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A SEARCH FOR THE MAMMALIAN BRAIN SITE(S)
MEDIATING OCCASION SETTING LEARNING

BY

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A Thesis submitted in conformity with the requirements for the Degree of Master of Science, Graduate Department of Anatomy and Cell Biology,
University of Toronto

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1998
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ABSTRACT

Using a conditional contextual discrimination paradigm, I have been able to simultaneously assess two types of learning: A complex occasion setting learning and a simple Pavlovian learning. An occasion setter is a discriminative cue (feature) that predicts whether or not a target stimulus is followed by a reinforced event (US). Behavioural evidence has demonstrated that discriminative control of the occasion setter does not rest with simple excitatory associations between the feature and the US or between the feature and the target stimulus. In all of the experiments presented here, one context (feature) predicted that access to a saccharin solution (target) would be followed by a LiCl injection (US), while a second context predicted that the identical saccharin solution would be followed by a saline injection. All control animals learn to suppress fluid consumption in the first relative to the second context (occasion setting) and all acquire an aversion to the LiCl paired context on a subsequent place test (Pavlovian learning). Pavlovian aversions to the “danger” context can be abolished and the occasion setting properties remain intact. Having demonstrated a behavioural double dissociation between the types of learning, the focus of this thesis is to identify the neuroanatomical loci mediating the two separate learning systems. Rats with combined hippocampal plus
global amygdala lesions blocked context aversions but not the ability of contexts to serve as occasion setters. Ibotenic acid lesions of the dorsal striatum and the ventrolateral prefrontal cortex produced negative results, all lesioned animals performed similar to controls. Rats with the central nucleus (CN) of the amygdala lesions and rats with the basolateral nucleus (ABL) of the amygdala lesions both demonstrated a partial block of aversiveness to the danger context, but were able to acquire the discrimination task normally. These findings demonstrate that the amygdala mediates the simple excitatory learning and reiterates previous conclusions that simple Pavlovian conditioning and occasion setting are controlled by different neural systems.
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Let me begin by saying that my stay with the laboids has been a very interesting and pleasurable experience. I would first like to thank Derek van der Kooy for his guidance. His supervising style made my stay here a very memorable one and it was this style that enabled me to survive my time in the Zombie Zone.

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I would also like to express my appreciation to my family (Mom, Dad, and Dane). Mom and Dad for their support throughout the entire journey. Thanks for all of the typing Mom. Dane you were a soldier with the flyers! Finally, thanks to Sarah who always kept me grounded (or at least tried to) while I was straying from the path.

As I am writing this paragraph a day after my defense, I would like to thank Rob McDonald, Mike Wiley, Martin Ralph, John Roder, Martin Wojtowicz and Derek (My defense committee). Thank you for one of the most humbling experiences of my life and giving me a new found appreciation for academia. All I can say is “Thanks for the PASS!!”

I have learned a lot of life lessons over the past couple of years and I will arm myself with this knowledge as I embark on the next phase of my life. Thanks again to everyone because it is now time to go and bash down some more obstacles.
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CHAPTER 1
GENERAL INTRODUCTION
The ability to learn and remember allows animals and humans to acquire, retain and retrieve many types of different information. Theorists believe that learning and memory capabilities are adaptive specializations brought about by natural selection (Sherry and Schacter, 1987). Using their learning and memory capacities, animals can solve specific problems posed by their environment. Memory capacity endows animals the ability to recognize familiar situations, landmarks or events and navigate to previous places. Animals appear to be innately predisposed to associate certain tastes (food) with illness. It has been postulated that this type of “primitive” learning was one of the first to evolve (Gaston, 1978). Over the years, theorists have grappled with the question: Is there one unitary memory system, or are there multiple memory systems involved in deciphering several types of information? Today, the popular view concerning learning and memory is one that incorporates multiple memory systems. Using a Conditioned Taste Aversion Paradigm and several contextual stimuli, we have been able to behaviourally identify two distinct types of learning: Pavlovian Learning and Occasion Setting. The research that I have undertaken has sought to determine the neuroanatomical locus(i) involved in mediating these two distinct types of learning.

**ASSOCIATIVE LEARNING**

**Classical Conditioning**

Learning is a process by which an organism benefits from experience so that it's future behaviour is better adapted to its environment (Rescorla, 1988). In Associative Learning a
biologically neutral stimulus (Conditioned Stimulus (CS)) is temporally paired with a biologically significant stimulus (Unconditioned Stimulus (US)). Eventually, the Conditioned Stimulus comes to acquire biologically significant meaning. This occurs after repeated pairings or can occur after only one trial as in the case of Conditioned Taste aversions. Ivan Pavlov’s (1927) pioneering experiments are an excellent demonstration of classical conditioning/Pavlovian learning. His well known experiments with dogs, were the first to demonstrate that animals could learn the temporal significance between a conditioning stimulus and an unconditioned stimulus. When Pavlov presented his dogs with meat powder (the unconditioned stimulus or US), the dogs reliably salivated (the unconditioned response or UR). Using a bell (conditioned stimulus or CS) to signal the presentation of the meat powder, a contingency was established so that on subsequent testing, the presentation of the bell was sufficient to elicit the unconditioned response (salivation). One could infer that, the bell had acquired some biologically significant meaning. Pavlovian conditioning became a model for associative learning, in which various relations were learned among various stimuli and/or events.

**Occasion Setting (Higher Order Associations)**

Behavioural observations demonstrated that animals could not only learn that a conditioned stimulus predicted the presence of an unconditioned stimulus (reinforcement), animals could also acquire the ability to use certain stimuli to predict when other conditioned stimuli would be associated with a US (Holland, 1983; Rescorla, 1987). In other words, a
given stimulus A (the target stimulus) is paired with a US but only when preceded by X (the feature). In the absence of X, A is not followed by the US. Therefore, X comes to modulate the association of reinforcement between the US and A (the target stimulus). In other words X “sets the occasion” for the reinforcement of A. It has become accepted that X serves as an “occasion setter”. The occasion setting function of X is believed to be independent of simple excitatory associations between X and the US (Holland, 1989a,b; 1991; Rescorla, 1986). Typically theorists (Konorski, 1967) have assumed that organisms form internal representations of CS’s and US’s involved in conditioning experiences. The formation of associations between representations of the CS and US endow the CS with the ability to activate the US representation. However, certain conditional discrimination (e.g. Occasion Setting) paradigms would establish a control element (the feature (X)) intermediating the representations of the CS and US. There are several lines of evidence from traditional conditioning procedures that demonstrate a dissociation between occasion setting and simple associative conditioning (Holland, 1989a,b.; Rescorla, 1986). This evidence will be examined in the discussion to follow.

Conditioned Taste Aversions: Anomaly or Associative Learning

A Conditioned Taste Aversion (CTA) is a type of learning that protects an animal against the consumption of a toxic fluid or food. It has been postulated that this type of learning is an adaptive specialization (Rozin & Kalat, 1971; Sherry & Schacter, 1987, Revusky, 1984). In other words, taste aversion learning was shaped by natural selection to
solve a specific problem or fulfill a niche in the animal's environment. Taste aversions are a unique form of learning because they can be acquired after a single pairing of the CS and the US, with long delays between the exposure to the food/taste and the feeling of sickness (Garcia et al., 1974). At first glance, this type of learning seems to have very different properties, given what is known about animal learning and memory in laboratory paradigms. (Schwartz, 1984).

Revusky (1984) stated that a Conditioned Taste Aversion was a type of associative learning because the process of selective association was involved. Selective association, associative predisposition, or belongingness of CS to US must play a role in the learning of conditioned taste aversions. If this were not so, associations of the sickness with the many extraneous events were bound to occur over a delay of several hours between the taste and sickness would cancel out the taste-illness association (Revusky, 1984).

I will be embarking on an examination of the existence of multiple memory systems in the mammalian brain. Sherry and his colleagues (1987) claim that memory systems are defined as systems in terms of 'rules of operation'. In functional terms, memory systems are different if making an adequate solution of a problem using one system, renders that system unable to solve a different problem using the same set of rules. Due to the above phenomenon, solving simple environmental problems is believed to have been a highly selective process which has developed or evolved into a more general associative learning process (Revusky, 1984).
idea fits with Sherry and Schacter's belief (1987) that by virtue of evolutionary exaptation, memory systems which had arisen to solve a specific problem, came to serve other memory function. For example, the evolutionary purpose of human memory was not that of remembering telephone numbers. Although taste aversion learning appears to occur after a single CS/US pairing and is resistant to long interstimulus intervals, it does appear to be a form of associative learning. Several studies have demonstrated that CTA's are stronger with a stronger CS (i.e. stronger-taste) and with a stronger US (i.e. higher dose of LiCl) (Garcia et al., 1974). Like other forms of associative learning, taste aversion learning demonstrates blocking (Martin et al., 1989), extinction (Kraemer et al., 1992), overshadowing (Schachtman et al., 1992) and second order conditioning (Stefurak et al., 1990). Finally, I will be using the Conditioned Taste Aversion framework in this thesis to examine a higher order conditioning task. There has been evidence demonstrating that conditioned taste aversions can be used as a good index of drug discrimination learning (Skinner et al., 1992; Jaeger et al., 1990).

The Memory Literature: A Historical Perspective

Aristotle was one of the first philosophers to postulate the possible location of our learning and memory faculties. He proposed that our memory faculties reside in the heart. Our modern day expression "learn by the heart" may have been derived from this early idea concerning the storage site of memory. Later thinkers, such Thomas Willis identified the brain as being the most viable anatomical candidate for memory. Centuries of investigation had led to little in the way of further progress. A breakthrough finding in the 1950's (Scoville and
Milner, 1957) implicated the medial temporal lobes of the mammalian brain as being an important site mediating learning and memory. The original line of thinking was that the hippocampal formation represented the unitary memory system of the mammalian brain. However, as is the case with human amnesics, hippocampal lesioned animals were impaired on certain tasks but were normal on others. The above evidence, along with recent findings, have formed the basis for one of the most intriguing and enduring debates in the history of neuroscience: Is there a unitary memory system, and if not, how many memory systems are there?

The Hippocampus: The Unitary Memory System

Historical observations of the ability of many animals to find their way back to their home nests over large distances and the ability of many South Sea Islanders to navigate open waters to locate various land marks, suggested that some type of mapping system was involved in memory. Driven by the findings of Scoville & Milner (1957), which implicated the medial temporal lobes in memory, decades of research produced several theories concerning the neuroanatomical basis of learning and memory. O’Keefe and Nadel (1978) proposed one of the most specific and enduring theories concerning the role of the hippocampus and memory. They proposed the cognitive mapping theory (O’Keefe & Nadel, 1978), which states that the hippocampus is the spatial learning and memory center of the brain. Their theory distinguished between a locale and a taxon system, with only the locale system being dependent on the hippocampus. The term ‘locale’ was chosen to denote the fact that this system processed spatial information. The term ‘taxon’ was chosen to denote the fact that processing within
this system(s) was based on the fact that processing was based on the taxonomic principles of category inclusion and generalization.

Electrophysiological recordings from cells in hippocampus demonstrated selective firing of specific cells when an animal was in particular areas of an environment (O'Keefe, 1976). The cells became known as place cells (units) and came to signal an animal's position in an environment (place field) (O'Keefe & Conway, 1978). Unit recordings from place cells demonstrated that although the same cells fired in different environments, there was a significant difference in the place fields and firing patterns of the place cells (O'Keefe & Conway, 1978) depending on the environment in which the animal was placed. Therefore, the specific firing patterns of place fields in certain environments, linked together in the appropriate fashion, provided the cognitive map for the hippocampus.

Over the years, considerable support for the cognitive mapping theory has accumulated from many animal studies (Morris et al., 1982; Sherry & Vaccarino, 1989; Bingman & Yates, 1992). One of the most frequently used procedures for testing spatial learning has been the water maze task (Morris et al., 1982). In this task, a submerged platform is placed in a circular pool filled with opaque water. Rats must use distal cues in the room to locate the hidden platform. Animals with hippocampal lesions are severely impaired on the learning of this task. Conversely, if the platform is visible the hippocampal rats learn the tasks as well as controls. This evidence was used to suggest that place learning could be derived from using the spatial relationships between (allothetic) distal cues. By demonstrating hippocampal deficits in
the Morris Water maze, the rodent literature has produced evidence in support of the cognitive mapping theory.

Although there has been evidence directly supporting the cognitive mapping theory, this support has been derived from a limited domain of experimental data. Many theorists began to attempt to amalgamate the animal literature with the human literature. It was becoming apparent that spatial learning and memory was probably not the only function of the hippocampus. Over the years, a considerable amount of data has been collected on the famous patient H.M. and various other amnesics. Cohen and Eichenbaum (1991) argue that the cognitive mapping theory cannot explain human amnesia, because memory deficits found in the amnesic patients are not limited to the spatial domain. Damage to the hippocampal formation often produced a gradient type of retrograde amnesia, in which memory for recent, but not remote memories, were lost (Nadel and Moscovitch, 1997). Although impairments of the right hippocampus impairs recall of spatial location (Smith & Milner, 1987) in humans, the specific role of the left hippocampus is unresolved. This situation has left Nadel (1991), no choice but to admit that "at least in the case of the human hippocampal system, there is more than spatial mapping going on".

In light of evidence demonstrating the inadequacies of the cognitive mapping theory to handle all aspects of literature concerning hippocampal damage, the theories concerning hippocampal involvement in memory evolved. If the cognitive mapping theory could be
changed from the claim that hippocampal representation is for spatial relations, to the position that the hippocampus is crucial for representing configurations among perceptually independent cues, both spatial and non-spatial relations alike (Cohen & Eichenbaum, 1991), then place learning could be synthesized with accounts that proposed that the hippocampus was able to process and synthesize configural cues from cue elements (or visa versa). Perhaps a configural system was involved in solving spatial tasks that required the learning of relationships between distal cues. Researchers began to explore the possibility of multiple memory systems. Many theories began to emerge most postulating that, in the very least, two separate systems were involved in memory.

**Configural Association Theory**

One theory that directly addresses this line of thinking was the configural association theory postulated by Sutherland and Rudy (1989). The configural association theory posits that there are two distinct learning and memory systems: a simple association system (SAS) that operates independently of the hippocampus and a configural association system (CAS) that is dependent on an intact hippocampus. If an animal has to remember one stimulus or cue, it requires only a simple association system. For tasks that require an animal to remember the relationship among two or more cues, the configural association system (i.e. hippocampus) is essential. The simple association system records the organisms experiences as changes in the strength of associations between elementary stimulus events. For example, the
relationship of a stimulus X and an unconditioned stimulus (US) can be strengthened or weakened depending on its contingency with the US. Thus the SAS system would be able to explain simple excitatory or associative learning paradigms as Pavlovian conditioning (i.e. direct pairings of a stimulus X with an unconditioned stimulus). The configural association system combines the representations of elementary stimulus events to construct unique representations and allows for formation of associations between configural representations, and other elementary representations. This theory provided an excellent model describing the results of the Morris water maze invisible platform task. The theory suggests that the hippocampal system is involved in place learning, because rodents learned the spatial location of the platform by using configural (distal) cues and their relationships in the maze room for orientation (Sutherland & Rudy, 1989). The SAS explained the lack of impairment shown by hippocampal lesioned animals in the visible platform task. A key point made by the authors of this theory was the fact that, in any situation, information about the organism’s experience is stored in both systems. In other words, both systems operate in parallel. This view that memory systems operate in parallel has become widely accepted and will be looked at more closely in the discussion concerning other theories of multiple memory systems. According to the configural association theory, in any paradigm, if one system were to be lost (i.e. CAS), the other system (SAS) would guide responding.

Sutherland and Rudy (1989) have used the configural association theory to explain results form tasks such as place learning, recognition memory, latent inhibition, Pavlovian serial
compound learning, discrimination reversal learning and stimulus selection. Solutions to all of
the above tasks are mediated through simple associations and/or hippocampal dependent
configural associations. Thus, the researchers put forth a prototypical configural, negative
patternning task, which they argued could only be solved configurally. This task has no
obvious simple association solution. In the negative patterning task each of two cues is followed
by a US when presented singly (A+ and B+), but the US is withheld when the two cues are
presented together (AB-). Control animals acquire this task, however, hippocampal lesioned
rats are typically impaired on this task (Rudy & Sutherland, 1989). Further experiments using
this paradigm have cast doubt on the solely hippocampal dependency for solving this task.

Skinner and van der Kooy (1997) have demonstrated that hippocampal lesioned animals can in
fact acquire the negative patterning task. Various other studies have questioned Sutherland and
Rudy’s (1989) configural theory by demonstrating that animals with hippocampal lesions can
acquire other configural tasks such as a visual-tactile transwitching task (Wishaw & Tomie,
1995). In light of the fact that tests of the configural theory have yielded conflicting results,
and that recent evidence has been presented which demonstrated that damage to extra-
hippocampal structures cause impairments in configural learning (Wishaw et al., 1992),
Sutherland & Rudy’s simplistic dichotomous system of learning and memory has undergone
severe scrutiny.
CURRENT THEORIES:

Parallel Multiple Memory Systems

As one ventures into the memory based literature of the current decade, one begins to see a trend in memory-based literature. The role of the hippocampus is no longer that of the only memory module (system), nor does its role seem to be limited to one of only two main memory streams. Instead of being the potential site of all learning and memory (1950’s), or the configural (relational) system of memory processing potentially all spatial and non-spatial complex or multiple (1970’s and 1980’s), many researchers now argue and agree that the experimental literature has pointed to a more limited role for the hippocampus in learning and memory.

While the idea of multiple memory systems is quite old, the systematic analysis and discussion of the issue has occurred much more recently. Behavioural dissociations of different types of learning and memory have allowed for identification of the neuroanatomical basis of various functionally dissociable systems. Researchers have identified at least five functionally dissociable memory systems in the mammalian brain which may be capable of processing different kinds of information. It has been proposed that the dorsal striatum, the amygdala, the cerebellum, the (Peri)rhinal cortex and the hippocampus are the distinct anatomical loci involved in the processing of the various types of information. Evidence has pointed to the involvement of the cerebellum in the learning of classical conditioning tasks using the rabbit eye blink paradigm (Swain et al., 1992). The rhinal cortex has become a major focus in much of the primate literature. Lesions of various areas of the rhinal cortex have lead to various
impairments in visual recognition tasks and the ability to form complex associations of various objects (Murray, 1996). The dorsal striatum seems to mediate the formation of reinforced stimulus-response associations. This system could be considered a simple associative learning system in which neutral stimuli come to elicit specific motor responses because the association between these stimuli and response was repeatedly reinforced (McDonald & White, 1993). Years of research had directed researchers to examine the role that the amygdala played in emotional behaviour. McDonald (1993) and other researchers (Davis, 1992; Sutherland & McDonald, 1990) have accumulated evidence that suggests that the neural system that includes the amygdala, may mediate the formation of behaviours based on the association of neutral stimuli with biologically significant events that have affective properties. Finally, the hippocampus still appears to be necessary for tasks that require the use of information about relationships among stimuli; however, this multiple memory system view appears to limit its function solely to these types of associations. It can be interpreted that the above definition of the role of the hippocampus would still aid in identifying and remembering multiple spatial locations (place learning).

By no means is this an exhaustive list of all possible memory systems in the mammalian brain. However, in support of the above multiple memory system models, a triple behavioural dissociation has been demonstrated by McDonald and White (1993) concerning the functions of the dorsal striatum, the hippocampus, and the amygdala. Using three tasks developed for an 8 arm radial maze, (McDonald & White 1993) the above researchers supplied evidence demonstrating that the role of the hippocampus was the processing of
spatial (stimulus - stimulus) relationships to guide behaviour, the role of the amygdala was acquiring a representation of conditioned reward (identifying incentive properties; Stimulus-Reward) in the absence of a response, and the role of the dorsal striatum was acquiring stimulus response contingencies. These parallel memory systems put forth by McDonald & White (1993) will be of great interest in the chapters to follow.

**Experimental and Theoretical Background**

Having briefly examined the history of the multiple memory system literature, I will now present the experimental results and the behavioural background upon which my thesis work has been based. Drug discrimination learning using the taste aversion framework is similar to occasion setting (Martin, Gans, & van der Kooy, 1990). Since taste aversions are easily learned, they have been used in drug discrimination procedures and found to be faster and more sensitive than traditional drug discrimination paradigms (Skinner & Martin, 1992). Therefore, using a drug/contextual discrimination (i.e. Occasion Setting) paradigm within the conditioned taste aversion framework, results from our lab have been able to demonstrate a behavioural dissociation between two types of learning: Occasion Setting and Pavlovian Learning. As described earlier, in an occasion setting paradigm, a target stimulus A, predicts the presentation of the US, only when preceded by X (the feature). Previous results (Skinner et al., 1994; Skinner et al., 1995) demonstrated that rodents have reliably come to acquire the ability to modulate consumption of a saccharin solution (the target) based on the presence of a feature cue (an occasion setter) which sets the occasion for the presence of
sickness due to the consumption of the saccharin solution paired with a Lithium Chloride (LiCl) injection. Both internal (i.e. morphine, saline injections) cues, as well as external cues (flavours, tastes) have been used effectively as occasion setters in our paradigm (Skinner & Martin 1992; Skinner et al., 1994, 1995).

As stated earlier, the occasion setting function of X is believed to be independent of simple excitatory associations between X and the US (Holland, 1989a,b; Rescorla 1986). There are several lines of evidence that support a dissociation between occasion setting and simple associative conditioning (Holland 1989; Rescorla 1986, 1991). First, manipulations of the feature (X) alone have no impact on its control over responding to the target (A). In other words, when X predicts that A will be followed by reinforcement, giving unreinforced exposure to X (extinction trials) does not reduce its control over responding to A. Also, when X precedes A, in the absence of the reinforcer, separate reinforcement of X does not change responding to A (Holland 1989a, Rescorla, 1991a). Therefore, we see that once the occasion setting framework has been laid down, extinction trials, as well as, isolating either the feature or the target have no effect on the ability of X to control responding to A. Skinner and her colleagues (1994) have been able to demonstrate X-US pairings (direct pairings of the feature cue and the US (i.e. context and LiCl)) are not sufficient to produce control over responding to the target in the contextual discrimination paradigm. The second line of evidence is that, if our contextual/drug discrimination task involved a direct association between context/morphine and Lithium Chloride, blocking results should be observed using Kamin’s (1967) blocking procedure. However, Martin and his colleagues (1990) showed
that after drug discrimination training, morphine did not block the formation of a novel flavour-LiCl association. Hence, a simple direct association of morphine and LiCl did not underlie these conditional discriminations. Third, transfer tests demonstrate that occasion setters only show transfer of control to elements trained in a similar occasion setting paradigm (Skinner et al., 1994), whereas, simple excitors and inhibitors often show generalized transfer across many CS’s.

**Our Behavioural Index of Learning**

Using our contextual discrimination paradigm, the ability for rodents to reliably learn to modulate consumption of target saccharin stimulus has been successfully demonstrated time and time again (Skinner et al., 1994; Skinner et al., 1994a; Skinner et al., 1995b; Martin et al., 1990). Various internal, as well as, external cues have been used as features in order to demonstrate this Occasion Setting Learning. While acquiring the ability to solve this paradigm, rodents simultaneously acquire Pavlovian aversions to the various contexts trained as the occasion setters, that predicts when saccharin will be followed by a LiCl injection. Pavlovian aversions are measured by allowing the animals free (10 minutes) access to the training environments. Control animals develop aversions to the context (feature cue) which predicts sickness and thus spend significantly less time in that context on a subsequent place test. Skinner and her colleagues (1994) have been able to extinguish Pavlovian learning in the rodents without affecting Occasion Setting. She has also been able to extinguish Occasion Setting in our paradigm without affecting Pavlovian Learning. This kind of behavioural
double dissociation presents strong evidence for the existence of two separate learning systems and strengthens the evidence supporting the fact that Occasion Setting Learning and Pavlovian Learning are fundamentally different.

Having found the behavioural evidence supporting a dissociation between Occasion Setting and Pavlovian Learning, the next step was to identify the neuroanatomical locus(i) which mediated each type of learning. While attempting to identify the loci mediating the two distinct types of learning, I postulate that upon finding these loci I may also be uncovering the specific mechanisms supporting both types of learning. However, I realize that two or three different loci do not necessarily denote two or three different neuronal mechanisms. One locus may contain several different mechanisms, as well, similar mechanisms may underlie the learning in several different anatomical structures. Skinner and her colleagues began with lesioning the hippocampus and using neonatal decorticated rats to assess their performance on our task. Although the rats with the aspiration lesion of the hippocampus were slower compared to controls, both groups learned the conditional discrimination task as well as controls, (Skinner et al., 1995). Amygdala Lesions have been found to block the Pavlovian aversions to contexts, but not the ability of contexts to serve as Occasion Setters (Skinner, unpublished). This piece of data implicates the amygdala as being the neuroanatomical locus mediating Pavlovian Learning in our Paradigm. This will be discussed in greater detail in the chapters to follow. The following chapters are a series of lesion studies aimed at identifying the neuroanatomical locus(i) which mediate the more complex higher order Occasion Setting Learning demonstrated in our Paradigm.
CHAPTER 2

COMBINED HIPPOCAMPALE PLUS AMYGDALA LESIONS BLOCK CONTEXT AVersions BUT NOT THE ABILITY OF CONTEXTS TO SERVE AS OCCASION SETTERS.
ABSTRACT

An occasion setter is a discriminative cue (feature) that predicts whether or not a target stimulus is followed by a reinforced event (the US). We previously postulated that discriminative control of the occasion setter does not rest simply with Pavlovian associations between the feature and the US or between the feature and the target stimulus. In this occasion setting task, one context predicted that access to saccharin will be followed by an injection of LiCl while a second context predicted that saccharin will be followed by a saline injection. Acquisition of this conditional discrimination was shown not to be dependent on hippocampal processing. Past work has also demonstrated that amygdala lesioned rats learned to suppress fluid intake in the first relative to the second context (occasion setting), however, they later failed to avoid the context associated with LiCl on a choice test after both Pavlovian and occasion setting training. In the present study, I attempted to identify the neuroanatomical loci of the complex learning using a double lesion. Rats with combined ibotenic acid lesions of the amygdala and aspiration lesions of the dorsal hippocampus were compared to sham controls on the contextual occasion setting task. Both groups learned to suppress saccharin intake in the LiCl paired context (occasion setting). However, sham controls showed a significant aversion for the LiCl associated context, whereas lesioned rats showed no such aversion on a choice test. Both groups were tested on the Morris Water task. On a probe trial, lesioned rats spent significantly less time in the quadrant which previously contained the platform compared to controls, thus demonstrating a hippocampal deficit. The evidence showing that the amygdala plus hippocampal lesioned
rats could acquire the occasion setting function of contextual cues in the absence of a context aversion further strengthens the suggestion that the Pavlovian aversive properties of the context are not necessary for acquisition of occasion setting. This anatomical dissociation supports previous behavioural data suggesting that simple Pavlovian conditioning and occasion setting learning are fundamentally different.
INTRODUCTION

The rationale for performing these series of experiments is to identify the neuroanatomical locus(i) involved in the acquisition of our conditional (contextual) discrimination. A previous study has been performed in which rats with global amygdala lesions (i.e. both the Central Nucleus (CN) and the basolateral amygdala (ABL) have been destroyed using ibotenic acid) have acquired the ability to modulate the consumption of a saccharin solution in our paradigm (Skinner, unpublished). These animals demonstrate the ability to consume significantly more of the target solution on a “safe” versus a “danger” day. The very interesting finding in this study was that the amygdala lesioned animals lost their aversion (fear) of the danger context on a subsequent place test. Perhaps, the amygdala is the site that mediates the simple Pavlovian Learning. Our conditional discrimination task has a taste aversion component. Many studies have suggested that lesions to the amygdala impair taste aversion learning (Gallo et al., 1992; Kesner, et al., 1992). Also amygdala lesions are known to impair performance on other aversive paradigms (Davis, 1992; LeDoux 1993), such as those that involve pairing visual, auditory or contextual cues with a mild footshock. After conditioning in the above paradigms, freezing behaviour in amygdala lesioned animals is severely impaired (Fanselow & Kim, 1994; Phillips & Le Doux, 1992). Animals with amygdala damage are also impaired on appetitively motivated tasks where previously neutral stimuli are paired with reward (Gaffan & Murray 1990; Brown & Fibiger 1993). In light of the above evidence and their own results,
McDonald and White (1993) proposed that the amygdala is involved in learning about the emotional significance of stimuli (Stimulus-Reward) learning.

Many theories exist which postulate that the hippocampal formation is responsible for forming relational or configural associations (Sutherland & Rudy, 1989). Sutherland and McDonald (1990) have also claimed that the hippocampus is involved in fear conditioning to context. Due to the contextual nature of this task it is possible that the hippocampus might also play a role (Good & Honey, 1991; Kim & Fanselow, 1992). In a previous experiment Skinner and her colleagues (1995) demonstrated that while hippocampal lesioned rats were slower to acquire the conditional discrimination task, they eventually reached control levels of performance. These animals also demonstrated a significant aversion to the danger context paired with the LiCl injection on a subsequent place test. This suggests that although the hippocampus may play some role in the processing of contextual cues, it may not be critical for the acquisition of conditional learning.

In an interesting study (1995), McDonald and White provided convincing evidence to support the view that memory systems are "on line" at all times and simultaneously process information in parallel. Depending on the behavioural demands of a particular task, these systems may work synergistically or may compete for information and possibly impair the "correct" processing. If as postulated by McDonald and White (1993), the amygdala is involved in learning the emotional significance of stimuli (Stimulus-Reward Learning) and the
hippocampus is necessary for stimulus - stimulus (S-S) associations, perhaps in the absence of one system the other was sufficient to acquire the contextual discrimination task. In other words, in the absence of an intact hippocampus, the emotional significance of the context cues might have been sufficient for discrimination learning, and likewise, in the absence of the amygdala, the sensory properties of the context might be sufficient for the discrimination learning seen in our previous experiments. Therefore, perhaps eliminating both memory systems would destroy the ability for the animals to learn the complex Occasion Setting Learning. If this is the case, I would expect to see no evidence of differential consumption in the two training contexts.
MATERIALS AND METHODS

Subjects

Thirteen adult male Wistar rats, weighing 350-400 g at the beginning of behavioural training, were used as subjects. All rats underwent surgery within one or two weeks of their arrival in the lab. After recovery from surgery, rats were adapted to a water deprivation schedule consisting of 60 minutes access to tap water each afternoon. One week later behavioural training began.

Surgery

Seven rats underwent ibotenic acid lesions of the amygdala. Rats were anesthetized with sodium pentobarbital (65 mg/kg ip) and placed in a stereotaxic instrument. After a scalp incision was made, holes were drilled in the skull to permit microinjections of the ibotenic acid. Using a 1.0 μl Hamilton syringe mounted on the stereotaxic instrument, injections of 2% ibotenic acid were made at each of two sites, one site per hemisphere (AP -2.3; ML ± 4.6; DV from dura -7.4) in a volume of 0.5 μl/site. Ibotenic acid was injected at a rate of 0.05 μl/min. and the needle was left in place for an additional 5 min. following infusion. In addition, these rats were given aspiration lesions of the dorsal hippocampus using the method described previously (Skinner et al., 1995). Aspiration was accomplished using a glass pipette attached to a vacuum aspirator. At each of four sites (coordinates: AP from bregma -2.5, L ± 2.0, DV from dura -2.5; AP -3.5, L ± 2.0, DV -2.5), the pipette was lowered to the dorsoventral coordinate, the aspirator was turned on, and the pipette was slowly raised back
up to the dura. Six control rats underwent the same procedure but only the cortex overlying the dorsal hippocampus was removed. In addition, these control rats received saline injections in the amygdala exactly as described for the sham lesioned rats in the previous experiment.

Conditional Discrimination

After recovery from surgery and adaptation to water deprivation, the two groups of rats were trained on a conditional contextual discrimination task. (Skinner et al., 1995a, b). This phase of the experiment was divided into danger and safe days. On danger days rats were placed in a novel context 15 min prior to 15 min access to a novel 0.1% saccharin solution. Removal of the saccharin solution was followed by an i.p. injection of 60 mg/kg LiCl dissolved in 3 ml/kg physiological saline and rats were returned to the home cage. On safe days, rats were placed in a second novel context 15 minutes prior to 15 minutes access to the saccharin solution. Saccharin removal was followed by an i.p. injection of physiological saline and rats were returned to the home cage. The training contexts were wooden boxes measuring 41 x 41 x 38 cm. For half the rats the danger context was a black box with a plexiglass floor wiped with 2% vinegar solution prior to each trial. The safe context was a white box with mesh floor covered with wood chips. The other half of the rats had these contextual cues reversed. This phase of the experiment consisted of 14 cycles of alternating safe and danger days for all groups; with a string of 10 safe days between cycles 7 and 8. For the purpose of statistical analyses, the data were organized into 14 cycles of safe days followed by danger days.
Vinegar Transfer Test

At the end of discrimination training all rats were given a two-day transfer test with a novel 2% vinegar solution. Rats were exposed to both training contexts, in a counterbalanced order, 15 minutes prior to a 15 minute access to the vinegar solution. No injections were given after removal of the flavoured solution.

Place aversion testing

Two days after the vinegar transfer test, all rats were given a place choice test. The test procedure consisted of placing a rat in a neutral gray zone between the black and white contexts used during training. The amount of time spent in each of the two contexts was recorded in seconds for a 10 minute period. Since the contextual cues were counterbalanced (half the rats had the black box as the LiCl-paired context and half had the black box as the saline-paired context) we did not measure the initial preference of the individual rats prior to training. However, considerable previous work using identical contextual cues has shown that naïve rats, on average, show a 50:50 split of their time in the two contexts on a place choice test (Bechara and van der Kooy, 1992; Mucha et al., 1982).

Morris Water Task

Upon completion of the place test rats were trained on the hidden platform version of the Morris water task. The apparatus was a white circular pool with 105.5 cm diameter and
76.0 cm wall height. The water was made opaque by the addition of Carnation powdered milk. A circular, plastic platform 10.0 cm in diameter and 49.5 cm in height was placed in one quadrant of the pool just below the surface of the water. Rats were given 5 blocks of 4 trials over a four-day period, for a total of 20 trials. On each trial, rats were placed in the pool from one of three starting locations (rats were not started from the platform quadrant) and the time to locate the platform was recorded in seconds. If the rat failed to locate the platform in 90 s, the experimenter placed the rat on the platform. On all trials the rat was left on the platform for 30 s. After completion of the last trial, all rats were given a probe trial for 90s with the hidden platform removed. The time spent in the quadrant that had previously contained the platform was recorded in seconds.

**Histology**

After completion of behavioural experiments, all rats were deeply anesthetized with sodium pentobarbital and perfused through the heart with isotonic saline followed by 10% formalin. The brains were removed and stored in 25% sucrose, and then 30u cryostat sections were cut. Sections were mounted on slides and stained with cresyl violet in order to verify the placement and extent of the lesions.
RESULTS

Histology

All lesioned rats had extensive damage to the dorsal hippocampus and amygdala (Figure 1). The majority of rats had near complete ablation of the central, lateral, basolateral, and basomedial nuclei of the amygdala. Most rats had some extra-amygdala damage including the most ventral portion of the caudate-putamen, the piriform cortex, and the dorsal and ventral endopiriform nuclei. While ibotenic acid usually spares fibers of passage, the lesioned rats in this experiment sometimes had cavities at the injection site that obviously disrupted some fibers of passage. Bilateral damage to the dorsal hippocampus was extensive in all lesioned rats. All rats had near complete ablation of the CA fields, dentate gyrus, and fimbria in the dorsal region, as well as damage to the overlying cortex and corpus callosum. Two rats had damage that extended into the ventral hippocampus. Sham lesioned rats had damage to the cortex overlying the hippocampus and two rats had minor damage to the most dorsal region of the hippocampus. Sham lesioned rats had no damage to the amygdala other than the saline needle tracts.

Conditional Discrimination Learning

Both sham lesioned rats and rats with combined lesions of the dorsal hippocampus and amygdala learned the conditional discrimination with contextual cues, consuming significantly less in the danger context than in the safe context. A $2 \times 14 \times 2$ (Groups x Cycles x Days)
ANOVA over the acquisition phase revealed a significant Cycles x Days interaction (F(13, 182) = 24.97, p < .05), reflecting acquisition of the discrimination and a significant Groups x Cycles interaction (F(13, 181) = 5.85, p < .05), reflecting higher consumption by lesioned animals on earlier cycles (Figure 2). Further ANOVAs performed on the last cycle, the last three cycles and the last five cycles demonstrated no significant interactions of group with any other variable.

**Vinegar Transfer Test.**

Both lesioned and sham rats showed transfer to a novel vinegar solution after conditional discrimination training with a saccharin solution (Figure 3). All rats suppressed consumption of the novel vinegar in the danger context relative to the safe context, as reported in the first experiment and in previous published work (Skinner et al., 1994 a, b). A 2 x 2 (Groups x Days) ANOVA over the transfer test revealed only a significant main effect of Days (F(1, 13) = 41.66, p < .05).

**Place Test**

As in previous experiments (Skinner et al., 1994a,b; Skinner, 1995) sham lesioned rats showed large aversions to the context associated with LiCl. However, like the amygdala lesioned rats (Skinner, unpublished), rats with combined lesions of the dorsal hippocampus plus amygdala did not avoid the context associated with LiCl (Figure 4). A 2 x 2 (Groups x Context) ANOVA revealed a significant two-way interaction (F(1, 25) = 13.91, p < .05).
Newman-Keuls tests revealed that the sham lesioned group showed a significant aversion to the LiCl associated context \(p < .05\), while the lesioned group did not avoid the LiCl associated context \(p > .05\).

**Morris Water Maze**

Aspiration lesions of the dorsal hippocampus were sufficient to produce an impairment in the water maze task. A 2 x 20 (Groups x Trials) ANOVA over the acquisition phase revealed a significant effect of Group \(F(1,12)=5.58, p < .05\) and Trials \(F(19,247)=2.28, p<.05\). A t-test on the last trial revealed that lesioned rats had significantly longer latencies to find the hidden platform than did the sham lesioned controls \(t(11) = 2.61, p < .05\) (data not shown). On the probe trial (hidden platform removed), control rats spent significantly more time searching in the correct quadrant than did the lesioned rats \(t(11) = 3.47, p<.05\) (Figure 5).
Figure 1. A low power photomicrograph of a coronal section through the forebrain of a representative rat with combined neurotoxic lesions of the amygdala and aspiration lesions of the dorsal hippocampus, showing a loss of the dorsal hippocampus. The extent of the amygdala damage is not clearly visible under this low power (A). A higher power photomicrograph of a representative amygdala lesion. The arrows indicate the outer edges of the gliotic reaction (B).
Figure 2. Saccharin consumption (ml) by the sham (n=6) and lesioned (n=7) contextual discrimination groups during the 14 cycles of discrimination training. S=Safe Day Trials where rats receive context 1 paired with saccharin-saline; D=Danger Day Trials where rats receive context 2 paired with saccharin-LiCl.
Figure 3. Mean (± sem) vinegar consumption (ml) by the lesioned and control groups during the 2-day transfer test at the end of training.
Figure 4. Mean (± sem) times (in seconds) spent in each of the two training contexts during the 10-min place test by rats in the lesioned and control groups. Safe= context associated with saline during training and Danger= context associated with LiCl during training.
Figure 5. Mean (± sem) duration of time spent in the platform quadrant of the water maze during a 90 s probe trial with the platform removed. Data represent means (± sem) for 6 control and 7 lesioned rats.
TIME (S) SPENT IN PLATFORM QUADRANT

CONTROL

LESION
DISCUSSION

Data from the sham lesioned rats in this experiment once again demonstrated that in non-lesioned rats contextual cues could serve as occasion setters to modulate a taste aversion and that this occasion setting function transfers to a novel flavour (Skinner et al., 1994). Unlike the controls, the hippocampal and amygdala lesioned rats demonstrated a lack of a place aversion to the context that predicted the LiCl injection. One may argue that the lack of this place aversion in the lesioned rats was due to the hippocampal damage and not to the damage to the amygdala. However, a previous study (Skinner, 1994) has demonstrated that hippocampal lesioned animals perform similar to controls avoiding the context associated with LiCl on a place test. Also, the lesioned rats in the present experiment were able to use contextual information to solve the conditional discrimination, suggesting that the hippocampus was not important in processing the contextual cues. Given that such a robust Pavlovian aversion to the context associated with LiCl occurred in this study and that the identical finding was observed with amygdala lesioned rats, the most salient conclusion is that the simple Pavlovian aversion to the context is mediated by the amygdala.

Previous behavioural data has demonstrated that simple direct Pavlovian associations between the context and LiCl were not sufficient to produce Occasion Setting Learning (i.e. discrimination learning/modulation of consumption) (Skinner et al., 1994 a,b). In the present experiment, lesioned rats also demonstrated that the acquisition of a context-LiCl association is not necessary for the context to serve as an occasion setter. This finding would imply that
the contribution of a context-LiCl direct association is negligible in this type of contextual discrimination learning, since rats with the combined lesions were not impaired on the conditional discrimination task and still demonstrated a complete lack of a place aversion. The results of the transfer data also demonstrate that animals are not forming a compound cue of context and saccharin to modulate their drinking response. If rats were learning about a compound cue, then presenting the novel vinegar solution should disrupt this compound cue and impair performance on our task. The above pieces of evidence support Holland's view of occasion setting where he states that, the modulation of a target-US association is independent of any direct associations between the occasion setter and the US (Holland, 1989).

Although, the combined lesions were sufficient to produce a significant impairment in the Morris water task, which is due to the dorsal hippocampal lesion, the combined lesioned animals did not display any deficits on the learning of our configural task. The fact that the more simple learning (i.e. Pavlovian Aversions) would be easier to disrupt than the more complex type learning (Occasion Setting) may seem counterintuitive. However, similar results have been reported in humans (Bechara et al., 1995; LaBar et al., 1995) and rats McDonald and White, (1993). In the human studies, conditioned autonomic fear responses were impaired in subjects with damage to the amygdala. These same subjects, however, were able to declare the contingencies that were currently active (Bechara et al., 1995). McDonald and White (1993) showed that rats with amygdala damage could learn a spatial version of the win-shift task in a radial arm maze. Amygdala lesioned rats could not learn the intuitively simpler task
of approaching a single visual cue that had previously been paired with reward. In essence, these amygdala lesioned rats could learn about a configuration of cues but not that a single element predicted food. Data such as these (presence of more complex learning in the absence of simple learning) are probably the best support for the idea that the mammalian brain has multiple memory systems. Since the simplest type of learning may only be mediated by one anatomical locus, it could be disrupted by one specific lesion. The acquisition of a higher order occasion setting learning may be harder to eliminate, because several memory systems may be able to acquire the discrimination simultaneously.
CHAPTER 3

ACQUISITION OF SIMPLE AND CONDITIONAL DISCRIMINATION IN DORSAL STRIATUM LESIONED RATS
ABSTRACT

This experiment examined whether ibotenic acid lesions of the dorsal striatum would disrupt the acquisition of the Occasion Setting Learning (i.e. the modulating of the drinking response). The findings of the previous chapter strengthened the hypothesis that the discriminative control of an occasion setter does not rest simply with Pavlovian associations between the feature and the US or between the feature and the target. Rats with dorsal striatal lesions were compared to sham controls on the contextual discrimination task. On some days, rats were exposed to context A (danger) for 15 minutes prior to a 15 minute presentation of a saccharin solution followed by a LiCl injection. On alternate days, rats were exposed to context B (safe) for 15 minutes followed again by the identical saccharin solution for 15 minutes after which they were given a saline injection. Both groups learned to suppress consumption of the saccharin solution in the LiCl paired context. Also, both groups demonstrated transfer of the occasion setting properties of the context to a novel vinegar solution, and both groups acquired a Pavlovian aversion to the LiCl paired context as demonstrated on a subsequent place test. The dorsal striatal lesioned animals behaved as control animals in our Contextual Discrimination Paradigm.
INTRODUCTION

The results of the previous experiment seem to suggest that neither the sensory nor the motivational aspects of the contextual cues were critical for their occasion setting properties. Perhaps the sensory/perceptual properties of the context are stored in another area of the brain such as the rhinal cortex. Animals with lesions to the rhinal cortex have demonstrated various deficits in conditional learning tasks (Parker et al., 1998; Buckley, 1997).

Skinner and her colleagues (1994b) have postulated that perhaps what the animals are learning during training in our paradigm is the modulation of a motor response (fluid consumption/licking the spout). When conditioned rats are given a series of extinction trials in which they are presented with tap water in both contexts, the discriminative performance of the rats disappear when the target flavour is re-introduced.

According to McDonald and White (1993) the dorsal striatum mediates the formation of reinforced stimulus motor response associations. Since the animals may be learning to modulate a motor (drinking) response in our paradigm, I decided to examine the Dorsal Striatum as the site that may be the neuroanatomical locus for our contextual discrimination learning.

The rat striatum can be divided into patch and matrix components. In the adult striatum, opiate receptors, opiate peptides and various cortical termination occur in the patchy distributions (Herkenham et al., 1981; Lanca et al., 1986). These groups form a mosaic of
holes (cellular islands / patches) in the striatal matrix (Goldman-Rakic, 1982). Heimer and coworkers (1975), distinguished between a "neocortical" system that includes the dorsal striatum and an "allocortical" system that includes the ventral striatum. Most of the patchy innervations of the striatum seem to occur in the ventral striatum. Massive innervation from the amygdala reaches all parts of the striatum, except the dorsolateral quadrant (White, 1989). Many researchers (Goldman-Rakic, 1982; Gerfen, 1984; Velley et al., 1988) have pointed out that there are relatively few patches in the dorsolateral striatum and observed that the nucleus accumbens (i.e. ventral striatum) consists of many patches.

The anatomical dissociations described above are also based on several behavioural findings. Although I present the striatum as being subdivided into two major systems, Reading and his colleagues (1991) have been able to further dissociate the striatum into three functional units based on differential performance of variations to a complex visual stimulus response habit task. Since the ventral striatum has an abundant supply of limbic system inputs, it is believed that this patchy system is involved in the mediation of emotion or reward (White, 1989).

Several lines of evidence implicate the dorsal striatum in the mediation of memory. Kesner (1974) demonstrated that electrical stimulation of the dorsal striatum directly after training disrupts the acquisition of various memory tasks. Rats with lesions of the dorsal striatum are impaired on brightness discriminations (Schwatzbaum et al., 1968), runway
learning (Kirby et al., 1981), reversal learning (Hannon et al., 1974) go / no go olfactory discrimination (Eichenbaum et al., 1988) and cued radial arm maze learning (Packard, et al., 1989) to name a few for the successful performance of the tasks just mentioned, the dorsal striatum must be able to create the permanent sensory-motor (Stimulus-Response) connections on the basis of experience.

Given the fact that the literature had evolved to the acceptance of the possibility of multiple memory systems, Packard and his colleagues (1989 & 1992) began to postulate that the dorsal striatum was responsible for mediating certain behavioural tasks which were unaffected by hippocampal lesions. Further analysis by McDonald and his colleagues (McDonald et al., 1993; McDonald et al., 1994; McDonald et al., 1995) has led to the elaboration of the role of the dorsal striatum and the type of learning and memory that this structure mediates. These researchers state that the dorsal striatum is necessary for acquisition of reinforced stimulus - (motor) response associations (McDonald et al., 1993). For example, upon seeing a light, an animal performs the motor response of walking towards the stimulus, hence the Stimulus-Response relationship is strengthened. Since the dorsal striatal lesions should disrupt the reinforcement of the licking response in our taste aversion paradigm, I would expect to see no evidence of differential consumption in either of the training contexts. Rats should either consume large amounts of saccharin or suppress the consumption of this fluid in both contexts.
METHODS

Subjects

Twenty adult male Wistar rats, weighing 350 - 400g at the beginning of behavioural training, were used as subjects. All rats underwent surgery within one or two weeks of their arrival in the lab. After a two week recovery from surgery rats were adapted to a water deprivation schedule consisting of 60 minutes access to tap water each afternoon. One week later behavioural training began.

Surgery

Twelve rats underwent ibotenic acid lesions of the dorsal striatum. These rats were divided into two groups. All rats were anesthetized with sodium pentobarbital (65 mg/kg ip) and placed into a stereotaxic instrument. After a scalp incision was made, holes were drilled in the skull to permit microinjections of the ibotenic acid. Three holes were drilled on each side of the skull to permit three injections on each side of the head. Using a 1.0 ul Hamilton syringe, the first six (striatal 1) rats, in injections of 2% ibotenic acid were made at each of six sites, three per hemisphere (A.P. + 1.6, ML ± 3.0, DV - 6.2; A.P. + 0.5, ML ± .37, DV - 6.6; A.P. - 0.7, ML ± 4.5, DV - 7.5) in a volume of .13 ul/site. Ibotenic acid was injected at a rate of 0.05 ml/minutes and the needle was left in place for an additional 5 minutes following infusion. The second six (striatal 2) rats were given surgery using the exact procedure, however, .2 ul of 2% ibotenic acid was injected per site. Eight lesioned rats were treated in
the same manner, but injected with sterile saline (0.9% NaCl) at a volume of .1 ul per site.

**Discrimination Training**

The second striatal group (Striatal 2) was operated two weeks after the first striatal lesioned group and the sham lesioned animals began training. After recovery from surgery the three groups of rats were trained on a conditional contextual discrimination task, similar to that used in the last chapter. This phase of the experiment was divided into danger and safe days.

On danger days rats were placed in a novel context 15 minutes prior to 15 minutes access to a novel 0.1% saccharin solution. Removal of the saccharin solution was followed by an i.p. injection of 60 mg/kg LiCl dissolved in 3 ml/kg physiological saline and rats were returned to the home cage. On safe days, rats were placed in a second novel context 15 minutes prior to 15 minutes access to the same saccharin solution. Saccharin removal was followed by an i.p. injection of physiological saline and rats were returned to the home cage. The training contexts were wooden boxes measuring 41 x 41 x 38 cm. For half the rats the danger context was a black box with a plexiglass floor wiped with 2% acetic acid solution prior to each trial. The safe context was a white box with a wire mesh floor covered with wood chips. The other half of the rats had these contextual cues reversed.

The animals were trained for a total of 7 cycles, after which, a string of 11 safe days were implemented in order to increase the overall consumption of the animals. All rats received 16 cycles by the end of training.
Vinegar Transfer Test

Following the acquisition phase of the conditional discrimination, all rats were given a two day transfer test with a novel 2% vinegar solution as in the previous chapter. Rats were exposed to both training contexts, in a counterbalanced order, 15 minutes prior to a 15 minute access to the vinegar solution. No injections were given after removal of the flavoured solution.

Place Aversion Testing

Two days after the vinegar transfer test, all rats were given a place choice test identical to that given in the previous Chapter.
RESULTS

Histology

All lesioned rats had extensive damage to the dorsolateral striatum. The majority of rats also had some damage to the overlying frontal cortex. Rats were removed from the study, if the lesion site examined was too small, in the wrong place, or unilateral. Although ibotenic acid lesions usually spares fibres of passage, the lesioned rats in this experiment had some damage to the fibres of the internal capsule. Rats that were injected with sterile saline showed little damage to the overlying cortex; in some cases there were signs of blood along the needle track. Figure 6 is a schematic demonstrating the extent of the area damaged in a typical striatal lesion.

Contextual Discrimination Phase

The dorsal striatum lesioned rats and the sham lesioned rats learned the contextual discrimination task, consuming significantly less in the danger context than in the safe context. A 3 x 16 x 2 (Groups x Cycles x Days) ANOVA over the acquisition phase revealed a significant Cycles x Days interaction (F(15,272) =22.5, p<.05) reflecting acquisition of the discrimination. There was no effect between groups (p>.05) showing that all groups performed similarly on our paradigm (Figure 7). A series of ANOVAs performed on the last cycle, the last three cycles and the last five cycles also demonstrated no interactions of group with any other variable.
**Vinegar Transfer Test**

Two days after the last cycle of discrimination training with the saccharin solution, all animals were given a transfer test with a novel vinegar solution. As reported in previous findings (Skinner et al., 1994) and in the last chapter, both the lesioned groups and the sham animals suppressed consumption of the novel vinegar solution in the danger context relative to the safe context (Figure 8). A 3 x 2 (Groups x Context) ANOVA over the transfer test revealed only a significant effect of Context ($F(1,17) = 70.3, p < .05$).

**Place Aversions**

Both groups showed a large aversion to the danger context which was paired with LiCl (Figure 9). A 3 x 2 (Groups x Context) ANOVA revealed a significant main effect of context only ($F(1,17) = 59.6, p < .05$).
Figure 6. Schematic diagrams of a typical dorsal striatal lesion in the animals from this experiment.
Figure 7. Saccharin consumption (ml) by the sham (n=8), striatal 1 (n=6), and striatal 2 (n=6) contextual discrimination groups during the 16 cycles of discrimination training. S=Safe Day Trials where rats receive context 1 paired with saccharin-saline; D=Danger Day Trials where rats receive context 2 paired with saccharin-LiCl. A string of safe trial was implemented between cycle 7 and 8.
Figure 8. Mean (± sem) vinegar consumption (ml) by the all groups during the 2-day transfer test at the end of training.
Figure 9. Mean (± sem) times (in seconds) spent in each of the two training contexts during the 10-min place test by rats in the various groups. Safe= context associated with saline during training and Danger= context associated with LiCl during training.
DISCUSSION

Once again, data from all three groups of rats demonstrated that contextual cues could serve as occasion setters which modulate a taste aversion. Lesions of the dorsal striatum did not affect the transfer of the occasion setting learning to a novel vinegar solution, nor did the lesions impair the rat’s ability to acquire an aversion to the LiCl paired context. Based on the present findings, it seems that the Dorsal Striatum plays no role in the acquisition of either the simple Pavlovian learning or the complex occasion setting learning which are simultaneously acquired in our paradigm.

Each behavioural task may have some unique feature or features that make it sensitive to the function of a particular system or systems. In this situation, we postulated that the motor response of licking the saccharin spout was the behaviour which may have been critical to the acquisition of our conditional discrimination task. Since the neural system mediating stimulus-response learning is believed to be the dorsal striatum, (McDonald et al., 1993) lesions to this brain structure should have disrupted the acquisition of the modulating response. Several explanations could be made in order to understand our findings. First, the motor drinking response may not play a critical role in the learning of the modulatory behaviour. Although this seems unlikely, it would account for the negative findings demonstrated here. A more plausible explanation is that the complex Occasion Setting learning is mediated by multiple memory systems. In a remarkable follow up study to their
triple dissociation paper (McDonald et al., 1993), McDonald and his colleagues, using active and passive versions of their conditioned-cue preference (CCP) task (McDonald et al. 1995) provided convincing evidence to support the view that memory systems are “on line” at all times and simultaneously process information in parallel. If this were the case, it would not be difficult for several structures to be able to solve a configural task simultaneously. Other structures such as the hippocampus (Morris et al., 1982), the entorhinal cortex (Parker et al., 1998), the medio dorsal thalamus (Hunt et al., 1991) and/or the prefrontal cortex may be realistic neuroanatomical candidates which should be examined as possible sites that mediate this complex type learning.

Past evidence (Skinner, unpublished) as well as the findings of the previous chapter demonstrate that the amygdala mediates the simple Pavlovian learning. Since the motor response may be a single component of the neural network mediating the complex Occasion Setting learning, other circuits in the brain were probably able to compensate for the loss of the dorsal striatum.
CHAPTER 4

ACQUISITION OF SIMPLE AND CONDITIONAL DISCRIMINATIONS IN VENTROLATERAL PREFRONTAL CORTEX LESIONED RATS
ABSTRACT

The present experiment examined the role of the ventrolateral prefrontal cortex on the acquisition of our Contextual Discrimination Paradigm. Previous work (Skinner et al., unpublished) has demonstrated that the ventromedial prefrontal cortex plays no role in the acquisition of our task. Rats were again trained on our contextual discrimination paradigm. Both rats with ibotenic acid lesions to the ventrolateral prefrontal cortex and sham lesioned rats acquired the ability to modulate their drinking consumption, consuming significantly more saccharin in the safe relative to the danger context. Both groups showed transfer to a novel vinegar solution and avoided the LiCl paired context on a subsequent place test. The neuroanatomical site mediating Occasion Setting learning in our contextual discrimination paradigm is not the ventrolateral prefrontal cortex.
INTRODUCTION

There are various theories of learning and memory which suggest that specific brain sites modulate particular types of learning. It is widely believed that the prefrontal cortex is involved in working memory (Goldman-Rakic, 1987). Working memory refers to the ability to hold information "on line" to guide behaviour in the absence of explicit cues for action selection. In most working memory tests, the same non unique stimuli are used trial after trial in delayed response and various delayed alternation tasks (Rushworth et al., 1997). The focus of most of the prefrontal cortical research has been on the medial regions. Delayed response type tasks and responding to spatial cues have been shown to be disrupted by medial prefrontal lesions (Aggleton et al., 1995; Kolb et al., 1974). Based on the above findings and the literature at that time, Skinner and her colleagues lesioned the medial prefrontal cortex in an attempt to localize the neuroanatomical locus of occasion setting in our paradigm. The lesioned animals acquired the contextual discrimination task as well as controls (Skinner, unpublished). The focus of this experiment now shifts to ventrolateral prefrontal cortex.

There are at least ten (10) anatomically dissociable subregions of the rat's prefrontal cortex. (Groenewegen et al., 1990). Unlike the medial regions of the prefrontal cortex, the ventrolateral regions do not seem to affect delayed response tasks and minimally affect spatial learning and memory (Kolb et al., 1990). The ventrolateral prefrontal cortex include areas such as the orbitofrontal, the granular and agranular insular regions. Many investigations
into visceral (ventrolateral prefrontal) cortex have implicated the above sub areas in the mediation of conditioned taste aversions induced by Lithium Chloride (Gaston et al., 1978). Cranial nerves VII, IX and possibly X carry taste sensations to the solitary tract, ultimately the neutral message is relayed to the taste cortex via the ventromedial thalamic nucleus (Norgren, et al., 1975). Our lesions overlap the taste cortex which as postulated by Mackey and his colleagues (1986) is the site at which taste information and aversive information is associated. Perhaps the prefrontal cortex stores information regarding incentive properties of various stimuli.

It has been demonstrated that dopamine innervation of the visceral cortex mediates the aversive, but not the rewarding aspects of opiates (Zito et al., 1988). Electrical stimulation of the prefrontal cortex while the animals are in a neutral environment, produces a significant preference for that environment on a subsequent place test. This effect can be blocked using dopamine antagonists (Duvauchelle, 1991). Also, lesioning the medial prefrontal cortex causes impairments in a visual discrimination in which primates must learn to associate visual stimuli with food reward, (Gaffan, et al., 1990). In light of the above findings and of recent finding by Wishaw and his colleagues, there is evidence to suggest that the ventrolateral prefrontal cortex may mediate the processing of certain configural tasks in which stimulus reward relationships are acquired.

Wishaw and his colleagues developed a Tactile Olfactory Configural task which
required a solution using conditional or relational learning as opposed to simple stimulus-response/reward learning (Wishaw et al., 1992). In this paradigm, a short string (S1) would signal food (R1) and a long string (S2) would signal the absence of food (R2), depending on the presence or absence of an odor (O). In other words, in the presence of an odor (O+) S1 signals R1 and S2 signals R2, whereas in the absence of an odor (O-), the meanings of S1 and S2 are reversed. Therefore, S1 and S2 are each associated with reward and it is their relationship to the odor (O) that determines when a cue will be reinforced. In essence, one could say that this paradigm somewhat resembles an occasion setting paradigm. For example, the odor or absence of the odor may "set the occasion" for the presence of a reward dependent on which of the strings (long or short) was pulled. Wishaw and his colleagues (1992) found that ventrolateral prefrontal cortex lesioned rats and control rats were able to acquire a simple odor discrimination in which they were required to discriminate between two cues. Both groups were also able to learn the reverse of this problem. However, ventrolateral prefrontal cortex lesioned rats were impaired compared to controls on the tactile olfactory task described above. Since there exists a possibility that our contextual discrimination task may be similar to Wishaw's configural task, we trained ventrolateral prefrontal cortex lesioned rats using our paradigm.
METHODS

Subjects

Twenty adult male Wistar rats, weighing 350 - 400g at the beginning of behavioural training, were used as subjects. All rats underwent surgery within one week of their arrival in the lab. After one week of recovery from surgery the rats were adapted to a water deprivation schedule consisting of 60 minutes access to tap water each afternoon. One week later behavioural training began.

Surgery

Ten rats underwent ibotenic acid lesions of the ventrolateral prefrontal cortex. Rats were anesthetized with sodium pentobarbital (65 mg/kg ip) and placed in a stereotaxic instrument. After a scalp incision was made, holes were drilled in the skull to permit microinjections of the ibotenic acid. Using a 1.0 ul Hamilton syringe mounted on the stereotaxic instrument, injections of 2% ibotenic acid were made at each of two sites, one site per hemisphere (AP + 1.0; ± 4.7, DV from dura - 5.4), in a volume of .13 ul/site. Ibotenic acid was injected at a rate of 0.05 ul/min and the needle was left in place for an additional 5 minutes following infusion. Ten sham lesioned rats were treated in the same manner but injected with sterile saline (0.9% NaCl).
Conditional Discrimination

After recovery from surgery the two groups of rats were trained on a conditional contextual discrimination task, similar to that used in the previous two chapters. Again, this phase of the experiment was divided into danger and safe days. On danger days rats were placed in a novel context 15 minutes prior to 15 minute access to a novel 0.1% saccharin solution. Removal of the saccharin solution was followed by an i.p. injection of 60 mg/kg LiCl dissolved in 3 ml/kg physiological saline and rats were returned to the home cage. On safe days, rats were placed in a second novel context for 15 minutes prior to a 15 minute access to the same saccharin solution. Saccharin removal was followed by an i.p. injection of physiological saline and rats were returned to the home cage. The training contexts were wooden boxes measuring 41 x 41 x 38 cm. For half the rats the danger context was a black box with a plexiglass floor wiped with 2% acetic acid solution prior to each trial. The safe context was a white box with a wire mesh floor covered with wood chips. The other half of the rats had these contextual cues reversed. The groups were trained for 15 cycles of alternating safe and danger days.

Vinegar Transfer Test

Following the acquisition phase of the conditional discrimination, all rats were given a two-day transfer test with a novel 2% vinegar solution as in the previous experiment. Rats were exposed to both training contexts, in a counterbalanced order, 15 minutes prior to a 15
minute access to the vinegar solution. No injections were given after removal of the flavoured solution.

**Place Aversion Learning**

Two days after the vinegar transfer test, all rats were given a place choice test identical to that given in the previous chapters.
RESULTS

Histology

Schematic drawings representing a typical ventrolateral prefrontal lesion is presented in Figure 10. Rats were removed from the study, if upon histological examination, the lesion site was not similar to the figure presented. In all rats the ibotenic acid lesions produced bilateral destruction of the area around the rhinal sulcus. This area included most or all of the agranular insular cortex and large parts of the claustrum. Some animals also had minor damage to the granular insular cortex.

Discrimination Training

The ventrolateral prefrontal cortex lesioned rats and the sham lesioned rats both learned the contextual discrimination task. Both groups consumed significantly less in the danger context than in the safe context (Figure 11). A $2 \times 15 \times 2$ (Groups x Cycles x Days) ANOVA over the entire acquisition phase revealed a significant Cycles x Days interaction ($F(14,270) = 10.8$, $p<.05$) reflecting acquisition of the discrimination. A closer examination of the last cycle, the last three cycles and the last five cycles demonstrated no significant interactions of group with any other variable.

Vinegar Transfer Test

Two days after the last cycle of discrimination training with the saccharin solution, all
animals were given a transfer test with a novel vinegar solution. As reported in precious findings (Skinner et al., 1991) and in the last two experiments, both the lesioned groups and the sham animals suppressed consumption of the novel vinegar solution in the danger context relative to the safe context (F(1,18) = 43.4, p < .05) (Figure 12).

**Place Aversions**

Both groups demonstrated a large aversion to the danger context, spending significantly more time in the safe context on the place test (Figure 13). A 2 x 2 ANOVA revealed a significant main effect of context (F(1,18) = 180, p < .05).
Figure 10. Representative drawings of a typical ventrolateral prefrontal cortex lesion.
Figure 11. Saccharin consumption (ml) by the sham (n=10) and ventrolateral prefrontal cortex lesioned (n=10) contextual discrimination groups during the 15 cycles of discrimination training. S=Safe Day Trials where rats receive context 1 paired with saccharin-saline; D=Danger Day Trials where rats receive context 2 paired with saccharin-LiCl.
Figure 12. Mean (± sem) vinegar consumption (ml) by the lesioned and control groups during the 2-day transfer test at the end of training.
Figure 13. Mean (± sem) times (in seconds) spent in each of the two training contexts during the 10-min place test by rats in the lesioned and control groups. Safe = context associated with saline during training and Danger = context associated with LiCl during training.
DISCUSSION

Results from this experiment reiterated that rats could use contextual cues as occasion setters to modulate a taste aversion. Once again, this occasion setting function of the cues transfers to a novel flavour and both groups of animals demonstrated significant Pavlovian aversions to the LiCl paired context on a place test. Thus, rats were able to acquire both the simple and conditional learning aspects of the contextual discrimination paradigm.

There is a great deal of evidence supporting a role for the ventrolateral regions of the prefrontal cortex in olfactory processing. The agranular insular component receives olfactory projections from the piriform cortex, periamygdaloid complex, entorhinal cortex and olfactory tubercle (Groenewegen et al., 1990). Eichenbaum and his colleagues have demonstrated that lesions to the ventrolateral prefrontal cortex produce deficits in odour discriminations (Eichenbaum et al., 1980; Eichenbaum et al., 1983). Since the above evidence suggests a role for the ventrolateral prefrontal cortex in olfactory conditioning, it should not be surprising that Wishaw’s lesioned rats demonstrated a learning impairment on his tactile-olfactory configural task. Perhaps the lack of an olfactory component to our paradigm was the critical factor which enabled the lesioned rats to acquire both the simple and complex types of learning.

Although certain aspects of Whishaw’s task (1992) resembled our contextual
discrimination paradigm, his configuration lacked certain critical aspects which would have rendered it an Occasion Setting Task. Although one could perceive either the odour or the string size as being an occasion setter which would modulate when the presence of the other cue predicted reward, both cues were presented simultaneously, without a temporal interval. Furthermore, all subjects could solve the tactile-olfactory tasks by simply committing to memory all of the possible stimulus arrangements. Although a similar argument could be made for our paradigm, the data presented from our transfer tests suggests that in fact a higher order conditional learning is taking place.

Based on the finding of the present experiment, the ventrolateral prefrontal cortex plays no role in mediating either the simple Pavlovian learning or the complex Occasion Setting learning demonstrated in our paradigm. If the visceral cortex was the site at which taste information and aversive information about CTA’s is stored, one would have expected to see a disruption on the modulation of the drinking response. Mackey and his colleagues (1986) postulated that perhaps the ventrolateral prefrontal cortex was the crucial site for the storage of memory that relays information between novel tastes and stimuli such as morphine. The results of this study (Mackey et al., 1986) demonstrated that the visceral cortex was critical only for the establishment of a morphine induced CTA, not for mediating gross taste discrimination or the aversive aspects of LiCl. Given that the animals in the above study still learned a LiCl mediated conditioned taste aversion with lesions to the prefrontal cortex but lost
the aversiveness to morphine, brings to the forefront, the possibility that there are multiple memory and storage sites within the mammalian brain that mediate specific tasks. Perhaps there are multiple memory systems and storage sites in a structure such as the amygdala, which we know mediates the pavlovian taste aversion effects in our paradigm. Occasion setters occur prior to the presentation of the target which it modulates. (Holland, 1983). In the case of the tactile-olfactory configural task, the animals received both cues simultaneously, which would lead to increased ambiguity. Since there is no temporal separation between the two cues in Wishaw’s task, his task cannot be considered an Occasion Setting task.
CHAPTER 5

SELECTIVE CENTRAL NUCLEUS (CN) AND BASOLATERAL NUCLEUS (ABL) AMYGDALA LESIONS AND THEIR EFFECT ON THE ACQUISITION OF THE CONTEXTUAL DISCRIMINATION PARADIGM
ABSTRACT

In our occasion setting paradigm, contextual discriminative stimuli predict whether or not a target stimulus (saccharin) will be followed by an unconditioned stimulus (sickness). Past work has demonstrated that the neuroanatomical locus of the simple Pavlovian learning in our paradigm can be localized to the amygdala. Recent studies have demonstrated that discrete lesions of the amygdala produce different effects compared to those of global lesions other behavioural tasks. Perhaps discrete lesions of selective amygdala nuclei will elucidate specifically which area mediates the various types of learning in our paradigm. Lesions of the central nucleus (CN) of the amygdala (Experiment A) and Lesions of the basolateral nucleus (ABL) of the amygdala (Experiment B) were examined in an attempt to further elaborate on their specific role in our Paradigm. Rats with CN lesions and ABL lesions were compared to sham lesioned animals in two separate experiments. In one distinct context (danger) a saccharin solution was followed by a LiCl injection, while in the second context, the same saccharin solution was followed by a saline injection. In both experiments, lesioned animals as well as the shams, learned to suppress fluid consumption in the first, relative to the second context. Also, all groups demonstrated transfer of the occasion setting properties of the contexts. On place tests performed after the 12th cycle and at the end of training, the CN and ABL lesioned animals, although they demonstrated significant aversions for the danger context, the difference of the time spent in the two contexts (danger-safe) was significantly lower in comparison to the SHAM lesioned animals. These findings support the previous anatomical evidence that the amygdala mediates the simple Pavlovian learning in our
contextual discrimination paradigm.
INTRODUCTION

As described in chapter two, global amygdala lesions in rodents, as well as primates, have been found to produce learning impairments in both appetitive as well as, aversive learning tasks (Fanselow and Kim, 1994; Dunn and Everitt, 1988; White and McDonald, 1993; Gaffan and Murry, 1990). Amygdala lesions in humans have also led to results that postulate a role for this brain structure in fear (emotional) learning (Adolphs et al., 1995; Bechara et al., 1995). For example, bilateral damage to the amygdala in humans compromises the recognition of fear in facial expressions while leaving intact recognition of face identity (Adolphs et al., 1994). It is now widely accepted that the neural system underlying emotional memory critically involves the amygdala.

The amygdala can be subdivided into many subregions, such as, the central, basolateral, lateral, basal, cortical and medial nuclei. In terms of memory function and the fear learning literature, there has been a great deal of emphasis placed on the central and basolateral nuclei of the amygdala. At first, many lesion studies examined the role of global lesions to the amygdala and their role in the learning of emotional learning tasks (Blanchard et al., 1972; Cahill & McGaugh, 1990). Having demonstrated effects of global lesions, extensive evidence came to the forefront demonstrating that effects of a particular amygdala nucleus lesion will differ from those of a discrete lesion of another nucleus or of a large gross lesion (Salinas et al., 1996; Hattfield et al., 1996; Kentridge et al., 1991; Kesner et al., 1989).
With respect to memory function, the central nucleus of the amygdala (CN) has played a critical role in classical conditioning of heart rate (Kapp et al., 1982) identifying reductions in reward magnitude (Kesner et al., 1989) and expression of conditioned fear (conditioned freezing) (LeDoux, 1993; Killcross et al., 1997). The basolateral nucleus of the amygdala (ABL) has been identified as the sensory interface of fear conditioning (LeDoux et al., 1990) and lesions to this area have demonstrated significant effects on the acquisition of multiple trial inhibitory avoidance, taste potentiated odor aversion, and visual discrimination (Hatfield et al., 1992; Peinado-Manzano, 1989; Tomaz et al., 1992). Until recently, the processing of fear mediating (emotionally significant information (stimuli)) information would have been described in relatively straightforward terms. The flow of sensory information through the amygdala which mediated various fear responses such as conditioned freezing, defecation, suppression of ongoing behaviour, autonomic and endocrine changes was believed to be a serial process. That is information was sent from the basolateral nucleus (ABL) of the amygdala onward to the to the central nucleus (CN) which then by way of output projections to other brain areas mediated the responses described above (Davis et al., 1992; Nader et al., 1997)

In contrast to this single fear mediating system described above, Killcross and colleagues (1997) have demonstrated a controversial finding that two independent fear learning systems exist within the amygdala. These researchers postulate that the central nucleus dependent learning system mediates the expression of classical fear responses such as
freezing, and defecation. While the basolateral amygdala learning system is responsible for mediating the ability of a cue that has been paired with a threatening event to influence ongoing behaviour. This powerful double dissociation has cast doubt over the serial processing theory of the amygdala nuclei and has become the impetus for a great many debates.

Given that a global amygdala lesion could not disrupt occasion setting learning (i.e. chapter two), how would it be possible that separately lesioning the discrete nuclei would disrupt both types of learning in our paradigm? According to Killcross and his colleagues, both nuclei receive information about the contextual cues simultaneously and in parallel. If this is the case, and the CN has mutual connections with the ABL, information could not only be shared, it could be shunted to the other nucleus in the event of one being damaged. Using Killcross' theory as a framework, if the CN were to be eliminated, the ABL would still be able to mediate the ongoing responses to the various contexts leaving the occasion setting properties intact without the fear response to the context being evident. On the other hand, if the ABL were to be eliminated, the CN would still mediate the conditioned fear response demonstrated on the place test, however one would expect to see a disruption in the drinking modulation (the occasion setting learning). In chapter two, both the ABL and the CN were damaged, in this event, some other brain area(s) were able to mediate the occasion setting learning.
Given the fact that global amygdala lesions disrupted the Pavlovian learning in our paradigm, and that the literature cites differing roles for the central vs the basolateral nuclei of the amygdala, in various paradigms, I examined discrete lesions of the central nucleus and the basolateral nucleus of the amygdala in comparison to control animals. In keeping with Killcross’ (1997) finding, perhaps lesioning the central nucleus (experiment A) of the amygdala would be sufficient to impair the animals’ response on the Place test (the measure of Pavlovian fear expression to the context), while the ABL lesions (experiment B) may disrupt the animal’s ability to modulate the consumption of the saccharin solution in the safe relative to the danger context (occasion setting learning).
EXPERIMENT A

In the first experiment, rats with ibotenic acid lesions of the central nucleus of the amygdala (CN) are compared to sham lesioned rats on our contextual discrimination task.

METHODS

Subjects

Twenty adult male Wistar rats, weighing 350-400 g at the beginning of behavioural training, were used as subjects. All rats underwent surgery within one or two weeks of their arrival in the lab. After recovery from surgery rats were adapted to a water deprivation schedule consisting of 60 minutes access to tap water each afternoon. One week later behavioural training began.

Surgery

Ten rats received ibotenic acid lesions of the central nucleus (CN) of the amygdala. After a scalp incision was made, holes were drilled in the skull to permit micro injections of the ibotenic acid. Bilateral lesions of the CN were made using the following stereotaxic coordinates: 2.0 mm posterior from bregma and 4.1 mm lateral from the midline, with one injection site 8.0 mm (0.2 µl) ventral from the skull surface (Paxinos and Watson, 1986). The ibotenic acid was injected at a rate of 0.2 µl/30 seconds and the Hamilton 1.0 µl syringe
injector needle was left in place for 5 minutes. Ten control rats received the same procedure, but were injected with sterile saline (0.9% NaCl).

**Conditional Discrimination and Pavlovian Training**

After recovery from surgery the groups of rats were trained on our conditional contextual discrimination task, similar to that used previously (Skinner et al., 1994a, b). This phase of the experiment was divided into danger and safe days. On danger days rats were placed in a novel context 15 minutes prior to a 15 minute access to a novel 0.1% saccharin solution. Removal of the saccharin solution was followed by an i.p. injection of 60 mg/kg LiCl dissolved in 3 ml/kg physiological saline and rats were returned to the home cage. On safe days, rats were placed in a second novel context 15 minutes prior to a 15 minute access to the same saccharin solution. Saccharin removal was followed by an i.p. injection of physiological saline and rats were returned to the home cage. The training contexts were wooden boxes measuring 41 x 41 x 38 cm. For half the rats the danger context was a black box with a plexiglass floor wiped with 2% acetic acid solution prior to each trial. The safe context was a white box with a wire mesh floor covered with wood chips. The other half of the rats had these contextual cues reversed.

This experiment consisted of seventeen cycles of alternating safe and danger days for both groups. Both groups of rats were given a Place Test after the 12th cycle. At the end
of training both groups were given a two-day transfer test with a novel 2% vinegar solution, followed by a final place test.

Vinegar Transfer Test

Following training, both groups of rats were given a two day transfer test. Rats were exposed to both training contexts, in a counterbalanced order, 15 minutes prior to a 15 minute access to the vinegar solution. Consumption was measured and no injections were given after removal of the flavoured solution.

Place Aversion Testing

The rats were given a Place Test after the 12th cycle and at the end of training to assess acquisition of a Pavlovian aversion to the context associated with the LiCl injections. The procedure consisted of placing a rat in a neutral gray zone between the black and white contexts used during training. The rat’s movement was monitored for 10 minutes, and the amount of time spent in each environment was recorded.
RESULTS

Histology

Schematic drawings of a typical CN lesion are shown in figure 14. Histological analysis of the central nucleus lesions demonstrated that most of the damage from the ibotenic acid was restricted to the central nucleus, and in all cases involved in the behavioural analysis the majority of the CN was destroyed. Only in the odd case did any damage to areas such as the ABL occur, and in these cases only a minor portion of the ABL was damaged.

Discrimination Training

Both CN lesioned rats and sham control rats learned the contextual discrimination task, consuming significantly less saccharin in the danger context than the safe context (Figure 15). A $2 \times 17 \times 2$ (Groups x Cycles x Days) ANOVA over the acquisition phase revealed a significant Cycles x Days interaction ($F(16,306) =13.7$, $p<.05$) demonstrating acquisition of the modulating behaviour. ANOVAs performed on the last cycle, the last three cycles and the last five cycles failed to demonstrate a significant group interaction with any other variable.
Vinegar Transfer Test

A transfer test with a novel vinegar solution was performed a day after discrimination training. Both the sham and CN lesioned groups showed significant transfer of occasion setting to the novel solution. The two groups suppressed their vinegar consumption in the danger context relative to the safe context ($F(1,18) = 39.9$, $p < .05$) (Figure 16).

Place Aversions

The first place test given after the 12th cycle of discrimination (Figure 17) revealed that both CN lesioned and sham lesion animals significantly avoided the LiCl paired context ($F(1,18) = 57.1$, $p < .05$). However, a 2 x 2 (Group x Context) ANOVA also revealed an interaction between group x context demonstrating that the difference of the time spent in the two contexts (danger-safe) was less in the CN animals compared to controls ($F(1,18) = 4.8$, $p < .05$).

The final place test (Figure 18) also demonstrated that both CN lesioned and sham lesioned animals significantly avoided the LiCl paired context ($F(1,18) = 114.4$, $p < .05$). Another 2 x 2 (Groups x Context) ANOVA performed on this test, revealed a Group x Context interaction reaffirming that the difference of the time spent in the two contexts (danger-safe) was less in the CN group ($F(1,18) = 9.1$, $p < .05$).
Figure 14. Schematic drawings of a typical lesion of the central nucleus (CN) of the amygdala.
Figure 15. Saccharin consumption (ml) by the sham (n=10) and CN lesioned (n=10) contextual discrimination groups during the 17 cycles of discrimination training. S=Safe Day Trials where rats receive context 1 paired with saccharin-saline; D=Danger Day Trials where rats receive context 2 paired with saccharin-LiCl.
Figure 16. Mean (± sem) vinegar consumption (ml) by the CN lesioned and control groups during the 2-day transfer test at the end of training.
CONSUMPTION (ML)

CONTROL

LESION

SAFE
DANGER
Figure 17. Mean (± sem) times (in seconds) spent in each of the two training contexts during the 10-min place test by rats in the CN lesioned and control groups after the 12th cycle. Safe= context associated with saline during training and Danger= context associated with LiCl during training.
Figure 18. Mean (± sem) times (in seconds) spent in each of the two training contexts during the 10-min place test by rats in the CN lesioned and control groups at the end of training. Safe= context associated with saline during training and Danger= context associated with LiCl during training.
EXPERIMENT B

In this experiment, rats with ibotenic acid lesions of the basolateral nucleus of the amygdala (ABL) are compared to sham lesioned rats on our contextual discrimination task.

METHODS

Subjects

Seventeen adult male Wistar rats, weighting 350-400 g at the beginning of behavioural training, were used as subjects. All rats underwent surgery within one or two weeks of their arrival in the lab. After recovery from surgery rats were adapted to a water deprivation schedule consisting of 60 minutes access to tap water each afternoon. One week later behavioural training began.

Surgery

Nine rats received ibotenic acid lesions of the basolateral nucleus (ABL) of the amygdala. After a scalp incision was made, holes were drilled in the skull to permit micro injections of the ibotenic acid. Bilateral lesions of the ABL were made using the following stereotaxic coordinates: 2.6 mm posterior from bregma and 5.0 mm lateral from the midline, with one injection site 8.0 mm (0.3 μl) ventral from the skull surface (Paxinos and Watson, 1986). The ibotenic acid was injected at a rate of 0.2 μl/30 seconds and the Hamilton 1.0 μl syringe injector needle was left in place for 5 minutes. Eight control rats received the same
procedure, but were injected with sterile saline (0.9% NaCl).

**Conditional Discrimination**

After recovery from surgery both groups of rats were trained on our conditional contextual discrimination task, similar to that used previously (Skinner et al., 1994a,b). This phase of the experiment was divided into danger and safe days. On danger days rats were placed in a novel context 15 minutes prior to a 15 minute access to a novel 0.1% saccharin solution. Removal of the saccharin solution was followed by an i.p. injection of 60 mg/kg LiCl dissolved in 3 ml/kg physiological saline and rats were returned to the home cage. On safe days, rats were placed in a second novel context 15 minutes prior to a 15 minutes access to the same saccharin solution. Saccharin removal was followed by an i.p. injection of physiological saline and rats were returned to the home cage. The training contexts were wooden boxes measuring 41 x 41 x 38 cm. For half of the rats, the danger context was a black box with a plexiglass floor wiped with 2% acetic acid solution prior to each trial. The safe context was a white box with a wire mesh floor covered with wood chips. The other half of the rats had these contextual cues reversed.

This experiment consisted of seventeen cycles of alternating safe and danger days for both groups. Both groups of rats were given a Place Test after the 12th cycle. At the end of training both groups were given a two-day transfer test with a novel 2% vinegar solution, followed by a final place test.
Vinegar Transfer Test

Following training, both groups of rats were given a two day transfer test. Rats were exposed to both training contexts, in a counterbalanced order, 15 minutes prior to 15 minutes access to the vinegar solution. Consumption was measured and no injections were given after removal of the flavoured solution.

Place Aversion Testing

The rats were given a Place Test after the 12th cycle and at the end of training to assess acquisition of a Pavlovian aversion to the context associated with the LiCl injections. The procedure consisted of placing a rat in a neutral gray zone between the black and white contexts used during training. The rat’s movement was monitored for 10 minutes, and the amount of time spent in each environment was recorded.
RESULTS

Histology

Figure 19 is a schematic representing a typical basolateral nucleus (ABL) lesion of the amygdala. All lesioned animals had significant damage to the basal, as well as, the lateral nucleus; in all cases involved in the behavioural analysis, the majority of each nucleus was destroyed. In some animals damage was also evident in the ventral endopiriform nucleus and occasionally on the edge of the central nucleus.

Discrimination Training

Both ABL lesioned rats and sham control rats learned the contextual discrimination task, consuming significantly less saccharin in the danger context than the safe context (Figure 20). A $2 \times 17 \times 2$ (Groups x Cycles x Days) ANOVA over the acquisition phase revealed a significant Cycles x Days interaction ($F(16,255) =17.3$, $p<.05$) demonstrating the acquisition of drinking modulation. ANOVAs performed on the final cycle, the last three cycles and the last five cycles failed to find a significant interaction of group with any other variable.
Vinegar Transfer Test

A transfer test with a novel vinegar solution was performed a day after discrimination training. Both the sham and ABL lesioned groups showed significant transfer of occasion setting to the novel solution (Figure 21). The two groups suppressed their vinegar consumption in the danger context relative to the safe context (F(1,15)=36.7, p<.05).

Place Aversions

The first place test given after the 12th cycle of discrimination (Figure 22) revealed that both ABL lesioned and sham lesioned animals significantly avoided the LiCl paired context (F(1,15) =40.8, p<.05). An 2 x 2 (Group x Context) ANOVA revealed an interaction between Group x Context demonstrating that the difference in time spent in the danger context vs. the safe context was less in the ABL group (F(1,15) =10.8, p<.05).

The final place test (Figure 23) also demonstrated that both CN lesioned and sham lesioned animals significantly avoided the LiCl paired context (F(1,15) =37.3, p<.05). A 2 x 2 (Groups x Context) ANOVA performed on this test revealed a Group x Context interaction reaffirming that the time difference for the two contexts (danger-safe) was significantly less in the ABL group compared to the SHAM lesioned animals (F(1,15)= 2.8, p>.05).
Figure 19. Schematic drawings of a typical basolateral nucleus (ABL) lesion of the amygdala.
Figure 20. Saccharin consumption (ml) by the sham (n=8) and lesioned (n=9) contextual discrimination groups during the 17 cycles of discrimination training. S=Safe Day Trials where rats receive context 1 paired with saccharin-saline; D=Danger Day Trials where rats receive context 2 paired with saccharin-LiCl.
Figure 21. Mean (± sem) vinegar consumption (ml) by the ABL lesioned and control groups during the 2-day transfer test at the end of training.
Figure 22. Mean (± sem) times (in seconds) spent in each of the two training contexts during the 10-min place test by rats in the ABL lesioned and control groups after the 12th cycle.

Safe= context associated with saline during training and Danger= context associated with LiCl during training.
Figure 23. Mean (± sem) times (in seconds) spent in each of the two training contexts during the 10-min place test by rats in the ABL lesioned and control groups at the end of training. Safe = context associated with saline during training and Danger = context associated with LiCl during training.
DISCUSSION

Consistent with results thus far, all groups of rats trained on this conditional discrimination task showed that contextual cues could serve as occasion setters to modulate a taste aversion and that this occasion setting transfers to a novel flavour. The surprising results of these two experiments were the partial effects demonstrated by both the CN and ABL lesioned animals on the place tests performed both after the 12th cycle and at the end of training. The results of experiment A and experiment B together confirm previous findings by Skinner and her colleagues (unpublished), as well as, earlier findings put forth in chapter 2 demonstrating that the amygdala is the brain site mediating the simple Pavlovian learning.

In experiment A, a partial effect was demonstrated on both place tests by CN lesioned animals. Although the direct association between the danger context and LiCl was partially blocked, lesioned animals were still able to modulate fluid consumption. I postulated based on the Killcross (1997) data that the CN may mediate the Pavlovian fear aversion to the context because of its postulated role as the site mediating the acquisition and expression of classical fear responses. On the other hand, the ABL was postulated to be the site mediating the complex occasion setting learning because according to Killcross, this site mediated the ability of a cue that has been paired with a threatening event to influence ongoing behaviour. The results of experiment B were identical to that of experiment A, ABL lesioned animals had no effect on occasion setting (drinking modulation), however, as with the
Our findings presented in these experiments support the role of the amygdala as being the motivational learning (stimulus-reward) centre of the brain (McDonald and White, 1993; LeDoux 1993; Kentridge et al., 1991). However, which processing theory does our data support? The answer to this question lies in future experiments. If animals received combined ABL and CN lesions, one could postulate that the two partial effects demonstrated in our experiments would sum up to completely impair the place (fear) learning. This summation effect would give support to independent parallel processing by these nuclei as postulated by Killcross and his colleagues (1997). If combined lesions demonstrate a similar partial effect, as seen here, perhaps my lesions of the CN and the ABL did not destroy the entire nuclei. If this is the case, the animals would still be able to acquire partial associations between the context and the LiCl injections as described earlier. This pattern of results would suggest serial processing of place aversions through the ABL and the CN, with the serial pathway being only partially destroyed in each nucleus. A third processing possibility is one in which the subnucleii of the amygdala actually interact synergistically in order to acquire the simple place learning. If this were the case, by lesioning each of the subnucleii we may have uncovered partial effects as demonstrated by both lesions in this study. In order to verify how processing of information which leads to simple learning actually takes place in the amygdala, future studies must be performed.
Once again, the findings presented here produce evidence supporting a dissociation between the two types of learning in our paradigm. The amygdala is the neuroanatomical site mediating the simple Pavlovian learning. The quest continues for the neuroanatomical site or sites which mediate the more complex occasion setting learning. Perhaps lesioning other single or multiple areas of the brain may disrupt the drinking modulation seen in our paradigm or perhaps a paradigm in which one eliminates the drinking component altogether may be necessary in order to produce an effect using the combined lesion approach. These possibilities will be examined in greater detail in the General Discussion to follow.
CHAPTER 6

GENERAL DISCUSSION
Using our conditional contextual discrimination paradigm, animals have been able to learn two different types of learning using a single behavioural task. Our paradigm assesses simultaneously a simple Pavlovian learning and a complex occasion setting learning using the same number of trials, and identical cues arranged in a slightly different manner. In a classical occasion setting paradigm, the occasion setter (feature cue) modulates a target-US association. A context serves as the occasion setter in all of the experiments discussed herein and the saccharin solution serves as the target cue.

Previous findings from our lab have demonstrated the ability of internal cues (drug states), flavours, and contexts to serve as occasion setters. (Skinner, 1995). Using contexts as occasion setters, we have been able to directly assess the Pavlovian (fear) aversion to the LiCl (danger) paired context. Directly pairing context and LiCl is not sufficient to produce discriminative performance (Skinner, 1994). Animals acquire the Pavlovian aversion as training occurs as measured by the place test. Holland and others (1983), have stated that extinction of occasion setting occurs if the occasion setter is extinguished in the presence of the response it comes to modulate. Skinner and her colleagues (1994) have extinguished the drinking response using tap water, however the Pavlovian aversion to the context remained. Likewise, as demonstrated previously (Skinner 1994) Pavlovian aversions can be abolished and the occasion setting properties of the context remain in tact. This is behavioural evidence supporting a dissociation between simple Pavlovian and complex Occasion Setting Learning.
Having ruled out a direct feature US association as mediating the complex learning, are the occasion setter and target cues forming a complex configuration that can be associated with a conditioned response that is distinct from the associations of either element alone (Pearce, 1987)? Although our transfer data seems at first glance controversial, it argues against the formation of a complex cue between the occasion setter (context) and the saccharin. If animals were responding to a complex/compound cue, introducing a novel cue such as vinegar should disrupt discriminative performance. However, this does not happen, time and time again, the occasion setting properties of the contexts transfer to the novel solution. Even the account that occasion setting is due to the summation of two Pavlovian associations: the first between the occasion setter and the US and the second between the target stimulus and the US cannot stand up in light of our transfer results. Therefore, the context in our paradigm follows Holland’s (1983) description of an occasion setter which modulates the association between the target (saccharin) and the US (LiCl).

Having behaviourally dissociated our Pavlovian learning from Occasion Setting, our efforts turned to identifying the neuroanatomical locus(i) underlying the two forms of learning. Before I examine the results of the hippocampal, the amygdala, the combined hippocampal plus amygdala, dorsal striatal, and the discrete lesions of the subnuclei of the amygdala, I will
first discuss the cortical lesions that produced little or no effects.

Many current Occasion Setting theories (Gluck & Myers, 1993; Schmajuk & Dicarlo, 1992), postulate a role for cortical areas in either storage of configural information or perhaps in the mediation of occasion setting responding. Several attempts at cortical lesions were done and these animals were run in our paradigm. First, neonatal decorticated rats did not block occasion setting with drug cues when these rats were trained as adults (Skinner, 1995). Perhaps these animals had sufficient time to make the necessary ‘neuronal modifications’ in order to recover any behavioural deficits that may have been demonstrated as neonates. It would be interesting to see if decortication lesions performed at adulthood would produce deficits in our paradigm. Previous unpublished data demonstrated that medial prefrontal cortical lesioned animals did not impair acquisition of our contextual discrimination. Likewise, the data presented in chapter 4 demonstrates that ventrolateral prefrontal cortical lesioned animals also did not impair acquisition of our context as discrimination task. Since in all of the above lesions, the rate of acquisition was the same as the control animals, we concluded that these areas played little or no role in the acquisition of occasion setting learning.

Since Scoville and Milner (1957) the medial temporal lobes have been implicated in learning and memory. With the impetus behind theories such as the configural association
theory implicating the hippocampus in configural learning. Skinner and her colleagues turned their attention to the hippocampus as the site that mediated occasion setting/conditional discrimination learning. Several neural network theories arising from the occasion setting/Pavlovian learning literature have also implicated the hippocampus as playing a critical role in the mediation of this complex type learning (Gluck & Myers, 1993; Schmajuk & Dicarlo, 1992). In light of the above theories, one would postulate that hippocampal lesions should have disrupted the learning of our paradigm.

Previous findings Skinner (1995), demonstrated that hippocampal lesioned rats could learn our occasion setting task at the same rate as controls. Since place test and transfer data were also identical to controls, the hippocampus seemed to play no role in the learning of our paradigm. In chapter two we verified the above results by demonstrating that combined hippocampal and amygdala rats showed no deficit in acquisition of the occasion setting learning. The above findings suggest that occasion setting learning is different from simple Pavlovian learning and not dependent on hippocampal processing.

Having ruled out the hippocampus as mediating our contextual discrimination, researchers had identified at least five functionally dissociable memory systems in the mammalian brain which are capable of processing different kinds of information. Taking this
multiple memory system analysis into consideration, Skinner and her colleagues (unpublished) lesioned the amygdala as a candidate which may mediate the acquisition of our paradigm. While amygdala lesioned animals learned to modulate their drinking consumption in the various contexts, and demonstrated transfer to a novel solution, these animals demonstrated a lack of an aversion to the LiCl paired context. This finding was similar to others which suggested that the amygdala was important for attaching emotional significance to conditioned stimuli (Phillips & LeDoux, 1992). McDonald and White's (1995) finding provided convincing evidence to support the view that memory systems are "on line" at all times and process information in parallel. Memory systems that are simultaneously on line can interact in a number of different ways. First, depending on the demands of a particular task, memory systems may compete for information and possibly impair the "correct" processing. Competition among memory systems was evidenced by the fact that hippocampal lesioned animals demonstrated enhanced acquisition of a CCP task (McDonald & White, 1995) and likewise demonstrated enhanced performance of a win-stay task which was a dorsal striatal based task (McDonald & White, 1993). In above situation, the parallel processing of each memory system overlapped with the behavioural demands of the CCP task, thus by eliminating the system which was less suited to the learning of these demands enhanced the animal's acquisition of the task. In essence, eliminating the hippocampus relieved the inhibitory interference with the dorsal striatum.

Second, if multiple memory systems are "on line" and process information simultaneously they may cooperate to successfully achieve the behavioural demands of a particular task. Each memory system would each play it's own part in the acquisition of a behavioural task. Thus,
the elimination of multiple sites at once may be necessary if one is to identify the neuroanatomical loci mediating our complex occasion setting learning.

McDonald (1993) has postulated that the hippocampus mediates learning about the relationships among contextual cues (stimulus-stimulus associations) and the amygdala mediates stimulus reward (attaching emotional significance to cues) learning. A combined lesion which eliminated both brain systems, should disrupt learning in our paradigm. Results from chapter 2 demonstrated that these animals could learn to suppress consumption in the danger context relative to the safe context. These animals were able to learn the occasion setting properties of the context, however, they demonstrated no aversion to the danger context. As in purely hippocampal lesioned rats (Skinner, 1995) these animals had a severe deficit on the Morris Water Maze task. The critical finding from this study was that animals could use contextual cues as occasion setters without having an aversion for the context. This finding provided more evidence supporting an anatomical dissociation between the two types of learning with the amygdala mediating the simple Pavlovian learning.

Following the McDonald multiple memory system hypothesis, I lesioned the dorsal striatum of several animals and examined the performance on our contextual discrimination task. We had always maintained that what the context may be modulating in our paradigm is a
drinking response. McDonald (1993) postulated that the dorsal striatum mediates stimulus-(motor) response learning. If the animals were learning a licking response, lesions to this area would have disrupted the modulation of drinking. Dorsal striatum lesioned animals performed as well as controls in our paradigm, this finding supports the conclusion that this structure by itself cannot disrupt/control occasion setting learning.

Recently, Killcross and his colleagues (1997) were able to dissociate two independent fear learning systems in the amygdala. This line of evidence suggested that the amygdala processed information in a parallel "on line" manner. The popular belief at this time is that the amygdala processes information serially, with the ABL being the sensory interface receiving many projections from the thalamo-cortical areas and then sends information forward to the CN which serves as the output nucleus for the expression of classically conditioned defensive responses. Killcross' (1997) data fit well with our multiple memory system view. If the subnuclei of the amygdala were responsible for two different types of fear learning (the CN mediates the acquisition and expression of classical fear responses (i.e. freezing, suppression, defecation); the ABL mediates the ability of a cue that has been paired with a threatening event to influence ongoing behaviour), it would seem to follow that perhaps these subnuclei would have had differential effects in our paradigm. Although we did not find the dissociation that we had postulated, we did uncover an interesting finding. Both the CN lesioned and ABL lesioned rats demonstrated a partial block of the Pavlovian fear to the LiCl associated context.
This finding further strengthens the evidence implicating the amygdala as the brain site mediating the Pavlovian fear response. The effect demonstrated by the global lesioned animals, may be due to the summation of the results demonstrated in chapter 5. Which of the two theories concerning the amygdala does our data support? At first glance, one may conclude that since both lesions had the same effect, the data supports a serial processing view of the amygdala. This may be possible if the lesions of the two nuclei were only partial lesions, however, this does not appear to be the case. The lesions did appear to destroy the majority of the CN and the ABL. This does not rule out a partial lesion effect because the necessary areas for correct processing of our paradigm may be larger than the anatomically defined ABL or CN. As stated earlier, further studies using subjects with the combined discrete lesions are necessary to shed light on this debate. Once again, the neuroanatomical brain site(s) mediating the occasion setting learning has evaded us.

What Next? Other Possibilities or Candidates

Consistent in the McDonald literature was that it was the difference in the behavioural demands of the task that made each task sensitive to the various memory systems. Based on the premise outlined above, the authors proposed three categories of tasks that would determine how memory systems interacted in a given paradigm:
Tasks in category A fit precisely the mnemonic function of a single system, and the other systems may interfere with or have no influence on the acquisition of category A tasks. Tasks in category B can be acquired by more than one of the three hypothesized memory systems, with each one acting alone. Tasks in category C may require the function of two or more of the systems for accurate performance. (p.17, McDonald & White, 1993).

Therefore, according to the above, by systematically eliminating neuroanatomical sites or memory systems, one should be able to identify the sites mediating any behavioural task.

All of the tasks presented in the triple dissociation paper (McDonald, 1993) were category A tasks that were mediated by only one system. The Pavlovian aspect of our paradigm is mediated by the amygdala. This would make Pavlovian fear learning a category A task. Although more work is required, I am postulating that occasion setting learning is a category B or C type task. What other brain sites could be candidates for site(s) that mediate the occasion setting learning in our paradigm?

A site that may mediate various configural and conditional discriminations that involve associating multiple cues varying in sensory modality is the rhinal cortex. The entorhinal cortex receives and projects many types of polysensory information. The rhinal cortex has become a major focus in the primate literature. Lesions of various areas of the rhinal cortex have led to various impairments in visual recognition tasks and the ability to form
complex associations of various objects (Murray, 1996). Deficit in flavour-visual associative memory has also been demonstrated with lesions to the rhinal cortex (Parker et al., 1998). Since it is possible that complex learning such as occasion setting might depend on areas of the brain that receive multimodal sensory input, the rhinal cortex is an attractive candidate.

An attempt to localize occasion setting in the rhinal cortex would speculate that this site solely mediates our complex behavioural task. Since I have postulated that our contextual discrimination is most likely a category B or C type task, perhaps a triple lesion of the amygdala, the hippocampus and the dorsal striatum would disrupt the discriminative ability in our paradigm. When beginning to consider these kinds of lesions, viability of the rats and their ability to survive post surgery must be taken into consideration. Perhaps lesioning three, four or five sites may never eliminate this type of learning because information necessary to acquire occasion setting learning is widely dispersed through many more sites in the mammalian brain.

In following with the above thought process, a close examination of each lesion done to this point, reveals a small impairment in the acquisition of the contextual discrimination. Although not statistically significant, control animals discriminate slightly better on safe vs. danger days in all experiments. Perhaps adding all of the lesions together, one would
completely block occasion setting. This would confirm our paradigm as being a category C task, in which occasion setting learning is mediated by many separate sites dispersed throughout the brain, each doing their own small separate part.

In recent work, we have decided to develop an occasion setting paradigm which does not have a taste-response component. Using drug cues as occasion setters and a context as the target cue, drug states come to modulate when a context predicts illness. Rats place tested under the influence of the drug states, demonstrate an aversion for the context when tested under the drug that “sets the occasion” for illness, and shows no preference when tested under the “safe” drug (unpublished data). The rationale behind the new paradigm, is that by taking out the strong response (licking) component of our paradigm, perhaps we will now be able to see deficits produced by hippocampal and/or amygdala lesions, which were masked in our contextual discrimination paradigm due to the inclusion of the drinking motor response in the learned response.

An Occasion Setting and A Neural Network Model

In my analysis of occasion setting, I have always assumed that the feature cue was modulating the target-US association. However, there are multiple ways in which a feature
might modulate responding to a target. Perhaps the occasion setter acts directly on the target CS, or on the production of the response, or finally on the modulation of the target-US relationship itself (Rescorla, 1985). We have ruled out the first two possibilities systematically. Our transfer data demonstrates a separation between the feature and the target because the cues are not treated as a compound cue and a novel vinegar solution does not disrupt the configural association. Demonstrating occasion setting learning without the direct Pavlovian fear eliminates the second possibility. Having attempted dorsal striatal lesions without producing an effect, provides evidence against the occasion setter modulating the specific drinking response. Also, the “response-less” paradigm should provide further information regarding this possibility. In light of the above findings, I conclude that occasion setters do modulate the association between the target and the US.

In Holland's hierarchical view, he posits that the occasion setter controls a particular CS-US association (Holland, 1983; 1985). Occasion setters are very specific to the trained target. He supported this hypothesis by demonstrating that a cue could serve as an inhibitor of a target, as well as, a facilitator for another target. This strengthens the view that occasion setters modulate target-US associations.

The modulatory properties of occasion setting have been described in a neural network
Gluck and Myers (1994) have demonstrated that their trial level model of hippocampal mediation of classical eyeblink conditioning can account for the occasion setting properties of contexts. They demonstrate that their feed forward network learns the discrimination and transfer tasks in the same way as described by Bouton and Swartzentruber (1986). They conclude that this hippocampal computational description can account for the occasion setting abilities of contextual cues.

The above theory would not have predicted the results of the hippocampal lesioned rats and Holland's occasion setting theory would not have predicted the general transfer seen in all of the experiments thus far. Holland would have predicted transfer only to elements that were trained in a similar occasion setting paradigm. There are several possible reasons why our paradigm showed general transfer. The context temporally overlapped the target cue in our paradigm, as opposed to the occasion setters used by Holland (1983). Since the animals had to make a response in order to access the target cue, the association being formed may have been between the response and the US. Previous data (Skinner, 1994) supports this possibility because extinguishing occasion setting in the presence of the response abolished discriminative performance. If the response component of our paradigm caused such a different result, perhaps by eliminating it, we would see a disruption of behavioural discrimination caused by a combined hippocampal and amygdala lesion. This study is currently under way.
The data presented in this thesis confirms the previous behavioural evidence suggesting that the modulation of simple associations and occasion setting is fundamentally different. Although I was not able to identify the neuroanatomical locus(i) of the occasion setting learning, I did identify and confirm that the amygdala is the site that mediates the simple Pavlovian aversion to the LiCl paired context. Future experiments must be carried out to determine where the acquisition of occasion setting learning takes place. The evidence presented thus far suggests that this complex learning cannot be reduced to simple Pavlovian associations. Whatever the aspects of the contextual cues that mediate the fear in this paradigm may be, they are not the properties that endow the same contextual cue the ability to modulate fluid consumption (acquire occasion setting learning).
REFERENCES
A


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**D**


**E**


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