THE DEVELOPMENT OF A RAT MODEL OF BRAIN-DAMAGE-PRODUCED AMNESIA

by

DAVID GERALD MUMBY

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Department of PSYCHOLOGY

The University of British Columbia
Vancouver, Canada

Date 23 April, 1992

ABSTRACT

The nonrecurring-items delayed nonmatching-to-sample (DNMS) task is an integral part of contemporary monkey models of brain-damage-produced amnesia. This thesis began the development of a comparable rat model of brain-damage-produced amnesia. First, a DNMS task for rats was designed by adapting key features of the monkey task. Then, the rat DNMS task was studied in three experiments; each assessed the comparability of the rat DNMS task to the monkey DNMS task. Experiment 1 determined the rate at which the rat DNMS task is learned and the asymptotic level at which it is performed, Experiment 2 assessed the memory abilities that it taps, and Experiment 3 investigated the brain structures that are involved in its performance.

In Experiment 1, rats were trained on the DNMS task and their performance was assessed at retention delays of 4, 15, 60, 120, and 600 s. All of the rats learned the DNMS task, and their performance was comparable to that commonly reported for monkeys in terms of both the rate at which they acquired the nonmatching rule at a brief retention delay and their asymptotic accuracy at delays of up to 120 s. These results establish that rats can perform a DNMS task that closely resembles the monkey DNMS task and that they can approximate the level of performance that is achieved by monkeys.

Experiment 2 examined the effects of distraction during the retention delay on the DNMS performance of rats. Rats were tested at retention delays of 60 s. On half of the trials, the rats performed a distraction task during the retention delay; on the other half, they did not. Consistent with findings from monkeys and humans, distraction during the retention delay disrupted the DNMS performance of rats. This suggests that similar memory abilities are involved in the DNMS performance of rats, monkeys, and humans.

Experiment 3 investigated the effects of separate and combined bilateral lesions of the hippocampus and the amygdala on DNMS performance in pretrained rats. Rats were tested both before and after surgery at retention delays of 4, 15, 60, 120, and 600 s. Each experimental rat received bilateral lesions of the hippocampus, amygdala, or both. There were no significant differences among the three experimental groups, and the rats in each of the three experimental groups were significantly impaired, in comparison to no-surgery control rats, only at the 600-s delay. In contrast, rats that had sustained inadvertent entorhinal and perirhinal cortex damage during surgery displayed profound DNMS deficits. These results parallel the results of recent studies of the neural basis of DNMS in monkeys. They suggest that, in contrast to one previously popular view, neither the hippocampus nor the amygdala play a critical role in the DNMS of pretrained animals and that the entorhinal and perirhinal cortex are critically involved.

On the basis of these findings, it appears that the rat DNMS task may prove to be a useful component of rat models of brain-damage-produced amnesia. This conclusion is supported by the preliminary results of several experiments that are currently employing the task.

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GENERAL INTRODUCTION

Bilateral damage to medial-temporal-lobe or medial-diencephalic structures causes an amnesic syndrome in humans that is characterized by an impairment of the ability to form new memories. Over the past decade, the study of monkey models of brain-damage-produced amnesia has begun to shed light on the nature of human brain-damage-produced amnesia and its neural bases. The development of comparable rodent models would benefit the study of brain-damage-produced amnesia in two general ways: (1) it would facilitate the conduct of large-scale parametric experiments -- the cost of large-scale monkey research is prohibitive for most researchers -- and (2) it would provide a broader comparative basis for drawing inferences about the anatomical bases of brain-damage-produced amnesia. This thesis constitutes the first stages in the development of a rat model of brain-damage-produced amnesia.

An integral feature of the monkey models of human brain-damage-produced amnesia is the nonrecurring-items delayed nonmatching-to-sample (DNMS) memory task. Human amnesics have difficulty performing the DNMS task (Squire, Zola-Morgan, & Chen, 1988), and so do monkeys with bilateral medial-temporal-lobe (Mishkin, 1978; Murray & Mishkin, 1984; Zola-Morgan & Squire, 1985a; Zola-Morgan & Squire, 1986; Zola-Morgan, Squire, Amaral, & Suzuki, 1989) or medial-diencephalic (Aggleton & Mishkin, 1989a, 1989b; Zola-Morgan & Squire, 1985b) damage.

The present experiments were conducted (1) to develop a DNMS task for rats that resembles the DNMS task for monkeys, and (2) to determine whether it parallels the monkey DNMS task in terms of the memory abilities and the brain structures that it engages. Accordingly, the General Introduction deals with the following three topics: (1) a description of human brain-damage-produced amnesia, (2) an historical account of attempts to model human brain-damage-produced amnesia in laboratory

animals, and (3) a description of the preeminent monkey models of human brain-damage-produced amnesia.

1. CHARACTERISTICS OF THE AMNESIC SYNDROME

In some brain-damaged individuals, impaired memory occurs in a relatively pure form, that is, in the absence of other primary symptoms. The major characteristic of this classic "amnesic syndrome" (Baddeley, 1990) is an inability to form new long-term memories. It occurs following bilateral damage to either one of two brain areas -- the medial diencephalon or the medial temporal lobe. Accordingly, depending on the hypothesized location of their brain damage, amnesic patients are classified as either medial-diencephalic or medial-temporal-lobe amnesics.

H.M.'s amnesia

Remarkably, much of what is known about brain-damage-produced amnesia has come from the study of a single patient. This patient is H.M., who has been amnesic since 1953, when he received bilateral medial-temporal-lobe resections for the treatment of intractable epilepsy (Scoville, 1954). H.M.'s surgery removed the anterior two-thirds of the hippocampus, the parahippocampal gyrus, the uncus, and the amygdala of both hemispheres (Milner, 1959). Despite his amnesia, H.M. is normal in many respects. He displays normal intelligence and intact perceptual and attentional abilities, and he suffers no apparent emotional or personality disorders (Scoville & Milner, 1957). H.M.'s deficits are, for the most part, limited to his memory functions, and this is why he has been a particularly useful case for the study of medial-temporal-lobe amnesia (Milner, 1968).

The core symptoms of H.M.'s medial-temporal-lobe amnesia are typical of the amnesia of other patients with bilateral medial-temporal-lobe damage (e.g., Milner, 1959; Victor, Angevine, Mancall, & Fischer, 1961) and of the amnesia of patients with bilateral medial-diencephalic damage (see Victor & Yakovlev, 1955; Victor, Adams, & Collins, 1971). Accordingly, the following description of H.M.'s amnesia serves as a general introduction to the predominant symptoms of human brain-damage-produced amnesia.

Impaired memory abilities. Following his bilateral temporal-lobe resection in 1953, H.M. exhibited a severe anterograde amnesia, which has not diminished to this day; he has extreme difficulty forming memories of events that he has experienced since his brain surgery. The devastating impact that H.M.'s anterograde amnesia has had on his life is apparent from the anecdotal accounts of Scoville and Milner (1957). For example, H.M. is unable to recognize doctors and nurses whom he has seen many times, and he often reads the same magazines over and over again without realizing it. H.M. can remember small amounts of information -- such as numbers or word associations -- for several minutes, as long as he is allowed to maintain his attention on them. As soon as he is distracted, the information is lost.

H.M. also displays mild retrograde amnesia; he has difficulty remembering events that occurred during the year prior to his surgery, but his memory for events that occurred earlier remains largely intact (Corkin, 1968).

H.M.'s memory impairments include both verbal and nonverbal information, and information in all sensory modalities (Scoville & Milner, 1957). The term "global amnesia" is often used to refer to amnesic syndromes, such as H.M.'s, in which memory for information in all sensory modalities is affected.

There have been many experimental demonstrations of H.M.'s difficulty in forming long-term memories. The following are three of them:

1. H.M. was severely impaired on a digit-span + 1 test. In this version of the digit-span test, one new digit is added to the previous sequence each time that the subject gets a sequence correct. For example, if a subject were able to correctly repeat the sequence " 4, 6, 3, 8," on the next trial the sequence might be " 4, 6, 3, 8, 5 " -- and this sequence would be repeated on each trial until the subject recalled it correctly, at which point another digit would be added. Normal subjects recalled sequences of about 15 digits after only 25 digit-span + 1 trials. In contrast, H.M. was unable to progress beyond his initial 6-digit memory span (Drachman & Arbit, 1966).

2. H.M. was also severely impaired on a block-tapping memory-span + 1 test -- a nonverbal version of the digit-span + 1 test (Milner, 1971). Several blocks were spread out in front of H.M., who watched as the experimenter touched several of them in sequence. H.M. was then asked to repeat the same sequence of touches. H.M. was unable to learn a sequence that was one block greater than his initial block-tapping span.

3. H.M was impaired on a nonverbal matching-to-sample task. H.M. was presented with a sample item (i.e., one of several different ellipses), and then, following a retention delay, the same item and several other similar ones were simultaneously presented. H.M.'s task was to select the item that he had seen before. He was unable to perform this task, even when the retention delay was only 5 s (Sidman, Stoddard, & Mohr, 1968). However, in the same study, H.M. performed normally on the matching-to-sample

task at delays of up to 40 s when verbal stimuli were used (i.e., sequences of three consonants). The experimenters concluded that H.M. can perform the matching-to-sample task only when it is possible for him to rehearse the stimuli during the retention delay.

Spared memory abilities. Patients with medial-temporal-lobe and medial-diencephalic amnesia can form some kinds of long-term memories. Schneider (1912) conducted the first systematic studies of preserved memory abilities in amnesic patients (see Parkin, 1982). Schneider showed his amnesic subjects a picture of an object and later tested their retention by presenting a fragment of the original picture. The subjects displayed an enhanced ability to identify the object from the picture fragment even though they could not recall having seen the picture before. Clearly, the experience of seeing the picture had been retained in some sense although the subjects were not consciously aware of it.

Despite Schneider's early demonstration, the extent of spared memory abilities in brain-damage-produced amnesia did not start to be appreciated until the 1960s. The first widely cited studies of spared memory abilities were studies of H.M., but it was subsequently shown that the abilities that were spared in H.M. also tended to be spared in other patients with medial-temporal-lobe amnesia and in patients with medial-diencephalic amnesia (Brooks & Baddeley, 1976, Cohen & Squire, 1980; Teuber, Milner, & Vaughan, 1968).

It was apparent at the outset that H.M.'s short-term memory abilities had been unaffected by the surgery -- his postsurgery digit span was 6 (Drachman & Arbit, 1966), well within the normal range. In 1962, Milner (cited in Murray, 1990) reported that some aspects of H.M.'s long-term memory had also been spared. She reported that H.M. could learn a mirror drawing task and retain it from session to session. Since then, this finding has been extended to several other perceptual and motor skills. For example, H.M.'s performance on the rotary-pursuit task and on a bimanual tracking task improved at a normal rate with practice, although his absolute level of performance on these tasks was inferior to that

of normal subjects (Corkin, 1968). H.M. also learned and retained the pattern-analyzing skills that are involved in reading mirror-deflected text (Cohen & Squire, 1980), and he performed almost as well as normal subjects on the incomplete-picture task (Milner, Corkin, & Teuber, 1968). H.M.'s good retention over successive sessions on these tasks contrasted with his persistent inability to recall previous sessions.

Are there multiple dissociable memory systems?

The fact that H.M. and other patients with brain-damage-produced amnesia can display long-term memory impairments in some situations but not in others suggests that there are at least two kinds of long-term memory, one which is impaired in brain-damage-produced amnesia and one which is not.

Several descriptive schemes have been proposed to distinguish the long-term memory abilities that are impaired in brain-damage-produced amnesia from those that are spared. One scheme distinguishes between explicit and implicit memory (Graf & Schacter, 1985). *Explicit memory* refers to the conscious recollection of previous experiences at the time of retrieval, whereas *implicit memory* refers to the retrieval and expression of information stored from previous experiences in the absence of conscious recollection. The explicit-implicit distinction is not based on assumptions about the quality of the stored information (Schacter, 1987a, 1987b). Patients with brain-damage-produced amnesia are impaired on explicit memory tasks, but they exhibit normal or near normal performance on implicit memory tasks (e.g., Graf & Schacter, 1985).

Another influential scheme for distinguishing between the long-term memory abilities that are impaired in brain-damage-produced amnesia and those that are spared posits separate systems for declarative memory, which is assumed to be impaired, and procedural memory, which is assumed to be spared. Unlike the explicit-implicit distinction, the declarative-procedural distinction is based on

assumptions about the nature of stored information. *Declarative memories* are said to be neural representations of previously experienced perceptions, thoughts, or facts, that can be described verbally by the individual who possesses them (Squire, 1986)[1]. *Procedural memories* are said to be inherent in the performance of skilled actions and revealed by changes in the quality of those actions. Other dichotomies that have been applied to the dissociation between lost and spared memory abilities in amnesia include "memory" versus "habit" (Mishkin et al., 1984), "episodic memory" versus "semantic memory" (Schacter & Tulving, 1982), and "working memory" versus "reference memory" (Honig, 1978). Although different in detail, these hypothetical dichotomies are conceptually similar to the declarative-procedural distinction.

The notion that there are at least two distinguishable types of long-term memory, one of which is impaired in brain-damage-produced amnesia and the other of which is not, suggests that there are at least two anatomically distinct long-term memory systems. In the following section, I provide an historical account of attempts to uncover these systems through the conduct of experiments on animal models.

2. EARLY ATTEMPTS TO MODEL BRAIN-DAMAGE-PRODUCED AMNESIA IN LABORATORY ANIMALS

The discovery of H.M.'s amnesia in the 1950s came at a time when most theorists accepted Karl Lashley's proposition that memory traces are widely distributed throughout the cortex (i.e., the concept of *equipotentiality*), rather than localized within particular structures. Lashley's experiments with

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1 By this definition, nonverbal animals cannot have declarative memory. Instead, the term "representational memory" is often used to refer to memory functions in animals that correspond conceptually to a subtype of declarative memory. Representational memories are neural representations of the attributes of a stimulus (Murray, 1990).

animals had suggested that the degree of impairment on complex learning tasks is proportional to the amount of cortical damage (i.e., the concept of *mass action*), but is unrelated to the particular area of cortical damage. H.M.'s devastating and selective memory impairment following the removal of his medial temporal lobes challenged Lashley's equipotentiality and mass action views[2]. H.M.'s case resulted in renewed support for localizationist views of memory function, and many experimenters began to search for the neural substrate of long-term memory. They began by focusing on the structures of the medial temporal lobes.

Early studies of the effects of hippocampal lesions in laboratory animals

H.M.'s amnesia was originally attributed to the removal of his hippocampus for three reasons: (1) There were previous reports of patients who had suffered from amnesia following bilateral hippocampal damage (Bechterev, 1900, cited in Victor et al., 1961; Glees & Griffith, 1952, cited in Victor et al., 1961), (2) there appeared to be a correlation between the extent of hippocampal damage and the severity of amnesia in a group of eight amnesic patients with bilateral medial-temporal-lobe resections (Scoville & Milner, 1957), and (3) patients with bilateral damage that was largely limited to the amygdala did not have amnesia (Scoville & Milner, 1957). However, in the 1950s and 60s, hippocampal lesions were found to have inconsistent effects on the performance of learning and memory tasks by rats and monkeys.

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2 Although there was already evidence from postmortem examination of Korsakoff amnesics that damage in relatively small areas of the brain, namely the mammillary bodies and the walls of the third ventricle, could cause impaired memory, both the suddenness and the severity of H.M.'s amnesia made his case more compelling.

Experiments with monkeys. Mishkin (1954) observed normal performance of preoperatively learned visual discriminations in a monkey with bilateral hippocampal removals. Orbach, Milner, and Rasmussen (1960) tried to duplicate H.M.'s medial-temporal-lobe lesions in monkeys by removing the hippocampus and the amygdala from both hemispheres; however, the lesions produced no visual-discrimination deficits, even when the trials were widely separated in time and the monkeys performed irrelevant discriminations during the intertrial intervals. Mishkin (1954), Mishkin and Pribram (1954), and Orbach et al. (1960) found that monkeys with bilateral hippocampal lesions were not impaired on a delayed-response task, and Correll and Scoville (1965) and Drachman and Ommaya (1964) found that monkeys with bilateral hippocampal lesions were not impaired on a delayed matching-to-sample task. By the end of the 1960s, the only memory task on which monkeys with bilateral hippocampal lesions had been found to be impaired was the delayed-alternation task (Pribram, Wilson, & Conners, 1962; Orbach et al., 1960).

Experiments with rats. In the 1960s, rats with bilateral hippocampal lesions were shown to have difficulty performing successive-brightness-discrimination (Kimble, 1963), maze-learning (Kaada, Rasmussen, & Kveim, 1961; Kimble, 1963), and passive avoidance (Kimble, 1963) tasks; however, they had no difficulty performing a simultaneous-brightness-discrimination task (Kimble, 1963). The performance of rats on an active avoidance task was improved by bilateral hippocampal lesions (Isaacson, Douglas, & Moore, 1961).

Why early attempts to model brain-damage-produced amnesia failed

The lack of consistent learning and memory impairments in laboratory animals with bilateral hippocampal lesions led many investigators to conclude that different neural systems subserve memory

in humans than in nonhumans. However, others questioned the adequacy of the tasks that had been used to assess the effects of hippocampal lesions on memory in laboratory animals (e.g., Drachman & Ommaya, 1964; Orbach et al., 1960). Unfortunately, during the 1950s and 1960s human brain-damage-produced amnesia itself was not well understood, and therefore it was not clear which type of memory tasks would be suitable for modelling it.

By the late 1960s, the study of H.M. and other amnesic patients was beginning to shed light on the nature of the spared memory abilities in human brain-damage-produced amnesia. Some of these insights suggested possible explanations for why lesions that impair memory in humans might not impair the performance of certain memory tasks by laboratory animals. In particular, theories were proposed to explain why bilateral hippocampal lesions did not disrupt the performance of the two kinds of tasks that had been used to assess their amnesic effects: (1) visual-discrimination tasks and (2) delay tasks.

Visual-discrimination tasks. Evidence that procedural-learning abilities are spared in humans with brain-damage-produced amnesia provided an explanation of why bilateral hippocampal damage did not impair visual discrimination learning in laboratory animals. On visual-discrimination tasks, it is not necessary for the subject to remember what happened on individual trials because the information that is relevant to successful performance is presented repeatedly over many trials. That is, visual discrimination tasks are not tests of explicit memory (or declarative memory); rather, they are similar to the tests of implicit memory (or procedural memory) that amnesic patients are capable of learning, and therefore, they are unlikely to be sensitive to brain-damage-produced amnesia.

> "In everyday human learning there are no strict counterparts of discrimination tasks in which the same piece of information is presented *ad nauseum*. In humans, motor learning perhaps comes closest to this..." (Iversen, 1976; p.16)

Thus, in Mishkin's (1954) and Orbach et al.'s (1960) early monkey experiments, bilateral hippocampal lesions may have produced amnesia that went undetected by the visual-discrimination tasks that they used to assess it (see Zola-Morgan et al., 1982).

Delay tasks. Evidence that short-term memory abilities are largely spared in patients with brain-damage-produced amnesia provided an explanation of why bilateral hippocampal damage did not impair the performance of laboratory animals in early experiments that employed various delay tasks. For example, the longest retention delays that Correll and Scoville (1965) and Drachman and Ommaya (1964) used to test delayed matching-to-sample performance in monkeys with hippocampal damage were only 5 and 12 s, respectively, well within the range of short-term memory. Similarly, Mishkin (1954), Mishkin and Pribram (1954), and Orbach et al. (1960), who did not observe delayed-response deficits in monkeys with bilateral hippocampal lesions, all used delays of 10 s or less. Bilateral hippocampal damage may have produced amnesia in all of these delayed matching-to-sample and delayed-response experiments that went undetected because of the retention delays were too brief. In support of this interpretation, Zola-Morgan and Squire (1985a) recently found that monkeys with bilateral lesions of the hippocampus and amygdala displayed a marked impairment on a delayed-response task at delays of 15 and 30 s, but performed normally when the delay was only 8 s.

3. THE PREEMINENT MONKEY MODELS OF BRAIN-DAMAGE-PRODUCED AMNESIA

By the end of the 1960s, it was becoming apparent that the development of animal models of brain-damage-produced amnesia would first require the development of memory tests for laboratory animals that amnesic patients would be expected to fail. In the mid 1970s, such a task was developed --

the nonrecurring-items delayed nonmatching-to-sample (DNMS) task by Mishkin and Delacour (1975). The DNMS task would later become a key component of monkey models of brain-damage-produced amnesia. This section describes the monkey DNMS task and the effects of medial-temporal-lobe and medial-diencephalic lesions on DNMS performance in monkeys.

The nonrecurring-items delayed nonmatching-to-sample (DNMS) task

On each trial of the DNMS task, a sample object is presented to the subject. Then, following a delay, during which the sample object is hidden from view, it is presented again, along with an unfamiliar object. The subject is rewarded for selecting the unfamiliar object from this pair. Monkeys quickly learn to perform this task with few errors at retention delays of only a few seconds, and once they have done so, their performance is almost as good as that of humans at delays of up to several minutes. Medial-temporal-lobe and medial-diencephalic lesions disrupt DNMS performance in both human and nonhuman primates (Squire, 1987).

When it was developed, the DNMS task was unique among memory tests for laboratory animals because it resembled human recognition-memory tests. In recognition-memory tests, subjects must decide which test items have been previously encountered. In typical human recognition-memory tests, a list of items is presented to the subject (e.g., a list of pictures, words, or nonsense syllables). Later, a test list, which includes items from the first list and some new items, is presented, and the subject must identify the items that appeared in the first list (e.g., Postman, 1950; Strong, 1912). The DNMS task is identical in principle to such tests of human-recognition memory; on each trial, the subject must distinguish between an object that was presented earlier and one that was not.

In most tests of animal memory, the subjects learn stimulus-reward or response-reward associations, and the same stimuli recur over many trials. These tasks fall into two categories: (1)

reference-memory tasks and (2) working-memory tasks. *Reference-memory* tasks are those in which the relations among stimuli, responses, and reward remain constant over trials; *working-memory* tasks are those in which the relations among stimuli, responses, and reward change from trial to trial (Olton et al., 1979).

The DNMS task is a working-memory task, but it differs from most other working-memory tasks for laboratory animals in one important respect: It involves nonspatial stimuli. In this respect, it resembles most human memory tasks. Prior to the development of the monkey DNMS task, laboratory animals had frequently been shown to have difficulty performing nonspatial working-memory tasks, such as delayed matching-to-sample, at delays of more than a few seconds. This led to the view that nonhuman animals are poor at remembering nonspatial information (see Iverson, 1976; Nissen, Riesen, & Nowlis, 1938). However, it is now clear that the poor performance of laboratory animals in early studies of nonspatial working memory was a methodological artifact. In conventional nonspatial working-memory tasks, a small set of test stimuli are presented trial after trial (e.g., Alexinsky & Chapouthier, 1978). Accordingly, after a few trials, all the test items are familiar, and the recognition task in effect becomes a recency-memory task (Mishkin & Delacour, 1975) -- on each trial, two familiar objects are presented, and the subject must remember which of the two has been encountered more recently. Laboratory animals appear to have difficulty making such recency discriminations after retention delays of more than a few seconds. The DNMS task is a test of recognition memory because it makes use of nonrecurring items[3]; with nonrecurring-items, the subject can solve the task by distinguishing between an object that it has seen before and one that it has not. Because the DNMS task is a test of recognition memory that can be readily performed by normal monkeys, it has provided

3 The term "trial unique" is typically used in place of "nonrecurring-items" in reference to the monkey DNMS task. The advantage of the term "nonrecurring-items" is that a simple antonym exists (i.e., "recurring-items"), which can be used to refer to versions of the task in which the same stimuli are presented repeatedly over several trials. Throughout this dissertation, "DNMS" refers to the nonrecurring-items version of the delayed nonmatchaig-to-sample task.

researchers with an appropriate test for determining whether medial-temporal-lobe and medial-diencephalic lesions cause amnesia in laboratory animals.

The origins of the preeminent monkey models of brain-damage-produced amnesia

The development of the monkey models of brain-damage-produced amnesia began with a serendipitous finding. Mishkin and Spiegler (cited in Mishkin & Appenzeller, 1987) found that monkeys with bilateral lesions of the amygdala had difficulty performing one-trial visual-discriminations, and they tried to accentuate the impairment by making bilateral lesions that included both the hippocampus and the amygdala. Monkeys with amygdalo-hippocampal lesions were so severely impaired on the one-trial visual-discrimination task that the experimenters wondered whether they could remember the stimuli from one trial to the next. Mishkin tried to answer this question by assessing the effects of amygdalo-hippocampal lesions on DNMS (Mishkin, 1978). He found that monkeys with bilateral lesions to both the hippocampus and the amygdala were profoundly impaired on the DNMS task, whereas monkeys with bilateral lesions to the either the hippocampus or the amygdala alone were only mildly impaired. The severe impairment of DNMS in monkeys with amygdalo-hippocampal lesions appeared to be a good animal model of medial-temporal-lobe amnesia for two reasons: (1) because the brain damage in monkeys with amygdalo-hippocampal lesions was similar to the brain damage in patients with medial-temporal-lobe amnesia, and (2) because accurate DNMS performance requires the kinds of memory functions that are impaired in patients with medial-temporal-lobe amnesia.

The effects of bilateral medial-temporal-lobe lesions on DNMS performance in monkeys

Since Mishkin's demonstration that bilateral amygdalo-hippocampal lesions disrupt DNMS in monkeys, several studies have been conducted to determine which medial-temporal-lobe structures must be damaged in order to produce such a disruption. Four different hypotheses have received support: (1) the hippocampus, (2) the hippocampus and the amygdala, (3) the temporal stem, and (4) the rhinal cortex.

Hippocampus. There is controversy over whether lesions limited to the hippocampus produce a recognition deficit in monkeys that is comparable to the profound recognition deficit that is displayed by patients with medial-temporal-lobe amnesia. Mahut, Zola-Morgan, and Moss (1982) and Zola-Morgan and Squire (1986) found severe DNMS deficits in monkeys with bilateral hippocampal lesions, whereas Mishkin (1978) and Murray and Mishkin (1984, 1986) found only mild DNMS deficits. Whether or not monkeys with lesions limited to the hippocampus display severe DNMS deficits appears to depend upon whether or not they are trained on the DNMS task prior to surgery. In experiments without presurgery training, bilateral hippocampal lesions have produced severe DNMS deficits (e.g. Mahut, Moss, & Zola-Morgan, 1981; Mahut et al., 1982; Zola-Morgan & Squire, 1986), whereas in experiments with presurgery training, bilateral hippocampal lesions have produced only mild DNMS deficits (e.g., Mishkin, 1978; Murray & Mishkin, 1984).

Hippocampus-Amygdala. Bilateral lesions of the hippocampus (Mahut et al., 1982; Mishkin, 1978; Zola-Morgan & Squire, 1985a; Bachevalier & Mishkin, 1989) or the amygdala (Mishkin, 1978; Murray & Mishkin, 1984) have been shown to produce mild DNMS deficits in monkeys. Combined bilateral lesions of both the hippocampus and the amygdala have been shown to produce a more severe

impairment -- one that is greater than would be expected from a simple summation of the effects of bilateral lesions to either structure alone (Mishkin, 1978; Murray & Mishkin, 1984; Zola-Morgan & Squire, 1985a). The synergistic effect of bilateral lesions of the hippocampus and amygdala led to the proposal that these structures are critical links in parallel neural circuits that are involved in DNMS performance and that each circuit can partially compensate for the loss of the other (Mishkin, 1982; Murray, 1990). These circuits are presumed to involve medial-diencephalic structures. Combined bilateral damage to the pathways through which the hippocampus and amygdala communicate with medial-diencephalic structures -- the fornix and the amygdalofugal pathway, respectively -- caused a severe DNMS deficit in monkeys, whereas bilateral damage to only one of these two pathways caused only a mild deficit (Bachevalier, Parkinson, & Mishkin, 1985).

Like the memory impairments of patients with brain-damage-produced amnesia, the memory impairments of monkeys with medial-temporal-lobe lesions are not specific to a single sensory modality. Bilateral hippocampal (Mahut et al., 1981) and amygdalo-hippocampal (Murray & Mishkin, 1984) lesions produced DNMS deficits in monkeys when either visual or tactual stimuli were used.

Temporal stem. Horel (1978) pointed out that the surgical technique that was used for removing medial-temporal-lobe structures in H.M. and other patients must also have damaged the temporal stem -- a fiber pathway that links the temporal cortex with the amygdala and the orbital frontal cortex (Cirillo, Horel, & George, 1989). He hypothesized that bilateral temporal-stem damage, not bilateral hippocampal damage, was the cause of medial-temporal-lobe amnesia. Horel based his hypothesis on the following three lines of evidence: (1) Bilateral temporal-stem lesions in monkeys disrupt the performance of some memory tasks that are not disrupted by bilateral hippocampal lesions (e.g., visual-discrimination tasks; Horel & Misantone, 1976). (2) The positive correlation between the extent of hippocampal damage and the severity of memory deficits, which was reported by Milner (1974), can

be accounted for by the fact that more extensive hippocampal resection is likely to damage more of the temporal stem. (3) Temporal cortex lesions in monkeys produce symptoms similar to those produced in humans by medial-temporal-lobe lesions. In fact, Gol and Faibish (1967) reported that memory deficits in a group of amnesic patients were more highly correlated with the extent of temporal neocortical damage than with the extent of hippocampal damage.

Could temporal-stem damage account for the severe DNMS impairment that Mishkin (1978) and Murray and Mishkin (1984, 1986) observed in monkeys with bilateral amygdalo-hippocampal lesions? In a test of the temporal-stem and hippocampus-amygdala hypotheses of medial-temporal-lobe amnesia, Zola-Morgan, Squire, and Mishkin (1982) found that monkeys with bilateral amygdalo-hippocampal lesions displayed a severe DNMS impairment, whereas monkeys with bilateral temporal-stem lesions were unimpaired. However, Cirillo et al. (1989) recently found that temporal-stem lesions placed anterior to those made by Zola-Morgan et al. (1982) produced a severe impairment on a delayed matching-to-sample task in monkeys. The anterior temporal-stem lesions made by Cirillo et al. (1989) damaged the portions of the temporal stem that would be expected to be damaged in monkeys and humans with amygdalo-hippocampal lesions; the more posterior temporal-stem lesions made by Zola-Morgan et al. (1982) did not.

Rhinal cortex. Recent evidence suggests that the DNMS deficits that are produced in monkeys by lesions of the hippocampus and amygdala may result from incidental damage to the rhinal cortex (i.e., the perirhinal and entorhinal cortices). In monkeys, hippocampal and amygdalar lesions are usually made by aspiration, and thus, portions of the overlying cortex must first be removed to gain access to the hippocampus and amygdala. Hippocampal lesions typically include the parahippocampal gyrus and the posterior half of the entorhinal cortex; amygdalar lesions typically include the piriform and periamygdaloid cortex, the anterior half of the entorhinal cortex, and, in some cases, the perirhinal

cortex (Murray, in press). In Mishkin's (1978) and Murray and Mishkin's (1984) studies, the DNMS deficits may have been more severe in monkeys with amygdalo-hippocampal lesions than in monkeys with separate hippocampal or amygdalar lesions because only the amygdalo-hippocampal lesions resulted in the removal of the entire entorhinal cortex.

Monkeys with bilateral lesions of the entorhinal and perirhinal cortex (Murray, Bachevalier, & Mishkin, 1989) or of the perirhinal cortex (Meunier, Murray, Bachevalier, & Mishkin, 1990; Zola-Morgan et al., 1989c) have been shown to be severely impaired on the DNMS task. Consistent with these findings, Horel, Pytko-Joiner, Voytko, and Salsbury (1987) observed a severe impairment in the delayed matching-to-sample performance of monkeys following either ablation or reversible cooling lesions of the inferior-temporal gyrus, which includes much of the perirhinal cortex.

Zola-Morgan et al. (1989a) have argued that the recent evidence that implicates the rhinal cortex in the performance of DNMS suggests that the amygdala does not contribute to recognition memory. Their conclusion is based on the following findings: (1) Radiofrequency lesions of the amygdala that spare the surrounding cortex do not produce an impairment in DNMS (Zola-Morgan et al., 1989a), (2) aspiration lesions of the amygdala that do include the surrounding cortex produce an impairment in DNMS (Mishkin, 1978; Murray & Mishkin, 1984), and (3) radiofrequency lesions of the amygdala do not exacerbate the DNMS deficits that have been produced by hippocampal aspiration (Zola-Morgan et al., 1989a). However, in view of the report that bilateral ablation of the amygdala plus rhinal cortex produces a more severe DNMS impairment than does bilateral ablation of the rhinal cortex alone (Murray & Mishkin, 1986), it is premature to conclude that the amygdala plays no role whatsoever in recognition memory.

Recently, Murray (in press) has suggested that the additional DNMS deficit that is produced when amygdalar lesions are added to rhinal lesions may result from damage to perirhinal efferent fibers that course just lateral to the amygdala. This idea is consistent with Cirillo et al.'s (1989) finding

that anterior temporal-stem lesions cause a greater memory impairment than do posterior temporal-stem lesions. Posterior lesions disconnect only the posterior half of the entorhinal cortex; anterior lesions disconnect most of the entorhinal cortex.

Murray's hypothesis is consistent with Horel's temporal-stem hypothesis of medial-temporal-lobe amnesia -- both predict that DNMS performance will be impaired following lesions of the temporal-lobe white matter. However, Murray's hypothesis differs from Horel's in attributing the DNMS deficit following temporal-stem lesions to disruption of the projections from perirhinal cortex, whereas Horel's hypothesis attributes the deficit to disruption of the projections from the anterior inferotemporal cortex.

Synopsis. None of the four interpretations of the effects of medial-temporal-lobe lesions on DNMS in monkeys -- hippocampus, amygdala-hippocampus, temporal stem, or rhinal cortex -- can be discounted on the basis of existing evidence. The difficulty in deciding among them is that they are not mutually exclusive: Damage to any one of the four areas may be sufficient to produce deficits under the appropriate conditions, whereas maximal deficits may be produced through combined damage to some subset of the four. Hippocampal lesions appear to cause deficits that are more severe in unpretrained monkeys than in pretrained monkeys. This finding suggests that the hippocampus plays a role in learning how to perform well on the DNMS task, but that it is less important once high levels of DNMS performance have been achieved. Nevertheless, a model of the severe memory deficits suffered by medial-temporal-lobe amnesics, such as H.M., should involve pretrained monkeys because H.M. is deficient in the performance of everyday memory functions that he had naturally acquired and overlearned in the years prior to his surgery.

Current evidence suggests those medial-temporal-lobe lesions that are most likely to cause severe DNMS deficits in pretrained monkeys involve the rhinal cortex. Severe DNMS deficits have been found

in pretrained monkeys with amygdalo-hippocampal lesions, but in all cases, the amygdalo-hippocampal lesions have also involved rhinal cortex damage. Conversely, damage to the rhinal cortex that spares the hippocampus and amygdala can produce DNMS deficits in both pretrained and unpretrained monkeys. Although additional damage to the hippocampus (Zola-Morgan et al., 1989c) or amygdala (Murray & Mishkin, 1986) can exacerbate the deficits produced by rhinal cortex damage, this may occur because the amygdalar and hippocampal lesions disconnect remaining rhinal cortex from other areas (Murray, in press). The anterior temporal stem lesions that have been shown to produce delayed matching-to-sample deficits in monkeys (Cirillo et al., 1989) disrupt many of the efferent connections of the rhinal cortex (Murray, in press).

The effects of medial-diencephalic lesions on DNMS performance in monkeys

There have been only a few studies of the effects of medial-diencephalic lesions on DNMS performance in monkeys. The mediodorsal thalamic nuclei and the mammillary bodies are the two most consistently and extensively damaged brain areas in Korsakoff amnesics (Victor et al., 1971). Aggleton and his colleagues found that bilateral lesions of the mammillary bodies did not produce DNMS deficits in monkeys (Aggleton & Mishkin, 1985), but that bilateral lesions of the mediodorsal thalamic nuclei and the adjacent anterior nuclear complex (Aggleton & Mishkin, 1983a) or of the mediodorsal nuclei alone (Aggleton & Mishkin, 1983b; Zola-Morgan & Squire, 1985b) did. Korsakoff amnesics have been shown to display similar impairments on a DNMS task (Squire et al., 1988) and on a nonrecurring-items delayed matching-to-sample task (Aggleton, Nicol, Huston, & Fairbairn, 1988).

GENERAL RATIONALE

This thesis constitutes the initial stages of an attempt to develop rat models of brain-damage-produced amnesia that are directly comparable to the monkey models. Such rat models could contribute to the study of brain-damage-produced amnesia in two important ways: (1) They could facilitate the conduct of large-scale parametric experiments -- the cost of large-scale monkey research is prohibitive for most researchers. (2) They could provide a broader comparative basis for drawing inferences about the anatomical bases of brain-damage-produced amnesia.

The first purpose of this thesis was to design and develop a rat version of the monkey DNMS task. The second was to assess the comparability of the rat DNMS task to the monkey DNMS task in terms of (1) the rate at which it is learned, (2) the asymptotic level at which it is performed, (3) the memory abilities that it taps, and (4) the brain structures that it engages.

First, I designed a DNMS task for rats that resembles the monkey DNMS task. Then, I used it in three experiments. Experiment 1 assessed the ability of intact rats to learn and perform the DNMS task. Experiment 2 examined the effects of distraction during the retention delay on the DNMS of intact rats -- distraction interferes with DNMS performance in monkeys (Zola-Morgan & Squire, 1985a; Zola-Morgan, Squire, & Amaral, 1989a, 1989b) and humans (Squire et al., 1988). Experiment 3 examined the effects of separate and combined bilateral lesions of the hippocampus and the amygdala on the DNMS performance of rats.

Although the primary purpose of this thesis was to assess the comparability of the rat and monkey DNMS tasks, the present experiments accomplished more. Because, together, they suggested that the rat DNMS task is a valid test of object recognition, each experiment also provided information about the mnemonic abilities of rats or the neural bases of their ability to recognize objects. Experiment 1 provided comparative data on the object recognition of rats; Experiment 2 provided data on the effects

of distraction on object recognition in rats; Experiment 3 provided evidence concerning the role of the hippocampus and amygdala in object recognition in rats.

A NEW DNMS TASK FOR RATS

In preparation for designing a rat version of the monkey DNMS task, I analyzed two DNMS tasks that had already been developed for rats (Aggleton, 1985: Rothblat & Hayes, 1987). Both tasks bear some resemblance to the monkey DNMS task, but both differ from it in major respects. My general strategy was to incorporate features of the existing rat DNMS tasks that are part of the monkey DNMS task and to eliminate features from them that either introduce a cognitive demand that is not present in the monkey DNMS task or make the task particularly difficult for rats. Accordingly, the first two sections in this chapter describe the two previous rat DNMS tasks and discuss their strengths and weaknesses. The third section outlines the specific considerations that guided the design of my rat DNMS task. The fourth and final section of this chapter describes the task.

1. AGGLETON'S Y-MAZE DNMS TASK

Aggleton (1985) developed a Y-maze DNMS task for rats. In the Y-maze DNMS task, 40 distinctive goal boxes serve as the test stimuli. On each trial, the rat is first enclosed for 20 s in a sample goal box that is attached to one of the arms of a Y maze. Then, the rat is removed from the sample box, the sample box is removed from the Y maze and replaced by a featureless goal box, and the rat is placed in the featureless goal box. Following a delay, the door to the featureless goal box is opened to provide the rat with access to the other two arms of the Y maze. At the end of both of these arms are distinctive goal boxes; one of them matches the sample goal box, and the other one, an unfamiliar goal box, does not. The rat is rewarded if it enters the unfamiliar goal box. That goal box then serves as the sample for the next trial. Aggleton's (1985) study was the first to demonstrate that rats can perform

well on a DNMS task -- once they had mastered the task at short delays, Aggleton's rats averaged approximately 80% correct at delays of 120 s.

Although the rat Y-maze DNMS task resembles the monkey DNMS task, it differs from it in key respects. The following are five of them:

1. The rat Y-maze DNMS task uses a much smaller set of test stimuli than the monkey DNMS task -- 40 distinctive goal boxes in the Y-maze DNMS task versus several hundred different objects in the monkey DNMS task. Thus, repeated exposure to individual stimuli is more frequent in the rat Y-maze DNMS task than in the monkey DNMS task.
2. In the rat Y-maze DNMS task, the 20-s duration of exposure to the sample is controlled by the experimenter. In the monkey DNMS task, the duration of exposure to the sample is controlled by the subjects -- the monkeys can respond to the sample with whatever latency they choose, which is typically within 2 or 3 s.
3. In the rat Y-maze DNMS task, the experimenter handles the subjects during trials; in the monkey DNMS task, the subjects are not handled.
4. In the rat Y-maze DNMS task, the unfamiliar goal box on one trial serves as the sample goal box on the next trial; in the monkey DNMS task, two new objects serve as the sample and unfamiliar items on each trial.
5. In the monkey DNMS task, the subjects must physically manipulate the test stimuli; in the rat Y-maze DNMS, the subjects enter the test stimuli.

These differences between the rat Y-maze DNMS task and the monkey DNMS task might make the cognitive demands of the two tasks substantially different, and thus, they make it difficult to generalize between them.

2. ROTHBLAT AND HAYES'S DNMS TASK

The rat DNMS task that was developed by Rothblat and Hayes (1987) involves a straight runway with a start area at one end and a goal area at the other. The goal area contains three recessed food wells. The start area is separated from the runway by a door. On each trial, the experimenter baits the central food well and positions a sample object over it. Then, the door is opened, and the rat runs down the runway to the goal area, where it displaces the sample object from the food well and retrieves the food. Then, the experimenter closes the door and returns the rat to the start area for the retention delay. During the delay, the experimenter places the sample object and an unfamiliar object over the lateral food wells. At the end of the delay, the door is opened, and the rat runs to the goal area. If it displaces the unfamiliar object, it is rewarded; if it displaces the sample object, it is not rewarded. Different sample and unfamiliar objects are used on each trial within a session.

Each rat in Rothblat and Hayes's (1987) study received 12 trials per day at delays of 10 s. Over the first 10 sessions, their scores increased at a statistically significant, but unimpressive, rate; first-session scores averaged 68%, and tenth-session scores averaged only 75%. After they reached the criterion of at least 75% correct over three consecutive sessions, each rat received additional sessions at delays of 120 s. Their mean score at delays of 120 s was 63%. Monkeys typically score between 85% and 95% at delays of 120 s (e.g., Bachevalier et al., 1985; Mishkin, 1978; Murray & Mishkin, 1984).

Several features of Rothblat and Hayes's rat DNMS task make it more similar to the monkey DNMS task than is Aggleton's Y-maze DNMS task. The following are three of them:

1. Rothblat and Hayes's task uses objects as test stimuli.
2. In Rothblat and Hayes's task, the subjects physically manipulate the test objects; they displace the unfamiliar object from a food well to obtain food.

3. Rothblat and Hayes's task employs a large pool of test stimuli (approximately 250 objects).

Although Rothblat and Hayes's task bears a closer resemblance to the monkey DNMS task than does Aggleton's Y-maze task, it differs from the monkey DNMS task in key respects. The following are three of them:

1. The subjects in Rothblat and Hayes's task are handled during the retention delay.
2. Rats do not easily learn Rothblat and Hayes's DNMS task.
3. The asymptotic DNMS performance of rats on Rothblat and Hayes's task does not compare favorably to that typically reported for monkeys.

The slow learning in Rothblat and Hayes's rats might have been partly due to the fact that initial DNMS sessions were conducted at retention delays of 10 s; shorter delays may have led to quicker learning. Handling the rats during the retention delay might also have contributed to their poor performance. Be that as it may, the poor performance of Rothblat and Hayes's rats is a major shortcoming for two reasons. First, if a rat DNMS task is to be considered to be comparable to the monkey DNMS task, then it is important that normal rats display similarly high baseline levels of performance. Second, low baseline levels of performance make it difficult to demonstrate statistically significant deficits.

3. REQUIREMENTS OF AN EFFECTIVE RAT DNMS TASK

My first step in designing a rat DNMS task that closely resembles the monkey DNMS task was to compile the following list of desirable features, which was based on my analysis of Aggleton's (1985) and Rothblat and Hayes's (1987) rat DNMS tasks and the monkey DNMS task:

1. The test stimuli should be objects.
2. A large pool of test objects should be used and two new objects should serve as the sample and novel objects on each trial within a session.
3. The rats should not be handled during sessions.
4. The operant response should be the displacement of an object from over a food well.
5. The duration of exposure to the sample object should be brief, and this duration should be controlled by the subject, not by the experimenter.
6. It should be possible to train subjects at retention delays of only a few seconds.

Then, I designed a rat DNMS task that satisfied each of these requirements.

4. THE NEW RAT DNMS TASK

This section describes the rat DNMS paradigm that I designed. The first subsection describes the apparatus, and the second outlines the general training procedure that was used in each of the three experiments in this thesis.

The apparatus

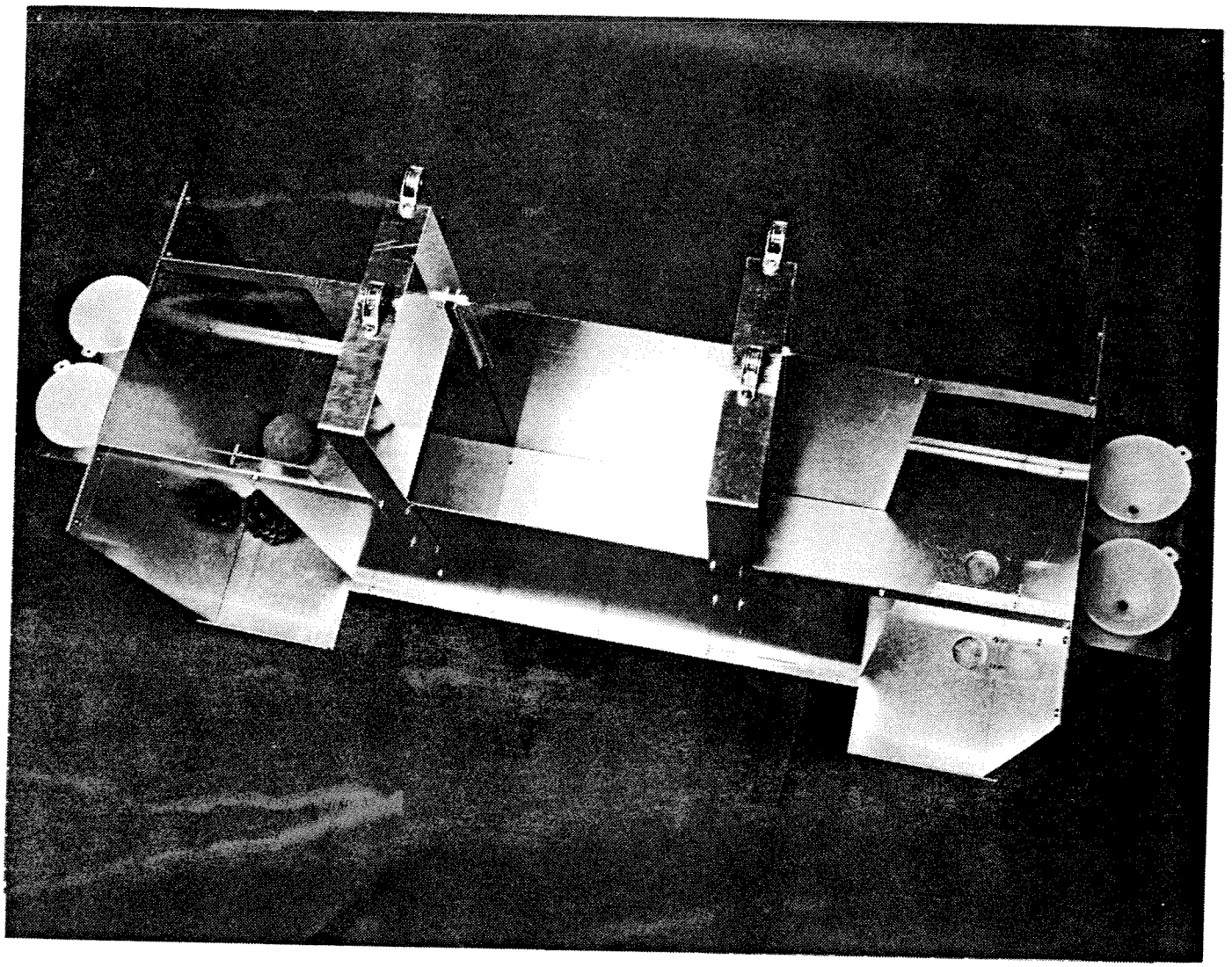
The apparatus, which was constructed of sheet aluminum (thickness = 0.127 cm), was a straight runway that was mounted 70 cm above the floor (see Figure 1). The apparatus was 60 cm long, 20 cm wide, and 40 cm high. There were two identical goal areas, one at each end of the runway. Each of the goal areas was separated from the central starting area by an opaque guillotine door; both doors were located 30 cm from the nearest end wall. Both goal areas had two recessed food wells, 3.5 cm in diameter and 2.0 cm deep. The food wells were separated by a short divider wall (9 cm x 9 cm), which protruded at a 90-degree angle from the center of the end wall. The food wells were centered 5 cm from the divider wall, and 3 cm from the end wall. The sides of the goal areas were open to allow the experimenter to easily position stimulus objects over the food wells and to quickly remove them. Food pellets (45 mg; Bio-Serv Inc., Frenchtown, NJ) were delivered to the food wells via funnels that were mounted on the outside of the apparatus and connected to the food wells with vinyl tubing.

The test stimuli were 350 test objects of various shapes, textures, and colors, comparable to the "junk" objects that have been used in the monkey DNMS paradigm (see Mishkin & Appenzeller, 1987). Each object was large enough to cover a food well but small enough and light enough to be easily displaced by a rat.

General training and testing procedures

The general training procedure for the rat DNMS task comprised three phases: (1) habituation to the apparatus, (2) object-discrimination training, and (3) acquisition of DNMS. Each rat received no more than one session per day and no fewer than four sessions per week. The rats' body weights were reduced to 85% of ad-lib values by limiting their daily ration of laboratory chow, and their weights were

Figure 1. The DNMS apparatus. (Photograph by Jack Wong.)



maintained throughout the experiment at a level that was 85% of the typical weight of rats of the same age, sex, and strain that are maintained with continuous access to laboratory chow. Training commenced after the rats had been on the restricted feeding regimen for 14 days. They were housed individually with continuous access to water, and they were maintained on a 12:12 hr light-dark cycle, with light offset at 11:00 p.m. All testing occurred during the light phase of the light-dark cycle. The rats were not handled during a session once they had been placed in the apparatus unless they urinated or defecated, in which case they were removed briefly so that the floor could be wiped clean.

Habituation. The habituation phase consisted of six 20-min sessions. During the first two sessions, the guillotine doors remained open, and each of the four food wells was baited with three or four food pellets. The wells were rebaited once the pellets in all four wells had been consumed.

On the third and fourth habituation days, the rats were shaped to run back and forth between the goal areas by alternately baiting a single well in each one. Food appeared equally often in all four wells. The guillotine doors remained open during these two sessions.

The operation of the guillotine doors was introduced during the fifth and sixth habituation sessions. The food wells were unbaited at the start of both of these sessions. When the rat entered one of the goal areas, the experimenter lowered the door at the other end and baited one of the food wells at that end with a single pellet. When the rat approached to within about 3 cm of the lowered door, it was raised to provide the rat with access to the baited food well. As soon as the rat found the food, the far door was lowered, and one of the wells behind it was baited. This door was opened as soon as the rat approached it. This cycle was repeated for the entire 20 min of the fifth and sixth habituation sessions.

Object discrimination. Following the six habituation sessions, each rat received four two-choice object-discrimination sessions; each session comprised 25 trials. These object-discrimination sessions were designed to accomplish two goals: (1) to teach the rats to displace objects from over the food wells, and (2) to eliminate any side preferences, that is, preferences for either the right or left food wells (cf. Rothblat & Hayes, 1987).

For a particular subject, the same two objects served as the stimuli for all 100 of its object-discrimination trials. One of the objects was randomly designated the S+ (reward); the other object was designated the S- (no reward). To begin each session, the rat was placed in the center of the apparatus. One door was open, and one was closed. The S+ and S- were each positioned over one of the food wells behind the closed door -- the position of S+ (left or right) varied from trial to trial according to an irregular, but balanced, pattern. Then, the door was raised to expose the two objects. When the rat approached and displaced an object, the far door was lowered behind it. If the rat displaced the S+, a food pellet was delivered to that food well; if it displaced the S-, no reward was delivered. Correction was allowed during the first object-discrimination session; if the rat first chose S-, it was permitted to then displace S+ to obtain a reward before the experimenter removed the objects. During the remaining object-discrimination sessions, correction was not allowed. As soon as an object had been displaced, the experimenter removed the S+ and S- and placed them over the wells at the other end of the apparatus in preparation for the next trial. The duration of the intertrial interval (i.e., the interval between the displacement of an object on one trial and the opening of the door to provide the rat with access to the objects on the next trial) varied, but it was typically 15 to 20 s.

Acquisition of DNMS. For DNMS training, the pool of objects was divided into seven sets of 50 objects each. For each rat, a different set of objects was used on each consecutive session (i.e., a particular set was used, on average, once every seven sessions). Different pairs of objects were used for

each of the trials within a session; one was randomly designated the sample, and the other the novel object.

To begin a DNMS session, the rat was placed in the apparatus with the doors raised, and it was allowed to explore the apparatus for approximately 1 min. Then, the doors were lowered to enclose the rat in the central starting area. Before each trial, a single food pellet was placed in one of the four food wells, and the sample object was placed over it. The location of the pellet and the sample object varied according to an irregular, but balanced, schedule; they appeared at each of the four food wells with equal probability. Once the sample object was in place, the novel object was placed over one of the food wells at the other end of the apparatus; its position, left or right, varied according to an irregular, but balanced, schedule.

To begin a trial, the experimenter raised the door to provide the rat with access to the sample object, which the rat approached and displaced from the food well. While the rat ate the food pellet, the experimenter removed the sample object and positioned it over the vacant food well at the other end of the apparatus. The other door was then raised, and the rat approached and displaced either the sample object or the novel object. A food pellet was delivered to the exposed food well if the novel object was displaced; no pellet was delivered if the sample object was displaced -- rats were considered to have displaced an object only if they moved it enough to expose the food well. As the rat ate the pellet, the experimenter removed the objects and lowered the door farthest from the rat. When the rat finished eating, it entered the central starting area and the experimenter lowered the other door to enclose it there.

The next trial began as soon as the rat was enclosed in the starting area, the new sample and novel objects were positioned, and the door was raised to provide the rat with access to the new sample object. Most intertrial intervals (i.e., the interval between the displacement of an object on one trial and the opening of the door to provide the rat with access to the sample on the next trial) were 30 to 40 s in

duration. If a particular rat was consistently slow to return to the starting area, it was occasionally rewarded with a food pellet as it entered the starting area. The rats were permitted to make corrections during the first two DNMS sessions, but not thereafter; if the rat first chose the sample object, it was permitted to displace the novel object to obtain a reward before the experimenter removed the objects.

The retention interval, or delay, was the time between the removal of the sample object and the raising of the second door to provide access to the sample and novel objects. The shortest delay that could be easily employed in this DNMS task was approximately 4 s; this is the delay that was employed on each trial of the DNMS training phase.

EXPERIMENT 1: DNMS PERFORMANCE IN RATS[4]

The two main objectives of Experiment 1 were (1) to demonstrate that intact rats can learn my DNMS task and (2) to show that they can perform it over a wide range of retention delays.

1. METHODS

Subjects

The subjects were 14 experimentally naive, male Long-Evans rats (Charles River, Quebec), 8 weeks old at the beginning of the experiment.

DNMS training

The rats were habituated and trained to perform the DNMS task as described in the preceding section. For each subject, training continued at the 4-s delay until it reached the criterion of at least 21 out of 25 correct choices on two consecutive sessions, whereupon the delay was increased to 15 s. The delay was subsequently increased to 30 s, to 60 s, and to 120 s whenever a rat reattained the criterion (two consecutive sessions of at least 21 correct trials) or completed eight sessions at a particular delay without reattaining the criterion. Each rat received four sessions at a 600-s delay after training at the 120-s delay was completed.

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4 This experiment has been published (Mumby, Pinel, & Wood, 1990).

Measuring the retention function

This phase of testing was designed to define each rat's retention function. It consisted of five mixed-delay DNMS sessions, each of which consisted of 25 trials. In each of these sessions, five trials were conducted at each of the following delays: 4 s, 15 s, 60 s, 120 s, and 600 s. These delays appeared in the following order in each session : 4 s, 15 s, 60 s, 120 s, 600 s, 600 s, 120 s, 60 s, 15 s, 4 s, 4 s, 15 s, and so on.

2. RESULTS

All 14 rats progressed successfully through the habituation and object-discrimination phases of the training, and all 14 learned the DNMS task. Once they had learned the task, they performed significantly better than chance at all delays.

Habituation

By the end of the final habituation session, all rats readily approached closed doors to gain access to food pellets on the other side.

Object discrimination

Although there was some initial hesitation, all of the rats quickly learned to displace objects from food wells. On the second object-discrimination session (i.e., the first session on which they were not permitted to make corrections), the mean number of correct trials was 65%, (ranging from 44% to

88%, SE = 3.30%), and on the fourth, and final, session the mean was 91% (ranging from 72% to 100%, SE = 2.08%).

Acquisition of DNMS

Figure 2 illustrates the performance of the DNMS task at each delay during the acquisition phase. Illustrated are the mean levels of performance on the first and last sessions at each delay. Performance during the first training session at the 4-s delay was significantly above chance (M = 59%, $t(13)$ = 4.14, $p < .005$, two-tailed; a two-tailed test was used because there is evidence that species differ in their intial propensities for selecting either the matching or the nonmatching stimulus. At the 4-s delay, 13 of the 14 rats achieved criterion within 16 sessions, not including the final two criterion sessions (M = 9.4 sessions or 235 trials). The 1 rat that did not achieve criterion at the 4-s delay scored as high as 88% on some sessions, but was inconsistent; this rat was switched to the 15-s delay after 20 sessions.

As illustrated in Figure 2, when rats were switched to delays longer than 15 s, their performance initially declined and then improved over sessions at the new delay. The number of rats reattaining criterion within the maximum of eight sessions at the 15-, 30-, 60-, and 120-s delays was 8, 12, 9, and 2, respectively. No rats achieved the criterion within the four sessions that were administered at the 600-s delay.

Retention functions

Figure 3 illustrates the mean retention functions of the 14 rats; these were calculated from the rats' performance on the five mixed-delay DNMS sessions. It should be noted that the use of an appropriate log scale for the abscissa in Figure 3 would change the shape of the retention function,

making it more linear. However, I chose to use the present scale in order to facilitate direct comparison with typical illustrations of monkeys' DNMS retention functions, many of which use the same delays and the same scale. The results of a repeated measures analysis of variance indicated that their performance declined significantly with increases in the retention delay; repeated measures $F(4, 52) = 75.72, p < .001$. However, performance at the 600-s delay was still significantly better than chance; $t(13) = 2.77, p < .05$, one-tailed. Performance at all delays was stable over the five sessions; that is, the mean scores on the first mixed-delay session were not significantly different from those on the fifth mixed-delay session.

3. DISCUSSION

All of the rats in this experiment learned the DNMS task. The rats' performance of the DNMS task was comparable to that commonly reported for monkeys in terms of both the rate at which they acquired the nonmatching rule at a brief retention delay and their accuracy at longer delays. The rats required a mean of 235 trials to achieve the initial criterion of 84% on two consecutive sessions, whereas, rhesus monkeys (Mishkin & Delacour, 1975), cynomolgus monkeys (Aggleton & Mishkin, 1983), and squirrel monkeys (Overman, McLain, Ormsby, & Brooks, 1983) required means of 90, 150, and 785 trials, respectively, to achieve a slightly more stringent criterion (e.g., at least 90% correct on two consecutive sessions or at least 90 correct on 100 consecutive trials). During the final mixed-delay test sessions, the rats in the present experiment averaged 90%, 91%, 81%, and 77% at delays of 4, 15, 60, and 120 s, respectively (see Figure 3). These levels of asymptotic performance compare favorably with the asymptotic levels observed in monkeys at comparable retention delays. The asymptotic scores of monkeys typically range between 90% and 100% at delays of about 10 s and between 85% and 95% at delays of 120 s (e.g., Aggleton & Mishkin, 1983a, 1983b; Murray & Mishkin, 1986). At the 600-s

Figure 2. Mean percent correct on the first and last session at each delay during acquisition training on DNMS. Performance improved between the first session and the last session at most delays (* $p < .05$, ** $p < .01$, ** $p < .001$). Error bars show SEMs.

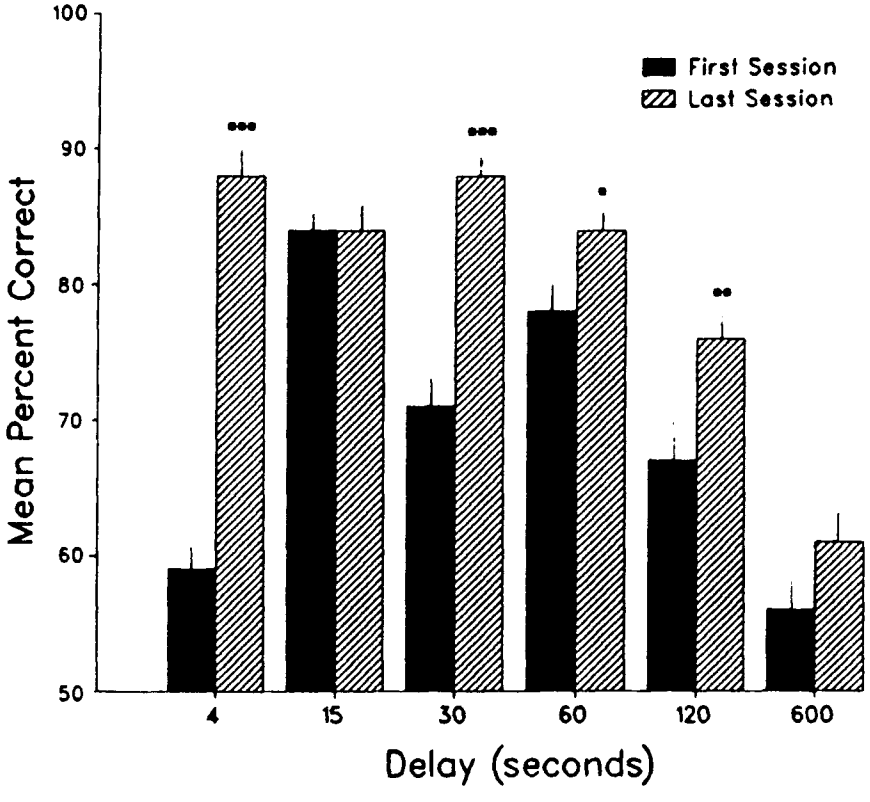
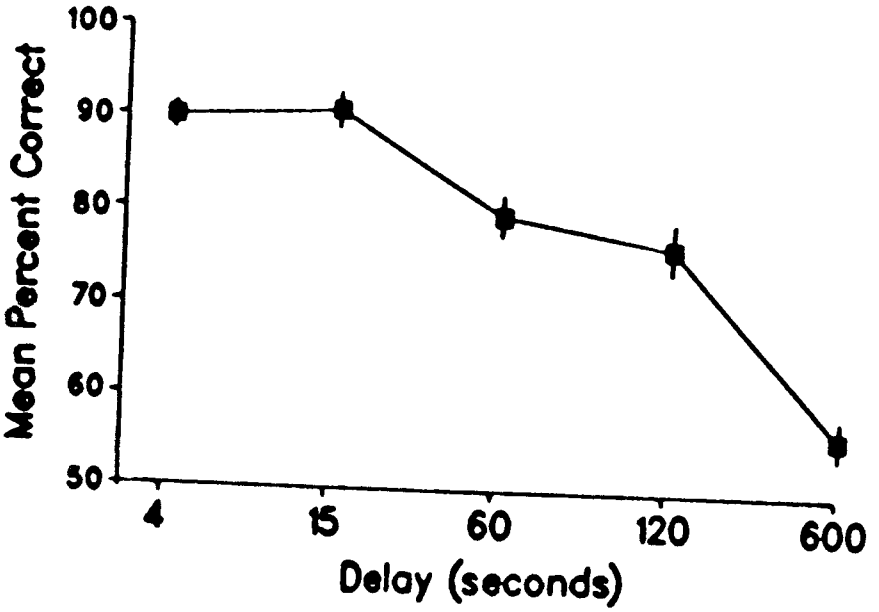

100
90
80
70
60
50
Mean Percent Correct
First Session
Last Session
***
***
*
**
4
15
30
60
120
600
Delay (seconds)

Figure 3. Mean percent correct on mixed-delay DNMS sessions. Retention was statistically significant at each delay (all ps < .01). Error bars show SEMs.


Mean Percent Correct
100
90
80
70
60
50
4
15
60
120
600
Delay (seconds)

delay, the rats scored 57% correct; although this level of performance is significantly above chance, it is considerably lower than the 80% that has been reported for monkeys (Zola-Morgan, Squire, & Amaral, 1986).

The better-than-chance first-session DNMS performance (i.e., 59%) confirmed previous observations in both rats (Aggleton, 1985; Rothblat & Hayes, 1987) and monkeys (Mishkin & Delacour, 1975). It presumably reflects the tendency of rats and monkeys to approach unfamiliar stimuli (cf. Ennaceur & Delacour, 1988).

The acquisition of the nonmatching rule was reflected in the significant improvement in the rats' performance as the training sessions progressed. The improvement in performance following the disruptive effect of lengthening the retention delay suggests that rats' DNMS abilities continued to improve with additional training after they had reached the performance criterion at the 4-s delay. There are several cognitive or perceptual abilities that may have continued to improve; for example, it is possible that the rats gradually learned to avoid distraction for increasing periods of time or that they became more efficient at encoding the physical attributes of the sample objects, or both.

To respond correctly, the rats had to learn the nonmatching principle and recognize the sample objects. They may have recognized the visual, tactual, or olfactory properties of the sample objects, or they may have circumvented the mnemonic demands of the task by odor-marking the sample objects during the sample phase of each trial. However, the following observations suggest that they based their choices primarily on their memory of the visual properties of the sample objects: (1) Rats rarely contacted objects without displacing them, which suggests that they were not responding on the basis of tactual differences between the test objects. (2) The rats frequently veered towards the correct object before reaching the goal area, which suggests that they were using visual cues. (3) During a separate series of control tests, two identical objects were used as the sample on each trial, one during the

sample phase of the trial and the other during the choice phase of the same trial; the rats performed as well on these trials as they did on conventional trials in which the same object served as the sample on both phases of the trial. This suggests that the rats were not performing the task by marking the sample objects.

The apparatus has two notable features that may have accounted for the rats' excellent performance. First, the two separate goal areas permitted the experimenter to position the sample and novel objects before the start of a trial, so that initial training could be conducted at brief retention delays (i.e., 4 s; cf. Rothblat & Hayes, 1987). Second, the presence of a central starting area eliminated the need to handle the rats during the retention intervals (cf. Aggleton, 1985; Rothblat & Hayes, 1987); distraction during retention intervals has been shown to disrupt DNMS in monkeys (Zola-Morgan & Squire, 1985a; Zola-Morgan, Squire, & Amaral, 1989a, 1989b) and humans (Squire et al., 1988).

This experiment was the second to demonstrate high levels of nonspatial working memory in rats -- the rats in Aggleton's (1985) Y-maze experiment performed almost as well. However, this experiment was the first to demonstrate impressive levels of object recognition in rats. The potential utility of this paradigm stems from the fact that it was expressly designed to mimic the widely studied monkey object-recognition DNMS task.

EXPERIMENT 2: THE EFFECTS OF DISTRACTION ON DNMS IN RATS

In order for the DNMS task to serve in rat models of brain-damage-produced amnesia that are comparable to the monkey models of brain-damage-produced amnesia, it must be shown that rats solve the DNMS task using memory abilities that are similar to those used by monkeys and humans. A comparative task analysis is a nonempirical way of determining that likelihood. Such an analysis suggests that both the rat and monkey DNMS tasks could be solved using either of two different strategies -- subjects could make their choices on the basis of (1) explicit memory for the initial presentation of the sample object, or (2) the relative familiarity of the two test objects. The former strategy would involve explicit memory, whereas the latter strategy would involve implicit memory.

The results of Experiment 1 provide some empirical evidence that rats, humans, and monkeys employ similar memory abilities when performing the DNMS task; like monkeys and humans, rats perform the DNMS task better at brief delays than at long delays. The purpose of Experiment 2 was to further examine whether rats, monkeys, and humans employ similar memory abilities when performing the DNMS task by assessing the effects on the DNMS of rats of another task manipulation (i.e., a task manipulation other than delay) that has been shown to influence the DNMS of humans and monkeys. Experiment 2 assessed the effects of distraction during the retention delay on the DNMS performance of rats.

In the late 1950s, Brown (1958) and Peterson and Peterson (1959) observed that humans display rapid forgetting of small amounts of verbal material if they engage in another cognitive task (e.g., counting backwards by threes) during the retention interval. Milner (1972) and Sidman et al. (1968) subsequently observed that introducing distraction during the retention delay produced a severe memory deficit in patients with brain-damage-produced amnesia, even when no deficits were apparent in the absence of distraction. There is controversy over whether distraction during the retention delay

has greater effects on the performance of amnesic patients than on the performance of normal subjects. Cermak, Butters, and Moreines (1974) found that Korsakoff patients performed significantly worse than control subjects on the Brown-Peterson task, whereas Baddeley and Warrington (1970) found that they did not.

Etkin (1972) observed that the performance of monkeys on a delayed matching-to-sample task was significantly better when the testing room lights were extinguished during the retention delay than when they were not. He concluded that these results reflected decreased retroactive interference from visual input during the darkened retention delays. Using an approach that was more similar to the Brown-Peterson paradigm, Zola-Morgan and Squire (1985) and Zola-Morgan et al. (1989a, 1989b) observed that the DNMS of monkeys was poorer when distractor objects, which the monkeys could displace from food wells in order to receive a food reward, were presented during the retention delay. This distraction procedure was adapted for use with rats in the present experiment.

1. METHODS

The methods were identical to those of Experiment 1, except where otherwise noted.

Subjects

The subjects were 8 male Long-Evans rats, all of which had previously served as control rats in other DNMS experiments -- each had previously received between 1000 and 1700 DNMS trials. Two of them had received bilateral sham lesions of the mediodorsal thalamus; electrodes had been lowered into the mediodorsal nuclei and withdrawn, without the passage of current. Two of them had received a sham bilateral hippocampectomy; a portion of posterior parietal cortex and corpus callosum overlying

the dorsal hippocampus had been aspirated bilaterally, without damaging the hippocampus. One of them had been a control subject in an ischemia experiment; ligatures had been placed around both carotid arteries, but they had not been constricted, and one of the femoral arteries had been cannulated but no blood had been withdrawn. Three of them were intact control rats from Experiment 3. None of the 8 subjects had displayed any DNMS deficits prior to the commencement of Experiment 2.

Procedure

Each rat received 10 DNMS sessions of 20 trials each. The delay was 60 s on each trial. All of the trials during odd-numbered sessions were ordinary DNMS trials (no-distraction trials), whereas all of the trials during even-numbered sessions included a distraction task during the retention delay (distraction trials).

On each distraction trial, 20 s after the rat had displaced the sample object, the far door was raised to reveal a single distractor object over a baited well at the other end of the apparatus. As the rat displaced the distractor object, the far door was lowered. Then, 20 s after the first distractor object had been revealed, the far door was raised to reveal another distractor object over a baited food well. As the rat displaced the second distractor object, the far door was lowered behind it. Then, 20 s after the second distractor object had been revealed (i.e., 60 s after the rat had displaced the sample object), the door was raised to reveal the sample object and a novel object. A food pellet was delivered to the exposed food well if the novel object was displaced.

The two different distractor objects that were used on each trial were different than the distractor objects that were used on other trials within a session. Accordingly, 80 different objects -- 20 samples, 20 novel objects, and 40 distractor objects -- were used during each session. They were selected from the pool of 350 objects.

2. RESULTS

Figure 4 illustrates the mean scores for each of the 8 rats on the 100 distraction trials and on the 100 no-distraction trials. Each of the 8 rats obtained a lower average score on the distraction trials than on the no-distraction trials ($p < .005$). Their mean score on distraction trials was significantly lower than on no-distraction trials; $t(7) = 4.76, p < .01$.

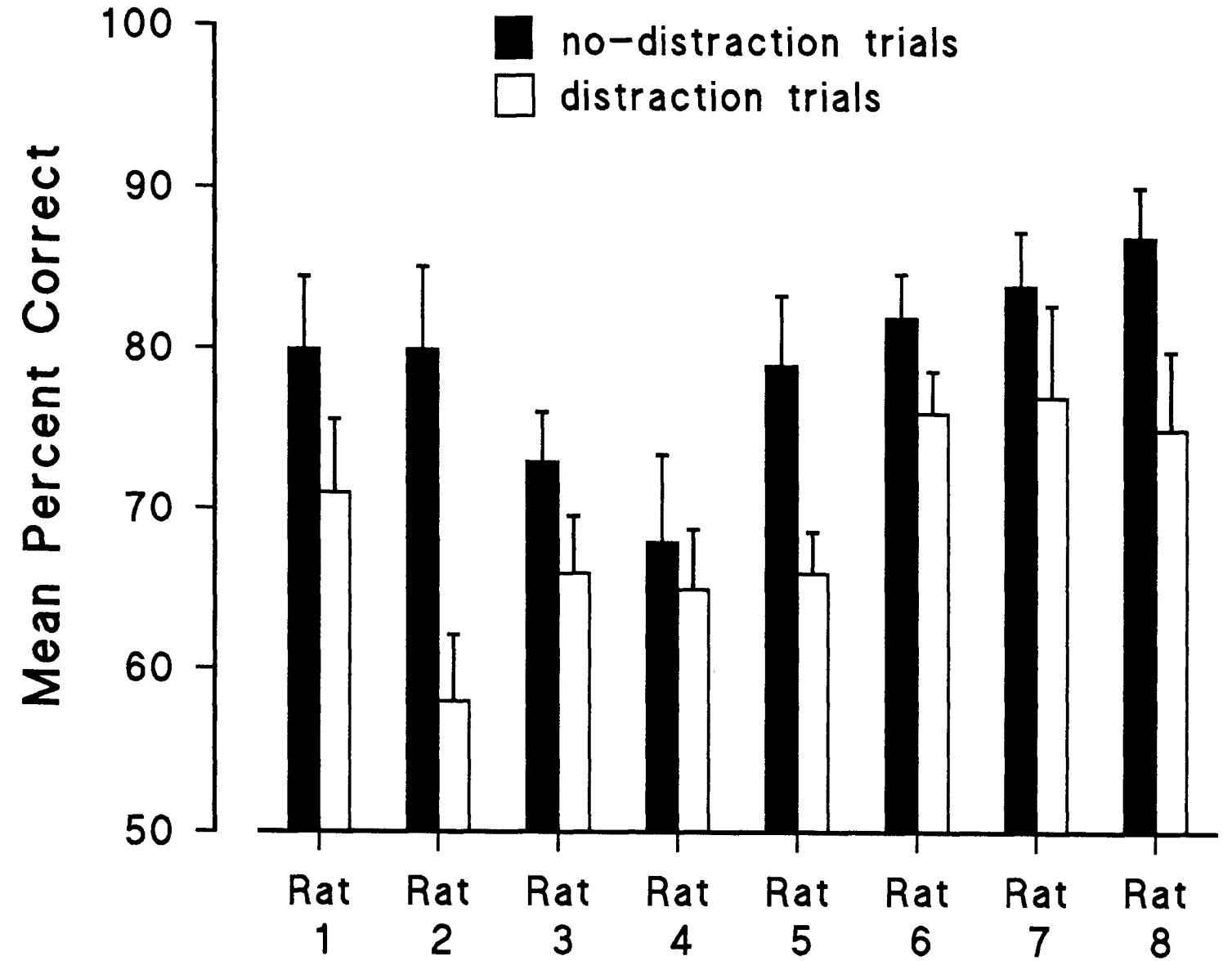
The results of two repeated measures analyses of variance indicated that the scores on neither the distraction trials ($F[4, 28] = 1.53, p = .219$) nor the no-distraction trials ($F[4, 28] = 1.41, p = .255$) changed significantly over the five sessions.

The performance of the rats on no-distraction trials ($M = 79\%$) was similar to that of the rats in Experiment 1, when they were tested at the same (i.e., 60-s) delay ($M = 81\%$).

3. DISCUSSION

In this experiment, the DNMS performance of rats was disrupted by the inclusion of a distraction task during the retention delay. The present experiment was the first to assess the effects of an interpolated activity during the delay on the DNMS performance of rats. My observation of disruptive effects of distraction during the delay on the DNMS performance of rats is consistent with reports of similar findings in monkeys (Zola-Morgan & Squire, 1985a; Zola-Morgan et al., 1989a, 1989b) and humans (Squire et al., 1988).

Figure 4. Mean scores on no-distraction and distraction trials. Error bars show SEMs.


100
90
80
70
60
50
Mean Percent Correct
no-distraction trials
distraction trials
Rat 1
Rat 2
Rat 3
Rat 4
Rat 5
Rat 6
Rat 7
Rat 8

The rats' performance of the distraction task may have interfered with the mnemonic processing of the sample objects, the novel objects, or both. It is possible that the rats' DNMS performance was worse on distraction trials than on no-distraction trials because the novel procedure that was used on distraction trials confused them. Two observations suggest that this was not the case: First, the rats' DNMS performance on distraction trials did not show any signs of improvement over the five sessions; if the poor performance on distraction trials was caused by the novelty of the procedure used on those trials, then this effect should have lessened as the rats gained experience with it. Second, none of the rats showed any indication that they were confused on any of the distraction trials -- all of them readily approached and displaced objects whenever they were revealed.

It is likely that the distraction task that was used in the present experiment and the distraction task that is used in monkey DNMS experiments disrupted performance through more than distraction *per se*. Because the distractor stimuli were similar to the test stimuli (i.e., they were objects of similar size), the performance of the distraction task may have disrupted mnemonic processing through proactive or retroactive interference. Be that as it may, the fact that the present findings parallel those of monkey experiments that employed the same distraction task suggests that rats, monkeys, and humans employ similar memory abilities when performing the DNMS task.

In humans, distraction during the retention delay disrupts performance of explicit-memory tasks but has little or no effect on the performance of implicit memory tasks (Graf & Schacter, 1987; Sloman, Hayman, Ohta, Law, & Tulving, 1988). Thus, the present finding suggests that the memory abilities that rats use when performing the DNMS task resemble those that humans use when performing explicit-memory tasks, but not those that humans use when performing implicit-memory tasks.

EXPERIMENT 3: THE EFFECTS OF SEPARATE AND COMBINED LESIONS OF THE HIPPOCAMPUS AND AMYGDALA ON DNMS IN RATS[5]

Bilateral damage to the medial temporal lobes has been shown to produce DNMS deficits in both monkeys and humans, but there is controversy over which medial-temporal-lobe structures must be damaged to produce these deficits. Impaired DNMS has been found in monkeys with hippocampal (Mahut et al., 1982; Zola-Morgan & Squire, 1985a; Bachevalier & Mishkin, 1989) or amygdalar (Murray & Mishkin, 1984) damage; however, in other studies, impairments in the DNMS performance of monkeys have been observed only if both the hippocampus and amygdala (e.g., Mishkin, 1978) or their primary efferents (Bachevalier et al., 1985) have been damaged. Recent evidence suggests that the impairments of DNMS following lesions of the hippocampus and amygdala in monkeys may result from incidental cortical damage (Murray et al., 1989; Murray & Mishkin, 1986; Zola-Morgan et al., 1989a, 1989c).

Recently, the controversy over the role of the hippocampus and the amygdala in recognition memory has extended to research on rats. Olton and Feustle (1981) found impaired recurring-items DNMS in rats with hippocampal lesions, whereas Aggleton, Hunt, and Rawlins (1986), Kesner (1991), and Rothblat and Kromer (1991) found no DNMS impairment in rats with hippocampal lesions. Aggleton, Blindt, and Rawlins (1989) found no impairment of DNMS following amygdala lesions, but they observed a substantial impairment following amygdalo-hippocampal lesions; however, they emphasized that collateral pyriform-cortex damage may have contributed to the impairment displayed by the rats with amygdalo-hippocampal lesions.

The amount of presurgery training on the DNMS task seems to influence the magnitude of postsurgery deficits. For example, hippocampal lesions in monkeys appear to have a less disruptive

5 This experiment is currently in press (Mumby, Wood, & Pinel, in press)

effect on their DNMS performance when they receive presurgery training (e.g., Mishkin, 1978; Murray & Mishkin, 1984) than when they do not (e.g., Zola-Morgan & Squire, 1986). Presurgery training has advantages and disadvantages. On one hand, postsurgery testing of subjects that have not had presurgery training is more likely to reveal an effect of the lesion. On the other, presurgery training allows one to establish stable baselines of performance in individual subjects to which their postsurgery performance can be compared. More importantly, presurgery training reduces postsurgery deficits that are due to impaired acquisition of the skills that are required for successful performance, rather than to impaired retention of the test objects, thus making postsurgery performance deficits more easy to interpret.

The purpose of Experiment 3 was to assess the effects of hippocampal, amygdalar, and combined amygdalo-hippocampal lesions on DNMS in rats. Rats were tested at retention delays of 4, 15, 60, 120, and 600 s both before and after receiving either bilateral hippocampal, amygdalar, or amygdalo-hippocampal lesions. After postsurgery testing, some of the rats with hippocampal lesions and some of the rats with amygdalar lesions received an additional bilateral lesion in order to give them combined amygdalo-hippocampal lesions; then, they were retested. Thus, some of the rats with amygdalo-hippocampal lesions received one-stage lesions, and others received two-stage lesions.

I chose to make aspiration lesions of the hippocampus rather than neurotoxic lesions because the former method, while not as selective as the latter, enables more complete lesions of the hippocampal formation -- in my view, it was best to begin studying the effects of hippocampal lesions on the performance of the new DNMS task by making complete lesions. Moreover, one of the major aims in this study was to compare the effects of similar lesions in rats and monkeys -- hippocampal lesions have been made by aspiration in most of the comparable monkey studies.

1. METHODS

The methods were identical to those of Experiment 1, except where otherwise noted.

Subjects

The subjects were 22 experimentally naive, male Long-Evans rats that were between 8 and 12 weeks old at the beginning of training, and 7 of the rats that had served in Experiment 1.

Habituation, object-discrimination, DNMS training, and measuring the presurgery retention function

Habituation, object-discrimination training, DNMS training, and measurement of the presurgery retention functions were conducted in the same way as in Experiment 1 -- with two exceptions: (1) For DNMS training, the number of trials per session was 25 for the 7 subjects that were used in Experiment 1, but it was reduced to 20 for the other 22 subjects so that more rats could be run each day. The performance criterion was 84% of the trials correct on two consecutive sessions (21 out of 25 or 17 out of 20). (2) Some of the rats' received 5 mixed-delay sessions, whereas the others received 10 mixed-delay sessions; although performance was stable over the 5 mixed-delay sessions in Experiment 1, I thought that it might improve if the rats received more testing. The data from Experiment 1 for the 7 rats that had served in that experiment comprised their presurgery data for the present experiment.

Surgery

Following presurgery testing, each rat received either bilateral aspiration lesions of the hippocampus ($n = 11$), electrolytic lesions of the amygdala ($n = 7$), or combined amygdalo-hippocampal lesions ($n = 4$), or it was assigned to a no-surgery control group ($n = 6$). Hippocampal aspiration required the removal of posterior parietal neocortex; a control group of rats with posterior parietal neocortex damage was not included because a pilot experiment had indicated that such lesions do not affect DNMS performance in pretrained rats. The 2 rats in this pilot experiment were trained and tested in the same way as those of the present experiment, except that the longest delay was 300 s instead of 600 s. Their mean presurgery scores were 95%, 85%, 75%, 78%, and 78%, at delays of 4, 15, 60, 120, and 300 s, respectively; their mean postsurgery scores were 92%, 85%, 80%, 82%, and 75%.

All surgery was performed under pentobarbitol anesthesia (60 mg/kg). In preparation for hippocampal aspiration, the scalp was incised and holes were drilled on each side of the skull. Each hole extended from approximately 2 mm posterior to the coronal suture to 2 mm anterior to the lamboid suture, and from 2 mm lateral to the sagittal suture to within 1 mm of the temporal ridge. Then, the exposed dura mater was cut, and the underlying neocortex and white matter were aspirated with a glass Pasteur pipette to expose the dorsal hippocampus. Next, the dorsal hippocampus and part of the lateral hippocampus were aspirated and the cavity was filled with Gelfoam (Upjohn Co., Don Mills, Ontario).

The bilateral amygdalar lesions were made with a bipolar stainless steel wire electrode, which was insulated with Teflon except for approximately 1 mm at its tip. The lesions of each amygdala were made at three sites; the following were the coordinates relative to bregma of the three sites: (1) AP -2.3, ML -4.5, DV -9.8; (2) AP -3.3, ML -4.5, DV -10.0; (3) AP -4.3, ML -4.5, DV -10.0. At each site, 2 mA of current was passed for 20 s.

The above procedures for hippocampal and amygdala surgery were combined for the rats that received one-stage amygdalo-hippocampal lesions. The amygdalar lesions were made first, then the hippocampal lesions.

After surgery, the rats that received hippocampal or amygdalo-hippocampal lesions were placed under a heat lamp in a recovery room for 1 day. Diazepam (10-15 mg/kg, IP) was administered as soon as they began to regain consciousness, and for the next 24 hr smaller doses were periodically administered to control convulsions that we have sometimes observed in rats following hippocampal lesions. Few convulsions were observed. The rats that received only amygdalar lesions were returned to their home cages immediately after surgery.

All experimental rats were allowed at least 14 days to recover from surgery before the commencement of postsurgery testing. Similarly, control rats were not tested for at least 14 days following their final presurgery mixed-delay session. During the first 10 days each experimental and control rat had continuous access to food, after which they were returned to a restricted feeding regimen for at least 4 days before testing recommenced.

Reacquisition of DNMS

Following recovery, rats were tested on the DNMS task at 4-s delays until they either reattained the original performance criterion (i.e., at least 84% of the trials correct on two consecutive sessions) or completed 20 sessions without reattaining it.

Postsurgery retention functions

Next, each rat's postsurgery retention function was determined in the same way that its presurgery retention function had been determined -- with either 5 or 10 mixed-delay sessions.

Stage-two lesions and testing

Some of the rats then received a second operation; 5 rats with amygdalar lesions received hippocampal lesions and 7 rats with hippocampal lesions received amygdala lesions. Following recovery from their second lesion, these rats were treated and tested as they had been following their first lesion.

2. RESULTS

The main result was that reacquisition of the DNMS task and postsurgery performance at delays of up to 120 s were unimpaired following either separate or combined bilateral lesions of the hippocampus and amygdala. Minor, but statistically significant, impairments were observed in all three experimental groups at delays of 600 s. Four rats with hippocampal lesions sustained inadvertent damage to entorhinal, perirhinal, or temporal association cortex; they were profoundly impaired at all delays. The results of the various phases of the experiment are described in the following subsections.

Habituation

By the end of the final habituation session, all rats readily approached closed doors to gain access to food pellets on the other side.

Object-discrimination training

All rats quickly learned the object discrimination task. On Session 2, the first session on which they were not allowed to correct their errors, scores averaged 68% (ranging from 44 to 88%), and on the final session, scores averaged 92% (ranging from 76 to 100%).

DNMS training

On the DNMS task, the rats required a mean of 280 trials (SE = 22.7) to reach the performance criterion at the 4-s delay. There were no significant differences in the number of trials to criterion or in the presurgery percent-correct scores between rats that received 20 trials per session and those that received 25 trials per session, so the data from all of the rats were pooled for the purpose of statistical analysis. Most rats' scores dropped each time that the delay was lengthened, and then recovered over subsequent sessions. Excluding the first two sessions at each delay, the mean-percent-correct scores at each delay during acquisition training were almost identical to those obtained during the subsequent mixed-delay sessions.

Presurgery retention functions

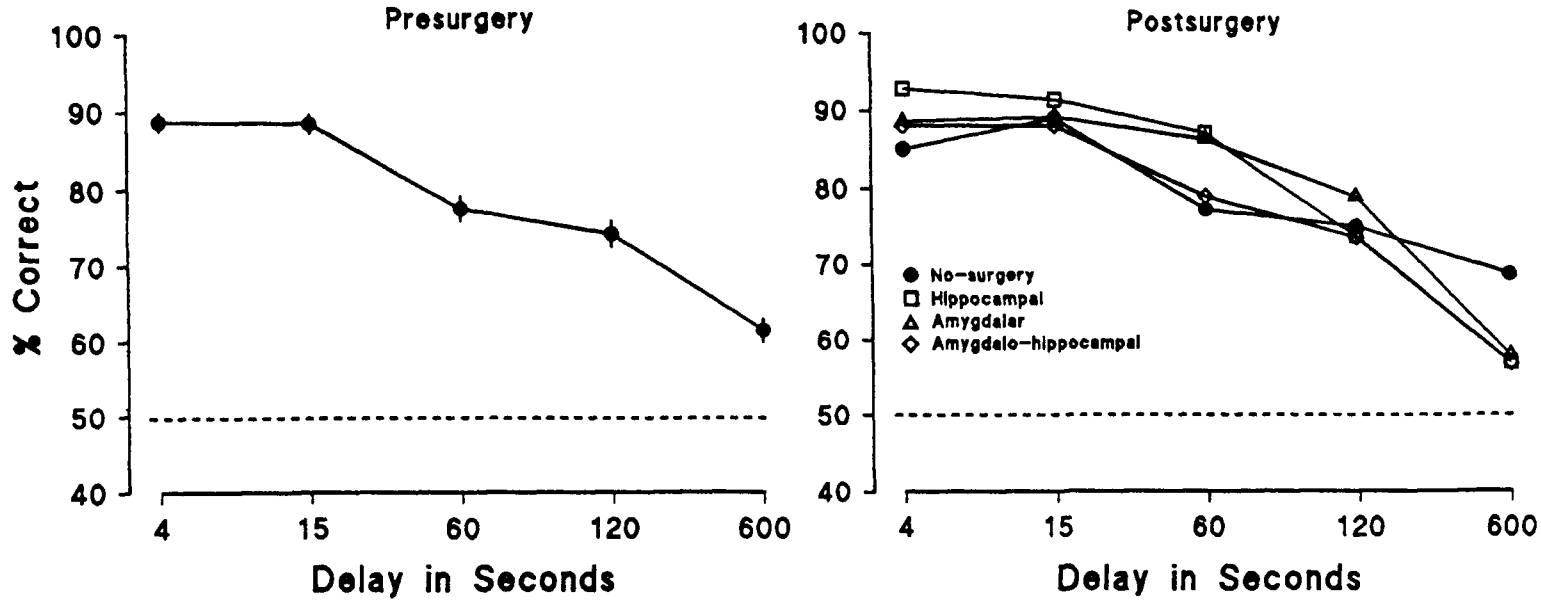
As shown in Figure 5, scores on the presurgery mixed-delay sessions declined significantly as the delay was lengthened ($F[4,115] = 52.6, p < .001$). There were no significant differences among the groups' presurgery scores at any delay. One-sample t tests revealed that the scores in each group were significantly better than chance at all delays (all ps $< .05$; one-tailed). Performance at all delays was stable over the mixed-delay sessions regardless of whether rats received 5 or 10 of these sessions; that is, the mean scores from the first mixed-delay session were not significantly different from those from the final mixed-delay session.

Histology

Figure 6 shows the location and the extent of the largest and smallest amygdalo-hippocampal lesions. In several of the rats with two-stage amygdalo-hippocampal lesions, the amygdalar and hippocampal lesions overlapped to form a continuous lesion, thus making it impossible to determine the boundaries of the damage sustained during each surgery. Accordingly, Figure 6 also shows the damage sustained by one of the rats with hippocampal lesions that did not subsequently receive amygdalar lesions and by one of the rats with amygdalar lesions that did not subsequently receive hippocampal lesions; the damage in these two rats was representative of that sustained by the other rats with hippocampal and amygdalar lesions.

Most of the hippocampal lesions included the entire dorsal hippocampus, most of the lateral hippocampus, and a portion of the neocortex and corpus callosum that overlies the dorsal hippocampus. The ventral extent of most of the hippocampal lesions spared small portions of the

Figure 5. The presurgery (left) and postsurgery (right) retention functions that were determined on mixed-delay sessions. Error bars show SEMs for presurgery scores. The SEMs for postsurgery scores were similar and ranged from 0.97 to 3.70.


Presurgery
Postsurgery
% Correct
100
90
80
70
60
50
40
4
15
60
120
600
Delay in Seconds
No-surgery
Hippocampal
Amygdalar
Amygdalo-hippocampal

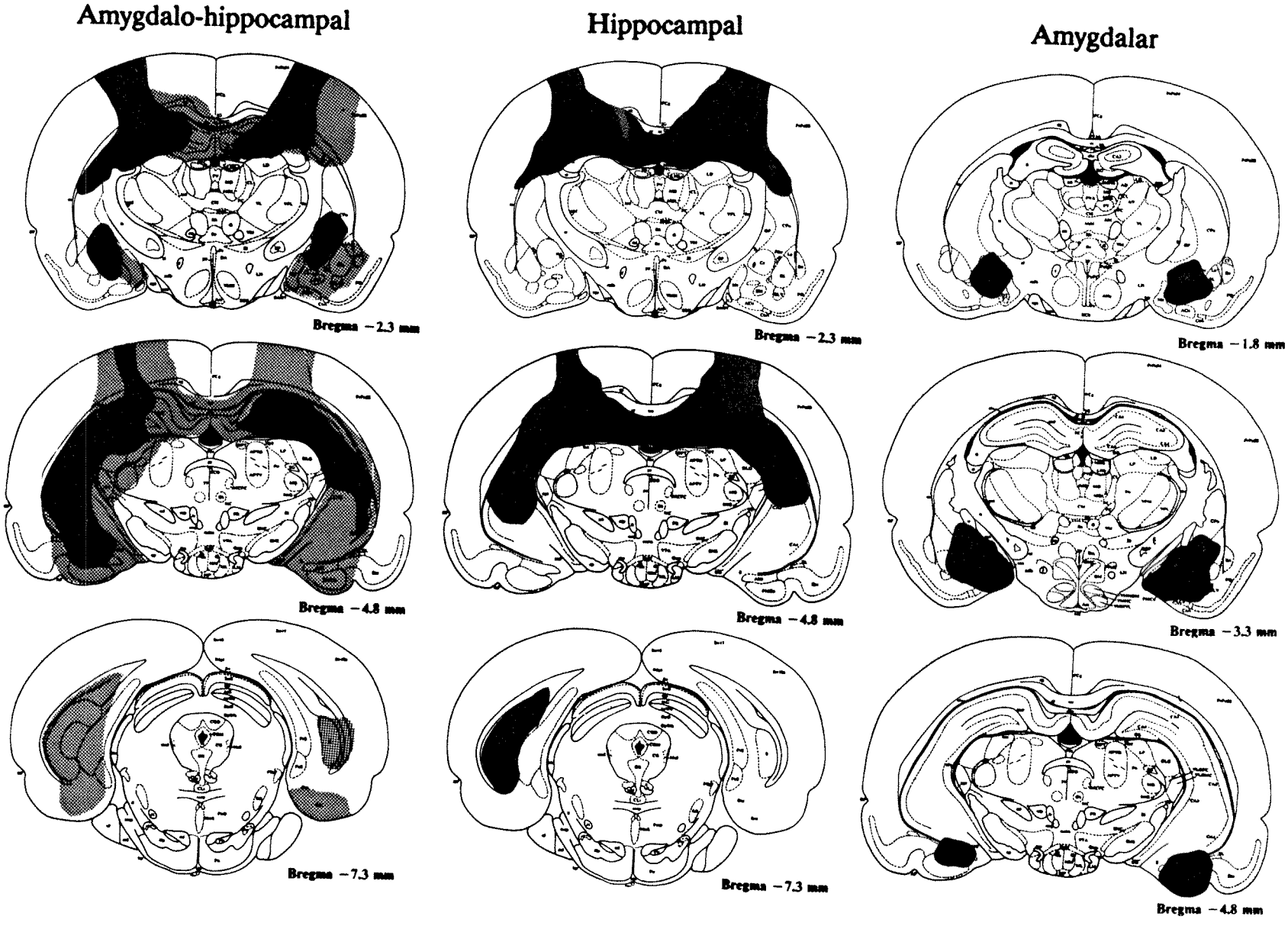
dentate gyrus and subiculum. The caudal extent of these lesions varied in the amount of presubiculum and parasubiculum that was removed. Each lesion extended rostrally to include the fimbria fornix, and two of them included slight unilateral damage to dorsal portions of the lateral septal nucleus. Some rats also sustained small unilateral lesions in the caudate nucleus.

Small infarcts were also present in the dorsal thalamus of some of the brains with hippocampal lesions. Most of these were unilateral and involved the habenular nuclei, lateral dorsal nucleus, lateral pulvinar nucleus, lateral geniculate, and medial geniculate nucleus; damage to these thalamic nuclei appeared to be unrelated to the behavioral results. Some of the brains with hippocampal lesions also received partial unilateral damage to the tectum.

Four of the rats with hippocampal lesions sustained cortical damage that was much more extensive than in the other rats in that group (see Figure 8). This damage was unintended and it included portions of entorhinal, perirhinal, and temporal association cortex (area Te2; Zilles, 1985). Accordingly, the behavioral data from these 4 rats were excluded from the overall statistical analyses -- they are dealt with separately at the end of this section. The extent of damage to the thalamus and tectum in these 4 rats was not unlike that found in the other rats with hippocampal lesions; however, one of them sustained large bilateral lesions of the lateral septal nucleus.

The amygdala lesions varied in the extent of damage to specific nuclei, but the most consistent damage was to the medial two-thirds of the amygdaloid complex. No specific amygdaloid nuclei were consistently spared. The caudal extent of some of the amygdala lesions included small portions of medial entorhinal cortex (see the largest amygdalo-hippocampal lesion in Figure 6), but the presence of such damage did not appear to be related to the behavioral results.

Figure 6. Reconstructions of (a) the largest (grey) and smallest (black) amygdalo-hippocampal lesions, (b) the lesions from one of the rats with hippocampal lesions that did not subsequently receive amygdalar lesions, and (c) the lesions from one of the rats with amygdalar lesions that did not subsequently receive hippocampal lesions. The drawings were adapted from the atlas of Paxinos and Watson (1982).


Amygdalo-hippocampal
Hippocampal
Amygdalar
Bregma −2.3 mm
Bregma −2.3 mm
Bregma −1.8 mm
Bregma −4.8 mm
Bregma −4.8 mm
Bregma −3.3 mm
Bregma −7.3 mm
Bregma −7.3 mm
Bregma −4.8 mm

Reacquisition of DNMS

On the first few trials of the first postsurgery testing session, many of the lesioned rats were slow to approach the goal areas when the doors were raised, but by the end of that session, most of them approached the goal areas without hesitation. Each rat readily displaced test objects from the food wells during the first postsurgery session and continued to do so thereafter. The mean number of postsurgery trials at the 4-s delay that was required to reattain the criterion by the no-surgery controls and the rats with hippocampal, amygdalar, and amygdalo-hippocampal lesions was 66.7 (SE = 66.7), 53.6 (SE = 10.1), 40.7 (SE = 19.8), and 66.2 (SE = 22.4), respectively. None of the differences among these means was statistically significant (F[3,32] < 1).

Postsurgery retention functions

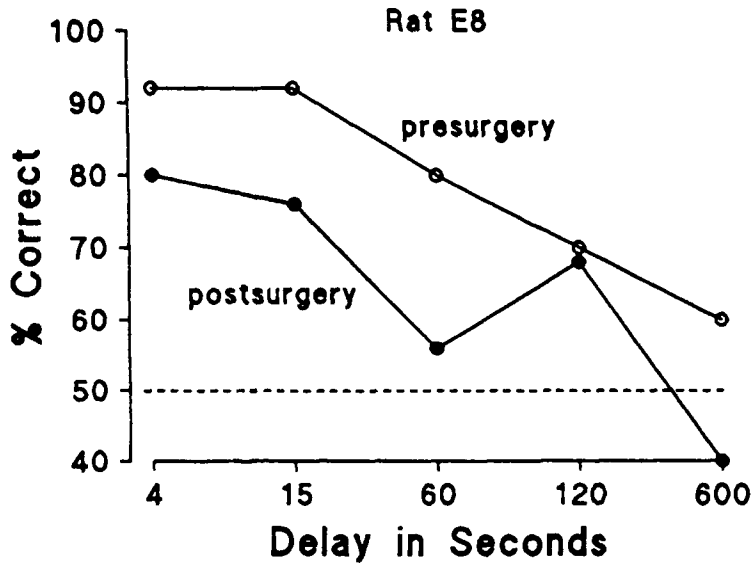
The mean postsurgery scores for each group on the mixed-delay sessions are illustrated in Figure 5. Analysis of variance indicated that the postsurgery performance of rats with one-stage amygdalo-hippocampal lesions was similar to that of rats with two-stage amygdalo-hippocampal lesions on mixed-delay sessions (F[1,14] < 1). Therefore, the data from all 16 rats with amygdalo-hippocampal lesions -- 4 with one-stage lesions and 12 with two-stage lesions -- were pooled for the purposes of statistical analysis and the presentation of results in Figure 5.

There were no statistically significant differences between the presurgery and postsurgery scores of any of the groups at any delay. For multiple planned comparisons between the groups' postsurgery scores at each delay, the critical confidence level was .01. Relative to the performance of the no-surgery control rats, postsurgery scores were significantly lower in all three experimental groups at the 600-s delay; hippocampal lesions $t(11) = 3.16$, amygdalar lesions $t(11) = 3.34$, and amygdalo-hippocampal

lesions $t(20) = 3.20$, all $ps < .01$). These differences occurred because the scores in each experimental group at the 600-s delay were slightly lower after surgery than before, while the scores in the no-surgery control group were slightly higher after surgery than before. One-sample t tests revealed that the postsurgery scores at the 600-s delay in each of the three experimental groups were still significantly better than chance (hippocampal, $t(7) = 3.41$; amygdalar, $t(7) = 2.92$; amygdalo-hippocampal, $t(16) = 2.49$; all $ps > .05$; one-tailed). There were no statistically significant differences among the four groups at any of the delays less than 600 s.

The 4 rats with hippocampal lesions that also sustained unintended damage to temporal association, perirhinal, and lateral entorhinal cortex displayed profound postsurgery impairments. One of them (E3) averaged 80% correct over the last 5 postsurgery sessions at the 4-s delay (i.e., Session 15 to 20) but could not achieve the criterion, and another one (E8) required 120 postsurgery sessions to reach the criterion, which was more than was required by any of the hippocampal-lesioned rats without damage to these areas of cortex. The presurgery and postsurgery retention functions for these 2 rats are illustrated in Figure 7, and reconstructions of their lesions are shown in Figure 8. Both of them displayed postsurgery deficits at all delays. The other 2 rats with unintended damage to entorhinal, perirhinal, and temporal association cortex averaged less than 65% correct over the last 5 postsurgery sessions at the 4-s delay, and thus they were not subsequently tested on mixed-delay sessions; their lesions are illustrated in Figure 8.

Figure 7. Presurgery (filled circles) and postsurgery (open circles) retention functions for 2 rats (E3 & E8) that sustained inadvertent damage to entorhinal, perirhinal, and temporal association cortex. The data are from mixed-delay sessions.


Rat E8
100
90
80
70
60
50
40
% Correct
presurgery
postsurgery
4
15
60
120
600
Delay in Seconds

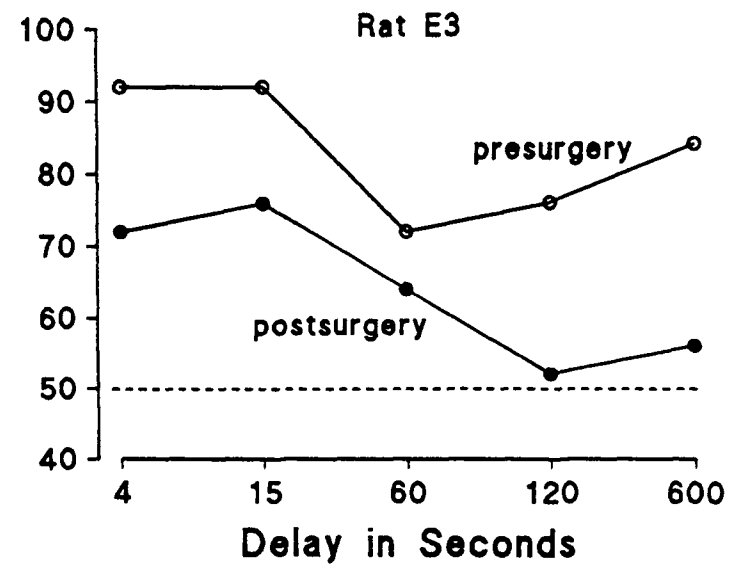
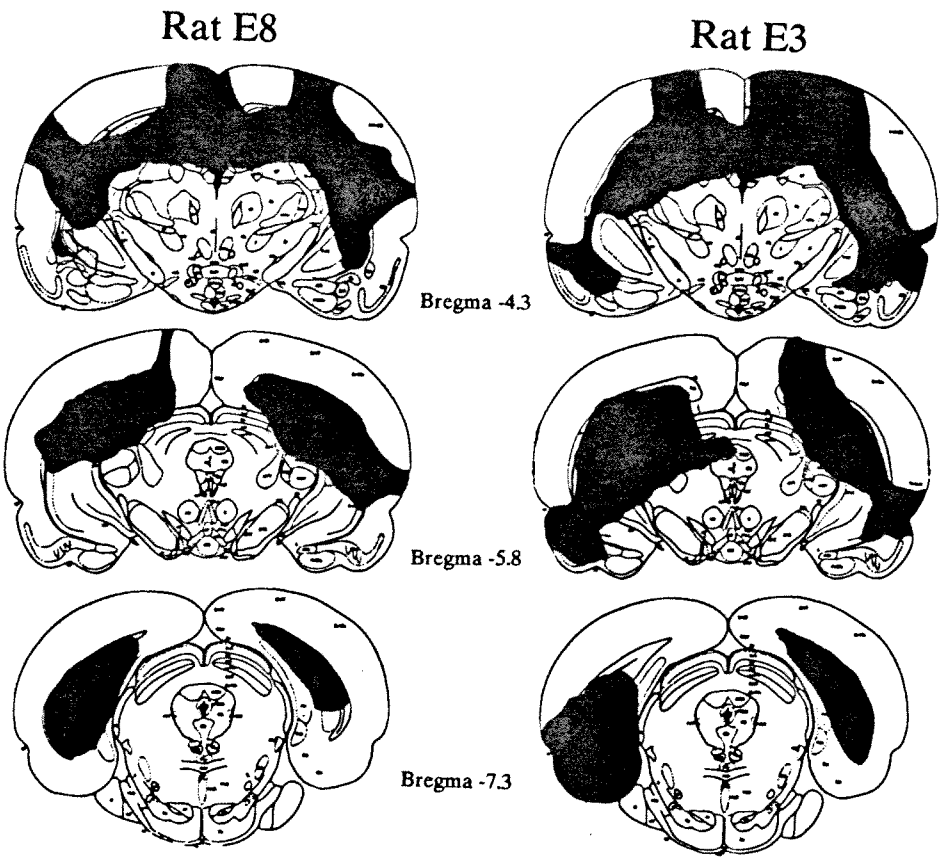
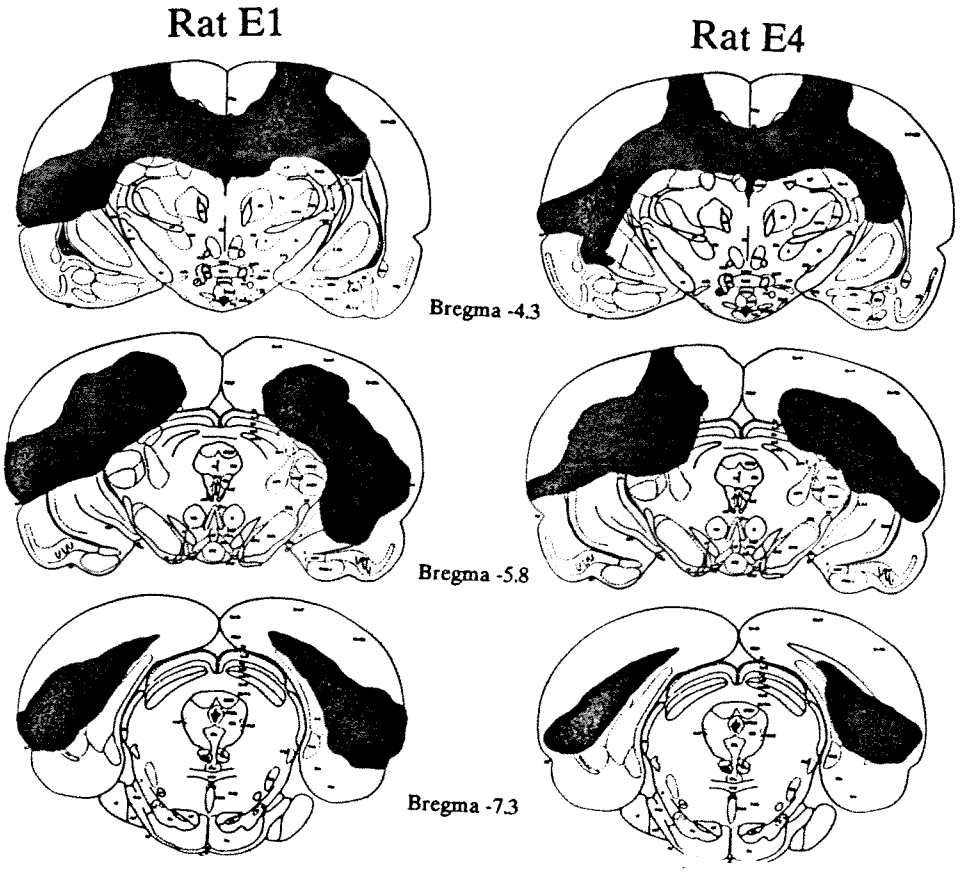

Rat E3
100
90
80
70
60
50
40
presurgery
postsurgery
4
15
60
120
600
Delay in Seconds

Figure 8. Lesions in 4 rats with hippocampal lesions that sustained inadvertent damage to entorhinal, perirhinal, and temporal association cortex.


Rat E8
Rat E3
Bregma -4.3
Bregma -5.8
Bregma -7.3


Rat E1
Rat E4
Bregma -4.3
Bregma -5.8
Bregma -7.3

3. DISCUSSION

All rats in this experiment received extensive presurgery training on the DNMS task. Then, the experimental rats received lesions of either the hippocampus, the amygdala, or both. All three groups displayed only mild postsurgery DNMS deficits; they were significantly impaired at delays of 600 s, but not at delays of 120 s or less.

The observation of only minor DNMS deficits in rats following bilateral lesions of the hippocampus alone or the amygdala alone are consistent with previous reports in both monkeys and rats. Mishkin (1978) and Murray and Mishkin (1984, 1986) observed only mild DNMS deficits in monkeys with hippocampal or amygdalar lesions; Rothblat & Kromer (1991) observed no DNMS deficits in rats with hippocampal lesions at delays of 30 s; Aggleton et al. (1986) observed no DNMS deficits in rats with hippocampal lesions or amygdalar lesions at delays of 60 s; Kesner (1991) observed no DNMS deficits in rats with hippocampal lesions on a DNMS task that was modelled after the one used in the present experiment. In contrast, the present finding of only mild DNMS deficits following combined amygdalo-hippocampal lesions is inconsistent with many previous reports. Profound DNMS impairments have been reported following amygdalo-hippocampal lesions both in monkeys (e.g., Mishkin, 1978; Zola-Morgan & Squire, 1985a) and in rats (Aggleton et al., 1989).

The results of recent studies with monkeys suggest a way of reconciling the present results with the numerous reports of major impairments of DNMS following combined lesions of the hippocampus and amygdala. They suggest that amygdalo-hippocampal lesions do not produce profound impairments on DNMS unless there is collateral damage to adjacent cortex. In primates, amygdalo-hippocampal lesions are usually created by aspiration, and thus they include damage to portions of the parahippocampal gyrus and the entorhinal cortex, and, in some cases, the perirhinal cortex (Murray & Mishkin, 1986). Impaired DNMS has been found in monkeys following bilateral lesions restricted to

these cortical areas (Meunier et al., 1990; Murray et al., 1989; Zola-Morgan et al., 1989c). Furthermore, monkeys with combined amygdalo-hippocampal lesions that spare the cortex surrounding the amygdala (i.e., entorhinal, perirhinal, and periamygdaloid cortices) perform no worse on a DNMS task than monkeys with lesions of the hippocampus and parahippocampal cortex (Zola-Morgan et al., 1989a). Thus, it is possible that the present rats with amygdalo-hippocampal lesions were only mildly impaired on DNMS because associated cortical areas were spared -- the dorsal approach that is used to aspirate the hippocampus in rats damages the posterior parietal association cortex (Kolb, 1990), and it spares the entorhinal cortex and the perirhinal cortex.

In the present experiment, 4 of the rats with hippocampal lesions sustained unintended damage to various amounts of entorhinal and perirhinal cortex. They were the only subjects that displayed severe postsurgery DNMS deficits. It is possible that the damage to entorhinal and perirhinal cortex was responsible for these deficits, but each of them also sustained damage to other structures that were not damaged in most of the rats with hippocampal lesions. Most notably, all 4 of these rats received damage to the lateral geniculate nucleus. Although this damage was not complete, it raises the possibility that visual impairment may have been responsible for their deficits.

Another possibility is that the DNMS deficits of these 4 rats resulted from damage to the temporal association cortex, which they all sustained. Kolb, Burhman, and McDonald (1989) found that rats with lesions of the temporal association cortex were unable to learn a Y-maze matching-to-sample task, whereas rats with lesions of the posterior parietal cortex displayed normal learning. In monkeys, lesions in the homologous brain region -- the inferotemporal cortex -- produce impairments on visual discrimination tasks and impairments across all delays of a delayed matching-to-sample task (Horel et al., 1987). In the present experiment, the 2 rats with perirhinal, lateral entorhinal, and temporal association cortex damage that were tested on mixed-delay sessions both displayed postsurgery deficits across all delays. Accordingly, the possibility cannot be ruled out that their postsurgery DNMS

impairments were due to perceptual rather than memory deficits, or to damage outside of the perirhinal and entorhinal cortex. For example, these rats all sustained extensive bilateral damage to the cingulum. Still, it seems unlikely that their hippocampal or amygdalar damage was a critical factor in their impairments because their hippocampal and amygdalar damage was no more extensive than that sustained by the other rats, which displayed only mild impairments.

There is a second factor--in addition to the lack of collateral damage to critical areas of cortex--that could have contributed to the good DNMS performance of the present subjects with amygdalo-hippocampal lesions. This second factor is the extensive presurgery training that they received. In monkeys, impairments on DNMS tasks following hippocampal lesions tend to be greater in subjects that receive no presurgery training (e.g., Bachevalier & Mishkin, 1989; Mahut et al., 1982; Zola-Morgan & Squire, 1986) than in subjects that do (e.g., Bachevalier et al., 1985; Mishkin, 1978; Murray & Mishkin, 1986). In the recent study of Rothblat and Kromer (1991), hippocampal lesions did not impair DNMS performance in rats that had received presurgery training. It is possible that the extensive presurgery training in the present experiment (M = 1211 presurgery trials; range = 825 to 1500) made the rats' DNMS performance relatively insensitive to large hippocampal lesions. But, such an explanation cannot explain why Aggleton et al. (1986) found no impairment on DNMS in rats with hippocampal lesions that did not receive presurgery training. Thus, it is possible that amygdalar, hippocampal, or amygdalo-hippocampal lesions would cause impairments in rats with no presurgery training in the DNMS paradigm that we used in this study, but there is no direct evidence to support this view.

Although the present findings suggest that lesions that are limited to the hippocampus and amygdala do not cause severe impairments of DNMS, the statistically significant impairments at the 600-s delay suggest that DNMS performance is sensitive to such lesions if the task is made sufficiently difficult. In support of this notion, other studies have found that mild DNMS impairments in monkeys

with hippocampal or amygdala lesions can be accentuated by requiring them to remember lists of several sample items at one time (e.g., Mahut et al., 1982; Mishkin, 1978; Murray & Mishkin, 1986). Similarly, Raffaele and Olton (1988) found impaired DNMS in rats with hippocampal lesions when only two stimuli were used throughout testing, thus increasing the degree of proactive interference over that which is present when different stimuli are used on each trial. Jagielo, Nonneman, Isaac, and Jackson-Smith (1990) reported a similar result using a two-stimuli matching-to-sample procedure.

Overall, the findings from this experiment strongly suggest that object recognition in rats does not require an intact hippocampus or amygdala, at least not when it is assessed by DNMS performance in pretrained rats. There were indications that DNMS performance is severely impaired in rats by damage to adjacent cortex, but this possibility requires systematic verification.

GENERAL DISCUSSION

This thesis constitutes the first stage in the development of a rat model of brain-damage-produced amnesia. Its first objective was to develop a DNMS task for rats: one that resembles the classic monkey DNMS task, one that can be readily learned by rats at short delays, and one that can be performed by rats, once learned, with a high degree of accuracy. This objective was clearly met. First, I designed a task that closely resembles the monkey DNMS task. Then, in Experiment 1, rats learned this DNMS task at a rate not substantially slower than the rate at which DNMS is learned by monkeys, and once they learned the task, they performed almost as well as monkeys at delays of up to 2 min. This was the first demonstration that rats can perform a nonrecurring-items object-recognition task at delays of more than a few seconds.

The second objective of this thesis was to provide a preliminary assessment of the potential of my rat DNMS task to serve as a component of rat models of human brain-damage-produced amnesia. The first two sections of this General Discussion focus on this issue. The third section describes ongoing research that is utilizing my rat DNMS task. The final section summarizes the main findings and conclusions of this thesis.

1. THE CORRESPONDENCE BETWEEN THE RAT DNMS TASK AND THE MONKEY DNMS TASK

The development of monkey models of brain-damage-produced amnesia has revealed much about the anatomical bases of human memory and amnesia -- see reviews by Squire (1987) and Murray (1990). One reason for their success is that they appear to be isomorphic models; that is, they mimic

both the underlying cause of human brain-damage-produced amnesia and its symptoms[6]. For example, the DNMS deficits that are displayed by monkeys with bilateral amygdalo-hippocampal lesions are isomorphic with human medial-temporal-lobe amnesia in the sense that (1) the brain damage in monkeys with amygdalo-hippocampal lesions is similar to the brain damage in patients with medial-temporal-lobe amnesia, and (2) the accurate DNMS performance requires the kinds of memory functions that are impaired in patients with medial-temporal-lobe amnesia. In fact, humans with medial-temporal-lobe amnesia display deficits on a DNMS task that is virtually identical to the classic monkey DNMS task (Squire et al., 1988).

In view of the success that monkey models of brain-damage-produced amnesia have had, my strategy for developing a rat model of human brain-damage-produced amnesia was to duplicate key features of the monkey models. Because the DNMS task is one of these key features, I chose to begin my attempt to develop a rat model of brain-damage-produced amnesia by developing a rat DNMS task.

I began by assuming that the most useful rat model of brain-damage-produced amnesia would be one that includes a DNMS task that corresponds to the monkey DNMS task in three general respects. (1) The test stimuli, the response requirements, and other key aspects of the protocol should be similar. Such similarities reduce the number of possible interpretations that could be made for differences in the effects of brain damage on the DNMS of rats, monkeys, and humans. (2) The asymptotic DNMS performance of rats should be comparable to that of monkeys and humans over a wide range of delay durations. In order to interpret differences in DNMS deficits among species, it is important that their baseline levels of performance be similar. In addition, low baseline levels of performance make it difficult to demonstrate statistically significant deficits. (3) The DNMS of rats must involve memory abilities that are similar to those involved in the DNMS of monkeys and humans,

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6 An isomorphic model is one that simulates both the symptoms of the disorder and their underlying cause (cf. Kornetsky, 1977).

that is, those involved in object recognition. A correspondence between the rat and monkey DNMS tasks in terms of these three criteria would allow a broader comparative basis for studying the effects of brain damage on memory -- one that includes rats as well as monkeys and humans. The following three subsections summarize the evidence that my rat DNMS task is similar to the monkey DNMS task in terms of the aforementioned three criteria.

The general protocol of the rat DNMS task resembles that of the monkey DNMS task

My rat DNMS task mimics the monkey DNMS task in terms of the test stimuli, the response requirements, and other key aspects of the protocol. A a large pool of objects serve as the test stimuli and two different objects are used on each trial within a session; the operant response is the displacement of the correct object to gain access to a food well; the duration of exposure to the sample object is brief and is controlled by the subject; it is possible to train rats at delays of only a few seconds and then to test them at a wide range of delays; and the rats are not handled during sessions.

The DNMS performance of rats is comparable to that of monkeys

In Experiment 1, the DNMS performance of rats compared favorably to that commonly reported for monkeys in terms of both the rate at which they learned the task and their asymptotic performance levels at delays of up to 2 min. The rats required a mean of 235 trials to achieve the initial criterion of 84% on two consecutive sessions, whereas rhesus monkeys (Mishkin & Delacour, 1975), cynomolgus monkeys (Aggleton & Mishkin, 1983), and squirrel monkeys (Overman et al., 1983) required a mean of 90, 150, and 785 trials, respectively, to achieve a slightly more stringent criterion (e.g., at least 90% correct on two consecutive sessions or at least 90 correct on 100 consecutive trials). During the final

mixed-delay test sessions, the rats in Experiment 1 averaged 90%, 91%, 81%, and 77% at delays of 4, 15, 60, and 120 s, respectively. The asymptotic scores of monkeys typically range between 90% and 100% at delays of about 10 s and between 85% and 95% at delays of 120 s (e.g., Aggleton & Mishkin, 1983a, 1983b; Murray & Mishkin, 1986).

The DNMS performance of rats, humans, and monkeys appears to involve similar memory abilities

The results of Experiments 1 and 2 support the view that rats, monkeys, and humans employ similar memory abilities when performing the DNMS task. Two variables -- the duration of the delay (Experiment 1) and presence of distraction during the delay (Experiment 2) -- affected the DNMS of rats in the same way that they affect the DNMS of monkeys and humans. The DNMS performance of rats declined when either the duration of the delay was increased or when a distraction task occurred during the delay (cf. Squire, 1987; Squire et al., 1988). These findings suggest, but do not prove, that similar forgetting processes occur in rats and primates during the delay. The observation of a disruptive effect of distraction during the delay suggests that the DNMS task involves explicit memory in rats, as it does in humans and monkeys. Distraction during the retention delay has been shown to disrupt the performance of humans on explicit-memory tests but not implicit-memory tests (Graf & Schacter, 1987; Sloman et al., 1988).

2. EVIDENCE FOR CORRESPONDENCE BETWEEN THE NEURAL SYSTEMS THAT UNDERLIE OBJECT RECOGNITION IN RATS, MONKEYS, AND HUMANS

In order for rat models of brain-damage-produced amnesia to be isomorphic with human brain-damage-produced amnesia and with monkey models of brain-damage-produced amnesia, it is essential

that similar neural systems mediate similar mnemonic functions in rats, monkeys, and humans. For example, object recognition should be mediated by the same structures in all three species. Accordingly, if the DNMS task is a valid test of object recognition in rats, as it appears to be in monkeys and humans, the DNMS performance of all three species should be sensitive to damage in the same brain areas.

The results of Experiment 3 suggest that the neural systems that are involved in the DNMS of rats are similar to those involved in the DNMS of monkeys in at least two ways: (1) Neither the hippocampus nor the amygdala appear to play a critical role in the DNMS of pretrained rats or monkeys. The observation in Experiment 3 of only mild DNMS deficits in pretrained rats following bilateral hippocampal or amygdalar damage is consistent with reports of only mild DNMS deficits in pretrained monkeys (Mishkin, 1978; Murray & Mishkin, 1984, 1986) and rats (Aggleton et al., 1986; Kesner, 1991; Rothblat & Kromer, 1991) with bilateral damage to these structures. There have been reports of more severe DNMS deficits following hippocampal damage in monkeys (e.g., Mahut et al., 1982; Squire & Zola-Morgan, 1985a; Zola-Morgan & Squire, 1986); however, in every one of those studies, the monkeys had not received presurgery training. It has not yet been determined whether hippocampal lesions produce severe DNMS deficits in rats without presurgery training. (2) The entorhinal cortex and perirhinal cortex appear to be critically involved in the DNMS of pretrained rats and monkeys. My observation in Experiment 3 of severe DNMS deficits in 4 rats with bilateral entorhinal or perirhinal damage is consistent with reports of severe DNMS deficits in monkeys with bilateral damage to these cortical areas (Meunier et al., 1990; Murray et al., 1989; Zola-Morgan et al., 1989c). It has not yet been determined whether lesions limited to the entorhinal and perirhinal cortex produce severe DNMS deficits in rats.

The 4 rats in Experiment 3 that displayed severe DNMS deficits following entorhinal and perirhinal cortex damage had also received damage to portions of temporal association cortex, and

therefore, it is possible that this damage contributed to their DNMS deficits. This, too, would be consistent with reports that bilateral lesions of the homologous cortical region in monkeys -- the inferotemporal cortex -- produce matching-to-sample deficits (Horel et al., 1987).

At first glance, my observation in Experiment 3 of only mild DNMS impairments in rats with bilateral amygdalo-hippocampal lesions appears to be inconsistent with reports of severe DNMS deficits in monkeys with bilateral amygdalo-hippocampal lesions (e.g., Mishkin, 1978; Zola-Morgan & Squire, 1985a). However, this inconsistency may reflect differences in the topography of telencephalic structures in rats and monkeys, rather than differences in their functions. In monkeys, amygdalo-hippocampal surgery usually involves the removal of most of the entorhinal cortex and parahippocampal cortex, and in some cases, parts of the perirhinal cortex. It has been proposed that the severe DNMS deficits following bilateral amygdalo-hippocampal lesions in monkeys are produced by this cortical damage (Murray, in press). In the rats that received amygdalo-hippocampal lesions in Experiment 3, these cortical areas were largely spared, and therefore, this could explain why their DNMS deficits were no worse following amygdalo-hippocampal lesions than following separate lesions of either structure. This illustrates one advantage of being able to address questions about the neural bases of brain-damage-produced amnesia in both rats and monkeys -- because many of the topographical relations among brain structures are different in rats and monkeys, the damage to structures other than the target structure that typically occurs during surgery may also be different in the two species.

3. OTHER DNMS EXPERIMENTS IN RATS

Although the present findings suggest that my rat DNMS task has potential to serve in isomorphic rat models of human brain-damage-produced amnesia, they do not, by themselves, provide sufficient

validation of such models. Accordingly, I have initiated several experiments to test the validity of the rat DNMS task as a component of isomorphic rat models of human brain-damage-produced amnesia and to use the models to answer questions about brain-damage-produced amnesia. These experiments are currently in progress; however, several of them are complete enough to warrant brief discussion here. The first two subsections describe these experiments. The first subsection describes results that suggest that the rat DNMS task and monkey DNMS task employ similar memory abilities. The second subsection describes results that suggest that there is continuity among rats, monkeys, and humans in terms of the neuroanatomy of their memory systems.

The third and final subsection describes my current efforts to develop a battery of tests for use in rat models of brain-damage-produced amnesia.

Do the rat and monkey DNMS tasks involve similar memory abilities?

The findings from Experiments 1 and 2 suggest that rats perform the DNMS task using memory abilities that are similar to those used by monkeys and humans. Task analyses also suggest that similar memory abilities are involved in the performance of the rat and monkey DNMS tasks. Converging lines of evidence from the following experiments support this conclusion.

DNMS with lists. In humans, the ability to later recognize items from a studied list of items decreases as the number of items in the list increases (e.g., Strong, 1912). A similar effect of list length on DNMS and matching-to-sample performance has been reported in monkeys (e.g., Bachevalier et al., 1985; Gaffan, 1974; Mahut et al., 1982; Mishkin, 1978; Murray & Mishkin, 1984, 1986). In this version of the DNMS task, a sequence of different sample objects is presented to the monkey. After a delay,

each sample object from the list is paired with a different novel object. The DNMS of monkeys is negatively correlated with the number of sample objects in the list.

I adapted the DNMS-with-lists procedure for use with rats. On each trial, a sequence of sample objects is presented to the rat at 20-s intervals. During the test phase, which begins 20 s after the presentation of the last object in the sequence, the sample objects are presented again in the same sequence, and each one is paired with a difference novel object; the rat is rewarded for selecting the novel object of each pair.

I have tested 5 rats on the DNMS task with lists of 3, 5, and 7 objects. Consistent with findings in monkeys, their mean scores declined substantially as the length of the list increased. The means were 72%, 65%, and 62%, respectively.

DNMS in anosmic rats. Monkeys appear to perform the DNMS task using visual cues. However, it is not as clear what sensory modality or modalities rats rely on when performing the DNMS task. In the Discussion of Experiment 1, I described some incidental observations that suggested that rats were also using visual cues when performing the DNMS task.

One way to test whether rats use a particular sensory modality, or subset of modalities, to solve the DNMS task is to make it impossible for them to use alternative modalities. In an ongoing experiment that is utilizing this approach, I have tested the DNMS performance of 4 anosmic rats. All of the rats had received extensive DNMS training and testing prior to the induction of anosmia, which was accomplished by flushing their nares with zinc sulphate; 2 of them were intact rats and had served as controls in other DNMS experiments, and 2 of them had bilateral amygdalo-hippocampal lesions and had served in Experiment 3. The posttreatment retention functions for these four rats were similar to their pretreatment retention functions. Thus, olfaction does not appear to play a critical role in the DNMS performance of rats.

Choice-response latency. The main dependent measure in most studies of memory in laboratory animals is the accuracy of responding, and studies of DNMS are no exception. However, in many contemporary studies of human memory, response latency is measured as well as accuracy, thus providing additional insights into the nature of the information-processing operations that underlie performance. In studies of recognition memory in humans, manipulations that increase the difficulty of the task tend to increase the subject's response latencies (e.g., Sternberg, 1966). Response latencies have not been measured in studies of DNMS in monkeys, but it is believed that the DNMS task is a test of recognition in monkeys. If the rat DNMS task involves cognitive processes that are similar to those involved in human recognition tests, then similar relations among task manipulations, response accuracy, and response latency should occur in both species.

The preliminary findings of an ongoing experiment suggest that response latency may be a useful dependent measure in studies of DNMS in rats. I videotaped several hundred of the DNMS trials of 3 rats at delays ranging from 4 s to 300 s. For each trial, I measured both response latency and response accuracy. Response latency -- the amount of time between when the rat's snout first entered the goal area and when the selected test object began to move -- was measured to the nearest .05 s. For each of the rats, mean response latency increased, and mean percent correct decreased, as the duration of the delay increased. These preliminary findings are consistent with the hypothesis that the DNMS task is a test of recognition in rats, as it is in humans and monkeys.

Do similar neural systems underlie DNMS performance in rats and monkeys?

Although the results of Experiment 3 suggest that there are some similarities in the neural systems that underlie the DNMS performance of rats, monkeys, and humans, many questions about the

extent of this similarity remain. I have initiated experiments that are aimed at answering some of those questions. These experiments take two general experimental approaches: (1) Lesion experiments ask whether surgical lesions affect the DNMS of rats in the same way that simlar lesions affect the DNMS of monkeys and humans. (2) Experiments designed to assess the etiological validity of the DNMS task ask whether nonsurgical treatments that produce amnesia in humans affect the DNMS of rats. Etiologically valid models are often used to delineate the pathology and to elucidate the pathogenesis of a disorder (Ridley & Baker, 1991).

Lesion experiments

The following experiments are being conducted to determine whether rats will display DNMS deficits following surgical lesions that cause DNMS deficits in monkeys and humans.

Rhinal cortex lesions. The results of Experiment 3 suggested that damage to lateral entorhinal and perirhinal cortex may cause severe DNMS deficits. However, in that experiment, the 4 rats with damage in these cortical areas also had extensive bilateral hippocampal damage. Thus, any single contribution that the cortical damage might have made to their deficits was unclear.

I have begun an experiment to determine the effects of bilateral entorhinal and perirhinal cortex damage on DNMS in rats. Rats are receiving bilateral entorhinal and perirhinal cortex lesions either alone or in combination with bilateral amygdalectomy or bilateral hippocampectomy. The training and testing procedures are identical to those of Experiment 3, so a direct comparison of the results with those of Experiment 3 will be possible. So far, I have tested 3 rats with bilateral entorhinal and perirhinal cortex lesions, 2 rats with similar cortical lesions combined with bilateral amygdalectomy, and 2 rats with similar cortical lesions combined with bilateral hippocampectomy. Another 6 rats are

currently undergoing presurgery DNMS training. So far, the two main results have been that (1) the rats in all three experimental groups required considerably more postsurgery trials to reattain the criterion at 4-s delays than did the rats in any of the groups in Experiment 3, and (2) their postsurgery scores were substantially lower than those of the control rats in Experiment 3 at delays of 120 s and 600 s, but not at shorter delays. These findings suggest that bilateral lesions of the entorhinal and perirhinal cortex produce DNMS deficits in rats, as they do in monkeys. It is not yet clear whether or not the effects of bilateral entorhinal and perirhinal cortex damage are accentuated by the presence of additional damage to the hippocampus or amygdala.

Hippocampal lesions in rats without presurgery training. As mentioned in the Discussion of Experiment 3, the DNMS deficits that are displayed by monkeys with bilateral hippocampal lesions are less severe if they have had presurgery training than if they have not (Murray, 1990). Consistent with these findings in monkeys (e.g., Mishkin, 1978; Murray & Mishkin, 1984, 1986), the results of Experiment 3 demonstrated that bilateral hippocampal lesions produce only mild DNMS deficits in rats that receive presurgery training. I have conducted a pilot experiment to determine whether the effects of bilateral hippocampal lesions are greater in rats that have not had presurgery training. Rats with bilateral hippocampal lesions ($n = 4$) and rats with control lesions of posterior parietal cortex ($n = 5$) were trained on the DNMS task at 4-s delays until they reached the criterion of at least 17 correct trials out of 20 on two consecutive sessions. After reaching the criterion, each rat received 6 sessions at delays of 15 s and 6 sessions at delays of 60 s. The rats with hippocampal lesions acquired the task at a normal rate, and they subsequently performed normally at delays of 15 s and 60 s. These findings suggest that bilateral hippocampal lesions do not produce severe DNMS deficits in rats that have not had presurgery training, and thus, they appear to be inconsistent with the findings from monkey experiments (e.g., Mahut et al., 1982; Squire & Zola-Morgan, 1985a; Zola-Morgan & Squire,

1986). However, they are consistent with Aggleton et al.'s (1986) observation in rats of normal acquistion of the Y-maze DNMS task and subsequent normal performance at delays of up to 60 s in rats with bilateral hippocampal lesions. This experiment must be replicated with more rats and with delays of longer than 60 s before it can be concluded that bilateral hippocampal lesions have similar effects on the DNMS of rats both with and without presurgery training.

Medial-diencephalic lesions. The mediodorsal thalamic nucleus and the mammillary bodies are the two most consistently and extensively damaged brain areas in Korsakoff amnesics (Victor et al., 1971). Bilateral lesions of the mammillary bodies have failed to produce DNMS deficits in monkeys (Aggleton & Mishkin, 1985) and rats (Aggleton, Hunt, & Shaw, 1990), but bilateral lesions of the mediodorsal thalamic nucleus have produced DNMS deficits in monkeys (Aggleton & Mishkin, 1983a, 1983b; Zola-Morgan & Squire, 1985b). Korsakoff amnesics display similar impairments on a DNMS task (Squire et al., 1988) and on a nonrecurring-items delayed matching-to-sample task (Aggleton et al., 1988).

I have initiated a series of experiments to determine the effects of bilateral mediodorsal nucleus and mammillary body lesions on the DNMS of rats. So far, I have tested only rats with bilateral mediodorsal nucleus lesions. Relative to rats with sham lesions, rats with electrolytic bilateral mediodorsal nucleus lesions displayed DNMS deficits whether they had received presurgery training or not. These results suggest that mediodorsal nucleus damage produces severe DNMS deficits in rats, as it does in humans and monkeys. The next stage in this study will be to examine the effects of mammillary-body lesions and combined lesions of the mammillary bodies and mediodorsal nucleus.

Experiments designed to assess the etiological validity of the rat DNMS task

The following experiments were designed to determine whether rats will display DNMS deficits following nonsurgical treatments that simulate known etiological factors in human brain-damage-produced amnesia.

Thiamine deficiency. A considerable amount of evidence links Korsakoff amnesia in humans to chronic thiamine deficiency (Butterworth, 1989). In laboratory animals, a period of thiamine deficiency can produce subsequent impairments on memory tasks, including impaired acquisition of DNMS in monkeys (Witt & Goldman-Rakic, 1983) and impaired spatial and nonspatial recurring-items delayed nonmatching-to-sample in rats (Knoth & Mair, 1991; Mair, Anderson, Langlais, & McEntee, 1988).

In an ongoing experiment that is utilizing my rat DNMS task[7], rats have been exposed to a period of thiamine deprivation lasting between 12 and 14 days, during which they were maintained on a thiamine-free diet and given daily injections of the antithiamine agent pyrithiamine. Following recovery from this thiamine deprivation, the experimental rats that had received pretreatment training required more trials to reattain the criterion at delays of 4 s than did untreated control rats, and once they did so, they displayed deficits at delays of 15 s, 30 s, 60 s, and 120 s. Experimental rats that had not received pretreatment training displayed acquisition deficits as well as subsequent retention deficits. Prelminary histological findings indicated that all of the experimental rats had damage to midline thalamic regions, but no apparent damage to either the mammillary bodies or the hippocampus.

Chronic alcohol consumption. Alcohol consumption reduces the absorption of thiamine from the gastrointestinal tract (Butterworth, 1989) and it interferes with thiamine metabolism in the brain

7 Mike Mana, Lisa Kalynchuck, and John Pinel have been major collaborators in this study.

(Rindi, 1989). There is also evidence that chronic alcohol exposure can cause memory impairments in humans (e.g., Oscar-Berman & Zola-Morgan, 1980a, 1980b) and laboratory animals (e.g., Beracochea & Jaffard, 1985), even in the presence of a diet replete with thiamine. Do rats that chronically consume substantial amounts of alcohol alcohol exhibit neuropathology and memory deficits if they are maintained on a thiamine-replete diet?

I used a schedule-induced-polydipsia paradigm to induce experimental rats to drink an average of approximately 2.5 g of ethanol per day over a 156-day period; control rats were rendered polydipsic for water, but they received no ethanol. Throughout the experiment, all of the rats were fed nutritionally-balanced laboratory rat chow. When later trained on the DNMS task, there were no clear differences between experimental and control rats in terms of either the rate at which they learned the task or their subsequent performance at delays of 15 s and 60 s. The brains of the rats that served in this pilot experiment have not yet been studied.

Transient forebrain ischemia. Cerebrovascular accidents often lead to impaired memory in humans (e.g., Glees & Griffith, 1952; Zola-Morgan, Squire, & Amaral, 1986). Zola-Morgan, Squire, and Amaral (1986) studied one patient who had suffered severe anterograde amnesia following a brief period of cerebral ischemia. The only brain damage that was observed in this patient and that could be reasonably linked to his memory impairment was complete bilateral infarction of the CA1 field of the hippocampus. In monkeys, Bachevalier and Mishkin (1989) found that a period of forebrain ischemia that produced damage to the CA1 and CA2 fields of the hippocampus also produced lasting DNMS deficits.

In an ongoing study in rats, a two-vessel-occlusion procedure for inducing transient cerebral ischemia has been found to produce bilateral CA1 lesions as well as severe DNMS deficits[8].

8 Emma Wood has been the principal investigator in this study.

Experimental rats that had received pretreatment training required more trials to reattain the criterion at delays of 4 s than did control (sham ischemia) rats, and once they did so, they displayed deficits at delays of 15 s, 30 s, 60 s, 120 s, and 300 s; experimental rats that had not received pretreatment training displayed acquisition deficits as well as subsequent performance deficits at the same delays.

The development of a test battery for use in rat models of brain-damage-produced amnesia

Any memory test can be failed for a variety of reasons, including some that have nothing to do with memory. Therefore, caution must be used when interpreting the findings from brain-damaged subjects on a single memory task. More information is available about the severity and the range of brain-damage-produced memory deficits when subjects are tested on more than one memory test. Accordingly, recent studies of brain-damage-produced amnesia in monkeys (Mahut et al., 1982; Murray & Mishkin, 1986; Zola-Morgan & Squire, 1985a; Zola-Morgan et al., 1989a, 1989b, 1989c) and humans (Aggleton et al., 1988; Squire et al., 1988) have employed a battery of tests, some of which are sensitive to brain-damage-produced amnesia in humans and some of which are not. By comparing patterns of spared and impaired performance across several memory tasks in subjects with discrete lesions to various parts of the brain, it may be possible to dissociate the mnemonic effects of damage to different areas.

I have recently developed a battery of memory tests for rats, all but one of which (i.e., a temporal-order recognition task) was designed to mimic a specific memory test that has been used in recent studies of brain-damage-produced amnesia in monkeys. Each of these tasks is similar to its monkey counterpart in terms of the test stimuli, the response requirements, and other key aspects of the protocol. Each of them uses the same apparatus and test stimuli as my rat DNMS task. Intact rats as well as rats with bilateral lesions of either the hippocampus, the amygdala, or the posterior parietal

cortex are currently being tested on this test battery. The battery includes the DNMS task and five other tasks; the tasks are administered in the following order:

Task 1: Object discrimination. This task assesses the ability of rats to make object-reward associations. The methods are similar to those that were used in the pretraining phase for the rat DNMS task -- the only exception is that in this task, each rat is trained with a single pair of objects until it reaches the criterion of at least 22 correct trials out of 25 on two consecutive sessions.

Task 2: Reversal of object discrimination. In this task, the contingency that was operative in the the object discrimination task, is changed; that is, the previous S- becomes S+ and *vice versa*. The rats are trained to the criterion of at least 22 correct trials out of 25 on two consecutive sessions.

Task 3: Concurrent object discrimination. This task assesses the ability of rats to learn several object-reward associations concurrently. Eight pairs of objects are used. In each pair, one object is designated S+ and the other S-. Each pair is presented five times per session in an intermixed order. The rats are trained until they reach a criterion of at least 36 correct trials out of 40 on two consecutive sessions. In an earlier pilot experiment, rats with bilateral amygdalo-hippocampal lesions required significantly more trials to reach this criterion than did rats with control lesions of the posterior parietal cortex.

Task 4: DNMS. This task assesses the ability of rats to learn the nonmatching principle, and to recognize, over a wide range of retention delays, objects that they have experienced in a single recent episode. The general procedures are identical to those that have already been described for this task. After reaching the criterion at delays of 4 s, the rats receive six sessions at delays of 15 s, 60 s, and finally 120 s.

Task 5: DNMS with lists. This task was described on page 81. It assesses the ability of rats to hold several items in memory over a delay. The rats are tested on lists of 3, 5, and 7 objects.

Task 6: Temporal-order recognition. This task assesses the ability of rats to judge which of two previously presented objects was presented before the other. It is an adaptation of DNMS with a 5-object list. A list of 5 objects is presented to the rat, and then, 20 s after the presentation of the final object in the list, 2 of the 5 objects are presented together; the rat is rewarded for choosing the object that appeared earlier.

3. SUMMARY AND CONCLUSIONS

The study of monkey models of brain-damage-produced amnesia has begun to elucidate the neural bases of memory and amnesia. The development of comparable rodent models would benefit the study of brain-damage-produced amnesia in two general ways: (1) it would facilitate the conduct of large-scale parametric experiments, and (2) it would provide a broader comparative basis for drawing inferences about the anatomical bases of brain-damage-produced amnesia -- one that includes rats as well as humans and monkeys.

This thesis took the first steps in the development of rat models of brain-damage-produced amnesia that are comparable to the monkey models. First, the monkey DNMS task, which plays a central role in monkey models of brain-damge-produced amnesia, was adapted for use with rats; the rat DNMS task that was designed is similar to the monkey DNMS task in several key respects. Then, the rat DNMS task was used in three experiments, which were designed to determine the comparability of the rat DNMS task to the monkey DNMS task in terms of the (1) the rate at which it is learned

(Experiment 1), (2) the asymptotic level at which it is performed (Experiment 1), (3) the memory abilities that it taps (Experiment 2), and (4) the brain structures that it engages (Experiment 3). The following were the six main findings of those experiments:

1. Intact rats readily learned the DNMS task.
2. Once they learned the task, intact rats performed at high levels of accuracy at delays of up to 120 s.
3. The performance of intact rats was better at shorter delays.
4. The DNMS of rats was disrupted by distraction during the delays.
5. Separate or combined lesions of the hippocampus and amygdala produced only a mild DNMS deficit in rats.
6. Lesions of the lateral entorhinal cortex, perirhinal cortex, and temporal association cortex appeared to produced a severe DNMS defict in rats.

Because each of these six results parallels the results of experiments on monkeys, they suggest that converging evidence from rat and monkey DNMS models of brain-damage-produced amnesia may help to elucidate the nature of human amnesia and its underlying causes. Ongoing experiments are providing additional support for this view.

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