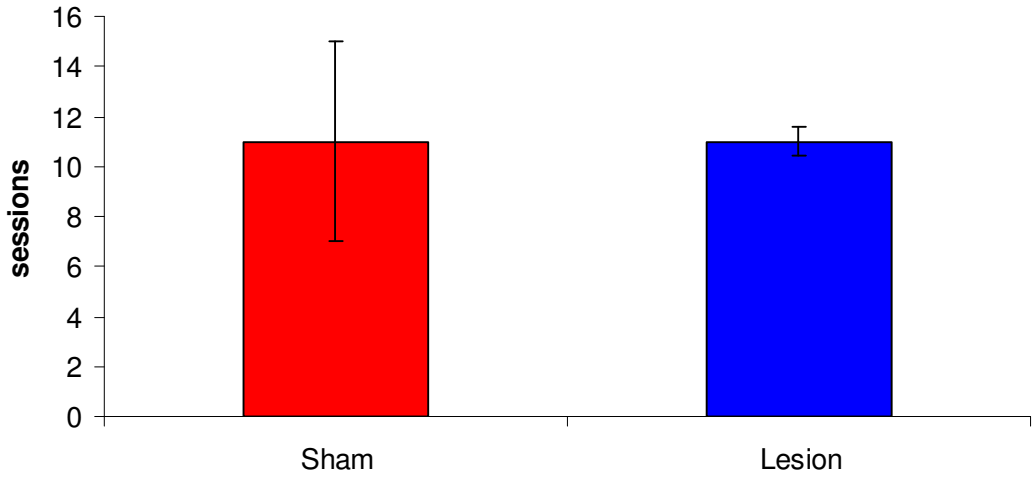


Figure 2-18: Average number of sessions taken to reach criterion on the new odours task. (Sham n = 2, Lesion n = 3)

The average performance level on the 4 testing sessions was above criterion for all rats (see Figure 2-19). An independent t-test showed there was no difference between the performance of the two groups ($t < 1$) on the context dependent task. All animals got their probe trials correct, indicating they were not using the odour of the reward itself to guide their behaviour.

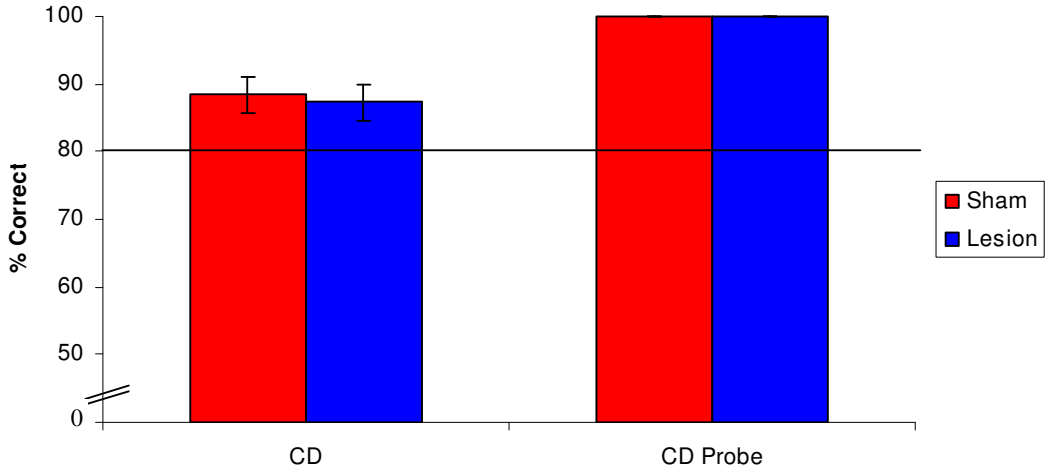


Figure 2-19: Average performance on the new odours task for the four testing sessions (expressed as % correct). CD = context dependent task. The rats received 12 context dependent trials per session as well as 1 context dependent probe. (sham n = 2, lesion n = 3)

2.4.1. Performance on new context task

The hippocampal lesioned animals performed just as well as sham animals on the 'new odours' task. The hippocampus does not appear to be necessary for the acquisition or performance of a new context dependent odour discrimination when it occurs within a familiar environment. The follow on question is hence, what if the new discrimination were to occur in an unfamiliar environment. Therefore, the next discrimination task took place on new apparatus in a different physical room.

One rat fell ill and died before completion of this task, so data in section 2.4.1 is based on sham n = 1, lesion n = 3. Due to the very low animal numbers only descriptions of data are given here.

All animals showed a similar acquisition and performance pattern during the 'new context' task.

The sham animal did not appear to differ from the lesioned animals in its initial performance on this task. Figure 2-20 shows that its acquisition graphs follow the same pattern as the one showing the averaged lesioned animal data - and even crosses it at one point.

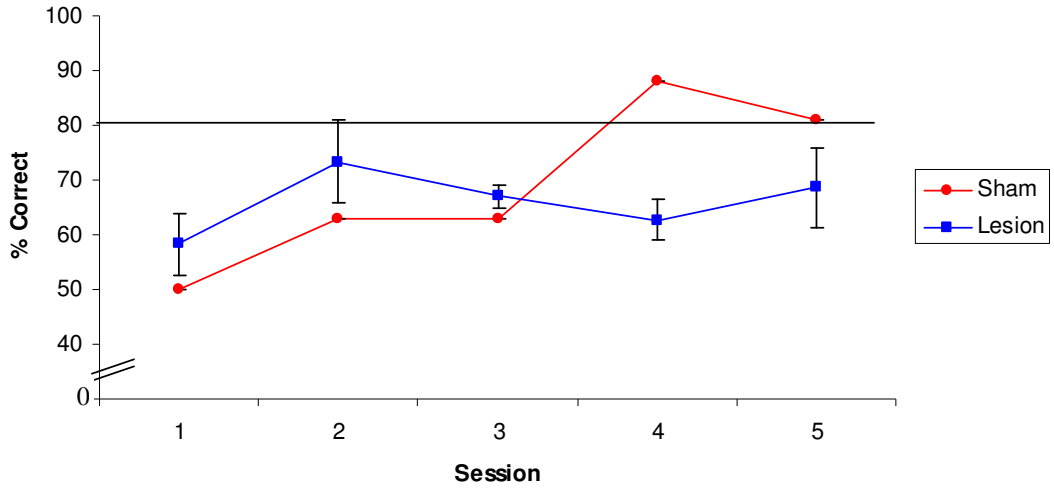


Figure 2-20: Average performance on sessions 1-5 of the new context task (expressed in terms of % correct). (Sham n = 1, Lesion n = 3)

There was no apparent difference between the sham animal and the lesioned animals for number of sessions to reach criterion on 'new context' task. The sham animal took 7 sessions to reach criterion, whereas the lesioned animals took an average of 12±2 sessions. Thus the hippocampal lesion group appears to be able to learn the new odours in a new context task just as well as the sham animal – but the animal numbers were very low.

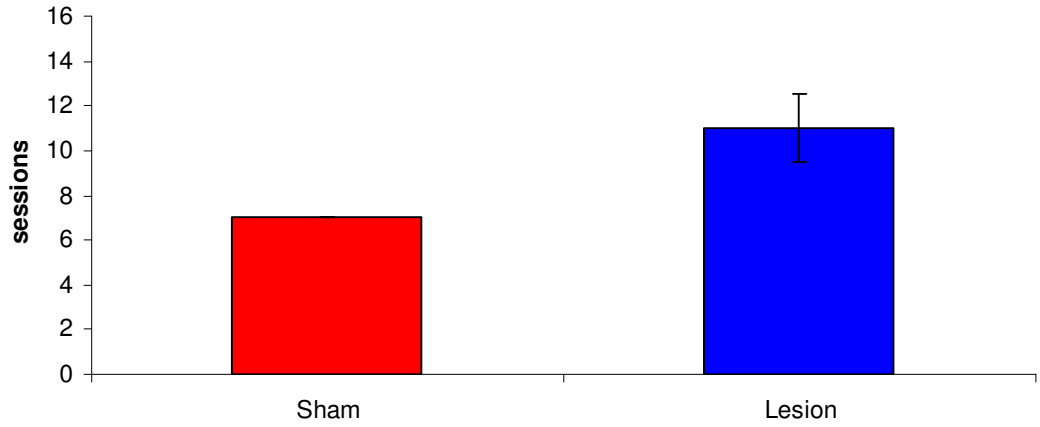


Figure 2-21: Average number of sessions taken to reach criterion on the new context task. (Sham n = 1, Lesion n = 3)

Post-criterion testing sessions illustrate that all animals can perform the 'new context' task at a consistently high level and there does not appear to be any difference between the performance of the lesioned animals compared to the sham animal (see Figure 2-22) Thus the hippocampus is not necessary to perform this 'new context' task.

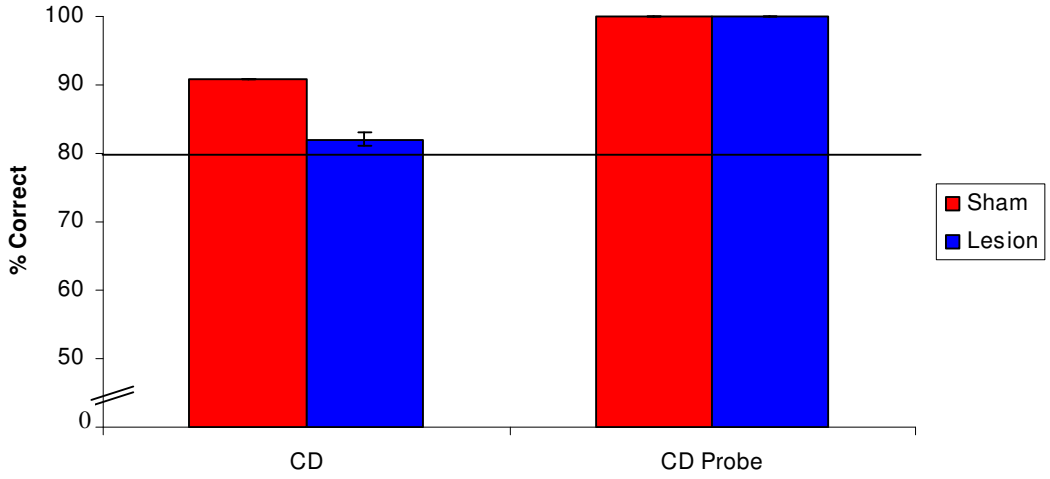


Figure 2-22: Average performance on the new context task for the four testing sessions (expressed as % correct). CD = context dependent task. The rats received 12 context dependent trials per session, as well as 1 context dependent probe. (sham n= 1, lesion n = 3)

2.5. Discussion

This task development study allowed valuable refinements to be made to the training regime used to teach the context dependent and context independent odour discrimination tasks. With time and extensive training all animals acquired accurate performance levels.

The second aim of this phase was to generate some initial results. Despite the low animal numbers (n = 6), a clear trend was observed. While sham operated animals performed the two tasks at above criterion level right from the start of post-surgery training, hippocampal lesioned animals showed an initial deficit in performance on the context dependent (but not the context independent) task. Nevertheless, this performance deficit in lesioned animals was overcome by further training.

In addition, hippocampal lesions did not affect new learning. All animals (sham operated and hippocampal lesioned) learnt the new versions of the context dependent odour discrimination task at the same rate and to a satisfactory level.

2.5.1. Development of Revised Task Protocol

The context dependent task proved relatively difficult for the rats to learn. However, by observing the behaviour of the animals and responding to this with gradual protocol changes, all animals did acquire the context dependent task.

Before starting the task, the rats were simply exposed to the experimenter and the apparatus in a habituation procedure. In addition to this, the rats were pre-trained to dig in pots of unscented sand task – first in their homecage and then on the apparatus. In this way the animals were shaped to perform the basic behaviours prior to commencing odour training (see also pre-training to dig in pots Wood et al 1999; nose poke Eichenbaum et al 1987). The penultimate stage of pre-training involved the rats learning to discriminate odours and appoint a reward value to them. This was done using a simple discrimination task whereby odour A was always rewarded and odour B was never rewarded (similar to the first stage of Good et al 1998). The final stage of pre-training taught the rats that odour discrimination could be used to predict the presence or absence of reward (i.e. A^+, B^+, X^-, Y^-). This section also shaped the animals into performing the correct *no touch* response for the unrewarded trials. Thus by the end of pre-training the rats were running around the apparatus and had learnt to both dig in rewarded pots and to with-hold their responding to non-rewarded pots.

Nevertheless, despite pre-training, several further task refinements were found to be necessary for subsequent acquisition of the context dependent odour discrimination task. Thus the main points that were carried forward from the task development stage into the revised training protocol were:

- **The use of blocks of 8 trials**. These made a dramatic difference to the performance of the animals (see Figure 2-10) and for the rest of the study, were introduced immediately after the pre-training stage.

- **The progressive movement from blocks of 8 trials, through blocks of 4 trials to random order**. This advancement onto increasingly more difficult variants of the task was a successful training strategy.

- **Correction Trials (maximum of 2 per non-dig trial).** These improved the rats performance on the non-dig trials, by increasing the energy cost of an incorrect digging motion. Limiting these to two ensured the necessary reinforcement was given without wasting time.

- **Retaining Rosemary and Tarragon 'non-dig' trials.** The first instinct of the rats was to dig in every pot they encountered. However, they quickly learnt that Rosemary and Tarragon were *not* associated with a reward and stopped digging in them. This odour / no reward association was constant, so keeping these trials present throughout acquisition training acted as a reminder that not all odours were rewarded, and that a behaviour other than digging could be correct.

The context independent task proved relatively easy to learn (taking just 4-5 sessions). The only post-pilot adaptation to this phase of the protocol was to balance the trial types to allow for more accurate comparisons between tasks (i.e. the full task now involved 12 CD trials and 12 CI trials, instead of 16CD and 8CI that was used previously). This revision in trial numbers was also used for the post-surgery testing sessions.

The overall result of this task development phase is shown in Table 2-5 as a revised training protocol for the rest of the study. Based upon the pilot study, the estimated time for completion of the acquisition and testing period was 35 sessions.

Table 2-5: Revised Pre-surgery Training Protocol

Prior to Habituation, animals get 1 week of food restriction, handling and digging pots in cages.

	Pre 1	Pre 2-3	Pre 4-7	Pre 8	Training	CRITERION
TRAINING	1 Session	2 Sessions	4 Sessions	1 Session		15/16 Correct for 2 Consecutive Sessions
	Habituation to platform	Habituation 8 plain pots (All rewarded) on platform	Habituation 8 plain pots (All rewarded) on platform with barriers	12 dig trials random order 6 each of A^+, B^+	16 trials 5 each of A^+, B^+ 4 x E^-, F^-	
ACQUISITION	Blocks of 8 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- up to 2 Correction Trials	CRITERION 6/8 correct Non-rewarded A/B Trials for 2 consecutive sessions	Blocks of 4 trials 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- up to 2 Correction trials	CRITERION 6/8 correct Non-rewarded A/B Trials for 2 consecutive sessions	Random Order 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- Up to 2 correction trials	
TESTING	CRITERION 6/8 correct Non-rewarded A/B Trials for 2 consecutive sessions	4 Sessions 4 sessions with: 21 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- & 1 randomly placed A/B Probe trial per session up to 2 Correction trials	2 Sessions 2 Sessions to get back to criterion after Probe Trials 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- up to 2 Correction trials	4 Sessions FULL TASK Random Order 24 Trials 6 each of A^+, B^-, A^-, B^+ 6 each of C^+, D^- up to 2 Correction trials	4 Sessions FULL TASK Random Order 26 Trials 6 each of A^+, B^-, A^-, B^+ 6 each of C^+, D^- & 1 A/B and 1 C/D Probe Trial up to 2 Correction trials	

2.5.2. Involvement of the hippocampus in context dependent memory

Although the pilot study was relatively small (n = 6) the results have interesting implications upon the involvement of the hippocampus in this context dependent odour discrimination tasks. All animals could perform both tasks well before surgery. However, after surgery the hippocampal lesioned animals displayed a performance impairment on the context dependent task, that could not be explained by any physical inability to perform the task. They were not impaired on the context independent task. Intriguingly, the impairment was only transient, and within 3 sessions the lesioned animals were performing as well as the shams. Once the initial impairment had been overcome, the lesioned animals consistently performed as well as the sham animals (as shown on the testing sessions). This suggests that an intact hippocampus is not *necessary* to perform this context dependent odour discrimination task, but if it is present, it will be used.

Nevertheless, although the hippocampus was involved in retention of the context dependent odour discrimination task when it was learnt before surgery, an intact hippocampus did not appear to be necessary for accurate performance. Thus the next question that arose was whether it was necessary for the acquisition of new discriminations post-surgery? The initial results from the pilot study suggest that it is not. Both the lesion and the sham groups learnt the 'new odours' and 'new context' task at the same rate as the shams, and could perform it consistently well when tested. (This topic is discussed further in chapter 5). However, the number of animals used in this pilot study was very small (lesion n = 3, sham n = 2) and training on new versions of the task took extended training. These factors may have masked mild lesion impairments or differences in performance. Hence it is worth examining this facet of the experiment further with a larger number of animals.

2.5.3. Addition to protocol: What cues are rats using to solve task?

The task development data suggested that the context dependent odour discrimination task could be performed accurately both with an intact hippocampus and without it. Furthermore, the fact that the lesioned animals can still perform a context independent task suggests they do not have a problem with identifying the odours or in retrieving odour-reward associations. However, a temporary impairment was still observed in lesioned animals, so perhaps the hippocampus contributes to either the representation of the context itself or to the rat's ability to combine context information with reward associations. The aim of this addition to the protocol was to determine the effects of hippocampal lesions on how the rats encode their environment and how they define which context they are in. Are the lesioned animals using different cues to sham animals to distinguish between the contexts? In order to address this question, a set of tasks were designed:

a) Placement Manipulation	**To determine whether the rats were using self-motion cues.** For these trials the rats were physically 'placed' into the centre of the contexts instead of being allowed to 'run' in from bridge under their own self-motion.
b) Context swap Manipulation	**To determine whether rats would be impaired if intra-maze cues were put into conflict with self-motion cues.** For these trials the cues that defined the context were swapped around, (i.e. those that usually appeared around the left-hand platform would now appear around the right-hand platform and vice versa). Rats were rewarded according to the intra-maze cues, not its physical location.

c) Combined Manipulation	**To determine whether the conflict above can be resolved upon removal of self-motion cues.** These trials were a combination of a) and b). The intra-maze cues that define the context are in their swapped configuration (i.e. those that are normally on the left appear on the right), but the animals are physically placed into each 'context' (i.e. they cannot use self-motion cues).

(For a more visual representation of these manipulations, please see the PowerPoint file on the CD in the appendix of this thesis)

These manipulations involved only the context dependent task and were inserted between the Retesting and 'New odours' phase of task (see Table 2-6).

Table 2-6: Post-surgery Testing Protocol

Prior to post-surgery testing, animals get 48hrs of food deprivation and 2 sessions of digging pots in their cages.

TESTING

5 Sessions		CRITERION	4 Sessions
Retest FULL TASK Random Order 24 Trials 6 each of A^+, B^-, A^-, B^+	**Retest** FULL TASK Random Order 24 Trials 6 each of A^+, B^-, A^-, B^+	22/24 correct for 2 consecutive sessions	**Retest** FULL TASK Random Order 26 Trials 6 each of A^+, B^-, A^-, B^+
6 each of C^+, D^-	6 each of C^+, D^-		6 each of C^+, D^-
Up to 2 Correction trials			& 1 A/B and 1 C/D probe trial
Animals must complete 5 sessions	up to 2 Correction trials		up to 2 Correction trials

MANIPULATIONS

8 Sessions	2 sessions	8 Sessions	2 Sessions	8 Sessions
Placement Manipulation Context Dependent Task only 8 Standard Run Trials (4 rewarded, 4 non-rewarded) then 8 Placement Trials (4 rewarded, 4 non-rewarded)	Context Dependent Task only 12 Standard Run Trials (6 rewarded, 6 non-rewarded)	**Context swap Manipulation** Context Dependent Task only 8 Standard Run Trials (4 rewarded, 4 non-rewarded) then 8 Context swap Trials (4 rewarded, 4 non-rewarded)	Context Dependent Task only 12 Standard Run Trials (6 rewarded, 6 non-rewarded)	**Combination Trials** Context Dependent Task only 4 Standard Run Trials 4 Context swapped Run Trials then 4 Standard Placement Trials 4 Context swap Placement Trials

NEW LEARNING

	CRITERION	4 Sessions		CRITERION	4 Days
New odours Context Dependent Task only Random Order 16 trials 6 each of E^+, F^-, E^-, F^+ up to 2 Correction trials	14/16 correct for 2 consecutive sessions	New odours Context Dependent Task only Random Order 17 trials 6 each of E^+, F^-, E^-, F^+ (8 rewarded, 8 non-rewarded) & 1 probe trial up to 2 Correction trials	New context Context Dependent Task only Random Order 16 trials 6 each of G^+, H^-, G^-, H^+ (8 rewarded, 8 non-rewarded) up to 2 Correction trials	14/16 correct for 2 consecutive sessions	New Context Context Dependent Task only Random Order 17 trials 6 each of G^+, H^-, G^-, H^+ (8 rewarded, 8 non-rewarded) & 1 probe trial up to 2 Correction trials

2.5.4. New experimental apparatus design

A new experimental apparatus was designed for use in the remainder of the study (see Figure 2-23). This was based upon the platforms used by Muller et al (1987) for place cell recordings.

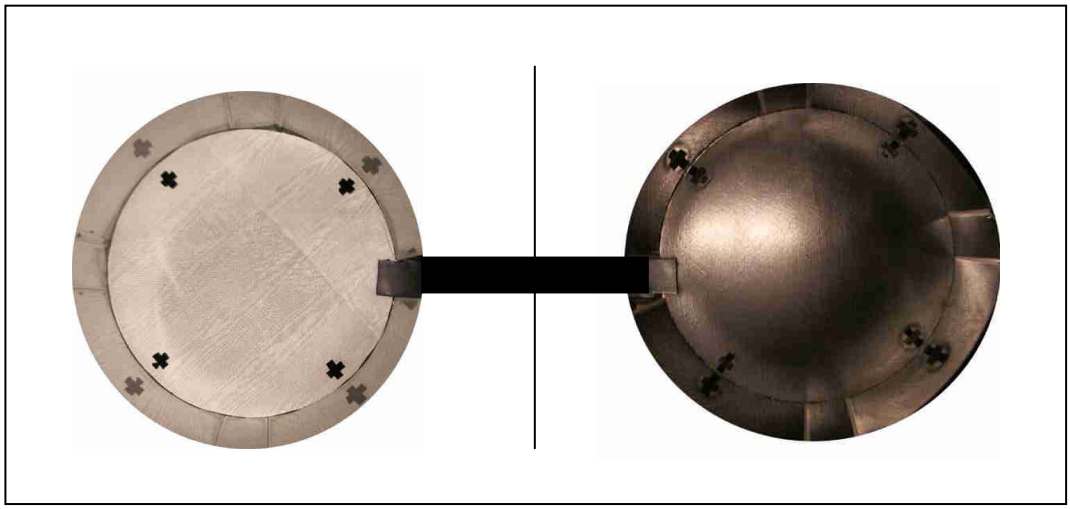


Figure 2-23: New experimental apparatus

It was decided that in order to make the apparatus more 'context' focused and less 'location in room' based, the contexts themselves should contain all the necessary cues for the task. One context was 'coloured' white and the other black for maximum contrast. The square platforms were exchanged for circles to minimize geometric cues, and clear 40cm high walls were added. Fabric attached to the outside of the clear walls gave the contexts their colour and allowed the context configuration to be easily changed during the cue manipulations. A soft padded floor insert was added to one platform to give each a different textural feel (hard wood vs soft padded vinyl). Controlled distal cues were removed, and uncontrolled distal cues were minimized

by surrounding the apparatus in black curtains. Furthermore, the home cages were moved away from the experimental apparatus.

The ‘new context’ apparatus was also modified (as illustrated in Figure 2-24). To make the new context distinguishable from the first set of platforms, the wall colour and floor texture was changed, but the geometric shape and size stayed the same.

(The construction of the new apparatus is described in more detail in the methods section of chapters 3 and 5.)

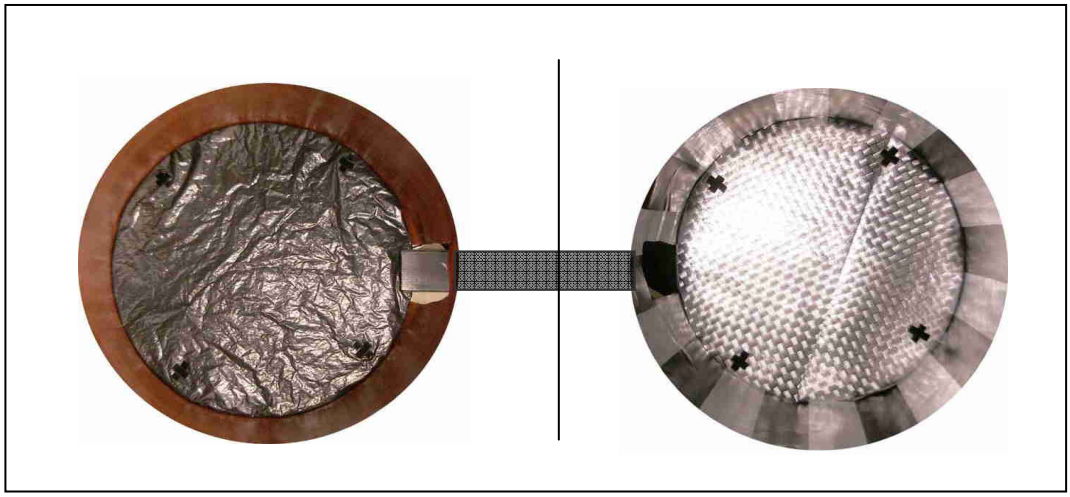


Figure 2-24: Modified 'new context' apparatus

2.6. Conclusion

The task development study provided an opportunity for task and training protocol refinements before the main study began. It demonstrated that rats can learn this context dependent odour discrimination task if they are trained in an appropriate way. Furthermore, it implied that the hippocampus may play a role in memory for this context dependent odour discrimination task, but that an intact hippocampus is not absolutely necessary for performance or further acquisition of new variants of the task.

3. The effect of hippocampal lesions on the retention of context dependent and context independent odour discriminations.

3.1. Introduction

Retrograde amnesia is the loss of information that was acquired before the onset of the amnesia. It can be extensive and extend across large portions of a subjects life. Retrograde amnesia is often observed after damage to the hippocampus (Lehmann and Lacanilao et al. 2007). However, the degree of impairment can depend upon the type of memory being assessed and the position of the damage. In spatial tasks, hippocampal damage leads to severe retrograde amnesia for almost all past spatial episodes (Nadel and Moscovitch 1997). On the other hand, memory for object discrimination tasks is usually unaffected (Nadel and Moscovitch 1997). Is contextual information sensitive to hippocampal removal? Evidence from contextual fear conditioning suggests that it is (e.g. Anagnostaras and Maren et al. 1999; Lehmann and Lacanilao et al. 2007) – but perhaps the use of aversive stimuli is a special case? This chapter explores the effect of hippocampal abolition on retention of a previously learnt context-dependent odour discrimination problem.

3.1.1. Experimental Aims

This chapter aims to explore the role of the hippocampus in the retention of a context dependent odour discrimination task. If, as expected, the hippocampus is necessary for the retention of context, post-training excitotoxic hippocampal lesions will result in a severe impairment in post-surgery performance (a retrograde amnesic effect).

In the last chapter, initial data suggested that perhaps an intact hippocampus is not necessary for performance of this context-dependent task. However, the number of

animals used in that study was small and the lesions were incomplete. Hence this chapter aims to address both of these issues, and examine behavioural performance with a larger data set.

3.2. Methods

This chapter follows on from the pilot study, and the methodology was largely the same. Therefore, only an overview will be given unless the details changed.

The protocol described in this chapter involved pre-surgery training, followed by complete-hippocampus lesion / sham surgery. Post-surgery testing took place after a recovery period of 14 days.

3.2.1. Subjects and Housing

The subjects were 23 Male Lister-hooded rats (Charles River UK) weighing 250-300g at the start of pre-surgery training. As in the pilot study, they were individually housed and kept on a 12h light/dark cycle with ad libitum access to water, but food restricted to 90% of their free feeding weight. (For further details see Chapter 2)

All procedures were carried out in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986 and European Communities Council Directive of 24 November 1986 [86/609/EEC], and all efforts were made to minimise suffering.

3.2.2. Apparatus

Contexts

Discrimination learning took place on two structurally identical platforms that could be defined as separate contexts by changing the colour of the fabric behind the clear polycarbonate walls and by using floor inserts. Photographs of the apparatus are shown below (see Figure 3-1) and can also be observed in videos contained on the context dependent in the appendix of this thesis. The platforms were circular (76cm in diameter) and made of 18mm exterior plywood painted black. They sat approx 75 cm above floor level on a frame. Walls of 40cm high were constructed from 1 mm thick clear polycarbonate, screwed to the outside of the wooden platform (see Figure 3-1). This was attached as four 48 cm by 40 cm lengths, with space between them for 4 doors (12 cm x 40 cm) on sliding attachments. The polycarbonate walls were 'coloured' using lengths of black or white blackout fabric fixed to the outside of the polycarbonate using Velcro and clips. The entire apparatus was washable and symmetrical. It was cleaned with soapy water between animals and was randomly rotated during training to reduce the impact of any uncontrolled cues (i.e. the platform was rotated so that bridge came out a different door, but orientation of overall apparatus was kept exactly the same).

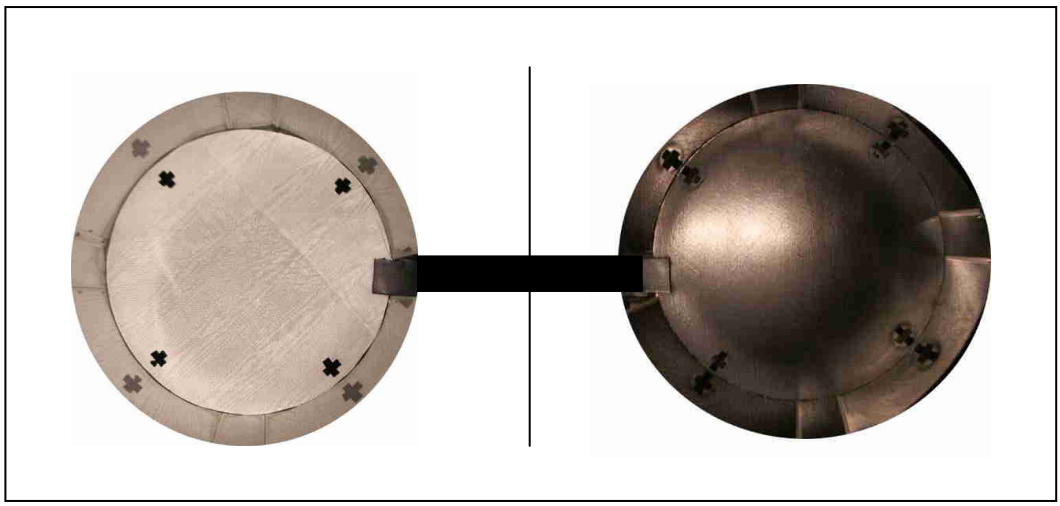


Figure 3-1: Diagram of polycarbonate apparatus used in the task. The black lines illustrate the black curtains that surround the apparatus and minimize extra-maze cues. The four crosses of Velcro can be seen on the platforms.

Crosses of velcro were evenly spaced around the perimeter of the platforms (approx 2 cm from the edge) to allow for the attachment of pots of scented sand (see Chapter 2 and Figure 3-1). A floor insert for the white platforms was constructed from soft non-slip tray covering and covered in white wood-grain effect sticky backed vinyl. This utilised velcro to hold it in place. Further crosses of velcro were fixed to the upper side of the floor insert for pot attachment. The two circular platforms were connected in the centre by the same bridge as used in the pilot study and the two large wooden barriers were again used to control access to the platforms (i.e. placed at either end of bridge to hold rat in the centre of the bridge for the inter-trial interval).

The entire apparatus was surrounded by thick black curtains to minimize extra-maze cues. Additional material was hung across the centre of the bridge to prevent the animals from observing one context while located in the other. A hole was cut in this to prevent it from obstructing the passage of the rats along the bridge (see Figure 3-1).

Odour Pots

The reward pots were the same clear Nalgene pots filled with a mixture of150g playsand and 0.8g household herbs / spices (as used in the pilot study; see chapter 2). Buried chocolate wheetos (Nestle) were used as the food reward (approx 2.5cm deep).

3.2.3. Behavioural Protocol

Handling

The rats were habituated to handling (5 sessions; 5 mins per day). During this time the rats were also given pre-task digging pots in their homecages. These contained chocolate wheeto cereal rewards and the animals food ration buried within approx 150g of plain sand.

General Protocol

As for the pilot study, testing took place on weekdays (Mon-Fri) between 8.30am and 8.30pm.

The rat was carried from the animal housing room into the testing room in its home cage. A transferral container (blue bucket) was used to carry the animal to the apparatus where it was placed on the centre of the bridge between the two barriers. At the end of the testing session the rat was carried in the transferral container back to its homecage.

The experimenter remained within the curtained area at all times during the task. To minimise experimenter effects, their exact location was unpredictable and bore no relevance to the site of the reward or to the behaviour expected.

At the end of their trials, the rat was lifted off the bridge and carried in the transferral container back to their homecage.

Habituation, Shaping and Training.

(For an overview see Table 3-1; further detailed explanations can be found in the methods section of the pilot study - chapter 2. For moving representations of the task see the PowerPoint file and videos on the context dependent in the appendix of this thesis).

Rats were habituated to the empty platform for 1 session (15 minutes) and then shaped to dig in pots of unscented sand (8 per session) for rewards over the next 6 sessions; for the first three of these sessions the rats had freedom to roam as they desired over the platforms, for the last three of these sessions barriers were introduced to restrict movement to one platform at a time (experimenter controlled). Thus, all trials began and ended on the bridge between the platforms. An inter-trial interval of 20-30sec allowed the experimenter to remove spilt sand and set-up next trial while the rat waited on the bridge. On pre-training session 8, odours were mixed with the sand (see above) and 12 rewarded trials (6 x A^+/ B^+) presented that were in accordance with the task rules that would appear later (i.e. odour A rewarded on white platform; odour B rewarded on black platform).

In this experiment all the odour pots were presented successively i.e. only one pot was ever available to the rat at any point in time.

Acquisition and Testing Phase

(The protocol for acquisition and testing is detailed in Table 3-1 and videos / a PowerPoint file are available to view on the context dependent in the appendix of this thesis. The protocol of moving from blocks of 8, to blocks of 4 and finally onto

random order, was established as a result of the pilot study and further details are available in chapter 2).

On a normal trial, the rat was placed on the centre of the bridge, between the two barriers (i.e. initially it only had access to the centre of the bridge). One pot of scented sand was placed in one of the contexts. The appropriate barrier was removed to give the rat access to the platform. The rat ran towards the pot, sniffed the odour and for this 'go / no-go' procedure, the correct response was either to dig in the pot and find the reward (+) or to turn away from it (-) according to the rules of the task.

In order to train the animals in the concept of unrewarded trials, two completely unrewarded foils (odours X and Y; rosemary and tarragon) introduced. Thus for the next stage of training the rats received 16 pseudorandomised trials per session (5 x A^+ and 3 x X^- on one platform; 5 x B^+ and 3 x Y^- on the other - see Figure 3-2). Rats were trained in this way until they reached a criterion of 15/16 trials correct for 2 consecutive sessions.

At this point the rats were introduced to the context dependent odour discrimination task. To solve the context dependent task, the animals had to pay attention not only to the odour of the sand, but to the spatial context it was in. Following the training protocol established during the pilot study, the rats began with blocks of 8 trials. Thus they received 20 trials per session; 16 context dependent trials (4 trials each of A^+, B^-, A^-, B^+) in blocks of 8 (i.e. 8 odour A followed by 8 odour B), as well as 4 randomly interspersed foils X^-/Y^-. On each trial, rats had 2 additional attempts to correct themselves before being moved onto the next trial (i.e. up to 2 repetitions of trial should rat get it wrong). However, only the first attempt at each trial was counted for scoring purposes. Rats were trained in this way and rewarded according to the rules shown in Figure 3-2, until they reached a criterion of 6/8 non-rewarded odour A / B trials for 2 consecutive sessions. Upon successful completion of the 'blocks of 8' phase, the rats were moved onto blocks of 4 trials (i.e. 4 odour A trials

followed by 4 odour B trials). This pattern was repeated twice per session alongside 4 randomly interspersed X^- / Y^- trials (total of 20 trials per session; 16 context dependent, 4 foils). In each session the trials were balanced but pseudo-randomised for position and reward properties, and up to two corrections were allowed per trial. Training continued in blocks of 4 until the rats reached a criterion of 6 out of 8 correct non-rewarded odour A/B trials for 2 consecutive sessions. The next training phase involved running the trials in pseudo-randomised order. Twenty context dependent trials were presented (4 each of A^+, B^-, A^-, B^+ and 2 each of X^- and Y^- with up to 2 corrections per trial). Again criterion was set at 6 out 8 correct non-rewarded odour A / B trails for 2 consecutive sessions.

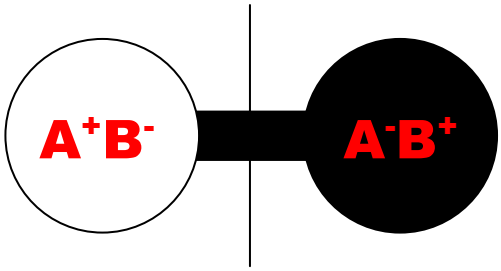


Figure 3-2: Rules of context dependent task

Testing Phase – Context Dependent Task

Having reached criterion on the context dependent task when it was presented in a pseudo-randomised manner, the rats underwent a period of testing. Testing sessions were exactly like the last stage of training (4 trials each of A^+, B^-, A^-, B^- and 2 each of X^- and Y^- with up to 2 corrections per trial), except for the addition of 1 randomly placed odour A or B probe trial. These were used to verify that the animals were using the scent of the sand to solve the task and not detecting the scent of the cereal reward itself. Probe trials were presented as rewarded trials (i.e. A or B in their

appropriate context), however no cereal reward was available within the pot. The reward was only dropped in after the animal dug for 5 sec. (For a demonstration see Probe Trial video on the context dependent in the appendix of this thesis). Following probe trial sessions, the rats were given two more standard acquisition sessions (4 trials each of A^+, B^-, A^-, B^+ and 2 each of X^- and Y^- with up to 2 corrections per trial), to ensure they were performing at criterion level before adding the next task.

Context Independent Task

The end training stage (Full task) involved the rats performing a context dependent task and context independent task at the same time. For the context independent task, odour E is always rewarded and odour F is never rewarded, regardless of the context they were presented in (odours E /F were cinnamon and cumin; for rules see Figure 3-3). At this stage the X/Y non-reward 'reminder' trials were stopped, and the E^+ / F^- trials were randomly interspersed with the context dependent trials. The rats were performing 24 trials per session – 12 context dependent trials (3 each of each of A^+, B^-, A^-, B^+) and 12 context independent trials (6 each of E^+, F^-) with up to 2 corrections per trials (as described above).

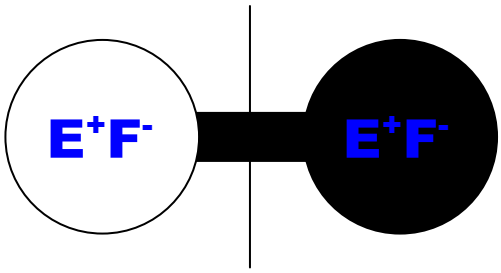


Figure 3-3: Rules of context independent task

The rats were trained for a fixed training period of 4 sessions. Thus after 4 sessions of training on the 'full task', the rats underwent a further period of testing.

Testing Phase – Full Task (Context Dependent and Context Independent).

The four testing sessions followed the same protocol as the full task described above. The rats received 24 trials per session, 12 context dependent trials (3 each of each of A^+, B^-, A^-, B^+) and 12 context independent trials (6 each of E^+, F^-) with up to 2 corrections per trials. In addition they also received 1 additional context dependent probe trial and 1 context independent probe trial per session (i.e. rats performed 26 trials/testing session). Probe trials were performed to check the rats were not using the odour of the cereal reward itself to perform the task

This marked the end of pre-surgery Training.

Table 3-1: Pre-surgery Training Protocol

Prior to Habituation, animals get 1 week of food restriction, handling and digging pots in cages.

	Pre 1	Pre 2-3	Pre 4-7	Pre 8	Training	CRITERION
TRAINING	1 Session	2 Sessions	4 Sessions	1 Session		15/16 Correct for 2 Consecutive Sessions
	Habituation to platform	Habituation 8 plain pots (All rewarded) on platform	Habituation 8 plain pots (All rewarded) on platform with barriers	12 dig trials random order 6 each of A^+, B^+	16 trials 5 each of A^+, B^+ 3 x X^-, Y^-	
ACQUISITION		CRITERION		CRITERION		
	Blocks of 8 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- up to 2 Correction Trials	6/8 correct Non-rewarded A/B Trials for 2 consecutive sessions	Blocks of 4 trials 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- up to 2 Correction trials	6/8 correct Non-rewarded A/B Trials for 2 consecutive sessions	Random Order 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- Up to 2 correction trials	
TESTING	CRITERION 6/8 correct Non-rewarded A/B Trials for 2 consecutive sessions	4 Sessions 4 sessions with: 21 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- & 1 randomly placed A/B Probe trial per session up to 2 Correction trials	2 Sessions 2 Sessions to get back to criterion after Probe Trials 20 trials 4 each of A^+, B^-, A^-, B^+ 2 x X^-, 2 x Y^- up to 2 Correction trials	4 Sessions FULL TASK Random Order 24 Trials 6 each of A^+, B^-, A^-, B^+ 6 each of E^+, F^- up to 2 Correction trials	4 Sessions FULL TASK Random Order 26 Trials 6 each of A^+, B^-, A^-, B^+ 6 each of E^+, F^- & 1 A/B and 1 E/F Probe Trial up to 2 Correction trials	

3.2.4. Hippocampal Lesion Surgery

Surgery was done in exactly the same way as described in the pilot study (see chapter 2 for further methodological details).

Surgery took place the day after the last pre-surgery testing session, after animals had been given ad libitum access to food and Rimadyl in their water overnight. 10 animals received full bilateral hippocampal lesions and the other 7 received sham surgery.

Surgery was done under Halothane (Merial Animal Health, UK) anaesthesia. After exposing the skull, a bilateral craniotomy was carried out using a dental drill. Sham surgery involved piercing through the dura with a needle (23G) to simulate the mechanical damage cause by needle entry during injections.

Lesion surgery on the other used multiple injections of ibotenic acid (Biotechnology, CA) that was dissolved in phosphate buffered saline (pH 7.4) at 10mg/ml following the protocol of Jarrard (1989). Injections of 0.05 or 0.1µl ibotenic acid were made into each hippocampi at different rostrocaudal and dorsoventral levels. Co-ordinates were calculated from bregma and are shown in Table 3-2. In a slight change to the pilot protocol, the dura was pierced with a 23G needle before lowering the syringe down into the brain. This was to try to avoid clogging the needle or compressing the brain.

After analysing the histology, a large amount of sparing was found (32-45%), thus the decision was take to use slightly adjusted surgery co-ordinates from that point forward. Thus 3 rats included in this chapter received the co-ordinates listed in Chapter 2, whereas the other 7 lesioned animals received the altered co-ordinates as

shown in Table 3-2. The initial set of co-ordinates were designed for rats of 300-400g at time of surgery. Since my rats had an extended pre-surgery training protocol, they averaged 576g when operated on. Other people in the facility were also having similar problems with heavy rats, so the co-ordinates were adjusted by Livia deHoz in an attempt to rectify this problem. I performed two test lesions with these 'new' co-ordinates and these both produced sparing of approximately 10%. Hence these 'new' surgery co-ordinates were used for the remaining 7 lesion surgeries.

Once injections were completed, the area was cleaned and the skin sutured back together across the top of the head. Injections of Rimadyl and saline were given immediately post-operatively.

Table 3-2: Stereotaxic co-ordinates for larger rats (calculated from bregma). These are exactly as before, except that four additional dorsal injections sites have been added on each side. The new co-ordinates are shown in blue at the bottom of the table.

Right Side				Left side			
AP	**ML**	**DV**	**ul**	**AP**	**ML**	**DV**	**ul**
-2.4	-1.0	-3.0	(0.05)	**-2.4**	1.0	-3.0	(0.05)
-3.0	-3.0	-2.7	(0.10)	**-3.0**	3.0	-2.7	(0.10)
	-1.4	-2.1	(0.05)		1.4	-2.1	(0.05)
		-2.9	(0.05)			-2.9	(0.05)
-4.0	-3.7	-2.7	(0.10)	**-4.0**	3.7	-2.7	(0.10)
	-2.6	-1.8	(0.05)		2.6	-1.8	(0.05)
		-2.8	(0.05)			-2.8	(0.05)
-4.3	-4.0	-7.0	(0.05)	**-4.3**	4.0	-7.0	(0.05)
-5.0	-3.0	-3.0	(0.10)	**-5.0**	3.0	-3.0	(0.10)
	-3.9	-7.0	(0.10)		3.9	-7.0	(0.10)
	-5.4	-5.1	(0.10)		5.4	-5.1	(0.10)
-6.1	-3.9	-3.6	(0.05)	**-6.1**	3.9	-3.6	(0.05)
		-7.0	(0.05)			-7.0	(0.05)
	-5.0	-4.5	(0.05)		5.0	-4.5	(0.05)
-6.6	-4.6	-4.6	(0.05)	**-6.6**	4.6	-4.6	(0.05)

AP: anterior-posterior (mm)
ML: medial-lateral (mm)
V: ventral from dorsoventral origin (mm)
µl: volume of ibotenic acid injected

The animals were given 14 days to recover from surgery before post-surgery behavioural testing began. Food restriction was re-started 48hrs before testing resumed. During this time food rations were hidden within digging pots in the rats' homecages.

3.2.5. Post-surgery Behavioural Testing

Retest on Full Task

Post-surgery testing was exactly the same as the full-task stage of pre-surgery and followed the same rules (see Figure 3-2 and Figure 3-3). The rats received 24 counterbalanced but pseudo-randomised trials per session; 12 context dependent trials (3 each of each of A^+, B^-, A^-, B^+) and 12 context independent trials (6 each of E^+, F^-), with up to 2 corrections per trials. The rats performed a minimum of 5 sessions of training before moving onto probe trials, so that an accurate measure of post-surgery performance level could be attained. Once the rats were again performing at criterion level (22/24 trials correct for 2 consecutive sessions), 4 testing sessions were given (i.e. minimum of 5 sessions pre-testing; if performing at criterion then, post-surgery testing began on session 6). Testing sessions were exactly the same as those given pre-surgery i.e. 24 trials per session: 12 context dependent, 12 context independent, with up to 2 correction trials + 1 A/B probe trial and 1 E/F probe trial).

Table 3-3: Post-surgery testing schedule

5 Sessions	CRITERION	4 Sessions
FULL TASK Random Order 24 Trials 3 each of A^+, B^-, A^-, B^+ 6 each of E^+, F^- up to 2 Correction trials	22/24 correct For 2 consecutive Sessions	FULL TASK Random Order 26 Trials 3 each of A^+, B^-, A^-, B^+ 6 each of E^+, F^- & 1 A/B and 1 E/F Probe Trial up to 2 Correction trials

3.2.6. Histology

Rats carried on to do further training / testing as described in the rest of this thesis. However, in order to fully understand the results the histology is described in this chapter.

Details of the histological procedure are given in Chapter 2 and technical sheets for staining slides has been included in the appendix. This section is therefore given by way of an overview.

Rats were euthanised using Euthatal (Sodium Pentobarbitol, Merial Animal Health, UK). They were then perfused intracardially using first saline and then 4% formalin. The brain was then removed and placed in a jar containing 4% formalin for at least 24hrs to ensure full fixation. To stabilise the tissue before slicing and to maintain their shape, the brains were embedded in egg yolk. 30µm coronal sections were cut using a cryostat, with every fifth section mounted onto gelatine coated slides and

stored for histological analysis. The slides were stained with cresyl violet acetate and mounted with DPX. (The protocol for this is included in the appendix of this thesis)

The extent of the lesions was assessed by transferring an image of each slice to the computer using a camera (Leica), mounted on a makroscope (Wild M420, Switzerland) and the Lecia Qwin Program. The image was then opened using Image J and the area measurement tool used to calculate the area of spared tissue for each animal. This was converted to a percentage sparing for each animal by dividing it by the average area of the hippocampus as found in the sham operated animals. 3D reconstructions of the lesions were then performed using a trial version of 3D doctor (version 4.0.061025). To produce these pictures, 3 areas were manually outlined on each slice photo within the software a) outline of cortex, b) outline of spared hippocampal tissue c) region of tissue devoid of cells (this would include hippocampal and non-hippocampal areas that had been affected by the toxin). These pictures can be observed in Table 3-4. The software then uses the boundaries from each slice to perform a triangulation procedure and reproduce a 3D model.

3.2.7. Data Analysis

Data analysis was done using SPSS as is described in the pilot study (see chapter 2). All numerical values and graphs are reported as $\pm$ SEM. Criterion was 80% and is marked with a black horizontal line on the graphs (where appropriate).

Independent samples t-tests were used to compare the performance of the groups (hippocampal lesioned and sham operated) throughout.

A Repeated measures ANOVA followed by simple effects analysis (with bonferonni corrections) was used to compare performance across the first 5 sessions of retesting.

It was also used to compare initial post-surgery performance on the context dependent task to post-surgery performance.

Paired t-tests were used to compare performance before and after surgery on the context dependent task.

Pearson's correlations were used to investigate the relationship between lesion size and performance on the context dependent task.

3.3. Results

23 animals began the training protocol. Unfortunately 4 animals died from illness / seizures before completion of pre-surgery training. One further animal stayed alive despite taking several seizures, but consequently seemed unable to learn the task even with extended training. This animal was also excluded from the data. The issue of seizures was an acknowledged problem with Lister Hooded rats from our supplier (CRUK) and these cases were reported to them for their investigations.

18 animals therefore completed pre-surgery training, and underwent surgery, during which another rat died. Hence the data given in this chapter represents 17 rats (split into 7 sham animals and 10 lesioned animals as appropriate).

3.3.1. Acquisition of Task

The adjusted protocol developed during the pilot study (see chapter 2) was an effective way of training the animals. An example acquisition graph for this protocol is shown in Figure 3-4. In this example the rat took 45 sessions to learn the task.

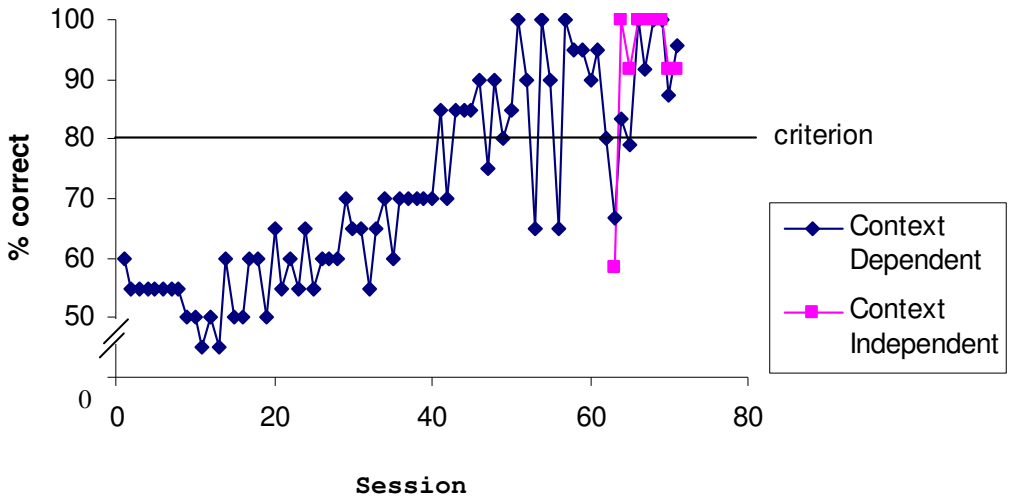


Figure 3-4: Typical performance of a rat trained every day on the protocol. Performance is expressed in % correct. This rat took 71 sessions to learn the task

A dip in performance is seen at session 53 and 56 where blocks of 4 and random order respectively were introduced. A further dip is seen at session 63 where the full task was introduced (context dependent and context independent together). The rats were run in batches of 6-8 animals and performance patterns were very similar across all batches.

3.3.2. Pre-surgery Performance

No difference in performance of groups before surgery

On average it took 60 ±5 sessions for rats to reach criterion level (n=17). Acquisition time did not differ between the allocated groups prior to surgery ($t < 1$).

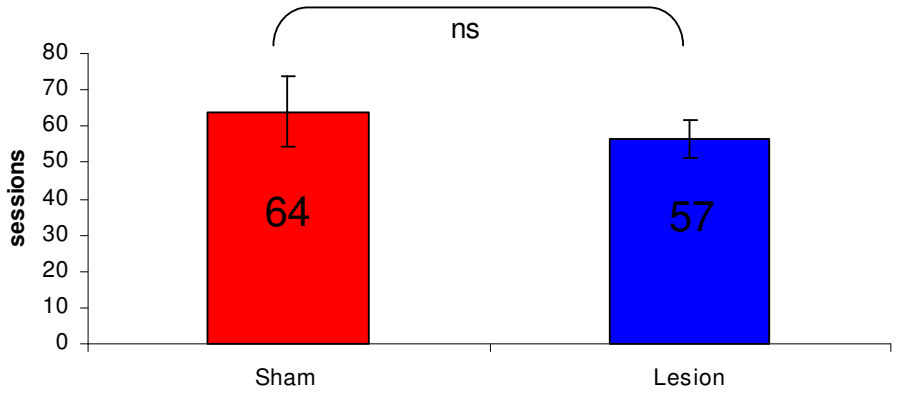


Figure 3-5: Average number of sessions taken to reach criterion before surgery (counted from the start of blocks of 8). Animals are split according to the surgery they would receive (sham n = 7, lesion n= 10).

Once the rats have learnt the tasks they all performed consistently well as shown by the 4 testing sessions in Figure 3-6. All animals perform the context dependent task at above criterion level, and perform the context independent task at near perfection by this stage of training. This lack of variability in the context independent task means it lacks the Gaussian distribution that is required for parametric tests. However, independent samples t-tests on the context dependent task showed no pre-surgery differences between testing groups (sham or lesion to be; $t < 1$). The final performance levels for each task strongly reflected the ease with which the rats

acquired and performed the context independent task compared to the context dependent task.

Probe tests indicate that the rats were not using the odour of the buried cereal to determine whether or not to dig. When the groups were combined, the rats performed 68 probe trials between them during this part of the protocol. Only 3 of these were incorrect (2 x context dependent task probes, 1 x context independent task probe).

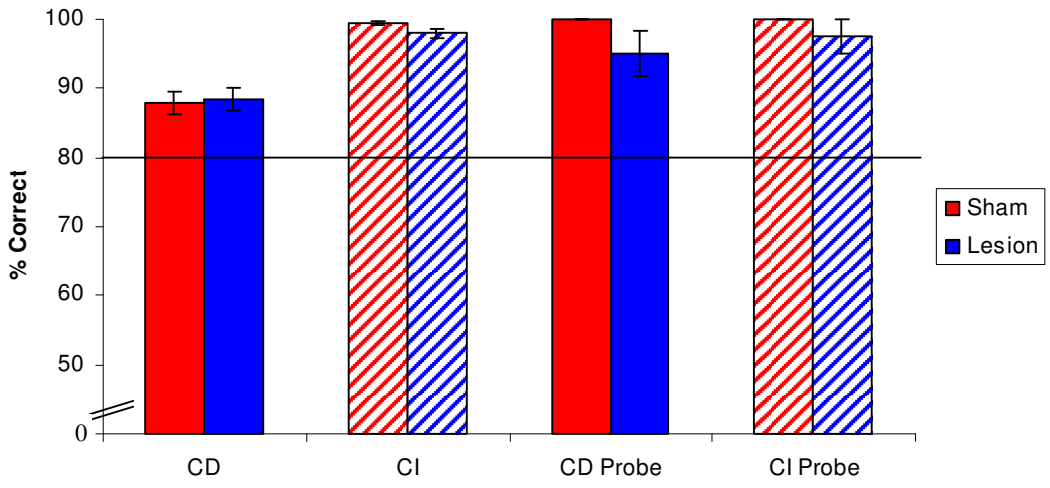


Figure 3-6: Average performance on the four pre-surgery testing sessions expressed as % correct. CD = context dependent task, CI = context independent task. The rats received 12 context dependent and 12 context independent trials per session, as well as 1 context dependent probe and 1 context independent probe per session. (sham n = 7, lesion n = 10)

3.3.3. Context dependent vs context independent tasks

Performance on the context dependent task was consistently lower than on the context independent task

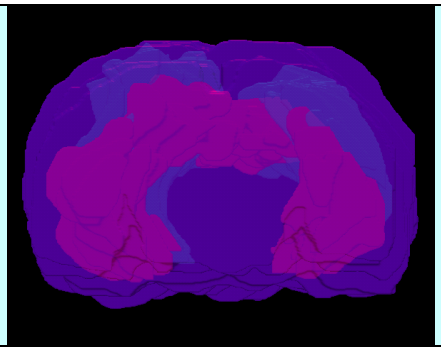
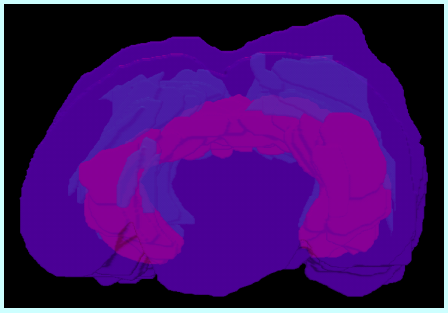
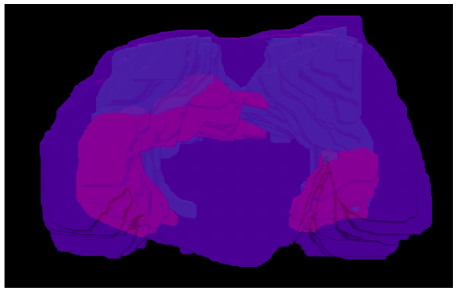
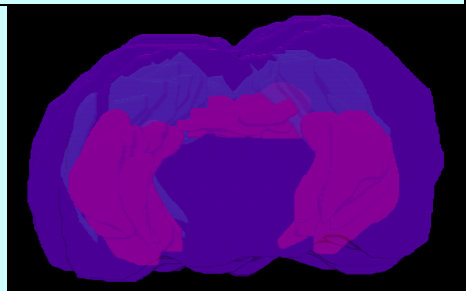
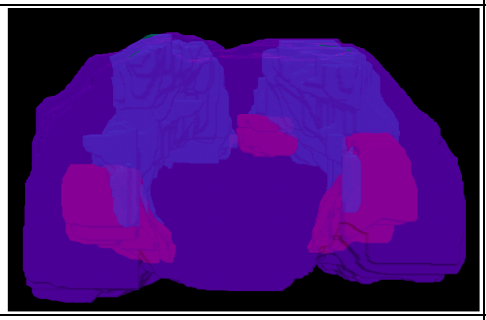
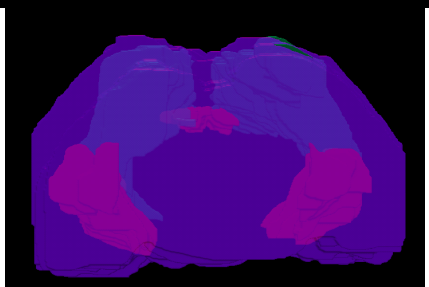
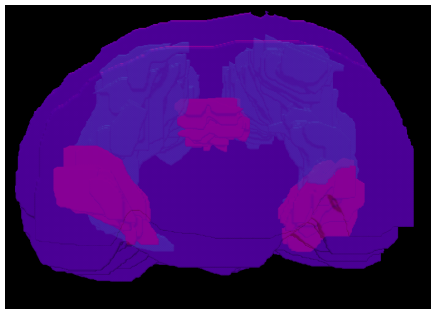
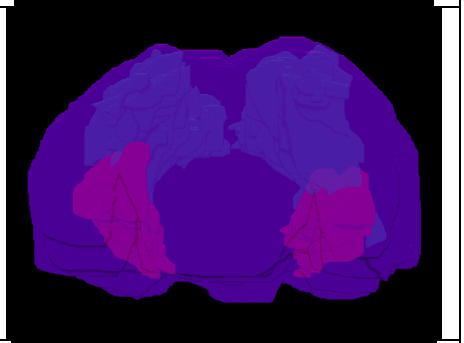
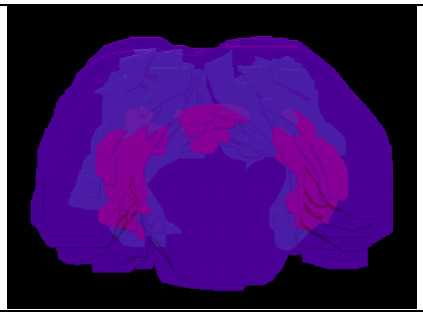
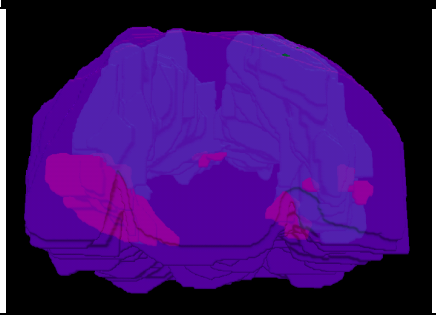
The context independent task is a simple discrimination task in which context is irrelevant. Rats can learn this distinction very rapidly. Conversely all rats took considerably longer to acquire the context dependent task, and their performance did not reach the same level as on the context-independent task. This divergence is reflected in their performance throughout this protocol - in acquisition, testing and post-surgery performance. Once learnt, the average performance on the context independent task was 99 % $\pm$1.2, whereas for the context dependent task it was only 88 % $\pm$0.4.

3.3.4. Histology

Size of lesions

The lesions varied in size, with hippocampal sparing ranging from 10% to 45% (Average 26.8 % $\pm$3.9) Sparing was consistently observed in the deepest and most posterior regions of the hippocampus (at the temporal pole) and sometimes additionally at the septal pole (see Table 3-4). Some entorhinal cortex damage was observed in all lesioned animals. The subiculum and post-subiculum showed extensive bilateral damage that was consistent in position across the lesioned group.

Table 3-4: Lesion size and 3D reconstruction. Blue highlighted boxes show the lesions that were done using the 'original' set of co-ordinates. For the 3D pictures, purple = cortex shape and outline, red = sparing, blue = area that was devoid of all cells (size of hole in brain)

Sparing		Sparing	
45%		43%	
34%		32%	
32%		22%	
20%		18%	
13%		10%	

Extra-hippocampal Damage

Cortical damage, (visible as a hole on the surface of the intact brain), was present in every lesioned animal. The damage to the neocortex was very consistent in position and size across all the lesioned animals. Using Paxinos Rat Atlas (Paxinos 1998) the areas affected were identified as the primary and secondary visual cortex, the parietal association cortex and the hindlimb area of the somatosensory cortex. However, only a small portion of each region was damaged. Despite damage to the visual cortex, the lesioned animals showed no signs of visual impairment and were able to discriminate between the two contexts during testing.

The reason behind the cortical damage was never discovered. The cortical damage was not present in sham animals, suggesting the problem was not the surgical procedure (i.e. not a drilling artefact or similar mechanical effect), but related to the lesioning itself. A possible explanation could have been impurities in the ibotenic acid, but as this was made up in several batches and each of these was used by other researchers in the laboratory without problems, this seems unlikely. Even when a more experienced colleague performed one of the lesion surgeries for me, the cortical damage was still evident. Thus the most likely cause of cortical damage was leakage of ibotenic acid into the cortex, (despite precautions that were taken to avoid it e.g. pausing after each injection to allow dispersion of toxin, cleaning needle between injections etc).

3.3.5. Post-surgery Performance

Initial deficit in performance of hippocampal lesion group on context dependent task (but not context independent task) post-surgery.

On completion of the pre-surgery training, the rats were given either hippocampal lesion surgery (n = 10) or sham surgery (n = 7), and left for 14 days to recover. They were then re-introduced to the context dependent and context independent tasks and their performance on the first 5 sessions of post-surgery testing is shown in Figure 3-7.

Overview

The context independent task was performed consistently well from the start of post-surgery testing. This confirmed that the rats did not have any problems with running, sniffing, discriminating odours or digging. However, the ceiling performance prevented the data from being used for parametric tests. Repeated measures ANOVAs were performed on the context dependent task data for the first 5 sessions of post-surgery testing. These revealed a significant effect of session ($F_{(4,60)} = 3.81$, $p = 0.008$) reflecting the gradual improvement in performance as the task progressed. A significant effect of group ($F_{(1,15)} = 26.01$, $p < 0.001$) indicated that the lesioned animals were performing significantly worse than the sham animals in the first 5 sessions of retesting. However, there was no session by group interaction ($F_{(4,60)} = 0.80$, $p = 0.530$)

Post-hoc simple effects (with Bonferroni correction) showed that the performance of the sham and lesioned groups were significantly different on days 1 to 3 (and on day 5 (day 1: $p < 0.001$, day 2: $p = 0.003$, day 3: $p = 0.027$, day 5: $p = 0.031$. No significant difference was observed on day 4 ($p = 0.332$) two groups differed

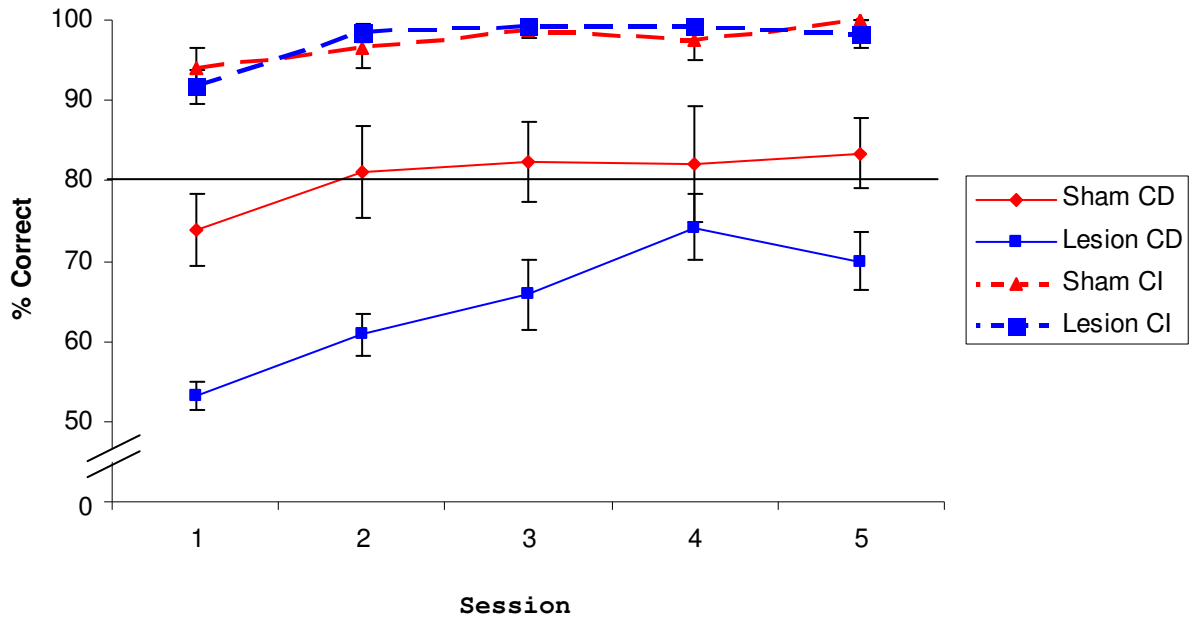


Figure 3-7: Average performance on sessions 1-5 of re-test expressed as % correct on each task. (sham n = 7, lesion n =10). All animals can immediately perform the context independent (CI) task well. However it takes 4 sessions for the average lesion performance to reach criterion level on the context-dependent task (CD), whereas the average sham performance re-attains criterion by the second session.

Animals with lesions take significantly longer than sham operated animals to regain criterion performance post-surgery.

Following on from the initial performance impairment seen in the lesion group, it was found that these animals also took longer to reach criterion level after surgery than sham animals – an average of 10 sessions for lesioned animals compared to 6 session for sham animals ($t_{(15)} = 2.13$, $p = 0.050$; see Figure 3-8)

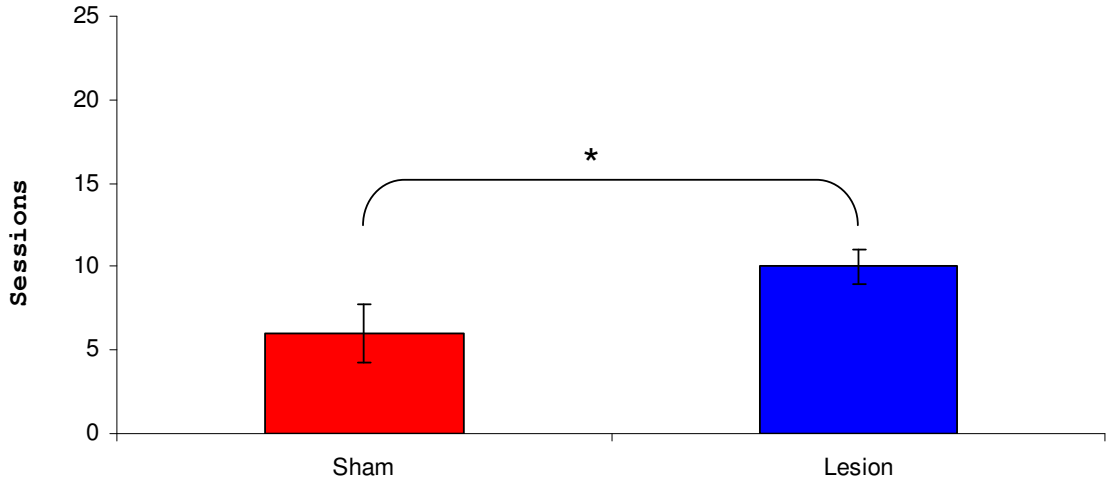


Figure 3-8: Average number of sessions taken to regain criterion on the original task after surgery. (sham n = 7, lesion n = 10)

Consistently high performance in both lesion and sham groups post-criterion

Once the animals have re-attained criterion performance on the context dependent task, both groups perform consistently well (see Figure 3-9). Probe tests confirmed the rats were using only the prescribed odours and not the odour of the reward to guide performance. In fact all rats scored 100% correct on their probe trials. Again a very high ceiling effect was observed for the context independent task. Similarly, an independent samples t-test on the context dependent task data revealed no effect of group ($t < 1$)

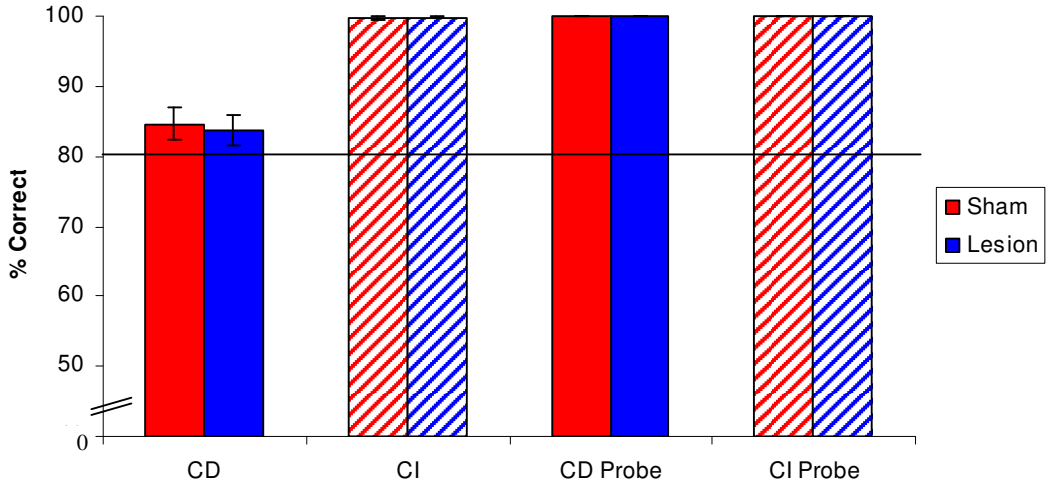


Figure 3-9: Average performance on the four post-surgery testing sessions after reaching criterion (expressed as % correct). CD = context dependent task, CI = context independent task. The rats received 12 context dependent and 12 context independent trials per session, as well as 1 context dependent probe and 1 context independent probe. (sham n = 7, lesion n = 10)

Animals take less training to regain criterion post-surgery than they do to learn the task pre-surgery

A repeated measures ANOVA on the pre and post surgery days to criterion data for the context dependent task revealed a significant effect of surgery ($F_{(1, 15)} = 114.20$, $p < 0.001$). This reflects the fact that the animals took much less time to re-acquire the context dependent task post-surgery than they did to learn it before surgery (Sham pre-surgery 64 $\pm$10 sessions, post-surgery 6$\pm$2 sessions; lesion pre-surgery 57$\pm$5 sessions, post-surgery 10$\pm$1 sessions; See Figure 3-10). However, there was no surgery by group interaction ($F_{(1,15)} = 1.33$, $p = 0.267$).

Post-hoc simple effects (with Bonferroni corrections) confirmed that both groups took significantly longer to attain criterion pre-surgery than they did post-surgery (Lesion $p < 0.001$, sham $p < 0.001$)

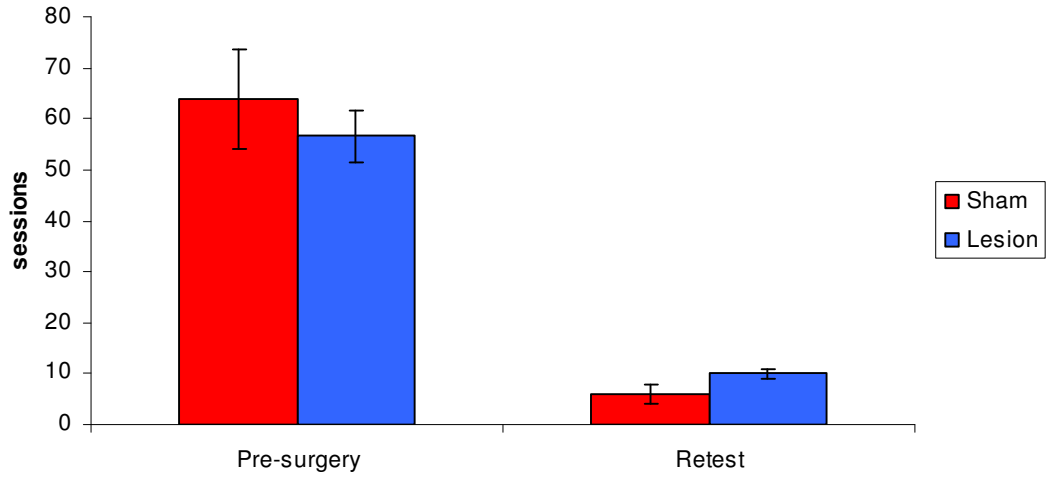


Figure 3-10: Average number of sessions taken to gain criterion on the context dependent task before and after surgery. (sham n = 7, lesion n = 10).

The animals perform just as well at the end of the post-surgery phase as they did at the end of the pre-surgery phase.

A repeated measures ANOVA on the pre and post surgery testing data for the context dependent task showed no effect of surgery ($F_{(1,15)} = 2.86$, $p = 0.111$) and no surgery by group interaction ($F_{(1,15)} = 0.114$, $p = 0.740$). This demonstrates that once the animals had re-attained criterion level, they could perform the task at the same consistently high level as they did before surgery (see Figure 3-11).

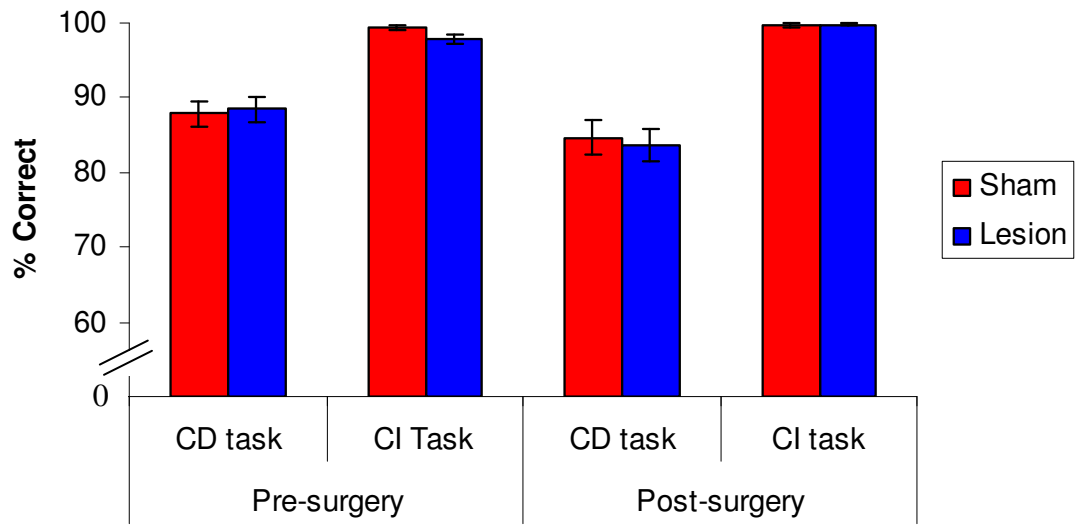


Figure 3-11: Comparing performance on context dependent and context independent task testing sessions, pre and post surgery. (sham n = 7, lesion n = 10)

3.3.6. Correlation between lesion size and performance

The spared hippocampal tissue varied in its extent, but was consistently at the temporal pole of the hippocampus and sometimes additionally but physically unconnected at the septal pole. As lesion size was variable some additional statistical examinations were carried out to see if this would have any bearing on behavioural performance. A Pearson's correlation confirmed that the average performance on the context dependent task for the first 5 sessions of post-surgery testing was related to the percentage of hippocampal sparing ($r(9) = 0.865$ $p = 0.001$), i.e. the more hippocampal tissue remained, the better the performance on the task (see Figure 3-12).

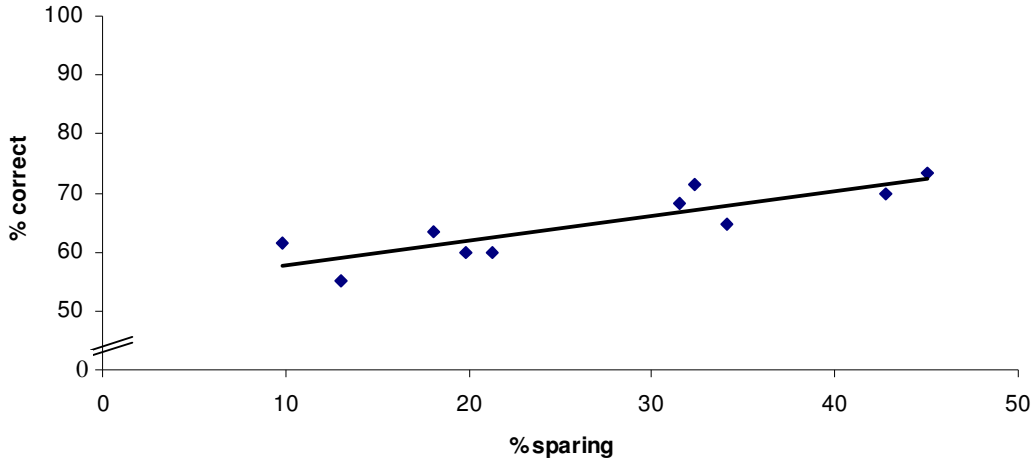


Figure 3-12: Correlation between % sparing and average % correct on over first 5 sessions of retest

Thus the residual hippocampus appears to be at least partially functional, and as the cortical damage is similar in all rats, this correlations appears to confirm that the hippocampus is indeed involved in the retention of this task.

Following on from this, correlations were performed on other aspects of the data. A Pearson's correlation showed that the average days to criterion for the context dependent task was almost correlated to lesion size ($r(9) = 0.612$ $p = 0.060$). Although not quite statistically significant, this result corresponds to the pattern shown above, i.e. the larger the volume of spared hippocampal tissue, the better the behavioural performance (in this case, the quicker criterion was regained on the context dependent task).

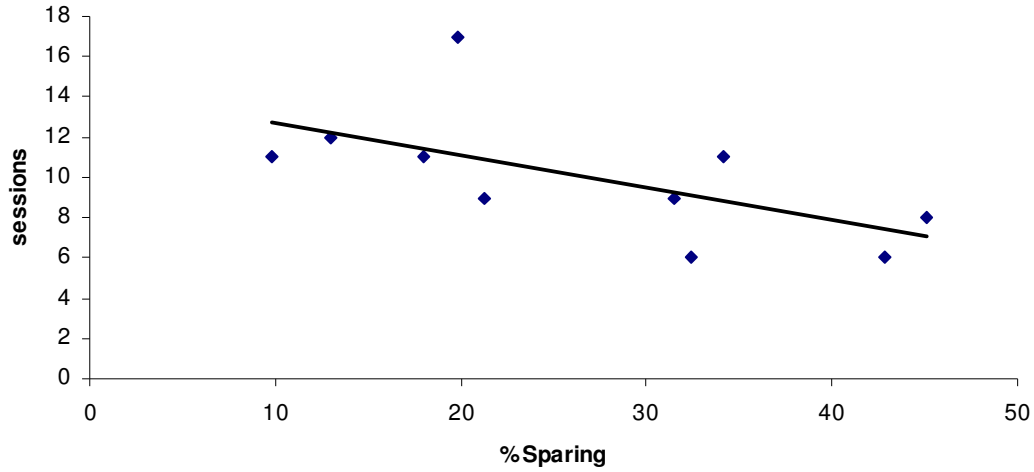


Figure 3-13: Correlation between % sparing and days to criterion for context dependent task post-surgery.

A final correlation was performed on the post-surgery testing data for the context dependent task. However, this time no correlation was found between performance and lesion size ($r(9) = 0.342$; $p = 0.333$). This demonstrates that although lesion size has a bearing on initial accuracy, once criterion level has been reattained; all animals are performing the task at the same consistently high level – regardless of the size of their hippocampal lesion.

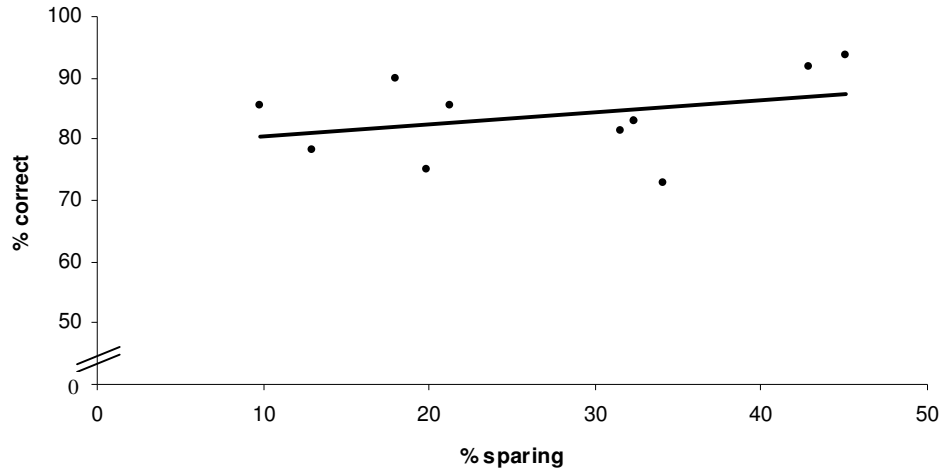


Figure 3-14: Correlation between % sparing and performance of context dependent task on post-surgery testing days.

3.4. DISCUSSION

The first main finding of this experiment is that rats with hippocampal lesions showed impaired performance on the first 5 days of post-surgery testing on a context-dependent odour discrimination problem compared to sham lesioned animals. The degree of this initial impairment was positively correlated with the volume of hippocampus removed. No impairment was observed on a concurrently run context-independent odour discrimination problem. As the animals were trained on both problems prior to surgery, these data suggest that the hippocampus plays a role in retention and/or retrieval of the context-dependent odour discrimination problem. The possible nature of this role will be discussed further below.

The second main finding was that, with continued training, the lesioned rats re-attained criterion levels of performance, after which they performed as well as shams on the context-dependent task. One interpretation of these data is that the lesioned animals learnt the context-dependent discrimination task as if it was a new task. This

would suggest that the hippocampus is required for retention of a previously acquired context-dependent odour discrimination, but that if the hippocampus is damaged, other structures can be used to acquire the task. Although this possibility cannot be completely ruled out, it seems unlikely considering that it took the lesioned animals only 10 sessions (average) to regain criterion as opposed to the 60 sessions (average) to learn the task initially. Nevertheless, further experiments are required to confirm this. An alternative possibility is that, having previously learned the task with an intact hippocampus, rapid relearning can occur, supported either by the spared hippocampal tissue, or by extra-hippocampal structures. Both of these possibilities will be considered further below.

3.4.1. Comparison to initial pilot data

The results in this chapter are very similar to the initial pilot data described in chapter two. Despite having only 3 rats in the lesion group, the pilot data nevertheless demonstrated both the initial post-surgery impairment in the context dependent task and the quick recovery back to criterion level, which was observed and statistically proven in this chapter. In addition, although the data has been pooled for statistical analysis, the data for this chapter was in fact collected in several smaller repetitions. Nevertheless, each batch of lesioned animals showed the same trend for initial post-surgery impairment in only the context dependent task, followed by complete recovery to criterion level. Performance on the context independent task was never adversely affected. Thus this task has been shown to be both robust and highly repeatable. Every lesioned animal demonstrated the same trends in behaviour following hippocampal lesion.

3.4.2. Discussion of functionality of spared hippocampal tissue

The volume of tissue spared after hippocampal lesions in this experiment varied in extent from 90% to 55% damaged tissue. Sparing was consistently at the temporal pole of the hippocampus and sometimes additionally at the septal pole (see Table 3-4 above). Ibotenic acid lesions spare the fibres of passage within the hippocampus, so it is highly likely that where there are large areas of sparing, the projections to and from the intact portion are maintained. Indeed, Moser and Moser (1995) have shown normal electrophysiological responses and acetyl-choline esterase distribution present in residual tissue after partial lesions. Furthermore, deHoz et al (2003) demonstrated with retrograde tracers, that the spared pole of the hippocampus maintains its normal pattern of inputs. Hence, where sparing was reasonably extensive, the residual hippocampus would probably have retained some connectivity – but what about functionality? Could residual tissue have had a behavioural relevance?

In 1998, Moser and Moser demonstrated that even with small amounts of hippocampal sparing, animals could still demonstrate retention of a watermaze task learnt pre-surgery. Furthermore, they observed a relationship between the volume of spared dorsal tissue and time spent in the platform zone during the probe trials, i.e.: more sparing led to improved performance. Similarly, in this experiment a correlation was found between lesion size and degree of initial impairment in performance. As the cortical damage is similar in all rats, these correlations confirm that the hippocampus is involved in the retention of this task and that residual hippocampal tissue in this experiment was at least partially functional. The larger the lesion the worse the initial performance (first 5 sessions) and the longer the animals took to regain criterion level.

On the other hand, the same study by Moser and Moser (1998) also demonstrated that rats with complete hippocampal lesions showed no preference for the target quadrant. Thus it is impossible to rule out the possibility that if the lesions had been

complete, the rats may have been more severely impaired, such that they were completely unable to solve the task. Nevertheless, this interpretation seems unlikely, as there was no significant relationship between the amount of spared tissue and the final level of performance. Furthermore, even the two rats with the largest volumes of sparing (10% and 13% sparing) were able to re-attain criterion and perform as well as controls, and as well as pre-surgery. Thus for performance to be maintained by spared tissue, we would have to propose that just 10% of hippocampal tissue is sufficient for this purpose.

3.4.3. Elemental and Configural solutions to the context dependent odour discrimination task.

The lesioned animals showed an initial post-surgery deficit in retention of this context dependent odour discrimination task, which suggests hippocampal involvement – but what role does the hippocampus actually play in this task? Accurate performance on the concurrently run context independent task demonstrates that the rats do not have a problem in determining odour identity or in retrieving odour-reward information. However, the impairment could be in storage or retrieval of the either context itself or of the configuration of context and odour.

The hippocampus has a special role in encoding configural associations between elemental stimuli (Rudy and Sutherland 1989). This region should therefore be essential for linking the 'odour' and 'context' stimuli in this context dependent odour discrimination task. However, since Rudy and Sutherland's original theory was published in 1989, it has been shown that not all nonlinear problems actually require the hippocampus. Indeed, although this context dependent odour discrimination task requires an association to be made between 'odour' and 'context', it can still be performed accurately when large portions of the hippocampus are absent.

Initial post-surgery impairment in lesioned animals

Although not critical for performance, a temporary deficit in performance was observed. Thus the hippocampus must play some role in supporting this context dependent odour discrimination in the intact brain. To this end the revised configural theory proposed by Rudy and Sutherland in 1995, may provide an explanation. They proposed that the hippocampus *contributes* to configural processing occurring in other areas of the brain. They postulate that conjunctive representations are actually constructed in non-hippocampal regions of the brain. If this is the case, then it may be these extra-hippocampal configural units that allow the lesioned rats to achieve accurate performance on the context dependent task. On the other hand, if configural units exist *outside* the hippocampus, how can the hippocampus 'contribute' towards their processing?

Rudy and Sutherland (1995) proposed that the role of the hippocampus is to increase learning rate by selectively enhancing the configural units and partially inhibiting elemental ones. According to this theory, during the pre-training phase of this task, both configural (context as a whole) and elemental (colour, texture etc) representations would have been created in the cortex. As the hippocampus was intact, enhancement signals would have ensured that behaviour was controlled by the configural units only. When the hippocampus was removed however, both elemental and configural units would have been released from their hippocampal influence. The incoming sensory stimuli would therefore have activated both the elemental and configural units equally. Without hippocampal mediation, no clear winner would emerge hence the performance of the lesioned animals was impaired. By contrast, the sham animals with their intact hippocampus would have retained the configural unit enhancement and therefore their accurate performance.

Rudy and Sutherland's theory (1995) also provides a theoretical background for the partial lesion results. The lesion correlation data demonstrated that the larger the lesion, the larger the initial (first 5 sessions) post-surgery performance impairment. If the role of the hippocampus is to send out signals to enhance configural encoding,

then the result of partial hippocampal lesions would be to reduce this output - not remove it altogether. Thus an intermediate situation arises; intact tissue sends out a partial enhancement / inhibition signal that is weaker than normal, but nevertheless strong enough to aid the associative strengthening of the configural unit. This partial signal is proportional to the quantity of hippocampal tissues spared after lesioning. Although this signal is not strong enough to fully support the use of only the configural unit, (or to completely inhibit the elemental one), it nonetheless assists the configural unit, thus an intermediate performance level is observed.

Re-attainment of criterion in lesioned animals

Despite their initial performance impairment on the context dependent odour discrimination task, the lesioned rats did re-attain criterion level performance. Above it has been suggested that (as the reacquisition was much faster than initial learning) the post-surgery the deficit in lesioned animals was due to impaired recall; however a relearning or reminding effect cannot be entirely ruled out.

As extra-hippocampal regions can support performance of this context dependent task, initial learning may have been taking place not just in the hippocampus but also in other structures. The cortex learns slowly by a trial and error process which extracts generalities about experiences. Conversely, the hippocampus has a much faster learning mechanism whereby co-occurring elements are extracted and encoded very rapidly. The long pre-surgery acquisition time may make this task more consistent with a cortical representation rather than a hippocampal representation.

Indeed, Rudy and Sutherland (1995) proposed that configural units actually reside outside the hippocampus – possibly in the cortex. They propose that the role of the hippocampus is to increase the rate of learning by enhancing configural encoding and inhibiting elemental encoding. However, this theory does not preclude the ability for accurate performance of a biconditional task in the absence of the hippocampus. Even without hippocampal enhancement, training and experience would still favour

the configural representation. Each time a trial was presented the appropriate configural unit would be strengthened. Conversely, the firing pattern of the individual elements would be unstable; sometimes gaining strength as the element was rewarded, but equally as often being involved in a non-reinforced situation. Thus new learning cannot be ruled out. However, given the timescale involved in reacquisition of criterion level performance, it seems more likely that existing configural representations were strengthened, rather than new ones created.

Similarly, if the second role of the hippocampal enhancement signal is to encourage the configural unit to gain associative strength, then a partial lesion should lead to an intermediate level of reacquisition. Indeed, in this experiment, a trend was observed for faster criterion re-attainment in animals with more spared hippocampal tissue. This again suggests that the enhancement signal generated by the hippocampus may be proportional to the volume of hippocampal tissue present (i.e. the larger the volume of hippocampal tissue spared, the larger the configural enhancement signal and the quicker criterion is regained).

Interim Conclusion

The data in this experiment support the hypothesis that configural units can exist in the cortex and that there are two ways in which they can be strengthened 1) by the hippocampus 2) by experience dependent changes. When the hippocampus is removed a temporary deficit is observed until sufficient experience has resulted in sufficient associative strength to support behaviour.

3.4.4. Contextual vs Configural

In this experiment, the variables have been presented as ‘context’ and ‘odour’. However, the term ‘context’ is a complicated variable. The task was designed so that each ‘context’ consisted of several elements (i.e. wall colour, floor colour, floor texture). Indeed, one hypothesised role for the hippocampus is in processing all

incoming stimuli and registering co-occurrences (Rudy and Robert 1995). In this way 'context' could be a configural stimuli in itself (i.e. the neural representation of a 'context' may encode for example black walls, smooth floor and black floor). However, these experimental results do not preclude the idea that all or some of the rats used a simpler or more elemental version of context. After the lesion, the hippocampal solution would no longer have been available, so a more elemental solution may have taken over. This switch between representations may take time and thus have been observed as a temporary impairment.

A complete association of all multi-modal stimuli was not necessary to solve this context dependent odour discrimination task. A neural representation consisting simply of 'black walls', would have been sufficient to discriminate the two contexts. Thus it could be argued that this task is not entirely contextual. Nevertheless, not-with-standing this potential simplification of 'context', the necessity for a configural representation is not so simply eliminated. A conjunction between 'black walls' and 'mint odour' would still have been required in order to solve the task. Thus, although it could be argued that this task was not strictly 'contextual', it was most definitely configural.

3.4.5. Multiple memory systems

In the paragraphs above, it has been suggested that regions of the brain other than the hippocampus may be able to encode configural stimuli. Indeed, what we call memory is actually the product of a number of separate but interacting systems, each of which has different rules and processes. However, it is very difficult (if not impossible) to devise a task that engages only one system. When a lesion inactivates one system, the detrimental effects of the lesion may be masked by the functioning of a secondary or parallel system. It is possible that something similar to this may be occurring in this task. Although the hippocampus is normally used in the intact brain, a secondary or parallel non-hippocampal system can take over and support normal

looking behaviour its absence. This secondary system may be cortical or may reside in areas like the amygdala which is thought to process reward associations, the perirhinal cortex which processes objects, or the entorhinal cortex which holds some spatial and path integration information. However, even if a second system can support accurate performance on this task, an initial impairment was still observed. Thus either the second system required time to establish links to extra-hippocampal representations made prior to surgery, or it learnt the task as it would a new one. Nevertheless, the new learning proposition seems unlikely due to the high speed of reacquisition observed post-surgery for the lesioned animals. If new learning was taking place, it would have to be proposed that this second system was more efficient than the original hippocampal system. Thus it seems more likely that the secondary system established control using existing representations.

(The concept of competing systems will be explored further in chapter 4 and a discussion of alternative areas for representations can be found in the General Discussion – Chapter 7).

3.5. Conclusions and further questions

The hippocampus *contributes* to the retention of context-dependent odour discriminations. Initial performance deficits in lesioned animals correlated positively to lesion size and a trend towards increased task recovery time was observed. Nevertheless, with experience all animals performed the context dependent task reliably and accurately, demonstrating that an intact hippocampus was not *necessary* for performance of this task.

As mentioned above, this context dependent odour discrimination task could be solved using either a multi-modal representation of context or a simple elemental

representation of one aspect of context. Although the data in this chapter show that the lesioned animals can reattain a criterion level performance on the context dependent task in the absence of an intact hippocampus, it does not show if the lesion has had any effect on the technique the animals used to solve the task. Thus, in the absence of the hippocampus a secondary or parallel system may have taken over. Eichenbaum (1994) suggested that even if a representation is being successfully used to solve a problem, it could still be highly abnormal. However, these abnormalities may not be revealed until the flexibility of the representation is challenged. To this end, chapter 4 aims to use cue manipulations to investigate the strategies the rats are using to solve the task.

In this chapter, after surgery lesioned animals re-attained a criterion level of performance very rapidly despite an initial impairment. As the speed of learning was much steeper than that observed prior to surgery, I would hypothesise that what was observed was merely a reconfiguration of existing connections and not the formation of new associations. However, the possibility of completely new learning cannot be fully ruled out. For this reason, further investigation into post-lesion learning is addressed in Chapter 5, where rats are challenged to learn two sets of new odour discriminations – in a familiar environment and one in a new environment. This will test their abilities for new learning post-surgery and allow for a comparison with the data found in this chapter.

4. Effect of hippocampal lesions on task strategy to solve a context dependent odour discrimination task – a study using cue manipulations.

4.1. Introduction

The previous chapters showed that the hippocampus played a role in the retention of the context dependent odour discrimination task, but was not essential for performance. Furthermore, the successful performance of the concurrently run context independent task by lesioned animals suggests that the hippocampus does not play a role in determining odour identity, or in retrieving odour-reward associations. Thus, it is likely that the hippocampus contributes to either:

a) a representation of the context;

b) to combining context information with information about the odour cues;

c) to retrieving the reward associations for particular context-odour combination.

The aim of this chapter is to determine the effects of hippocampal lesions on how rats encode their environment, and how they define which context they are in. The contexts used in the previous experiments were distinguishable by intra-maze cues (colour and texture). However they could also have been defined by their absolute or relative positions in the environment (i.e. one context to the left of connecting stem, and the other context to the right).The fact that the animals were running in different directions from the bridge to access the two platforms may have emphasized the spatial differences. It is possible therefore that the animals could have used self motion or directional information (running east versus west) to solve the task. Indeed head direction cells (found in extra-hippocampal areas such as the dorsal presubiculum, anterior thalamic nuclei, lateral dorsal thalamus, striatum and

retrosplenial cortex;) encode just such directional information (Etienne and Jeffery 2004). Similarly, the Grid Cells of the medical entorhinal cortex (Hafting and Fyhn et al. 2005) are ideal for path-integration (McNaughton and Battaglia et al. 2006). Both of these may still be available to hippocampal lesioned animals.

It is possible that sham and lesioned animals may have been using different sets of cues. As they lacked their hippocampus, it is predicted that lesioned animals could not use traditional spatial information (as encoded by place cells), but may still have had some ability to use directional information. Controls animals on the other hand may have been using a combination of intra-maze and spatial cues. In order to test these predictions, a set of manipulations were designed for this context dependent odour discrimination task:

- The first manipulation removed self motion cues.

  If removal of the hippocampus eliminated the lesioned rats ability to use spatial information, it is predicted that they will be unaffected by this manipulation. If on the other hand, non-hippocampal directional information was sufficient to support performance, then this manipulation may impair performance in the hippocampal lesioned animals.

  Intact animals have both spatial and directional information available to them. However, it is unknown how much the accurate performance of the sham animals depends upon each them – and whether impairments will result at their removal.

- The second manipulation randomised the location of the two contexts.
  This put intra-maze cues into conflict with directional and self-motion cues.

  It is predicted that lesioned animals will be unimpaired due to their lack of integration in spatial information.

  Sham animals on the other hand may be impaired as spatial cues and intra-maze cues are in conflict and the spatial cues can no longer be used to differentiate between contexts.

- The third manipulation both removes self-motion cues and randomises the locations of the two environments.

  This should remove the conflict. Therefore both groups should be able to use the intra-maze cue information to accurately guide their behaviour.

(Distal cues were minimized by thick black curtains, so for the purposes of this chapter are taken to have had minimal influence on behaviour).

4.1.1. Aims

The aim of this series of manipulations was to assess the relative contributions of:

a) self-motion cues

b) intra-maze cues

to the performance of the context dependent odour discrimination task, and how this may differ between sham operated and hippocampal lesioned animals.

4.2. Methods

This chapter follows on directly from the previous chapter and the same animals were used. Thus the animals involved had already completed all of the previous protocols (pre-surgery training, lesion / sham surgery, post-surgery testing).

This chapter describes the manipulations that were performed in order to investigate differences in strategies between the sham and lesion groups.

4.2.1. Subjects and Housing

The subjects were the same 17 Male Lister-hooded rats that had completed the protocols in chapter 3 who had received either sham surgery (n = 7) or bilateral ibotenic acid hippocampal lesions (n = 10). They were individually housed, on a 12h light/dark cycle with ad libitum access to water, and slight food restriction (For further details see chapter 2)

All procedures were carried out in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986 and European Communities Council Directive of 24 November 1986 [86/609/EEC], and all efforts were made to minimise suffering.

4.2.2. Apparatus

The manipulations used the same apparatus as described in chapter 3. This consisted of two structurally identical circular (76cm diameter) black wooden platforms. Clear polycarbonate walls (40cm high) with fabric behind them (black or white) allowed the platforms to be defined as separate contexts. Four evenly spaced clear polycarbonate sliding doors were also present in the walls. (Photographs of the

apparatus are shown in chapter 3). The whole apparatus sat approx 75 cm above floor level on a frame.

Odours were presented in clear nalgene pots of sand (150g) scented with household herbs (0.8g basil or mint). Buried chocolate wheetos (Nestle) were used as the food reward.

4.2.3. Behavioural Protocol

General Protocol

As previously, Testing took place on weekdays (Mon-Fri) between 8.30am and 8.30pm, and the rat was transferred to and from the apparatus in a cylindrical container.

The protocol for the manipulations is detailed in Table 4-1 and a dynamic representation is available as a PowerPoint file on the CD in the appendix of this thesis. Only the context-dependent task was run and the rules remained the same as previously; in the white context, odour A is rewarded, but odour B is not. In the black context, odour B is rewarded, but odour A is not. The order of the rewarded and non-rewarded trials was pseudo-randomised.

Placement Manipulation to remove self-motion cues

The placement manipulation was designed to determine whether the rats were using self-motion cues to solve the task. Testing involved 16 trials per session (4 each of A^+, B^-, A^-, B^+; 2 trials at each of the 8 pot locations)

a) **8 'standard run' trials (4 rewarded, 4 non-rewarded).** These followed the normal protocol, whereby the rat ran off the bridge into the context and responded to the odour of the pot. Between trials the rat resided on the bridge between the two barriers.

followed by

b) **8 'placement' trials (4 rewarded, 4 non-rewarded).** For these trials the bridge was removed and the polycarbonate door replaced, making an entirely circular environment. The rat was then physically placed by the experimenter into the centre of the context, allowed to respond to the pot as normal, then lifted out of the context again. Between trials, the rat resided in the transferral container (blue bucket) and the experimenter mildly disorientated the rat by walking slowly around the platforms and rotating the blue bucket.

Testing lasted for 8 sessions and results were combined and averaged across sessions.

Retrain

2 sessions of standard testing (16 'running' trials: 4 each of A^+, B^-, A^-, B^+ in randomised order) were given immediately following the placement manipulation, to reaffirm the standard rules and procedures of the task before the next set of manipulations began. This reaffirmation was continued for a period longer than two sessions in the rare cases where the animals had not performed at, or above 80% correct during this time.

Context swap manipulation to put intra-maze cues in conflict with self-motion cues

These were used to assess the impact on performance of putting intra-maze cues in conflict with self-motion cues. Up until this point, the white platform had always appeared on the left-hand side and the black platform on the right-hand side of the testing arena (standard configuration). During this set of trials, however, platform position was randomised, with the white platform occuring as often on the right-hand side, as it did on the left (and vice versa for the black). Rats received 16 trials per session (4 each of A^+, B^-, A^-, B^+):

a) **8 standard configuration trials (4 rewarded, 4 non-rewarded).**

b) **8 swapped configuration trials (4 rewarded, 4 non-rewarded).**

These trials were randomly intermixed and rats were rewarded according to the intra-maze cues (i.e. black / white) and not the position of the platform in the room. The rats were tested for 8 sessions, and results collapsed across sessions.

Retrain

Again, 2 sessions (+) of reaffirmation of the standard protocol (16 running standard configuration trials per session: 4 each of A^+, B^-, A^-, B^+) were given, before the next manipulation was begun.

Combination Manipulation to assess resolution of cue conflict by removal of self-motion cues

These trials were a combination of the above two trial types (placement and context swap). They were used to determine whether the conflict really was between self-motion and intra-maze cues or if some unidentified factor was playing a role. If the conflict was as expected between self-motion and intra-maze cues, resolution of performance should be observed when animals are placed into the context swapped condition (i.e. removal of the self-motion cue while retaining intra-maze context swap). At this stage the rats received 16 counterbalanced but randomized trials per session (4 each of A^+, B^-, A^-, B^+):

a) **8 'run' trials** 4 in the standard configuration
4 in the swapped configuration

followed by

b) **8 'placement' trials** 4 in the standard configuration
4 in the swapped configuration

These trials were run for 8 sessions, and results collapsed across sessions for analysis. The context swapping and placement were done as described above. The order of the rewarded and non-rewarded trials was randomised.

Table 4-1: Protocol for manipulations

8 Sessions	2 sessions	8 Sessions	2 Sessions	8 Sessions
Placement Manipulation Context Dependent Task only 8 Standard Run Trials (4 rewarded, 4 non-rewarded) then 8 Placement Trials (4 rewarded, 4 non-rewarded)	Context Dependent Task only 12 Standard Run Trials (6 rewarded, 6 non-rewarded)	Context swap Manipulation Context Dependent Task only 8 Standard Run Trials (4 rewarded, 4 non-rewarded) then 8 Context swap Trials **(4 dig, 4 non-dig)**	Context Dependent Task only 12 Standard Run Trials (6 rewarded, 6 non-rewarded)	Combination Manipulation Context Dependent Task only 4 Standard Run Trials 4 Context swapped Run Trials then 4 Standard Placement Trials 4 Context swap Placement Trials All counterbalanced for rewarded / non-rewarded

4.2.4. Histology

Rats carried on to do further training / testing as described in the rest of this thesis, thus histology was not done until all experiments were completed. However, the method and results of the histology were described in chapter 3.

Rats were euthanized and perfused intra-cardially with 4% formalin. The brain was then removed, fixed in formalin and embedded in egg yolk before being sliced into 30µm coronal sections using a cryostat. Every fifth section was mounted onto gelatine coated slides and stained with cresyl violet acetate. The extent of the lesion was assessed and the area of hippocampal sparing calculated.

4.2.5. Statistical Analysis

Statistical analysis was performed on SPSS for windows version 12.0. All numerical values and graphs are reported as $\pm$ SEM.

The data was examined using repeated measures ANOVAs, with simple effects comparisons with Bonferroni corrections where as appropriate. Homogeneity of covariance was tested for using a Mauchly sphericity test.

Post hoc independent samples t-tests were used to compare groups

Pearson's correlations were used to investigate the relationship between lesion size and behaviour.

4.3. Results

These trials were run to establish what cues each of the groups were using to solve the task, and if there were any differences in methodology between groups.

The images shown under the graphs indicate the orientation of the platforms e.g.  indicates that the white context was on the left and the black on the right.

4.3.1. Performance on Placement Manipulation

Removing self-motion cues did not impair performance.

Repeated measures ANOVA showed that placing the rat into the contexts instead of letting them run did not cause an impairment in performance (No effect of placement $F_{(1,15)} = 3.50$, $p = 0.081$; see Figure 4-1). In addition, there was no effect of group ($F_{(1,15)} = 2.01$, $p = 0.177$) and no group by placement interaction ($F_{(1,15)} = 0.16$, $p = 0.696$). The animals can still perform task when placed into context instead of running in (i.e. ideothetic cues are not crucial for differentiating between the two contexts).

The slight dip in overall performance (compared to the previous chapters) is probably related the change of protocol. The rats received a lot of additional handling by the experimenter during the placement manipulation (i.e. they were lifted into and out of the contexts) and spent a period of time between each trial in the transfer container. Furthermore, upon exiting the transfer container the rats would ordinarily (in the rest of this thesis) have found themselves to be on the connecting bridge, not inside a context as occurred during this manipulation. Thus, although the rats were accustomed to both human contact and to the transfer container, the deviation in protocol may still have affected performance.

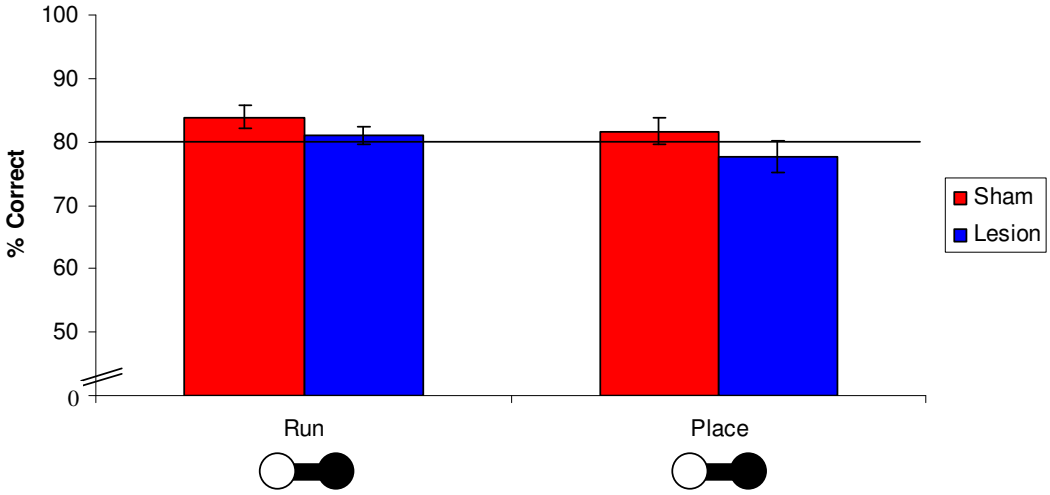


Figure 4-1: Average performance on placement manipulation. During Run trials, the rats ran into the context from the bridge. For the place trials, rats were manually placed into the centre of the context, hence self-motion cues were unavailable. (sham n = 7, lesion n = 10)

No correlation was observed between lesion size and performance on placement manipulation.

Following on from the correlations between performance and lesion size observed in chapter 3, Pearson's Correlations were performed on the place manipulation data. However, no significant correlation was observed between lesion size and performance on either the standard ($r(9) = 0.23$, $p = 0.516$) or swapped ($r(9) = 0.110$, $p = 0.763$) placement trials. This may not be entirely surprising as the lesioned animals are performing at criterion level throughout this task.

4.3.2. Performance on context swap manipulation

Sham animals were impaired relative to hippocampal lesioned animals on the context swap condition

In this task the contexts occurred randomly on both the left and right, so that self motion cues would be put into conflict with intra-maze cues. However, the reward status of the odours continued to be related to intra-maze cues.

An overall ANOVA shows a main effect of context swap ($F_{(1,15)} = 46.46$, $p = <0.001$) and of group ($F_{(1,15)} = 30.27$, $p < 0.001$) and a context swap by group interaction ($F_{(1,15)} = 7.88$, $p = 0.013$).

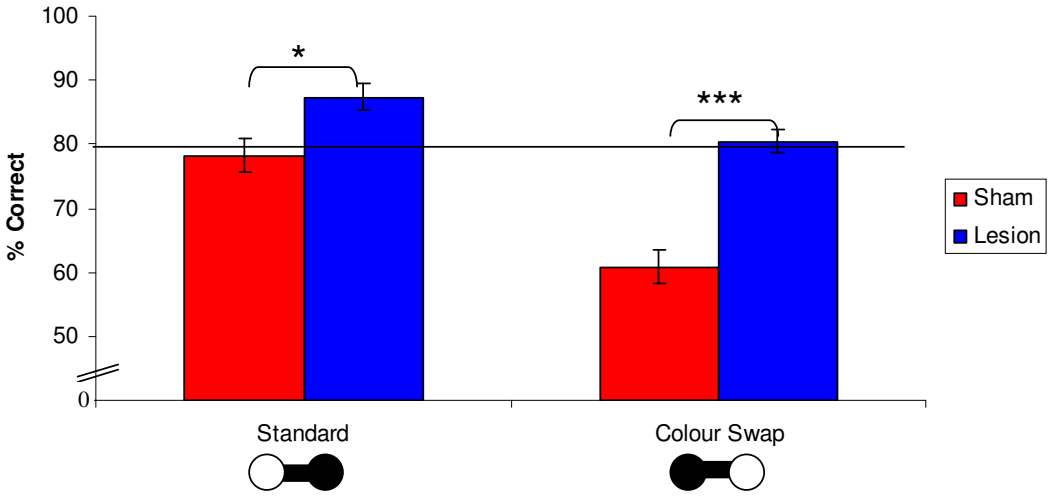


Figure 4-2: Average performance on context swap manipulation. The standard configuration is white on the left, black on the right. The context swap configuration is black on the left, white on the right. (sham n = 7, lesion n = 10)

Sham animals were impaired relative to hippocampal lesioned animals on the context swap condition

The main effect of task reflects the fact that all groups perform worse on the context swapped condition than the standard condition. Indeed, simple effects analysis confirmed that performance on the two parts of the task was significantly different for both experimental groups (Lesion $p = 0.007$, sham $p < 0.001$). Nevertheless, a task by group interaction was also observed. Further post-hoc simple effects showed there was a significant difference between the group in both configurations (standard $p = 0.012$, swapped $p<0.001$). In spite of this, one striking result stands out - lesioned animals performed at or above criterion on both tasks. Sham animals by contrast, performed at around criterion for the standard trials, but were below criterion on the context swapped condition. Thus, although impaired on both trial types, the sham group was particularly impaired on the context swap trials.

No correlation was observed between lesion size and performance on context swap manipulation.

Pearson's Correlations were performed on the context manipulation data. However, no significant correlation was observed between lesion size and performance on either the standard ($r(9)= 0.39$, $p = 0.256$) or swapped ($r (9)= 0.187$, $p = 0.606$) context trials. This may not be entirely surprising as the lesioned animals are performing at (or above) criterion throughout this task.

4.3.3. Performance on Combined Placement and Context swap Manipulation

Sham animals were impaired on the context swap condition when they 'ran' into the context. Performance was restored if the animal was 'placed' into the context swapped context.

The overall ANOVA revealed a significant effect of group ($F_{(1,15)} = 13.12$, $p = 0.003$), a placement by context swap interaction ($F_{(1,15)} = 15.23$, $p < 0.001$), and a placement by context swap by group interaction ($F_{(1,15)} = 4.17$, $p = 0.029$). There was also a significant effect of placement when comparing the sham and lesion groups ($F_{(1,15)} = 5.39$, $p = 0.003$). *(All other factors were non-significant)*

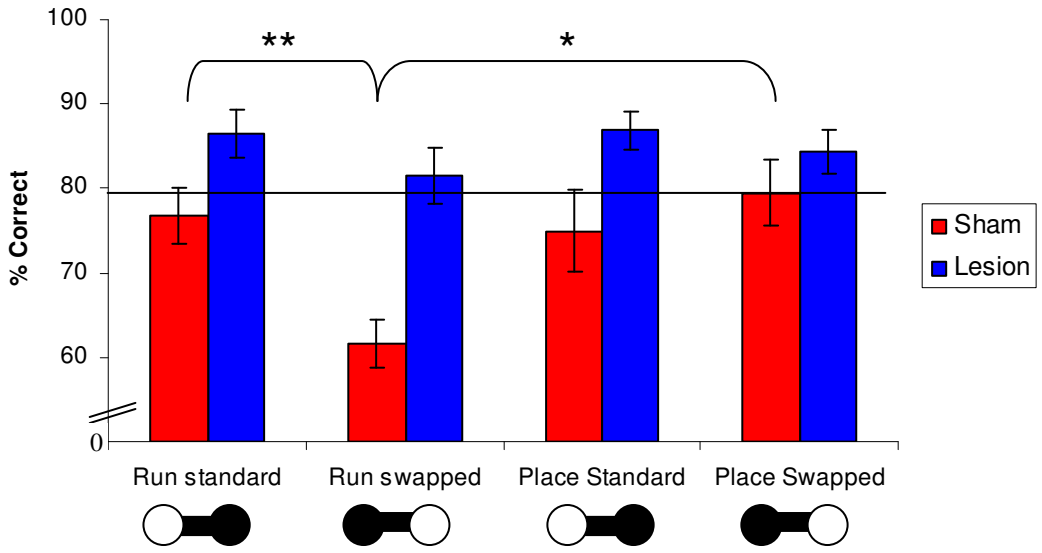


Figure 4-3: Average performance on the combined manipulation. The standard configuration is white on the left, black on the right. The swapped configuration is black on the left, white on the right. (sham n = 7, lesion n = 10)

Replication of results seen in manipulation 1 (placement)

The first analysis demonstrated that the results of manipulation one (removal of self-motion cues) was repeated. The simple effects analysis (with Bonferroni corrections) showed no change in accuracy for either lesioned animals ($p = 0.885$) or sham animals ($p = 0.607$) when they were placed into the contexts instead of being allowed to run in themselves.

Replication of results seen in manipulation 2 (context swap)

The second analysis aimed to see if the results of manipulation two were repeated. Again, it was shown that lesioned animals showed no changed in performance between the standard arrangement and the swapped one ($p = 0.051$). Conversely, the performance of the sham animals was badly affected by the conflict between self-motion and intra-maze cues ($p < 0.001$)

Thus both the earlier manipulations are both robust and repeatable. Furthermore, behaviour has not changed as result of experience on these tasks.

Sham animals were impaired on the context swap condition when they 'ran' into the context. Performance was restored if the animal was 'placed' into the context swapped context.

In this third manipulation, the aim was to investigate the interactions that occur between self-motion cues and intra-maze cues. Indeed, in addition to repeating the finding of the other manipulations, this set of experiments showed that placing the rat into the swapped arrangement (i.e. context swapped but NO self-motion cues) could rescue the performance of the sham animals back towards criterion level. Simple effects analysis (with Bonferroni corrections) confirmed that no difference in accuracy was observed between the standard and swapped arrangements if the rats were placed into the contexts (lesion $p = 0.546$, sham $p = 0.375$). However, a difference was observed for the sham animals when they 'ran' into the configurations (Lesion $p = 0.051$, sham $p < 0.001$)

Indeed in the standard arrangement neither the sham nor the lesioned animal showed any change in performance between the running and placement versions of the task (lesion $p = 0.885$, sham $p = 0.607$). However, in the swapped arrangement, although the lesioned animals still showed no change ($p = 0.444$), the sham animals perform significantly differently when they are placed into the context instead of being able to run in themselves ($p = 0.001$)

Simple effects analysis was also carried out to compare the groups on each task. The lesion and sham animals actually showed a significant difference in accuracy on almost all of manipulations (Run into standard configuration $p = 0.043$, Run into swapped configuration $p = 0.001$, Place into standard configuration $p = 0.027$). However, notably, the groups did not differ in performance when placed into the swapped configuration. Thus, the idea of a 'rescue' in performance when intra-maze cues and self-motion cues are in conflict is upheld. The difference between groups for the other measures may reflect a general level of confusion for the sham animals. Lesioned animals performed at or above criterion on all tasks. Sham animals by contrast, performed at around criterion for the standard arrangement trials and the combined context swap and placement trials, but were below criterion on the running context swapped condition. Thus, although impaired on both trial types, the sham group was particularly impaired on the context swap trials.

4.4. Discussion

In the face of manipulations to the context dependent odour discrimination task, hippocampal lesioned animals actually performed better than sham animals. Lesioned animals maintained above criterion performance throughout all manipulations. Sham animals on the other hand, performed the task accurately whenever they were placed into the contexts, but displayed a performance deficit when moving independently between contexts whose relative positions had been manipulated. Nevertheless, recovery of performance (irrespective of platform configuration) occurred when the sham rats were moved passively by the experimenter into the contexts.

These data suggest that at least in some way, the information used by the sham and lesion rats to solve this task is not equivalent. The experiments were designed to remove either self-motion cues (placement) or to put self-motion cues into conflict with intra-maze cues (context swap), or to remove the conflict (placement and context swap). The data suggest that sham animals use a combination of self-motion and intra-maze cues, such that when the two came into conflict with each other, performance was impaired. Lesioned animals on the other hand depended more on intra-maze cues, so performed consistently well throughout all manipulations.

4.4.1. Multiple Memory Systems

Within the brain there are several parallel or competing systems active all the time (White and McDonald 2002). When the activity in one system is reduced or removed, another system may take over to maintain behavioural patterns. Thus, although accurate performance was possible post-surgery (see chapter 3), this does not mean that the hippocampus is not normally involved in memory for this context

dependent odour discrimination task. Instead, another system may have taken over and masked the impairment effect of the lesion.

Nevertheless, it is possible to probe task performance in order to reveal the difference in processing occurring between the primary and secondary system. Eichenbaum (1994) suggested that to disproportionately impair the hippocampal lesioned animals, tasks should selectively emphasise comparisons between items. Conversely, preserved learning should occur when separate representations of stimuli are important. Indeed in their 1988 paper Eichenbaum et al demonstrated that fornix lesioned animals were impaired at learning a successive go left / go right odour discrimination, which emphasised comparisons. Conversely, on a successive go/no-go task that emphasised the significance of individual stimuli, fornix lesioned animals actually learnt quicker than sham animals. Thus, the absence of a relational strategy that compares outcomes across trials may actually have conferred an advantage to these lesioned animals. Similarly, as the context dependent odour discrimination task used here is a go/no go task that can be solved using simple representations of context, perhaps employing a relational strategy was equally as disadvantageous to these sham operated animals.

Conversely, other situations exist where the ability to encode complex or indirect relationships between stimuli is vital. In the watermaze for example, complex representations allow normal animals to navigate to the platform even from novel starting locations (Eichenbaum et al 1990). On the other hand, it has been demonstrated that animals with hippocampal lesions show an abnormal dependence on cues within a direct line view of the platform to guide their behaviour. They are unable to use the cues flexibly in order to navigate to the platform from a novel starting position (Eichenbaum et al 1990). Thus intact and lesioned animals encode and use cues quite differently. Furthermore, if animals in the watermaze used only directly related cues, it seems unlikely that the lesioned animals in this chapter will have paid any attention to background cues that had no predictive value. Thus it may not be surprising that changing these parameters did not affect their performance.

Eichenbaum, Matthews and Cohen (1989) showed that sham operated animals could use inferential assumptions to solve a problem where familiar odour stimuli were recombined into novel pairs. Fornix lesioned rats were impaired at this task, behaving at chance level as if the task was completely new. Eichenbaum, Matthews and Cohen (1989) suggested that a relational representation allowed the sham animals to encode all the stimuli into an 'organised scheme'. This permitted comparison between stimuli that had not previously been experienced together. On the other hand, the representation system of fornix lesioned animals encoded the two stimuli into one configuration. This type of representation could only support performance when the stimuli appeared in the same way as it had done in training. Single elements could not be interpreted individually. The manipulations in this chapter would only have affected animals that had stored the stimuli representations within a larger network; alongside the features of the environment (i.e. the sham operated animals). This detailed representation would have allowed them to compare present sensory data to the stored expectation and to compute the differences. If the changes were interpreted as discordant, the task may have been interpreted as different and an impairment in behaviour observed. Indeed, if the sham animals could not reconcile the changes in the context swap manipulation to their internal representation of environment, it is not surprising that performance impairments were observed. Conversely, when a set of independent representations were used for encoding context, as long as each cue was present somewhere in the apparatus, the change in their organisation would not recognised. Thus the animal would continue with the task as normal and no impairment would be observed (as was seen in the lesioned animals). In some tasks, this kind of unchanging behaviour may have been disadvantage. However, in this set of manipulations it may have helped the lesioned animals maintain accurate performance. Lesioned animals don't deal with competing objectives. Their encoding is more straight forward, and unlikely to include details that are irrelevant to reward (like the relative locations of each platform). Thus these animals would not even recognise that a change has occurred, and a modification in behaviour would *not* be expected.

Overall, this combination of data and literature review shows that even when learnt performance is apparently normal, animals with hippocampal lesions may have a different representation of the task that only becomes apparent that in novel situations (Eichenbaum 1994).

4.4.2. Lesion

The hippocampal lesioned animals in this experiment actually maintained criterion level performance throughout all manipulations. This proves that the lesioned animals were not suffering from any kind of generalised impairment. Furthermore, it suggests that, although all animals (intact and lesioned) can perform the original context-dependent odour discrimination task post-surgery, they may not be processing the stimuli in the same way.

Other investigators have demonstrated that hippocampal damage can lead to performance facilitation. Eichenbaum et al (1986) trained rats post-surgery on a 2 odour discrimination task which required a go/no-go response. Although both the lesion and sham animals acquired the task at the same rate, the lesioned rats acquired the reversal much more rapidly than sham animals (see also Zola & Mahut, 1973 for data in monkeys). Thus, just as in chapter 3, the fact that both groups learnt to perform the discrimination task accurately, did not mean they were using the same kind of memory representation. When manipulations taxed the representations, they turned out to be qualitatively different. By way of an explanation for this facilitation, Eichenbaum et al (1986) suggested that during reversal training, control animals experienced a conflict between their present lack of reinforcement and their former association of that odour to reward. As a reversal has taken place, the relationship between the old and new reward statuses are incompatible and confusion / deficits in performance result. Conversely, the fornix lesioned animals do not experience this conflict and simply learnt the reversal as a new set of problems. Without the need to extinguish an old memory, learning occurs much faster. Similarly, Means, Walker

and Isaacson (1970) demonstrated that rats with hippocampal system damage learn a go/no-go single alternation task much quicker than controls. Thus in situations where comparisons with previous reward status can be unhelpful, loss of hippocampal tissue may actually confer an advantage. In this chapter, instead of a conflict between past and present reinforcement attributes, the sham operated animals may have experienced a conflict between past configurations of cues and the present structure of their environment. Lesion animals on the other hand may have held a more simplistic representation of the two contexts, which lacked information regarding their relative relationship. Thus changing these un-encoded aspects had no effect on behaviour. On the whole, the representations available to normal and lesioned rats may be differentially advantageous depending upon the varying task demands (Eichenbaum et al 1988). In this particular set of manipulations, the representation held by the lesioned rats was superior.

4.4.3. Incidental versus explicit encoding

The lack of deficit in the hippocampal lesioned animals could be related to the fact that these tasks were learnt prior to surgery and after many repetitions. This may have allowed the animals to encode a fixed representation of the context dependent odour discrimination task which was (in the absence of the hippocampus) impervious to additional modifications. The revised configural learning theory of Rudy and Sutherland (1995) suggests that the brain encodes stimuli both as elements and as configurations. This next section looks at how using each of these could have ensured accurate performance for the lesioned animals in this set of manipulations.

In terms of fear conditioning, Phillips and Ledoux (1994) showed that if contextual cues were seen as background cues (i.e. another CS was more predictive of shock), then animals with dorsal hippocampal lesions would not encode them and hence would display a context fear impairment. By contrast, if the explicit CS was removed, the context became the only stimuli that could be associated with the

shock. Under these conditions, context became a foreground cue and lesioned animals encoded this just as well as intact animals. Good et al (1998) also discussed the fact that if context was incidentally encoded (as a non-vital background) then hippocampal lesioned animals would not recognise when it changed and thus would not show contextual control of responding. On the other hand, if contextual stimuli were explicitly encoded as vital for determining the reward properties, then animals with hippocampal lesions showed no deficit in performance. Thus, it may not be whether the stimuli are contextual or not that determines whether lesioned animals will encode them. It may depend more on how vital contextual stimuli are to resolving the task in hand. The animals in this experiment received a large amount of pre-surgery training. During this time, simple extra-hippocampal associations could have occurred in the cortex (Rudy and Sutherland 1989). When the hippocampus was removed, these links may have been sufficient to allow accurate performance to be regained. However, these extra-hippocampal links would only have been made between the most salient foreground features (e.g. colour) and the reward status of the odour. Links with more peripheral features (like motion cues) are unlikely to have been encoded (Good et al 1998). Thus during post-surgery testing, although the lesioned animals showed accurate performance, they were actually reacting in a reasonably simplistic way. As a result, during these manipulations, their performance was found to be impermeable to influence from background features considered less salient or important during pre-surgery training. Hence changing the relative positions of the platforms had no effect on their performance.

An alternative explanation is that during pre-surgery training, configural units may have formed in the cortex (Rudy and Sutherland 1995).These would have allowed many aspects of the task to be encoded together as one unit. Thus the animal could react accurately when the stimuli are presented in exactly the same way as during training, but were unable to use the stimuli flexibly (Eichenbaum, Matthews and Cohen 1989). Consequently, when the stimuli are manipulated, the influence on behaviour would depend upon how important or relevant the manipulated feature was perceived to be and whether this particular feature had been encoded explicitly

in the original pre-surgery configural representations of the task. In this particular set of manipulations, it would be presumed that the relative context positions were not perceived as important enough to be included within the original configuration. Thus, changes in this parameter had no effect on behaviour.

Nevertheless, if the cortical representations are sufficient to control behaviour, and stable enough not to be affected by other influences, why is there a deficit in the intact animals? The answer may lie in the automatic encoding of relationships in the hippocampus. The cortex is a relatively stable, slow changing system. The hippocampus by contrast, is a lot more adaptable. The hippocampus receives inputs from adjacent structures regarding self-motion and orientation, as well as strong visual and geometric inputs. It attempts to rapidly integrate information from several different modalities into one fluid representation. Due to this integration function, it cannot remain impervious to discrepancies, and quickly recognises conflicts between previously acquired knowledge and the present situation. If the discrepancies are large, the place cell representations and hence the cognitive map can become disorganised, and confusion results. To recognise this situation as different to those previously encountered, the hippocampus may establish a new representation of this space, essentially wiping the slate clean with regard to behavioural rules that applied previously.

Thus, how the task was initially encoded and how much the existing representations can be influenced by new information will determine the behavioural consequence of cue manipulations.

4.4.4. Residual hippocampal tissue

In this set of experiments, although the animals have been divided into sham and lesion groups, the size of the lesion varied from 55% to 90% of hippocampal tissue removed. In spite of this, correlation data was not significant, suggesting that the animals showed the same pattern of performance irrespective of lesion size. Thus, two hypotheses arise. Either the residual hippocampal tissue was not functional or it was insufficient to change behaviour towards that of the intact animals. The first hypothesis is somewhat unlikely, as the residual tissue did appear to improve initial post-surgery performance (see chapter 3). Thus we are left with the idea that the residual tissue was somehow insufficient to change behaviour. If the hippocampus actually stores the complex representations of background cues, (e.g. Rudy and Sutherland 1989), then removal of tissue would reduce the volume available for encoding, and lead to the loss of extraneous information. Conversely, if representations are actually stored outside of the hippocampus (Rudy and Sutherland 1995), then the loss of hippocampal tissue would lead to a decrease in the signal emphasising configural associations. As the configural associations of background cues are not imperative to task performance, without the hippocampal reinforcement, their accessibility and influence on behaviour may be lost. Either way, it appears that accurate performance can be retained in the face of conflicting background stimuli without an intact hippocampus.

4.4.5. Use of Self-motion cues

The aim of this set of experiments was to investigate one of the strategies the lesioned animals could have been using to solve the context dependent odour discrimination task – namely the use of self-motion cues. However, the lesioned animals showed no impairments in performance during the manipulations, thus it can be concluded that they were not relying upon self-motion cues to guide their

behaviour. Instead, it appears that the lesioned rats were indeed using the intra-maze cues to guide their responses.

However, the fact that cue conflict had no effect on their behaviour does suggest they are not representing the task in exactly the same way as sham animals. Above it was suggested that their representation of the environment may be more simplified than that of sham operated animals. Indeed, it seems likely that lesioned animals simply jumped between the two context representations as they encountered them. They showed no evidence of recognizing their relationship or predicting which they might enter next.

By contrast the sham operated animals displayed severe impairments in sections of this chapter. Their impairment in the face of conflicting self-motion and non-spatial cues suggests that their intact hippocampus enables them to encode a detailed representation of their environment - including how the different parts are inter-connected. Furthermore, as these animals move through their environment they update this representation and compare their internal representations to the reality they encounter. Thus when disparity occurs between cues the animals rapidly detect this and attempt to integrate it into their existing representation. Several predictions have been made by previous researchers as to what the outcome of a conflict situation should be in an intact animal. However, the impaired performance of the sham operated animals in the face of conflict suggests that they **do not**:

a) Have a hierarchy of information which prioritises visual (colour) information over both odour and motion based information (Etienne and Jeffrey 2004)

b) Disregard self-motion cues as unreliable (Taube 1995, Gothard and Skaggs et al 1996, Etienne et al 2000, McNaughton and Battaglia et al 2006, Etienne and Jeffrey 2004).

c) continually jump between representations (i.e. continually flip their internal representation each time a discrepancies is encountered) (Taube 1995, Gothard and Skaggs et al 1996, Skaggs and McNaughton 1998).

Instead it seems likely that the sham operated animals either:

a) regress to using simple path integration information (Etienne and Jeffrey 2004) - an unsuccessful technique in this case;

b) create a new representation of space based upon current conditions that does not have task rules associated to it (Eichenbaum, Matthews an Cohen 1989);

c) attempt to use some other as yet uninvestigated strategy that is equally as unsuccessful in supporting task performance.

4.5. Conclusion and further questions

This set of manipulations showed that the lesioned animals were relying on intra-maze cues for accurate performance of the context dependent odour discrimination task. Sham operated animals, on the other hand, had a more wide-ranging set of influences, which included self-motion and intra-maze cues. The difference suggests that even though accurate performance on the task was possible for both groups, they were achieving this in a slightly different way.

This task was learnt before surgery when all animals had an intact hippocampus, yet results in a difference in processing between lesion and sham animals. How then would this difference impact on new learning? Would the lack of an all encompassing relational representation aid or hinder the acquisition of new variants of this task? This question was addressed in chapter 5.

5. The effect of hippocampal lesions on acquisition of new context dependent odour discriminations

5.1. Introduction

In humans, retrograde amnesia is almost always accompanied by some anterograde amnesia (inability to learn new information; Squire and Alvarez 1995). However, the degree of anterograde and retrograde amnesia is not correlated. Therefore the presence or absence of one, tells us nothing about the extent of the other.

The previous chapters have indicated that the removal of the hippocampus causes a short-term retrograde amnesia for a previously learnt task. Hence the hippocampus seems to play a role in retention of a task learnt prior to surgery. However, what about new learning? The re-attainment of criterion in the previous chapter may have been possible by re-establishing a route to non-hippocampal representations. However, the converse of this is whether such representations can be created from scratch in the absence of the hippocampus? In chapter 4 it was demonstrated that hippocampal lesioned animals were representing the contextual stimuli in a more simplistic way than the sham operated animals. Nevertheless, are these simpler forms of context representation actually sufficient to support learning? If new representations are to be established in the absence of the hippocampus, it is highly likely that they will be based on different cues or are made using different strategies than observed for the intact animals. Furthermore, can these new representations be stored in alternative, extra-hippocampal and undamaged sites (Squire and Alvarez 1995)? The focus of this section is to examine the effect of hippocampal lesions on the acquisition of new context dependent odour discriminations.

Hippocampal lesioned rats show no deficit in aquistion of contextual tasks.

Previous published literature does not provide a clear cut answer as to whether a lesion effect will be observed during new learning in this task. For example, Eichenbaum et al (1988) demonstrated that quite apart from causing impairments, a lack of hippocampal processing could actually facilitate performance of a successive two odor discrimination task with go/no go responses. Fornix lesioned animals actually outperformed sham operated animals on this task. Sham animals would attempt to combine the stimuli into a multi-factorial configural representation. However, in lesioned animals other brain systems were free to support behaviour using simpler cue associations.

Nevertheless, this literature did employ fornix transactions and not the hippocampal lesions that were used in this thesis. Similarly, Eichenbaum et al's (1998) data is based upon animals learning a completely new task with new rules. The tasks used in this section have the same rules as previously acquired – the rats are simply required to apply them in a new situation. Furthermore, by the time the animals in this thesis reach the post-surgery new learning phase, they have gained a significant amount of experience on this style of task. This may have an effect on their procedural ability during new learning – something which would not have been observed in the Eichenbaum experiment. With these experimental differences, it is difficult to predict whether hippocampal lesions will produce enhancement in learning on this context dependent odour discrimination task or not.

Hippocampal lesioned rats demonstrate temporary impairments in acquisition of contextual tasks.

Further contradictory results regarding the post-surgery acquisition of context conditioning are provided by Good et al (1991). They showed that normal animals conditioned to respond to a lever in one context (food reward) demonstrated reduced levels of responding when the lever was presented in a novel context. Hippocampal lesioned animals however, failed to show this automatic inhibition of the appetitive response (i.e. lesions showed more pawing / biting at lever than shams) and took

extended training to curtail their responding (even when the lever was no longer rewarded). This data would predict that post-surgery learning should be impaired for hippocampal lesioned animals. Nevertheless, with training the lesioned animals did learn the biconditional version of the task (i.e. rewarded in one context, non-rewarded in the other). Thus perhaps the anterograde effect was only temporary until an alternative learning strategy was embraced? As for Eichenbaum et al above, Good et al's (1991) animals were learning the task from scratch post-surgery. In addition, the two different modalities were used for the the discrimination (one visual cue and one auditory cue). Perhaps this integration put an extra emphasis onto a hippocampal method of processing? What happens if the biconditional stimuli are in the same modality (i.e. both odours as in this thesis)?

Hippocampal lesioned rats can demonstrate contextual conditioning, but only under certain circumstances.

Fear conditioning studies have also been used to study anterograde amnesia. Simple cued conditioning (e.g. to a tone) can be successfully expressed independently of the hippocampus. However, contextual conditioning is more controversial. Some studies find a mild anterograde amnesic effects after hippocampal lesions (e.g. Phillips and LeDoux 1992, Kim et al 1993, Maren and Fanselow 1997, Young et al 1994, Maren et al 1998) while others do not (Maren et al 1997, Phillips and LeDoux 1994, Frankland et al 1998). These differences may depend on how reliable an elemental solution is for the task. For example when a salient cue like a tone will predict a footshock, dorsal hippocampal lesioned animals appear to condition only to this salient cue, and thus have impaired contextual conditioning. Conversely, when context was the only variable available to encode, no deficit in contextual freezing was observed (Phillips and LeDoux 1994). Hence it seems that (under some conditions at least), hippocampal independent systems can acquire conditional associations (Fanselow 2000).

Thus these insights give mixed predictions about the acquisition of new context dependent odour discriminations post-surgery. If the task is encoded in the same way as contextual fear, it may be possible to learn it without a hippocampus. However, if associating the odour / reward relationship with contextual cues specifically requires the hippocampus, the necessary acquisition of rules would not occur in its absence.

Therefore, many factors including task, lesion type and involvement of context may all be critical to whether an anterograde amnesic effect will be observed during the learning of new discriminations. This chapter explores the effect of hippocampal lesions on the acquisition of a new context dependent odour discrimination in situations where either some aspects are familiar (i.e. new odours in a familiar context) or all cues are novel (new odours in a new context). It is hypothesised that lesioned animals show impaired acquisition of new task variants. Furthermore, sham animals will find the new odours task easier to learn than the new context task, as they will not have to make a new context representation, only integrate the new odours into an existing representation.

5.2. Methods

This chapter follows on directly from the previous chapters, and a lot of the methodology was the same. Therefore, I will only go into detail on the things that changed.

The protocol described in this chapter involved the animals that had already completed all the previous training regimes (i.e. pre-surgery training, lesion / sham surgery, post-surgery testing and manipulations). In this part, they had to learn new versions of the task.

5.2.1. Subjects and Housing

The subjects were 16 of the same Male Lister-hooded rats that had completed the protocols in chapters 2-4. (It would have been n = 17 except one rat died before completing this stage). 7 were sham operated animals and 9 had received bilateral ibotenic acid lesions of the hippocampus. They were individually housed, on a 12h light/dark cycle with ad libitum access to water, and slight food restriction (For further details see Chapter 2)

All procedures were carried out in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986 and European Communities Council Directive of 24 November 1986 [86/609/EEC], and all efforts were made to minimise suffering.

5.2.2. Apparatus

Contexts

The 'new odours' task used the same black and white contexts as were described in chapter 3. These consisted of two structurally identical circular (76cm diameter) black wooden platforms, with an adjoining bridge. The platforms had clear polycarbonate walls (40cm high) and four evenly spaced clear sliding doors. Fabric was placed behind the polycarbonate to colour it (black or white). (Photographs of the apparatus are shown in chapter 3). The whole apparatus sat approx 75 cm above floor level on a frame.

The 'new context' discrimination apparatus was very similar to that used previously, but was constructed from different materials. They consisted of two circular platforms (76cm in diameter) that sat approx 75 cm above floor level on a table. Walls of 40cm high were constructed from plastic garden netting, cardboard and then

either brown or white sticky backed vinyl. Stripes of black sticky backed vinyl were added to the white walls to conform them as a new and different context. A door was cut in the cylinder to allow for the bridge (for further details of bridge see chapter 3). Two new floor inserts were constructed, one from a black plastic bin-bag and the other from shiny silver textured sticky backed vinyl and card. These were affixed to the platform with velcro. Crosses of velcro were evenly spaced around the perimeter of the platforms to allow for the attachment of odour pots. The two barriers were again used to control access to the platforms and the entire apparatus was surrounded by thick black curtains to minimize extra-maze cues. (Photographs of the apparatus are shown below and can also be observed in the videos contained on the CD in the appendix of this thesis.)

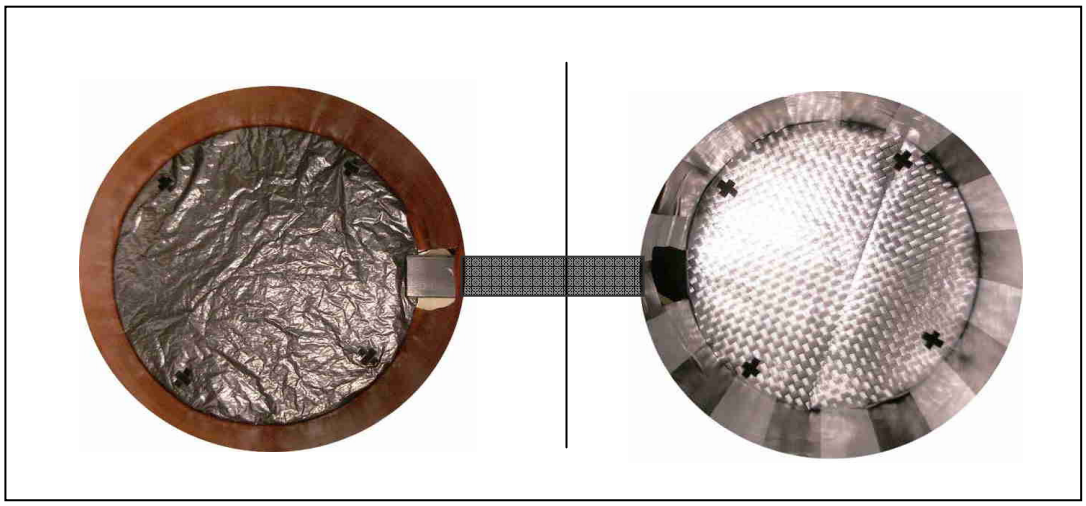


Figure 5-1: New context apparatus

Odour Pots

The reward pots were the same clear Nalgene pots filled with playsand (150g) and herbs / spices (0.8g). The new odours task used fennel and Allspice as odours, whereas the new context task used sage and parsley. Buried chocolate wheetos (Nestle) were used as the food reward.

5.2.3. Behavioural Protocol

General Protocol

As previously, testing took place on weekdays (Mon-Fri) between 8.30am and 8.30pm, and the rat was transferred to and from the apparatus in a cylindrical container.

New odours Task

The new odous task followed the same rules as the original context dependent task and training/testing took place in the **same** room and utilised the **same** apparatus. However, two new odours (G and H) were used (fennel and allspice) and **no** context independent task was run. Furthermore, as the rats were already very familiar with the apparatus and general protocol from completing the other sections of the experiment, no habituation or initial training phases were used (i.e. blocks of 8 etc were not used). The training protocol jumped straight to trials in pseudo-randomised order. (The protocol for new learning is detailed in Table 5-1. Further videos / a PowerPoint file are available to view on the CD in the appendix of this thesis).

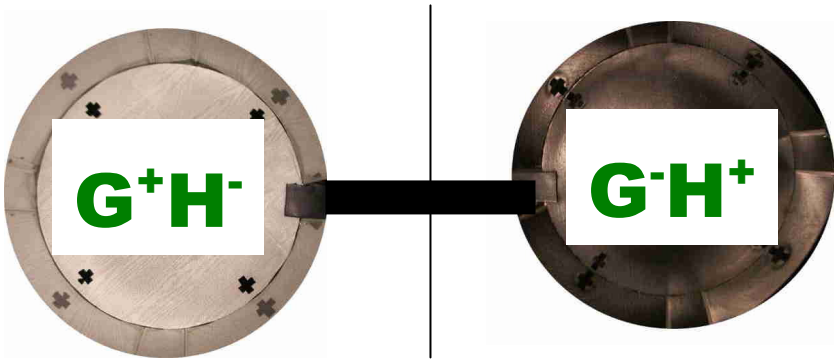


Figure 5-2: Rules and apparatus used for new odours task

Thus for the new odours task, the animals were trained on 16 counterbalanced, pseudo-randomised trials per session (4 each of each of G^+, H^-, G^-, H^+) with up to two corrections per trial. Training continued until the rats reached criterion (14/16 trials correct for 2 consecutive sessions). At this point 4 testing sessions were given. Within each testing session the rat received a standard set of trials (16 trials: 4 each of each of G^+, H^-, G^-, H^+) and a probe trial added in at a randomised point (17 trials in total). The probe trial consisted of a pot that although sited in a reward position, did contain the reward at the beginning of the trial. Instead once the rat had dug in the pot for 5 seconds, the experimenter dropped the reward onto the top of the sand. Probe trials were used to ensure the animal was using the odour of the sand to determine the reward properties of a pot, and not directly sensing the reward itself.

New Context Task

The new context training was run in immediately following completion of the new odours task using the same animals, and was essentially identical except for the use of a new set of apparatus (see description above) It again used the rules of the context dependent task, except that this time training and testing took place in a **new** room, with a **new** set of contexts and odours (sage and parsley; odours J and K counterbalanced across groups).

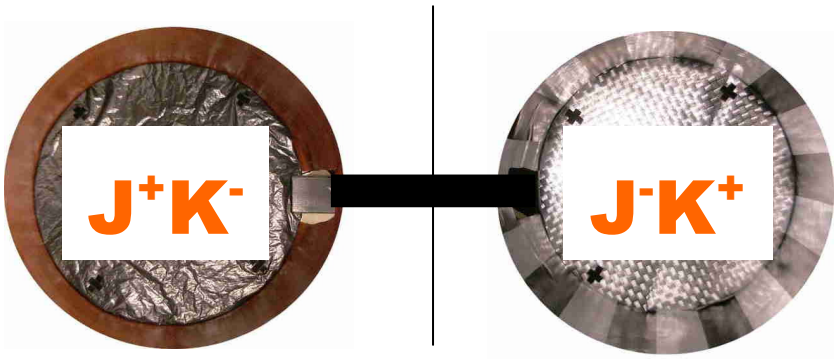


Figure 5-3: Rules for new context task

Rats were trained on the new context task for 16 randomised but counterbalanced trials per session (4 each of each of J^+, K^-, J^-, K^+) with up to two corrections per trial (for rules see Figure 5-3). Training continued until the rats reached criterion (14/16 trials correct for 2 consecutive sessions). At this point 4 testing sessions were given. Within each testing session the rat received a standard set of trials (16 trials: 4 each of each of J^+, K^-, J^-, K^+) with a probe trial added in at a randomised point (17 trials in total)

Table 5-1: Schedule for new learning post-surgery

	CRITERION	4 Sessions		CRITERION	4 Sessions
New odours Context Dependent Task only Random Order 16 trials 4 each of G^+, H^-, G^-, H^+ (8 rewarded, 8 non-rewarded) Up to 2 Correction trials	14/16 correct for 2 consecutive Sessions	New odours Context Dependent Task only Random Order 17 trials 4 each of G^+, H^-, G^-, H^+ (8 rewarded, 8 non-rewarded) & 1 probe trial up to 2 Correction trials	New context Context Dependent Task only Random Order 16 trials 4 each of J^+, K^-, J^-, K^+ (8 rewarded, 8 non-rewarded) up to 2 Correction trials	14/16 correct For 2 consecutive sessions	New Context Context Dependent Task only Random Order 17 trials 16 trials 4 each of J^+, K^-, J^-, K^+ (8 rewarded, 8 non-rewarded) & 1 probe trial up to 2 Correction trials

5.2.4. Histology

The histology for this chapter (due to the use of the same experimental subjects), has already been described in chapter 3. In real time however, the histological procedure was actually carried out upon completion of the new context task.

The general protocol for this was that the rats were euthanized and perfused with formalin. After removal, their brains were embedded in egg yolk, sectioned on the cryostat and then stained with cresyl violet acetate. The volume of hippocampal sparing in the lesioned animals was calculated using the area measurement tool in Image J, and compared to shams. 3D reconstructions were done using a program called 3D doctor. Full results including 3D reconstructions can be observed in chapter 3.

5.2.5. Statistical Analysis

Statistical analysis was performed on SPSS for windows version 12.0. All numerical values and graphs are reported as $\pm$ SEM.

Independent samples t-test were used to compare groups throughout.

Repeated measures ANOVA followed by simple effects analysis (with bonferonni corrections) was used to compare performance across the first 5 sessions of each task and to compare learning across all 4 tasks (pre-surgery, post-surgery, new odours, new contexts).

Pearson's correlations were used to investigate the relationship between lesion size and performance.

5.3. Results

5.3.1. New odours

No difference between groups on acquisition and performance of new odours task.

A repeated measures ANOVA on the performance data for the first 5 sessions of the new odours task showed a significant effect of session ($F_{(4,56)} = 8.94$, $p < 0.001$) reflecting the gradual learning of the task. However, no effect of group ($F_{(1,14)} = 0.57$, $p = 0.463$) and no session by group interaction ($F_{(4,56)} = 0.76$, $p = 0.554$) were observed. Therefore, both groups acquired the new odours task at the same rate.

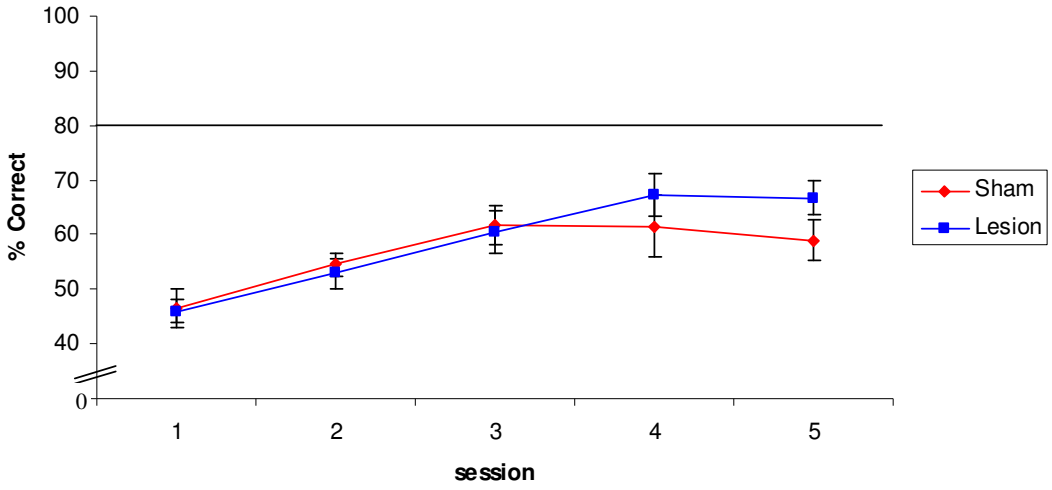


Figure 5-4: Average performance on sessions 1-5 of the new odours task, expressed as % correct. (Sham n = 7, lesion n = 9). Only the context dependent task was performed at this stage.

Independent samples t-tests showed that there was no significant difference between groups for the number of sessions it took them to reach criterion performance (sham average 16±3 sessions, lesion average 12±1 sessions; $t_{(14)} = 1.31$, $p = 0.209$). All animals acquired the new task at the same rate.

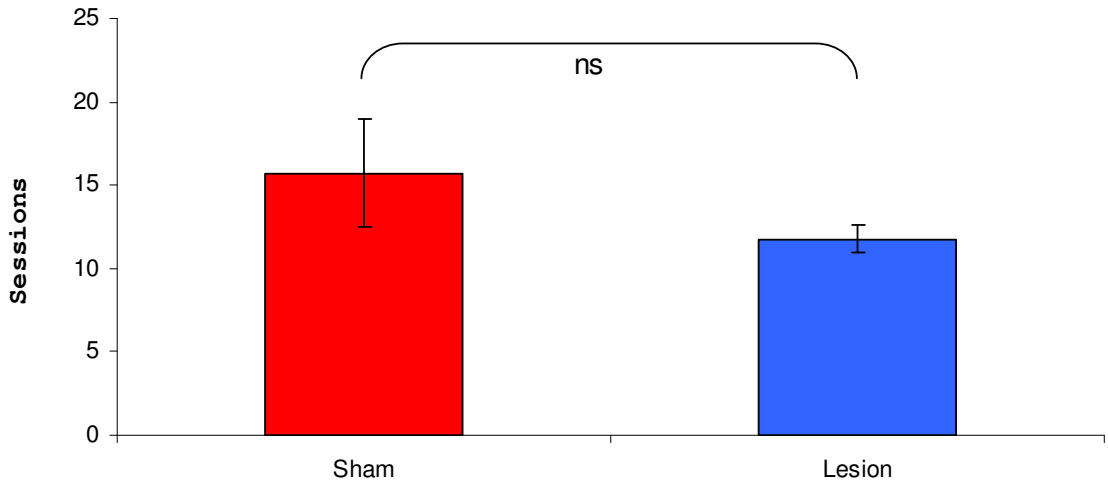


Figure 5-5: Average number of sessions taken to reach criterion on the new odours task. (Sham n = 7, lesion n = 9)

An independent samples t-test on the context dependent task for the post-criterion new odours testing sessions did not vary between groups ($t_{(14)} = -1.34$, $p = 0.203$). The lesion animals can learn and perform this new odours task as competently as sham animals. The probe trial data demonstrates that the animals were using the odour of the sand itself and not the odour of the reward to direct their behaviour.

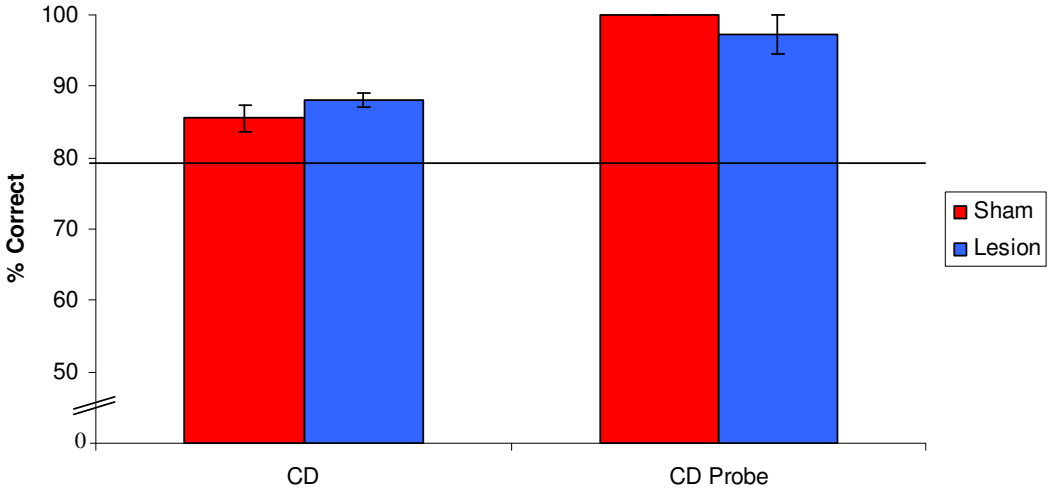


Figure 5-6: Average performance on the new odours task for the four testing sessions, expressed as % correct (Sham n = 7, lesion n = 9). The rats received 12 context dependent trials and 1 probe test per session.

5.3.2. Correlation between lesion size and performance

In chapter 3 it was shown that initial post-surgery performance correlated to the percentage of spared hippocampal tissue. Therefore, the correlation data for learning new odours was also calculated. Like the post-surgery data, a strong trend was observed for performance to improve with increased spared hippocampal tissue. The Pearson's correlation was extremely close to significance level ($r(8) = 0.67$ $p = 0.051$)

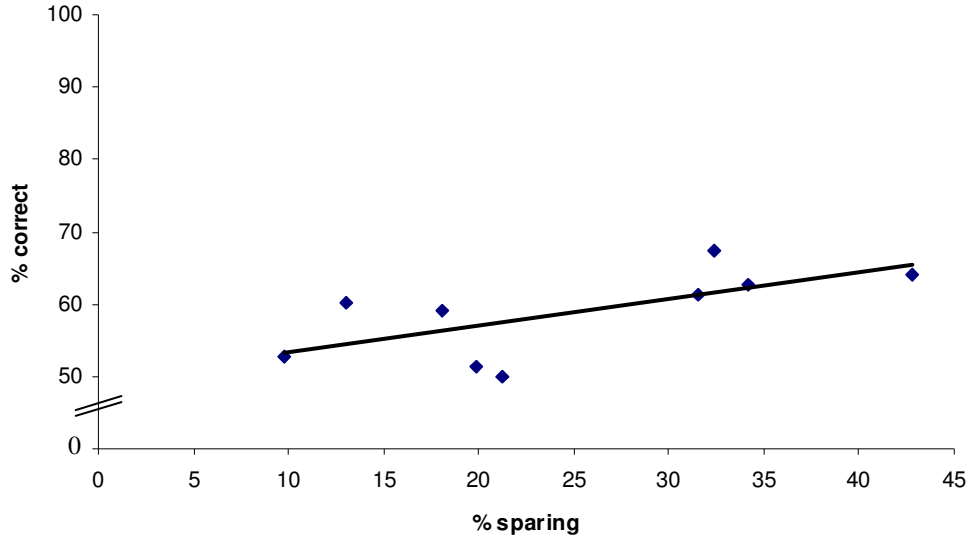


Figure 5-7: Correlation between % sparing and average % correct on over first 5 sessions of new odours.

Nevertheless, no correlation was observed between lesion size and sessions to criterion on the new odours task ($r(8) = -0.32$, $p = 0.404$). The data shows a wide spread.

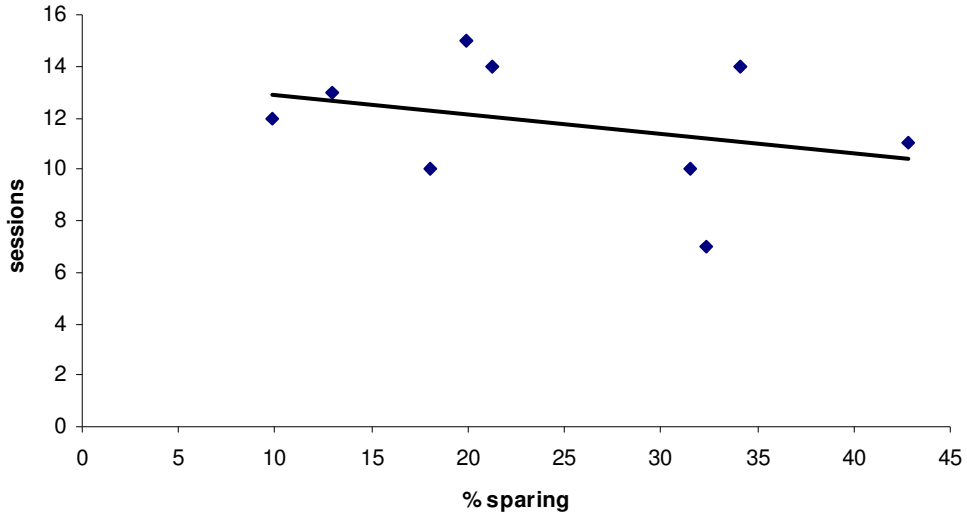


Figure 5-8: Correlation between % sparing and sessions to criterion on New odours task.

No correlation was observed between lesion size and performance on the testing days ($r(8) = -0.49$, $p = 0.178$). By the time they reached criterion and were allowed to proceed onto the testing days, all animals were performing at a consistently high level.

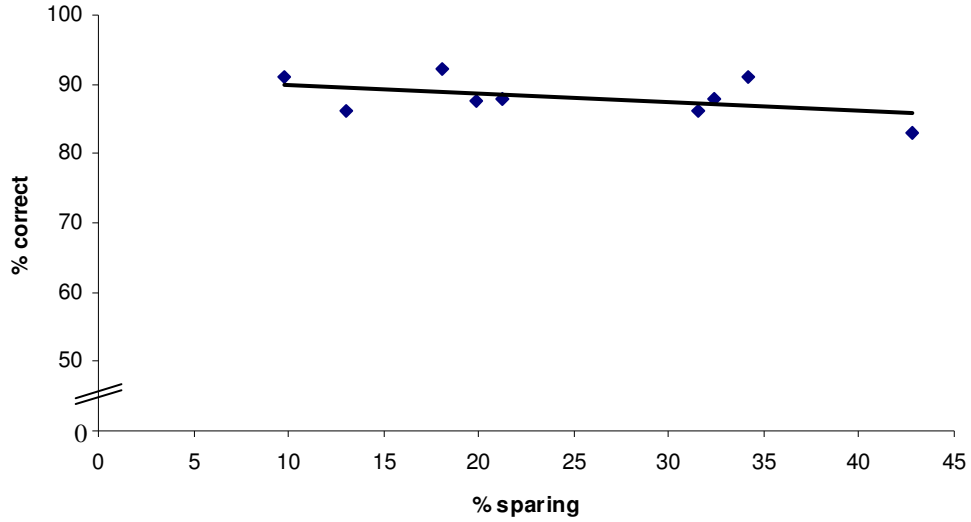


Figure 5-9: Correlation between % sparing and performance on new odours testing days.

5.3.3. New context

No difference between groups on acquisition and performance of new context task.

A repeated measures ANOVA indicated that there was a gradual learning process occurring across the first 5 sessions, as was reflected in a significant effect of session ($F_{(4,56)} = 6.80$, $p < 0.001$). However, no other differences were observed. No effect of group ($F_{(1,14)} = 0.43$, $p = 0.523$), or session by group interaction ($F_{(4,56)} = 1.07$, $p = 0.379$) was seen.

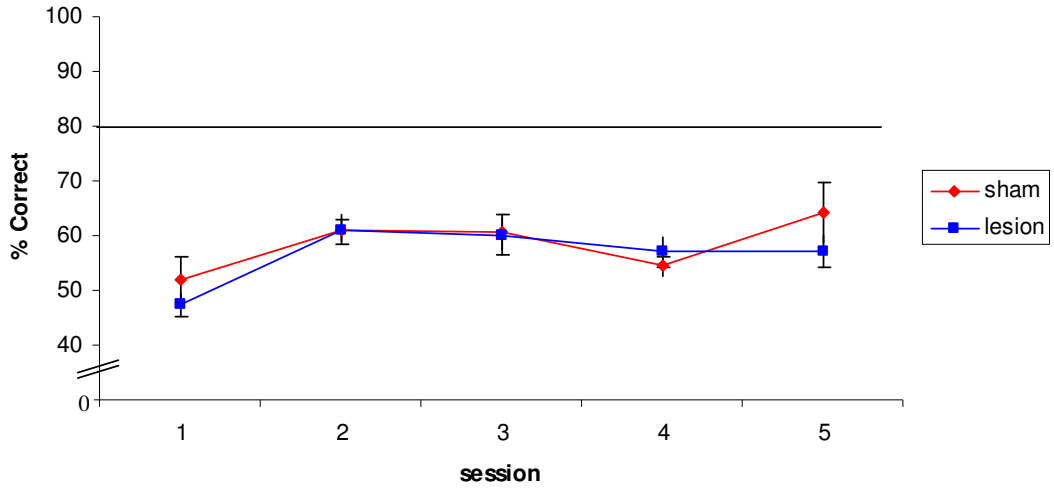


Figure 5-10: Average performance on sessions 1-5 of new context task, expressed as % correct. (Sham n = 7, lesion n = 9). Only the context dependent task was run at this stage.

The number of sessions taken to reach criterion did not differ significantly between groups ($t_{(14)} = 1.11$). All animals acquired the new task at the same rate (Average sessions to criterion: sham = 20 sessions, lesion = 15 sessions).

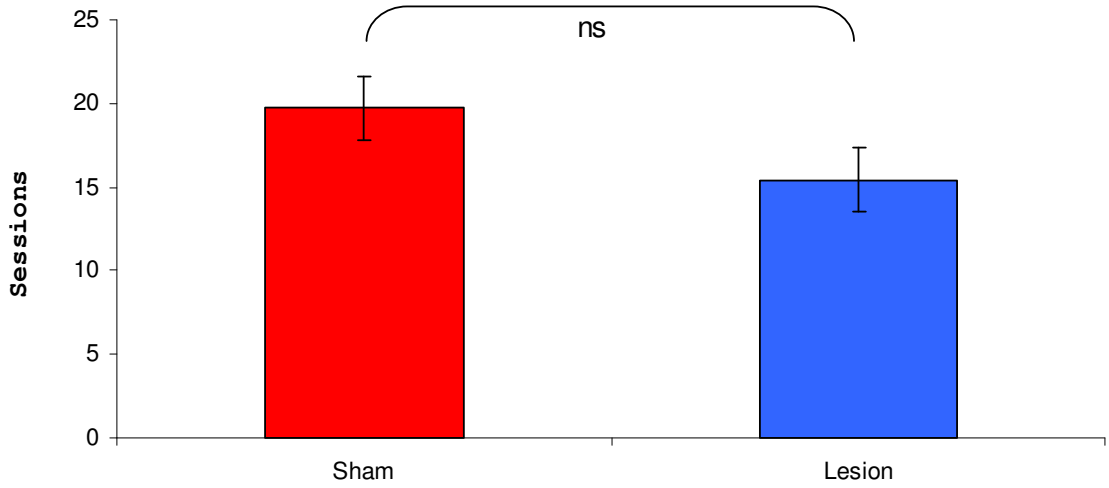


Figure 5-11: Average number of sessions taken to reach criterion on the new odours task. (Sham n = 7, lesion n = 9)

Performance during the new context testing sessions did not vary between groups ($t<1$). The lesion animals learn this task at the same rate as sham animals, and both groups achieved a consistently high performance level. The 100% correct probe trial data demonstrates animals were not using the odour of the reward to guide their behaviour.

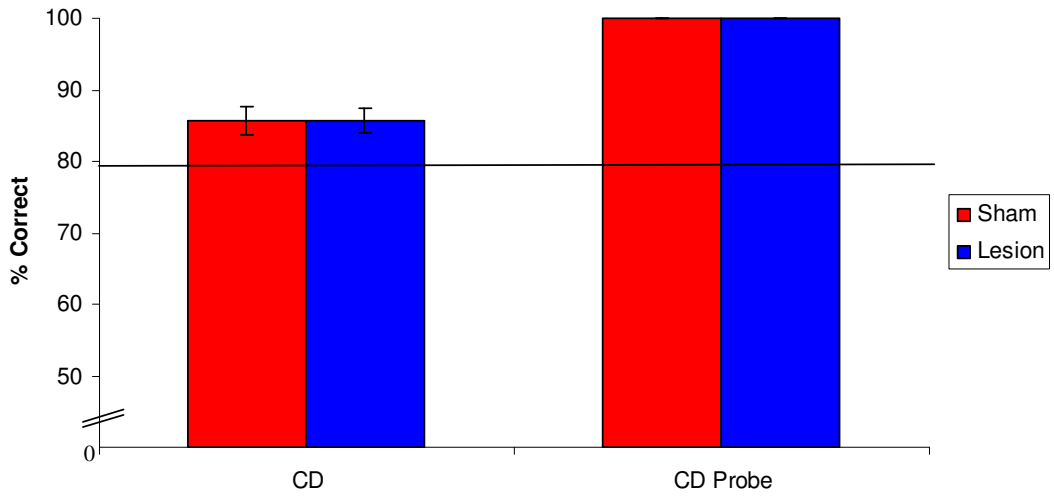


Figure 5-12: Average performance on the new context task for the four testing sessions, expressed as % correct. (Sham n = 7, lesion n = 9). The rats received 12 context dependent trials and 1 probe test per session.

5.3.4. Correlation between lesion size and performance

No significant correlation was observed between lesion size and performance on the first 5 days of new context training ($r(8) = 0.37$, $p = 0.331$). Thus the trend for lesion size to correlate to initial performance was not observed when the animals learnt new odours in a new context.

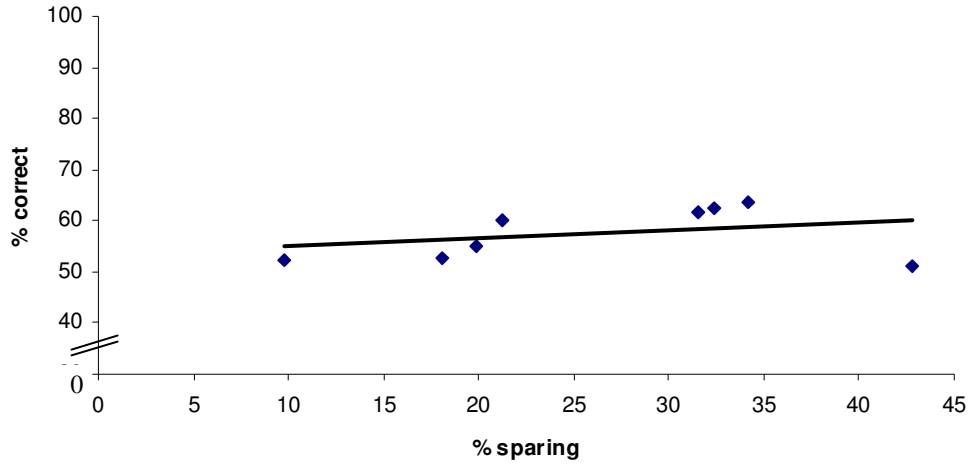


Figure 5-13: Correlation between % sparing and average % correct on over first 5 sessions of new context.

Similarly, no correlation was observed between lesion size and sessions to criterion in the new context ($r(8) = -0.23$, $p = 0.548$).

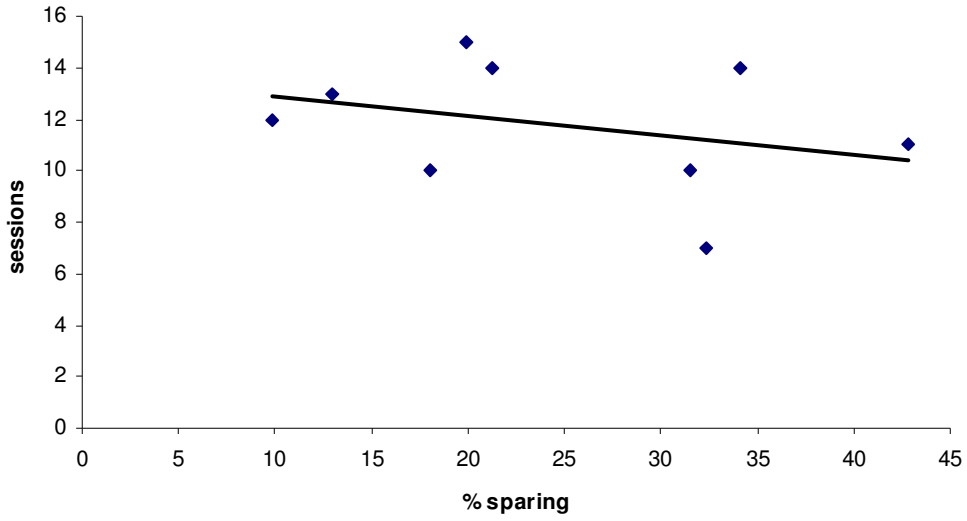


Figure 5-14: Correlation between % sparing and sessions to criterion on new context task.

Again, no correlation was observed between lesion size and performance on the testing days ($r(8) = -0.36$, $p = 0.340$). All animals were performing consistently above criterion by this point.

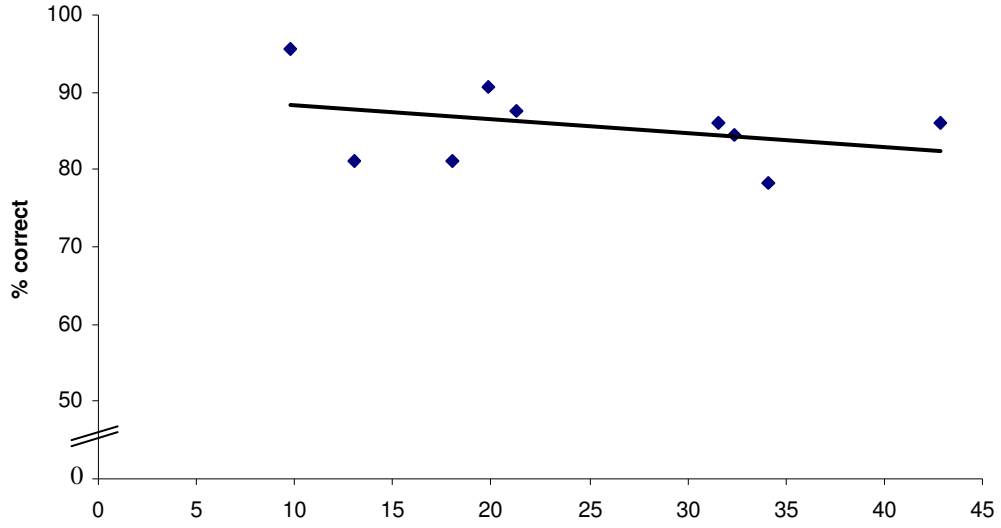


Figure 5-15: Correlation between % sparing and performance on new context testing days.

5.3.5. Compare performance on 'new odours' and 'new context and odours tasks'

It was hypothesised that the animals would find the new odours task easier to learn than the new context task, as they would not have to make a new context representation, only integrate the new odours into an existing representation – a function that in the intact animals may have involved the hippocampus.

No difference in initial average performance of new odours task compared to new context task.

A repeated measures ANOVA comparing the initial performance (1st 5 days) data for the new odours and new context tasks did not demonstrate an effect of task ($F_{(1,14)} = 0.02$, $p = 0.881$) and no task by group interaction ($F_{(1,14)} = 1.73$, $p = 2.09$). The animals performed no better on the new odours task than they did on the new context task, despite some elements of the apparatus being familiar in the first instance, and all being new in the second.

No difference in sessions required to reach criterion on new odours task compared to new context task.

A repeated measures ANOVA comparing the days to criterion data for the new odours and new context tasks showed no effect of task ($F_{(1,14)} = 4.01$, $p = 0.065$) , or group ($F_{(1,14)} = 1.73$, $p = 0.209$) and no task by group interaction ($F_{(1,14)} = 0.01$, $p = 0.932$). Hence, the new context task took *no* longer to learn than the new odours task.

5.3.6. Comparing and contrasting pre-surgery acquisition with post-surgery reacquisition and new learning

Part of the purpose of testing the acquisition ability of the hippocampally lesioned rats, was to compare the data to their re-test performance post-surgery. The relatively fast reacquisition of criterion performance post-surgery was interpreted in chapter 3 as some form of recall and not relearning. If this is the case, a difference in performance should be observed between post-surgery performance (chapter 3) and the new learning in this chapter.

To address this question, additional statistical tests were used to compare performance between the retest, new odours and new contexts tasks. For these experiments, a direct comparison of results across tasks is appropriate as they all used the same subjects, and the same experimental procedures (including performance criteria).

Initial performance during post-surgery training was different from the new odours or new context training for sham operated animals only.

The rats perform at a higher level of competence on the first 5 sessions of training immediately following surgery than they do on the first 5 sessions of the new odours or new contexts tasks (see Figure 5-16). A repeated measures ANOVA comparing performance on the first 5 sessions of post-surgery, new odours and new context tasks showed a significant effect of timing indicating a difference in competency between retest / new odours/ new context tasks, ($F_{(2,28)} = 42.43$, $p < 0.001$). There was also an effect of session reflecting the gradual learning process ($F_{(4,56)} = 16.13$, $p < 0.001$). An effect of group ($F_{(1,14)} = 8.98$, $p = 0.010$), and a task by group interaction ($F_{(2,28)} = 14.52$, $p < 0.001$) suggested that some of this difference in competency was related to the surgical group the animals were in.

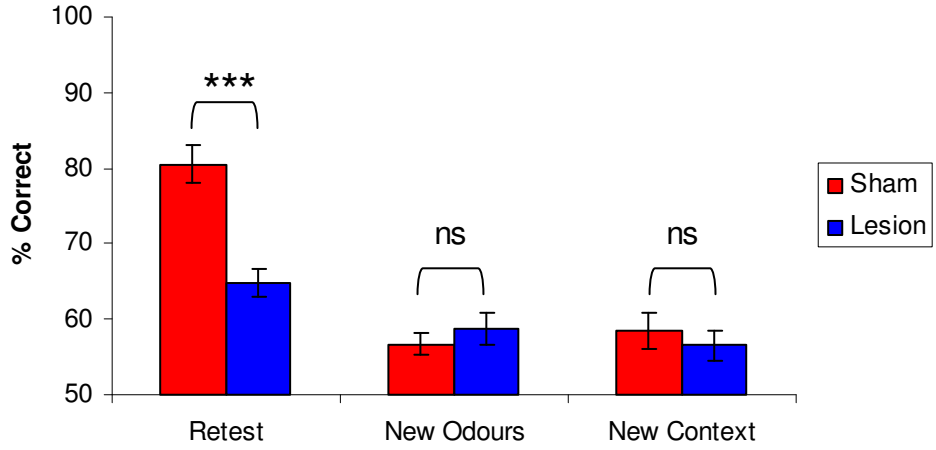


Figure 5-16: Average performance on the first 5 sessions of training on each task. (Sham n = 7 through out n= 6, lesion n =10 for pre-surgery and retest, n = 9 for new odours and new context)

Post-hoc simple effects (with Bonferroni corrections) demonstrated that the sham group performed significantly better on the first 5 days of re-test compared to either the new odours task ($p < 0.001$) or new context task ($p < 0.001$). However, their initial performance did not differ between the two new tasks (new odours vs new context: $p = 1.000$). The lesion animals however did not perform any better on the initial post-surgery retesting than they did on either the new odours ($p = 0.081$) or new context tasks ($p = 0.067$). However, these statistics are not very far from the 0.05 significance level. Sham operated animals were recalling information post-surgery and learning new information at all other points in the protocol, so a difference in initial performance was expected for them. However, it is interesting that the lesioned animals showed a trend towards this too (even if the statistical tests were non-significant).

Acquisition of the context dependent odour discrimination task was faster post-surgery than during new learning for the sham operated animals only.

Re-acquisition immediately after surgery (Chapter 3) is the only part of the protocol where there is a significant difference between the groups in the sessions to criterion measure (see Figure 5-17). However, there is some uncertainty as to whether the rats are displaying recall or new learning during this retesting phase. Conversely, new learning is definitely occurring during other parts of the protocol (pre-surgery, new odours, new context).

A repeated measures ANOVA on the whole 'Sessions to criterion' data set (Pre-surgery, retest, new odour and new context) revealed a highly significant effect of task ($F_{(3,42)} = 79.34$, $p < 0.001$) indicating that the rats were learning the tasks at different rates. However it did not find an effect of group ($F_{(1,14)} = 0.48$, $p = 0.502$) nor a task by group interaction ($F_{(3,42)} = 0.64$, $p = 0.594$), suggesting that although the tasks were different, all groups acquired / regained performance on them at a similar speed (see Figure 5-17).

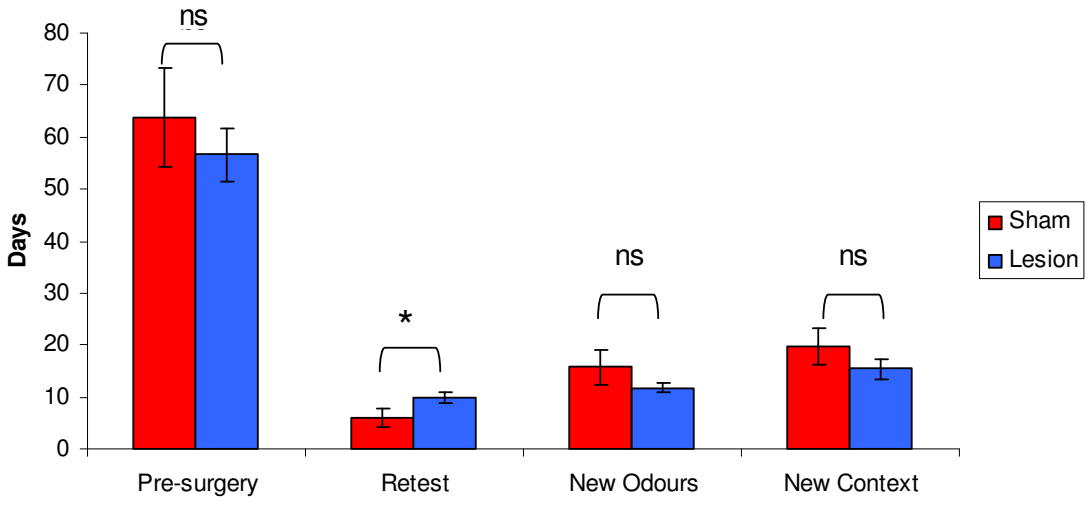


Figure 5-17: Average number of sessions taken to reach criterion on each part of the protocol. (Sham n = 7 through out n= 6, lesion n =10 for pre-surgery and retest, n = 9 for new odours and new context)

Post-hoc simple effects (with Bonferroni corrections) confirmed that the speed of reacquisition post-surgery was significantly different to learning new odours for the sham animals ($p = 0.003$) but not for the lesioned animals ($p = 1.00$). Similarly, learning in new contexts took longer than reacquisition post surgery for sham animals ($p = 0.003$) but not for lesioned animals ($p = 0.274$). The speed of acquisition did not differ for either group between the new odours and new context tasks (sham: $p = 0.556$, lesion: $p = 0.509$).

The statistical differences were expected for the sham operated animals as they were recalling information post-surgery, but learning new information at all other points in the protocol. Conversely, if the lesion had resulted in abolition of memory for the original pre-surgery task, then it should have taken the lesioned animals a lot longer to reacquire the context dependent odour discrimination after lesion surgery. This is difference in acquisition times was not observed, thus relearning cannot be ruled out.

Overall, the statistical analysis above cannot rule out the fact that new learning may have occurred for the lesioned animals during post-surgery re-attainment of criterion. However, a trend was observed for the initial (1^{st} 5 days) performance data for new learning to be a little less accurate than was observed post-surgery. Thus perhaps these measures are simply not sensitive enough to pick up the small differences.

5.4. Discussion

The main finding of this chapter is that hippocampal lesioned animals can learn new context dependent odour discriminations both a context they were familiar with prior to lesioning, and in a novel context. Furthermore, they learn these discriminations at a similar rate and perform them to the same degree of accuracy as sham operated animals.

5.4.1. Comparison to initial pilot data

The results described in this chapter are very similar to those found in chapter 2 (task development). They both demonstrate that removing sections of the hippocampus has no effect on the ability of rats to learn or perform the context dependent task. In addition, the data for this chapter was collected in several smaller repetitions. All repetitions demonstrated the same trends in performance. Thus again the task is shown to be robust and the results highly repeatable.

5.4.2. Appraisal of functionality of spared hippocampal tissue

The degree of spared hippocampal tissue varied quite considerably for the lesioned animals used in this experiment. In chapter 3, the issue of functional residual hippocampal tissue was discussed in relation to post-surgery performance. There it was concluded that the residual tissue was likely to be at least partially functional. Indeed a significant correlation was observed between lesion size and initial performance.

When these correlations were repeated for the data in this chapter (New odours / new context), no such significant correlations were observed. Nevertheless, a definite

trend was observed for initial performance (over sessions 1-5) on the new odours task to be better in animals with larger volumes of spared hippocampal tissue. This suggests that (as in chap 3) the remaining tissue may have retained some partial functionality. In the new odours task, the context was already familiar. The ability to recover an existing representation of the context may have conferred some learning advantage. Thus, (at least in the first few days of training), being able to even partially reinstate an existing representation of the context, may have conferred some advantage.

Nevertheless, this proposed ability to reinstate an existing representation of context did not confer any long term advantage. Although the average number of sessions to reach criterion level performance was slightly lower for the sham animals than for the lesioned animals, no significant difference between the groups was observed. Thus, it has to be concluded that all animals learnt the new odours task at a similar rate, regardless of whether they had an intact hippocampus or not. Furthermore, no correlation was observed between lesion size and sessions taken to reach criterion on the new odours in a familiar context task. Likewise, once the animals reached criterion performance, they all (lesioned and sham animals) displayed a high degree of consistent accuracy and no correlation was observed between lesion size and accuracy. Thus for the new odours in a familiar context task, the spared hippocampal tissue may only have been behaviourally advantageous for the first few sessions.

On the other hand, when performance on the new odours in a new context task was examined, none of the correlations came even close to significance level. Indeed, when examining the sessions to criterion data, it can be observed that the average is actually marginally less for the lesioned animals than it was for the sham animals. Similarly, the correlation data for lesion size vs initial performance (sessions 1-5) displays an almost horizontal line. Thus, why at this stage have the fully intact animals lost their advantage? The answer could be that for the new context task, the reinstatement of an existing representation of context could be a distracter rather than

an assistant. Although the new context shared certain features with the old one (e.g. size and shape of arenas), the use of the old representations would not have conferred any advantage to the sham animals. In fact, trying to fit the new context representation into the framework of the old one could theoretically have hindered performance (although there is little evidence for this in the data). Both sham and lesioned animals needed to create a new context representation in order to solve the task. However, there is some evidence in this thesis (see chapter 4) that the two groups may not have formed this representation in exactly the same way. For the lesioned animals, the context representation may have been simpler and less integrative of background features than for the shams (for further discussion see Chap 7). Nevertheless, both groups managed to successfully master and perform the new context task within a comparable length of time.

In 1995, Moser et al examined performance of a spatial watermaze task using a similar experimental structure to this thesis (i.e. pre-train the rats, hippocampal lesion surgery, test retention, then retrain the rats). They found that animals with partial lesions to the hippocampus could learn and perform the fixed platform watermaze task as accurately as sham animals. Similarly, in their 1995 paper Moser et al showed that pre-training hippocampal lesions had no effect on new learning. Thus it seems quite clear that partial lesions do not remove the rat's ability to learn a spatial watermaze task. Similarly, in this chapter it has been demonstrated that partial lesions do not prevent new learning on this context dependent odour discrimination task.

Even so, Moser et al (1995) did demonstrate a linear relationship between the degree of learning deficit and the volume of dorsal hippocampal tissue spared. A larger volume of spared hippocampal tissue led to a reduced latency to the platform and an increase in time spent in the target quadrant. This trend for improved new learning with hippocampal sparing was not statistically observed in this chapter – although a trend towards improved initial performance on new odours task was observed. This difference may be due to the differing training protocol. Moser et al (1995) did no

training prior to surgery. The animals had to learn from scratch post-surgery. Conversely, in this experiment, the animals had been performing this context dependent odour discrimination task for a number of months prior to initiating this new learning protocol – albeit with different odours/contexts. Therefore the procedural aspects and the general rules of the task may have been well established in non-hippocampal areas of the brain prior to new learning (Moser et al 1995). Thus, any learning trends could have been masked by the rapid acquisition of the new variants.

Nevertheless, Moser and Moser (1998) did demonstrate that complete lesions prevented the rats from learning the watermaze task, and reduced their performance to chance level. Therefore, it is not entirely clear what would have happened if the lesions had been complete in this experiment. On the other hand, it seems unlikely that the data would have changed significantly, since one of the animals had only 10% sparing and still learnt to perform the new odours and new contexts tasks at above criterion level.

5.4.3. Comparison to post-lesion learning on other tasks

In the introduction to this chapter, data from Good et al (1991) predicted that hippocampal lesions would cause impairments in performance, Eichenbaum et al (1988) predicted an enhancement, and fear conditioning studies predicted either an impairment or no effect of lesion on performance. The last of these predictions was the trend observed in the data from this chapter (i.e. hippocampal lesions did not affect acquisition of new context dependent odour discriminations). Thus it seems that new learning can still occur in a less intact hippocampus. Perhaps this is a result of the functioning of the residual hippocampal tissue (as suggested above) or perhaps extra-hippocampal areas can take over (as will be described below).

Although the ability to form the necessary configurations for this task has been attributed to the hippocampus, the result found above is not completely unprecedented. Similar abilities to perform contextual tasks without a hippocampus have been observed for object context recognition (Langston et al 2006, Norman and Eacott 2005) and contextual fear conditioning (e.g. Maren and Aharonov et al 1997, Gisquet-Verrier and Dutrieux et al 1999). However, (as described in chapter 4), it is believed that in the absence of the hippocampus, the task may be learnt and performed in a different way to that occurring in the intact brain.

Researchers in the field of contextual fear conditioning have suggested that in the intact brain there is a constant interaction of the elemental and configural systems (Rudy and Sutherland 1989, Fanselow 1999). In this task, the context may have been encoded as either a configural combination of cues, or alternatively just one aspect of the context may have been 'picked out' and use as an elemental cue. Nevertheless, regardless of whether the context itself is encoded elementally or configurally, a configuration would still be required to link 'context' to odour reward properties.

The role of the hippocampus according to Rudy and Sutherland (1995) is to selectively enhance the salience of conjunctions, and partially inhibit their elemental counterparts. This should speed up learning. Hence, in the absence of the hippocampus, this emphasis on configural representations would be lost and learning may be slower – but not necessarily prevented. In a new learning situation (like in this chapter) the lack hippocampal enhancement may not be detrimental to performance. The reward status of each element would be ambiguous – 50% of the time rewarded, 50% of the time unrewarded. Contrastingly, the configural unit would reliably predict reward and hence would still be capable of supporting task acquisition.

Nevertheless, in 2001, O'Reilly and Rudy adapted this theory to suggest that **both** the hippocampus and the cortex could encode configurations, but that the hippocampus did so in a rapid, automatic way, whereas the cortex did so in a more

slow changing way, recognising generalities. They suggested that non-linear problems (such as ours) are difficult to solve, and hence are always learnt via the establishment of slow task-driven conjunctions, not rapid hippocampal dependent ones as predicted previously. Thus, in this way the effect of hippocampal lesions in this context dependent odour discrimination task may simply have been masked by the nature of the task. Similarly, the extensive pre-lesion training period may have masked any potential lesion induced enhancement of the type that was observed by Eichenbaum et al in their 1988 paper. Either way, it can be concluded that the hippocampus is not necessary for post-surgery acquisition of new variants of a familiar context dependent odour discrimination task.

5.4.4. New odours were learnt at the same speed in a familiar context as in a novel context.

It was predicted that the new odour task would be easier to learn than the new context task. The familiar contexts that were used for the new odours task should have re-activated previous representations. Contrastingly, the new apparatus used for the new context task would have necessitated the establishment of completely new traces. However, no such learning artefact was observed, and both tasks were learnt with the same speed and competency for all animals. Nevertheless, the residual hippocampal tissue did appear to give some initial enhancement to the lesioned rats on the new odours task. Hence, although it seems likely that the new odours task actually produced new constructs reflecting the changed parameters, it may be that a simple swap of odour properties within an existing trace was attempted near the beginning of training.

5.4.5. Multiple Memory Systems

When one system (e.g. the Hippocampal system) is damaged, a second or parallel systems can take over its function and maintain behavioural accuracy. Indeed, despite the hippocampal involvement in post-surgery retention that was demonstrated in chapter 3, the lesioned animals were still capable learning new variants of this context dependent odour discrimination task. Although residual tissue may potentially play a role in this learning ability, even the animal that had lost 90% of its hippocampal tissue could learnt the task as fast and accurately as shams. As discussed in the previous chapter (chapter 4) it is highly likely that the new learning occurring in lesioned animals does not proceed in a way that is identical to that occurring in the intact brain. Nevertheless, this secondary or parallel non-hippocampal system is sufficient to support progressive acquisition and consistent above criterion performance – and may mask any lesion effects that would be observed due to hippocampal removal itself.

(The concept of a secondary / parallel system will be discussed further in the General Discussion - Chapter 7)

5.4.6. Implications for Retest Results

The data in this chapter give a strong demonstration of the pattern of performance and time required for new learning. It shows that new learning begins with chance performance, and continues at or just above chance for over 5 sessions. Conversely, the pattern observed during retesting (see Chapter 3) was of impairment for just 3 sessions and a complete recovery of performance in around 9 sessions (compared to an average of 13 sessions for new learning). These differences suggest that new learning may not have been occurring during the immediate post-lesion phase. Instead, these data support the hypothesis that after removal of the hippocampus,

temporary impairments are observed until new routes are established to existing information.

5.5. Conclusion

Rats can learn this task at an equivalent rate and accuracy irrespective of whether their hippocampus is intact or not. Thus it is clear that the hippocampus is not necessary to acquire this biconditional context dependent odour discrimination task.

These data do not rule out the idea that new learning may be occurring during re-attainment of criterion on a task learnt prior to lesion surgery.

6. The effect of hippocampal lesions on the retention of context dependent and independent odour tasks, when stimuli are presented simultaneously.

6.1. Introduction

Previous chapters have indicated that although involved in context dependent odour discrimination in the intact brain, an intact hippocampus is not *necessary* for performance. However, it is possible that this result could be related to the way the task was presented. Until now all odour cues have been presented individually. This successive presentation may have hindered the comparison between odours, and encouraged the rats to learn the significance of an individual stimuli instead of the entire conjunction (Eichenbaum and Fagan et al. 1988). Conversely, changing to simultaneous presentations of the two odours should facilitate comparisons among multiple stimuli, and encourage response choices that are based upon associations(Eichenbaum and Fagan et al. 1988). The hypothesis is that by encouraging the use of more relational / configural representations, the task will become more dependent upon the hippocampus.

Previous studies have suggested that altering task demands can alter the type of memory processing employed. In 1986, Eichenbaum et al observed that odour stimuli presented successively were easier to learn than those presented simultaneously. Furthermore, rats with fornix lesions were not only **unimpaired** at a successive cue go/no go odour discrimination, but actually learnt the task **quicker** than sham animals. Thus perhaps the successive variant of the context dependent odour discrimination task used until now was not the best for enlisting the hippocampus. By contrast, when Eichenbaum et al (1986) presented the same odours simultaneously, (with a movement towards the positive odour indicating the choice), an acquisition impairment *was* observed for the fornix lesioned animals. Thus,

perhaps simultaneous presentation of the odour stimuli in this context dependent task will lead to a similar post-lesion impairment?

Driscoll et al 2005 observed similar performance differences in their visual watermaze task. For this task, the rats had to move one of two visual cues (patterns) at the end of a trapezoidal tank. In their elemental task, each stimulus was uniquely paired with either reward or no reward (i.e. $A^{+}B^{-}$, $C^{+}D^{-}$, $E^{+}F^{-}$). Post-training hippocampal damage caused a significant performance impairment in this task. Their second task was a transverse patterning problem. In this task, all stimulus elements appeared as 2 different stimulus pairs, which were rewarded in one circumstance but not in the other. ($A^{+}B^{-}$, $B^{+}C^{-}$, $C^{+}A^{-}$). This variant was found to be even more dependent upon the hippocampus, with lesioning impairing both post-surgery performance and relearning. Without acknowledging the relationships between the stimuli, it is not possible to learn this complex task. Thus although the successive version of my experimental task revealed only an initial deficit, the simultaneous addition of a second pot, may make the task more hippocampal dependent.

Consequently, this evidence suggests that although animals without a functioning hippocampus may be able to perform a one pot version of the task (as shown previously), a two pot version may be more challenging.

6.2. Materials and Methods

A lot of the methods for the simultaneous (2 pot task) were very similar to those used in the previous chapters of this thesis for the successive (1 pot task). For that reason, I will overview the methodology and only go into detail on anything that has changed.

6.2.1. Subjects and Housing

New subjects were used for this chapter. These were 8 Male Lister-hooded rats (Charles River UK) weighing 250-300g at the start of pre-surgery training. They were housed in groups of 4 before surgery, and either singly or in pairs post-surgery depending on whether another rat was at the same stage of training. They were kept on a 12h light/dark cycle with ad libitum access to water, but food restricted to 90% of their free feeding weight.

All procedures were carried out in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986 and European Communities Council Directive of 24 November 1986 [86/609/EEC], and all efforts were made to minimise suffering.

6.2.2. Apparatus

Contexts

The training took place in the same testing rooms as before and the contexts were the same black and white contexts as described for the successive task (see chapter 3). These were two structurally identical circular (76cm diameter) black wooden platforms, with clear polycarbonate walls (40cm high) and four evenly spaced clear sliding doors. Fabric was placed behind the polycarbonate to colour it (black or

white). The whole apparatus sat approx 75 cm above floor level on a frame. However, for this new odours task, 2 new floor inserts were constructed and affixed to the platform floors using Velcro. A white floor insert was constructed from a circle of thick card, with soft non-slip tray covering affixed to one side. The whole insert was then covered in white wood-grain effect sticky backed vinyl. The black floor insert was a circle of thick card covered in black sticky backed vinyl to make a hard surface. Crosses of Velcro (x8) were evenly spaced around the perimeter of the inserts (approx 2 cm from the edge) to allow for the attachment of pots of scented sand (see Figure 6.1). The pots were always presented in pairs for this task, and these pairs could be positioned in 4 different locations (the same as in the successive task). These are shown by the numbers round the circumference of the circle in Figure 6.1.

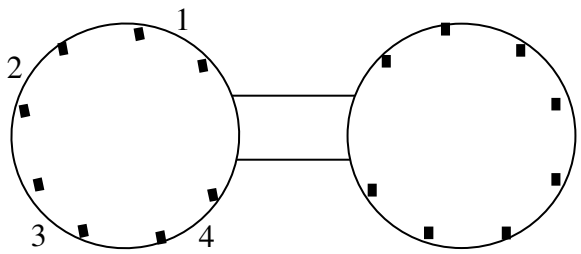


Figure 6.1: Schematic illustration showing the positions of the 8 crosses of velcro on the floor inserts. The numbers show the 4 possible positions for the pairs of pots.

The two circular platforms were connected in the centre by the same bridge as used in the pilot study and the same two barriers used to control access to the platforms. The entire apparatus was surrounded by thick black curtains to minimize extra-maze cues, with an additional piece in the middle to prevent the animals from observing one context while located in another.

Odour Pots

The reward pots were the same clear Nalgene pots filled with playsand (150g) and herbs (0.8g mint or basil) as used in the pilot study (see chapter 2). Buried chocolate weetos (Nestle) were used as the food reward. In this chapter, 2 odour pots were always presented next to each other for each trial – 1 was rewarded, the other unrewarded.

6.2.3. Behavioural Protocol

Handling

Rats were habituated to handling for 5 days (5mins/day) before training. Furthermore, during this time the rats were taught to dig by giving them pots of unscented sand in their homecages within the animal housing room. These pots contained chocolate wheeto cereal rewards and the animal's food ration. Each pot was affixed with Velcro to a wooden base for stability. The animals were group housed at this stage, so 1 pot per rat was placed in cage.

General Protocol

Testing took place on weekdays (Mon-Fri) between 8.30am and 8.30pm (as previously).

The rat was carried from the animal housing room into the testing room in its home cage. To move the rat to the apparatus it was placed into the same transferral container (blue bucket) as before. It was then placed on the centre of the bridge between the two barriers ready to begin the trials. At the end of the testing session the rat was carried in the transferral container back to it's homecage.

The experimenter remained within the curtained area at all times during the task, but their exact location was unpredictable.

Habituation, Shaping and Pre-Training.

Habituation, shaping and training was carried out as described in the training section of Table 6-1. Rats were first habituated to the empty platform (15 mins, 1 session), then given 2 sessions in which they were shaped to dig in unscented pots for rewards (1 pot per trial, 8 trials per session, randomised for position). The experimenter used the barrier to restrict the movement of the rat segregating the session into 'trials'). During the inter-trial interval of 20-30sec the rat would remain on the bridge and the experimenter would remove any spilt sand, before setting up for the next trial.

The final stage of pre-training involved introducing odours to the sand (mixed as describe above). For one session, twelve trials (6 A^+, 6 B^-) were presented to the rats (1 pot per trial). The trials were configured so that each odour was only encountered on one platform (e.g. A only on left platform, B only on right platform) to conform to task rules that would be introduced later (see Figure 6.2)

Training

The initial stage of training was designed to introduce the concept that some odours are rewarded and others are not. Thus, two completely unrewarded foils were introduced (odours X and Y; rosemary and tarragon). The rats received 16 pseudorandomised trials per session, with two pots presented on each trial; i.e. A^+ and X^- on one platform; B^+ and Y^- on the other. Thus rats were trained for 16 trials per session (8 A^+X^-, 8 B^+Y^-) until they reached a criterion of 14/16 trials correct for 2 consecutive sessions.

On all simultaneous trials, the rat began on the centre of the bridge between the two barriers. Two pots of scented sand were placed into one of the contexts. The appropriate barrier was removed to give the rat access to the platform. The rat ran towards the pot, sniffed the odours and chose one pot to dig in. Instantaneously when the rat had started to dig in one pot, the other pot was removed by the experimenter. This prevented the rats from correcting any mistakes they might have made, and encouraged them to discriminate the odours accurately enough to get the trial correct at the first attempt. Correction trials were also used. If a rat dug in an incorrect pot, the correct pot (i.e. the one containing the reward) was quickly removed to prevent the rat rectifying its mistake. The rat was hence left with only the non-rewarded pot on the platform. He was allowed to dig in this for as long as he desired, however, as soon as he turned away, this pot was also removed. The rat was then left on an empty platform – which usually resulted in them returning swiftly to the bridge. The trial was then repeated up to twice more to allow the rat the chance to learn which was the correct odour and hence the rewarded pot.

Table 6-1: Training protocol for simultaneous task

Prior to Habituation, animals get 1 week of food restriction, handling and digging pots in cages.

TRAINING	**Pre 1** 1 Day Habituation to platform	**Pre 2-3** 2 Days Habituation 12 plain pots on platform (All rewarded) 1 pot per trial	**Pre 8** 1 Day 12 dig trials random order A^+ / B^+ 1 pot per trial	**Training** 16 trials In context 1: A^+ X^- In context 2: B^+ Y^- 2 pots per trial	CRITERION 14/16 Correct For 2 Consecutive Sessions
ACQUISITION	16 trials 8 in context 1: A^+ B^- 8 in context 2: B^+ A^- 2 pots per trial	CRITERION 14/16 Correct for 2 Consecutive Days	4 Days 20 trials 8 in context 1: A^+ B^- 8 in context 2: B^+ A^- & 1 randomly placed A/B probe trial per day 2 pots per trial	FULL TASK 24 trials 6 in context 1: A^+ B^- 6 in context 2: B^+ A^- & 12 x E^+ F^- (6 in each context) 2 pots per trial	CRITERION 22/24 correct For 2 Consecutive days
TESTING	4 Days 26 trials 6 in context 1: A^+ B^- 6 in context 2: B^+ A^- & 12 x E^+ F^- (6 in each context) & 1 M/B and 1 E/F probe trial per day 2 pots per trial	**SURGERY**	5 Days + FULL TASK 24 trials 6 in context 1: A^+ B^- 6 in context 2: B^+ A^- & 12 x E^+ F^- (6 in each context) 2 pots per trial	CRITERION 22/24 correct For 2 Consecutive days	4 Days 26 trials 6 in context 1: A^+ B^- 6 in context 2: B^+ A^- & 12 x E^+ F^- (6 in each context) & 1 A/B and 1 E/F probe trial per day 2 pots per trial

Acquisition and Testing of context dependent task

Once the animals had reached criterion performance on the training stage, they were moved onto the acquisition phase where the context dependent odour discrimination task was introduced. (The protocol for acquisition and testing is detailed in Table 6-1 and videos / a PowerPoint file are available to view on the CD in the appendix of this thesis.)

Training for the context dependent task in this chapter did not involve the additional shaping steps (e.g. blocks of 8) that were used for the successive task in chapter 3. Instead training started straight away with trials presented in random order. Nevertheless, the rules were exactly the same as for the successive task (see Figure 6.2). In the white context, odour A is rewarded, but odour B is not; in the black context, odour B is rewarded, but odour A is not. Thus the rats received 16 trials per training day (8 in each context), with 2 pots (1 x odour A, 1 x odour B) presented on each trial.

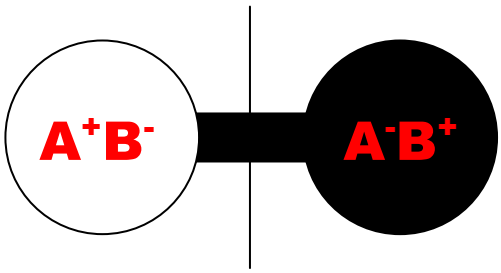


Figure 6.2: Rules of Context Dependent Task

Training continued until the rats reached a criterion of 14/16 trials correct for 2 consecutive sessions, whereupon 4 testing days were run. Testing days were exactly like the last stage of training (8 trials each of $A^{+}B^{-}$, $A^{-}B^{+}$ with up to 2 corrections

per trial), except for the addition of 1 randomly placed odour A or B probe trial. These were used to verify that the animals were using the scent of the sand to solve the task and not detecting the scent of the cereal reward itself. Probe trials were presented as normal (i.e. two pots presented, one odour A, one odour B), however no cereal reward was available within either pot. The reward was only dropped into the correct pot after the animal dug for 5 sec. If the animal got the probe trial wrong, it did not receive any reward and the trial was not repeated (i.e. no corrections on probe trials)

Acquisition and Testing of context dependent task

Once the rats had completed the context dependent testing days, they were moved onto the Full task (a combination of context dependent and context independent tasks). For the context independent task, odour C is always rewarded, odour D is never rewarded , regardless of the context they are presented in (odours are cinnamon and cumin; for rules see Figure 6.3). Again 2 pots were presented together in each context. The context dependent and context independent trials were inter-mixed. The rats received 24 trials per day at this stage (12 Context Dependent (6 each of A^+B^-, A^-B^+), 12 Context Independent (6 E^+F^- in each context) with up to 2 corrections per trials.

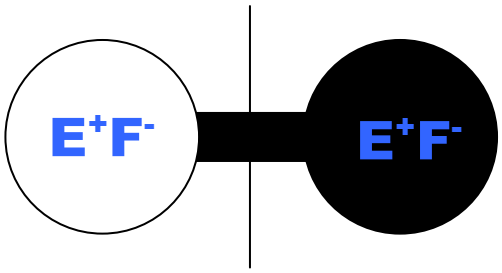


Figure 6.3: Rules of context independent task

Training continued in this way until the rats reached a criterion of 22/24 trials correct, at which point 4 testing days were run. The four testing days followed the same protocol as the full task described above. The rats received 24 trials per session, 12 context dependent trials (6 each of A^+B^-, A^-B^+) and 12 context independent trials (6 x $E^+ F^-$ in each context) with up to 2 corrections per trials. In addition they also received 1 context dependent probe trial and 1 context independent probe trial per session (i.e. rats performed 26 trials/testing day). The probe trials were set-up to look like rewarded trials, however the reward was not dropped into the pot until the animals had dug for 5 sec. This was to check that the animals were indeed using the odour of the sand to dictate behaviour and not sensing the reward itself.

This marked the end of pre-surgery Training.

6.2.4. Hippocampal Lesion Surgery

Surgery took place the day after the completion of the full task testing days. The rats were given ad libitum access to food and Rimadyl in their water overnight that night. Bilateral ibotenic acid hippocampal lesion surgery was given to 4 animals. The other 4 underwent sham surgery. The protocol was the same as described previously in chapters 2 (see there for further methodological details).

Surgery was done under Halothane (Merial Animal Health, UK) anaesthesia. After exposing the skull, a bilateral craniotomy was carried out using a dental drill. Sham surgery involved piercing through the dura with a needle (23G) to simulate the mechanical damage cause by the needle entry during the lesion's chemical injections.

Lesion surgery on the other used multiple injections of ibotenic acid (Biotechnology, CA) that was dissolved in phosphate buffered saline (pH 7.4) at 10mg/ml following the protocol of Jarrard (1989). Prior to injections, the dura was pierced in the appropriate place with a 23 gauge needle. Then injections of 0.05 or 0.1µl ibotenic

acid were made into each hippocampi at different rostrocaudal and dorsoventral levels (For co-ordinates see chapter 3 section 3.2.4)

Once injections were completed, the area was cleaned and the skin sutured back together across the top of the head. Injections of Rimadyl and saline were given immediately post-operatively to both groups.

The animals were given 14 days to recover from surgery before post-surgery behavioural testing began. Food restriction was re-started 48hrs before testing resumed. During this time food rations were hidden within digging pots in the rats' homecages.

6.2.5. Post-surgery Behavioural Testing

Post-surgery Testing was exactly the same as the full-task stage of pre-surgery and followed the same rules (see Figure 6.2 and Figure 6.3). The rats simultaneously performed the context dependent and context independent tasks (i.e. 24 trials per day: 12 context dependent, 12 context independent, with up to 2 correction trials). The rats performed a minimum of 5 days of training before moving onto probe trials, so that an accurate measure of post-surgery performance could be attained (see Table 6-1).

Once the rats had completed at least 5 days and were again performing at criterion level (22/24 trials correct for 2 consecutive days), 4 testing days were given. These were exactly the same as those given immediately before surgery (26 trials, 12 context dependent, 12 context independent, 2 probe trials per day.)

6.2.6. Histology

Histological analysis was done straight after post-surgery testing was completed, and was carried out in exactly the same way as described in the pilot study (chapter 2). Rats were euthanised using Euthatal (Sodium Pentobarbitol, Merial Animal Health, UK). They were then perfused intracardially using first saline and then 4% formalin. The brain was then removed and fixed with 4% formalin for at least 24hrs. The fixed brains were embedded in egg yolk. 30µm coronal sections were cut using a cryostat, with every fifth section mounted onto gelatine coated slides and stored for histological analysis. The slides were stained with cresyl violet acetate and mounted with DPX. (The protocol for this is included in the appendix of this thesis)

The extent of the lesions was assessed by transferring an image of each slice to the computer using a camera (Leica), mounted on a makroscope (Wild M420, Switzerland) and the Lecia Qwin Program. The area of spared tissue for each image was calculated using Image J for each animal. 3D reconstructions of the lesions were then performed using 3D doctor (version 4.0.061025)

6.2.7. Data Analsyis

Data analysis was done using SPSS as is described in the pilot study (see chapter 2). All numerical values and graphs are reported as ± SEM. Criterion was 80% and is marked with a black horizontal line on the graphs (where appropriate).

ANOVA's were used throughout, with the Mauchly sphericity test used to check homogeneity of covariance.

Independent samples t-tests were used to compare the performance of the two groups on individual days and on mean days to criterion.
Paired samples t-tests were used to compare the performance of the rats on the context dependent task compared to the context independent task.

Bonferroni adjustments for multiple comparisons were made as required.

Pearson's correlations were used to investigate the relationship between lesion size and behaviour.

6.3. Results

8 animals were trained on this protocol. 4 were given full hippocampal lesion surgery and 4 sham surgery. The results of the simultaneous (2 pot) task were very similar to those for the successive (1 pot) task in chapter 3.

6.3.1. Acquisition of Task

All animals successfully acquired both the context dependent and context independent tasks.

The simultaneous (2 pot) protocol was an effective way of training the animals with the rats taking an average of 22± 2 days to reach criterion performance. An example acquisition graph for this protocol is shown in Figure 6.4. In this example the rat took 21 sessions to complete pre-surgery training (from the start of the context dependent task to end of full task testing days). The rats took less time to learn the simultaneous 'choice' task than they did to learn the successive 'go/no go' task (Average days to criterion for successive: 60 ± 5; simultaneous 22± 2).

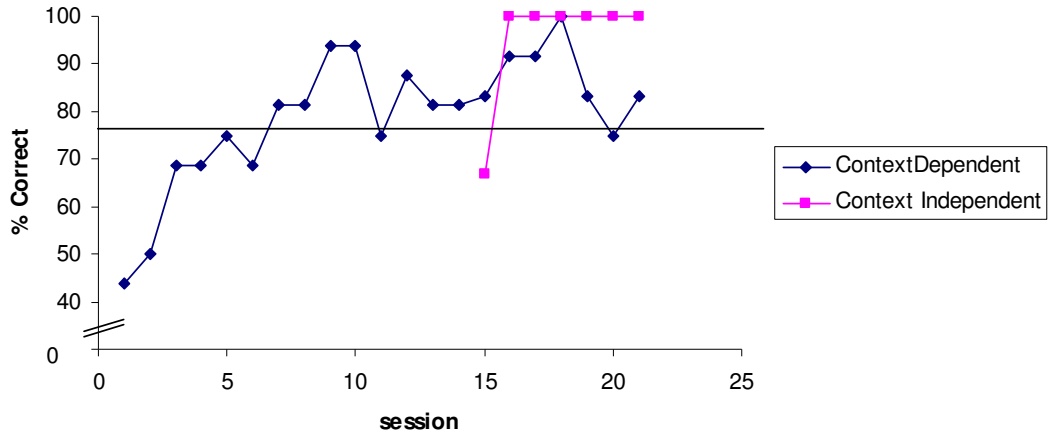


Figure 6.4: Typical performance of a rat trained every day on the protocol. Performance is expressed as % correct.

6.3.2. Pre-surgery Performance on Task

No difference in performance of groups before surgery

The average number of days taken to reach criterion did not differ between the prospective sham and lesion groups ($t_{(6)} = -5.45$, $p = 0.606$).

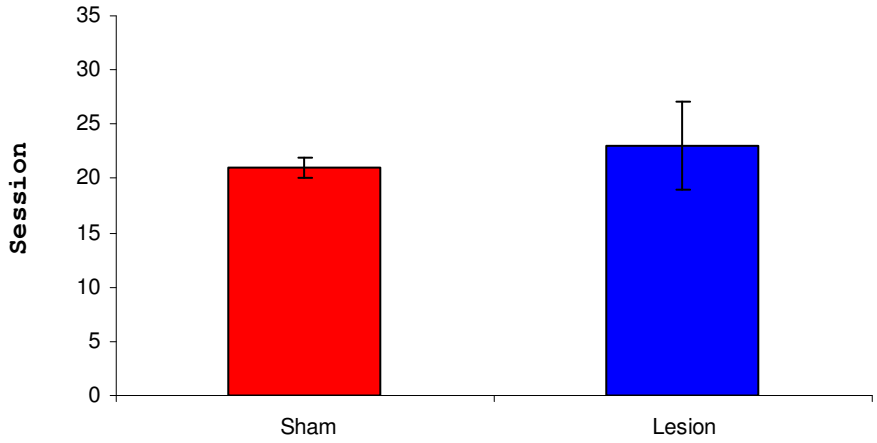


Figure 6.5: Average number of days taken to reach criterion before surgery (counted from the introduction of the context dependent task). Animals are split according to the surgery they would receive (sham n = 4, lesion n= 4).

Once the rats have learnt the tasks they all perform consistently well as shown by the 4 testing days in Figure 6.6. Independent samples t-tests on the context dependent task showed that there was no difference in performance between sham and lesion (to be) groups before surgery ($t_{(6)} = -1.28$, $p = 0.25$). Statistical tests were not performed for the context independent task due to the lack of variability in the data. This reflects the high degree of accuracy with which the rats performed the context independent task compared to the context dependent task. Probe tests indicate that

the rats were not using accessorily odours to solve the task. When combined, the rats performed 32 probe trials between them during this part of the protocol. Only 4 of these were incorrect (2 context dependent task probes, 2 context independent task probe). As there were always two pots available, and the rats were generally performing at around 80% correct, it is not surprising that some probe trials were incorrect. However, the vast majority were performed correctly, confirming that the rats were using the odour of the sand to execute the task, and not using the odour of the reward itself.

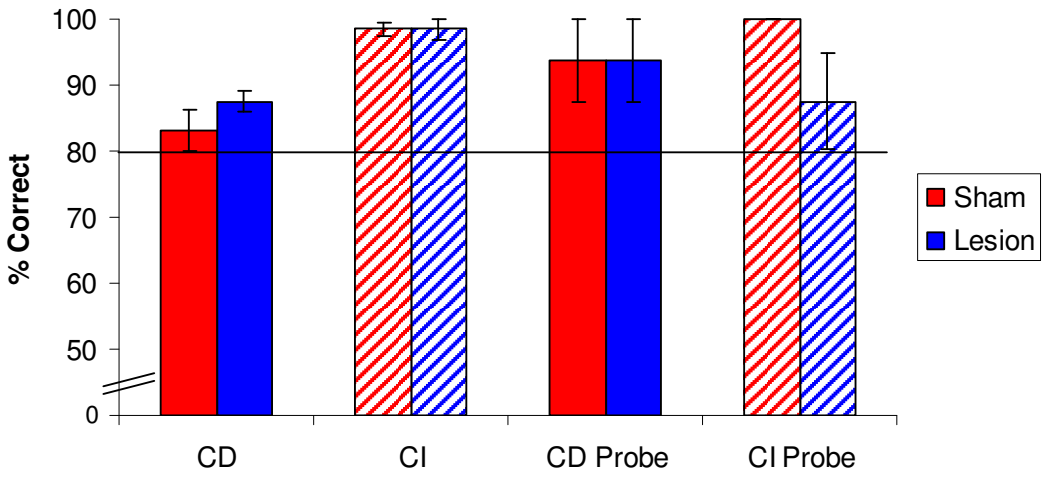


Figure 6.6: Average performance on the four pre-surgery testing days, expressed as % correct. (sham n = 4, lesion n= 4). CD = context dependent task, CI = context independent task. The rats received 12 context dependent and 12 context independent trials per session, as well as 1 context dependent probe and one context independent probe.

6.3.3. Context dependent vs context independent tasks

The Context Dependent task took longer to acquire than the Context Independent task

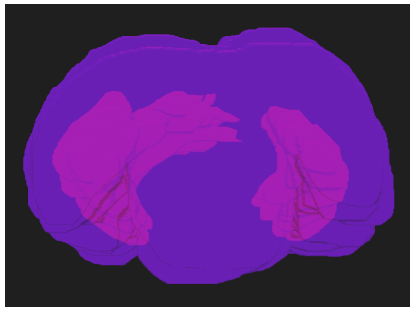
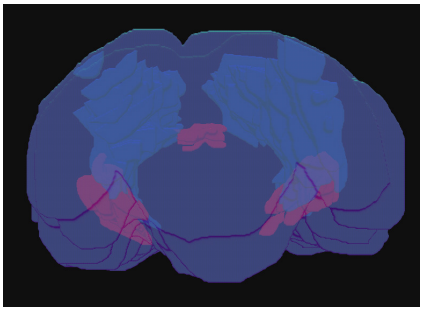
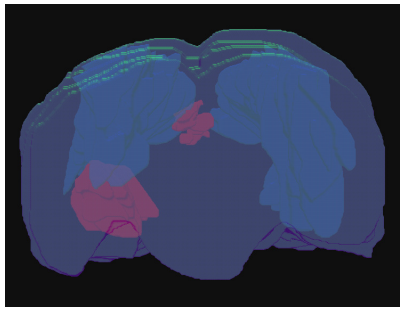
The context independent task is a simple discrimination task, where context is irrelevant. Rats can learn this distinction very rapidly. All rats took longer to learn the context dependent task (see section 6.3.1) and perform it accurately (section 6.3.2) than they did on the context independent task. This is reflected in their performance throughout this chapter, in acquisition, testing and post-surgery performance. Once learnt, the performance on the context independent task was usually around 100% correct, whereas for the context dependent task it was only around 80% correct.

6.3.4. Histology

The size of lesions was variable

The lesions varied in size with hippocampal sparing ranging from 3% to 53%.

Table 6-2: **Lesion size and 3D reconstruction**. purple = cortex shape and outline, red = sparing, blue = area devoid of cells.

Lesion Reconstructions for Simultaneous Task			
% Sparing		% Sparing	
53%		14%	
13%		3%	

The set of Surgical Co-ordinates designed for larger rats were successful in lesioning the majority of the hippocampus.

In chapter 3, the issue of surgical co-ordinates was discussed in relation to animal size. The histological results of this simultaneous task give further grounds to support the hypothesis that surgery co-ordinates need to reflect the weight of the rat. This task was easier to learn, and so the pre-surgery training time was reduced. As a result the rats weighed less at the time of surgery (though still more than 400g, so the large rat co-ordinates were still used). In 3 out of 4 rats, the hippocampal sparing was reduced to less than 15%. This suggests that there is nothing wrong with the surgery co-ordinates per se, they just need to be adapted to suit the weight of the rat. The final lesion was inexplicably very incomplete at 53% sparing. The sparing was mainly in the central to ventral regions of the hippocampus, and was especially pronounced the in the deepest most inferior areas.

Extra-hippocampal Damage

The entorhinal cortex showed some damage in all lesioned rats. The subiculum and post-subiculum showed extensive bilateral damage, that was consistent in position across the lesioned group. As in previous chapters, cortical damage was sustained to the regions above the hippocampus and was observable as a hole upon removal of the brain.

6.3.5. Post-surgery Performance on Task

Initial deficit in performance of hippocampal lesion group on context dependent task post-surgery.

On completion of the pre-surgery training, the rats were given either hippocampal lesion surgery (n = 4) or sham surgery (n = 4), and given 14 days to recover. They were then re-introduced to the 2 tasks (context dependent and context independent) and their performance on the first 5 days of post-surgery testing is shown in Figure 6-7 .

Overview

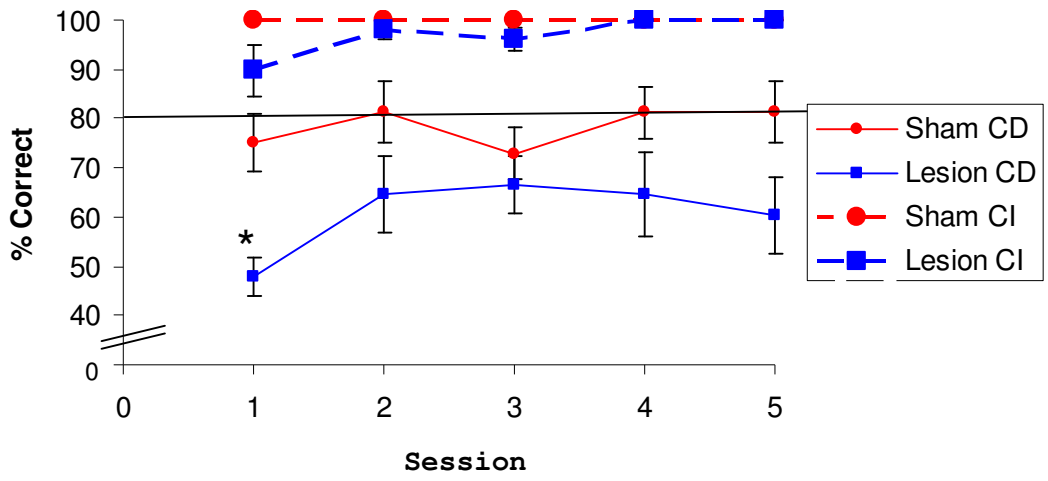
All animals immediately performed the context independent task at above criterion level. This reflects the ease with which the rats performed the context independent task (as discussed above) and confirmed that the rats were not suffering any problems with running, sniffing, discriminating or digging. A repeated measures ANOVA was performed on the context dependent task data. This revealed a significant effect of group ($F_{(1,6)} = 8.60$, $p = 0.026$) showing that the lesioned animals were performing significantly worse than the sham animals over the first 5 days of retesting*. There was no effect of session ($F_{(4,24)} = 1.51$, $p = 0.230$) showing there was no significant improvement in performance within the first 5 days of post-surgery testing. Furthermore, there were no significant group by day interaction ($F_{(4,24)} = 0.976$, $p = 0.439$). Post-hoc simple effects confirmed that the sham and lesion groups performed significantly differently across the first 5 days of retesting ($p = 0.026$) for the context dependent task. The lesion group took time to regain/relearn the context dependent task (i.e. the lesion of the hippocampus impairs initial performance on the context dependent task). Sham animals performed the context dependent task at around criterion level from the first post-surgery day. Thus,

* without animal 3 which had 53% sparing, the statistics for this effect of group rise to $F_{(1,5)} = 26.43$, $p = 0.004$

hippocampal lesioned animals were specifically impaired on the context dependent but not the context independent task (see Figure 6-7).

When the lesioned group was split into those that had small amounts of sparing (3 animals) and those that had large volumes of sparing (1 animal), it can be seen from the graph (Figure 6.8 b) that the animal the rat with a large amount of intact spared hippocampal tissue only behaved like the rest of the lesioned group in the first session. After that its performance was much more similar to that of the sham operated animals. Thus, this animal with an intermediate amount of hippocampal tissue displays an intermediate performance over the first 5 days of post-surgery training.

a)



b)

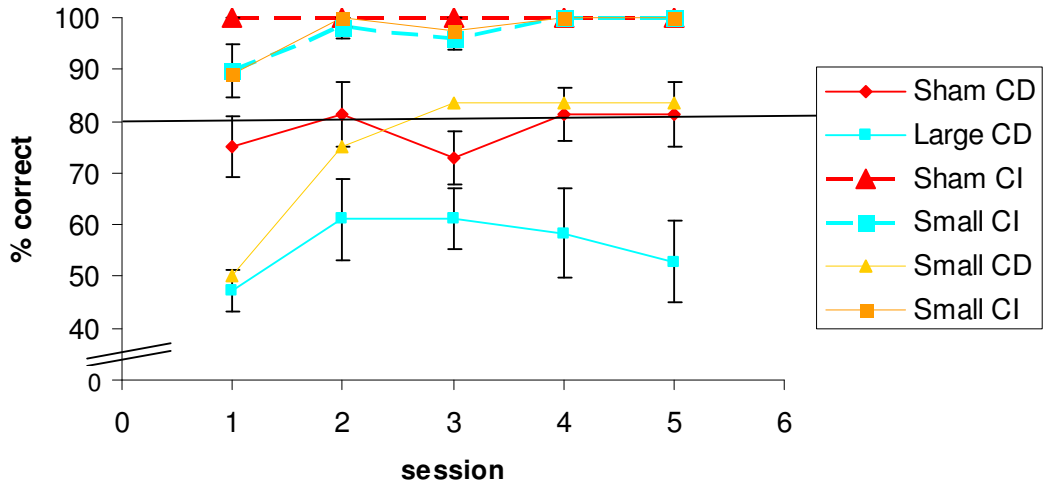


Figure 6.7: Average performance on days 1-5 of re-test expressed as % correct on each task.
a) Animals split into 2 groups – sham (red, n = 4) and average for all lesioned animals (blue, n = 4).
b) Animals split into 3 groups – sham (red, n = 4), rat with large amount of sparing (yellow, n = 1), rest of lesion group (blue, n = 3)

Lesioned animals take significantly longer than sham operated animals to regain criterion performance post-surgery.

Following on from the initial performance impairment seen in the lesion group, it was also found that these animals also took notably longer to reach criterion level again after surgery than sham animals ($t_{(6)} = -2.95$, $p = 0.026$). Lesioned animals took an average of 12±2 sessions to reach criterion level, as opposed to the 4±1 session it took sham operated animals. In addition, the scatter of data on the graph (Figure 6.8) clearly shows that one of the lesioned animals took less time to acquire the task than the others. This was the animal with the smallest lesion (53% spared tissue). The average days to criterion rose to 14±1 when this animal was removed from the analysis.

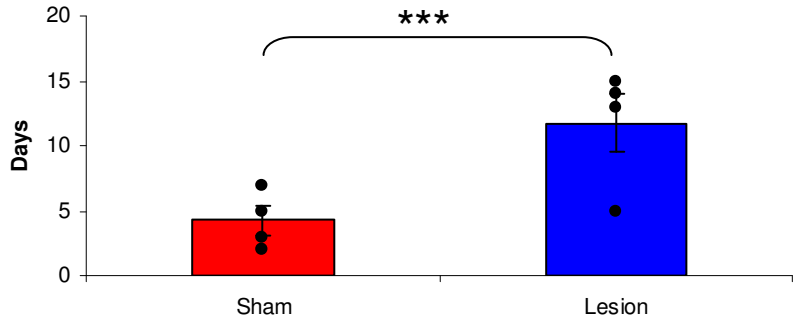


Figure 6.8: Average number of sessions taken to regain criterion on the original task after surgery. (sham n = 4, lesion n= 4)

Consistently high performance in both groups after criterion regained.

Once the animals have re-attained criterion performance on the context dependent task, both groups perform consistently well (see Figure 6.9). Probe tests show the rats are not using the odour of the reward when deciding whether to dig or refrain. Between them the rats receive 32 probe trials, and got only 2 context dependent probe trials wrong.

An independent samples t-test showed that there was no significant difference between groups on their performance of the context-dependent task on the post-surgery testing days ($t<1$)

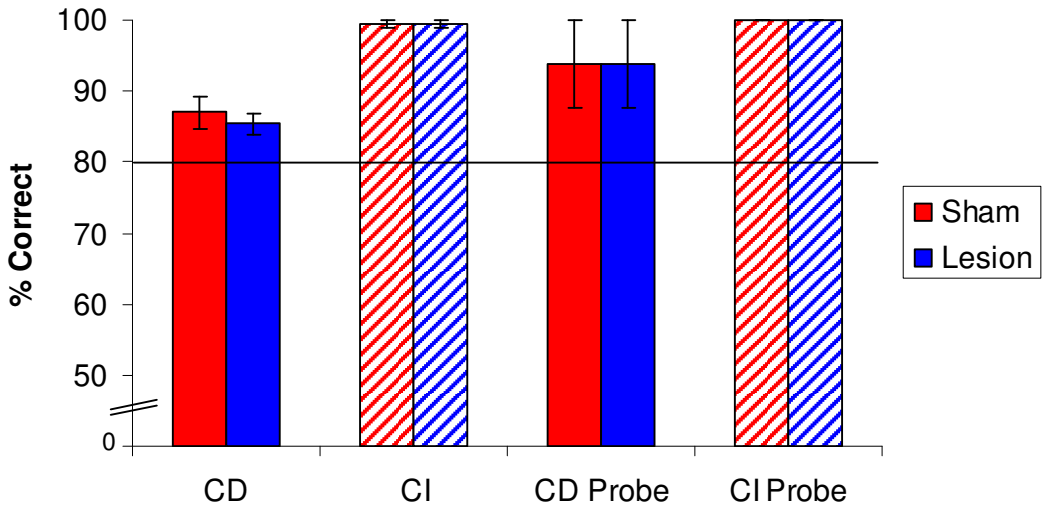


Figure 6.9: Average performance on the original task for the four post-surgery testing days, expressed as % correct. CD = context dependent task, CI = context independent task. The rats received 12 context dependent and 12 context independent trials per session, as well as 1 context dependent probe and 1 context independent probe. (sham n = 4, lesion n= 4).

The next set an analysis compares the pre-surgery acquisition data to the post-surgery data to see if there are any differences in learning rate or final performance level.

Animals take less training to regain criterion post-surgery than they do to learn the context dependent task pre-surgery

A repeated measures ANOVA on the pre and post surgery days to criterion data revealed a significant effect of surgery ($F_{(1,6)} = 94.12$, $p < 0.001$; see Figure 6.10), reflecting the fact that the animals re-attain criterion much quicker post-surgery than they do before surgery. However, no effect of group ($F_{(1,12)} = 3.251$, $p = 0.121$) and no group by surgery interaction was observed ($F_{(1,6)} = 3.251$, $p = 0.121$). Simple effects confirmed that the days to criterion measure differed pre vs post-surgery for both the sham ($p < 0.001$) and lesion ($p = 0.001$) animals.

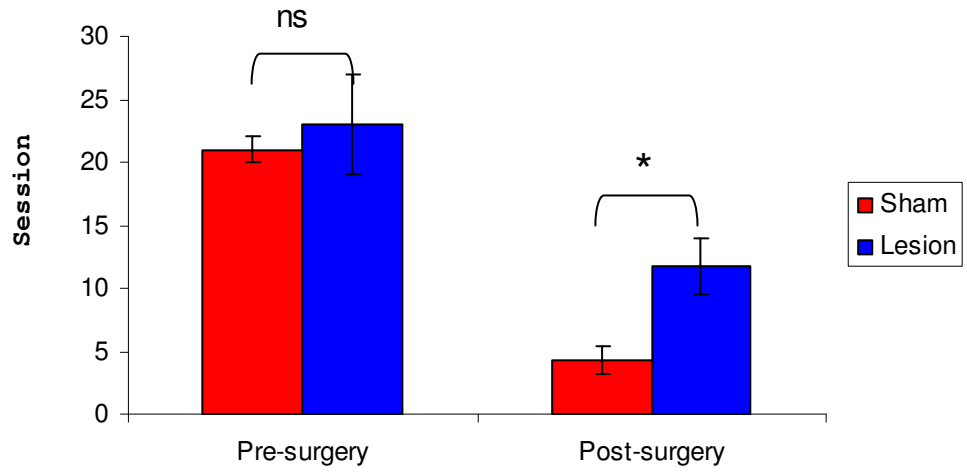


Figure 6.10: Comparing performance on context dependent and context independent task testing days, pre and post surgery. (sham n = 4, lesion n= 4)

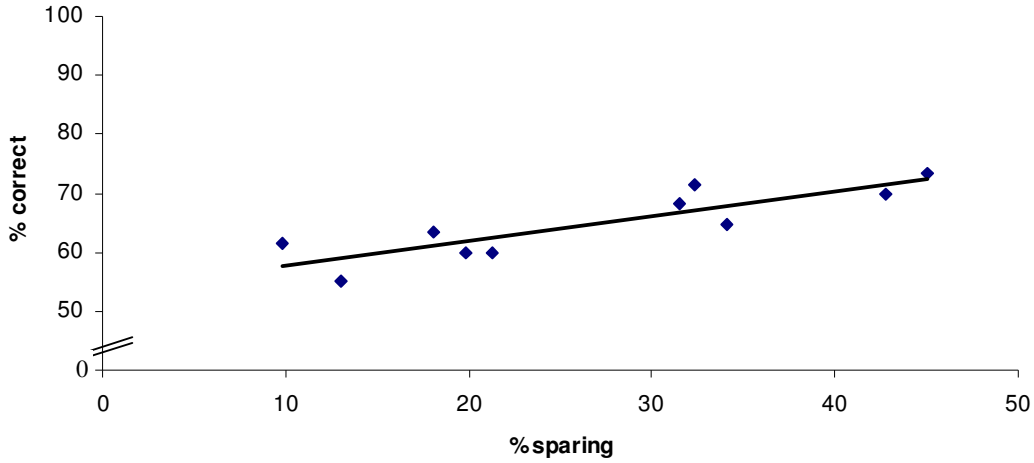
The animals perform just as well on the context dependent task post-surgery as they did pre-surgery.

An overall ANOVA comparing the pre and post surgery performance of the sham and lesion groups for the context dependent task showed no main effect of surgery ($F_{(1,6)} = 1.03$, $p = 0.759$) i.e. once animals had re-attained criterion level, they could perform the task at the same consistently high level as they did before surgery. Furthermore, no effect of group ($F_{(1,6)} = 0.33$, $p = 0.589$) and no group by surgery interaction was observed ($F_{(1,6)} = 1.270$, $p = 0.303$)

6.3.6. Correlation data for lesion size and initial post-surgery performance level.

In order to compare the data fully to that found in chapter 3, some additional examinations were carried out to see if lesion size had any bearing on behavioural performance. A Pearson's correlation analysis between lesion size and performance on the context dependent task averaged over the first 5 sessions of post-surgery training was found to be non significant ($r(4) = 0.92$, $p = 0.076$). This is in contrast to the highly significant correlation found for the successive (1 pot) task in chapter 3 This difference is probably due to a combination of factors including the small number of animals (only 4 in the lesion group) and the fact that lesion size was less variable in this chapter compared to chapter 3. Nevertheless, the angle of the line and the trend is very similar to that found for the one pot task. Furthermore, at 0.076, the significance level is not that far away from the 0.05 criterion level. For this reason I would suggest that, just like for the successive task, the lesion size was also correlated to behaviour over the first 5 days for the simultaneous task (i.e. the more hippocampal tissue that remained, the better the performance on the task)

a)



b)

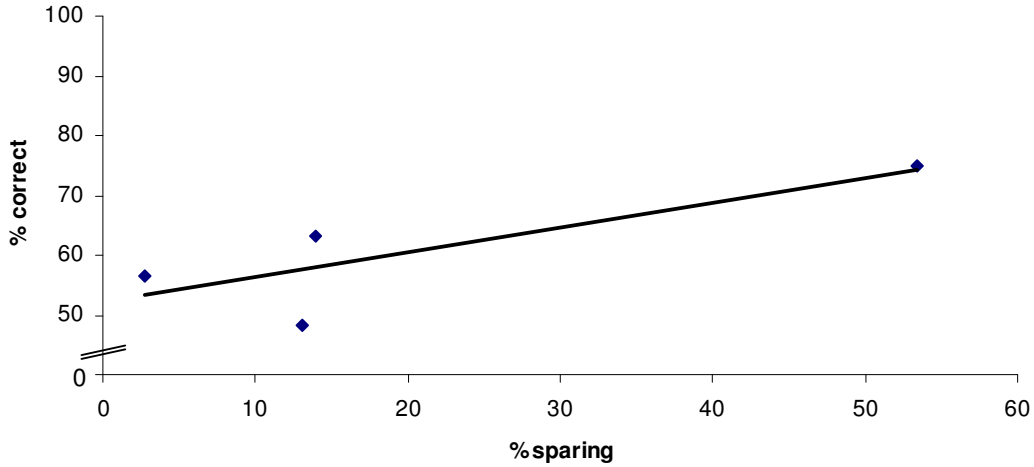
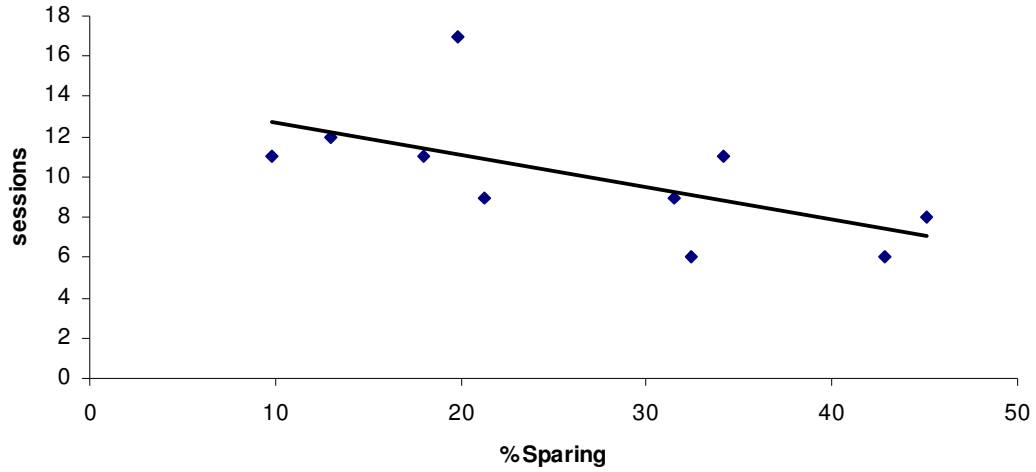


Figure 6-11: Correlation between % sparing and average % correct on over first 5 days of retest. a) for successive task (from chapter 3) b) for simultaneous task (this chapter)

Following on from this, correlations were performed on other aspects of the data. A Pearson's correlation showed no correlation between average days to criterion for the context dependent task and lesion size ($r_{(4)} = 0.82$ $p = 0.182$). However, again the angle of the line and the similarity to the results from chapter 3 suggest that the

larger the volume of spared hippocampal tissue, the better the behavioural performance (in this case, the quicker criterion was regained on the context dependent task). This interpretation must however with a little more caution than the initial impairment correlation, as no significant correlation was observed between days to criterion and lesion size in chapter 3 ($p = 0.06$).

a)



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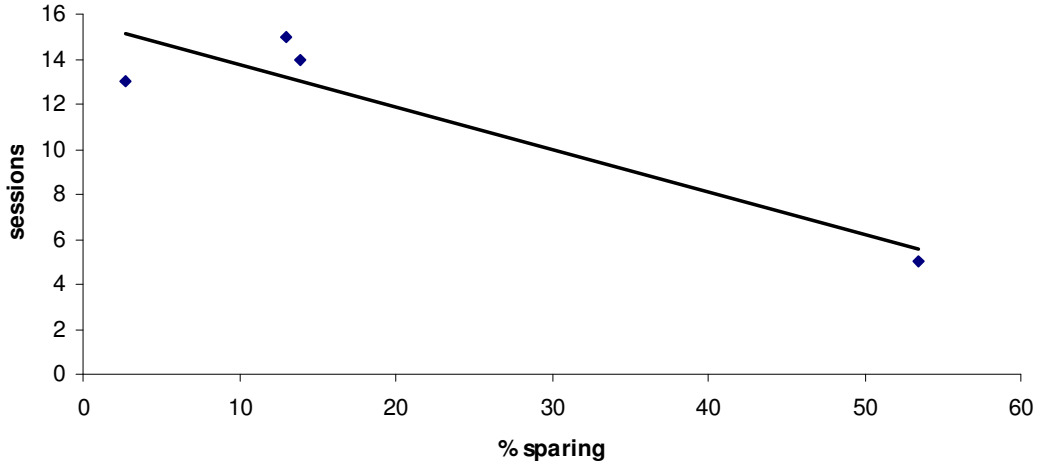
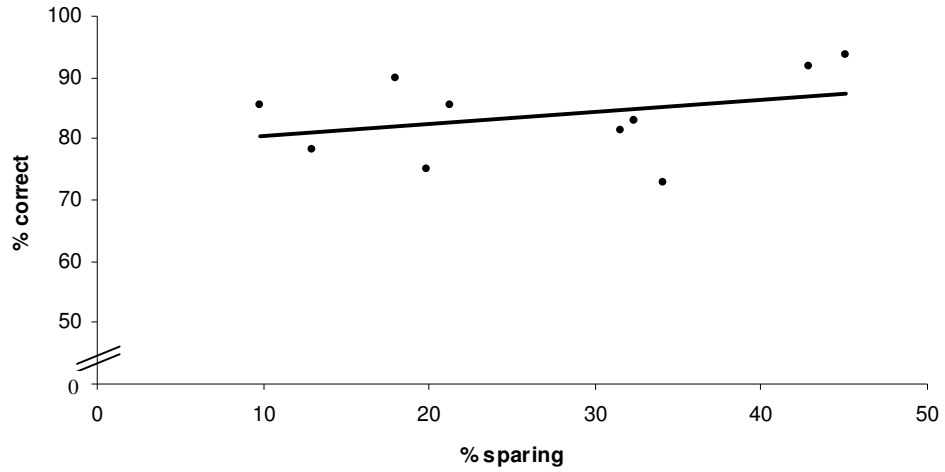


Figure 6-12: Correlation between % sparing and days to criterion for context dependent task post-surgery. a) for successive task (from chap 3) b) for simultaneous task (this chapter)

A final correlation was performed on the post-surgery testing data for the context dependent task. No correlation was found between performance and lesion size ($r(4) = 0.23$; $p = 0.773$). Thus, as in chapter 3 once criterion level has been regained, all animals are performing the task at the same consistently high level – regardless of the size of their hippocampal lesion.

a)



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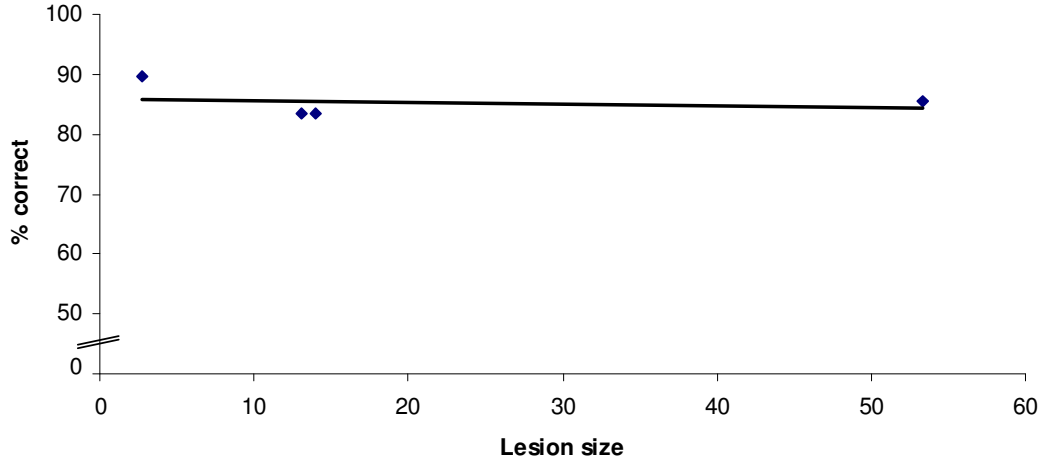

Figure 6-13: Correlation between % sparing and performance of context dependent task on post-surgery testing days a) successive task (from chap 3) b) simultaneous task (this chapter)

6.4. The simultaneous tasks displays the same pattern of results as the successive task

The results of the simultaneous version of the task demonstrate the same general trends as observed for the successive version described in chapter 3. Removal of the hippocampus causes an initial impairment in performance of the context dependent task, indicating some hippocampal involvement. However, this impairment is overcome by further training, and soon the lesioned animals can again perform as well on the context dependent odour discriminations as the sham operated ones. Furthermore a trend for lesion size to affect behaviour was observed. In general, the more hippocampal tissue spared, the easier the animals found initial training post-surgery and the faster the lesioned animals regained criterion. However, there were differences. The simultaneous discrimination task seems to be simpler and was certainly learnt more quickly than the successive discrimination task. Conversely, by merit of having 2 pots present, there were more errors on the probe trials. Similarly, the initial post-surgery impairment in performance seemed to be longer lasting for the simultaneous task than was observed for the successive task.

Conversely, the context independent task was very rapidly acquired, irrespective of parameters used to teach it, and hippocampal lesions had no effect on its expression.

Overall, the simultaneous task produced a very similar pattern of results to the successive task.

6.5. Discussion

The main result of this experiment is that the hippocampus *contributes* to the retention of this simultaneously presented context-dependent odour discrimination task if it is present, but a full hippocampus is not *necessary* for accurate performance. As the hippocampus was present during training, a temporary retrograde amnesic effect was observed post-lesion. The degree of this impairment tended to increase according to the volume of hippocampal tissue that was removed.. Nevertheless, with time and further training it was possible to overcome this impairment and for performance level return to normal.

No such impairment was observed on the concurrently run context independent task.

6.5.1. Comparison to previous data

Conversely to what was expected, the results in this chapter for the simultaneous (2 pot) task bear a striking resemblance to those observed when the stimuli were presented successively (i.e. 1 pot at a time; chapter 3 and the task development chapter 2). An initial post-surgery impairment in the context dependent task was observed, followed by a quick recovery back to criterion level, where performance was sustained. The context independent task however, was never adversely affected by the hippocampal lesion and performance was always above criterion level. Thus, these data add to assertion that this task is very robust and highly repeatable. Every lesioned animal demonstrated the same trends in behaviour following hippocampal lesion, irrespective of how the task was presented.

The successive and simultaneous tasks were similar in many ways. They were identical in cue and reinforcer modality (two odour discriminations and food reward respectively), and they both engaged reference memory capabilities (the ability to

hold or maintain information relevant to task performance, which does not change across trials). However, one big difference between the tasks was the time required for successful acquisition. The simultaneous task was learnt much more quickly than the successive go/no go version (average 22 days for simultaneous, 60 days for successive). Thus it seems (at least for this task) that choices are easier to acquire than the interruption of an ongoing behavioural response.

6.5.2. Appraisal of functionality of spared hippocampal tissue

In contrast to previous chapters, 3 out of the 4 hippocampal lesioned rats used for this task had large hippocampal lesions with very little sparing (<15%). This demonstrated quite clearly that the animals could indeed perform this task in the absence of the hippocampus. Even the rat with only 3% sparing managed to re-acquire and sustain above criterion performance on the context dependent odour discrimination task. It is highly unlikely that the 3% spared hippocampal tissue was supporting this behaviour alone. One final rat had large volumes of central-ventral sparing (53%). This added an extra dimension to the data, with this animal consistently performing at an intermediate level between that of the sham and lesions during post-surgery training – suggesting that the spared tissue did confer some behavioural advantage. Although the spread of lesion size was not sufficient to lead to statistically significant correlations, the presence of this small lesion animal allowed the trends to be explored within this simultaneous task data. Thus, just as for the successive task data (chapter 3) it as found that larger volumes of spared tissue resulted in improved initial post-surgery performance on the context dependent task and a quicker return to criterion level performance. As ibotenic acid lesions spare the fibres of passage within the hippocampus, it is highly likely that the spared tissue was at least partially functional, and the projections to and from it would have been intact. Thus, just as Moser and Moser (1998) demonstrated for the watermaze, these data show that the hippocampus is involved in retention of this context dependent odour discrimination task, even if it is not absolutely necessary for accurate performance.

One factor that does remain however, it is that 100% lesions were not achieved in this study. Thus, although highly unlikely, it is possible that the small volumes of residual tissue were sufficient to support task performance and that should that remaining 3% for example, have been removed a more long lasting impairment may have been observed in this experiment.

6.5.3. Multiple Memory Systems

Within the brain, several different systems act together to produce what we term 'memory'. Each system has different rules and processes. The hippocampal system is thought to be specialised for encoding large volumes of relational information, whereas the non-hippocampal systems encode more simplistic links between prominent stimuli. It was hypothesised that the change from successive to simultaneous presentations of the odour pots in this task would lead to a form of input that was more attune to the processing style of the hippocampus and less in line with the other systems. Indeed, Eichenbaum et al (1988) demonstrated that fornix lesioned animals were more impaired at acquisition of a simultaneous task that emphasised comparisons than a successive task that emphasised individual stimuli. However, although that might be the case for acquisition in Eichenbaum et al's (1988) task, it does not seem to apply to retention in this context dependent odour discrimination task. The lesioned animals displayed exactly the same pattern of behaviour regardless of how the task was presented.

Caution must be taken however in comparing this context-dependent biconditional task, to simple discrimination tasks like that of Eichenbaum et al (1988). Although on the surface they may seem very similar, a straight-forward discrimination task (A^+, B^-) is much easier to learn and may not require the hippocampus for retention. For example, Jonasson et al (2004) demonstrated that bilateral neurotoxic lesions of the hippocampus had *no* effect on retention of a simultaneous (2-pot) forced choice

discrimination paradigm. This task was very similar in style to the one used in this thesis. It used pots of sand scented with herbs and spices, with a buried food reward. However, as with Eichenbaum et al (1988) it was a simple discrimination task (A^+, B^-), thus was perhaps more akin to the context independent task in this thesis, than the context dependent task. A similar *lack* of impairment after hippocampal lesions was found for a two odour discrimination task by Kaut and Bunsey (2004). Thus, simply presenting the odours simultaneously does not seem to be enough to promote hippocampal involvement. Indeed, in this chapter (and throughout the thesis), the concurrently run context independent task (C^+, D^-) showed absolutely no effect of hippocampal lesions. Thus simply changing the method of presentation was not sufficient to change the demands of the task.

A further difference between this task and that of Eichenbaum et al (1988) is its biconditional nature. In the odour ports task (Eichenbaum et al 1988) the animals had simply to give a positive (or withholding) response to one odour stimuli. By contrast, in this task, the animal had to integrate the odour stimuli not only with its reward status but also with a 'context' cue. This ensured the task was configural and (in theory at least) would have pushed towards hippocampal relational encoding. Thus, although this task was designed to play to the integrative strengths of the hippocampus, it nevertheless demonstrates that it is very difficult (if not impossible) to devise a task that engages only one system.

Nevertheless, just because the animals can demonstrate accurate performance, does not mean that they are processing the stimuli in the same way (see chapter 4). Indeed, Eichenbaum, Matthews, Cohen (1989) demonstrated that fornix lesioned and intact animals can actually show a difference in behavioural tactics in an odour discrimination task. Normal animals approached one port, sniffed and either responded (nose poke) or moved on. Fornix lesioned animals, on the other hand sampled both odours before deciding upon a response - a comparative approach. This provides further evidence that different systems may guide behaviour in slightly different ways - even if in the end performance accuracy is similar. (Unfortunately,

this kind of tactical data was not collected for this study, but could offer an interesting avenue for future experimentation).

Overall, the data in this thesis do not support the assumption that changing the method of presentation of a context dependent task affects its dependence on the hippocampal system. Instead it seems that the secondary system that processes the tasks, performs similar computations, irrespective of whether the stimuli all arrive together (within the same timeframe) or discontinuously.

6.5.4. Configural Theory

The revised configural theory of Rudy and Sutherland (1995) has suggested that it is possible to encode configurations within the cortex. For this task, training took place in the presence of the hippocampus, hence the encoding of configurations was promoted, and the associational weights of the elements demoted. Strong configurations would thus have been established in the cortex prior to surgery. Following loss of the hippocampus, the inhibitory signal aimed at the elemental system would be lost, and it could proceed unimpeded. Thus upon presentation of the stimuli, both the elemental and configural systems would become active together and confusion would arise. However, as this is a biconditional task, the neuronal weights of each element are still not sufficient to allow for a solution. With experience, the configural unit with its high predictive value would still win the battle for associative strength. In this way an initial confusion or impairment could be resolved with time and experience – as was seen in this experiment.

6.5.5. Contextual vs Configural

In this context dependent odour discrimination task the contexts were presented as multi-modal stimuli, varying in both 'colour' and texture. Nevertheless, it would still have been possible for animals to build their association upon just one element of the context (e.g. Mint and Black will be rewarded, mint and white will be unrewarded). This kind of encoding would not necessarily have been 'contextual' but would still have required an association or configuration to be made between 'colour', odour and reward status. In the successive task, each odour pot was presented individually, which may have emphasised the encoding of elements. The rats could simply have learnt to approach or avoid a particular set of cues. This kind of encoding would not necessarily be dependent on the hippocampus. The two pot presentation used in the simultaneous task by contrast, would have emphasised relationships between cues and perhaps encouraged the encoding of a more relational representation (Eichenbaum et al 1988). As one hypothesised role for the hippocampus integration of multi-modal stimuli and the encoding of relationships, this second variant of the task was predicted to be more dependent upon relational / configural encoding and thus on the hippocampus. However, this dependence was not observed and exactly the same pattern of results was observed post-surgery for both the simultaneous and successive tasks. Thus, the idea that using a two pot presentation style would encourage the encoding of more complex configural relationships is not substantiated in this thesis.

6.6. One trial vs multiple trial learning

Although this simultaneous version of the task was learnt much quicker than the successive version, a significant amount of training was still required for the animals to reach criterion level before surgery. This training provided a lot of space for rehearsal and may have allowed non-hippocampal systems time to extract build up a representation that was capable of supporting task performance post-surgery. Nevertheless, the data observed was highly consistent between the two tasks, despite the successive (1 pot) task taking nearly three times as long to acquire as the simultaneous (2 pot) task. Thus in terms of experience needed for extra-hippocampal sites to acquire associative strength, it may be less about fixed timescales and more dependent upon the task parameters themselves. Indeed, Eichenbaum, Fagan, Matthews and Cohen (1988) demonstrated that simultaneous go left / go right tasks can be learnt in approximately half the time that it takes to learn a successive go left/go right task or a successive go/no go task (a similar result to that found here). Similarly, it has been shown that fear conditioning to a context (e.g. Bevins 1995, Fanselow 1986) and object-recognition (e.g. Ennaceur 1988, Dix and Aggleton 1999) can be acquired in just one trial. Thus the altered task demands may change the nature of memory processing, and determine the systems capable of executing it. This in turn alters the degree to which the hippocampus is involved in behavioural execution of the task.

6.7. New Learning

The possibility that the animals in this experiment simply relearn the task cannot be completely ruled out. However, considering it took the lesioned animals only 12 sessions (average) to regain criterion as opposed to the 22 sessions (average) it took to learn the task initially this seems unlikely. In addition, new learning data gained in chapter 5 suggests that, at least for the successive (1 pot) task, new learning takes much longer than re-attainment of criterion post-surgery. As the pattern of the results for this chapter are virtually identical to those found for the successive task (chapter 3), there is no reason to think that new learning on this simultaneous protocol would show any divergence in pattern to that observed in chapter 5. Thus it it would be predicted that if new learning should occur in this protocol, it would take longer than the post-surgery re-attainment time observed.

In conclusion, although it cannot be completely ruled out, new learning is unlikely to have occurred for the hippocampal lesioned animals post-surgery in this simultaneous version of the context-dependent odour discrimination task.

6.8. Conclusion

The results obtained with the simultaneous (2 pot) version of this task followed the same pattern as those found for the successive (1 pot) task. Thus contrary to the hypothesised outcome, a change from successive to a simultaneous presentation of odourous stimuli had no effect on behavioural outcome. The hippocampus, although not necessary for performance of this context dependent odour discrimination task, is still implicated in its encoding within the intact brain. Upon removal of the hippocampus, a temporary deficit is observed until a new route to the consolidated information is found. The size of the lesion affects the length and severity of this impairment. The context independent task was completely unaffected by hippocampal ablation.

This context dependent odour discrimination task is very robust and highly repeatable. Despite the fact that the results in this chapter were based upon just 8 rats, they have been shown to be both statistically significant, and highly consistent with those found earlier in this thesis. However, it is recognised that a larger sample size would be required to provide an entirely reliable conclusion.

7. General Discussion

This thesis set out to explore the role of the hippocampus in contextual learning and memory, using a novel behavioural task. It was hypothesised that the hippocampus would be essential for the encoding, retention and behavioural expression of this context dependent odour discrimination task. However, the data has demonstrated that this is not entirely the case. Although, when present during encoding, the hippocampus was subsequently found to *contribute* to retention, an intact hippocampus was not *necessary* for accurate performance of this context dependent odour discrimination task.

The first main finding of this thesis was that the hippocampus plays a role in retention and/or retrieval of a context-dependent odour discrimination problem learnt prior to surgery. Rats with hippocampal lesions showed impaired performance relative to sham animals on the first 5 days of post-surgery testing on the context-dependent odour discrimination problem. The degree of this initial impairment was positively correlated with the volume of hippocampus removed. No impairment was observed on concurrently run context-independent odour discrimination problem, so these deficits could not have been due to perceptual or procedural difficulties.

The second main finding was that an intact hippocampus is not necessary for accurate performance on this context dependent odour discrimination task. With training, rats with hippocampal lesions could re-attain criterion levels on the context dependent task learnt prior to surgery. Thereafter they showed the same consistent accuracy as sham operated animals.

One interpretation of this reacquisition data is that the lesioned animals learnt the context-dependent discrimination task as if it was a new task. Although further statistical comparisons did not rule out new learning, the lesioned animals did appear to show a lower degree of accuracy on the new tasks and took longer to attain criterion on them than was observed post-surgery. Furthermore, no correlation was found between lesion size and new learning performance. Thus it is proposed that

lesioned animals were performing some kind of recall (albeit an impaired one) during post-surgery reacquisition and were not relearning the entire task.

Furthermore, the pattern of initial post-surgery impairment followed by recovery to criterion level is highly robust for this context dependent odour discrimination task – even when task parameters change. After the task development stage, small changes were made to the experimental procedure and the contexts were exchanged for simpler ones, yet here, and right through each batch of animals, the pattern of results never changed. Furthermore, even when the method of presentation was switched from successive go/no go to simultaneous go left/ go right, although the speed of pre-surgery acquisition increased dramatically, the same pattern of temporary impairment and recovery to consistent accurate performance was observed post-surgery.

The next main finding was the sham and lesion rats seemed to solve this task differently. Sham animals appeared to use a combination of self-motion and intra-maze cues. Lesioned animals on the other hand depended upon intra-maze cues. Removal of self-motion cues had no effect on the performance of either sham or lesioned animals. However, when intra-maze cues came into conflict with self-motion cues (i.e. the contexts were located in opposite positions), sham animals displayed a performance deficit, while lesioned animals continued as normal - apparently oblivious to the inconsistency. A recovery of performance for the sham animals was observed when self-motion cues were again removed – despite the still swapped arrangement of the platforms. Thus it was indeed the self-motion cues that caused the conflict.

The final finding is that hippocampal lesioned animals could acquire new variants of the context dependent odour discrimination task at the same speed and perform them to the same degree of accuracy as sham operated animals. This occurred irrespective of whether the new odours were presented in a familiar or novel context.

Overall, this task provided a robust method for investigating hippocampal function

However, the hypothesis that the hippocampus would be necessary for retention of this context dependent odour discrimination task is unsupported by this thesis. Post-training hippocampal lesions did lead to retention deficits on the context dependent odour discrimination task, but these were only temporary and could be overcome by further training. Performance on the context independent task was unaffected by the lesion. Post-surgery acquisition of the context dependent and context independent odour discrimination task was unaffected by the hippocampal lesions.

Nevertheless, rats with hippocampal lesions were found to use more simplistic representations to solve the task and unlike sham operated animals did not respond to conflict occurring between cues that may have been encoded more implicitly (e.g. self-motion cues).

7.1. Developing the Task

The aim of the first section of this thesis was to develop a context dependent task that would be sensitive to hippocampal lesions. In order to study both retention and acquisition, it was necessary to pre-train the animals prior to lesions, and to teach them new variants of the task post-lesion. Chapter 1 of the thesis tracked the development of the behavioural task and the changes required to convert the initial idea into a suitable training protocol. It demonstrated that rats could learn the context dependent odour discrimination task if they were trained in an appropriate way. Furthermore, despite small animal numbers in this initial study (sham n = 2, lesion n = 3), a statistically significant post-surgery behavioural deficit after hippocampal lesions was observed. This implied that the hippocampus may play a role in memory for this task, and justified further investigation. The task development study also examined the ability of the animals to learn new variants of the context dependent odour discrimination tasks post-surgery. Hippocampal lesions did *not* appear to impair new learning, but further investigations were required to substantiate this. (This work was described in chapter 5 of the thesis). Hence the pilot study yielded useful data that set the path for the rest of the thesis.

7.2. Relevance of Lesion Size

The majority of the work in this thesis is based upon the behaviour of rats with partial ibotenic acid hippocampal lesions. The degree of sparing ranged from 3% to 53%. Despite this range not being the original intention, it did offer the opportunity for a correlation analysis.

In terms of retention of the task post-surgery, a high degree of correlation was observed between the volume of hippocampal tissue spared and the initial post-surgery performance level (i.e. the more complete the lesion, the worse the performance average was over the first 5 days of post-surgery testing). This trend was observed for both the successive go/no go task (chapter 3) and the simultaneous go left/go right variant of the task (chapter 6) – although the latter correlation was not significant (probably due to the low animal number in the study). Thus the hippocampus does play a role in retention of this context dependent odour discrimination task.

Nevertheless, with training, all animals did reacquire criterion level performance on the context dependent task. Furthermore, a trend was observed in both chapter 3 and 6 for animals with larger hippocampal lesions to take longer to regain criterion (although the correlations were not significant). Thus spared hippocampal tissue seems to aid both initial accuracy and time to reacquire criterion post-surgery.

However, the volume of spared hippocampal tissue gave no advantage to the animals when learning new variants of the context dependent odour discrimination task (chapter 5). Thus, hippocampal tissue is not vital for accurate performance on this task.

This reacquisition ability in the lesioned rats raises three possibilities:

The first is that the lesioned animals were relearning the task – but data from chapter 5 showed different trends during new learning.

The second possibility is that the spared hippocampal tissue contains sufficient information within itself for the reacquisition of the task (Moser and Moser 1998). This leads to the hypothesis that if the lesions had been complete, the animals would not have been able to re-attain criterion level performance (Moser and Moser 1998). However, this would imply that just 10% spared hippocampal tissue contains sufficient information to support performance on the successive go / no go task and just 3% spared hippocampal tissue is sufficient for the simultaneous go left/go right version. This level of functionality from a tiny volume of spared hippocampal tissue seems unlikely. Furthermore, the small hippocampal sparing was not continuous, but distributed in tiny patches across the area that previously contained the hippocampus. Thus the likelihood of such a high level of spared connectivity and functionality is reduced further still. Thus, in these cases it seems unlikely that the hippocampus was still fully supporting behaviour. It would be predicted that even 'complete' hippocampal lesioned animals would be able to reacquire this task post-surgery.

The third possibility is that the spared hippocampal tissue had some functionality and that this facilitated retrieval of the necessary information from other non-hippocampal areas. In 1995, Rudy and Sutherland suggested that the hippocampus plays two roles. The first is to enhance the activation or salience of configural / conjunctive representations formed in other parts of the cortex and the second is to decrease the salience of their elemental counterparts. If this is the case, then the spared hippocampal tissue in the lesioned animals may have produced a low level output, which aided the configural representation, but nonetheless was insufficient to completely block elemental encoding. The initial post-surgery confusion in the lesion animals may therefore have been the result of dual activation of both configural and elemental representations in the cortex. How active each became may partially

depend upon the size of the facilitation / inhibition signal received from the hippocampus, which in turn would be proportional to the volume of hippocampal tissue spared. Rudy and Sutherland's theory (1995) may also give a theoretical explanation of the recovery to criterion post-lesion with training. As the task is biconditional, the reward properties of the elements are not consistent. With experience / training the configural associations would gain more associative strength than the elements and thus enable the recovery of performance. How quickly this occurred may again depend upon the size of the enhancement signal from the hippocampus. Hence, an intermediate recovery of performance is observed for partially lesioned animals and the speed of this recovery is proportional to the volume of hippocampal tissue spared.

Another theory that may account for the partial lesion data is the Multiple Trace Theory (Nadel and Moscovitch 1997). This theory states that the hippocampus automatically encodes each experience as a new memory trace, such that in these experiments where time and repetition is abundant during pre-surgery training, each combination (i.e. odour A in the black context is rewarded) would be recorded as many memory traces. However, each of these traces would 'point to' and activate the same areas in the cortex. As the hippocampus is acting as the linking factor between several disparate parts of the cortex, it is always essential and required for recall. However, if each memory trace acts as an 'access road', then by destroying only part of the hippocampus, only a proportion the routes to the cortex would become useless. The others would still be viable and hence still be able to support the memory. The more hippocampal tissue was damaged, the more traces would be lost and the less chance there would be of finding a useful index to all the required parts of the cortex. However, where this theory comes unstuck is when it comes to explaining why the lesion effect is only temporary. If the linking traces are gone – no recovery should be possible, unless new learning occurs, (and that was branded as unlikely above).

Nevertheless, a further dichotomy exists within the data. Although chapter 3 and 6 suggested that the hippocampus played a role in the retrieval of this context dependent odour discrimination task, the data in chapter 5 demonstrated that accurate

and timely new learning can occur even when large portions of the hippocampus are absent (see also Moser et al 1995, 1998). At first these results seem incompatible - does this task require the hippocampus or not? However, this issue may be partially resolved using a theory from Moser et al (1998). This suggested that the volume of hippocampus required for retrieval of information and performance on a task is not fixed (Moser et al 1995). Instead it reflects the volume of the hippocampus present when the task was acquired (Moser and Moser 1998). If the hippocampus is intact, the normal rat will distribute information into a widespread network of hippocampal cells for encoding. When it comes to retrieval, Moser et al (1995) have suggested that 70% of dorsal tissue must be present to maintain performance. If on the other hand, only a small slab of hippocampal tissue is present during training, information will only be distributed to this small area. Subsequently, a reduced area becomes sufficient for later retrieval. One quarter or less of the hippocampus may be sufficient to allow encoding of a representation (Moser et al 1995). Thus in chapter 3, as the task was encoded with an intact hippocampus, a large volume of sparing was required for normal retrieval. However, as the lesion was already performed and the hippocampus already reduced, the encoding of the new tasks in chapter 5 was simply packed into the smaller spared area. Later retrieval could also proceed from these small regions. Nevertheless, although spared hippocampal tissue could be supporting performance, for the above theory to hold true for the data in chapter 5, new learning would have had to be encoded into a tiny volume of tissue – in one case just 10% of the original hippocampal volume. This level of functionality seems unlikely for a small distributed volume of sparing.

A more likely explanation for the conserved ability of the lesioned rats to learn new variants of the context dependent odour discrimination task is that they used extra-hippocampal regions to encode the specific task associations. This assumption is backed up by the data in chapter 4, which showed that the lesioned animals showed a different approach to the task. The fact that their representations were shown to be highly dependent on intra-maze cues and to be unaffected by conflict with self-motion cues suggests the rats had a simpler representation of the context, contusive to a non-hippocampal method of task resolution. Thus, lesioned animals were not

necessarily using a representation of 'context' for this task, but may instead have been associating the odour reward properties to just one element of the context. Nevertheless, although not necessarily contextual, this task was still configural. Thus the ability of the lesioned animals to perform this configural task is consistent with the suggestion by Rudy and O'Reilly (2001) that the neocortical system can configural representations without the hippocampus, albeit at a slow rate.

7.3. Comparison with Context Fear Conditioning

Contextual fear conditioning forms the basis of much of the current knowledge regarding contextual processing. The next section will describe how the results in this thesis compare to those with the context fear conditioning literature.

The hippocampus is necessary for retention of contextual fear

Post-training lesions resulted in performance impairments in both the simultaneous (chapter 3) and successive (chapter 6) versions of this context dependent odour discrimination task. This matches the result found within the contextual fear conditioning literature (e.g. Anagnostaras and Maren et al 1999, Lechmann and Lacanilao et al 2007). Associations / configurations formed with an intact hippocampus require a large percentage of the hippocampus for subsequent retrieval (Moser and Moser 1998).

However, this result is in opposition to that of McNish and Gewirtz et al who found no retention impairment for fear potentiated startle (1997) or contextual blocking (2000). They had suggested that the impairment in contextual fear conditioning was due to an interference with the expression of the freezing behaviour (see also Winocur et al 1987, 1997). However, the results in this thesis demonstrate that impairments can occur even when the fear stimulus is removed from the experiment and behavioural expression is measured in a completely different way.

The hippocampus is not absolutely necessary for the acquisition of contextual fear

Lesioned rats learnt new versions of this context dependent odour discrimination task at the same speed as sham operated animals. Subsequently, after reaching a criterion levels of performance, all animals continued to perform at the same consistent and accurate level on the task (chapter 5). Similarly, no deficit in acquisition of contextual fear conditioning is observed following hippocampal lesions (Maren and Aharonov et al. 1997; Gisquet-Verrier and Dutrieux et al. 1999; Anagnostaras et al 2001). It is hypothesised that instead of forming a configural representation of all the cues that make up the context, the lesioned rats instead condition their responses to one element of the context – a method that appears to be independent of the hippocampus. Thus, it appears that an intact hippocampus is not required for acquisition of either contextual fear or context dependent odour discriminations.

The hippocampus is more involved when contextual complexity is high.

Moses et al (2007) found that for contextual fear conditioning, a significantly greater impairment in freezing was observed when animals with hippocampal lesions were conditioned in a complex context instead of a simple context. Although context complexity itself was not tested in this thesis, the results were seen to similar between two different sets of apparatus. During the task development phase (chapter 2), the contexts were composed of two flat square black platforms surround by an array of context unique distal cues. For the rest of the thesis (Chapters 3 – 6), the contexts were round platforms with high walls, distinguishable as separate contexts by their intra-maze 'colour' (black / white) and their floor texture (hard / soft). Nevertheless, the same trends for impaired initial post-surgery performance followed by recovery to criterion level, was observed on both sets of apparatus. Similarly, neither set of contexts impaired the rat's ability to acquire new versions of the task post-surgery. Thus, for this context dependent odour discrimination task at least, changing the apparatus does not appear to change the experimental results. Nevertheless, it is possible that differences may have been observed if the contexts had been made more complex or overlapping. In these circumstances, conditioning to

one aspect of the context would not have been sufficient. A configural representation of context would have been essential for solving the task.

In terms of generalisation, Moses et al (2007) found that lesioned rats were more likely to generalise from complex context to a simple context rather than the other way around. The concept of generalisation was not directly tested in this thesis – however, the task manipulations performed in chapter 4 do suggest that the hippocampal lesioned animals have a simpler representation of their environment that includes intra-maze cues but not self-motion cues for example. This simpler representation would suggest that as long as the animal did not detect a change (as in the context swap / cue conflict manipulation) the lesioned rats would indeed generalise their responses to a similar context.

Configural representations of context are required for context fear conditioning

Rudy and O'Reilly (1999) demonstrated that pre-exposure to the elements of a context were not sufficient to rescue context fear from the immediate shock deficit. Only exposure to the entire context could produce the context pre-exposure facilitation effect (CPFE; see also Fanselow et al 1990). Thus contextual fear conditioning requires a configural representation of context. However, no such configural representation of context itself was absolutely necessary for this context dependent odour discrimination task. Accurate performance would have been possible by conditioning to one aspect of the context. Nevertheless, although a configural representation of context may not have been required, the task could not have been solved without a configural representation between a context cue, the odour and its reward properties. Thus although not necessarily contextual, this task was definitely configural. Therefore, the data is this task does not support the hypothesis that an intact hippocampus is necessary for configural processing. However, the manipulations (chapter 4) did suggest that the representations hippocampal rats form may be less detailed than hippocampal based representation. Without the hippocampus the rats appeared not to integrate secondary cues like self-

motion into their representations of the context. Thus, the role of the hippocampus in encoding 'extra' non-vital configurations is upheld.

Summary

Overall the data in this thesis fit well with the literature on contextual fear conditioning. They both agree that the hippocampus is required for retention of contextual information but may not be necessary for its acquisition if an elemental solution is possible. They also agree that configural representations can help the animals have a more all encompassing view of their environment.

7.4. Comparison to another configural task

The pattern of temporary post-surgery impairments following hippocampal lesioning was also observed by Wishaw and Tomie 1991. Their task was a simultaneous conditional discrimination that combined string size (Thin T_1 and Thick T_2) with position (Left P_1 and Right P_2). Two strings were presented – one hung off each side of a raised platform.

If the rat pulled up the correct string, it received the food reward that was tied to the end of it. As in this thesis, the rats took an extended period of time to learn this task (6 weeks for the string task). Following lesion surgery they displayed a temporary impairment in performance that was rapidly overcome with training. Furthermore, their task reacquisition showed significant savings compared to pre-surgery learning. Thus the trends observed in this thesis, as well as being replicable within this experimental setup (i.e. between batches and in both simultaneous and successive task variants), appear also to be more universal to the functioning of the hippocampus. Thus, configural contextual stimuli may be processed in a very similar way to other configural stimuli within the brain.

7.5. Comparison to theories of hippocampal function

7.5.1. Cognitive Map Theory

The cognitive map theory (O'Keefe and Nadel 1978) asserts that the hippocampus is only involved in tasks that require the processing of spatial stimuli. Although this task was set up to be non-spatial, as the contexts always appeared in one configuration (except during manipulations), it may have been possible for the animals differentiate the contexts in this way. Furthermore, despite the fact that the placement manipulation in chapter 4 demonstrating that awareness of spatial position was not vital for performance, the cue swapped manipulation also demonstrated that sham animals could recognize spatial inconsistencies when they occurred. Lesioned animals appeared to lack this ability. This suggests that when the hippocampus is intact, some spatial encoding does indeed occur in this task. Nevertheless, accurate performance on the context dependent task could not have been achieved using spatial encoding alone. Integration of odour, context (potentially spatial context) and reward properties was needed in order to solve the task. Furthermore, on each trial, the odour pot moved pseudo-randomly between eight locations, while its reward properties remained linked to non-geometric contextual cues. Thus, in term of absolute physical location on the platform, four positions within each context held the same reward properties. It is possible that each set of four locations became equivalent 'places' in the eyes of the rats, but nevertheless an association of this with odour would still be required in order to compute reward outcome. 'Place' was not sufficient on it's own. Thus although spatial processing in the hippocampus may have been occurring during this task, it alone was not sufficient to explain support behaviour. In addition, the removal of the hippocampus (and therefore presumably the spatial encoding) did not prevent the rat from accurately performing the odour discrimination tasks.

7.5.2. Declarative Memory Theory

The declarative theory suggests that hippocampal processing allows memories to be relational and flexible in the face of novel situations. Non-hippocampal processing on the other hand leads to rigid and inflexible representations. Within the cue manipulation section of this thesis (chapter 4), the rats were faced with novel situations. Hippocampal lesioned rats continued to follow intra-maze cues irrespective of what else was occurring. This could be regarded as rigid and inflexible behaviour. On the other hand, the animals with an intact hippocampus (sham animals) recognized the conflicts occurring between intra-maze and self-motion cues, and this had an effect on their behavioural accuracy. This could be seen as evidence that the sham animals had a relational view of the task, which integrated stimuli from all their senses in order to provide a unified view of the environment. When these comparisons produced conflicting results, a deficit in performance on the context dependent task was observed.

The second facet of the declarative hypothesis as stated by Squire et al (1983) is that the role of the medial temporal lobe is time limited. Over time memories are reorganized and consolidated to long term storage sites outside the hippocampus. As the pre-training stage of this context dependent odour discrimination task involved extensive training (chapter 3 and 6), it is possible that consolidation had occurred prior to surgery. In this way, although it played a role in the acquisition of the context dependent task, the hippocampus was not absolutely necessary for its retention.

Overall, the declarative theory fits well with the experimental data described in this thesis.

7.5.3. Explicit and Incidental Memory

Contingent reinforcement is the method of committing things to memory. Without this style of encoding, the animal would completely fail to learn a task. However, a second more subtle system also encodes background contextual stimuli automatically and incidentally. This form of incidental learning is not always immediately obvious in the behaviour of the animal (Good et al 1998). Nonetheless, it can affect the way knowledge is encoded or retrieved if conditions change. If the incidentally encoded information suddenly becomes relevant to task performance, behaviour can be affected. This phenomenon was exactly what was observed during the task manipulations in chapter 4 of this thesis. A feature that was reasonably irrelevant to task performance (i.e. the relative positions of the two platforms/contexts), suddenly appeared in conflict with other background cues (i.e. the rats internal representation of its environment as updated by self-motion cues). However, unlike intact rats, hippocampal lesioned rats failed to recognise this mismatch. Without the hippocampus they had no access to this incidentally encoded representation, thus they continued to perform the task on the basis of their contingent representation of primary predictive cues. Therefore the ambiguity only detrimentally affected the performance of the sham operated animals and not the hippocampal lesioned ones.

The incidental theory of hippocampal function therefore corresponds well with the cue manipulation data presented in this thesis.

7.5.4. Configural Association Theory

The configural theories of Sutherland and Rudy (1989; 1995; Rudy and O'Reilly 2001) gave special emphasis to the hippocampus in the encoding of configurations – of which context was thought to be a prominent example. The original Configural Theory (Rudy and Sutherland 1989) postulated that only the hippocampus could encode configural representations. However, this did not explain how tasks non-linear tasks (like the one in this thesis) could be learnt without the hippocampus. The revised configural theory (Rudy and Sutherland 1995) states that the main neural circuitry for configural associations lies outside the hippocampus in the cortex; thus extra-hippocampal areas are sufficient to support performance – as was found in this thesis. The role of the hippocampus then becomes a tool for increasing learning rate. It is thought to do this by
selectively enhancing the salience of configural units in the cortex and decreasing their similarity to the elements of which they are composed(Rudy and Sutherland 1995).

This revised configural theory fits well with the temporary post-surgery impairment observed in this thesis (chapters 3 and 6).

When the hippocampus was removed, a temporary deficit was observed as configural and elemental units competed for associative strength. Nevertheless, in a biconditional task (such as this), every element is both rewarded and non-rewarded in equal measure. Thus rehearsal and experience resulted in the attainment of sufficient associative strength for the configural representation to 'win' over the elemental one – even without the support of the hippocampus. Thus, after an initial period of impairment, performance was restored.

The configural theory therefore gives a good account of the data shown in this thesis.

7.5.5. An Elemental Solution?

This context dependent odour discrimination task requires a representation of context to become associated with odour and reward. Although the intention was that 'context' encoding would encompass all of the relevant stimuli present around the platform, as these features did not overlap between contexts, this may not have been the case. Accurate performance would still have been possible if the conditioning stimulus was reduced to just one element of the context (e.g. black + mint = reward). Indeed, from the results of the cue manipulations (chapter 4) it seems likely that although intact animals had a complex representation of context, the lesioned animals instead used a simpler version of context to perform the odour discrimination task.

Nevertheless, even with a simplified version of context, the task would still require a configuration to be formed between this 'context cue', odour and reward. This need for configurations originally put biconditional tasks on Rudy and Sutherland (1989) list of hippocampal dependent activities. Elemental solutions were seen as unfeasible due to the equally reinforced and non-reinforced nature of the elemental stimuli (Rudy and Sutherland 1995).

However, it may be possible to break a conditional discrimination down into discrete compounds (e.g. XA^+, XB^-, YA^-, YB^+). In a biconditional task, none of the elements are ever encountered individually. Thus no competition actually occurs between the representations of the elements and their configurations. If biconditional tasks can be broken down into chunks like this, then it would open the way for an elemental solution that would be independent of the hippocampus (Rudy and Sutherland 1995, Gallagher and Holland 1992). In addition, the use of an elemental strategy could explain how rats were able to accurately perform the task even in the absence of their hippocampus.

7.6. Alternative Brain Regions that may support performance

Performance on the context dependent odour discrimination task described in this thesis is not *dependent* upon the hippocampus, but nevertheless when present the hippocampus has shown to be participatory. Thus some other brain region must be able to support behavioural performance. This area must receive the appropriate sensory inputs, and it seems likely that it will be either connected to or associated with the hippocampus. But where could this be? Perhaps the amygdala or the grid cells of the entorhinal cortex can support task performance. As the entorhinal cortex receives *its* sensory inputs from perirhinal and postrhinal cortices, maybe it can support performance? The aim of this section is to explore these possibilities and examine how performance based upon activity in these areas would differ from that expected with an intact hippocampus.

7.6.1. Amygdala

The amygdala has been associated with a wide range of functions including emotion, learning and memory, attention and perception. Numerous experiments have emphasised the amygdala's role in learning and memory for negative emotions – for example the amygdala is thought to play a key role in the acquisition and expression of context fear (Phillips and LeDoux 1992, Fanselow and Kim 1994, Goosens and Maren 2001. But for opposing view see Selden and Everitt 1991)

In addition, the role of the amygdala in the processing of more positive emotions has also been explored. Some studies have reported that the amygdala is required for appetitive tasks (e.g. Kesner et al 1989, Everitt, Morris, O'Brien and Robbins 1991, Parkinson, Robbins and Everitt 2000). Similarly, Gilbert et al (2003) suggested that the amygdala may play a significant role in learning conditioned flavour preferences. Therefore, one role for the amygdala could be in the appropriating reward values to stimuli (Baxter and Murray 2002, White and McDonald 2002). If this is the case, then the amygdala may have had the ability to process the odour / reward association

that was required for the context-dependent odour discrimination task described in this thesis.

In addition, the amygdala (or at least the central nucleus of the amygdala) has been shown to be necessary for behaviours that are directed towards the conditioned stimulus itself (e.g. approach or avoidance; Parkinson et al 2000, Baxter and Murray 2002). In this thesis, a dig response was required when the odour cue was encountered within the correct context. Thus the amygdala may have been involved in the initiation of this digging behaviour.

Therefore, considering its role in stimulus response associations and cue response initiation, the amygdala appears to be an ideal candidate for supporting performance on the context dependent odour discrimination task in the absence of the hippocampus. Thus it may have been the presence of the amygdala that prevented hippocampal lesions from completely impairing performance on the context dependent odour discrimination task.

7.6.2. Entorhinal Cortex

The entorhinal cortex is one step away in the processing chain from the hippocampus and receives much of the same information. Indeed, the entorhinal cortex is the physical link between the hippocampus and nearly all the other association cortices (Fyhn and Molden et al. 2004). Spatially related grid cells (Fyhn and Molden et al. 2004) have been recorded in the medial region of the entorhinal cortex . Medial entorhinal neurons encode a regularly repeating grid of tessellating equilateral triangles that covers the entire environment (Hafting and Fyhn et al. 2005). Neighbouring grid cells are slightly out of phase with each other, thus, the position of the animal can be computed from the output of a group of medial entorhinal cells (Fyhn and Molden et al. 2004). Although grid cells respond to cue movement (rotation), their responses to non-geometric parameters such as colour or texture is yet to be proven. The grid cells have been hypothesised to play a modulatory role in

resetting the hippocampal map should it fall out of sync with landmarks in the external environment. Thus it could have been these cells that signalled the discordance between self-motion and intra-maze cues in the intact animals (chapter 4).

Thus, in light of the limited evidence available, it does **not** seem likely that the entorhinal cortex is sufficient to support performance of this context dependent odour discrimination task, in the absence of the hippocampus.

7.6.3. Perirhinal Cortex

The perirhinal cortex is both close to the hippocampus and reciprocally connected to it (directly and via the entorhinal cortex). It is the anatomical bottleneck for incoming sensory stimuli about objects (Suzuki and Amaral 1994). It is thought to be involved in perception of and memory for objects - representing and storing the complex conjunctions of features that make up each one (Murray 2001).

The task described in this thesis could potentially be solved by the perirhinal cortex if the stimuli involved could be viewed as objects. Indeed, even objectless scenes contain background information that could be processed as object-like configurations of visual features (Buckley 2005).

Murray et al (2005) suggested that the perirhinal cortex is sufficient to encode objects or static scenes that can be observed in one view. However, the hippocampus acts on a larger scale, integrating multiple views, scenes or locations into a single fluid representation. A practical example of this is the finding that hippocampal lesions impair performance on a match to location task if the animal is allowed to move about on a tether in a room (multiple views; Hampton 2004), but have no effect if the items are presented on a tray (i.e. single view; Murray and Mishkin 1998). Thus, for the experiments in this thesis, if the animals encoded each context

as a separate ‘object’ and the odourous cues as part of the ‘scene,’ then the perirhinal cortex may have been sufficient to support performance.

Furthermore, if each view is an independent scene, then conflict that occurred between intra-maze cues and path-integration during the task manipulations would not be detected, and hence would not affect performance – as was observed in Chapter 4 of this thesis. The lesioned animals lacked the overall world view or sense of connection that was present in the intact animals, and were instead solving the task with more simplistic reactions to associations between single views and/or cues.

7.6.4. Summary

This section has identified three regions other than the hippocampus which may be able to support performance of the context dependent odour discrimination task – the amygdala, the entorhinal cortex and the perirhinal cortex. Speculative reasons can be given for all of these could fit the data observed in this thesis. However, the only definitive way to decipher what region was responsible would be to do further combined lesion / temporary impairment studies.

7.7. Experimental Limitations

Incomplete hippocampal lesions

The results discussed in this thesis are based upon incomplete hippocampal lesion data. Although care has been taken to fully analyse these results with regard to the extent of tissue loss, generalisations have inevitably been made across subjects. Furthermore, the possibility of differential results with complete removal of all hippocampal tissue cannot be ruled out. Nevertheless, the data were highly consistent throughout the set of experiments and replications – even with slight changes in protocol and stimulus presentation method. Furthermore, care has been taken when applying statistical methods, to avoid over manipulating the data and to prevent unsubstantiated conclusions. The lesion placement was highly consistent throughout the thesis, removing all central hippocampal tissue and the majority of the dorsal portion. Sparing was mainly evident in the deep regions of the ventral hippocampus – and sometimes in the very septal pole of the dorsal hippocampus. The data for the animals with complete dorsal lesions did not differ substantially from the rest of the group. However, as all the rats had some degree of ventral sparing, the same test could not be applied to ventral lesions.

Damage to overlying cortex

Ideally hippocampal lesions should cause maximal damage to hippocampal tissue (DG and CA areas) but leave all extra-hippocampal structures intact. However, to chemically lesion the hippocampus, a needle tract through the overlying neocortex is unavoidable. It would be hoped, that only a small amount of cortical tissue would be lost during this procedure. However, despite changes in protocol, this was not the case during my study.

The areas of cortex affected by accidental damage were identified as the primary and secondary visual cortex, the parietal association cortex and the hindlimb area of the somatosensory cortex. Damage to the visual cortex did not appear to create visual problems for the lesioned animals, although this was never directly tested. Similarly,

the small amount of damage to the very large somatosensory cortex is unlikely to have had much of an effect on performance.

The evidence on the effect of this kind of cortical damage is mixed. Traditional aspiration lesions that resulted in small amounts of damage to the overlying cortical areas were shown to have no effect on spatial learning in the water-maze (Moser, Moser et al. 1993). However, other studies (with larger lesions) partially contradict this by suggesting that removal of the parietal associative cortex may cause specific deficits in spatial processing (DiMattia and Kesner 1988; Save and Moghaddam 1996, Save et al 1992)
Damage to the APC has been shown to impair the processing of proximal cues (e.g. objects) but not distal cues (Goodrich-Hunsaker et al. 2005). However, these studies had a much higher level of APC damage than was found in this thesis, thus may not be comparable. Overall, the literature is not clear as to whether the extra-hippocampal damage sustained would have affected behaviour.

Other issues
Alternative explanations for the results reported in this thesis include:

- The small number of animals in this study may have given a biased result. However, despite this limitation, statistically significant results have been obtained and stable trends observed between repetitions.

- The relationship between context and olfaction may be a special one that does not require the hippocampus. This seems unlikely due to the similarity of other results of this study to other studies that used different behavioural paradigms.

- The task took a long time to learn. Thus it may have been learnt in a way that did not require the hippocampus (i.e. a more gradual incremental learning process).

7.8. Overall Conclusions

Although the hippocampus contributes to the retention of this context dependent odour discrimination task if it is present during encoding, an intact hippocampus is not *necessary* for acquisition or accurate performance post-surgery. Other non-hippocampal areas can take over these functional demands in its absence.

Nevertheless, if any hippocampal tissue is spared, it will contribute to and improve initial retention ability and speed up reacquisition time.

Furthermore, although the context dependent odour discrimination task can be performed accurately in the absence of an intact hippocampus, the approach used by the lesioned rats differs from that of the sham rats, in that they make less use of self-motion cues.

7.9. Future Experiments

It would be very interesting to look for hippocampal firing correlates to the non-spatial task parameters explored in this task. Wood et al (1999) demonstrated firing correlates to odour, approach and match/non-match in a continuous non-match to sample (cNMS) task. Similarly, Jeffrey et al (2003) has demonstrated contextual firing correlates to context 'colour' and odour, as well as combinations of the two. In this task I would be looking for correlates to odour, context and reward properties and their combinations. My hypothesis would be that the encoding of the odours would be related to their reward properties. For the context independent task, an individual neuron would fire in response to a particular odour, no matter where in the environment it occurred. Whereas for the context dependent task, each odour would be encoded by two different cells – one in each context. This firing pattern would correspond to its pattern of reinforcement (See Figure 7-1).

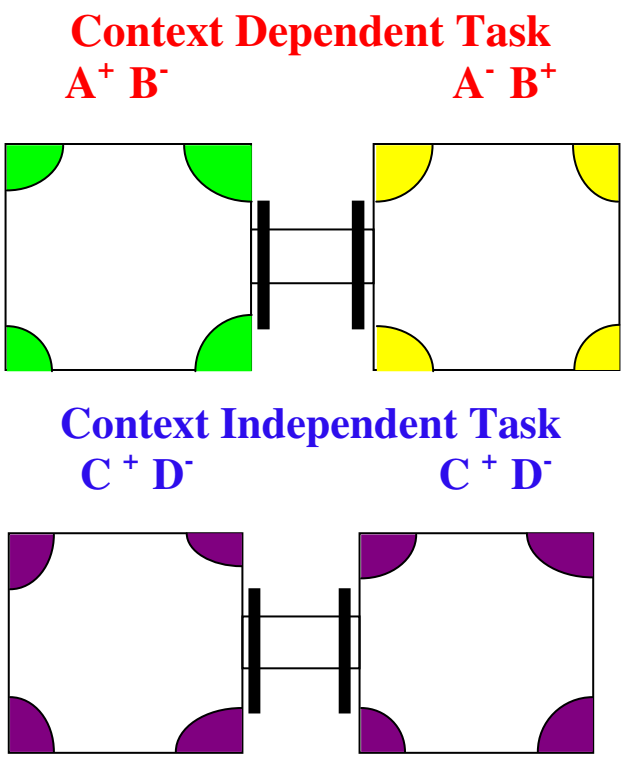

Figure 7-1: Hypothesised pyramidal firing patterns while rats perform context dependent and independent tasks. Letters represent odours; + is rewarded, - is unrewarded on equivalent side of apparatus. Each colour represents a different cell.

Of particular interest would be the pattern of pyramidal cell firing as rats learnt the task – in particular whether firing patterns represented (or even predicted) task errors. Furthermore, there may be subtle differences in firing between errors that are during initial acquisition of task, and errors made when animals are performing consistently at criterion level.

In addition it would be interesting to record from either place cells or head direction cells during the task manipulations, where self-motion cues are at odds with intra-maze cues in the contexts. Which cues do the cells follow? Does the entire neuronal population respond in the same way, or are there differential responses within / between rats i.e. is the remapping of place fields / head direction parameters partial or complete?

8. Appendices

8.1. Histology Protocol

Cresyl Violet Stain

Wear Lab coat and Gloves

Use fume cupboard for xylene and coverslipping

1. Place slides in rack, dehydrate sections through 70% alcohol to absolute alcohol by dipping rack 10 times in each solution

2. Take sections to xylene 1 until cleared

3. Hydrate sections by placing in rehydration alcohol 1 for 30 seconds

4. Place sections in acid alcohol 1 for 5 minutes

5. Wash in running water

6. Place in Cresyl violet for 4-5 minutes (depends on age of solution)

7. Wash in water

8. Differentiate by dipping in acid alcohol 2 for 10 seconds, place in water and check microscopically that the fibre tracts appear colourless, if not repeat this step until well differentiated. If over differentiated go back to step 6.

9. Dehydrate sections through 70% alcohol to absolute alcohol by dipping rack 10 times in each solution

10. Take sections to xylene 1 for 30 seconds, place in xylene

11. Mount with DPX from xylene 2

Solutions used

0.1% cresyl violet acetate, 70% alcohol with 0.5% acetic acid

8.2. CD of videos

Folder	Filename	File Type
Chap 2&3 – successive task	Successive Task Appatus Rewarded trial in black context Rewarded trial in white context Non-rewarded trial in black context Non-rewarded trial in white context Probe trial	Windows Media Audio/Video file
Chap 5 – New context	Rewarded trial in brown context Rewarded trial in striped context Non-rewarded trial in brown context Non-rewarded trial in striped context	Windows Media Audio/Video file
Chap 6 – simultaneous task	Simultaneous task apparatus Trial in black context Trial in white context	Windows Media Audio/Video file
	Powerpoint moving task representations	Powerpoint

References

References

Alheid, G. F. deOlmos., J.S.,Beltramino, C. (2004). Amygdala and extended amygdala. The Rat Nervous System. G. Paxinos. London, Elsevier-Academic Press**:** 495-578.

Alvarado, M. C., Rudy J.W (1995). "Rats with damage to the hippocampal-formation are impaired on the transverse-patterning problem but not on elemental discriminations." Behavioral Neuroscience **109**(2): 204-211.

Alvarado, M. C., Rudy, J.W. (1995). "A comparison of “configural” discrimination problems: Implications for understanding the role of the hippocampal formation in learning and memory." Psychobiology **23**(3): 178-184.

Amaral, D., Lavenex, P. (2007). Hippocampal Neuroanatomy. The Hippocampus Book.. Andersen, P. M.,.Morris. R.G. M., Amaral, D.; Bliss, T.; O'Keefe, J. USA, Oxford University Press Inc.

Anagnostaras, S. G., Maren, S., Fanselow, M.S. (1999). "Temporally Graded Retrograde Amnesia of Contextual Fear after Hippocampal Damage in Rats: Within-Subjects Examination." J. Neurosci. **19**(3): 1106-1114.

Anagnostaras, S. Gale. G. D., Fanselow, M. S. (2001). "Hippocampus and contextual fear conditioning: Recent controversies and advances." Hippocampus **11**(1): 8-17.

Anderson, M. I., Jeffery K. J. (2003). "Heterogeneous Modulation of Place Cell Firing by Changes in Context." J. Neurosci. **23**(26): 8827-8835.

Anderson, P., Morris., R. G. M.; Amaral, D., Bliss, T., O'Keefe, J (2007). Historical perspecitive: Proposed functions, biological characteristics, and neurobiological models of the hippocampus. The Hippocampus Book. P. M. Andersen, R.G.M.; Amaral, D.; Bliss, T.; O'Keefe, J. USA, Oxford University Press Inc.

Bannerman, D. M., Rawlins, J. N. P., et al. (2004). "Regional dissociations within the hippocampus--memory and anxiety." Neuroscience & Biobehavioral Review Festschrift in Honour of Jeffrey Gray - Issue 1: Anxiety and Neuroticism **28**(3): 273-283.

Bast, T., Wei-Ning, Z., Joram, F (2003). "Dorsal hippocampus and classical fear conditioning to tone and context in rats: Effects of local NMDA-receptor blockade and stimulation." Hippocampus **13**(6): 657-675.

Baxter, M. G. and. Murray, E. A (2002). "The amygdala and reward." Nat Rev Neurosci **3** (7): 563-573.

Bayley, P. J., Frascino, J. C, et al. (2005). "Robust habit learning in the absence of awareness and independent of the medial temporal lobe." Nature **436**(7050): 550-553.

Bergman, A. B., M; Hamlisch M (1973). The Way We Were. USA.

Bevins, R. A., & Ayres, J. J. B. (1995). "One-trial context fear conditioning as a function of the interstimulus interval." Animal Learning & Behavior **23**: 400–410.

Blanchard, R. J. and. Blanchard, D. C (1972). "Effects of hippocampal lesions on the rat's reaction to a cat." Journal of Comparative and Physiological Psychology **78**(1): 77-82.

Buckley, M. J. (2005). "The role of the perirhinal cortex and hippocampus in learning, memory, and perception." The Quarterly Journal of Experimental Psychology Section B **58**(3): 246 - 268.

Bussey, T. J., Dias, R. et al. (2001). "Perirhinal cortex and place-object conditional learning in the rat." Behavioral Neuroscience **115**(4): 776-785.

Coutureau, E., Killcross, A. S. et al. (2002). "Acquired equivalence and distinctiveness of cues: II. Neural manipulations and their implications." Journal of Experimental Psychology: Animal Behavior Processes **28**(4): 388-396.

Davidson, T. L, McKernan, M. G et al. (1993). "Hippocampal lesions do not impair negative patterning: A challenge to configural association theory." Behavioral Neuroscience **107**(2): 227-234.

de Hoz, L. Knox., J., Morris, R. G. M. (2003). "Longitudinal axis of the hippocampus: Both septal and temporal poles of the hippocampus support water maze spatial learning depending on the training protocol." Hippocampus **13**(5): 587-603.

DiMattia, B. V. and Kesner, R. P. (1988). "Spatial cognitive maps: Differential role of parietal cortex and hippocampal formation." Behavioral Neuroscience **102**(4): 471-480.

Dix, S. L. and Aggleton, J. P. (1999). "Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition." Behavioural Brain Research **99**(2): 191-200.

Driscoll, I., Howard, S.R., Prusky, G.T.; Rudy, J.W.; Sutherland, R.J. (2005). "Seahorse wins all races; Hippocampus participates in both linear and non-linear visual discrimination learning." Behavioural Brain Research **164**: 29-35.

Dusek, J. A. and Eichenbaum, H. (1998). "The hippocampus and transverse patterning guided by olfactory cues." Behavioral Neuroscience **112**(4): 762-771.

Eichenbaum, H. (1994). The hippocampal system and declarative memory in humans and animals: experimental analysis and historical origins. Memory Systems 1994. Schacter, D. L., Tulving, E., M.I.T. Press**:** Pages 147-202.

Eichenbaum, H., Fagan, A. et al. (1988). "Hippocampal system dysfunction and odor discrimination learning in rats: Impairment of facilitation depending on representational demands." Behavioral Neuroscience **102**(3): 331-339.

Eichenbaum, H., Kuperstein, M. et al. (1987). "Cue-sampling and goal-approach correlates of hippocampal unit activity in rats performing an odor-discrimination task." J. Neurosci. **7**(3): 716-732.

Eichenbaum, H., Mathews, P. et al. (1989). "Further studies of hippocampal representation during odor discrimination learning." Behavioral Neuroscience **103**(6): 1207-1216.

Eichenbaum, H., Stewart, C. et al. (1990). "Hippocampal representation in place learning." J. Neurosci. **10**(11): 3531-3542.

Eichenbaum, H. Fagan, A; Cohen, N.J. (1986). "Normal olfactory discrimination learning set and facilitation of reversal learning after medial-temporal damage in rats; Implications for an account of preserved learning abilities in amnesia." Journal of Neuroscience **6**: 1876-1884.

Ennaceur, A. and Delacour, J. (1988). "A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data." Behavioural Brain Research **31**(1): 47-59.

Etienne, A. S., Jeffery, K. J. (2004). "Path integration in mammals." Hippocampus **14**(2): 180-192.

Etienne, S., Poucet, B. (2000). "Hippocampal-parietal cortical interactions in spatial cognition." Hippocampus **10**(4): 491-499.

Everitt, B. J., Morris, K. A. et al. (1991). "The basolateral amygdala-ventral striatal system and conditioned place preference: Further evidence of limbic-striatal interactions underlying reward-related processes." Neuroscience **42**(1): 1-18.

Fanselow, M., S., (1990). "Factors governing one-trial contextual conditioning." Animal Learning & Behavior **18**(3): 264-270.

Fanselow, M. S. (1986). "Associative vs. topographical accounts of the immediate shock-freezing deficit in rats: Implications for the response selection rules governing species—Specific defensive reactions." Learning and Motivation **17**: 16–39.

Fanselow, M. S. (1999). "Learning theory and neuropsychology: Configuring their disparate elements in the hippocampus." Journal of Experimental Psychology: Animal Behavior Processes **25**(3): 275-283.

Fanselow, M. S. (2000). "Contextual fear, gestalt memories, and the hippocampus." Behavioural Brain Research **110**(1-2): 73-81.

Fanselow, M. S. and. Kim, J. J. (1994). "Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala." Behavioral Neuroscience **108**(1): 210-212.

Fortin, N. J., Agster, K.L.; Eichenbaum, H.B. (2002). "Critical role of the hippocampus in memory for sequences of events." Nature Neuroscience **5**(5): 458-462.

Frankland, P. W., Cestari, V. et al. (1998). "The dorsal hippocampus is essential for context discrimination but not for contextual conditioning." Behavioral Neuroscience **112**(4): 863-874.

Fyhn, M., Molden, S. et al. (2004). "Spatial Representation in the Entorhinal Cortex." Science **305**(5688): 1258-1264.

Gallagher, M., Holland, P.C. (1992). "Preserved configural learning and spatial learning impairment in rats with hippocampal damage." Hippocampus **2**(1): 81-88.

Gallagher, M. Holland, P C. (1992). "Preserved configural learning and spatial learning impairment in rats with hippocampal damage." Hippocampus **2**(1): 81-88.

Gilbert, P. E., Campbell, A. et al. (2003). "The role of the amygdala in conditioned flavor preference." Neurobiology of Learning and Memory **79**(1): 118-121.

Gisquet-Verrier, P., Dutrieux, G. et al. (1999). "Effects of lesions to the hippocampus on contextual fear: Evidence for a disruption of freezing and avoidance behavior but not context conditioning." Behavioral Neuroscience **113**(3): 507-522.

Gleitman, H., Fridlund, A.; Reisberg, D (1999). Psychology. New York, WW Norton & Company Inc.

Good, M. and Honey, R. C. (1991). "Conditioning and contextual retrieval in hippocampal rats." Behavioral Neuroscience **105**(4): 499-509.

Good, M. de Hoz, L., Morris, R.G.M. (1998). "Contingent versus incidental context processing during conditioning: Dissociation after excitotoxic hippocampal plus dentate gyrus lesions." Hippocampus **8**(2): 147-159.

Goodrich-Hunsaker, N. J, Hunsaker, M. R et al. (2005). "Dissociating the role of the parietal cortex and dorsal hippocampus for spatial information processing." Behavioral Neuroscience **119**(5): 1307-1315.

Goosens, K. A. and Maren, S. (2001). "Contextual and Auditory Fear Conditioning are Mediated by the Lateral, Basal, and Central Amygdaloid Nuclei in Rats" Learn. Mem. **8**(3): 148-155.

Gothard, K., Skaggs, W. et al. (1996). "Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task." J. Neurosci. **16**(2): 823-835.

Gray, J. A. (1979). The neuropsychology of anxiety. Oxford University Press.

Gray, J. A., McNaughton. N. (2000). The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septohippocampal System. Oxford, Oxford University Press.

Hafting, T., Fyhn, M. et al. (2005). "Microstructure of a spatial map in the entorhinal cortex." Nature **436**(7052): 801-806.

Hampton, R. R., Benjamin, H. M.; Murray, E. A. (2004). "Selective hippocampal damage in rhesus monkeys impairs spatial memory in an open-field test." Hippocampus **14**(7): 808-818.

Honey, R. C. and Ward-Robinson, J. (2002). "Acquired equivalence and distinctiveness of cues: I. Exploring a neural network approach." Journal of Experimental Psychology: Animal Behavior Processes **28**(4): 378-387.

Honey, R. C. and Watt, A. (1999). "Acquired relational equivalence between contexts and features." Journal of Experimental Psychology: Animal Behavior Processes **25**(3): 324-333.

Jeffery, K. J., Anderson, M. I. et al. (2004). "A proposed architecture for the neural representation of spatial context." Neuroscience & Biobehavioral Reviews **28**(2): 201-218.

Jeffery, K. J. Anderson, M. I. (2003). "Dissociation of the geometric and contextual influences on place cells." Hippocampus **13**(7): 868-872.

Jonasson, Z. Ballantyne, J. K., Baxter, M. G. (2004). "Preserved anterograde and retrograde memory of rapidly acquired olfactory discriminations after neurotoxic hippocampal lesions." Hippocampus **14**(1): 28-39.

Kaut, K. P., Bunsey, M. D. et al. (2003). "Olfactory Learning and Memory Impairments Following Lesions to the Hippocampus and Perirhinal-Entorhinal Cortex." Behavioral Neuroscience **117**(2): 304-319.

Kennedy, P. J. and Shapiro, M. L. (2004). "Retrieving Memories via Internal Context Requires the Hippocampus" J. Neurosci. **24**(31): 6979-6985.

Kesner, R. P. (1993). "Memory for spatial locations, motor responses, an d objects: Triple dissociation amoung the hippocampus, caudate nucleus and extrastriate visual cortex." Exp. Brain Res. **93**: 462-470.

Kesner, R. P., Walser, R. D. et al. (1989). "Central but not basolateral amygdala mediates memory for positive affective experiences." Behavioural Brain Research **33**(2): 189-195.

Kim, J. and Fanselow, M. (1992). "Modality-specific retrograde amnesia of fear" Science **256**(5057): 675-677.

Langston, R. F. (2006). Integrated memory for “what”, “where” and “which” is dependent on the hippocampus in a spontaneous recognition paradigm in the rat. FENS, Vienna, FENS Forum Abstracts.

Lehmann, H., Lacanilao, S. et al. (2007). "Complete or partial hippocampal damage produces equivalent retrograde amnesia for remote contextual fear memories." Eur J Neurosci **25**(5): 1278.

Lehmann, H. Carfagnini, A.,Yamin, S., Mumby, D. G. (2005). "Context-dependent effects of hippocampal damage on memory in the shock-probe test." Hippocampus **15**(1): 18-25.

Leutgeb, J. K. and Moser E. I. (2007). "Enigmas of the Dentate Gyrus." Neuron **55**(2): 176-178.

Maren, S. (1999). "Neurotoxic or electrolytic lesions of the ventral subiculum produce deficits in the acquisition and expression of Pavlovian fear conditioning in rats." Behavioral Neuroscience **113**(2): 283-290.

Maren, S., G. Aharonov, et al. (1997). "Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats." Behavioural Brain Research **88**(2): 261-274.

Maren, S., Anagnostaras, S. G. et al. (1998). "The startled seahorse: is the hippocampus necessary for contextual fear conditioning?" Trends in Cognitive Sciences **2**(2): 39-42.

Maren, S. and Fanselow M. S. (1997). "Electrolytic Lesions of the Fimbria/Fornix, Dorsal Hippocampus, or Entorhinal Cortex Produce Anterograde Deficits in Contextual Fear Conditioning in Rats." Neurobiology of Learning and Memory **67**(2): 142-149.

Matus-Amat, P., Higgins, E. A. et al. (2004). "The Role of the Dorsal Hippocampus in the Acquisition and Retrieval of Context Memory Representations" J. Neurosci. **24**(10): 2431-2439.

McDonald, R. J., Murphy, R. A., Guarraci, F. A., Gortler, J. R., White, N.M., Baker, A.G. (1997). "Systematic comparison of the effects of hippocampal and fornix-fimbria lesions on acquisition of three configural discriminations." Hippocampus **7**(4): 371-388.

McNaughton, B. L., Battaglia, F. P. et al. (2006). "Path integration and the neural basis of the 'cognitive map'." Nat Rev Neurosci. **7**(8): 663-678.

McNish, K. A., Gewirtz, J. C.et al. (1997). "Evidence of Contextual Fear after Lesions of the Hippocampus: A Disruption of Freezing But Not Fear-Potentiated Startle." J. Neurosci. **17**(23): 9353-9360.

McNish, K. A., Gewirtz, J. C., et al. (2000). "Disruption of contextual freezing, but not contextual blocking of fear-potentiated startle, after lesions of the dorsal hippocampus." Behavioral Neuroscience **114**(1): 64-76.

Means, L.W., Walker, D. W. et al. (1970). "Facilitated single-alternation go, no-go acquisition following hippocampectomy in the rat." Journal of Comparative and Physiological Psychology **72**(2): 278-285.

Mogenson, G. J., Jones, D. L. et al. (1980). "From motivation to action: Functional interface between the limbic system and the motor system." Progress in Neurobiology **14**(2-3): 69-97.

Morris, R. G. M. (2006). "Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas" European Journal of Neuroscience **23**(11): 2829-2846.

Morris, R. G. M. (2007). Theories of Hippocampal Function. The Hippocampus Book. P. M. Andersen, R.G.M.; Amaral, D.; Bliss, T.; O'Keefe, J. USA, Oxford University Press Inc.

Moser, M., Moser, E.et al. (1995). "Spatial Learning with a Minislab in the Dorsal Hippocampus" PNAS **92**(21): 9697-9701.

Moser, M.B. and Moser, E. I. (1998). "Distributed Encoding and Retrieval of Spatial Memory in the Hippocampus." J. Neurosci. **18**(18): 7535-7542.

Moses, S. N. Winocur, G.R., Jennifer D.; Moscovitch, Morris (2007). "Environmental complexity affects contextual fear conditioning following hippocampal lesions in rats." Hippocampus **17**(5): 333-337.

Muller, R. and Kubie, J. (1987). "The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells." J. Neurosci. **7**(7): 1951-1968.

Mumby, D. G., Gaskin, S. et al. (2002). "Hippocampal Damage and Exploratory Preferences in Rats: Memory for Objects, Places, and Contexts" Learn. Mem. **9**(2): 49-57.

Murray, E. A., Graham, K. S. et al. (2005). "Perirhinal cortex and its neighbours in the medial temporal lobe: Contributions to memory and perception." The Quarterly Journal of Experimental Psychology Section B **58**(3): 378 - 396.

Murray, E. A. and Mishkin, M. (1998). "Object Recognition and Location Memory in Monkeys with Excitotoxic Lesions of the Amygdala and Hippocampus." J. Neurosci. **18**(16): 6568-6582.

Murray, E. A., Richmond, B. J. (2001). "Role of perirhinal cortex in object perception, memory, and associations." Current Opinion in Neurobiology **11**: 188-193.

Nadel, L. and Moscovitch, M. (1997). "Memory consolidation, retrograde amnesia and the hippocampal complex." Current Opinion in Neurobiology **7**(2): 217-227.

Nadel, L. Willner, J. (1980). "Context and conditioning: a place for space." Physiol Psychol **8:.** 218–228.

Norman, G. and Eacott, M. J. (2005). "Dissociable Effects of Lesions to the Perirhinal Cortex and the Postrhinal Cortex on Memory for Context and Objects in Rats." Behavioral Neuroscience **119**(2): 557-566.

O'Keefe, J., Conway, D.H (1978). "Hippocampal Place Units in the Freely Moving Rat:

Why They Fire Where They Fire." Exp. Brain Res. **31**: 573-590.

O'Reilly, R.C. Rudy, J.W. (2001). "Conjunctive Representations in Learning and Memory: Principles of cortical and hippocampal function." Psychological Review **108**: 311-345.

O'Reilly, R. C., Rudy, J.W. (2000). "Computational principles of learning in the neocortex and hippocampus." Hippocampus **10**(4): 389-397.

Parkinson, J. A., Robbins, T. W. et al. (2000). "Dissociable roles of the central and basolateral amygdala in appetitive emotional learning." European Journal of Neuroscience **12**(1): 405-413.

Paxinos, G., Watson., C. (1998). The Rat Brain in stereotaxic coordinates. San Diego, California, Academic Press, Inc.

Phillips, R. and LeDoux, J. (1994). "Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning." Learn. Mem. **1**(1): 34-44.

Phillips, R. G. and LeDoux, J. E. (1992). "Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning." Behavioral Neuroscience **106**(2): 274-285.

Rolls, E. T. and. Kesner, R. P (2006). "A computational theory of hippocampal function, and empirical tests of the theory." Progress in Neurobiology **79**(1): 1-48.

Rudy, J. W. and. O'Reilly, R. C. (1999). "Contextual fear conditioning, conjunctive representations, pattern completion, and the hippocampus." Behavioral Neuroscience **113**(5): 867-880.

Rudy, J. W. and O'Reilly, R. C. (2001). "Conjunctive representations, the hippocampus, and contextual fear conditioning." Cognitive, Affective, & Behavioral Neuroscience **1**: 66-82.

Rudy, J. W. and Sutherland R. J. (1989). "The hippocampal formation is necessary for rats to learn and remember configural discriminations." Behavioural Brain Research **34**(1-2): 97-109.

Rudy, J. W., Sutherland, R. J. (1995). "Configural association theory and the hippocampal formation: An appraisal and reconfiguration." Hippocampus **5**(5): 375-389.

Rumelhart, D. E., Hinton, G. E.; Williams, R.J. (1986). "Learning representations by back-propagating errors." Nature **323**: 533 - 536.

Sanders, M. J., Wiltgen, B. J. et al. (2003). "The place of the hippocampus in fear conditioning." European Journal of Pharmacology **463**(1-3): 217-223.

Save, E. and Moghaddam, M. (1996). "Effects of lesions of the associative parietal cortex on the acquisition and use of spatial memory in egocentric and allocentric navigation tasks in the rat." Behavioral Neuroscience **110**(1): 74-85.

Save, E., Poucet, B. et al. (1992). "Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation." Behavioral Neuroscience **106**(3): 447-456.

Scoville, W. B. Milner, B. (1957). "Loss of recent memory after bilateral hippocampal lesions." Journal of Neurology, Neurosurgery and Psychiatry **20**: 11-21.

Selden, N. R. W., Everitt, B. J. et al. (1991). "Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues." Neuroscience **42**(2): 335-350.

Skaggs, W. E. and McNaughton, B. L. (1998). "Spatial Firing Properties of Hippocampal CA1 Populations in an Environment Containing Two Visually Identical Regions." J. Neurosci. **18**(20): 8455-8466.

Spence, K. W. (1952). "The nature of response in discrimination learning." Psychological Review **59**: 89-93.

Squire, L. R. (1992). "Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans." Psychological Review **99**(2): 195-231.

Squire, L. R. and Alvarez, P. (1995). "Retrograde amnesia and memory consolidation: a neurobiological perspective." Current Opinion in Neurobiology **5**(2): 169-177.

Squire, L. R., Zola-Morgan, S. (1983). The neurology of memory: The case for correspondence between the findings for human and nonhuman primates. The psychological basis of memory. Deutsch. J. A., New York, Academic Press**:** Pages 199-268.

Stefanacci, L., Buffalo, E. A. et al. (2000). "Profound Amnesia After Damage to the Medial Temporal Lobe: A Neuroanatomical and Neuropsychological Profile of Patient E. P." J. Neurosci. **20**(18): 7024-7036.

Suzuki, W. and Amaral, D. (1994). "Topographic organization of the reciprocal connections between the monkey entorhinal cortex and the perirhinal and parahippocampal cortices." J. Neurosci. **14**(3): 1856-1877.

Taube, J. S. Burton, H.L. (1995). "Head direction cell activity monitored in a novel environment and during cue conflict situation." Journal of Neurophysiology **74**: 1953-1971.

Thammaroj, J., Santosh, C. et al. (2005). "The Hippocampus: Modern Imaging of its Anatomy and Pathology" Practical Neurology **5**(3): 150-159.

Tulving, E. (1972). Organization of Memory. New York, Academic Press.

Waugh, N. C. Norman, D.A. (1965). "Primary Memory." Psychol Rev **72**: 89-104.

Whishaw, I. Q. and. Tomie, J.A (1991). "Acquisition and retention by hippocampal rats of simple, conditional, and configural tasks using tactile and olfactory cues: Implications for hippocampal function." Behavioral Neuroscience **105**(6): 787-797.

White, N. M. and McDonald, R. J. (2002). "Multiple Parallel Memory Systems in the Brain of the Rat." Neurobiology of Learning and Memory **77**(2): 125-184.

Winocur, G., Rawlins, J. N. P. et al. (1987). "The Hippocampus and Conditioning to Contextual Cues." Behavioral Neuroscience **101**(5): 617-625.

Wood, E. R., Agster, K. M. et al. (2004). "One-Trial Odor-Reward Association: A Form of Event Memory Not Dependent on Hippocampal Function." Behavioral Neuroscience **118**(3): 526-539.

Wood, E. R., Dudchenko, P. A. et al. (1999). "The global record of memory in hippocampal neuronal activity." Nature **397**(6720): 613-616.

Young, S. L., Bohenek, D. L. et al. (1994). "NMDA Processes Mediate Anterograde Amnesia of Contextual Fear Conditioning Induced by Hippocampal Damage - Immunization against Amnesia by Context Pre-exposure." Behavioral Neuroscience **108**(1): 19-29.

Zola-Morgan, S. and Squire, L. (1984). "Preserved learning in monkeys with medial temporal lesions: sparing of motor and cognitive skills." J. Neurosci. **4**(4): 1072-1085.

Zola-Morgan, S. and Squire, L. R. (1985). "Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia." Behavioral Neuroscience **99**(1): 22-34.