THE FUNCTIONS OF AMYGDALA AND HIPPOCAMPUS IN CONDITIONED CUE PREFERENCE LEARNING

by

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ABSTRACT

The experiments in this thesis examined the roles of stimulus configuration on conditioned cue preference (CCP) learning by asking what information is processed and by which neural substrates. Results from Experiments 1 and 2 showed that lesions of the lateral nucleus of the amygdala (LNA) but not of fimbria-fornix (FF) impaired CCP learning when the cues paired with food during training were distinct from those not paired with food in either of two different apparatuses. In Experiments 3 and 4 LNA lesions increased the size of the CCP when the cues paired with food and no food were ambiguous in two different apparatuses. Learning the ambiguous cue CCP required at least one session of unreinforced pre-exposure to the cues and was eliminated by FF lesions. In the last series of experiments, a latent learning effect of unreinforced pre-exposure on ambiguous cue CCP learning on the radial maze was found in normal animals that received at least 3 sessions of unreinforced pre-exposure. FF lesions made before, but not after, pre-exposure eliminated the latent learning effect. Hippocampus lesions made either before or after pre-exposure eliminated the CCP learning. Taken together, the results are consistent with the hypothesis that distinct cue CCP learning is based on conditioned approach responses to cues paired with food, mediated by a neural system that includes the LNA. The results also suggest that ambiguous cue CCP learning takes place in two phases. First spatial learning occurs during unreinforced pre-exposure, a process that requires an intact FF. Subsequently, information about the location of the reinforcer is added to the spatial information during the reinforced training trials by a process of "reconsolidation". An intact hippocampus is required for this process. The implications of these results and interpretations for latent learning and latent inhibition are considered.
Résumé

Cette thèse vise la mise en lumière du traitement de l’information et les substrats neuronaux relatifs à la configuration de stimuli à la tâche d’apprentissage de préférence conditionnée indicée (CCP). Les expériences 1 et 2 montrent qu’une lésion du noyau latéral de l’amygdale (LNA), mais non du fimbria-fornix (FF), altère l’apprentissage CCP dans deux appareils différents lorsque les indices appariés à la nourriture se distinguent de ceux non appariés. Les résultats des expériences 3 et 4 montrent qu’une lésion du LNA provoque une augmentation du CCP dans deux appareils différents lorsque les indices appariés à la nourriture ne comportent aucune ambiguïté. Le CCP à indices ambigus recquiert au moins une session de pré-exposition non renforcée aux indices laquelle s’élime par une lésion au niveau du FF. Chez les sujets normaux, au labyrinthe radial, dans les conditions de pré-exposition non renforcée aux indices et de CCP à indices ambigus, au moins 3 sessions s’avèrent nécessaires pour produire un effet d’apprentissage latent. Une lésion au niveau du FF faite avant la pré-exposition, mais non après, élimine cet apprentissage latent. Les lésions de l’hippocampe faites avant ou après la pré-exposition suppriment dans les deux cas l’apprentissage CCP. Les résultats supportent l’hypothèse voulant que l’apprentissage CCP à indices distinctifs se base sur des réponses conditionnées aux indices appariés à la nourriture, lesquelles sont médiées par un système neuronal incluant le LNA. Aussi, l’apprentissage CCP à indices ambigus a lieu en deux phases. D’abord, l’apprentissage spatial survient pendant la pré-exposition non renforcée, processus exigeant un FF intact. Ensuite, l’information concernant l’emplacement du renforceur est ajoutée à l’information spatiale pendant les essais renforcés par un processus dit de reconsolidation, lequel exige un hippocampe intact. Les implications de ces résultats ainsi que l’interprétation de l’apprentissage latent et de l’inhibition latente sont considérées dans cette thèse.
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PREFACE

In previous experiments for my Master’s thesis, I showed that the morphine conditioned cue preference (CCP) on the radial arm maze was impaired by lesions of the lateral nucleus of amygdala (LNA). However, Olmstead and Franklin (1998) reported that LNA lesions did not impair the morphine CCP in an enclosed box apparatus. This difference was confirmed in an unpublished experiment in which the same rats with LNA lesions failed to show a morphine CCP in the separate arm version of the task on the radial maze, but then showed a large morphine CCP in the box apparatus. It was hypothesized that this disparity might be due to differences in the stimulus configurations available to the rats in the two apparatuses and I therefore decided to look at the effects of changing stimulus configurations on CCP learning.

The basic principle of all CCP experiment is the exposure of the subjects to one cue or set of cues in the presence of a reinforcer (the paired trials) and an equal amount of exposure to some equivalent cue or set of cues in the absence of the reinforcer (the unpaired trials). Some form of learning occurs involving effects of the reinforcer and the cues that are paired with it. When given a choice between the two sets of cues with no reinforcers present, the subjects exhibit a preference for the cues previously paired with the reinforcer. In CCP experiments with rats this preference is measured as a difference in the amount of time the rats choose to spend in the presence of each set of cues.

This preference is attributed to the learning that occurs during the paired trials. The purpose of the present thesis was to study the effects of stimulus configuration on the learning that occurs during these trials. The learning was examined on the behavioral level and in terms of the neural substrates that mediate it.
Two basic stimulus configurations were defined: distinct and ambiguous. In distinct cue paradigms the subjects can see only one set of cues at a time. This was the situation in the two morphine experiments described above. In the separated arm radial maze paradigm the rats are confined on the end of one arm after a morphine injection (the “paired” trials) and on the end of another arm on the other side of the maze after a saline injection (the “unpaired” trials). They see completely distinct sets of cues from the two arms, so there is no ambiguity about which cues are paired with the reinforcer. In the box apparatus two compartments with distinctive cues separated by an opaque partition are used. The rats are confined in one compartment after a morphine injection and in the other compartment after a saline injection. Again the cues paired with the reinforcer are distinct from the cues not paired with the reinforcer.

If the stimulus configurations during the training trials in both apparatuses were the same, how can this variable account for the differences in the morphine CCPs observed? I hypothesized that this may have been due to stimulus configuration differences during the pre-exposure and testing phases of the procedure. In most CCP experiments the rats are placed in the apparatus and allowed to explore freely for one or more “habituation” sessions before the start of training. In the separated arm radial maze paradigm the rats are placed on the center platform of the maze with the two arms open and available for exploration. From the platform the rats can see the cues visible from both arms at essentially the same time. In contrast, in the box apparatus, the two compartments are connected by a tunnel that prevents the rats from seeing the two sets of cues at the same time. These differences also applied to the test trials in both apparatuses.
These considerations led to two hypotheses. First, these stimulus configuration differences result in different forms of learning that involve different neural structures, and that these factors could, in principle, account for the differences in the effects of lesions on the morphine CCP in the two apparatuses. Second, although CCP learning occurs during the training trials when the cues are paired with the reinforcer, learning, memory and retrieval processes also occur during unreinforced pre-exposure, exposure to the unpaired cues during training, and during the test trial. Therefore, processes occurring in all three phases of the CCP paradigm must be considered.

In the present experiments, situations in which the rats cannot see both the paired and unpaired cues at the same time are defined as “distinct”. Situations in which the rats can see both sets of cues at the same time are defined as “ambiguous”. The most general form of the hypothesis tested was that these distinct and ambiguous stimulus configurations engage different memory systems which result in different forms of learning. The different forms of learning nevertheless result in similar CCPs because this is the behaviour measured in these behavioral paradigms.

Although the impetus for the present studies came from an investigation of the morphine CCP, it was decided that a drug reinforcer was not ideal for trying to understand the learning processes that result in conditioned preferences. Therefore, food was used as the reinforcer in the present studies to examine effects of changing stimulus configurations on CCP learning.

In the experiments described in Chapter 3, a standard box apparatus with a wood (opaque) partition between the two large compartments was modified to simulate the stimulus characteristics of the separated arm radial maze apparatus. A removable
Plexiglas (clear) partition was used to separate the paired and unpaired compartments so that the rats could see the cues in both compartments at the same time. By using the clear partition during the pre-exposure and test trials, and the standard opaque partition during the training trials, the stimulus configurations of the separated arm paradigm on the radial maze could be duplicated.

When the opaque partition (distinct cues) was used in all three phases of the procedure (the standard box apparatus procedure) the food CCP was blocked by lesions of LNA, but unaffected by fimbria-fornix lesions. The same pattern of effects was observed with the opaque partition (distinct cues) during training and the clear partition (ambiguous cues) during pre-exposure and testing.

In previous experiments, McDonald and White (1995b) studied a radial maze CCP in which the food-paired and unpaired arms were adjacent to each other. In this configuration, the rats can see most of the same cues from both arms during all three phases of the procedure. This form of ambiguous cue CCP learning was duplicated in the box apparatus by using the clear partition in all three phases of the procedure. Neither LNA nor FF lesions blocked the CCP in this paradigm. Moreover, there was clear evidence that this form of CCP learning was facilitated by LNA lesions. This is the first evidence that lesions of the amygdala can improve CCP learning instead of impairing it.

These results with the clear partition led to the idea that lesions of the amygdala facilitate ambiguous stimulus CCP learning. This idea was further examined in the adjacent arm CCP paradigm on the radial maze in Chapter 4. These experiments showed that ambiguous cue CCP learning on the radial maze was facilitated by LNA lesions, but
only if the rats had been pre-exposed to the maze environment. FF lesions eliminated the CCP observed in pre-exposed rats with LNA lesions.

These new findings led to several hypotheses. First, they suggested that the ambiguous cue CCP on the radial maze is dependent on an intact fimbria-fornix, a part of the hippocampal memory system, implicating spatial learning in this behaviour. Second, they suggested that a form of amygdala-based conditioned approach learning may occur simultaneously with and independently from the spatial learning. It is suggested that the ambiguous nature of the conditioned cues results in undifferentiated conditioned approach responses to the adjacent maze arms, interfering with expression of the CCP based on spatial learning. Lesions of the amygdala impair this form of conditioning, eliminate the interference and result in a facilitated CCP.

In Chapter 5 the idea that the ambiguous cue CCP on the radial maze is dependent on hippocampus-based spatial learning was examined. Previous experiments (McDonald and White, 1995b) had shown that rats must be allowed to move around on a radial maze to learn a discrimination between adjacent arms, and that they cannot learn this discrimination (within 8 training trials) in the CCP paradigm because they are confined in the arms during the training trials. Based on the findings in Chapter 4, it was shown for the first time that rats can learn the ambiguous cue CCP on the radial maze if they are given least three 10 min sessions of unreinforced pre-exposure to the maze, during which they move around freely. Lesions of FF made before the pre-exposure sessions prevented this form of CCP learning, but similar lesions made after pre-exposure had no effect. Lesions of the hippocampus impaired CCP learning when made either before or after pre-exposure.
These new findings are important for several reasons. They apparently provide a method for separating two forms of learning that normally occur simultaneously. It is hypothesized that the rats acquire spatial information about the maze environment while moving around on the maze during the unreinforced pre-exposure sessions, and subsequently add information about the location of food in that map during the training trials. It is suggested that this reorganization of the memory is accomplished by a hippocampus-based process of reconsolidation similar to that described by Nadel and Moscovitch (1998).

An intact fimbria-fornix is required for the acquisition of this information, but is not required for its subsequent use in learning the CCP. The present findings do not permit localization of the hippocampus effect to a specific phase or phases of the learning process. However, based on previous findings on the effects of hippocampus lesions on separated arm CCP learning (White and Wallet, 2001; White, Holahan and Goffaux, in press) it is suggested that the hippocampus is involved in the recall and reorganization of the spatial information during training and in the recall of the reorganized information to produce the ambiguous cue CCP during the test trial.

The findings in this thesis also have implications for understanding the behavioural phenomena of latent learning and latent inhibition. Just as different learning processes produce CCPs in different situations, these phenomena may also be based on different learning processes in different situations. These implications are discussed in the thesis.
CHAPTER ONE

"The old horses know the way"

"One spring around 2000 B.C., Kuang-Chung traveled with the Emperor Huan to conquer the Ku-Chu province. When winter came they set out for home but could not find the way back. Kuang-Chung advised the Emperor to rely on the old pack horses they had brought with them. They released the horses and followed them and soon realized that they were on the correct path back to their home province."

Choon-Chiu

GENERAL INTRODUCTION

Navigation

All organisms spend much of their time traveling from place to place; accordingly, the ability to navigate - to move efficiently from one place to another - is an important function for all individuals. Navigation involves storing relevant information about previously visited places and how to move among them. This can include what a certain place means to an individual, where it is, how to get there from another place, the geometrical relationships among places, what cues exist in the vicinity of the places and along the route, and how to recognize and identify the place when it is reached (Evans, 1980). Thus, the ability to navigate requires a number of complex cognitive functions based on the cooperation of multiple neural systems (Aguirre & D'Esposito, 1999).

In general, navigation is the result of certain kinds of information stored in memory and a strategy for using that information. The information and strategy used in any specific navigation task depends on the cues available in the environment (Winocur, 1980) and experience (Kolb & Whishaw, 1998).
Cues available in the environment

Navigation is based on knowledge about routes and locations; in other words, information that can be stored as a cognitive map in memory. Selection of the navigating strategy is affected by information available in the environment, such as allocentric or egocentric stimuli (Galati, Lobel, Vellar, Berthoz, Pizzamiglio, & Le Bihan, 2000). For example, Suzuki and her colleagues reported that in a stimulus-rich environment, animals tended to use the topographical relationships among the surrounding stimuli to navigate in an eight arm radial maze. In contrast, in a stimulus-poor environment, they used a stereotyped response strategy (Suzuki, Augerinos, & Black, 1980). In addition, motivational states (Hsiao & Isaacson, 1971) as well as the purpose of the behavior (Tolman, 1932) may also play a role in the selection of navigational strategies. Although salient landmarks and spatial cognitive maps contribute to successful navigation behavior, the neural mechanisms that allow them to do so are different (Weiskrantz, 1990; Kesner, 1998).

Experience

Experience enables animals to find their geographical goals faster and more accurately, and to change the style of their navigation behavior if required. One can easily find one’s home without difficulty, think of 10 possible routes to get there, and easily recognize the destination when one arrives. In contrast, when one is looking for a building in an unfamiliar environment for the first time, at least two new representations have to be acquired simultaneously: the look of the building and its location. The former representation involves object or scene recognition (Magliano, Cohen, Allen, & Rodriguez, 1995; Strasser & Bingman, 1997; Rosenbaum, Priselac, Kohler, Black, Gao, Nadel, & Moscovitch, 2000) while the latter involves spatial or topographical learning (Barrash, Damasio, Adolphs, & Tranel, 2000; Bohbot, Kalina, Stepankova, Spackova, Petrides, & Nadel, 1998).

Navigation memory

Normal navigation behavior requires storage of spatial and object information in memory systems and subsequent retrieval of this information. Impairment of either of
these abilities, due to damage to the related brain areas, could lead to impairment in navigation ability, disorientation, and several different kinds of agnosia (Barrash, Damasio, Adolphs, & Tranel, 2000; Maguire, Frackowiak, & Frith, 1996; Aguirre, Zaraeh, & D'Esposito, 1998). The ability to remember places and navigate among them is known as topographical memory in humans and spatial memory in animals (Squire, 1992; Olton & Samuelson, 1976).

Sometimes, even in the same situation, individuals may use different behavioral strategies to solve the same task. Findings to be discussed show that this results from the preferential activation of different brain memory systems. Thus, an observed failure in navigation or topographical memory may be attributed to defects in one or more independent learning and memory systems in the brain, or could be due to the competition that exists among the behavioral tendencies promoted by different memory systems.

Learning and memory

Like all forms of memory, spatial memory involves three stages: encoding, consolidation, and retrieval (Spear & Riccio, 1994). Encoding refers to the active process of representing stimulus information so it can be stored by a memory system (Hebb, 1949). Consolidation refers to the idea that neural processing, occurring after the initial registration of information, contributes to the permanent storage of memory (McGaugh, 2000). Retrieval, which is the active processes of locating and using information stored in memory (Spear, 1973), is a process of recovering information from long-term memory and bringing it into working memory (Hebb, 1949).

Memory and its neural basis have been studied by philosophers, biologists, psychologists and neuroscientists for hundreds of years (Rosenzweig, 1996). Modern memory research began in the in the early 20th century when Lashley published one of his most famous papers, "In Search of Engram." (Lashley, 1950). He defined the engram as the neural network that represents a fragment of past experience encoded in the brain as "a memory trace".
Search for the engram - Karl Lashley

In Lashley’s experiments, memory functions remained intact even with large lesions made in the cortical areas of the brain. Before making lesions in various discrete regions of their cortex, he had taught rats to run a maze. After examining the effects of the lesions, Lashley concluded that there was a positive correlation between behavioral deficits observed and lesion size, regardless of the location of the lesion. The derived principle was called the “law of mass action” (Lashley, 1929). This idea implies that it is not possible to demonstrate the isolated localization of a memory trace anywhere in the nervous system. Limited regions may be essential for learning and retention of a particular activity, but within such regions the parts are functionally equivalent and the engram is represented throughout the region. A further concept, “equipotentiality”, was proposed by Lashley based on his failure to induce impairments in memory functions after discrete cortical lesions. This concept states that all parts of the cortex are equally important for all memory functions. Therefore, Lashley rejected the phrenological view that individual memories are stored in distinct locations in the brain. He believed that the brain is a “unitary processor” which consists more or less of equipotential components. These components are equipotential because they can be substituted for each other (Lashley, 1938).

It is now clear that Lashley’s behavioral methods were not sensitive enough to detect specific, lesion-induced alterations in memory processes. Numerous subsequent studies using lesion techniques similar to those of Lashley showed that specific memory functions are linked with specific brain areas (Chiba, Kesner, & Reynolds, 1994; Squire & Zola-Morgan, 1991; Nagahara, Otto, & Gallagher, 1995). If a particular memory task is impaired by a particular lesion, the specific characteristics of the task can tell us something about the function of the lesioned area. Lashley’s erroneous conclusion was due to the fact that the converse is not true: if a task is unimpaired by a lesion, the result does not tell us which part of the brain mediated the task. It seems that Lashley used tasks unrelated to the functions of the cortical areas he ablated, saw no effects, and erroneously concluded that the cortex was equipotential. In contrast to Lashley, current memory theorists argue that memory is not a single brain function. Rather, the brain
processes, stores, and retrieves information in several distinct ways in several different places (Squire, 1992; Sherry & Schacter, 1987; Teuber, 1955).

**Single versus Multiple Memory Systems**

An issue in memory research is whether diverse phenomena of memory rely on a single memory system or whether they are processed in several distinct systems. The unitary theory of brain function was first proposed by Flourens in the 19th century; he believed that the lower centers were for sensation and motion, while the cortex was a unitary organ for a unitary mind. The idea that all knowledge is spread across the entire cortex (Fancher, 1988), was an early version of equipotentiality. The Faculty psychologists who followed the unitary view of Flourens and Lashley held that apparent differences in types of memory observed when different tasks are learned are due to variations in the strength and accessibility of the memories, which are processed in a single psychological and biological entity (Farah, 1994; Plaut & Shallice, 1993). The modern version of this Faculty psychology is that memory and information are processed and stored in all parts of the brain as neural networks (McClelland, McNaughton, & O'Reilly, 1995; Hasselmo & McClelland, 1999). Information processing arises from the interaction of large numbers of simple elements or nodes, and knowledge is expressed due to the interaction patterns among these elements. This is essentially a modern version of the notion of equipotentiality (Hasselmo & McClelland, 1999). This school of memory theorists suggests that differences among memories are due to differences in levels of processing (Cermak & Craik, 1979), strength of synaptic connections, nature of synaptic transmission, and patterns of neural activation within the equipotential field (McClelland, McNaughton, & O'Reilly, 1995; Anderson, 1983).

**Phrenologists and Modern Multiple Memory Systems View**

The second major idea about how memory functions are organized in the brain is the hypothesis of multiple memory systems, presaged by the 19th century phrenologist Gall (Heeschen, 1994), who put forward the idea that distinct psychological functions (personality characteristics) are mediated by specialized areas in the brain. This idea was supported by findings of Paul Broca (Eling, 1994) and Carl Wernicke (Keyser, 1994) who
used aphasic patients to show that different aspects of language were localized in different areas in the brain and were therefore processed by different neural substrates (Sherry & Schacter, 1987). Also, instigated by research on H.M., a neurological patient (Scoville & Milner, 1957), since 1950 a growing number of physiological psychologists and neuroscientists have argued for the existence of multiple memory systems in the brain. The theory of multiple memory systems holds that different kinds of memory are processed in different parts of the brain (Squire, Knowlton, & Musen, 1993; Polster, Nadel, & Schacter, 1991; Kesner, 1980). Hence, each memory system is a distinct anatomical structure (or series of structure) that processes and stores a distinct type of information (Kesner, 1998; White & McDonald, 2002).

A Case of Amnesia - H.M.

The original report on H.M. by Scoville and Milner (1954) describes a patient with a bilateral medial temporal lobectomy who suffered from memory dysfunctions, which included topographical disorientation:

Ten months before I examined him, his family had moved from their old house to a new one a few blocks away on the same street. He still had not learned the new address (though remembering the old one perfectly), nor could he be trusted to find his way home alone (Milner, 1965).

The hippocampus was the main structure bilaterally removed in surgery from H.M.’s brain for his epilepsy. Immediately following surgery, he suffered a severe loss of ability to form new memories. He could store new information briefly, but had difficulty in recalling the information, especially when distracted. The case study of H.M. led memory researchers to focus on the role of the hippocampus in learning and memory.

Hippocampus

These reports initiated extensive empirical research on the role of hippocampus in memory and other behaviors. Damage to the hippocampus in animals has been reported
to produce deficits in behavioral inhibition (Douglas, 1967); it also increases activity and exploration of familiar environments (Morris, 1983), and results in deficits in spatial and relational memory (Olton & Papas, 1979; Eichenbaum, 1999).

In all species tested, hippocampus lesions impair spatial memory (Olton & Papas, 1979; Becker, Walker, & Olton, 1980), relational learning (Gilbert, Kesner, & DeCoteau, 1998), and declarative learning (Eichenbaum, 1999). Several brain memory theories have been proposed with emphasis on the functions of the hippocampus. Among these, the cognitive map theory (O'Keefe & Nadel, 1978) and the contextual retrieval theory (Hirsh, 1974) have been two of the most influential.

**Cognitive Map Theory**

The cognitive map theory (O'Keefe & Nadel, 1978) suggests that there are two kinds of memory systems in the brain: the locale and taxon systems. The locale system is dependent on the hippocampus, the taxon system is not. The term "taxon" was chosen to denote the fact that processing within the nonhippocampal systems was based on taxonomic principles of category inclusion and generalization. Memories formed by the taxon system are simple stimulus response (S-R) tendencies which are acquired incrementally, strengthening with each reinforced occurrence. O'Keefe and Nadel (1978) used the terms guidance and orientation to refer to stimulus and response functions. Both of these rely on egocentric spatial systems. Guidance is a specific cue attached to a positive or negative valence that is able to induce approach or avoidance. Orientation is a specific form of behavior that is to be executed in the presence of a cue.

Locale memory refers to a hippocampus dependent S-S type of learning that appears to be acquired rapidly in an all-or-none manner (O'Keefe & Nadel, 1978b). O'Keefe and Nadel incorporated Tolman's (1932) cognitive map into their theory of locale learning. The concept and terminology of the cognitive map, as originally proposed by Tolman (Tolman, 1932) suggested that animals can form cognitive maps which represent relationships among stimuli or landmarks in the environment. This cognitive map allows animals to anticipate routes and locations to be visited as well as to take short cuts toward a goal (Tolman, 1948). Reinforcement is purely informational in the locale.
information processing system. Biological reward is encoded in the same way as other specific stimuli in the environment.

The idea that the hippocampus is involved in spatial memory was strongly supported by the discovery of place cells in the hippocampus (Dostrovsky & O'Keefe, 1971). Place cells are individual neurons recorded in the rat hippocampus when the animal is exposed to a specific location in an environment. These cells consistently fire when the animals return to the same location (Dostrovsky & O'Keefe, 1971). Further experiments confirmed that place cells respond to specific locations in a multiple stimulus environment. Removal of a single stimulus or a pair of stimuli does not affect the firing of place cells in that context (O'Keefe & Conway, 1978).

**Contextual Retrieval Theory**

In his contextual retrieval theory, Hirsh (1974) suggested that there are two distinct types of memory: hippocampus-dependent memory that subserves contextual retrieval and hippocampus-independent memory that subserves habit formation. Contextual retrieval is defined as "retrieval of an item of stored information initiated by a cue which refers to but is not necessarily described within the information that is retrieved." (Hirsh, 1974, pg.422). The role of the hippocampus is to retrieve such information from an independent memory storage area and place it on the "performance line". Hirsh defines the performance line as "a system mediating the series of events or processes initiated by the overtly observable stimulus and resulting in the occurrence of the overtly observable response. It is considered to exist in real time and real space, and ultimately to be physiologically observable." (Hirsh, 1974, pg.422)

The performance line also subsumes a type of memory. This is habit memory, which comprises a series of S-R associations stored directly on the performance line. Unlike hippocampus-dependent memory, this type of memory does not have access to information stored in other brain areas. "It suffices to say that the occurrence of the stimulus causes the occurrence of the response. Contextual retrieval has played no role in such a system (Hirsh, 1974, pg.423).

"In the absence of the hippocampus, associative retrieval operates. Behavior is completely controlled by external stimuli and learning is a matter of habit formation."
Readers familiar with learning theory will realize that the behavior of normal animals is treated in a neo-Tolmanian framework, while that of hippocampally-ablated animals is held to be everything for which early S-R theorists could have wished” (Hirsh, 1974, pg.439).

**Relational learning**

Recent work by Eichenbaum and his colleagues (Eichenbaum & Cohen, 2001; Cohen & Eichenbaum, 1993) suggest that the hippocampus supports a relational representation of items, integrating discrete sensory representations, and linking them to create some sort of representational whole. This sort of relational processing can be utilised to link the various aspects of a stimulus, to learn about the relationship among different stimuli and to relate various components of a spatial environment or event. Spatial learning is one example of relational memory. Furthermore, hippocampus dependent relational memory processing plays a role in contrasting and comparing discrete stimuli from representations of different situations. This hippocampus dependent system is capable of making inferences or generalizations across facts derived from different resources. This kind of function is known as representational flexibility.

The relational representational theory of hippocampus function is similar to the cognitive map and contextual retrieval theories in that all the theorists propose that the hippocampus system supports the encoding of configurations of cues, such as the cues that compose places, and scenes, and items in context. However, it differs from the cognitive map and contextual retrieval theories in other ways. In contrast to O’Keefe and Nadel, the relational representation hypothesis suggests that the hippocampus is involved in processing of the representation of multiple cues, and these are not restricted to memory for environmental space or spatial memory. O’Keefe and Nadel do not explicitly emphasize representational flexibility or inference in their model. The relational representational hypothesis differs from contextual retrieval theory because the latter theory emphasizes involvement of the hippocampus in utilization of contextual cues to retrieve the appropriate associations. However, it does not explain how cue and context interact during the retrieval process (Eichenbaum, 1994).
Conditioning

In addition to spatial or relational learning, lesions of the dorsal hippocampus, dentate gyrus, fornix/fimbria, and entorhinal cortex also produce deficits in nonspatial forms of contextual fear conditioning. Hippocampus lesions reduce conditioned freezing to context (McNish, Gewirtz, & Davis, 1997), auditory trace fear conditioning (McEchron, Bouwmeester, Tseng, & Weiss, 1998), negative occasion setting (Holland, Lamoureux, Han, & Gallagher, 1999b), and illness-induced context aversion (Best & Orr, 1973). Of particular interest, Phillips and LeDoux (1994) showed a selective effect of hippocampal lesions on conditioning of freezing to “background” stimuli (contextual cues) as opposed to “foreground” stimuli (phasic tone CS). The authors proposed that the tone-US association are mediated by the amygdala and that the hippocampus is involved in forming contextual associations between the background stimuli and the US (Phillips & LeDoux, 1994).

Physiology of hippocampus memory – LTP

A physiological basis for the mnemonic functions of the hippocampus has been proposed based on the demonstration of the hippocampal long-term potentiation phenomenon (Bliss & Lomo, 1973). Long-term potentiation alludes to the fact that if two inputs to the hippocampus are activated at the same time, the output produced by one of them may be potentiated. This potentiation can last from minutes to hours. This may be the basis for more permanent changes, such as the construction of new connections (synapses or synaptic mechanisms) between the neurons (Bliss & Lomo, 1973; Holscher, 1997; Bliss & Collingridge, 1993), and it has been suggested that these changes may be the basis of memory storage in the hippocampus. This hypothesis has been the subject of intensive recent research (Hall, Thomas, & Everitt, 2000; Sacchetti, Lorenzini, Baldi, Bucherelli, Roberto, Tassoni, & Brunelli, 2001)

Hippocampus and motivation

Although hippocampus-dependent cognitive learning does not necessarily depend on the presence of an incentive goal (Hirsh & Krajden, 1982), it has been implicated in the processing of internal motivational stimuli (Kimble & Coover, 1966; Tamura, Ono,
Fukuda, & Nishijo, 1991). Davidson and Jarrard (1993) found that hippocampal-lesioned rats were impaired on interoceptive discrimination based on food deprivation level (Davidson & Jaffard, 1993). Hsiao and Isaacson (1971) trained the animals to run to one arm for food and the other arm for water in a T-maze. Normal control animals chose the food arm when food deprived, and the water arm when water deprived. Hippocampus lesion animals were impaired in this kind of learning (Hsiao & Isaacson, 1971; Hirsh, Leber, & Gillman, 1978). This result is consistent with H.M.'s difficulty in identifying his state of hunger/satiation (Hebben, Corkin, Eichenbaum, & Shedlack, 1985).

The hippocampus system may also acquire representations of the relationships among internal and external stimuli (Hock & Bunsey, 1998), such as the internal affective states produced directly by drug use or by conditioned stimuli based on drug use (White, 1996). In this type of multiple representation relational learning, the hippocampus system may be critical for incorporating affective information into an otherwise affectively neutral cognitive map.

**Memory systems in the brain**

Although the hippocampus plays a critical role in certain forms of learning and memory, it is not the only structure related to memory in the brain. A large number of other learning and memory functions are unaffected by lesions of the hippocampus and are therefore thought to be independent of this structure.

**Dissociation of Memory Systems in the Brain**

Most of the evidence for differences in the functions of memory systems was acquired using dissociation methods (Bechara, Tranel, Damasio, Adolphs, Rockland, & Damasio, 1995; Packard, Hirsh, & White, 1989; Dunn & Everitt, 1988; Bussey, Muir, Everitt, & Robbins, 1997). To show that a particular kind of memory is uniquely mediated by a particular system, a critical structure in each system is selected for study. The logic of the dissociation requires that lesions to brain area A impair behavioral function X, but have no effect on behavioral function Y. At the same time, lesions to structure B should impair behavioral function Y, but have no effect on X. This pattern of results is taken to suggest that A is required for function X, and B for function Y. Both
X and Y have at least one component that is not shared by the other and these unique components, at a minimum, can be attributed to structures A and B respectively. These kinds of findings have suggested that several more-or-less independent neural systems acquire and store different types of information with different types of processes (Polster, Nadel, & Schacter, 1991; Knowlton, Mangels, & Squire, 1996; Sherry & Schacter, 1987).

**Multiple Memory Systems Theorists**

More explicitly, several theorists have proposed that there are multiple independent memory systems in the brain. Examples of those proposals include declarative versus non-declarative memory (Eichenbaum, 1999; Cohen & Squire, 1980), configural associations versus simple associations (Sutherland & Rudy, 1989), implicit vs explicit memory (Graf & Schacter, 1985), working vs reference memory (Shapiro & Olton, 1994), and an “attribute” model of memory (Kesner, 1980; Kesner & DiMattia, 1987; Kesner, 1998).

Most of these multiple memory theories, like the cognitive map and contextual retrieval theories, are focused on hippocampus-dependent memory. The authors of these theories also proposed the existence of hippocampus-independent forms of memory, such as procedural or habit memory - but the neural substrates for these types of memory are seldom considered. The neural substrates of non-hippocampal memory have been considered in two theories: Kesner and colleagues (Kesner, 1980) have proposed a memory attribute model, and a triple dissociation experiment was conducted by McDonald and White (1993) to test the hypothesis of multiple parallel systems with functions based on memory types.

**Attribute Model of Memory**

In his theory, Kesner (Kesner, 1980; Kesner & DiMattia, 1987; Kesner, 1998) proposes that the organization of memory is based on several functionally separate attributes which are classified into data-based and knowledge-based types. Data-based memory is a temporary representation of incoming information in specific internal and external contexts. Knowledge-based memory is composed of different attributes of memory, processed in independent systems that include space, time, response, affect and
sensory perception in animals, plus language in humans. This theory suggests that
different neural substrates subserve different memory attributes within an extensive
neural circuit. For example, spatial location memory is represented in the hippocampus,
entorhinal cortex, parietal cortex, fimbria-fornix and infra- and prelimbic cortex. Specific
attributes can operate independently and in parallel to each other, but their interactions are
generally specified in this model. For example, the hippocampus mediates spatial location
and temporal attributes, the caudate nucleus mediates response attributes, and the
amygdala mediates affect attributes. These ideas suggest that one needs to analyze the
neural circuits that mediate each attribute. If indeed there are different neural regions that
represent, for example, spatial location information in memory, then it is important to
uncover whether and how these neural regions contribute differentially to the
representation of this information.

Multiple Parallel Memory Systems

A study by McDonald and White (1993) provided information about the
relationships among three proposed memory systems by demonstrating a triple
dissociation (McDonald & White, 1993). These workers used three different memory
tasks on a radial arm maze: conditioned cue preference (CCP), win-shift learning, and
win-stay learning, each of which was thought to require a different type of memory.
McDonald & White used these tasks to dissociate the neural structures that correspond to
each of these types of memory: amygdala, hippocampus, and dorsal striatum, respectively.
They showed that lesions to each of these structures impaired performance on a different
one of the tasks, but did not impair the other two tasks. This led to the conclusion that the
neural system functionally centered on the amygdala mediates conditioned emotional
learning, such as Pavlovian conditioning (Pavlov, 1927), a specific example of which is
stimulus-reward learning, represented by the conditioned cue preference task (Cador,
Robbins, & Everitt, 1989; White & Hiroi, 1993). The second neural system is the one
centered on the hippocampus. Lesions of this structure or of the fimbria/fornix, a major
input-output pathway of the hippocampus, impaired win-shift learning, an instance of
cognitive or relational learning (Olton, Walker, & Gage, 1977; Olton & Werz, 1978). The
third neural system is centered on the dorsal striatum, lesions of which impaired
performance on the win-stay task, an instance of habit, or S-R learning (Gaffan & Davies, 1981). Based on this experiment and subsequent work in White’s laboratory, a multiple parallel memory systems model has been proposed (White & McDonald, 2002). The model suggests that different memory systems are able to work in parallel and independently or together with each other to affect behavioral output.

Damage to a specific brain area not only impairs its dependent memory function but could also affect other kinds of memory. A single brain memory system may interact with other systems to determine the final behavioral outcome. There are at least three possible types of interactions among brain memory systems: facilitation, cooperation and competition. The observation that lesions of the fimbria-fornix improved or facilitated amygdala dependent radial maze CCP (White & McDonald, 1993) is an example of facilitation.

Co-operation between two memory systems is suggested by the finding that lesions of both systems impair a task while lesions of either structure alone have no effect. McDonald and White (1995) trained rats to run from the center platform of a radial maze into either of two arms separated by at least 135 degrees. One arm always contained food, and the other never contained food. Separate lesions of the fimbria-fornix or dorsal striatum failed to block this type of active place discrimination. However, combined lesions of the fimbria-fornix and dorsal striatum impaired it. This was explained by the hypothesis that both systems normally learned the discrimination. If one of the systems was impaired, the behavior could be maintained by the other system (McDonald & White, 1995).

Competition was demonstrated in a water maze where rats were trained to swim to both visible and hidden platforms in the same location. On the test trial, the visible platform was moved to a new location. Rats with fimbria-fornix lesions tended to swim to the visible platform in the new location suggesting they were guided by explicit cues. Rats with dorsal striatum lesions tended to swim to the original location of the platform suggesting they were guided by the spatial cues. When both systems were intact, these systems competed with each other during the choice of platform (Devan & White, 1999).
**Amygdala**

The present thesis is focused on the amygdala and hippocampus memory systems. The literature on the hippocampus has already been reviewed, and amygdala system is reviewed in detail here.

**Affective memory**

Since it was first reported that bilateral temporal lobe lesions in monkeys produced changes in affective reactions (Brown & Schaefer, 1888) and a similar report 50 years later by Kluver and Bucy (1939), the amygdala has been considered a critical site for emotional responding (Rosvold & Pribram, 1954). Weiskrantz (1956) suggested that the behavioral changes following amygdala lesions may indicate a role for the amygdala in the association of environmental stimuli with biologically important events. And, indeed, considerable evidence suggests that the amygdala system is involved in both positive and negative conditioned affective learning. Lesions of the amygdala have been shown to impair both appetitive and aversive conditioning (Burns, Everitt, & Robbins, 1999; Cador, Robbins, & Everitt, 1989; Maren, 1999; Goddard, 1964; Phillips & LeDoux, 1992).

**Positive affective learning**

Lesions of different subregions of the amygdaloid complex impair different types of Pavlovian conditioning tasks (Gallagher & Holland, 1994; Parkinson, Robbins, & Everitt, 2000). It seems that the lateral and/or basolateral nuclei and the central nuclei play different roles in mediating conditioned appetitive behavior (Whitelaw, Markou, Robbins, & Everitt, 1996; Alderson, Robbins, & Everitt, 2000; McIntyre, Ragozzino, & Gold, 1998). Lesions of the basolateral nuclei impair operant responding for a stimulus paired previously with food (Hatfield, Han, Conley, Gallagher, & Holland, 1996; Robledo, Robbins, & Everitt, 1996), sucrose (Burns, Robbins, & Everitt, 1993), water (Cador, Robbins, & Everitt, 1989), and a receptive female (Everitt, Cador, & Robbins, 1989). Post-training lesions of the central, but not basolateral nuclei, impair memory for magnitude of reinforcement on a 8 arm radial maze task (Kesner, Wasler, & Winzenried,
Lesions of the central nuclei of amygdala also impair increments of CS associability in a Pavlovian conditioning task (Holland & Gallagher, 1993).

Temporary inactivation or permanent lesions of the lateral/basolateral amygdala (LNA/BLA) block both acquisition (White & McDonald, 1993) and expression (Kantak, Green-Jordan, Valencia, Kermin, & Eichenbaum, 2001) of a food conditioned cue preference (CCP) in the radial maze. LNA lesions also blocked the amphetamine CCP in a closed apparatus with separate compartments (Hiroi & White, 1991). Pre-training injections of lidocaine into the basolateral amygdala did not block a CCP trained in this apparatus (Schroeder & Packard, 2000). The present evidence indicates that the amygdala is a critical structure for processing of the association of positive affective information with neutral stimuli, even if the functional distinction among different nuclei of the amygdala is still not well defined.

Negative affective learning

Amygdala lesions impaired inhibitory avoidance learning (Parent, West, & McGaugh, 1994; Parent, Quirarte, Cahill, & McGaugh, 1995), conditioned taste aversion (Bermudez-Rattoni & McGaugh, 1991; Dunn & Everitt, 1988; Morris, Frey, Kasambira, & Petrides, 1999; Aggleton, Petrides, & Iversen, 1981), conditioned place aversion (Kesley & Arnold, 1994), fear potentiated startle (Kim & Davis, 1993; Falls & Davis, 1995), and many manifestations of conditioned “fear” responding (Killcross, Robbins, & Everitt, 1997; Fendt & Fanselow, 1999; Kim & Fanselow, 1992; Helmstetter, 1992; Maren & Fanselow, 1995). An intact amygdala is necessary for both acquisition and expression of conditioned freezing (Maren, Aharonov, Stote, & Fanselow, 1996). Killcross et al. (1997) dissociated the functions of central and lateral nuclei of the amygdala in fear conditioned tasks. They showed that lesions of the central, but not basolateral, nuclei of amygdala impaired conditioned suppression of ongoing responses by shock, and lesions of the basolateral, but not central nuclei of amygdala impaired the ability of a shock paired CS to influence ongoing operant responses or choice behavior. Similar to the evidence from positive affective learning, no consensus exists concerning differences in the functions of different nuclei of amygdala in conditional aversions (Killcross, Robbins, & Everitt, 1997).
Memory functions of the amygdala – modulation or storage?

There are two major hypotheses regarding the role of amygdala in memory function (Cahill, Weinberger, Roozendaal, & McGaugh, 1999; Fanselow & LeDoux, 1999). One hypothesis suggests that amygdala modulates memory storage in other brain areas (Frysztak & Neafsey, 1994; Handwerker, Gold, & McGaugh, 1974; Packard, Cahill, & McGaugh, 1994; Packard & Teather, 1998). A role for the amygdala in memory modulation was first proposed by Goddard who showed that post-training electrical stimulation of the amygdala after aversive learning induced retrograde amnesia (Goddard, 1964). Extensive studies from McGaugh's laboratory show that post-training treatments, such as microinjection of drugs or hormones or electrical stimulation, affect amygdala based time- and dose-dependent modulation of memory storage (for reviews, see McGaugh, 2000; McGaugh, Introini-Collison, Cahill, Castellano, Dalmaz, Parent, & Williams, 1993). These studies also show that post-training injections of stimulants, such as amphetamine or β-adrenoreceptor agonists enhance memory consolidation. All these effects were blocked by lesions of the stria terminalis and blockade of brain β-adrenoreceptors.

Liang and et al (1982) showed that bilateral basolateral amygdala lesions blocked one-trial inhibitory avoidance when made before training or within two days after training. Basolateral amygdala lesions made more than two day after training did not block the inhibitory avoidance response (Liang, McGaugh, Martinez, Jensen, Vazquez, & Messing, 1982). Also, Parent et al. (1994) showed that retention of inhibitory avoidance was impaired by immediate microinjections of lidocaine into the amygdala but not by microinjections given 24 hours later (Parent, Quirarte, Cahill, & McGaugh, 1995; Parent, West, & McGaugh, 1994). Amygdala lesions induced after training did not block memory for footshock-motivated escape training (Parent, West, & McGaugh, 1994). These findings suggest that the amygdala has a time limited role in affecting memory storage in other brain memory systems.

The other hypothesis proposes that the amygdala is the site where CS-US associations are formed and permanently stored. Research supporting this hypothesis shows that basolateral amygdala lesions abolish conditioned freezing when made 1, 14 or
28 days after Pavlovian fear conditioning (Maren, Aharonov, & Fanselow, 1996). These results suggest that the amygdala is a brain area that stores the specific memory information, since lesions of the amygdala impair that specific memory regardless of when they are made. Taken together this evidence suggests that the amygdala may have both memory modulation and storage functions. According to this idea, the amygdala can be involved in storage of a specific memory, and also in modulating other types of memory during its formative stages in other brain areas.

Anatomical basis of memory systems

The main assumption of multiple memory system theories is that several functionally and anatomically distinct memory systems acquire and store information in the brain. Therefore, it is necessary to understand the anatomical basis of these conceptual memory systems. As the present thesis focuses on the hippocampus and amygdala, their anatomy, afferents and efferents, as well as connections between the two structures will now be described.

Hippocampus

Anatomically, the hippocampus is probably the single most salient structure in the brain of the rat. The hippocampal formation includes the entorhinal cortex, the subicular complex, the hippocampus proper and the dentate gyrus. Lorente de No (1934) divided the hippocampus into four subregions: CA1, CA2, CA3, and CA4. There are two major afferents of the hippocampus: the first, consists of inputs from the neocortex, which converge on the entorhinal cortex and then enter the hippocampus via the perforant path (Amaral & Witter, 1987). Entorhinal cortex is the main site to receive extrinsic sensory information from the neocortical areas (Insausti, Amaral, & Cowan, 1987; Amaral & Amaral, 2000). The second set of afferents is subcortical, which reach the hippocampus via the fimbria-fornix (FF) bundle (O'Mara, Commins, Anderson, & Gigg, 2001). FF is the main route connecting the hippocampus and other subcortical structures such as medial and lateral septum, nucleus accumbens, ventral striatum, anterior thalamus, mamillary nuclei, locus coeruleus, and raphe nucleus (Amaral & Witter, 1995). It provides the main cholinergic as well as the serotonergic and adrenergic inputs (McMahon & Kauer, 1997). Because of these connections, the effects of FF lesions on
memory functions are often said to be similar to those of hippocampus lesions (van der Staay, Raaijmakers, Lammers, & Tonnaer, 1989; Olton, Walker, & Wolf, 1982). Although this is not always the case (Zola-Morgan, Squire, & Amaral, 1989; White & Wallet, 2000; Ferbinteanu & McDonald, 2001), FF lesions have been used as a model of hippocampal damage (Cassel, Duconseille, Jeltsch, & Will, 1997).

Most of the hippocampal efferent neurons originate in the subiculum. There are two main output projections from the hippocampal formation: cortical and subcortical projections. The subcortical projections are via subiculum and the fimbria-fornix into the ventral striatum and mammillary body (Swanson and Cowan, 1977). However, subcortical projections from hippocampus are not entirely governed by the fimbria-fornix (van Groen & Wyss, 1990). There is also a direct projection from CA1 to amygdala. The cortical projections are from CA1 and subiculum to the entorhinal cortex. CA1 also has direct projection into the prefrontal cortex (Swanson, 1981). The projections of hippocampus to prefrontal cortex reach both medial and lateral prefrontal cortex (Verwer, Meijer, & Witter, 1997).

**Amygdala**

The amygdala in all mammals consists of numerous nuclei that often merge with their neighbors, as well as with adjacent brain regions (McDonald, 1998; McDonald, 1991). These nuclei can be classified into three main groups. First, the basolateral nuclear group, which includes various subdivisions of the lateral, basal, and accessory basal nuclei. These nuclei receive sensory information from primary sensory cortex, association cortex, thalamus and hippocampus; and send projections to the ventral striatum, dorsomedial nuclei of the thalamus, prefrontal cortex and the central nucleus. Secondly, there is the superficial cortex-like nuclear group, which includes the cortical nuclei and nucleus of the lateral olfactory tract. And thirdly, the centromedial nuclear group which includes the central and medial nuclei. The central nucleus receives projections from the basolateral division and sends projections to a wide variety of regions in the brain, such as ventral tegmental area, locus coerulus, hypothalamus, pons, midbrain, and medulla. On the other hand, the medial nucleus receives information on odors and pheromones (Petrulis
Johnston, 1999; Hess, Gall, Granger, & Lynch, 1997), and relays it to the medial basal forebrain and hypothalamus.

**Interactions between Amygdala-Hippocampus systems**

**Anatomical connections**

Projections from the hippocampus to amygdala originate in the ventral subiculum and adjacent portions of CA1. The projections divide into lateral and medial divisions (McDonald & Mascagni, 1997; Swanson & Cowan, 1977). Most of the medial ventral subiculum projections reach the amygdala via the accessory basal nucleus; there are also moderate projections to the anterodorsal and posteroventral parts of the medial nucleus, the intra-amygdaloid portion of the bed nucleus of the stria terminalis, and the amygdalohippocampal area. Direct projections from the subiculum to the lateral and basolateral nucleus are sparse, other indirect projections from subiculum reach the bed nucleus of the stria terminalis. The lateral projections from subiculum are mainly to the lateral nucleus, medial portions of the basal nucleus and the medial subdivision of the central nucleus (Amaral, Price, Pitkanen, & Carmichael, 1992). In addition, there is a robust projection to the caudal part of the anterior hypothalamic area. Afferents from CA1, originating from the portion adjacent to the ventral subiculum, reach the basal nucleus, lateral nucleus, and posterior cortical nucleus (McDonald, 1998).

Projections from the amygdala nuclei to the hippocampal formation are segregated, projecting to non-overlapping regions of the hippocampus (Pikkarainen, Ronkko, Savander, Insausti, & Pitkanen, 1999). The largest input from the amygdala in the lateral nucleus followed by the accessory basal nucleus (Amaral, Price, Pitkanen, & Carmichael, 1992) which, in turn sends substantial projections to the entorhinal cortex, the CA1 and the parasubiculum. Most of the projections from the lateral nuclei are directed to the entorhinal cortex and parasubiculum (Amaral & Witter, 1995; Pikkarainen, Ronkko, Savander, Insausti, & Pitkanen, 1999). The entorhinal cortex receives inputs from different nuclei of the amygdala, such as the lateral nucleus, accessory basal nucleus, perimaygdaloid cortex, anterior cortical and medial nuclei, and the paralaminar nucleus.
Physiological evidence

There is evidence of functional interactions between the hippocampus and amygdala systems in the brain. Electrophysiological recordings in cats demonstrated that the electrical activity of cells in the lateral and basal amygdaloid nuclei oscillate in phase with neuronal activity in the entorhinal cortex (Pare & Gaudreau, 1996). Other evidence showed that basolateral amygdala lesions impair hippocampal long-term potentiation in rats (Ikegaya, Saito, & Abe, 1994). Furthermore, high-frequency stimulation of the basolateral amygdala facilitates the induction of long-term potentiation in the dentate gyrus (Ikegaya, Saito, & Abe, 1995; Ikegaya, Saito, & Abe, 1996). On the behavioral level, Packard and his colleagues showed that drug injections into the amygdala produced facilitation of different forms of hippocampus and caudate dependent memory (Packard, Cahill, & McGaugh, 1994; Packard & McGaugh, 1996; Packard & Teather, 1998).

Maren and his colleagues showed that single-pulse stimulation of the ventral agranular bundle evoked extracellular field potentials in the basolateral amygdala (Maren & Fanselow, 1995). This evidence shows that bi-directional interactions between these two systems exist in the brain; however, their interactions and mutual influences may or may not translate into explicit behavior.

Behavioral Interactions

While evidence indicates that both the amygdala and hippocampus play independent roles in learning and memory, it is possible that they may also interact with each other in certain situations (McDonald & White, 1994). These structures may also synergistically influence the memory function of each other at the physiological and behavioral levels.

Among the evidence for these interactions is a report by White and McDonald (1993) that unreinforced pre-exposure to an environment interferes with subsequent CCP learning in the same environment. The CCP learning was impaired by amygdala lesions (as already described), and the interfering effect of pre-exposure on this form of learning was impaired by fimbria-fornix lesions. The latter finding was replicated by White and Wallet (2000), who also reported that hippocampus failed to eliminate the interference produced by unreinforced pre-exposure.
Latent inhibition was first demonstrated by Lubow and Moore (1959). They exposed four goats and four sheep to either 10 trials of a moving rotor or a flashing light. Half of the animals exposed to the rotor received pairings of the rotor with a footshock (which elicited leg flexion); the other animals received light-shock pairings. The same pattern, counterbalanced, was used for the animals initially exposed to the light. Unreinforced pre-exposure to either of the stimuli, paired with shock retarded acquisition of the conditioned leg flexion response, compared to pre-exposure to the other stimulus (Lubow & Moore, 1959).

Latent learning

One of the earliest latent learning experiments was done by Tolman and his students (Tolman & Honzik, 1932). Three groups of animals were trained in a complex maze. The first group always received food reward at the end of the maze. The second group was placed in the maze without food until the eleventh day. The third group of animals never received food in the maze. During the first 10 trials, the second and third groups, which were not reinforced made more errors than the reinforced group. However, when food was introduced to the second group on the eleventh day, their errors decreased significantly in a single day’s training to the level of the first group that had been reinforced from the first trial. This result suggested that learning took place during the 10 unreinforced trials, but that this learning was “latent”: it was not expressed since there were no goals or reinforcers in the environment (Tolman & Honzik, 1932).

Perceptual learning

Another illustration of learning facilitated by unreinforced stimulus pre-exposure is perceptual learning. In Gibson and Walk’s (1956) classic demonstration of perceptual learning, experimental animals were bred from birth to 90 days in standard colony cages fitted with white cardboard walls. Round or triangular metal shapes were fixed to the walls and their positions were changed daily. When the rats were 90 days old, both the experimental and the control animals, which had never been exposed to the shapes, were trained to discriminate between a circle and a triangle. The pre-exposed rats performed the discrimination significantly better than the control group, showing that the animals
had learned something during prolonged exposure to the shapes (Gibson & Walk, 1956; Gibson, Walk, Pick, & Tighe, 1958; Walk, Gibson, Pick, & Tighe, 1958)

Factors affect unreinforced learning

Number of unreinforced pre-exposures

The observation that exploration facilitates learning in some situations (Shanab, 1965), and retards it in others (Lubow & Moore, 1959), is still not understood (McLaren & Mackintosh, 2000). Prados showed that 4 pre-exposure sessions facilitated subsequent learning in a water maze, but this facilitatory effect was abolished when the animals were given 8 pre-exposure trials, and latent inhibition effects were observed with 16 pre-exposure trials (Prados, 2000). The number of exposure trials appears to be a critical factor that leads to this biphasic latent impact when reinforcers are introduced at a later stage of the experiment.

Task complexity

The complexity of the discrimination task is another factor that affects the consequences of unreinforced learning. Oswalt (1972) reared rats with easy, intermediate and simple visual patterns for 50 days and then trained them to discriminate those patterns in a maze. Pre-exposure facilitated the discrimination only on the difficult pattern discrimination (Oswalt, 1972). Trobalon et al (1991) reported similar results when they pre-exposed rats to the extra-maze landmarks of radial maze arms they were subsequently required to discriminate. Such pre-exposure facilitated the learning of a spatial discrimination, in which the two arms were separated by an angle of 45 degrees. In contrast, the pre-exposure retarded the learning of a spatial discrimination between the two maze arms separated by an angle of 135 degrees. The researchers argued that the critical factor determining whether pre-exposure facilitates or retards subsequent learning is the proportion of common features or elements shared by the reinforced and non-reinforced cues. In adjacent arm discriminations, pre-exposure reduces the associability of common elements and facilitates subsequent discrimination learning, whereas pre-exposure retarded separate arm discriminations by reducing the associability of unique elements (Trobalon, Sansa, Chamizo, & Mackintosh, 1991).
Role of context in unreinforced learning

At least three types of context manipulation affect unreinforced learning: First, latent inhibition is context specific. Latent inhibition is attenuated when CS pre-exposure and conditioning occur in different contexts (Hall & Channell, 1986; Honey & Good, 1993; Lovibond, Preston, & Mackintosh, 1984; Westbrook, Jones, Bailey, & Harris, 2000). However, latent learning is not always context specific, it can also be produced by pre-exposure to the stimulus only or to the context itself (Trobalon, Sansa, Chamizo, & Mackintosh, 1991; Gibson & Walk, 1956; Crist, Kapadia, Westheimer, & Gilbert, 1997).

Second, contingency between context and stimulus pre-exposure is a critical factor in producing effective unreinforced learning (Channell & Hall, 1983; Killcross, Kiernan, Dwyer, & Westbrook, 1998). Killcross et al. (1998) showed that latent inhibition was eliminated by a delay between conditioning and test sessions, and also reduced by a delay between pre-exposure and conditioning sessions. However, perceptual learning was unaffected by either of the intervals (Killcross, Kiernan, Dwyer, & Westbrook, 1998).

Third, re-exposure to the context alone after pre-exposure of a CS in the context did not affect latent inhibition. In a conditioned suppression experiment, animals first received 40 CS+context pre-exposure trials, then they were re-exposed to the context only (an extinction trial), and later the CS was paired with shock in the same context. The interpolation of an extinction trial between pre-exposure and conditioning did not extinguish the latent inhibition. However, when the number of CS-US conditioning trials was reduced, context re-exposure reduced the latent inhibition (Baker & Mercier, 1982; Hall & Minor, 1984). In contrast, latent learning was not affected by additional context or stimulus exposures (Walk, Gibson, Pick, & Tighe, 1958; Gibson, Walk, Pick, & Tighe, 1958).

This result implies that context manipulation leads to different consequences in latent inhibition and latent learning. One possible explanation for these differences is that different types of information involving different neural systems could be acquired in parallel during the pre-exposure session, and this could produce latent learning and latent inhibition independently.
Neural basis of Unreinforced Learning

Unreinforced exploration is a behavior with no explicit observable goal. One of the features of exploration is increased locomotor activity. But locomotion (pure motor behavior) is not equivalent to exploration because exploration is a true response to novel stimuli or environments. It is important to distinguish between exploratory and locomotor behavior. While the hippocampus is activated during unreinforced exploration (Dringenberg, Kornelsen, Pacelli, Petersen, & Vanderwolf, 1998), lesions of the hippocampus do not affect genuine exploratory behavior, although they do affect locomotor activity (Corey, 1978).

C-fos activation has been observed in the hippocampus when animals were first exposed to an unreinforced environment (Sotty, Sandner, & Gosselin, 1996; Radulovic, Kammermeier, & Spiess, 1998). Similarly, a recent study showed that unreinforced pre-exposure to the context increased c-fos expression in basolateral and medial nuclei of amygdala, hippocampus and parietal cortex in mice (Radulovic, Kammermeier, & Spiess, 1998). These activations did not occur when the animals were subsequently exposed to the same context. In addition, pre-exposure could activate location-specific place cells in the hippocampus that are not triggered by any single stimulus or by any specific pair of stimuli (O'Keefe & Conway, 1978).

With no specific goal, information acquired during unreinforced explorations may not be reflected in the behavior of animals, but may affect the acquisition of new learning involving the same stimuli. This new learning can be seen as a reference task that is used to detect unreinforced learning.

Hippocampus and latent inhibition

The effects of hippocampus lesions on latent inhibition have been examined during several different tasks, but the results have been mixed. Werka et al (2001) showed that dorsal hippocampal lesions impaired latent inhibition of a two-way shuttle avoidance tasks that is sensitive to amygdala lesion. In their studies, pre-exposure to CS (tone) produced latent inhibition of the task in both control and neocortical lesioned, but not in hippocampal lesioned rats. In fact, hippocampus/CS pre-exposure animals appeared to
learn better than the hippocampus. No pre-exposure rats, since the pre-exposure group needed more trials to reach extinction criteria.

Latent inhibition has also been shown in the conditioned taste aversion (CTA) paradigm. Pre-exposure to a flavor (CS) retarded subsequent conditioned taste aversion learning with a specific flavor (Hall & Channell, 1986). McFarland et al. (1978) found that dorsal hippocampus lesions failed to block latent inhibition of a lithium chloride induced conditioned taste aversion, but the lesion impaired conditioned taste aversion per se. This finding was confirmed by a recent report that dorsal hippocampal lesions failed to affect latent inhibition of a conditioned taste aversion (Gallo, Bielavska, Roldan, & Bures, 1998). These results, however, contradicted an earlier study showing that latent inhibition of conditioned taste aversion was augmented following hippocampal lesions (Reilly, Harley, & Revusky, 1993). All these findings suggest that hippocampus may be involved in some cases of latent inhibition of conditioned taste aversion, but the actual mechanism or mechanisms of these effects are not understood.

Kaye and Pearce (1987) examined the effects of dorsal hippocampal lesions on latent inhibition of appetitive conditioning in rats. In the first phase of the experiment, half of the animals received pre-exposures to a discrete light. During the second phase, all groups received pairings of light with condensed milk. The rats pre-exposed to the light acquired the conditioned orienting response to the light slower than the control rats. The hippocampus lesioned animals with pre-exposure learned at the same rate as those that had received no pre-exposure. Similar findings were obtained by Han et al. (1995) using a conditioned appetitive task. In their experiments, animals pre-exposed to a visual CS learned slower in a later CS-food association task. This latent inhibition was impaired by pre-training ibotenic acid lesions of the hippocampus (Han, Gallagher, & Holland, 1995).

On the other hand, Honey and Good (1993) reported a similar experiment, using auditory stimuli and the same ibotenic acid method to damage the hippocampus, which failed to block latent inhibition. In addition, hippocampus lesioned animals showed substantial latent inhibition even when they were pre-exposed and trained in different contexts, whereas the latent inhibition of intact rats was specific to the pre-exposure context. It appears that the hippocampus lesions abolished context related, but spared latent inhibition of the discrete auditory stimulus.
Finally, pre-exposure to the maze environment has been shown to interfere with the separate arm conditioned cue preference (CCP) in a radial maze. This interference was disrupted by fimbria-fornix (White & McDonald, 1993), but not by hippocampus lesions sustained prior to pre-exposure (White & Wallet, 2000). If the fimbria-fornix lesions were made after the pre-exposure, but prior to training and testing, they did not affect the latent inhibition (McDonald & White, 1995).

The function of the hippocampus in latent inhibition has not yet been resolved. Some of the evidence suggests that the hippocampus may be not involved in latent inhibition induced by unreinforced pre-exposure. However, lesions of the parahippocampal areas such as entorhinal cortex (Shohamy, Allen, & Gluck, 2000), or fimbria-fornix (White & McDonald, 1993) impair unreinforced learning with different behavioral tasks.

**Hippocampus and latent learning**

Compared to latent inhibition, fewer studies have focused on the neural mechanism of latent learning. Kimble and his colleagues (Kimble & BreMiller, 1981; Kimble, Jordan, & BreMiller, 1982) demonstrated that latent learning was preserved on a Hebb-Williams maze task in hippocampus lesioned rats. Rats were given either an unreinforced maze or field pre-exposure before food or water finding tasks. Maze pre-exposure was given in the Hebb-Williams maze using pattern number 11 of the Rabinovitch-Rosvold series. Field pre-exposure used the same table top with no maze walls. Rats with hippocampus lesions were impaired on learning to find the reinforcer in both pre-exposure conditions; however, unreinforced pre-exposure to the maze environment improved reinforced maze learning about equally in both the normal and lesioned rats.

This evidence suggests that hippocampus may not be directly involved in latent learning that occurs during unreinforced pre-exposure, although the reinforced learning tasks used in latent learning experiments were impaired by hippocampal lesions. Although several models have been proposed, the neural mechanisms of the unreinforced and reinforced learning and their interactions are still not well understood (Bouton, 1993; Weiner & Feldon, 1997; Holland, 1997).
Amygdala

Reported effects of amygdala lesions on latent inhibition are mixed. Schauz and Koch (2000) showed that microinfusions of the N-methyl-d-aspartate (NMDA) receptor agonist d,l-2-amino-5-phosphonopentanoic acid into the basolateral amygdala before preexposure of rats to the CS prevented latent inhibition of fear-potentiated startle (Schauz & Koch, 2000). Similar findings were reported in a recent study using an appetitive magazine approach task; pre-exposure to an auditory CS retarded acquisition of the approach behavior. Lesions of the basolateral nucleus of amygdala reduced this latent inhibition (Coutureau, Blundell, & Killcross, 2001).

In contrast, Weiner and his colleagues found that electrolytic lesions of the basolateral amygdala failed to disrupt latent inhibition of a conditioned emotional response (Weiner, Tarrasch, & Feldon, 1996). Holland et al. (1993) concluded that lesions of the central amygdala did not impair latent inhibition, since amygdala does not play a role in decrements in CS associability, which were impaired by hippocampus lesions. According to the attentional hypothesis (Holland & Gallagher, 1999), when multiple stimuli are presented simultaneously in a trial, attention to a stimulus increases when that stimulus is a more accurate predictor of reinforcement (the US) than other stimuli. Similarly, attention to a stimulus decreases when that stimulus is not an accurate predictor of reinforcement (the US). Therefore, in latent inhibition, attention to the pre-exposed stimulus and the context decrease, since the pre-exposed stimulus and the context are both poor predictors of reinforcement (Mackintosh, 1975).

Holland and his colleagues showed that lesions of the central nucleus of amygdala impaired increments in CS associability, such as occur in unblocking procedures, but not decrements in CS associability, such as occur in latent inhibition procedures (Holland & Gallagher, 1993).

Nucleus accumbens

The nucleus accumbens is part of a brain system usually linked to rewarding and motivated behaviors (Mogenson, Jones, & Yim, 1980; Di Chiara, 2000; Wise & Bozart, 1984). Considerable evidence shows that nucleus accumbens also plays a critical role in
latent inhibition. Tai et al. (1995) showed that both electrolytic and excitotoxic NMDA lesions of the nucleus accumbens blocked latent inhibition in a conditioned emotional response paradigm (Tai, Cassaday, Feldon, & Rawlins, 1995). In vivo microdialysis in unrestrained rats showed that pre-exposure to the CS abolished the potentiation of dopamine release during conditioning and subsequent conditioned dopamine release (Young, Joseph, & Gray, 1993). The role of dopamine in latent inhibition has further been confirmed by the effects of preconditioning bilateral injections of the dopamine agonist amphetamine into the nucleus accumbens, which abolished latent inhibition of the conditioned emotional response. In contrast, 6-Hydroxydopamine-induced lesions of dopamine terminals in the nucleus accumbens resulted in potentiation of latent inhibition. Bilateral local injections of the dopamine antagonist haloperidol into the nucleus accumbens before conditioning also potentiated latent inhibition (Joseph, Peters, Morgan, Grigoryan, Young, & Gray, 2000). Pre-exposure to the CS did not change dopamine release in the nucleus accumbens, but it prevented the potentiation of dopamine release during conditioning. The pre-exposure reduced conditioned dopamine release in the shell, but not in the core of the nucleus accumbens in subsequent conditioning trials. In summary, the results of these experiments indicate that lesions of the nucleus accumbens blocked latent inhibition, furthermore, there is a negative correlation between dopamine transmission in the nucleus accumbens and latent inhibition (Gray, Moran, Grigoryan, Peters, Young, & Joseph, 1997). The neural mechanism of this inhibition of dopamine release mechanism during conditioning is still not fully understood.
CHAPTER TWO
PRESENT STUDIES

The conditioned cue preference (CCP) is thought to be an instance of Pavlovian or Classical conditioning, mediated by an amygdala-based memory system. As already described, the standard CCP apparatus consists of a two compartment box with distinctive cues in each, connected by a tunnel at the rear of the apparatus. When the rats acquire a CCP in this apparatus, they are assumed to have acquired a conditioned response to the conditioned cues in the paired (with the reinforcer) compartment. CCPs are also acquired when the cues visible from the arm of a radial maze are used as the CS. McDonald and White (1995) showed that rats acquired a conditioned preference for the food-paired arm if it was separated from an unpaired arm by at least 135 degrees (separated arm CCP); however, rats failed to acquire a CCP (with up to 8 training trials) if the two arms were separated by only 45 degrees (adjacent arm CCP). In other situations, however, rats easily learn to discriminate between adjacent arms of a radial maze (Olton & Pappas, 1979; McDonald & White, 1995b). One goal of the present studies was to discover the conditions, if any, that permit adjacent arms CCP learning.

In the box apparatus the rats cannot see the cues in the two compartments at the same time during any phase (pre-exposure, training, testing) of the procedure. In contrast, with separated radial maze arms the rats can see the cues visible from both the paired and unpaired arms at the same time during both pre-exposure and testing, although they can see only one set of cues at a time during the training trials. With adjacent radial maze arms the rats can see the cues visible from both arms during all three phases of the procedure.

These differences led to the question of whether the CCPs acquired in these conditions are based on the same or different kinds of learning and, in parallel, what neural structures mediate this type - or types - of learning. To examine these questions a box apparatus was modified to simulate the stimulus characteristics of the radial maze. The opaque partition that normally separates the two compartments was replaced with a system that allowed a clear Plexiglas or an opaque wood partition to be placed interchangeably between the compartments. With the clear partition in place the rats could see the cues in both compartments from either one, the same situation that prevails
on the maze during pre-exposure and testing with the separated arm CCP procedure, and
during all three phases with the adjacent arm CCP procedure. Thus, the stimulus
conditions that prevail during the separated arm CCP are duplicated by using the clear
partition during pre-exposure, the opaque partition during training and the clear partition
during testing. The conditions of the adjacent arm CCP are duplicated by using the clear
partition during all three phases of the procedure.

Evidence reviewed shows that an intact amygdala is required for separated arm
CCP learning. There is also evidence that the hippocampus/fimbria-fornix system, that
has been linked to relational memory or spatial discriminations involving multiple stimuli
that constitute a context or a spatial environment (Olton & Papas, 1979; Barnes, 1988),
may also influence learned behavior in this task. It is also possible that the
hippocampus/fimbria-fornix system is involved in CCP learning with adjacent maze arms
and with the clear partition in the box apparatus because the cues associated with the
reinforcer are ambiguous in those situations. Accordingly, another purpose of the present
studies was to examine the roles of these learning and memory systems in the various
forms of CCP learning.

The extent to which the predictions about the duplication of stimulus conditions
proved to be correct demonstrated the degree to which the factors determining the
involvement of different types of learning in each form of the CCP are understood. In
addition to manipulating stimulus conditions during the three phases of the CCP
procedure the involvement of different brain systems was studied using lesions. The
results of these stimulus and brain manipulations were used to infer the types of learning
involved in the different forms of CCP learning, to attribute these learning types to neural
systems, and to understand the functions and interactions of the memory systems.

GENERAL METHODS

Materials and Methods

Subjects

Subjects were male Long-Evans rats, purchased from Charles River, Canada,
weighing 300-375 grams at the start of each experiment. The rats were individually
housed in single cages in a temperature-controlled room with the lights on from 7 a.m. to
7 p.m. They had free access to water and food, except as indicated in the procedure. All subjects were treated in conformity with the guidelines of the Canadian Council on Animal Care. All procedures used in these experiments were reviewed and approved by the appropriate McGill Facility Animal Care Committees (FACC).

**Apparatus**

**Radial Arm Maze**

An eight-arm radial maze made of wood and painted flat gray was used (see Figure 1). The maze had an octagonal center platform 40 cm in diameter. Eight arms, 60 cm long and 9 cm wide, were attached to the platform. Rectangular wooden blocks (35 x 19 x 8.5 cm) were used to obstruct six of the eight arms. Two similar blocks had wooden panels (31 x 28.5 cm) attached to the end farthest away from the center of the maze. These blocks were used to restrict the rats to their assigned paired and unpaired arms on the training days of the CCP task. The maze was located in the center of a windowless 2.9 x 2.9 m room that contained a variety of distal cues. A TV camera was suspended from the ceiling above the center of the maze. The camera was connected to a monitor in a room near the testing room.

**Conditioned cue preference box**

Two 3 compartment conditioned cue preference boxes were used (see Figure 2). The boxes were made of wood with Plexiglas front walls. Two of the compartments were identical in size (45x45x30cm). One of these compartments was painted gray, and the other was painted with vertical black and white stripes. Both compartments had wood chips on the floor, which were replaced before each trial for each rat.

The gray compartment was on the left side of one of the two boxes used, and on the right side of the other box. The two compartments were divided by either a clear plexiglas partition or an opaque wooden partition. The partitions were interchangeable. The third compartment was an unpainted tunnel (36x18x20cm) protruding from the rear of the two large compartments connecting their entrances, which were located at the back of both compartments next to the partitions.
Overhead view of a radial arm maze illustrating one configuration of the maze used for adjacent arm CCP learning.
Radial Maze CCP
Figure 2  CCP box

(A) Front view of Conditioned Cue Preference (CCP) apparatus with a clear (left) or an opaque (right) partition inserted between the two large compartments.

(B) Overhead view of a CCP apparatus. A smaller compartment connected the two large compartments at the back of the apparatus. Entrances to the two big compartments is controlled by a guillotine door.
Figure 2A

Clear Partition

Opaque partition

Figure 2B

Guillotine door

Plexiglas wall

Back

Front

Partitions
Electrodes

Nichrome electrodes (0.25 mm in diameter) with enamel insulation were used for electrolytic lesions. The insulation at the tips of the electrodes was removed with Strip X (GC Electronics).

Surgery

Rats undergoing surgery were anaesthetized with sodium pentobarbital (50 mg/kg, ip). Since these experiments originated as part of a project on the morphine conditioned cue preference, no analgesic treatment was administered. All lesions were stereotaxically placed, bilaterally, with coordinates based on Paxinos and Watson (1998) measured in relation to bregma and the skull surface, using standard stereotaxic techniques (Paxinos & Watson, 1998).

Lateral nuclei of Amygdala lesions. Electrolytic current (1.5mA, 20 s) was passed through an electrode with 1.0 mm exposed at the tip. A single lesion was made on each side of the brain. The stereotaxic coordinates were 3.5 mm posterior; 5.5 mm lateral, and 8.5 mm below the skull surface.

Fimbria/Fornix lesions. Radio-frequency current (6mA, 30 s) was passed through electrodes with 0.8 mm exposed at the tip. Two lesions were made on each side of the brain. The stereotaxic co-ordinates were 1.5 mm posterior; 0.8 and 2.2 mm lateral, and 4.5 mm below the skull surface.

Controls. Control animals did not receive any surgical procedures.

Histology

After the completion of behavioral testing, the rats were deeply anesthetized with an injection of 30 % chloral hydrate and perfused with 0.9% saline followed by 10% formol-saline solution. The brains were stored in 10% formol-saline for more than a week before sectioning. Following fixation, they were frozen and cut into 30 um sections, and every fifth section through the lesion site were mounted on glass slides and stained with formol thionin.
Histological analysis

The histological material for rats with lesions of the fimbria-fornix and lateral nucleus of the amygdala in each experiment were examined separately and found to be characterized primarily by their similarity (note that the same procedures were used to make the lesions in all experiments). In addition to the targeted structures, minor damage was also produced in adjacent structures in some rats in each experiment. The behaviour of rats sustaining such damage was compared with that of rats with lesions confined to the target structures, and no consistent differences were found in any experiment. (Given the small numbers of subjects involved, such differences would be difficult to detect.) Accordingly, information on the fimbria-fornix and lateral nucleus of the amygdala lesions were combined across experiments, and this material is summarized here.

Lateral nucleus of amygdala. An electrolytic lesion of the lateral nucleus of the amygdala representative of that sustained by rats in all experiments is shown in Figure 3. The reconstruction of the largest and smallest lesions for the subjects in all experiments is shown in Figure 4. Electrolytic current damaged the anterior and the large, posterior portions of the nucleus. Subjects were discarded from the analysis if the lesions were too small (less than 60% of the damage in all sections) or limited to one side of the brain. In some animals the lesions invaded the adjacent basolateral and central nuclei and the endopyriform nucleus, as well as small portions of ventral hippocampus in animals with more posterior LNA lesions. Thirty-six percent of the subjects with these lesions sustained damage to adjacent perirhinal and entorhinal cortex. The behavior of rats with this damage was indistinguishable from that of rats in which the lesions did not involve these structures.

Fimbria-fornix. A radiofrequency lesion of the fimbria-fornix representative of that sustained by rats in all experiments is shown in Figure 5. The reconstruction of maximum and minimum lesioned areas from the rats in all experiments is shown in Figure 6. Subjects were included in the statistical analysis only if all fimbria-fornix fibers were interrupted in at least one brain section examined. In some rats, the damage extended to the posterior portions of the septum and the anterior part of dorsal hippocampus. In most cases there was also some damage to the corpus callusom and a
minor portion of cortex. Thirty-three percent of these subjects sustained damage to thalamic areas ventral to the FF. The behavior of rats with these lesions was indistinguishable from that of rats that did not sustain these lesions.

**Behavioral procedure**

**Handling**

Handling and food deprivation of the rats began seven days after surgery. Food was removed from all home cages. All rats were handled for 4 days before starting the CCP task. During the handling sessions, 6-8 rats were put into a large wooden handling box. After about 10 minutes, each rat was picked up in turn 5 times and handled for 1 minute each time. The rats were then returned to their home cages where they were given two to three food pellets plus 10 pieces of Kellogg's Froot Loops cereal. The animals were weighed daily to ensure that they maintained at least 80% of their free-feeding weights. Water was freely available in all home cages.

**Maze Conditioned Cue Preference**

All CCP procedures involved three phases: pre-exposure, training and testing. In each phase, the experimenter placed the rat on the maze, left the room, closed the door and observed the animals on the TV monitor. The maze was cleaned with germicidal detergent and deodorant Quatricide PV before each trial for each rat. Each rat in each experimental group was assigned a unique set of radial maze arms: a food paired arm and a no food arm, the two arms assigned to each rat were adjacent to each other. The first phase was pre-exposure phase. All rats were placed on the maze with no food present. The food and no food arms assigned to each rat were open; all other arms were obstructed with the wooden blocks. Animals were placed on the center platform of the radial maze and allowed to explore the open arms freely for 10 min. The number of pre-exposure trials and the context in which they took place varied according to the experiment.

The second phase was the training phase. In each session, the animals were confined to the end of an arm using a wooden block. On food paired sessions, 50 Kellogg's Froot Loops were placed at the end of the arm. The rats remained on the arm for 30 min. The
**Figure 3**  Representative amygdala lesions

Coronal sections showing a representative lateral nucleus of amygdala lesion. The anterior-posterior distance from Bregma (in mm) is shown at the bottom of each brain. The limits of the lesion are indicated with a thin black line.
Lateral Nuclei of the Amygdala Lesion

-2.30

-2.80

-3.14

-3.60

-3.80

-4.16
Figure 4  Summary of amygdala lesions

Drawings of electrolytic lesions of the lateral nucleus of amygdala sustained by rats in all experiments. The lighter areas indicate the maximum extent of all lesions and the darker areas indicate the minimum damage sustained by any rat. Numbers on the right are distances from Bregma (in mm).
Lateral Nuclei of the Amygdala Lesions

-2.45

-2.85

-3.25

-3.70

-3.90
Figure 5  **Representative Fimbria-Fornix lesions**

Coronal sections showing a representative fimbria-fornix lesion. The distance from Bregma (in mm) is shown at the bottom of each section.
Fimbria-Fornix Lesion
Figure 6  Summary of Fimbria-Fornix lesions

Drawings of radio-frequency lesions of the fimbria-fornix sustained by rats in all experiments. The lighter areas indicate the maximum extent of all lesions and the darker areas indicate the minimum sustained by any rat. Numbers on the right are distances from Bregma (in mm).
Fimbria-Fornix Lesions

-0.83
-1.33
-1.78
-2.00
procedure was the same for food unpaired sessions, except that no food was placed in the arm. Half of the animals in each group were placed on their food arms on the first session, the other half were placed on the no food arms on the first session. These placements were reversed on the second day of the training phase. The two days constituted a single training trial. The number of training trials varied according to the experiment.

The third phase was the test phase. This session was identical to the pre-exposure session except that animals were placed on the maze for 20 minutes. Both the paired and unpaired arms assigned to each subject were open, while the other arms were blocked. The time at which each rat entered and exited each arm was recorded. A rat was considered to be in an arm if its front feet crossed the threshold from the center platform.

Box CCP

The procedure for the box CCP was similar to the procedure used for the maze CCP. In each phase of the experiment the partition between the two large compartments was either Opaque or Clear, depending on the requirements of the experiment.

During the pre-exposure phase, the entrances to the tunnel from the large compartments remained open. The rats were placed in the tunnel and allowed to explore the three compartments for 10 min.

During the training phase, the rats were confined to one of the large compartments. For food paired sessions, 50 Froot Loops were placed in the corner of the appropriate compartment and the animals remained there for 30 minutes. For unpaired sessions, they were placed in the other large compartment with no food for the same amount of time. These two sessions constituted a single trial, and their order was counterbalanced within all groups. The number of trials was determined by the requirements of each experiment.

For the test phase, the entrances to the connecting tunnel were open. Each animal was placed in the tunnel and the times at which the rat entered and exited each compartment were recorded.

Data Analysis

The goal of these experiments was to determine the kinds of information rats (both normal and brain damaged) require to learn to discriminate between two locations. Rats were provided with different kinds of information in each experiment and a
determination of whether or not they had learned the discrimination was made. The conditioned cue preference (CCP, also called conditioned place preference) paradigm was used to make this determination. This paradigm takes advantage of rats’ natural tendency to search, or forage, for food when they are hungry. The rats are given identical experience of the two locations to be discriminated, except that they are fed in one (the “paired” location) and not in the other (the “unpaired” location). When tested without food, a tendency for a rat to spend more time in the paired than in the unpaired location is taken as evidence that the discrimination has been learned.

In addition to the two locations to be discriminated, the CCP paradigm involves a third location. This is the area that connects the paired and unpaired locations: the centre platform on the maze apparatus (Figure 1) and the tunnel at the rear of the compartments in the box apparatus (Figure 2). The rats spend some time in this third area during pre-exposure and test sessions, but it is always inaccessible during the training trials, making it a “neutral” area. On maze test trials the rats usually spend about 60 percent of the total time on the centre platform; in the box apparatus they usually spend about 50% of the total time in the tunnel. For reasons that are unclear, the amount of time spent in the third area often varies across experiments but this does not affect the comparison of the time spent in the paired and unpaired locations used to determine if the rats have learned to discriminate between them.

The fact that the rats usually spend at least half of each test session in the neutral area, making occasional relatively short trips to the locations to be discriminated, means that the amounts of time they spend in those two locations are largely independent of each other. Accordingly, decisions about whether or not a group of rats learned any particular discrimination were made by comparing the group mean times spent in the food and no-food locations using pre-planned pairwise comparisons (Cohen, 1988, Kirk, 1969). In each experiment the food and no-food means for all groups were entered into a two-way, repeated measures ANOVA. The first factor was Group, with the number of levels equal to the number of groups. The second factor was Time in the paired and unpaired arms, a repeated measure with two levels. For each group the significance of the difference in time spent in the paired and unpaired locations was tested using the following formula:

\[
F_{(paired-unpaired)} = \left[ \frac{N^* (X_{paired} - X_{unpaired})^2}{MS_{Interaction}} \right]
\]
This analysis is based on the ratio of the difference between the mean times spent in the two locations to the mean square error of the interaction term from the ANOVA. The degrees of freedom associated with the F value is \( N - 2 \) where \( N \) is the number of rats in each group. Since the comparisons were pre-planned, this statistic is considered to provide adequate protection from errors associated with multiple comparisons.
CHAPTER THREE
CONDITIONED CUE PREFERENCE IN THE BOX APPARATUS

Several lines of evidence reviewed suggest that the amygdala is important in associative learning involving biologically salient and emotional stimuli (Weiskrantz, 1956; Cador, Robbins, & Everitt, 1989; Peinado-Manzano, 1994). Lesions of the basolateral and lateral nuclei of the amygdala impair learning tasks involving simple associations with reinforcers, but have no effects on spatial learning tasks (Sutherland & McDonald, 1990; Wan, Pang, & Olton, 1994). The conditioned cue preference (CCP) is an example of a stimulus-reinforcement learning task that is impaired by lesions of the amygdala (Hiroi & White, 1991).

The experiments in this Chapter were intended to test some ideas from previous research on the 8-arm radial maze (McDonald & White, 1993; White & McDonald, 1993; White & Ouellet, 1997; White & Wallet, 2000) about how rats learn the conditioned cue preference when the stimuli available for discriminating between spatial locations are either distinct or ambiguous.

Previous CCP experiments, especially those using drugs as reinforcers (Carr, Fibiger, & Phillips, 1989; Tzschentke, 1998; White, 1996) have been done with an apparatus consisting of two large boxes with distinct interior stimuli. The standard procedure is to pair the reinforcer with the stimuli in one of the boxes and some control treatment with the stimuli in the other box, and then to give the rat a choice between the two boxes with no reinforcer present. During this free choice, or preference, test the rats move between the two boxes freely. In some experiments (Carr & White, 1983; White, Packard, & Hiroi, 1991) they move between the two boxes through a tunnel that prevents them from seeing the stimuli in both boxes at the same time, creating an unambiguous discrimination task. In other experiments (Nader, Bechara, Roberts, & van der Kooy, 1994) the rats move between the two boxes by crossing an open space that allows them to see the stimuli in both boxes at the same time, creating an ambiguous discrimination task. In the present experiments using a box apparatus, this difference was compared to the separated arm (distinct stimuli) and adjacent arm (ambiguous stimuli) CCP learning paradigms previously used on the radial maze. The more general idea tested was that the two CCP learning paradigms result in different forms of learning in both apparatuses.
To test this idea a CCP box apparatus with an opaque partition and a tunnel between the two large boxes was modified so that a clear Plexiglas partition could be substituted. With this transparent partition in place, the cues in both boxes could be seen from both boxes although from different angles in each case. In this way the stimuli associated with the reinforcer were ambiguous. The effects of LNA and FF lesions on CCP in these situations were examined.

**Experiment One**

In this experiment, the effects of LNA and FF lesions on CCP learning in the box apparatus with an opaque partition during training were studied. Two conditions of opaque partition CCP learning were examined. In the OOO condition, the opaque partition was used in all three phases of the CCP procedure: pre-exposure, training, and testing. The OOO condition is the “standard” CCP box apparatus used in many (but not all) laboratories.

In the COC condition, the clear partition was used during pre-exposure, the opaque partition was used during training and the clear partition was used during testing. The COC condition is similar to the CCP on the radial maze with separate arms (sCCP). In that maze condition, the rat can see the sets of environmental stimuli visible from the two arms at essentially the same time during the pre-exposure and testing phases, as is the case in the present COC condition. However, during the training phase in both apparatuses, the rats can see only one set of stimuli during the food-pairing and no-food sessions. Therefore, only one set of stimuli is consistently associated with food during the training sessions. The other set of stimuli is consistently paired with no food.

Once the association has been formed, animals can discriminate the food-no food locations in the opaque partition condition by simply approaching the food paired stimuli. In the clear partition condition they can see both sets of stimuli at the same time. Nevertheless, expression of an existing conditioned approach response to the cues in one of the compartments may still be possible, producing a CCP.
Methods and procedures

Ninety-six Long Evans rats were randomly assigned to 6 pre-training and 4 post-training lesion groups. Pre-training lesion groups consisted of 8 animals each in 2 control groups, 10 animals each in 2 fimbria-fornix (FF) lesion groups, and 12 animals each in 2 lateral nuclei of amygdala (LNA) lesion groups. Post-training lesion groups included: 8 animals each in 2 control groups and 10 animals each in 2 LNA lesion groups. Rats with each type of lesion were assigned to the OOO (Opaque pre-exposure, Opaque training, and Opaque testing) or COC (Clear pre-exposure, Opaque training, and Clear testing) conditions. All groups of rats received one day of pre-exposure, and 2 training trials.

The rats in the pre-training lesion groups were allowed to recover for 7 days after surgery before the start of behavioral testing. Rats in the post-training LNA lesion groups underwent electrolytic LNA lesions the day after the training trials were completed and were tested seven days post surgery. The rats in the control groups were also tested 8 days after the last training trial.

Results and Discussion

The results for the CCP tests are shown in Figures 7 (OOO) and 8 (COC). In both conditions, the control and the fimbria/fornix (FF) lesion groups exhibited robust CCPs, but the rats with both pre- and post-training LNA lesions displayed no preferences for their food-paired compartments. In the pre-training OOO groups, 8 FF and 8 LNA pre-training lesion rats were retained for analysis. The statistical analysis revealed that the mean time spent in the paired and unpaired compartments differed significantly for the rats in both the control \[F(1,21)=10.86; p<0.01\], and FF lesion \[F(1,21)=17.88; p<0.01\] groups, but it was not significantly different for the rats in the LNA lesion \[F(1,21)=1.38\] group.

In the OOO post-training lesion group, 6 LNA post-training lesion rats were retained for analysis. The statistical analysis revealed that the mean time spent in the paired and unpaired compartments differed significantly for the rats in the control \[F(1,12)=32.46; p<0.01\] group, but it was not significantly different for the LNA lesion \[F(1,12)=3.2\] group.
Figure 7  OOO CCP

(Top) *Pre-training lesions.* Mean total amount of time (± SE; seconds) spent in the compartments by control rats, rats with pre-training lesions of the LNA or fimbria-fornix in the OOO condition.

(Bottom) *Post training lesions.* Mean total amount of time (± SE; seconds) spent in the compartments by control rats, rats with post-training lesions of the LNA in the OOO condition.

**Note:** At the bottom of each figure showing the results of CCP experiment with a series of icons summaries the procedure. These show the apparatus used and the conditions that applied during each of the 3 phases of the procedure.
(Top) **Pre-training lesions.** Mean total amount of time (± SE; seconds) spent in the compartments by control rats, rats with pre-training lesions of the LNA or fimbria-fornix in the COC condition.

(Bottom) **Post training lesions.** Mean total amount of time (± SE; seconds) spent in the compartments by control rats, rats with post-training lesions of the LNA in the COC condition.
PRE-TRAINING LESIONS

POST-TRAINING LESIONS

PE

Training

Test

Clear

Opaque

Clear
The results for the COC pre-training lesion groups are shown in Figure 8. Eight FF and 10 LNA pre-training lesion rats were retained after histological analysis. The statistical analysis revealed that the mean time spent in the paired and unpaired compartment differed significantly for the rats in both the control \[F(1,23)=34.94; p<0.01\], and FF lesion \[F(1,23)=64.84; p<0.01\] groups, but was not significantly different for the rats in the LNA lesion \[F(1,23)=0.03\] group.

In the COC post-training lesion groups, 6 LNA post-training lesion rats were retained after histological analysis. The statistical analysis revealed that the mean time spent in the paired and unpaired compartment differed significantly for the rats in control \[F(1,12)=15.27; p<0.01\] group, but was not significantly different for the rats in the LNA lesion \[F(1,12)=0.84\] group.

Blocking of the distinct cue CCP with pre-training LNA lesions is consistent with previous findings that these lesions block the amphetamine (Hiroi & White, 1991) and sucrose (Everitt, Morris, O'Brien, & Robbins, 1991) CCPs in the same type of apparatus and the food CCP in the separate arms paradigm on the radial maze (McDonald & White, 1993). In addition, several previous studies have reported that amygdala lesions block learning involving conditioned approach responses (Peinado-Manzano & Martinez-Martin, 1987; Peinado-Manzana, 1988). As long as food was paired with a distinct set of stimuli during training, there were no differences between animals pre-exposed and tested with distinct or ambiguous stimuli. This is consistent with the idea that distinct stimuli formed conditioned associations with food during the training trials. These CSs elicited conditioned approach responses during the test session. Lesions of the LNA impaired the acquisition, expression or both of this conditioned approach response.

The present findings are also consistent with the recent demonstration that temporary inactivation of the basolateral amygdala immediately after training blocked the food CCP (Schroeder & Packard, 2000). This suggests that the present lesions of the LNA may have blocked acquisition of the opaque partition CCP. However, lesions of the LNA may also block expression of the CCP. In the present study LNA lesions made 24 hours after the training trials impaired the distinct cue CCP. Similar findings were reported by Hiroi and White (1991) who showed that post-conditioning lesions of the LNA impaired the amphetamine CCP in a distinct CCP compartment and by Everitt et al. (1991), who
showed that pre-testing bilateral quinolinic lesions of the basolateral amygdala prevented the expression of a sucrose CCP in a distinctive environment. These findings indicated that the LNA is involved in the expression of this form of CCP.

LNA lesions blocked CCP learning regardless of whether the opaque or clear partition was used during pre-exposure or testing; that is even when the rats were pre-exposed and tested in situations where the conditioned stimuli were parts of a large array of stimuli. In this case, an ambiguous stimulus condition did not prevent the LNA dependent conditioned approach response from controlling behaviour during the test session.

FF lesions failed to block food CCP learning, suggesting that the system of which this structure is a part may not be involved in this form of learning. This finding is consistent with previous reports that lesions of the FF do not impair distinct cue CCPs (McDonald & White, 1995; Hiroi & White, 1991) or other forms of the conditioned approach response (Sutherland & McDonald, 1990; Peinado-Manzano, 1990; Wan, Pang, & Olton, 1994).

**Experiment Two**

In Experiment One LNA lesions impaired CCP learning in both the OOO and COC conditions, showing that this structure is critical for CCP learning when the stimuli paired with food are unambiguous: that is, when these stimuli are clearly differentiable from the stimuli presented in the no-food condition. Experiment Two was designed to examine the effects of LNA lesions on CCP learning when the stimuli associated with food are ambiguous: in effect, when stimuli from both compartments are visible to animals during both food and no food training trials. Based on previous findings with the radial maze apparatus (McDonald & White, 1995) it was predicted that the rats would have difficulty in learning a CCP with a clear partition in the box apparatus.

Three conditions of ambiguous cue CCP learning were examined. In the CCC condition, the clear partition was used in all 3 phases of the procedure. The NCC condition was similar, except that no pre-exposure to the apparatus was given. The CCC and NCC conditions were similar to the adjacent arm CCP condition on the radial maze (McDonald & White, 1995) because the rats could see the cues in both boxes during all
phases of the procedure. In the OCO condition the rats received food training with the clear partition but were pre-exposed and tested with opaque partitions.

The experiment tested the hypothesis that the LNA is involved in learning the ambiguous stimulus CCP with pre-training electrolytic lesions of the LNA. A group of animals with FF lesions was also tested in the NCC condition to examine the hypothesis that this structure is critical for CCP learning with the clear partition.

**Methods and Procedures**

Sixty-eight rats were randomly assigned to 7 groups, with 8 rats in each of three control groups. There were 12 LNA lesion rats in each of the CCC and OCO groups, and 10 LNA rats in the NCC group. A group of FF lesion rats (N=10) was also tested in the NCC condition. All lesions were made before the behavioral procedures started. Different groups received 0 (NCC) or 1 pre-exposure trial with either clear (CCC) or opaque (OCO) partitions inserted between the two compartments. All groups of rats were given two food training trials with the clear partition, and were tested with the same partition used for the pre-exposure session. The rats in the no pre-exposure groups were also tested with the clear partition.

**Results and Discussion**

The results for the CCC, OCO, and NCC groups are shown in Figure 9. In the CCC groups, 8 LNA lesioned rats were retained after histological analysis. The statistical analysis revealed that the mean times spent in the paired and unpaired compartments did not differ significantly for the rats in control [F(1,12)=0.07] group, but the mean times were significantly different for the rats in the LNA lesion [F(1,12)=63.3] group.

In the OCO condition, 8 LNA lesioned rats were retained after histological analysis. The statistical analysis revealed that the mean times spent in the paired and unpaired compartments differed significantly for the rats in both the control [F(1,12)=22.50; p<0.01] and LNA lesion [F(1,12)=26.17] groups.

In the NCC condition, 8 LNA and 8 FF lesioned rats were retained after histological analysis. The statistical analysis revealed that the mean time spent in the paired and unpaired compartments differed significantly for the rats in the control
Figure 9 Clear Partition CCP

(Top) **CCC condition.** Mean total amount of time (± SE; seconds) spent in the compartments by control rats, rats with pre-training lesions of the LNA in the CCC condition.

(Middle) **OCO condition.** Mean total amount of time (± SE; seconds) spent in the compartments by control rats, rats with pre-training lesions of the LNA in the OCO condition

(Bottom) **NCC condition.** Mean total amount of time (± SE; seconds) spent in the compartments by control rats, rats with pre-training lesions of the LNA or fimbria-fornix in the NCC condition
CCC

![CCC Graph](image)

OCO

![OCO Graph](image)

NCC

![NCC Graph](image)

PE
- No, Clear
- Opaque

Training
- Clear

Test
- Clear
- Opaque
The effects of LNA lesions were also examined by comparing the size of the preferences in the individual groups. A preference score was obtained by subtracting the total time spent in the unpaired compartment from the total time spent in the paired compartment for each rat. In the CCC condition the mean preference score for the LNA lesioned rats was significantly larger than the preference score for the controls ($t(14)=5.31; p<0.01$); but there was no significant difference between the two corresponding groups in the OCO condition ($t(14)=0.95$). In the NCC condition the mean preference score for the LNA rats was significantly larger than that for the controls ($t(14)=3.05; p<0.01$), however, the score from the FF group did not differ from the score for the controls ($t(14)=0.50$).

**Latent Inhibition**

When the clear partition was used during the food training trials a CCP was observed in the normal control rats that were not pre-exposed to the apparatus (NCC), but there was no CCP in normal rats pre-exposed with the clear partition (CCC). This appears to be a form of latent inhibition (Lubow, 1973). Pre-exposing the rats to a different condition from that used during training (OCO) did not result in latent inhibition, a possible context effect.

**Amygdala Function in Clear Partition CCP Learning**

Although LNA lesions consistently impaired CCP learning with an opaque partition in Experiment One they failed to do so in the present experiment when a clear partition was used during training. On the contrary, lesions of the LNA facilitated the ambiguous stimulus CCP. In the CCC condition, control rats did not show a CCP but rats with LNA lesions exhibited a large CCP. In the NCC condition the control rats showed a modest (but significant) CCP but the CCP exhibited by the LNA lesioned rats was significantly larger. These observations mean that the LNA is not involved in learning the ambiguous cue CCP.
The hypothesis that LNA lesions facilitated CCP learning in the CCC condition by blocking latent inhibition can be rejected on the grounds that these lesions also increased the size of the CCP in the NCC condition, in which there was no pre-exposure. Instead, this case of lesion-produced facilitation appears to be the result of a process limited to the training and test trials.

A close analysis of the acquisition and expression of information during these two phases of the clear partition CCP paradigm suggests a possible mechanism for the observed facilitation. With the clear partition in place no distinctive stimuli were identified with either compartment, so the cues on the two sides of the apparatus constituted a single context. During the food-pairing training trials normal rats would have acquired conditioned approach responses to these contextual cues. However, as the cues were undifferentiated as to compartment, they were equally likely to elicit approach responses to either one and would not have produced a preference for either compartment. On the contrary, these undifferentiated approach responses could be expected to interfere with the expression by another system of learned information about the location of the food.

Since all the contextual cues were visible from both compartments any associations between them and a reinforcer available in one compartment might tend to be weakened by exposure to the same cues with no reinforcer in the other compartment. However, evidence that stimulus-reinforcer conditioning extinguishes very slowly or not at all (Bouton, 1993; Wilson, Brooks, & Bouton, 1995; Bouton & Sunsay, 2001) suggests that even if these undifferentiated associations were weaker than normal they were probably able to elicit undifferentiated approach responses during the test trials, resulting in interference with expression of a CCP by a different neural system.

Lesions of the LNA would eliminate these undifferentiated approach responses allowing free expression of the CCP acquired by a different system, resulting in a larger preference for the food-paired compartment.

Amygdala lesions did not increase the size of the CCP in the OCO procedure. Although these rats would have acquired an undifferentiated conditioned approach response during training trials with the clear partition, testing with the opaque partition presented a different context to which the undifferentiated conditioned response
apparently did not generalize. Since there was no interference from these amygdala mediated responses in the normal rats, lesions of that structure did not increase the size of the CCP in the OCO condition.

Although there was no generalization from the ambiguous cue training to the distinct cue testing situation, there is apparently generalization between distinct cue training and ambiguous cue testing, as shown by the presence of a CCP in the COC condition in Experiment 1. This comparison suggests that the context as a whole rather than individual cues may constitute the CS in this form of conditioned approach learning. Thus, generalization occurs when the conditioned context forms part of a larger context presented on the test trial (COC), but does not occur when only part of the conditioned context is presented on the test trial (OCO).

Ambiguous Cue CCP Learning in the Box Apparatus

Use of the clear partition creates a single context consisting of the cues in both compartments. Locations within this context can be specified relative to the cues that make up the context. During the training trials rats can learn the location of food relative to these cues, a form of spatial learning. On the test day the rats can return to this location, (which has no food), a behaviour that is expressed as a CCP.

The hippocampus–FF complex has been associated with spatial learning of this type (Becker, Walker, & Olton, 1980; Rawlins & Olton, 1982). However, lesions of FF had no effect on CCP learning in the NCC condition. This finding suggests two possible conclusions: 1) The FF is not involved in ambiguous cue CCP learning. Although this suggestion is inconsistent with previous suggestions concerning ambiguous cue learning on the radial maze (see next section for discussion) it is consistent with another previous finding (McDonald, Murphy, Guarraci, Gortler, White, & Baker, 1997) that FF lesions failed to block 3 different types of non-spatial relational learning, although some of these tasks were impaired by hippocampus lesions; or 2) The ambiguous cue CCP is learned in parallel by two (or more) neural systems. If one of these systems includes FF, lesioning it alone would have no effect because the behaviour would be maintained by the other system. Only lesioning both systems simultaneously would eliminate the CCP in this
situation. The roles of FF and hippocampus in ambiguous cue learning will be examined further in Chapter 5.

**Comparison of Box and Maze CCPs**

In the present experiments CCP learning in the radial maze with separated and adjacent arms was simulated in the box apparatus with the COC and CCC conditions, respectively. Both forms of CCP learning require rats to discriminate between two locations after receiving food in one but not the other.

On the training trials of both the separated arm and COC tasks the cues associated with food are completely or almost completely different from those not associated with food, creating unambiguous discrimination tasks. CCPs on both of these tasks can result from simple conditioned approach responses to the cues paired with food. Consistent with this analysis and with previous findings that such conditioned responses require an intact amygdala (Weiskrantz, 1956; Peinado-Manzano, 1988; Peinado-Manzano & Martinez-Martin, 1987), LNA lesions impaired the opaque partition CCP in the present experiments and have been reported previously to impair the separated arm CCP (White & McDonald, 1993). In the box apparatus, this result was also obtained when the clear partition was used for pre-exposure and testing, suggesting that the CCP acquired with the opaque partition generalizes to the clear partition situation.

On the training trials of both the adjacent arm CCP in the maze and with the clear partition in the box apparatus most or all of the same cues are visible from both the food and no-food locations, creating ambiguous discrimination tasks. LNA lesions failed to block the clear partition CCP in the present experiment, and do not affect the adjacent arm CCP (McDonald & White, 1995b). McDonald & White (1995b) found that the adjacent arm CCP was impaired by lesions of the FF, and suggested that learning the location of the food in that ambiguous cue situation requires that it be specified in terms of its relationships to other cues in the environmental context, a form of spatial learning. However, the clear partition CCP in the present experiment was not affected by FF lesions, and the neural substrate mediating this task remains unknown.

On the ambiguous cue discrimination tasks in both the maze and box apparatuses normal rats failed to acquire the CCP when pre-exposed to the ambiguous cues with no
food present. Normal rats failed to learn the clear partition CCP after a pre-exposure trial (CCC condition), but did acquire a clear partition CCP with no pre-exposure (NCC condition), an apparent instance of latent inhibition. On the radial maze, normal animals failed to learn the adjacent arm task after a single pre-exposure trial and up to 8 training trials, but learning in this situation has not been examined without pre-exposure, so it is unclear if the maze finding is also an instance of latent inhibition. This issue will be examined further in Chapter 4.

The Role of Movement in Ambiguous Cue CCP Learning

Previous findings on the radial maze suggest that movement is required to learn ambiguous cue discriminations. McDonald & White (1995b) found that rats could not learn to discriminate between adjacent arms if they were confined in the ends of the arms during the food training trials (the CCP paradigm); however they learned the same discrimination easily if they were allowed to run into the arms from the centre platform on the training trials. White & Oullete (1997) found that rats could learn the adjacent arm discrimination while confined to the ends of the maze arms if they were moved back and forth between them by the experimenter several times during each training trial.

In contrast, in the present experiments in the box apparatus, normal rats in the OCO and NCC conditions and rats with LNA lesions in all three ambiguous cue conditions tested acquired the ambiguous cue CCP while confined in the boxes and prevented from moving between them. This difference is probably due to the fact that the area in which the rats are confined on the maze arms is much smaller than the area in which they are confined in the box apparatus. Therefore, while ambiguous cue learning is prevented on the maze, some such learning may occur in the box apparatus because the size of the boxes permits the animals to move among locations that permit views of the cues that are sufficiently different to allow the acquisition of information about the location of the food. This may explain how animals could learn the clear partition CCP in the boxes even with no pre-exposure but could not learn the adjacent arms CCP in on the maze even though the CCP procedure was used in both cases. This issue will be examined further in Chapter 4.
Determination of Neural Substrate of Learning by Task Properties

The findings suggest that the involvement of neural systems in CCP learning is determined by the discriminability of the stimuli in the two environments. When the cues paired with food and not paired with food are clearly discriminable amygdala-based conditioned approach responses produce CCPs. When the food- and no food-paired cues are ambiguous they amygdala system fails to acquire information that discriminates among the ambiguous cues. In fact, the data suggest the hypothesis that non-discriminatory, amygdala-based conditioned approach responses may interfere with the expression of a CCP. This ambiguous cue CCP may be based on simultaneously acquired information about the spatial location of the food by an as yet unknown neural system (examined in Chapter 5).
CHAPTER FOUR
ADJACENT ARM CONDITIONED CUE PREFERENCE ON THE RADIAL MAZE: Role of the Amygdala

Experiments using the box apparatus in Chapter 3 showed that the amygdala is an essential part of the neural system that mediates CCP learning with unambiguous cues (opaque partition condition). Previous research (White & McDonald, 2002; McDonald & White, 1993) showed that the amygdala is also required for CCP learning with unambiguous cues when separated radial maze arms are used as the food and no food locations. Both of these findings suggest that the CCPs result from amygdala-based acquisition of conditioned approach responses to the cues visible from the food-paired location. The findings of Chapter 3 also suggest that when the cues defining the food and no food locations are ambiguous, a similar amygdala-based learning process results in undifferentiated conditioned approach responses to both the food and no-food locations. These responses may interfere with the expression of learned behaviors that produce a CCP by other neural systems. In the present chapter the possibility that a similar process occurs during ambiguous cue CCP learning on the radial maze was investigated.

McDonald and White (1995b) found that normal rats failed to learn an ambiguous cue CCP after as many as 8 training trials using adjacent arms on an 8-arm radial maze as the food-paired and unpaired locations. However, rats easily learned to discriminate between the same arms on the same maze when they were allowed to run into the arms from the center platform (McDonald & White, 1995), or when they were moved between adjacent radial maze arms by an experimenter several times during each training trial (White & Ouellet, 1997). Both of these adjacent arms discriminations were acquired in situations where the rats moved between the food and no food arms on the training trials, and both were impaired by FF lesions. Win-shift learning on the radial maze also requires adjacent arm discriminations. This learning situation permits movement among the arms and is impaired by FF or hippocampus, but not by LNA lesions (Becker, Walker, & Olton, 1980; McDonald & White, 1993; Packard, Hirsh, & White, 1989). As already discussed, this evidence implicates the spatial learning functions of FF and hippocampus (Kesner & Novak, 1982; Rasmussen, Barnes, & McNaughton, 1989; White & Ouellet, 1997) in learning to discriminate between adjacent arms of the radial maze. These
findings also suggest that one reason rats fail to learn the adjacent arms CCP is that they are confined on the maze arms during the training trials, preventing the movement that is required to learn the spatial location of the food.

The other factor that may contribute to the failure of rats to acquire the adjacent arms CCP is the possibility that that normal rats acquire amygdala-based undifferentiated conditioned approach responses during ambiguous cue CCP training. Expression of this response on the test day may interfere with expression of CCP learning by another neural system. Consistent with this suggestion, McDonald & White (1993) found that rats with amygdala lesion tended to make fewer errors than control animals on the win-shift task, although the effect was not statistically significant.

The experiments described in this chapter investigated the roles of amygdala-based learning and of movement in ambiguous cue CCP learning on the radial maze.

**Experiment Three**

This experiment was designed to test the hypothesis that a learning process mediated by the amygdala interferes with ambiguous cue CCP learning on the radial maze. The hypothesis predicts that pre-training LNA lesions will facilitate adjacent arm CCP learning.

**Methods and Procedures**

Forty-six rats were randomly assigned to 4 groups of animals given 2, 4, 6 or 8 training trials (N=8 in each group), and an amygdala lesion group (N=14) given 4 training trials.

**Results and Discussion**

After histological analysis, 11 LNA lesioned rats were retained for statistical analysis. The results are shown in Figure 10. Normal rats did not show CCPs with up to 8 training trials. Only the LNA lesioned rats given 4 training trials exhibited a CCP.

The statistical analysis revealed that the mean times spent in the paired and unpaired arms did not differ significantly for the rats in the 2 [F(1,38)=1.01], 4 [F(1,38)=1.03], 6 [F(1,38)=0.35] or 8 [F(1,38)=0.45] training trial groups, but was
Figure 10  LNA lesions facilitate adjacent arm CCP learning on the radial maze

Mean total amounts of time (± SE; seconds) spent in the arms by control rats with 2, 4, 6, 8 training trials, and rats with pre-training lesions of the LNA in the given 4 training trials.
LNA lesions facilitate adjacent arm CCP learning

![Graph showing the effect of LNA lesions on adjacent arm CCP learning. The graph compares paired and unpaired trials for different numbers of pairing trials per lesion. The x-axis represents the number of pairing trials per lesion (2, 4, 6, 8, and 4+ LNA), and the y-axis represents the time in seconds. The bars indicate the mean and standard error for paired and unpaired trials.](image)

PE (Lesions)

Training (2, 4, 6, 8 trials)

Test
significantly different for the rats with LNA lesions given 4 training trials \[F(1,38)=13.16; p<0.01\].

This experiment replicates McDonald and White's (1995b) finding that normal rats given up to 8 training trials fail to learn the adjacent arm CCP on the radial maze.

Rats with LNA lesions acquired the adjacent arm CCP in 4 training trials. This pattern of results is similar to that for ambiguous cue CCP learning in the box apparatus, described in Chapter 3. Since the LNA lesions in the present experiment were made before the pre-exposure session the results do not suggest which phase(s) of the procedure are involved when the normal amygdala blocks the CCP. However, the findings of Experiment 2 tend to rule out an interaction of the LNA with the pre-exposure trial. Therefore, some form of learning during the training trials may be the basis of the amygdala-based blocking of the CCP. As suggested in Experiment 2, this effect probably involves acquisition of an amygdala-based conditioned approach response to the ambiguous cues visible from both maze arms. These undifferentiated approach responses not only fail to discriminate between the food and no food arms, but also appear to interfere with expression of a discrimination between the adjacent arms by another neural system.

Kantak et al. (2001) showed that pre-testing inactivation of the basolateral amygdala (BLA) blocked separate arm CCP learning on the radial maze, suggesting that the LNA may be involved in the expression of conditioned approach responses. This may also be true for the hypothesized approach responses in the present ambiguous adjacent arm discrimination. The expression of this conditioned response may have interfered with the expression of an adjacent arm discrimination. Lesions of the LNA would have eliminated this undifferentiated conditioned response and eliminated this interference.

Evidence already discussed suggests that the adjacent arm CCP depends on the acquisition of spatial information about the location of food with respect to the ambiguous environmental cues visible from the maze arms. Other evidence suggests that movement is required for the acquisition of such a spatial map of an environment. Since the rats in the present experiment were confined in the maze arms during the training trials, the required spatial information could not have been acquired during that phase of the procedure. The following experiment investigates the hypothesis that this information
is acquired during the pre-exposure session, when the rats are allowed to move around freely on the maze.

**Experiment Four**

On the day before the first training trial in all experiments described so far, all rats were placed into the experimental apparatus - maze or box - with no reinforcers present and allowed to explore freely for 10 min. Free movement during unreinforced pre-exposure to an environment may permit acquisition of a spatial map of that environment. This may permit a rat subsequently given food while confined in a small area of the environment to learn the spatial location of the food even if it does not move around while actually finding and consuming it.

To test this hypothesis the pre-exposure phase of the adjacent arm CCP paradigm was manipulated. A group of rats pre-exposed to the same environment as subsequently used for training and testing (the normal procedure) was compared to groups that were either not pre-exposed or pre-exposed to a different environment than the one used for training and testing. It was predicted that normal rats would fail to acquire the adjacent arm CCP in any of these conditions, but that rats with LNA lesions that were pre-exposed to the same environment used for training and testing would learn the CCP. It was also predicted that amygdala-lesioned rats that were not pre-exposed, or were pre-exposed to a different environment would not learn the CCP because they would not have acquired the spatial information required to discriminate between the arms.

**Methods and Procedures**

Sixty-eight rats were assigned to 3 control and 4 lesion groups. The rats in three groups were given a normal 10 min pre-exposure session on the maze (same PE). These included a group of normal controls (N=8), a group with LNA lesions (N=10) and a group with combined LNA and FF lesions (N=12). Two groups were given a 10 min pre-exposure session on a similar maze in a different room containing different environmental cues (Diff PE). These included a normal control group (N=8) and a group with LNA lesions (N=12). The two final groups were not given any pre-exposure (No PE). These
included a normal control group (N=8) and a group with LNA lesions (N=10). All rats received 4 training trials.

**Results and Discussion**

After histological analysis of the rats with LNA lesions, 8 same PE, 8 No PE and 10 Diff PE rats were retained; 9 combined LNA-FF lesion same PE rats were also retained for analysis. The results are shown in Figure 11. None of the normal control groups showed CCPs. Among the rats with LNA lesions, only those in the same PE group showed a CCP. The combined LNA/FF lesion, same PE group also failed to show a CCP.

The statistical analysis revealed that the mean times spent in the paired and unpaired arms did not differ significantly for any group of normal controls: No PE [F(1,21)=0.20], Diff PE [F(1,21)=0.75], Same PE [F(1,21)=0.75]. These differences also failed to reach significance for the LNA lesioned rats in the No PE [F(1,31)=1.64] and Diff PE [F(1,31)=1.05] groups, but was significant for the Same PE group [F(1,31)=42.58; p<0.01]. The paired-unpaired difference was not significant for the LNA/FF Same PE group [F(1,31)=2.67].

The failure of the normal rats in all three pre-exposure conditions to exhibit CCPs is consistent with previous findings (McDonald & White, 1995) and the findings in Experiment 3 that normal animals do not learn the adjacent arms CCP with 4 training trials using the standard CCP procedure. The presence of a CCP in the rats with LNA lesions that were pre-exposed to the same environment replicates the finding of Experiment 4, and is consistent with the idea that some form of amygdala-based learning interferes with expression of the CCP by another neural system.

The failure to observe a CCP in amygdala-lesioned rats that were not pre-exposed or that were pre-exposed to a different environment shows that pre-exposure to the specific context subsequently used for CPP training and testing is necessary for adjacent arms CCP learning, and suggests that some form of learning about this context during pre-exposure is essential for acquisition of the adjacent arms CCP. The absence of a CCP in the rats with combined lesions of LNA and FF pre-exposed to the same environment is
Figure 11  Effects of Pre-exposure on adjacent arm CCP learning in rats with LNA lesions

(Top) Control groups. Mean total amounts of time (± SE; seconds) spent in the arms by control rats given different forms of pre-exposure.

(Bottom) LNA lesion groups. Mean total amounts of time (± SE; seconds) spent in the arms by rats with LNA lesions or combined LNA and FF lesions given different form of pre-exposure.
CONTROL

![Graph showing data for CONTROL](image)

LNA LESIONS

![Graph showing data for LNA LESIONS](image)

PE Training Test

Lesions No, Same, Different
consistent with the hypothesis that spatial learning by the FF/hippocampus system during pre-exposure is required for acquisition of the adjacent arms CCP.

**Adjacent Arm CCP Learning**

Two factors prevent normal rats from acquiring the adjacent arm discrimination with the CCP procedure. First, as previously shown (McDonald & White, 1995; White & Ouellet, 1997), both movement in the environmental context and an intact fimbria-fornix are required for spatial learning to occur and, more specifically, for rats to learn a discrimination between adjacent arms of an 8-arm radial maze. Rats cannot acquire the information required for this discrimination during the training trials of the CCP procedure because they are confined in the arms of the maze during training.

They can, however, acquire this information when they move around on the maze during the pre-exposure session, in effect a form of latent learning (Blodgett, 1932). According to this idea information about the spatial environment acquired during the pre-exposure session by a neural system that includes the fimbria-fornix is retrieved during the training trials and reorganized to include information about the location of food in the spatial environment. A mechanism of this type has been proposed for hippocampus-based learning by Moscovitch and Nadel (Nadel & Moscovitch, 1997; Nadel & Moscovitch, 1998; Nadel & Moscovitch, 2001) and others (Sara, 2000) is also consistent with recent evidence for the reconsolidation of memories in the hippocampus (Winocur, McDonald, & Moscovitch, 2001) and amygdala (Nader, Schafe, & LeDoux, 2000; Amorapanth, LeDoux, & Nader, 2000). Nader et al (2000).

In one experiment (Land, Bunsey, & Riccio, 2000), rats were trained on a signaled avoidance task that was not learned by rats with hippocampus lesions made before training, but was unaffected by similar lesions made 30 days after training. When the rats were exposed to the trained signal just before the lesions were made, subsequent performance of the task was severely impaired. These findings are interpreted to mean that an intact hippocampus was required for the initial consolidation of memory, but was not required for retention of the consolidated memory. Exposure to the trained signal is thought to have caused the memory to be recalled, making it vulnerable to interference, for at least some time after the exposure. The lesion result is thought to show that the
hippocampus is an essential part of the neural mechanism that normally recalls and then reconsolidates the memory.

These ideas suggest that in adjacent arm CCP learning the rats acquire pure spatial information about the maze environment during unreinforced pre-exposure. This memory would be retrieved each time the rats are exposed to the environment during a food training trial. Information about the location of food could be added to the spatial map before the reorganized memory is reconsolidated. During the test trial recall of this reorganized information would be expressed as a CCP as it guided the hungry rats in their search for food.

**Amygdala-Based Learning in the Adjacent Arm CCP**

As suggested by the effect of amygdala lesions in the experiments in this chapter, a second factor that prevents normal rats from learning the adjacent arm CCP may be amygdala-based learning that occurs during the training trials. As proposed in Chapter 3, during the training trials the normal amygdala may acquire conditioned approach responses to cues visible from both the food and no food arms. Expression of these undifferentiated conditioned responses on the test day may interfere with expression of hippocampus-based spatial information about the location of food, eliminating or reducing the size of the CCP observed.

Amygdala lesions eliminate this form of interference, permitting expression of the adjacent arm CCP by the hippocampus system. However, this can only occur if the conditions for acquiring the CCP by this system have been met: the rats must have been allowed to move around in the same environment subsequently used for training and testing, and their spatial learning system (FF and hippocampus) must be intact.

On an 8-arm radial maze, most cues visible from the end of one arm are also visible from the end of an adjacent arm, the locations used for the food and no food training trials in the ambiguous cue CCP procedure. However, there are a certain number of cues which are not visible from both arms, but which can be seen from only one or the other of the arms. It can be predicted that while rats are acquiring undifferentiated conditioned approach responses to the cues visible from both arms, they might also acquire similar responses to the set of cues visible only from the food arm.
The undifferentiated cues are paired with food on half the training trials, when the rats are on the food arm, and paired with no food on the other half of the trials, when the rats are on the no-food arm. The unique cues are paired with food an equal number of times but are never paired with no food. Consequently, it is possible that with repeated trials the conditioned responses to the unique cues could become stronger than the responses to the undifferentiated cues resulting in an amygdala-based ambiguous cue CCP. The number of trials required for this CCP to be observed would presumably vary as a function of the relative proportions and saliency of the unique and undifferentiated cues. In the maze environment used in the present experiment, more than 8 training trials would be required to demonstrate this form of CCP learning.

**Comparison of Ambiguous Cue CCP Learning in the Maze and Box Apparatus**

Amygdala lesions facilitated ambiguous cue CCP learning in both the adjacent arm radial maze paradigm (present experiment) and in the clear partition CCP in the box apparatus (Chapter Three, Experiment Two). In both cases this is thought to be due to the elimination of interference with the expression of hippocampus-based spatial learning about the location of the food by amygdala-based undifferentiated conditioned approach responses on the test day.

The finding that rats with amygdala lesions learn the adjacent arm CCP in the maze only if they have been pre-exposed to the maze environment contrasts with the observation in Experiment Two that rats with amygdala lesions can acquire the clear partition CCP with no pre-exposure in the box apparatus (NCC condition). As proposed in Chapter Three, this difference may be due to the relative sizes of the areas in which the rats are confined during training. The boxes are large enough to allow sufficient movement during the training trials for learning about the relationships among the cues (including the food) visible from the two boxes; the maze arms are small enough to preventing similar learning about the environmental cues visible from the two arms. This analysis predicts that rats would be unable to learn a clear partition CCP in a box apparatus that restricts movement.
Latent Learning and Latent Inhibition

The demonstration that only rats pre-exposed to the maze environment acquire the ambiguous cue CCP on the radial maze can be described as a case of latent learning: the unreinforced acquisition of information during pre-exposure contributes to the subsequent acquisition of a response when a reinforcer is introduced (Blodgett, 1932). In the original demonstration rats explored a maze without reinforcers and then learned the location of the reinforcer when it was introduced while continuing to move through the maze. In the present demonstration of latent learning the rats also explored the maze environment without reinforcers, but then learned the location of the food without any additional movement during the training trials. This methodological separation of the acquisition of the spatial information or map during pre-exposure from the acquisition of information about the location of the reinforcer within that map during the training trials offers the possibility of studying these two forms of learning separately.

Another possible effect of unreinforced pre-exposure to the experimental context might be latent inhibition of the acquisition of amygdala-based conditioned responses to the undifferentiated experimental context. Since this form of learning normally interferes with expression of CCP learning, retarding it might facilitate the CCP. If this process really occurs we might expect to see a CCP in the normal rats pre-exposed to the same environment. Since this did not happen, there is no evidence for this process in the present experiment. Increasing the number of pre-exposures might produce this form of latent inhibition, facilitating the CCP. If this latent inhibition of amygdala-based learning was the source of the CCP in rats with increased pre-exposure, amygdala lesions should eliminate the CCP in these conditions.

The other effect of increasing the number of pre-exposure sessions might be improved spatial learning by the fimbria-fornix - hippocampus system. If this effect is the source of the CCP amygdala lesions should not affect it, but lesions to the FF-HPC system should eliminate the CCP. If both the latent inhibition and spatial learning processes function in this situation, CCP learning should be blocked by either amygdala or FF lesions.
Mutual Interference of Behaviors Acquired by Different Neural Systems

A general assumption of the present analysis is that observed CCPs result from two kinds of learning, each mediated in a different neural system. These are spatial learning mediated by a system that includes the hippocampus and fimbria-fornix and classically conditioned approach responses mediated by a system that includes the amygdala. The systems acquire the two types of information independently during the pre-exposure and training trials. Expression of the resulting behaviors by the two systems on the test day may produce a CCP.

Disabling either system may eliminate the CCP in some cases, but in both the present and previous experiments lesions of one system or the other facilitates a CCP. The latter result is interpreted to mean that the two systems have independent influences on behavior. With any given CCP procedure, one system may express behavior that results in a CCP; disabling that system eliminates the CCP. The other system may express behaviors that are incompatible with a CCP; disabling such a system facilitates or increases the size of the CCP. This kind of independent parallel learning and subsequent competition of outputs from the systems for control of behavior is a central notion of multiple parallel memory system theory.

The improvement of adjacent arm CCP learning in the maze and of clear partition CCP learning in the box apparatus produced by amygdala lesions is an example of this phenomenon. As already discussed, this is thought to be due to the elimination of amygdala-based undifferentiated conditioned approach responses normally acquired during pairings of ambiguous cues with the consumption of food.

The present demonstration of latent learning is another example: the previously reported finding that learning the separated arm CCP is retarded by pre-exposure to the environment subsequently used for training and testing, but not by pre-exposure to a different environment (McDonald & White, 1995). The retarding effect of pre-exposure to the same environment on the unambiguous cue CCP was eliminated by lesions of the fimbria-fornix, suggesting that it may be due to the same form of spatial learning that produced latent learning in the present experiment.

In the separate arm CCP case it is possible that the acquisition of a spatial map during unreinforced pre-exposure to the maze environment included information about
two unambiguous locations that did not contain food. When the rats were confined in the separated arms on the training trials an amygdala-based conditioned approach response to the unambiguous food-paired cues was acquired. Because the cues visible from each separated maze arm represented only a part of the spatial environment that had been learned during pre-exposure, it can by hypothesized that the hippocampus-based spatial information was not recalled and reorganized. On the test day expression of information acquired by this system during pre-exposure could have interfered with expression of the CCP by the amygdala system. Therefore, separated arm CCP learning required more training trials (4) in rats pre-exposed to the maze environment than were required (2) by rats not pre-exposed to the environment, by rats pre-exposed to a different environment, or by rats with fimbria-fornix lesions pre-exposed to the same environment. The rats in each group that expressed a CCP after 2 training trials were prevented in a different way from acquiring spatial information about the maze environment during the pre-exposure session.

Note that this form of interference with separated arm CCP learning is not latent inhibition in its original sense, which refers to a reduction in the associability of conditioned stimuli (Hall, 1980; Honey & Hall, 1989). Rather it is a case of latent learning by one system, the expression of which interferes with the expression of a different behavior learned by another system.

McDonald & White (1995b) reported that although normal rats failed to learn the adjacent arms CCP in 8 training trials, rats with fimbria-fornix lesions given 8 training trials did exhibit a CCP. This finding can be understood as the result of two processes just discussed. First, the CCP could have been due to the amygdala-based acquisition of conditioned approach responses to cues visible from the food arm, which may have become stronger than the conditioned approach response to the undifferentiated cues after 8 training trials. At the same time, spatial information about the empty maze acquired during pre-exposure would have interfered with the expression of the discriminatory conditioned approach response. Since rats with fimbria-fornix lesions were unable to acquire this spatial information this form of interference was absent in these rats, permitting expression of the amygdala-based, unique cue CCP.
Although this is another learning situation in which pre-exposure appears to retard learning of a conditioned response, it is not a traditional case of latent inhibition. Instead, it is another instance where latent learning by one neural system interferes with expression of a conditioned response by another system.

**Summary**

Normal rats do not exhibit adjacent arm CCPs after up to 8 training trials; however, rats with amygdala lesions do exhibit this CPP. The amygdala lesions eliminate a form of inference thought to be due to undifferentiated conditioned approach responses acquired during the training trials when ambiguous environmental cues are paired with food. The elimination of these responses allows unimpeded expression of a CCP thought to be due to fimbria-fornix - and hippocampus - based acquisition of information about the spatial location of the food. This information is thought to be acquired in two phases. First a spatial map of the environment is acquired during the pre-exposure session when the rats explore the maze in the absence of reinforcers. Second, this spatial map is recalled during the training trials when the rats are re-exposed to the same spatial cues while given food on one of the arms. The map is reorganized to include information about the location of the food and re-stored (reconsolidated). A similar process may occur when the rats learn that there is no food on the other arm. On the test day this learned information influences the hungry rats’ search for food resulting in a CCP for the food-paired arm.

This analysis emphasizes latent learning of spatial information by a neural system that includes the fimbria-fornix and hippocampus during unreinforced pre-exposure to the maze. This form of learning is proposed as the basis of ambiguous cue CCP learning in both the maze and box apparatus. It is also suggested that this form of learning is the cause of the retardation of unambiguous cue learning in the two CCP paradigms. Chapter 5 describes experiments that investigate this form of learning and the role of the fimbria-fornix and hippocampus in it.
CHAPTER FIVE
ADJACENT ARM CONDITIONED CUE PREFERENCE IN THE RADIAL
MAZE: Roles of Fimbria-Fornix and Hippocampus

The first experiment in this chapter examines the contribution of pre-exposure to the maze environment on subsequent adjacent arm CCP learning on the radial maze, an apparent case of latent learning. The second experiment examines the functions of FF and hippocampus in adjacent arm CCP learning, including the roles of these structures in the effects of pre-exposure on this task.

It was shown in Chapter 4 that normal rats failed to learn the adjacent arm CCP with a single pre-exposure session and up to 8 training trials. However, rats with LNA lesions learned this task after a single pre-exposure with 4 training trials. Rats with combined LNA and FF lesions did not learn the task, showing that adjacent arm CCP learning (at least in rats with LNA lesions) requires an intact FF.

The adjacent arm CCP is thought to require rats to form a spatial map of the maze environment, which permits them to identify the locations of the two arms with respect to cues in that environment. This idea is consistent with many previous demonstrations that rats with FF lesions fail to exhibit behaviors thought to depend on the acquisition of spatial maps (Barnes, 1988; Eichenbaum, Stewart, & Morris, 1990; O'Keefe, Nadel, Keightley, & Kill, 1975; Packard, Hirsh, & White, 1989; Olton, Walker, & Gage, 1977).

Previous work (McDonald & White, 1995; White & Ouellet, 1997) showed that rats must move around within an environment to acquire such spatial information. Since the rats are confined at the ends of the arms during CCP training, they cannot acquire a spatial map of the environment during these trials. As suggested in Chapter 4, the rats may acquire this information while moving around on the maze during the pre-exposure session. Spatial information acquired at that time may be modified to include information about the location of food within the environment during the CCP training trials, leading to the CCP when the rats use this information to search for food during the test trial.

The facilitatory effect of LNA lesions on the performance of this task was attributed in Chapter 4 to the acquisition by normal rats of conditioned approach responses to the ambiguous cues visible from both arms. During the test trial, these conditioned approach responses would lead to equal tendencies to enter the food-paired
and no-food arms, interfering with expression of the CCP based on information about the spatial location of the food. LNA lesions would eliminate this kind of learning and the interference it produces, allowing the CCP to be expressed.

The question addressed in this Chapter is the neural basis of this CCP. As shown in Chapter 4, an intact FF is required for the learning that produces the adjacent arm CCP. It is unclear, however, if FF is required for acquisition of the spatial map during pre-exposure or for its reorganization during CCP training to include information about the location of the food, or both. Furthermore, the function of the hippocampus, a structure closely related to FF and spatial learning (Barnes, 1988), such as win-shift (Olton & Werz, 1978; Packard, Hirsh, & White, 1989; Rawlins, Maxwell, & Sinden, 1988) and Morris water maze task (Morris, Garrud, Rawlins, & O'Keefe, 1982; McDonald & White, 1994) in adjacent arm CCP learning has not been investigated at all.

In a previous series of experiments on the learning that takes place during pre-exposure the retardation (or latent inhibition) of separated arm CCP learning was eliminated by FF lesions made before but not after pre-exposure (McDonald & White, 1995). This finding suggested that FF is involved in the acquisition of information during pre-exposure, but not in its effect on subsequent CCP learning.

Lesions of the hippocampus made before pre-exposure had no effect on the latent inhibition of subsequent separated arm CCP learning (White and Wallet, 2000), suggesting that this structure is not involved in acquiring information during pre-exposure. This surprising conclusion is consistent with a previous report (Kimble & BreMiller, 1981) that, although lesions of the dorsal hippocampus impaired learning to find water in a Hebb-Williams maze, they had no effect on the improvement in performance produced by unreinforced pre-exposure to the maze (ie, on latent learning). This evidence suggests the possibility that the hippocampus may not be involved in pure spatial learning, when no reinforcers are involved. Rather it may be important for learning about reinforcers, either about their location or about how to find them.

**Experiment Five**

This Experiment examined the effects of unreinforced pre-exposure to the maze environment on subsequent adjacent arm CCP learning in normal rats. Although one pre-
exposure session did not produce adjacent arm CCP learning in Experiment 4, it was hypothesized that additional pre-exposure sessions might allow the effect to appear, for two possible reasons.

First, the observation of an adjacent arms CCP in rats with amygdala lesions in Experiment 4 suggested that an amygdala-based conditioning process interferes with expression of the CCP in normal rats. The CS in this conditioning process was hypothesized to be the environmental cues that are visible from both adjacent arms. Additional pre-exposure sessions would provide more unreinforced exposure to the ambiguous cue CS, possibly resulting in latent inhibition of the interfering, undifferentiated conditioned responses. As in the case of amygdala lesions, this could result in a larger CCP.

Second, additional unreinforced pre-exposure may produce a better spatial map of the maze environment, permitting the rats to form a more accurate representation of the location of food in that environment. This may result in a larger CCP on the test day. However, two points about this possibility should be noted. First, the results of experiment 4 suggest that a single pre-exposure is sufficient to produce an adjacent arm CCP based on an FF-dependent form of learning, probably a spatial map. The results of that experiment suggest that the CCP fails to appear in normal rats because of interference from amygdala-based learning. Second, it is also possible that if the number of pre-exposure trials exceeds a certain value, latent inhibition of spatial learning could occur (Prados, Chamizo, & Mackintosh, 1999). Accordingly, this experiment examined the effect of different numbers of pre-exposure trials on adjacent arm CCP learning.

Methods and Procedure

Six groups of 8 rats each were assigned to receive 0, 1, 2, 3, 4, or 6 pre-exposure sessions in the same maze used for subsequent adjacent arm CCP training and testing. A different pre-exposure (DPE) group (N=8) received three pre-exposure sessions in a similar maze in a different room from that used for CCP training and testing. All rats received 4 training trials.
Results and Discussion

Figure 12 shows the mean times spent in the paired and unpaired arms for the seven groups of rats. The statistical analysis revealed that the mean time spent in the paired and unpaired arms did not differ significantly for the rats in 0 \( F(1,49)=0.20 \), 1 \( F(1,49)=0.75 \), 2 \( F(1,49)=0.53 \) PE groups, but were significantly different for the rats in the 3 \( F(1,49)=15.13; p<0.01 \), 4 \( F(1,49)=7.29; p<0.01 \) and 6 \( F(1,49)=12.55; p<0.01 \) PE groups. The paired-unpaired difference was not significant for the 3 DPE group \( F(1,49)=0.27 \).

These findings show that giving normal rats a sufficient amount of unreinforced pre-exposure to the maze environment permits subsequent learning about the location of the food in relation to a set of ambiguous cues, and expression of this learned information as an adjacent arm CCP during the test trial. Rats given the same amount of pre-exposure to a different environment failed to show a CCP. This observation rules out possible influences of motivational factors such as fear or hunger on this form of learning. It also suggests that information about the intra-maze arm configuration that may have been acquired during pre-exposure was not sufficient for the rats to learn the adjacent arm CCP. Rather, this effect was specific to a particular set of environmental cues that defined the location of the food in the maze environment.

Since rats with LNA lesions learned the adjacent arm CCP with only a single pre-exposure session in Experiment 4, it seems likely that the observation of a CCP in the present experiment was due to latent inhibition of undifferentiated amygdala-based conditioned responses that interfere with expression of the CCP by another memory system. Three sessions of unreinforced pre-exposure to the ambiguous stimulus CS was apparently sufficient to eliminate or attenuate these undifferentiated responses so that they did not interfere with expression of the CCP.

Another possible reason for the observed CCP may have been an improved spatial map of the maze environment due to increased pre-exposure to the cues in a situation that permitted the rats to move around in the environment. Such an improved spatial map may have resulted in better learning about the location of food during the hypothesized second phase of the process by which, it is suggested, rats learn the adjacent arms CCP in these experiments. Better or more accurate information about the location of food might
Figure 12  Effects of pre-exposure on adjacent arm CCP learning

Mean total amounts of time (± SE; seconds) spent in the arms by rats given 0, 1, 2, 3, 4, or 6 sessions of unreinforced pre-exposure trial in the same room as training and testing and a group given 3 sessions of pre-exposure in a different room.
Effects of pre-exposure on adjacent arm CCP learning

![Bar graph showing the effects of pre-exposure on adjacent arm CCP learning.](image)

- **PE**: 0, 1, 2, 3, 4, 6 trials PE,
- **Training**: 3 DPE
- **Test**:
be expected to result in a larger CCP when the rats use this information to search for food during the test trial.

Although 3 pre-exposure sessions was the minimum required to produce an adjacent arms CCP, 6 pre-exposures had the same effect in the present experiment, providing no evidence for latent inhibition of spatial learning. Prados (2000) reported that 4 pre-exposure trials facilitated but 16 trials retarded subsequent spatial learning in a water maze (Prados, 2000). Considerable evidence shows that the number of trials required to observe latent inhibition is task dependent. Some learning tasks require 72 pre-exposure trials to produce latent inhibition (Ayres, Philbin, Cassidy, & Bellino, 1992), while on other tasks it occurs after only one pre-exposure trial (McDonald & White, 1995a). Therefore, it is possible that a larger number of pre-exposure trials might provide evidence for latent inhibition of spatial learning in the adjacent arms CCP learning paradigm.

**Experiment Six**

This experiment examined the effects of FF and hippocampus lesions on adjacent arm CCP learning. Previous experiments suggest that spatial learning is essential for this task, and evidence already reviewed suggest that these structures are important for spatial learning. A previous investigation of the latent inhibitory effect of unreinforced pre-exposure on acquisition of the separated arm CCP task (McDonald & White, 1995a) suggested that FF may be involved in the acquisition of information during pre-exposure, but not in the expression of that information either during the training trials or the test trial. Other previous studies (Kimble & BreMiller, 1981; White & Wallet, 2000) suggested that the hippocampus may not be involved in this form of learning at all. Accordingly, the present experiment examined the effects of FF and hippocampus lesions made before or after 3 pre-exposure sessions on subsequent adjacent arm CCP learning.

**Methods and Procedure**

Sixty-four rats were randomly assigned to groups which sustained lesions made before pre-exposure (Pre-PE) or after pre-exposure (Post-PE). Pre-PE lesion groups included a hippocampus sham lesion group (N=8), a neurotoxic hippocampus group
(N=12), and an electrolytic FF lesion (N=12) group. Post-PE lesion groups included a hippocampus sham lesion (N=10) group, a neurotoxic hippocampus lesion group (N=10), and an electrolytic FF lesion (N=12) group. Lesions in the post-PE groups were made on the day after the last day of pre-exposure followed by seven days to recover from the surgery. The sham control animals were subjected to the same surgical procedure and recovery period as the hippocampus lesion rats. All rats in this experiment received 3 pre-exposure sessions, 4 training trials and a test.

Surgery

**Hippocampus lesions.** Hippocampus lesions were made by injection of the neurotoxin NMDA in a concentration of 5 mg/ml of phosphate buffer (pH=7.4). The solution was injected using a Hamilton (Reno, NV) mini-pump and Silastic tubing connected to 30 ga cannulas aimed stereotaxically at each of the 10 injection sites on each side of the brain (see Table 1). Injections 6, 7, 9 and 10 were made by using the same holes in the skull; in each case, the more ventral injection was made first. For injections 1 to 8, 0.2 ul of NMDA solution was injected over 25 seconds. For injection 9 and 10, 0.3 ul was injected over 40 seconds. The cannula was left in place for 3 minutes after each injection was completed.

**Sham lesions.** All hippocampus-sham-lesioned rats were treated in the same way as the hippocampal lesioned animals with the injection of phosphate buffer. Sham animals in all other groups were anesthetized and placed into the stereotaxic apparatus. Their skulls were exposed, but no holes were drilled, and no electrodes or cannulas were lowered into the brain.

**Histological analysis**

Coronal brain sections stained with thionin from a representative hippocampus lesion animal are shown in Figure 13. The reconstruction of the largest (grey) and smallest (dark) lesions from the subjects in both hippocampus lesion groups is shown in Figure 14. All subjects included in the statistical analysis sustained bilateral lesions that produced complete loss of cells in all subfields of the dorsal hippocampus. Animals were discarded from analysis if they sustained only unilateral dorsal hippocampus lesions.
### Table 1. Coordinates for Hippocampus Lesions

<table>
<thead>
<tr>
<th>Injection site</th>
<th>Anterior/Posterior</th>
<th>Medial/Lateral</th>
<th>Ventra/Dorsal</th>
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<tbody>
<tr>
<td>1</td>
<td>-3.1 ± 1.0</td>
<td>± 1.0</td>
<td>-3.6</td>
</tr>
<tr>
<td>2</td>
<td>-3.1 ± 2.0</td>
<td>± 2.0</td>
<td>-3.6</td>
</tr>
<tr>
<td>3</td>
<td>-4.1 ± 3.0</td>
<td>± 3.0</td>
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<td>-4.1 ± 3.5</td>
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<td>5</td>
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<td>6</td>
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<td>10</td>
<td>-5.8 ± 5.1</td>
<td>± 5.1</td>
<td>-7.5</td>
</tr>
</tbody>
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*All injection sites are given with reference to bregma according to the Paxinos and Watson (1998) rat brain atlas. The injected volume was 0.2 μl at all sites except sites 9 and 10, at which the volume were 0.3 μl.*
Figure 13  Representative NMDA hippocampus lesion

Coronal sections of a representative hippocampus lesion. The distance from bregma (in mm) is shown at the bottom of each section.
Hippocampus Lesion

-2.30
-3.30
-3.60
-4.30
-5.20
-6.04
Figure 14  Summary of Hippocampus lesions

Drawings of NMDA neurotoxic lesions of the hippocampus sustained by rats in all experiments. The lighter areas indicate the maximum extent of all lesions and the darker areas indicate the minimum sustained by any rat. The numbers beside each section are the distance from Bregma (in mm).
Some of the rats also sustained ventral hippocampus damage, but no behavioural
effect of this damage could be detected. There was fairly extensive damage to the cortex
dorsal to the hippocampus in some rats, and medial dorsal thalamic nuclei damage were
sustained in 30% of hippocampus lesioned rats. The behavior of rats with these cortical
and thalamic lesions was indistinguishable from that of the rats that did not sustain these
forms of extra-hippocampal damage.

Results and Discussion

Pre-PE lesions. After histological analysis, 9 hippocampus, 9 fimbria-fornix and
8 sham lesioned rats were retained for analysis. As shown in Figure 15, only the pre-PE
sham lesioned animals exhibited a CCP. The statistical analysis revealed that the mean
times spent in the paired and unpaired arms did not differ significantly for the rats in FF
[F(1,24)=2.11] or hippocampus [F(1,24)=0.00] lesion groups, but were significantly
different for the rats in the sham [F(1,24)=6.59; p<0.02] lesion group. These finding show
that the adjacent arm CCP with 3 pre-exposure sessions is blocked by both pre-training
FF and hippocampus lesions.

Post-PE lesions. After histological analysis, 9 fimbria-fornix, 8 hippocampus and
10 sham lesion rats were retained for analysis. Statistical analysis showed that there were
significant differences between the times spent in the paired and unpaired arms by the
control [F(1,24)=9.54; p<0.01] and the FF lesion [F(1,31)=6.80; p<0.02] groups, but not
by the hippocampus [F(1,31)=0.78] lesion group. The adjacent arm CCP with 3 pre-
exposures is unaffected by post-pre-exposure FF lesions, but is impaired by post-pre-
exposure hippocampus lesions.

Fimbria-Fornix Lesions. Lesions of FF made before rats were given 3
unreinforced pre-exposure sessions impaired adjacent arm CCP learning; however,
similar FF lesions made after the pre-exposure sessions had no effect. Two
interpretations of this finding are possible. First, one effect of pre-exposure is thought to
be latent inhibition of undifferentiated conditioned approach responses that interfere with
the expression of CCP learning, and this form of learning could have been eliminated by
the FF lesions. Although this possibility cannot be ruled out categorically, there is no
evidence that amygdala-based learning is affected by FF lesions, so it would appear to be
unlikely. Second, the finding may suggest that FF is part of a neural system that acquires information about the maze environment during unreinforced pre-exposure. This is consistent with other evidence that FF is involved in acquisition but not in retention of spatial information (Sutherland & Rodriguez, 1989; Barnes, 1988). As has been suggested, this information is presumably stored when it is acquired and then retrieved during the training trials and reorganized to include information about the location of food. The fact that FF lesions made after pre-exposure did not affect acquisition or expression of the CCP suggest that FF is not involved in either the reorganization or expression of the spatial information.

This pattern of FF lesion effects is similar to that observed on the effect of unreinforced pre-exposure on separated arm CCP learning: FF lesions made before pre-exposure eliminated the retarding effect of pre-exposure, but FF lesions made after pre-exposure did not alter the retarding effect of pre-exposure (McDonald & White, 1995a). This suggests the possibility that information acquired during unreinforced pre-exposure results in latent learning of the adjacent arm CCP, but latent inhibition of the separated arm CCP. Although acquisition of the information requires an intact FF, this structure is not involved in either of its latent effects.

**Hippocampus Lesions.** Dorsal hippocampus lesions made before or after pre-exposure impaired the adjacent arm CCP. This finding is consistent with previous reports of the effects of these lesions on tasks such as win-shift learning in the radial maze (Olton, Walker, & Gage, 1977) and relational learning (Wood, Dudchenko, Robitsek, & Eichenbaum, 2000; Cohen & Eichenbaum, 1993), contextual conditioning (Phillips & LeDoux, 1992; Maren, Aharonov, & Fanselow, 1997), and place learning in the Morris water maze (Morris, Garrud, Rawlins, & O'Keefe, 1982; Eichenbaum, Stewart, & Morris, 1990). At least two possible hypotheses can explain the present findings.

First, an intact hippocampus could be required for acquisition of information during unreinforced pre-exposure to the maze environment. Although this possibility cannot be ruled out on the basis of evidence in the present experiment, White and Wallet (2000) found that dorsal hippocampus lesions similar to those in the present experiment made before pre-exposure had no effect on the retarding action of the pre-exposure on separated arm CCP learning. In that experiment, the task affected by the pre-exposure
Figure 15  Effects of fimbria-fornix and hippocampus lesions on adjacent arm CCP Learning

(Top) Pre-PE lesions. Mean total amounts of time (± SE; seconds) spent on the arms by sham control rats, rats with hippocampus or fimbria-fornix lesions made before pre-exposure.

(Bottom) Post training lesions. Mean total amounts of time (± SE; seconds) spent in the arms by sham control rats, rats with hippocampus or fimbria-fornix lesions made after pre-exposure.
depended on an amygdala-based conditioned approach response and so was not affected by the hippocampus lesions, leaving only the learning that occurred during pre-exposure as a possible process that could have been affected by the lesions. The fact that there was no such effect led to the conclusion that hippocampus lesions did not affect this form of unreinforced learning.

Even though they are both affected in the same way by FF lesions, it is not possible to conclude unequivocally that the learning occurring during pre-exposure is identical in situations where it produces latent inhibition and latent learning. Nor is it possible to conclude with certainty that hippocampus lesions had no effect on this form of learning in the present experiment. However, the findings do suggest this as a possible hypothesis.

On this hypothesis rats with hippocampus lesions in the present experiment acquired information about the environment normally during pre-exposure, so the lesions must have affected one or both of the subsequent phases of the CCP learning process: reorganization of the spatial map during training to include information about the effects of reinforcers, or expression of the learned information about the location of food during testing. This is the second hypothesis about the effects of hippocampus lesions in the present experiment.

In the latent learning study by Kimble and BreMiller (1981) hippocampus lesions that had no effect on latent learning impaired learning to find the reinforcer. In the present study, it is also possible that the hippocampus lesions had no effect on unreinforced spatial learning, but impaired learning about the location of the reinforcer during the training trials. Thus, the second hypothesis about the effects of these lesions is that they impair learning that involves the effects of reinforcers.

Other previous findings with latent inhibition suggest that the hippocampus may be involved in reinforced relational learning (Honey & Good, 1993; Coutureau, Galani, Gosselin, Majchrzak, & Di Scala, 1999). As already discussed in the Introduction section, Davidson and Jarrard (1993) found that hippocampus-lesioned rats were impaired on an interoceptive discrimination based on food deprivation level (Davidson & Jaffard, 1993). Hsiao and Isaacson (1971) trained rats to run to one arm for food and the other arm for water in a T-maze. Normal control animals chose the food arm when food deprived, and
the water arm when water deprived. Hippocampus animals were impaired on this task (Hsiao & Isaacson, 1971; Hirsh, Leber, & Gillman, 1978). Similar results have been reported in human studies; H.M. encountered difficulty in identifying his state of hunger/satiation (Hebben, Corkin, Eichenbaum, & Shedlack, 1985).

Other evidence for the idea that the hippocampus processes information that involves reinforcers comes from a report on the effect of food on the place fields of hippocampal neurons. These recordings require that the subjects move around the environment so that the effect of all locations on the neurons can be sampled. A standard technique for encouraging the rats to move around is to food deprive them and scatter food on the floor of the test cage. Recently, Kentros et al. (2001) reported that mice formed normal place fields that were stable from day-to-day using this technique. When no food was available in the test cage (a situation similar to unreinforced pre-exposure in the present experiment) place fields were formed but were unstable over days.

Finally, the hippocampus may have been involved in the retrieval of spatial information during the test session. Inactivation of the dorsal and ventral hippocampus after training disrupted performance in the watermaze (Moser & Moser, 1998). Similar findings that lesions of the hippocampus blocked the expression of spatial learning were reported in the radial maze (Jarrard, 1978).
CHAPTER SIX
GENERAL DISCUSSION

The main findings of the experiments described in this thesis, and their interpretations, are summarized below. The two most general findings are:

1. Lesions to parts of the amygdala and hippocampus memory systems have different effects on CCPs learned in different apparatuses. This suggests that although apparently similar CCPs are acquired, the configuration of the apparatus determines what kinds of learning produce this behavior. This is attributed to the fact that apparatus configuration determines which cues are available to the rat during different phases of the learning paradigm. The available cues and their relationship determine what is learned and which neural system learns it.

2. Unreinforced pre-exposure to the to-be-conditioned cues has different effects on different kinds of CCP learning, and these effects are affected differently by lesions to the amygdala and hippocampus memory systems. Strictly, CCP learning consists of pairing cues with a reinforcer. Unreinforced pre-exposure to the apparatus, often used as a standard laboratory technique to "habituate" rats and make them easier to handle, can result in more than one form of learning each of which interacts in its own way with the different forms of CCP learning.

More specific findings leading to these general conclusions are:

1. Lesions of the LNA impaired distinct cue (opaque partition) CCP learning in the box apparatus (Chapter 3) and have previously been shown to impair distinct cue (separate arms) CCP learning on the radial maze. Distinct cue CCP learning was not affected by FF lesions (Chapter 3). These findings are interpreted to mean that the distinct cue CCP is due to an amygdala-based conditioned approach response to the cues paired with the reinforcer. The FF is not involved in this type of learning (as distinct from the effects of pre-exposure on this type of learning, see below).

2. Lesions of the LNA did not impair ambiguous cue CCP learning when rats were trained with either the clear partition in the box apparatus (Chapter 3) or with adjacent arms on the maze (Chapter 4). This means that the amygdala system is not involved in ambiguous cue CCP learning.
3. Lesions of the LNA enhanced ambiguous cue CCP learning with the clear partition in the box apparatus (Chapter 3) and with adjacent arms on the maze (Chapter 4). This effect is attributed to the elimination of interference with expression of the CCP by undifferentiated conditioned responding to conditioned cues visible from both the paired and unpaired locations in the two apparatuses.

4. In the box apparatus unreinforced pre-exposure to the to-be-conditioned cues impaired ambiguous cue CCP learning by normal rats (Chapter 3). Rats with FF lesions that were not pre-exposed acquired a normal ambiguous cue CCP. Unreinforced pre-exposure in the box apparatus may latently inhibit acquisition of the ambiguous cue CCP. The neural basis of ambiguous cue CCP learning in the box apparatus remains unknown.

5. In the maze apparatus unreinforced pre-exposure to the to-be-conditioned cues facilitated ambiguous cue CCP learning in rats with LNA lesions. This facilitation was eliminated by FF lesions (Chapter 4). The facilitation of ambiguous cue CCP learning is attributed to latent learning: FF-mediated pure spatial learning during unreinforced pre-exposure leads to the acquisition of a spatial map of the environment. Information about the location of the food in that map is added during training. In this experiment the effect was only detectable when amygdala-mediated interference with expression of the ambiguous cue CCP has been eliminated with LNA lesions.

6. Additional unreinforced pre-exposure facilitated ambiguous cue CCP learning in normal rats, an instance of latent learning. Lesions of FF impaired the acquisition, but not the expression of unreinforced learning during pre-exposure (Chapter 5). This finding is similar to the previously shown effect of FF lesions on the retardation of distinct cue CCP learning.

7. Lesions of the hippocampus impaired ambiguous cue CCP learning (Chapter 5). It was not possible to determine if this effect was due to an impairment of unreinforced learning during pre-exposure or to impairment of learning about the location of the reinforcer during the training trials. Previous findings that hippocampus lesions had no effect on pure spatial learning, as measured by its retarding effect on distinct cue CCP learning, suggest the former possibility.
Two kinds of CCP learning

The present findings suggest a distinction should be made between two levels of analysis in discussing the CCP. On one level, a CCP consists of a learned preference for one area of an experimental apparatus over another area of the same apparatus. The other level examines the nature of the information that is processed to produce the CCP and the neural substrates that do this processing. The available evidence suggests that although the behavior defined as a CCP is the same, the information processed and the neural systems that process it are highly dependent on the cues available in the experimental apparatus and, in some cases, in its surrounding environment. If the cues visible from the food-paired and unpaired environments are distinct the CCP is blocked by lesions of the LNA (White & McDonald, 1993), but not by FF lesions or dorsal hippocampus lesions when there is no pre-exposure (White & Wallet, 2000). (Ferbinteanu and McDonald (2000) reported that hippocampus lesions impair the separated arm CCP in rats that have been pre-exposed to the maze environment. This issue is discussed further below.) In contrast, if the cues visible from the paired and unpaired locations are similar the CCP on the radial maze is impaired by FF lesions (made before pre-exposure) and hippocampus lesions, but not by lesions of the LNA. (The ambiguous cue CCP learned in the clear partition box paradigm was not impaired by LNA or FF lesions.) Information from the present experiments and from much previous work by many investigators suggests that different kinds of information are processed by these neural substrates. Therefore, the CCPs in these two situations result from different forms of learning.

Each of these forms of CCP learning is affected in a different way by information that is processed in both the amygdala and hippocampus neural systems during unreinforced pre-exposure to the apparatus. In the following sections, factors that affect these forms of learning are discussed and an attempt is made to specify exactly what kind of information is processed by these neural systems in each phase of each CCP paradigm.

Apparatus

Distinct Stimulus Conditions. The distinct stimulus conditions that result in an amygdala-based CCP were provided in the present thesis by the opaque partition condition in the box apparatus and by the separated arm paradigm on the radial maze,
because in both of these paradigms the rats could see only one set of cues on the food-paired trials, and a different set on the unpaired trials. On the radial maze task the rats could see both the paired and unpaired cues simultaneously during the pre-exposure and test sessions, and this condition was duplicated in the box apparatus by using the clear partition during these two phases of the procedure (COC). LNA lesions block CCP learning in both cases (Chapter 3 and White & McDonald, 1995). FF lesions do not block either form of CCP (Chapter 3 and McDonald & White, 1993).

The same pattern of lesion effects was found when the opaque partition was used during all three phases of the procedure in the box apparatus (OOO), suggesting that the critical feature of this amygdala-based task is the stimulus configuration during the training trials. Given that amygdala lesions do not impair CCP learning with adjacent arms or a clear partition during the training trials it appears that amygdala-based learning that produces a CCP only occurs when the stimuli paired with the reinforcer are distinguishable from other stimuli in the environment. This in turn suggests that amygdala system processes information about individual stimuli that are either salient features of the environment or "snapshots" that may include the entire scene visible from particular locations (Amsel, 1993). It appears that when conditioned, these distinctive cues can elicit approach responses even if they are presented together with other non-conditioned stimuli.

**Ambiguous Stimulus Conditions.** The CCP acquired in the adjacent arm paradigm on the radial maze was impaired by FF lesions made before pre-exposure, and by hippocampus lesions made either before or after pre-exposure (Chapter 5). In this paradigm most of the same cues can be seen from both the food-paired and unpaired arms, making them ambiguous with respect to the correct arm. These findings are consistent with the idea that the hippocampus system processes information about the relationships among the cues, and that this information can be used to identify specific locations with respect to more than one of the cues. LNA lesions did not impair this form of CCP learning.

In contrast, a CCP paradigm that was similar in this respect, the clear partition CCP in the box apparatus, was not affected by FF lesions. The area in which the rats are confined on the maze arms is much smaller than the area in which they are confined in
the box apparatus. This is true regardless of whether comparison is based on absolute or on the percentage of the total area of the visible environment in the two apparatuses. This difference may provide a partial explanation of the differences between the effects of the lesions in the two apparatuses. This explanation requires a consideration of the role of movement in CCP learning.

**Role of movement**

McDonald and White (1995b) showed that different information about environmental cues was acquired when rats were given food while confined on the end of a radial maze arm (ie, trained with the standard CCP procedure) compared to when they were trained by allowing them to move around on the maze searching for food. Although rats learned to discriminate between separated maze arms with either procedure, they learned an adjacent arm discrimination only with the active procedure. The passive separated arm discrimination was impaired by LNA lesions, but not by FF lesions. The active adjacent arm discrimination was impaired by FF lesions, but not by LNA lesions. These findings were interpreted to mean that the active, adjacent arm discrimination was based on the acquisition of a spatial map of the cues in the maze environment, and that movement in the environment was required for this kind of information to be acquired. Moving among locations in the environment may allow information about the cues acquired at different locations to be held simultaneously in working memory and used to compute the spatial map.

Experiments in the present thesis show that normal rats can learn the adjacent arm CCP on the radial maze only if they are allowed to explore the maze without reinforcement before the training trials, during which their movement is restricted to the ends of the maze arms. However, normal rats do not require pre-exposure to learn the clear partition CCP in the box apparatus. This difference may be due to the aforementioned differences in the area in which the rats are confined on the two apparatuses. It has been hypothesized that rats cannot learn the adjacent arm CCP during the training trials because they cannot move around sufficiently to acquire the type of information required to form a spatial map of the maze environment. However, the
comparatively larger area in which the rats are confined during training in the box apparatus may permit sufficient movement for the required information to be acquired.

What is Learned?

Pre-Exposure. On the radial maze, the pre-exposure conditions are identical in the separated and adjacent arm paradigms, in that the rats can move around and see the environmental cues visible from both arms at the same time. As they move around the apparatus freely during unreinforced pre-exposure the rats are able to obtain views of the apparatus from different locations. As suggested in the section on the role of movement, it is thought that the animals acquire a spatial map of the locations that make up the environment, including both the experimental apparatus and the cues in the room in which it is located, if these are visible.

Evidence from previous work and from the present thesis suggests that acquisition of such spatial information requires an intact FF, but not an intact hippocampus or amygdala.

In the box apparatus, the only cues visible are those inside the box, an area that is considerably smaller than the room in which the maze is located. As they move around in the clear partition pre-exposure condition the rats may be able to acquire a spatial map of the visual cues in the apparatus, all of which could be seen from both boxes. Although the visual cues in each box were different, they were homogeneous within each box, so they may not have provided much information about the relations among locations within each box. Moreover, the floors of the two boxes were different, and these cues could only be detected when the rat was actually in either box, so there was no possibility of relational learning concerning those cues while the rats moved around in the box apparatus.

Unreinforced pre-exposure to the apparatus and its environment may also produce latent inhibition of the amygdala-based classical conditioning process thought to occur during training (described in the next section).

Training. Two forms of learning are thought to occur during training. The first is impaired by amygdala lesions, originally labeled "stimulus-reward" by Jones and Mishkin (1972), and also observed by Weiskrantz (1956). In distinct cue CCP paradigms
this form of learning is sufficient to produce a CCP. Neutral cues visible from a location that contains a reinforcer are paired with the effects of that reinforcer. During the test trial, this association allows the now-conditioned stimulus to elicit conditioned responses similar to those responses primarily elicited by the reinforcer itself. As already described, these conditioned responses can result in the observed CCP. Pre-exposure to the environment can retard or eliminate the classical conditioning process that produces this CCP.

A similar form of learning occurs in ambiguous cue CCP paradigms but it has different consequences. During the training trial the conditioned cues can be seen from both the food-paired and unpaired locations. This means that elicited approach responses are almost equally likely to result in entries into both the food and no-food locations. The classical conditioning process that produces these undifferentiated conditioned approach responses is subject to latent inhibition by unreinforced pre-exposure to the maze environment.

As described in the discussion of the role of movement, spatial information cannot be acquired during the training trials because the rats are prevented from moving around in the environment. However, it is proposed that when rats are exposed to an environment in which a spatial map has already been acquired and stored, this information is recalled, reorganized with additional information about the location of food in the environment, and "reconsolidated (Nader, Schafe, & LeDoux, 2000; Nadel & Land, 2000). This hypothesis explains how unreinforced pre-exposure to the maze environment results in adjacent arm CCP learning on the radial maze when the rats are prevented from moving during the training trials. It provides an explanation for the classic phenomenon of latent learning (Blodgett, 1932).

Note that pre-exposure to the box environment was not required for ambiguous cue learning in that apparatus. As already discussed, this is thought to be due to the relatively large size of the boxes in which the rats are confined during training. These may permit sufficient movement to allow the rats to acquire simultaneously a spatial map of the apparatus and information about the location of food in that map. It is likely that these two kinds of information are normally acquired simultaneously, for example when a rat learns to find food in a maze by running through it repeatedly.
**Testing.** It is assumed that during testing learned information acquired by both the hippocampus and amygdala systems is expressed simultaneously. The resulting behavior is the result of a competition between the outputs of the systems to control behavior.

In the distinct cue learning situations the CCP is thought to be due an amygdala-based, classically conditioned approach response causing the rats to spend more time in contact with the cues that were paired with food. Two processes can interfere with this form of the CCP, both resulting from unreinforced pre-exposure. First, latent inhibition of the conditioning process could reduce or eliminate the CCP. This process has not actually been demonstrated with either the separated arm radial maze CCP or with the opaque partition box apparatus CCP.

Unreinforced pre-exposure has been shown to retard the separate arm CCP on the radial maze, and this retardation is eliminated by FF lesions. This suggests that although this case of retardation resembles latent inhibition, it may actually be due to the acquisition of spatial information by a different neural system during pre-exposure. This information would be acquired in the presence of all the environmental cues, but the fact that the rats are exposed to a subset of these cues during each training trial may prevent the proposed process of recall and reorganization of the map to include information about the location of the food. During the test trial, however, re-exposure to the same set of environmental cues that constituted the original spatial map would result in its recall. The recall of information that there is no food on the maze may interfere with expression of the amygdala-based classically conditioned CCP. Elimination of the initial acquisition of this spatial information by FF lesions made before pre-exposure would eliminate this form of interference and facilitate the appearance of a CCP.

In ambiguous cue learning situations the CCP is thought to be due to the recall of a spatial map of the apparatus and its environment, including information about the location of food, guiding the search of a hungry rat for food. Since the rats are prevented from moving during the training trials, this behavior depends on a minimum amount of unreinforced pre-exposure and on subsequent exposure to a location in the same environment that contains food. This explanation may apply to the adjacent arm paradigm on the radial maze, but it is unclear if it applies to the clear partition paradigm.
in the box apparatus because pre-exposure was not required for and FF lesions did not impair the latter form of learning.

Amygdala-based learning may also affect behavior during ambiguous cue CCP test trials on the radial maze. It has been suggested that since most of the cues visible from the food-paired arm are also visible from the unpaired arm the conditioned approach responses may result in entry into either arm, interfering with expression of a CCP by the hippocampus system. Amygdala lesions appear to eliminate this form of interference.

Unreinforced pre-exposure to the apparatus may result in latent inhibition of these undifferentiated conditioned responses. Three pre-exposure sessions were required to produce an adjacent arm CCP in normal rats, but only a single session was required in rats with LNA lesions. This suggests that the rats acquired a spatial map in only one session. The CCP resulting from the reorganization of this map on the test trials was apparent when interference from undifferentiated conditioned approach responses was absent in rats with amygdala lesions. This further suggests that the additional two pre-exposure sessions required to observe a CCP in normal rats was required to latently inhibit the acquisition of the undifferentiated conditioned approach responses rather than to improve the spatial map.

Undifferentiated, amygdala-based conditioned approach responses also interfered with the expression of ambiguous CCP learning in the box apparatus. When the rats were trained and tested with the clear partition, amygdala lesions facilitated the CCP. However, when the rats were trained with the clear partition and tested with the opaque partition, no facilitation by amygdala lesions was observed.

The Influence of Pre-Exposure on the Effects of Hippocampus Lesions

Ferbinteanu and McDonald (2001) used the separate arm radial maze paradigm with a single session of unreinforced pre-exposure. They found that lesions of FF facilitated CCP learning, and that either amygdala or dorsal hippocampus lesions impaired CCP learning. These effects of amygdala and FF lesions are consistent with the present findings and interpretations. However, White & Wallet (2001) reported that hippocampus lesions impaired, separated arm CCP learning when the rats had not been pre-exposed to the maze.
There are two major differences in the experimental paradigms that produced these opposite effects. First, Ferbinteanu & McDonald’s rats were pre-exposed; White & Wallet’s rats were not pre-exposed. Second, Ferbinteanu & McDonald’s hippocampus lesions were confined to the dorsal part of the structure; most of the rats in White & Wallet’s experiment sustained lesions to the entire structure. Ferbinteanu & McDonald also reported that lesions confined to the ventral striatum facilitated CCP learning, but no interaction of this effect with pre-exposure was demonstrated.

No principled explanation for Ferbinteanu & McDonald’s findings can be given here. Such an explanation will require further experiments with rats that have comparable lesions of the entire hippocampus and of its dorsal and ventral parts, and investigation of the interactions of the effects of these lesions with unreinforced pre-exposure to the maze.

**Interactions among memory systems in CCP learning**

The present explanations and hypotheses of CCP learning and the effects of unreinforced pre-exposure on it rely on three major concepts (White & McDonald, 2002): the idea that different kinds of information are processed and stored in independent neural systems; the idea that the outputs of these systems guide or control behavior; and the idea that these outputs interact with each other, in either a co-operative or competitive manner. It is also possible that one memory system could directly influence another, either promoting or retarding learning by the second system.

**Interaction of Outputs**

Anatomically, both the hippocampus and amygdala send projections to nucleus accumbens (Callaway, Hakan, & Henriksen, 1991; DeFrance & Yoshihara, 1975; Groenewegen, Wright, Beijer, & Voorn, 1999; Groenewegen, Mulder, Beijer, Wright, Lopes Silva, & Pennartz, 1999; Kelley & Domesick, 1982; Kelley, Domesick, & Nauta, 1982) and medial prefrontal cortex (Jay, Glowinski, & Thierry, 1989; McDonald, 1991; Swanson, 1981). These two areas may play a role in coordinating information projected from the hippocampus and amygdala and generate the behavioral result of this interaction.

**Nucleus Accumbens.** The nucleus accumbens is often linked to reward, motor integration, behavioral output, and goal directed behavior (Mogenson, Jones, & Yim,
1980; Annett, McGregor, & Robbins, 1989; Carr & White, 1983; Westbrook, Good, & Kiernan, 1997; Parkinson, Robbins, & Everitt, 1999). This brain area receives input from the hippocampus and amygdala and projects to the ventral pallidum, substantia nigra and ventral tegmental areas. Nucleus accumbens is believed to act as an interface between the limbic and motor system, and regulates information from both the amygdala and hippocampus dependent memory systems (Groenewegen, Mulder, Beijer, Wright, Lopes Silva, & Pennartz, 1999).

It appears that projections from amygdala to nucleus accumbens are mediated by a glutamatergic system (Mulder, Hodenpijl, & da Silva, 1998; Howland, Taepavarapruk, & Phillips, 2002), and activation of the amygdala may affect the release of dopamine in the nucleus accumbens (Fudge & Haber, 2000). In addition, projections from the hippocampus to the nucleus accumbens are mainly through the glutamatergic based subiculum-accumbens or FF pathway (Di Chiara, 2000). Activation of the hippocampus may affect the release of glutamate or ACh in the nucleus accumbens. This suggests that there are complicated interactions among different sources of innervations and their neurotransmitters in this area.

The present study showed that the fimbria-fornix, which connects the hippocampus and nucleus accumbens, is critical for acquisition of information during unreinforced pre-exposure. This evidence is consistent with the results of several studies suggesting that projections from the subiculum to the nucleus accumbens plays a role in exploration and the acquisition of spatial information about the environment (Groenewegen, Vermeulen-Van der Zee, & Te Kortschot, 1987; Martin, 2001). In addition, a recent study (Martin, 2001) showed that while animals navigated towards a goal location the synchronous firing of neurons recorded simultaneously in the nucleus accumbens and subiculum increased.

The other hippocampal efferents to the nucleus accumbens are via the entorhinal cortex (Krayniak, Meibach, & Siegel, 1981). Lesions of the entorhinal cortex disrupted both place and cue tasks in the radial maze (Jarrard, Okaichi, Steward, & Goldschmidt, 1984) and also conditioned freezing in fear conditioning tasks when a context serves as the CS (Maren & Fanselow, 1997). These findings suggest that contextual information,
including spatial information originating in hippocampus and cortex and reaching the entorhinal cortex may also influence the function of nucleus accumbens.

There is also evidence that nucleus accumbens is involved in spatial learning (Schacter, Yang, Innis, & Mogenson, 1989). Bilateral inactivation of the nucleus accumbens impaired performance in the win-shift task on the radial maze (Seamans & Phillips, 1994). Furthermore, electrophysiological evidence shows that some cells in nucleus accumbens preferentially respond to either amygdala or FF stimulation, but not to both (Martin, 2001). Lavoie & Mizumori (1994) showed that single unit activity in nucleus accumbens correlates with spatial, reward- and movement-related behavioral conditions. Firing consistent with both place and cue guided movements was recorded in a radial maze task. These findings indicate that nucleus accumbens may play a role in integrating spatial and reward-related information, which in turn affects voluntary motor output structures to achieve accurate spatial foraging behavior.

The findings also suggest the possibility that information from both amygdala and hippocampus converges in nucleus accumbens initiating competitive interactions that can explain certain behavioral phenomena such as some cases of latent inhibition. In general, the nucleus accumbens may play an integrative role in mediating the outputs of the memory systems to produce the observed behavioral interactions between reinforced and unreinforced learning in the CCP and other situations.

Prefrontal Cortex. The other possible anatomical site of amygdala-hippocampus interactions may be the prefrontal cortex. Both the amygdala and hippocampus have direct projections to the prefrontal cortex (Swanson, 1981; Thierry, Gioanni, Degenetais, & Glowinski, 2000; McDonald, 1991) and the output of this structure may affect the function of the nucleus accumbens (Seamans, Floresco, & Phillips, 1998). The prefrontal cortex may not directly influence specific behaviors, but it may affect or modulate ongoing learning (Kolb, Buhrmann, McDonald, & Sutherland, 1994; Williams & Goldman-Rakic, 1995).

The prefrontal cortex is involved in various high level cognitive functions, such as rule learning, the ability to shift between behavioral strategies, behavioral flexibility, and spatial and emotional learning (Fuster, 2001; Miller & Cohen, 2001). Electrophysiological studies show that the prefrontal cortex may be involved in the
integration of motivational information with spatial location (Pratt & Mizumori, 2001). Lesions of the medial prefrontal cortex blocked both the morphine and cocaine CCPs in the box apparatus. Although drug-induced CCPs may differ in many respects from those produced with food, these findings suggest that prefrontal cortex may be involved in CCP learning (Tzschentke & Schmidt, 1999; Tzschentke, 2000). Wise, Murray, & Gerfen (1996) proposed that the prefrontal cortex may mediate a type of object-affect association that results in approaching stimulus A or avoiding stimulus B, the basic measurement of CCP learning.

**A Hypothesis about Neural Mediation of Two Types of CCP Learning**

It is possible that spatial information mediated by the hippocampus medial prefrontal system may be important for the CCP that depends on spatial learning, while information mediated by the amygdala-nucleus accumbens system may be important for the CCP that depends on forming association between discrete individual stimuli and reinforcers.

Both anatomical and behavioral evidence support this suggestion. The major afferents of ventral medial prefrontal cortex are from the hippocampus (Thierry, Gioanni, Degenetais, & Glowinski, 2000). Lesions of the prefrontal cortex impaired radial maze and water maze tasks (Kolb, Buhrmann, McDonald, & Sutherland, 1994) and other spatial tasks involving learning about the location of reinforcers in spatial environments (Gemmell & O'Mara, 1999; Winocur & Moscovitch, 1990; Kolb, Buhrmann, McDonald, & Sutherland, 1994).

Amygdala-nucleus accumbens pathways are critical for stimulus-reward associations (Everitt, Morris, O'Brien, & Robbins, 1991; Everitt, Cador, & Robbins, 1989). In contrast, these structures may not be involved in spatial learning tasks: lesions of the LNA did not impair learning of the win-shift task in the radial maze (McDonald & White, 1993; Becker, Walker, & Olton, 1980) and lesions of the nucleus accumbens did not impair the water maze task (Floresco, Seamans, & Phillips, 1996). These findings are consistent with the idea that the two systems may mediate different types of learning, and that these types of learning may underlie the discrete and ambiguous cue CCPs examined in this thesis.
Direct interactions between amygdala and hippocampus

As reviewed in the General Introduction, there is considerable evidence for behavioral and physiological interactions between the amygdala and hippocampus systems. Activation in the amygdala may potentially influence hippocampal memory function through direct projections via the entorhinal cortex or subiculum (Amaral & Witter, 1995; Pikkarainen, Ronkko, Savander, Insaulste, & Pitkanen, 1999). Thus, Packard et al. (1994) showed that injections of amphetamine into the amygdala modulated hippocampus dependent memory in the water maze task (Packard, Cahill, & McGaugh, 1994).

White and McDonald (1993) showed that FF is critical for the acquisition of information acquired that interferes with amygdala-dependent separated arm CCP learning. Although the acquisition of this information does not require an intact hippocampus (White & Wallet, 2000) its effect on subsequent amygdala-based conditioning may be mediated by the hippocampus. This effect could occur directly on the lateral and basolateral nuclei of amygdala via the subiculum (O'Mara, Commins, Anderson, & Gigg, 2001). It is also possible that activity in the fimbria-fornix could affect neural plasticity in the amygdala via its projections to entorhinal cortex, retarding acquisition of the amygdala dependent conditioned approach during training.

The implications of present results for unreinforced learning

The present experiments show that unreinforced learning produces a latent learning effect on ambiguous cue CCP learning and a latent inhibition effect on the distinct cue CCP learning on the maze apparatus. This suggests that the effects of unreinforced pre-exposure are task dependent: the effect of pre-exposure is determined by interactions between the forms of learning that occur during unreinforced pre-exposure and the form of learning that occurs during subsequent reinforced exposure.

As already suggested, the present method of maze pre-exposure has two effects on CCP learning. First, it may reduce the associability of an individual CS. Reduction of associability would retard subsequent stimulus-reward learning. Second, it allows the formation of a spatial map. The recall of this information during training may facilitate
learning during subsequent reinforced exposure. Recall of the combined information during the test trial may result in an ambiguous cue CCP. In the distinct cue situation, recall of the pure spatial information may interfere with expression of the CCP. Thus the present findings provides the beginnings of a theoretical framework for understanding the interaction between reinforced and unreinforced learning. This includes the phenomena known as latent learning and latent inhibition.
CONCLUSIONS

The present results show that (1) more than one memory system is involved in CCP learning. (2) The stimulus configurations of the environment determine which systems are involved in each situation. (3) CCPs in distinct cue situations are based on conditioned approach responses acquired by a neural system that includes the lateral nucleus of the amygdala. (4) CCPs in ambiguous cue situations are learned by a system that includes fimbria-fornix and hippocampus, as well as some other, unknown structures. (5) Ambiguous cue CCPs include two separable types of information: a spatial map of the environment and information about the location of reinforcers in the environment. Although normally acquired simultaneously, these types of information can also be acquired sequentially. (6) Unreinforced pre-exposure to the environment results in at least two forms of learning: acquisition of spatial information and reduction of the associability of potential conditioned cues. (7) These forms of learning either facilitate or interfere with subsequent CCP learning.
REFERENCES


