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The role of thalamo-frontocortical mechanisms in measures of spatial learning and memory

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The role of thalamo-frontocortical mechanisms in measures of spatial learning and memory

Harrison, Loredana Maggiora, Ph.D.

University of New Hampshire, 1992

THE ROLE OF THALAMO-FRONTOCORTICAL MECHANISMS IN
MEASURES OF SPATIAL LEARNING AND MEMORY

BY

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DISSERTATION

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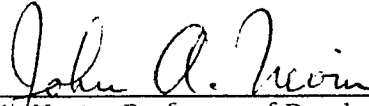
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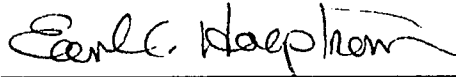
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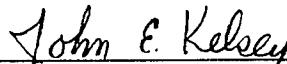
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November 17, 1992
Date

DEDICATION

This dissertation is dedicated to my dear family. To my grandparents, Louisa and Mario Maggiora, Elisabetta and Luigi Campagna: our time together was precious. You are a profound part of my history, and your wisdom will always be remembered. To my parents, Vittoria and Luciano Maggiora: together you set an extraordinary example for me to follow. Your love, sacrifice, and dedication set the stage for my achievements. Finally, to my husband, Jose Maria Harrison: I am so fortunate to have found you. You are my strength and my greatest inspiration.

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ABSTRACT

THE ROLE OF THALAMO-FRONTOCORTICAL MECHANISMS IN
MEASURES OF SPATIAL LEARNING AND MEMORY

by

Loredana Maggiora Harrison
University of New Hampshire, December, 1992

Human amnesia has been attributed to diencephalic lesions produced by

Wernicke-Korsakoff syndrome, tumor, trauma and infarct. Rodent models of

diencephalic amnesia suggest the involvement of three anatomic systems, namely the

nuclei of the brainstem and diencephalon, the mammillary bodies, and the

mediodorsal nucleus of the thalamus. In recent years our laboratory has investigated

these systems. Only radiofrequency (RF) lesions of the thalamus involving lateral

portions of the internal medullary lamina (L-IML) and including the mediodorsal

nucleus (MDn) of the thalamus have disrupted measures of learning and memory in a

manner that is qualitatively similar to that seen in human Korsakoff amnesics.

The purpose of the experiments reported here was to further examine the

neurologic basis of these deficits. The effects of RF lesions of the L-IML were compared

to lesions restricted to two areas of prefrontal cortex (PFC) found to be denervated by the

L-IML lesion, i.e. the shoulder of the medial wall (MW) and the cortex dorsal to the

rhinal sulcus (RS). Memory was assessed with three tasks: the radial arm maze (RAM),

spatial serial reversal (SSR), and spatial delayed nonmatching to sample (DNMTS)

tasks. Also, Wheat Germ Agglutinate - Horseradish Peroxidase (WGA-HRP) analyses

were done to verify the extent to which MDn projections to PFC were affected by these lesions.

Rodents with lesions of the L-IML were impaired on all three tasks, performing significantly worse than the cortical groups on RAM and DNMTS. Although they committed more errors during initial learning of SSR, like MWs and RSs they exhibited a preserved capacity to perform this task, and demonstrated positive transfer. The PFC groups were impaired only on DNMTS, suggesting that neither of these areas alone can account for the full complement of deficits observed in animals with L-IML lesions. WGA-HRP analyses verified that neither prefrontal lesion interrupted pathways from the thalamus to the other prefrontal area, indicating that these lesions successfully destroyed most of the intended targets.

INTRODUCTION

In spite of decades of human and animal research, much remains to be learned about the functional significance of the area of frontal cortex known as prefrontal cortex (PFC). This area is generally thought to engage in the high level processing that mediates self awareness, however research has also indicated a role in memory. In his book entitled <u>Memory and Brain</u>, Larry Squire (1987) asserts that general long term memory is not within the domain of the PFC. Rather this area appears to possess working memory capacities related to the performance of such specialized functions as the temporo-spatial organization of behavior. The following literature review investigates the evidence in favor of the participation of prefrontal cortex in these memory related processes.

<u>Anatomical Findings in Humans and Primates</u>

There is a certain degree of variability in the descriptions of prefrontal cortex found in the human literature. Squire (1987) describes it as a "large, heterogenous and supramodal association area" lying rostral to the precentral gyrus and the premotor area, and receiving projections from the mediodorsal nucleus of the thalamus.

Patricia Goldman-Rakic (1987a) provides a far more complete description of the anatomy of this division of the frontal lobe. She describes the PFC as an area lying anterior to the precentral and premotor cortices, i.e. Brodmann's areas 4 and 6 of frontal cortex. In humans, the prefrontal cortex is thought to correspond to Brodmann's areas 8, 9, 10, 11, 47, 46, 45 and 44. A similar organization is seen in primates, with the PFC corresponding to Walker's areas 8a & b, 9, 10, 12, 45 and 46. Goldman-Rakic notes that the PFC may further be distinguished from other frontal

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subdivisions on the basis of cytoarchitectonic differences. In both humans and primates, the PFC is characterized by an internal granular layer (Layer IV) that is either absent or less prominent in other areas of frontal cortex.

Goldman-Rakic also provides an extensive outline of the afferent and efferent connections of PFC. To summarize: this area is known to be widely connected with temporal, parietal, and occipital association areas. In addition it shares information with both premotor and limbic cortices. Finally, it is known to have extensive subcortical projections, some of which are unidirectional in nature, others bidirectional.

The relevance of these connections between PFC and subcortical structures has received a great deal of attention in the past 50 years. The early investigations of Rose and Woolsey (1948) and Akert (1964) uncovered major projections between PFC and the mediodorsal nucleus (MDn) of thalamus. In an extensive review of the literature on the anatomy and function of the thalamus, Markowitsch (1982) concludes that the existence of afferent and efferent fibers between the MDn and various frontal areas, including prefrontal cortex, has now been clearly established. In addition, Brodal (1981) notes that HRP analyses in primates have demonstrated the existence of reciprocal connections between the MDn of the thalamus and the PFC, and that these projections appear to be organized topographically. In fact, the organization of these projections has been demonstrated to be specific to the extent that it now serves as a criterion for defining prefrontal cortex and establishing homologies across species (Fuster, 1989). In a description of the anatomy and function of the frontal lobes, Damasio (1985) notes that afferent projections from thalamus to prefrontal areas tend to originate where PFC efferents terminate. Also, Markowitsch and Damasio assert that it is the magnocellular portion of the MDn that projects to orbitofrontal cortex, while the parvocellular portion of the MDn projects to dorsolateral frontal cortex in primates. However, Brodal makes the interesting observation that fibers projecting

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from thalamus to PFC not only originate in the MDn but also in neighboring thalamic nuclei, and Damasio specifically mentions a reciprocal relationship between PFC and the intralaminar nuclei of the thalamus.

Proposed Function in Humans

What purpose could such a closely related, topographically organized set of projections between the thalamus and PFC serve?

Markowitsch's 1982 paper reviews electrophysiologic, lesion, and stimulation studies of the MDn in animals and compares them to both pathologic analyses and studies of the destruction of this area in man for putative therapeutic purposes. The author concludes that a direct comparison between the two literatures is difficult at best, with the MDn appearing to play a far greater role in the processing of memory related information in humans than in animals. He notes however, that whether this difference in function is due to true differences in anatomy or to differences in the behavioral evaluations done in humans versus animals remains to be seen. In spite of the diversity of findings presented in Markowitsch's paper, he argues in favor of the MDn acting as a center for memory related processes. This argument is largely based upon anatomic and behavioral findings in human Korsakoff patients.

Between 1887 and 1889 S. S. Korsakoff wrote three papers describing what he referred to as "disturbances in psychic activity" related to alcoholism. His first paper was entitled "The disturbance of psychic activity in alcoholic paralysis and its relation to its disturbance of the psychic sphere in multiple neuritides of nonalcoholic origin". His second paper addressed "A few cases of peculiar cerebropathy associated with multiple neuritis". The third paper, entitled "Psychic disorder in conjunction with peripheral neuritis", (translated by Victor and Yakovlev, 1955) describes cases of both alcoholic and nonalcoholic origin and effectively lays the groundwork for our current understanding of the pathology of this disease and its resultant behavioral disorders.

It was Korsakoff who first reported that a profound impairment in memory appeared to develop following periods of malnutrition related to certain acute infections or chronic diseases. He described his patients as appearing to be in complete possession of their faculties, and specified that only after a long conversation with a patient would one begin to realize that the individual had forgotten everything that had happened during the illness and just prior to the onset of the illness. Additionally, Korsakoff reported that in some, the memory of remote events was disturbed while in others memory for facts, and even words, was lost.

By the early 1940's, researchers began to point to Vitamin B deficiency as the probable cause of Wernicke's Encephalopathy, i.e. acute hemorrhagic polioencephalitis (Campbell & Russell, 1941; Riggs & Boles, 1944). In addition, Campbell and Russell (1941) suggested that the clinical features of this disease are sufficiently similar to indicate a relationship between Wernicke's and Korsakoff's syndromes. Finally, DEWardener and Lennox (1947) published a review of 52 cases of the disorder, reporting recoveries following early diagnosis and treatment with thiamine injections. The authors argued that acute thiamine deficiency appeared to be the sole cause of the syndrome.

In a detailed analysis of the relationship between these two diseases, Malamud and Skillicorn (1956) concluded that Wernicke's and Korsakoff's syndromes are one and the same, with Wernicke's representing an acute phase and Korsakoff's representing a chronic phase. The authors also suggest that both phases result either directly or indirectly from thiamine deficiency most often related to chronic alcoholism. Following histologic examination of 70 Korsakoff cases, they reported that the disease manifested itself in degenerative changes in the periventricular and periaqueductal gray matter, as well as the mammillary bodies and the dorsomedial nucleus of the thalamus. No consistent cortical damage was found. They therefore argued that the mental deficiencies associated with Korsakoff's Psychosis, namely

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memory impairments, temporal disorientation, and confabulation could not be attributed to cortical pathology, but rather that research should focus on periventricular-hypothalamic regions and on the mammillary bodies (MB).

By 1971, Victor, Adams and Collins began to assert the importance of the MDn, and not the MBs in the amnesic component of Korsakoff's Disease. They also provided greater detail as to the memory impairments seen in conjunction with degeneration of the MDn. The authors describe immediate memory as being intact. However, long term memory impairments are both retrograde and anterograde in nature, with some retention of remote memory. They concur with Talland's (1965) suggestion that affective disorders also associated with this disease are partly responsible for the memory defects, however they insist that these memory deficits are largely due to abnormalities in consolidation.

In addition, Victor and colleagues (1989) address the question as to whether the memory loss seen in Korsakoff's is partial or global in nature. In doing so, the authors review the suggestion set forth by Squire (1986) that procedural memory (memory for skills and operations) is spared while declarative memory (memory for facts, episodes, lists - similar to working memory) is impaired in cases of amnesia resulting from damage to diencephalic structures including those involved in Korsakoff's pathology. The authors are not convinced by these procedural vs. declarative memory distinctions and thus argue that memory is globally impaired.

If, as Markowitsch and Victor et al. argue, the MDn plays a dominant role in long term memory processes, does this role extend to its projections to the prefrontal cortex? When discussing the PFC, the memory issue becomes an elusive one, and the tendency in the human literature is to refer to deficits in "higher cognitive processes", rather than making specific statements about memory. For example, Goldman-Rakic (1987b) alludes to the role of prefrontal cortico-thalamic pathways in the deficits seen in Korsakoff's patients. However, she describes the disorganized thought and behavior

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associated with this disease as most likely being due to a loss of the "cognitive capacity" for representational knowledge.

In fact, most patients with frontal lobe damage do not demonstrate chronic memory disturbances. Damasio (1985) provides a description of what is best described as a frontal lobe syndrome. He argues that all human frontal lobe patients share certain features in common including: "inability to organize future activity and hold gainful employment; tendency to present a favorable view of themselves; stereotyped but correct manners; diminished ability to experience pleasure and react to pain; diminished sexual and exploratory drives; lack of motor, sensory or communication defects; and overall intelligence within expectations based on educational and occupational background". Hence, basic memory function and intellectual capacity are spared in the face of major disturbances in personality. Damasio does, however, make one interesting exception. He explains that some frontal patients do exhibit an amnesic syndrome similar to that seen in Korsakoff's patients and pinpoints damage to the ventromedial aspects of the frontal lobe, or more specifically the basal forebrain, as the likely source.

While Damasio points to orbitofrontal areas as the likely cause of the frontal lobe syndrome, Goldman-Rakic (1987a) has suggested that the global nature of this syndrome reflects damage to multiple areas of prefrontal cortex, including dorsolateral and obitofrontal regions. A closer look at Damasio's review of the anatomical findings lends support to Goldman-Rakic's statements. In addition to the deficits described above, he reports instances of patients demonstrating impairments of high level cognitive ability, including attentional deficits and the failure to extract meaning from ongoing experience, in the absence of global memory impairments. Damasio suggests that in these cases damage to dorsolateral areas of the frontal lobe is the likely source of impairment.

Although Milner's 1982 paper on cognitive deficits following frontal lobe lesions in humans largely addresses issues of lateralization of function, in many ways it influenced both Damasio's and Goldman-Rakic's interpretations of the impairments seen following frontal lobe damage. In similar fashion, she describes the deficits seen in these patients as revealing "specific cognitive disorders that appear against a background of normal functioning on many intellectual, perceptual and memory tasks." In general she describes patients with left frontal lobe excisions as showing deficits in sequential ordering tasks and patients with right frontal lobe excisions as being impaired on tasks requiring the temporal sequencing of events. However, some of Milner's findings warrant a closer look.

Her paper reviews the performance of both frontal and temporal patients on a variety of conditional associative learning and sequential ordering tasks following unilateral excisions of cortical tissue for the treatment of focal epilepsy. Of interest here are her findings on the sequential ordering tasks. She discusses disturbances in the temporal organization of skilled behavior to obtain a goal, noting that while patients are capable of performing the individual actions involved in a sequence of behaviors, they are somehow unable to execute them in the correct temporal sequence, often performing them in the wrong order or omitting certain behaviors altogether. More specifically, Milner reports on the performance of frontal lobe patients on both verbal and non-verbal subject-ordered pointing tasks. Subjects examined stacks of cards imprinted with sets of 6, 8, 10 or 12 stimuli comprised of either high imagery words, low imagery words, representational drawings or abstract designs. Subjects were instructed to go through a stack, touching one item per card, while being careful not to point to a single item more than once. Among her findings, Milner reports difficulties experienced by frontal patients with the representational and abstract drawings as set size increased. She found that the left frontal group showed impairments on 8 item sets, whereas deficits became apparent in the right temporal

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group (large hippocampal lesions) with set sizes of 10 and the right frontal group with set sizes of 12. Nothing is reported for the left temporal groups.

In discussing the results for the temporal patients, she notes that the deficits emphasize the memory component of the task citing that "no patient in any group had difficulty with the 6-item sets, but the larger sets clearly make demands on an active working memory." However, she attributes the similar deficits seen in frontal patients to disorganized thought, i.e. an inability to group items together in a meaningful way due to some higher cognitive deficit. Why the differing explanations? Is it not possible that the deficits seen in these frontal patients are memory related as well?

Squire (1987) directly addresses research on patients with unilateral frontal excisions, citing much of Milner's work, and argues that these patients are not amnesic in the true sense of the word. They do not display any of the characteristic impairments of recall or recognition seen in amnesics. He asserts that it is working memory that is disrupted in these cases, because deficits are observed when the memory tasks require more than simple recall or recognition. Therefore, Squire concludes that frontal lesions "do not produce a straightforward, general memory impairment, rather they influence performance on memory tests".

At this point in time, the human literature remains undecided as to the role of the prefrontal cortex and its functional relationship to the various thalamic nuclei with which it shares reciprocal projections. Squire himself attests to the difficulties in using findings in human patients to determine just which areas of frontal cortex, and namely prefrontal cortex, are involved in which processes. However, if one allows for the variability in size and location of human PFC lesions, and the lack of bilateral lesions in most human cases, it can be said that the human literature is compatible with evidence in primates that cortical damage involving the PFC produces deficits in tasks that place demands on working memory.

Primate Literature

Prefrontal Cortex

In a 1985 paper, Passingham analyzes the lack of agreement regarding the role of the prefrontal cortex in memory not only in the human but also the primate literature. He cites Milner's self-ordered pointing tasks in humans and delayed response (DR) and delayed alternation (DA) tasks in primates, noting that neither literature resolves whether damage to the prefrontal cortex results in a genuine memory deficit. Although both lines of research provide examples of poor performance on tasks that make demands on memory, more often than not the impairments seen following PFC lesions are described as resulting from some form of perseveration or cognitive failure.

Descriptions of frontal lobe impairments in relation to perseveration are long standing. In the early 1960's, Mortimer Mishkin conducted a series of experiments to investigate whether or not perseveration provides an adequate explanation for the deficits seen after frontal lobe damage. In a comparison of auditory and visual differentiation tasks (go no-go tests in which correct no go responses were not reinforced), animals with lateral frontal damage demonstrated great difficulties in suppressing error tendencies. In a subsequent experiment, the performance of lateral frontal and temporal animals was compared on a series of two choice visual discrimination tasks. Two different versions of the task were run. In the first version, both objects of the test pair were baited on the first trial. The preferred object in the first trial was then designated the positive stimulus for the remaining discrimination trials. In the second version of the task, neither object was baited and the preferred object became the negative stimulus for the remainder of testing. Frontal animals outperformed temporal animals and closely approximated the performance of controls in the first set of discrimination problems. However in the second set, frontal animals committed considerably more errors than did the other groups. These difficulties in

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suppressing response/stimulus preferences were confirmed in a subsequent series of visual discrimination reversals, in which frontal animals demonstrated rates of discrimination comparable to normals during initial learning but severe impairments on reversals. Mishkin therefore took the findings of these three studies to indicate that the impairments seen in lateral frontal animals could best be described as perseverative interference.

On the basis of these results, Mishkin began to consider the possibility that deficits in delayed response performance might also be due to perseveration. While perseverative behavior had been observed in humans on delayed response tasks similar to those used with primates, attempts to explain these deficits in terms of disturbances in memory had failed. To further test this issue Mishkin introduced the use of delayed matching and delayed nonmatching to sample tasks. Frontal animals, that had been found to perform proficiently in the nonmatching to sample condition, demonstrated severe impairments in the matching condition. Given strong evidence of success in one aspect of this testing procedure and failure in the other, it was argued that the observed deficits could not be attributed to impairments in memory. In an attempt to reconcile the results of these experiments, (response perseveration in differentiation tasks, stimulus perseveration in tests of learning set, and abnormal preference for novelty in matching to sample tasks) Mishkin concluded that lateral frontal animals suffer from a single, generalized impairment, i.e. the perseveration of "central mediating processes" such that they persist on initial sets of learning.

Mishkin further assumed that if perseveration of central sets disrupted performance on object discriminations, it would also impair spatial discriminations. This hypothesis was tested with animals that had previously participated in the object reversal studies, and in fact similar impairments were observed on spatial reversals. He therefore concluded that the impairments seen in lateral frontal animals are not

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modality specific and that perseveration alone was sufficient to account for reversal deficits.

In spite of the preponderance of evidence in favor of Mishkin's hypothesis, problems did arise in subsequent studies. The literature at the time had also demonstrated severe impairments in DA and DR following lesions of lateral/dorsolateral areas of PFC. In order to confirm whether the behavioral deficits observed by Mishkin could be attributed solely to lateral damage, a study was done comparing performance of animals with dorsolateral versus orbitofrontal lesions on visual pattern differentiation and spatial delayed alternation (SDA) tasks. As expected, dorsolateral animals showed greater impairments than did orbitals on SDA. Contrary to the results found in his earlier study, the lateral group performed better than did orbitals on visual differentiation. The difference in performance of the dorsolateral animals was attributed to preoperative training, making this experiment a test of postoperative retention as opposed to postoperative learning. However, these same subjects were also tested on: object-quality learning set, discrimination reversals, spatial reversals, and matching and nonmatching to sample tasks. In short, larger deficits were found in orbital animals in all cases except spatial reversal, in which the performance was identical across groups. In analyzing the implications of the spatial reversal findings, Mishkin concluded that spatial reversal combines both a reversal element, on which orbitals are more severely impaired, and a spatial element, on which the literature had demonstrated laterals to be more impaired. He thus considered the possibility that both groups had failed on the spatial reversal task for two different reasons: orbitals failing due to reversal deficits, and laterals failing due to spatial deficits. Therefore, according to Mishkin, perseveration of central sets appeared to best account for the behavior of orbital animals whereas perseverative interference was secondary to spatial deficits in lateral animals. Explanations based upon some form of memory impairment remained out of the question.

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As recently as 1985, Passingham lamented the continued focus on perseverative interference, citing an overreliance upon DR and DA tasks as a potential deterrent to the analysis of memory impairments in PFC animals. Passingham called into question whether such tasks are even capable of assessing memory, given their reliance upon only two spatial positions throughout testing. He argued that testing PFC monkeys with tasks involving binary response choices does little else than determine whether or not delays influence performance. In addition, it makes comparisons with the human literature virtually impossible given the use of multiple lists of items in clinical testing.

Passingham therefore elected to test the spatial memory capacity of animals with prefrontal lesions by using a visual search task designed by Collin, Cowey, Latto and Marzi (see Passingham, 1985) in a series of three experiments. The task, similar to Olton's (Olton & Samuelson, 1976) Radial Arm Maze, was used to determine whether lesions of the sulcus principalis (SP) impaired working memory for lists of multiple spatial locations, the argument being that such a task would make clear distinctions between a true memory impairment and behavioral impairments resulting from perseveration.

Passingham reported that following excision of the sulcus principalis, performance is impaired on this task in which monkeys are required to retrieve peanuts from a vertical panel containing 25 food wells. He recalled the performance of human patients with unilateral frontal lobe lesions (Milner, 1982; Petrides & Milner, 1982) on qualitatively similar tasks and argued that the evidence clearly suggests that cortical damage involving the PFC produces deficits in tasks that place demands upon working memory. According to Passingham, explanations based upon perseveration do not sufficiently account for the deficits seen both in primates and humans. Therefore, the more plausible explanation is that damage to PFC impairs working memory. However, he qualified his statements by noting that the evidence is not

sufficient to determine whether excision of the SP in primates eliminates temporary memory stores or disrupts some "central executive mechanism" that drives both spatial and nonspatial working memory.

In a comprehensive discussion of the organization and function of the primate prefrontal cortex, Patricia Goldman-Rakic (1987a) responds to many of the questions raised by Passingham. Although she also views the PFC as a centralized mechanism for guiding some form of short term or working memory, the two diverge on which experimental procedures most accurately measure the activities performed by this area of frontal association cortex. In direct contrast to Passingham, Goldman-Rakic not only favors the use of DR and DA tasks to assess the types of information processed within PFC, but presents detailed evidence in support of the localization of spatial delayed response function in Walker's area 46 of the dorsolateral convexity, i.e. the cortex surrounding the sulcus principalis.

Following an extensive review of the behavioral literature, Goldman-Rakic reports that monkeys with lesions restricted to, or including the SP show sparing on a wide variety of tests with or without delays including visual discrimination problems, object learning set, object discrimination reversals, object alternations, matching to sample and cross modal matching tasks. Yet performance of delayed response tasks depends upon the "bilateral integrity" of the dorsolateral prefrontal cortex. She suggests that the feature that most clearly distinguishes delayed response from the other tasks is that it requires memory for the position of objects in space, and more specifically that it taps into "a synthetic capacity that requires at least three subfunctions: access to appropriate information, ability to hold that information 'on line' for the temporal interval over which a decision or operation is to be performed, and initiation of a motor command."

In a subsequent paper, she also provides electrophysiological findings indicating that neurons of the dorsolateral prefrontal cortex code the spatial locations

for cues presented during an oculomotor delayed response task. Funahashi, Bruce and Goldman-Rakic (1989) trained animals to fixate on a point that appeared in the center of a CRT while a visual cue appeared at one of several peripheral points on the screen. Animals were reinforced for maintaining fixation throughout the peripheral cue period and during a subsequent delay. At the end of the delay, the fixation point disappeared, serving as a signal to make a saccade to where the peripheral cue had been presented. Recordings of activity in the SP and the frontal eye fields indicated that these prefrontal neurons encode the location of cues in space. Furthermore, unit activity was found to be time locked to delay periods ranging from 1.0 to 6.0 seconds, terminating upon the execution of the behavioral response. The authors take these findings as evidence that the neurons of the dorsolateral PFC (including those of the SP) transiently store information in order to guide responses in the absence of external cues, thus the primate SP is part of a neural network that is essential to behaviors that are guided by stored representations of stimuli in space.

How do these findings relate to Mishkin's theory and Passingham's arguments regarding the role of PFC and the appropriate methods for measuring it's function? Goldman-Rakic describes primates and humans with frontal lobe damage as displaying an excessive dependence upon or sensitivity to external stimulus cues. This serves to explain why monkeys with lesions of the SP tend to repeat responses previously reinfor ed on a delayed response task, i.e. this occurs in response to the absence of external stimuli at the time of selection. Goldman-Rakic draws a comparison between this behavior and the perseveration seen in humans on the Wisconsin Card Sorting task, suggesting that the WCS resembles a delayed response task at the time of category shift. At this point, in spite of the multiple cues visible on the cards themselves, there are no external cues to aid the subject in making a shift, therefore they must rely upon internalized knowledge, i.e. concepts or instructions to successfully complete the task. It thus appears that lesions of the dorsolateral PFC disrupt the ability to generate and

maintain internal representations. Therefore, rather than providing an explanation for the deficits seen in PFC cases, perseveration is a symptom that reflects an excessive reliance upon external cues following the disruption of a mechanism that guides behavior by internal stimulus representations.

In regards to Passingham's insistence that DR and DA tasks fail to assess memory, several issues must be considered. First, an important distinction separates the arguments made by Passingham and Goldman-Rakic. Whereas his arguments are largely based on the failure of these tasks to assess recognition memory, hers underscore the ability of DR and DA to assess the integrity of representational memory. Second, Goldman-Rakic agrees that tasks such as those employed by Passingham and Petrides and Milner demonstrate that sequencing of behavior and recall of temporal order of events is impaired in both primates and humans with PFC damage. However, she argues that the successful recall of lists of previously visited locations and of the order of previous stimulus selections both depend upon representational memory. Hence, these tasks may be viewed as tapping into the same mechanism as is tested by delayed response tasks and, in fact, Goldman-Rakic and Passingham do come to the similar conclusion that lesions of this area of PFC disrupt a form of visual representational memory.

Lastly, in response to arguments that the findings from DR and DA are not directly comparable to the human literature, Goldman-Rakic asserts that delayed response type tasks were specifically designed to test the ability of animals, including humans, to respond to events on the basis of stored information, as opposed to external stimulation. In addition, human studies have been done employing these tasks. Freedman and Oscar-Berman (1986) compared the performance of patients with either frontal lobe damage or Korsakoff's disease to alcoholic controls on delayed alternation and delayed response tasks nearly identical to those used with primates. Subjects were tested in a modified Wisconsin General Testing Apparatus. For the delayed alternation

tasks, a penny was hidden under one of two black plaques while out of view of the subject. On the first trial, both plaques were baited with pennies. From the second trial on, the penny was placed under the plaque opposite the one that had just been chosen. In the delayed response task, four different delays were employed (0, 10, 30 and 60 s). Just as in primate experiments, the plaques were baited in full view of the subject and then responses were delayed. They report that subjects with bilateral frontal damage showed impairments on both DR and DA tasks analogous to those seen in primates. The authors also report deficits in Korsakoff's patients similar to those seen in an earlier study (Oscar-Berman et al., 1982). Whereas in the 1982 study Korsakoff patients were impaired on both DR and DA tasks, in 1986 impairments were found only on DA. They cite the inclusion of a larger number of trials to criterion in the former study and ceiling effects in the latter as the probable causes of discrepancy between the two studies. However, they argue that their more recent results support arguments that some of the deficits seen in Korsakoff's are due to associated frontal lobe pathology related to a disruption of pathways between the MDn and PFC. In addition, these findings are comparable to Isseroff et al.'s (1982) report that primates with lesions of the MDn demonstrate greater impairments on DA than DR.

Relationship Between Prefrontal Cortex and Mediodorsal Nucleus

At the same time Mishkin (1982) reported evidence supporting a role for medial thalamic nuclei in memory, Isseroff, Rosvold, Galkin and Goldman-Rakic (1982) investigated whether the primate mediodorsal nucleus participates in some of the same functions as those engaged in by its two major prefrontal cortical projection sites, namely dorsolateral prefrontal cortex (surrounding the SP) and orbitofrontal cortex. Although damage to the primate PFC had been demonstrated to impair spatial memory, a question remained as to whether or not primates with lesions of the MDn exhibited similar deficits.

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Radiofrequency lesions were done and performance measured on delayed alternation and delayed response tasks. In addition, animals were tested on object discrimination reversal and on visual pattern discrimination tasks not previously found to be impaired by PFC lesions. The authors report that, similar to PFC animals, MDn animals did not differ significantly from controls on either the object or visual discrimination tasks. Isseroff et al. conclude that the deficits observed after MDn lesions resemble those seen following damage to the dorsolateral PFC. They argue that the results provide evidence that circumscribed lesions of the MDn in monkeys do produce impairments on spatial tasks, thus indicating that the MDn and its projections to PFC participate in a system that is critical to spatial memory.

These results are interesting not only because they provide support for a functional relationship for the MDn and its connections with PFC in memory, but also because they relate to arguments set forth by Goldman-Rakic (1987a) regarding the specific type of memory involved. In discussing findings that monkeys with PFC lesions are impaired on DR while showing spared performance on visual discrimination tests, she suggests that these two classes of task test two different types of memory. More specifically, the former tests memory in the absence of external stimuli at the time of selection, while the latter tests memory in the presence of stimuli at the time of response. Hence, DR depends upon representational memory while discrimination tasks depend upon associative memory. Goldman-Rakic argues that associative memory is not one of the functions of PFC.

Obtaining a clear dissociation in deficits following the disruption of connections between MDn and its dorsolateral and orbitofrontal projection sites has remained difficult. Mishkin's (1964) paper demonstrated that both dorsolateral and orbitofrontal animals were impaired on object quality learning set, discrimination reversals, spatial reversals and matching and nonmatching to sample tasks. In all cases except spatial reversals, orbitofrontals performed more poorly. Both groups

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performed equally poorly on spatial reversals. Finally, dorsolateral animals showed greater impairments on spatial delayed alternation tasks. A review of the subsequent literature suggests that lesions disconnecting the dorsolateral PFC from the MDn produce impairments in DR and DA whereas lesions disconnecting orbitofrontal areas tend to be associated with deficits in the learning of spatial and nonspatial visual, tactile and auditory discrimination learning.

It is therefore possible that the pathways between the medial thalamus and dorsolateral and orbitofrontal areas of prefrontal cortex participate in the processing of different types of information. Deficits in DR and DA following dorsolateral lesions indicate that this area of PFC participates in the short term storage of information perhaps best described as visual representational memory. Although Goldman-Rakic has argued against localization of associative memory in PFC, deficits in discrimination performance following orbitofrontal lesions may indicate otherwise.

In conclusion, the human and primate literature leave many questions unanswered as to the role of the thalamus and its connections with prefrontal cortex in memory. However, a review of the rodent literature may shed further light on this issue.

Rodent Literature

Prior to the 1960's it was largely assumed that the rodent cortex did not possess areas analogous to the prefrontal cortex of primates. However, anatomical and behavioral studies done since that time have proven otherwise. The first major investigation into the anatomical organization of the cortical projections of the rodent MDn was done by Leonard in 1969. In many ways the study was an extension of comparative analyses of the mammalian thalamus and its relationship to cerebral cortex done by Rose and Woolsey in the late 1940's. Employing the Fink-Heimer silver staining technique, Leonard was able to trace degeneration of fibers following both thalamic and cortical lesions. She reported the existence of two major pathways, one

between the rodent MDn and the dorsal bank of the rhinal sulcus and the other between the MDn and medial cortex, also referred to as the medial wall. Subsequent research established that these pathways originate from two distinct divisions of the MDn, namely a medial and a lateral segment (Krettek & Price, 1977a), and that these subdivisions are analogous to the magnocellular and parvocellular divisions of the primate MDn (Jones, 1985). Consequently arguments have been made on the basis of this hodological evidence in favor of subdividing the rodent PFC into areas corresponding to those found in other species (Divac, et al., 1978; Divac et al., 1978, Fuster, 1989; Groenewegen, 1988). Therefore, a review of the anatomical literature indicates that the pattern of MDn subdivisions and their prefrontal projection sites are in fact quite similar in rats and monkeys. In primates, the medial magnocellular area of the primate MDn is known to project to orbitofrontal cortex, and the lateral parvocellular subdivision projects to dorsolateral prefrontal cortex. Quite similarly, in rats the central segment projects to the dorsal bank of the rhinal sulcus, while a lateral subnucleus projects to the medial wall area.

Eichenbaum et al. (1983) not only provide evidence in favor of anatomical homologies in rodent and primate thalamo-cortical projections, but also provide behavioral evidence of functional similarities between the two. They suggest that the primate literature provides a dissociation between dorsolateral and orbitofrontal areas, with dorsolateral cortex appearing to be involved in learning and memory of spatial location and orbitofrontal cortex playing a role in olfactory discrimination learning, and that in both cases the greatest deficits are seen in tasks requiring the inhibition or reversal of "prepotent responses". Thus their study makes a comparison of performance on a spatial learning task versus an olfactory discrimination task in an attempt to find a similar dissociation of function between medial wall (MW) areas and rhinal sulcal (RS) areas in rodents. They report that animals with MW lesions are impaired and show increased perseveration on the spatial task, whereas RS animals

exhibit deficits and increased perseveration on the olfactory discrimination task. The authors therefore conclude that functional parallels do exist between the subdivisions of primate and rodent PFC, and, similar to early human and primate researchers, they assert that deficits observed in both cases may be characterized as task specific perseveration.

A recent analysis of the rodent literature was presented by Kolb (1990). In it he provides an extensive review of the anatomical and behavioral findings over several decades and comes to three major conclusions. First, Kolb asserts that several anatomical regions exist in rodent prefrontal cortex each characterized by distinct cytoarchitectures and possessing distinct thalamic, cortical, and subcortical interconnections. Second, there is sufficient evidence to indicate that medial frontal and orbitofrontal regions are functionally dissociable, with medial frontal animals, but not orbitofrontal animals, showing deficits in memory. Similar to Squire's (1987) assessment of the human literature and Goldman-Rakic's (1987a) assessment of the primate literature, Kolb notes that medial frontal animals are not amnesic in the true sense, but rather they exhibit deficits in what might be described as working memory, short term memory, representational memory or temporary memory. Finally, a comparison of prefrontal anatomy and the effects of prefrontal lesions in rodents and primates reveals sufficient similarities in organization and function to suggest that rodents may provide a reasonable model for the study of the function of prefrontal cortex.

Areas of Future Research

The literature builds an interesting case in favor of a role for the mediodorsal nucleus of thalamus and its projections to medial prefrontal cortex in memory. However, there are a number of other interesting anatomical possibilities. The internal medullary lamina (IML), a system of myelinated axons which traverse the thalamus, are known to enclose the anterior nucleus and form a partition between the

medial and lateral groups of thalamic nuclei. The medial group is dominated by the MDn. Interspersed within the IML are small cell groups referred to collectively as the intralaminar, or nonspecific nuclei. These nuclei are known to project diffusely to PFC (cf. Brodal, 1981). Included in their projections are pathways to the same prefrontal target areas of MDn. Recent research done in the laboratory of Robert Mair at the University of New Hampshire indicates that the critical lesion for producing memory deficits is that of the Lateral-IML centered on the mediodorsal, central lateral and paracentral nuclei of thalamus.

Mair, Robinson and Koger (1992) briefly review the literature on diencephalic amnesia and conclude that three different systems have been scrutinized, namely the nuclei of the brainstem and diencephalon, the mammillary bodies, and the mediodorsal nucleus of the thalamus.

In the first case, it has been suggested that diencephalic amnesia results from the disruption of brain catecholamine systems (Joyce, 1987; Mair et al., 1985; McEntee & Mair, 1990). However, there is no conclusive evidence that amnesia can result from neurotransmitter deficits in the absence of other brain pathologies.

Our laboratory has taken a two pronged approach to investigating the role of the other two systems listed above. In the first phase, an animal model of Korsakoff's disease was developed in rodents. More specifically, pyrithiamine induced thiamine deficiency (PTD) was used to produce diencephalic amnesia. In phase two, the neurobiology of this model was further dissected via comparisons of the behavioral consequences of thiamine deficiency to those of stereotaxic lesions which reproduced the individual components of this pathology.

Performance of PTD animals has been assessed in several ways including: multimodal discrimination tasks (Mair et al., 1991), spatial serial reversal tasks (Mair et al., 1991) and delayed conditional discrimination tasks based on spatial cues (Mair et al., 1992. Robinson, 1991). Although PTD rats require more training than controls to

reach criterion, they perform at criterion or at least comparably to controls on discrimination tasks. Regarding spatial serial reversal learning (SSR), PTD animals require more trials to achieve criterion than do normals, however they also demonstrate positive transfer from one problem set to the next. In contrast, PTD animals show significant impairments in spatial delayed nonmatching to sample (DNMTS) performance.

Mair et al. (1991) have asserted that the spared performance on SSR, i.e. the ability of animals to learn to respond consistently and eventually demonstrate positive transfer, indicates that reference memory remains intact in these animals. On the other hand, impairments on DNMTS are taken as evidence of the abnormally rapid temporal decay of working memory. Thus they argue that PTD animals have "an impaired capacity for working memory with sparing of reference memory in a manner that is qualitatively similar to humans with Korsakoff's disease".

Histological analyses of PTD animals have consistently revealed the following lesions: a) a large bilateral medial thalamic lesion that includes the IML and denervates layer IV in several cortical areas involving all the projection sites of the MDn, and b) lesions of the mammillary bodies found in conjunction with enlargement of the mammillary recess of the 3rd ventricle.

Subsequent stereotaxic lesioning of these two sites of PTD pathology have produced interesting results. Our laboratory has compared the performance of animals with radiofrequency (RF) lesions of the medial portion of the IML (M-IML); the lateral portion of the IML (L-IML); the medial mammillary nuclei (MBs); the M-IML and MBs combined; and the fornix. DNMTS performance was impaired following either L-IML or fornix lesions, and only the L-IML lesion produced a deficit comparable in severity to that of recovered PTD animals (Mair et al., 1990). These same animals were also trained on a procedure in which retention intervals were varied according to performance to determine the critical delay at which individual animals were 75%

accurate on DNMTS. In this case, only L-IMLs were impaired, with performance dropping below 75% accuracy at significantly shorter intervals than controls. In addition, the performance of the L-IML group closely resembled that of PTD animals tested on this same procedure (Robinson, 1991). Lastly, Mair et al. (1992) compared the effects of anterior and posterior RF lesions to complete RF lesions of the L-IML. Only those animals with complete L-IML lesions were severely impaired on DNMTS, once again exhibiting deficits comparable to those seen in PTD animals (Knoth & Mair, 1991; Robinson & Mair, 1991). Taken together, these findings indicate that lesions of the L-IML site produce DNMTS deficits that are qualitatively similar to those observed in PTD animals.

Mair et al. (1992) consider a number of possible explanations for the effects of thalamic lesions on DNMTS. First, L-IML lesions may produce incidental damage to the mammillothalamic tract, impairing working memory by disconnecting the thalamus from hippocampal input. However, this argument is inconsistent with several observations: a) the mammillothalamic tract is spared in PTD animals (Knoth & Mair, 1991); b) both PTD and L-IML lesions tend to spare the anteromedial (AM) thalamic nuclei (Knoth & Mair, 1991; Robinson & Mair, 1991; Mair & Lacourse, 1992); c) DNMTS deficits produced by L-IML lesions were much greater than either MB or fornix lesions disrupting hippocampal inputs to AM (Mair & Lacourse, 1992); and d) lesions of the M-IML which destroy the AM have no effect on DNMTS performance. Second, amygdaloid pathways known to travel through the inferior thalamic peduncle and the IML to MDn (Krettek & Price, 1977b) might be disrupted by IML lesions. Although disruption might well occur with L-IML lesions, these fibers largely terminate in the medial MDn in areas corresponding to the M-IML lesions. Findings that M-IML lesions do not impair DNMTS performance (Mair & Lacourse, 1992) may be taken as evidence that amygdalo-thalamic pathways are not critical to the performance of this task. In addition, the finding that combined MB/M-IML lesions fail to affect DNMTS runs

counter to arguments that combined lesions of hippocampal and amygdalo-thalamic pathways produce thalamic amnesia (W. P. G. Mair et al., 1979; Mishkin, 1982).

A third possibility is that L-IML lesions interrupt the flow of information along fiber tracts within the IML. Cramon et al. (1985) have asserted the importance of the mammillothalamic tract and the IML in diencephalic amnesia. Once again, findings that M-IML lesions do not produce DNMTS deficits comparable to those seen following either lesions of the L-IML or PTD treatment argue against this. A fourth possibility is that destruction of the intralaminar nuclei produces the DNMTS deficit. If this is the case, then the findings of Mair et al. (1992) and Mair and Lacourse (1992) may be taken as an indication that this deficit requires nearly total destruction of the paracentral and centrolateral nuclei, which correspond to the complete L-IML lesion site.

Finally, it is possible that L-IML lesions disrupt DNMTS performance by extensively denervating prefrontal cortex. In the rat, bidirectional pathways between MDn and PFC are known to course through the location of the L-IML lesion (Krettek & Price, 1977a). In addition, PTD animals present evidence of cortical denervation in all the target areas of the MDn (Mair et al, 1989). This last explanation for the effects of thalamic lesions on DNMTS performance is of particular relevance to the experiments reported on here. The present review of the human and animal literature has discussed findings from a number of studies indicating that lesions of MDn projection areas in PFC disrupt performance on delayed response type tasks (DR, DA) based on spatial cues. However, what remains in question is whether lesions of these areas, i.e. the shoulder of the Medial Wall and cortex dorsal to the rhinal sulcus, can account for the full extent of behavioral deficits seen in PTD animals or from complete lesions of the L-IML site.

For this reason, a comparison of rats with discrete stereotaxic lesions of the L-IML, MW and RS on the Radial Arm Maze (RAM), Spatial Serial Reversal (SSR) and Spatial Delayed Nonmatching to Sample (DNMTS) tasks was done. Our laboratory has provided substantial evidence indicating that PTD animals demonstrate behavioral

impairments comparable to those seen in Korsakoff's patients. Until recently, the focus has been on the medial thalamic contribution to memory. The purpose of this series of experiments was to extend our analysis to the contributions of associated prefrontal areas employing a coherent set of spatial tasks already proven with thalamic lesions.

Radial Arm Maze

The Radial Arm Maze introduced by Olton and Samuelson (1976) has received a great deal of attention in recent years as a pure test of spatial working memory in rodents. Several variations of the original task have been done in animals with either thalamic or prefrontal lesions.

Kessler, Markowitsch and Otto (1982) outline deficits in the RAM performance of animals with lesions of the MDn as compared to animals with ventral tegmental lesions and to sham operated controls. They report no intergroup differences in error rates when trials were uninterrupted. However, MDn animals committed significantly more errors following the introduction of an imposed delay between trials, leading Kessler et al. to conclude that the deficits observed in the MDn group resemble those seen in humans with medial thalamic lesions. However, the RAM employed by Kessler et al. involved a system of concentric and hexagonal alleys that was more complex than that developed by Olton. It is possible that the deficits observed are due to the complexity of the maze, rather than true impairments in working memory.

Stokes and Best (1988) also report deficits in rats with lesions of the MDn in a task similar to that used in the present series of experiments. Animals were pretrained to a criterion of 7 correct responses out of 8 choices on 5 consecutive days, given bilateral lesions of the MDn, and then tested postoperatively on the same task. The authors report that MDn lesioned animals made significantly more errors as compared to controls. They also provide evidence of perseveration and response patterning postoperatively. Stokes and Best assert the importance of task difficulty, noting

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previous observations that MDn animals are only impaired following either long imposed delays or, as was done in this particular study, testing in cue impoverished environments. Recent findings in our laboratory indicate that task difficulty may not be the critical factor. Kivlihan (1992) compared postoperative acquisition by animals with stereotaxic lesions of either the MDn, L-IML, or fornix on an 8 arm task similar to that described by Stokes and Best. All three groups made more errors to criterion than did controls. Of particular interest , however, is the finding that L-IMLs were significantly more impaired than the MDn group. This observation raises the question whether the failure to observe deficits in animals with MDn lesions on RAM tasks without delays or cue restrictions is due to the limited or incomplete nature of their lesions. Previous findings in our laboratory that DNMTS performance is impaired only by extensive lesions of the thalamus (Mair et al., 1992) lend support to this argument.

Regarding the performance of prefrontal animals, Kesner and Holbrook (1987) report the results of various manipulations of the original 8 arm task. Rodents with medial frontal lesions were tested for item and order memory of the sequential presentation of 2, 4 and 8 spatial locations. The authors argue that these animals exhibit a failure to organize temporal information that is consistent not only with the primate literature, but also with findings in humans demonstrating spared recognition of objects but not of the orders in which they are presented. In a subsequent study, Kesner (1990) used the same apparatus to test for frequency memory of specific spatial locations. During a "study phase" rats were allowed to visit three different spatial locations once and one spatial location twice. During a subsequent test phase, rats were reinforced only for selecting the twice visited location. Impairments in frequency memory in medial prefrontal animals were once again taken to reflect temporal ordering deficits.

Finally, in a task in which 4 arms contained reinforcement, and 4 did not, Kolb et al. (1983) report acquisition deficits in both medial frontal and orbitofrontal animals. However, both groups eventually acquired the task. These results are consistent with Robinson's (1991) findings that PTD animals were eventually able to acquire a similar task in which 5 arms were always reinforced and 3 were not. In Kolb's study, medial frontals were somewhat slower to learn the task as compared to controls, whereas orbitals were seriously impaired. Both medial and orbital animals entered the unreinforced alleys more often than did controls, and orbital animals returned to arms in which they had previously been reinforced more often than either medial or control subjects.

Assessing the RAM literature remains difficult for two reasons. First, thalamic lesions intended to target the MDn, including those done in the studies discussed here, involve either stereotaxic or excitotoxic lesions that are often limited in size, and more specifically in their anterior/posterior (AP) extent. Second, PFC lesions are often done by either ablation or aspiration techniques, rather than finepoint stereotaxic lesions. Consequently, the true origins of the reported deficits remain in question.

Spatial Serial Reversals

In 1977, Kolb reported impaired performance of spatial reversals in animals with lesions of the MDn. Employing a Grice Box, Kolb assessed initial learning of a two choice spatial disc_mination and four subsequent reversals of the problem. Although the MDn group performed similarly to controls during initial learning, they were impaired on the first reversal and failed to show improvement over time. However, it should be noted that Kolb's lesions were large and frequently included other midline thalamic nuclei.

Mair et al. (1991) report that PTD animals made significantly more errors during acquisition of a two choice spatial discrimination than did controls, yet unlike Kolb's lesioned animals they achieved criterion levels of performance on seven

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subsequent reversals. In fact, PTDs demonstrated a pattern of transfer between problems similar to that of controls, with both groups committing the largest number of errors on the first reversal problem followed by steady improvement across subsequent reversals. Until the present set of experiments, our laboratory had not tested the performance of animals with stereotaxic lesions of the L-IML on SSR. Thus this experiment was expected to provide an important comparison to our findings in PTD animals.

The present set of experiments also provide the first assessment in our laboratory of the performance of animals with prefrontal lesions. The testing of animals with MW and RS lesions on an SSR task was of great interest in light of the anatomic relationship between the medial thalamus and the PFC. In an early study, Divac (1971) employed the Grice Box to compare frontal animals pretrained on 60 spatial reversals. Three surgical groups were compared: a frontopolar group (FP), a medial frontal group (MF), and an overtrained MF (MFov) group having completed 100 reversals prior to surgery. Animals with FP lesions demonstrated no deficits postoperatively. However, Divac reported transitory impairments on two of four postoperative reversals in both MF groups as compared to controls, with the overtrained MF group performing slightly better than the "undertrained" MF group. In 1975, Weir and Thomas replicated Divac's experiment with one exception. In this case, MF animals received limited presurgical training (initial learning followed by 4 reversals) but demonstrated no impairments postoperatively. Weir and Thomas concluded that although Divac's "undertrained" group may have appeared impaired relative to overtrained MFs, the deficits were subtle at best.

In 1974, Kolb et al. used the Grice Box to compare the performance of medial frontal and orbitofrontal animals to controls on a series of 5 spatial reversals. Although neither group was found to differ significantly from controls on initial learning, only orbitofrontal animals showed improvement across reversals. Almost

10 years later, Kolb et al. (1983) tested animals on a nearly identical task and reported that rats with medial or orbital lesions did not differ from controls on any measure.

A large proportion of the rodent literature has also employed olfactory discrimination tasks to assess the performance of prefrontal animals given ties between olfactory cortex, MDn and RS. More specifically, cells within the piriform cortex are known to project by way of the inferior thalamic peduncle to the central segment of the MDn, which in turn projects to the dorsal bank of the rhinal sulcus (Krettek & Price, 1977b; Price, 1985). Eichenbaum et al. (1983) have provided evidence of a dissociation in deficits between MW and RS animals on spatial delayed alternation and olfactory go, no-go discrimination tasks. While MW's were impaired only on the spatial task, RS's were impaired only on the discrimination task. According to Eichenbaum et al., whether or not this discrimination deficit extends to other stimulus modalities remains to be clearly demonstrated.

Delayed Nonmatching to Sample

Whereas the RAM task was selected for its capacity to assess the encoding of spatial information, SDNMTS was selected for the demands it places upon the ability to make temporal distinctions.

The performance of both PTD animals, and those with RF lesions of the thalamus on NMTS tasks has been studied extensively in our laboratory. Knoth & Mair (1991) report deficits in response latency and accuracy on an intensively pretrained (1, 345 trials) Nonmatching to Sample task. Of critical importance are the histological findings reported in this study, namely that PTD animals with confirmed lesions of the IML demonstrated a considerable drop in performance following treatment whereas PTD animals with sparing of the IML showed only transitory deficits.

In a related study, designed to investigate the preventative properties of the glutamate antagonist MK-801, Robinson and Mair (1991) demonstrate impaired DNMTS performance in PTD animals. In this case, the performance of animals

undergoing pyrithiamine-induced thiamine deficiency was compared to that of animals undergoing the same treatment paired with injections of MK-801. Almost all rats receiving MK-801 were unimpaired on DNMTS as compared to the "unprotected" group which was less accurate and tended to respond more slowly. Animals were pretrained on DNMTS at several retention intervals ranging from 0.1 to 6.0 seconds. Following treatment, animals were retrained on DNMTS at retention intervals ranging from 3.0 to 15.0 seconds using a staircase procedure. During staircase training, when an animal scores above 75% correct in a session the retention interval is increased in the subsequent training session. If an animal scores below 75% correct on 2 consecutive sessions at a specific retention interval, the delay is decreased on the subsequent training session. In this way retention intervals are varied as a function of performance to determine the critical delay at which the performance of individual animals stabilizes on DNMTS. Robinson and Mair report that performance of PTD animals decayed to a 75% accuracy level at abnormally short latencies. However, the capacity to meet the procedural demand of the task is spared at short delays. Once again, histological analyses uncovered thalamic lesions centered on the IML in all animals of the PTD group. Only one MK-801 animal displayed similar pathology.

Mair and Lacourse (1992) compared the performance of animals with RF lesions of the: a) lateral portions of the internal medullary lamina, b) medial portions of the internal medullary lamina (M-IML) , c) mammillary bodies, d) M-IML and MB combined, e) fornix, and f) shams. Only L-IML and fornix groups were significantly impaired on a SDNMTS task identical to that proposed here, and deficits in the fornix group were substantially smaller than those seen in L-IMLs. On the basis of these findings, Mair et al. (1992) compared performance following partial vs. complete lesions of the L-IML and found that only the lesions extending AP for most of the L-IML produced significant impairments in SDNMTS. In addition, the results of both these

studies corroborate findings in PTD animals of spared capacity to meet the procedural demands of the task with performance remaining dependent upon retention interval.

Finally, preliminary results from a pilot study testing SDNMTS performance in MW animals (R.G. Mair & E. E. Kivlihan, personal communication, May 7,1991) indicated impairments similar to those seen in L-IMLs. In response to these findings, and to the fact that the performance of RS animals had yet to be tested in our laboratory, a detailed analysis of the relative contributions of these two areas of prefrontal cortex to SDNMTS performance was done.

In summary, the purpose of the experiments described in the following pages was to assess and compare deficits following lesions of the L-IML of thalamus and the MW and RS of prefrontal cortex. The rodent literature indicates that the RAM is sensitive to impairments in spatial memory not only following lesions of the hippocampal system, but also of the medial thalamus and MDn projection sites in PFC. The task also appears to be capable of making distinctions in deficits between MW and RS animals. Our laboratory had previously reported RAM deficits in animals with lesions of the L-IML. In order to test the possibility that the impairments observed were due to acquisition deficits, another experiment was done in which animals were pretrained. It was expected that following pretraining, the performance of L-IML, as well as MW and RS groups, would provide an accurate measure of the ability to encode spatial information and hold it "on line" sufficiently long to complete the task.

The literature also presents divergent evidence regarding the degree of SSR deficits demonstrated by rodents with lesions of the MW. In addition, little still is known regarding the nature of the deficits in RS animals, namely whether discrimination deficits seen following lesions of the RS are modality specific or reflect a generalized discrimination deficit. Thus the SSR task was expected to provide further information regarding the nature of the discrimination deficits observed in rhinal sulcal animals.

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Last, the measurement of the performance of prefrontal animals on SDNMTS was expected to: a) provide further information as to the magnitude of the SDNMTS deficits following lesions of the MW and RS as compared to those following lesions of the L-IML; b) provide a direct comparison of the relative contributions of MW and RS to the performance of this task; and c) provide the first detailed analysis in our laboratory of the ability of prefrontal animals to make temporal distinctions.

Taken together it was expected that these three tasks would provide a systematic evaluation of spatial memory capacity in animals following lesions of the thalamus and prefrontal cortex.

EXPERIMENTS

General Plan

In Experiment 1, subjects were initially pretrained on a radial arm maze task. Upon achieving criterion performance, animals were matched for performance and assigned by block randomization to the following surgical groups: Medial Wall, Rhinal Sulcal, Lateral-IML or control. Following surgery, relearning of the RAM task was measured. Upon completion of the RAM task, the same animals were trained on a spatial discrimination and a series of 7 consecutive reversals of this task.

In Experiment 2, a second set of subjects received dipper and shuttle training followed by pretraining on a spatial delayed nonmatching to sample task. Upon achieving criterion performance, animals were matched for performance and assigned by block randomization to one of the following surgical groups: Medial Wall, Rhinal Sulcal, or control. A L-IML surgical group was not included in this study given the extensive SDNMTS data already collected from L-IMLs by our laboratory. Following surgery, relearning of this task was measured.

Experiment 1

Subjects

Forty male Long Evans rats, obtained from the Charles River Laboratories served as subjects. Animals were housed in individual wire mesh cages in a vivarium on a 12:12 light:dark schedule. Animals were placed on a 23-hr water deprivation schedule 5 days prior to behavioral training, and maintained daily on 1 hr of water for the duration of the studies. Animals were fed standard lab chow ad libidum. Rats were handled extensively both prior to and throughout the experiments.

<u>Apparatus</u>

Subjects were tested first on the Radial Arm Maze and then on a Spatial Serial Reversal task.

The RAM (92 cm in height), consisting of eight arms (62.5 cm in length X 12.5 cm in width), was constructed of wood according to specifications set forth by Olton and Samuelson (1976). One modification was made, however. The vast majority of RAM experiments reported in the literature employ food reinforcers. Our maze was fitted with wells for the containment of a water reinforcement (0.2 ml tap water). Wells were constructed of bottle caps (1 mm high X 2.5 mm wide) centered and placed 4 mm from the end of each of the radial arms. In addition, wells constructed of the same size bottle caps were affixed underneath each arm, directly beneath the reinforcement wells, to control for any possible odor cues released by the tap water used for reward. The RAM was located in a secluded room containing numerous visual cues including: a chemistry hood, a sink, relay racks, a Morris Water Maze set on its side, cabinets and chairs.

Behavioral testing and training for the SSR task took place in chambers similar to those described by Knoth and Mair (1991). Three Lafayette 8500 automated chambers measuring 31 cm in length X 21 cm in width X 20 cm in height were used. Each chamber was constructed of stainless steel side panels with a floor consisting of stainless steel bars measuring 0.4 cm in diameter (spaced 1.7 cm apart). A pan of pine shavings located beneath the floor was changed regularly. The top and ends of each chamber were constructed of Plexiglass. The top was outfitted with a houselight and buzzer. A single port was centered at the "start" end and two ports were placed 10.5cm apart at the "choice" end. Ports (5.5 cm in diameter and 7.5 cm in length) were constructed of PVC tubing. All three ports were mounted horizontally (3 cm above the floor), opening into the chamber. Each port was outfitted with a 6W instrument light. In addition, infrared photocells were positioned vertically in each port for the detection of nose pokes. Within each choice port stainless steel drinking spouts delivered 0.1 ml tap water

reinforcements following the brief (0.1 s) activation of miniature solenoid valves. Licks were recorded by drinkometer circuit. Finally, each chamber was placed in a sound isolating box constructed of wood (dimensions: 60 cm in length X 40 cm in width X 47 cm in height) which was ventilated by fans which drew and exhausted air through baffles.

Treatment

Subjects received water ad libitum beginning three days prior to surgery and throughout a 10-12 day recovery period.

Surgery was performed under sterile surgical conditions. Animals were anesthetized with a combined dose of Ketamine (60 mg/kg IM) and Rompun (6 mg/kg IM) and mounted on a Kopf Stereotaxic Instrument with incisors 3.3 mm below the interaural line (IA). In all groups, including the control animals the scalp was sectioned along the midline, retracted, and the periosteum scraped away from the skull. Following treatment, wounds were sutured and bathed in an antibacterial solution (Povodine Solution). In experimental animals, skull openings were made with a dental burr. Radio frequency lesions were made bilaterally. For Lateral-IML treatment, lesions were made 1.0 mm off midline. At AP 5.2 lesions were placed at DV 4.8 and 3.6. At AP 6.2 lesions were placed at DV 5.0 and 3.6. At AP 7.2 lesions were placed at DV 5.0 and 3.6. Lateral-IML lesions were made by heating an electrode to 70° C and maintaining this temperature for a period of 60 s. For Medial Wall treatment, lesions were made at 0.8 and 2.0 mm off the midline. At 0.8 mm off ML they were made at: a) AP 4.7/DV 1.0; b) AP 3.7/DV 1.0 and 2.2; c)AP 2.7/DV 1.0 and 2.2; d) AP 1.7/DV 1.0 and 2.2; and e) AP 0.7/DV 1.0 and 2.2. At 2.0 mm off ML they were made at: a) AP 4.7/DV 1.0; b) AP 3.7/DV 1.0; and c) AP 2.7/DV 1.0. For Rhinal Sulcal treatment lesions were made: a) 1.8 and 3.0 off the midline at AP 4.7/DV 2.2 ; b) 1.6, 2.8 and 4.0 off the midline at AP 3.7/DV 4.0; and c) 3.0 and 4.2 mm off the midline at AP 2.7/DV 5.1. Medial Wall and Rhinal Sulcal lesions were done at 75° C for 60 s also.

Body temperature was maintained by wrapping the animals in small blankets during surgery and during the first 24 hours of recovery in their home cages. Animals were allowed a 12 - 14 day recovery period before resuming behavioral testing and training.

Procedure

RAM. Subjects were given two daily adaptation sessions in which they were individually placed in the center of the RAM and allowed to explore the apparatus for a period of 10 minutes. Once adapted, animals were pretrained (25 sessions) on the RAM to a criterion of 7 of 8 correct choices on three consecutive sessions. We began pretraining with a pure RAM task. However, during initial training on this "Straight 8" task, response patterning (eg. running in a clockwise or counter clockwise pattern; the use of adjacent arm strategies) was observed in the animals. Therefore a 1 minute delay was introduced at various points in the task until the patterning behavior was eventually eliminated by what is best described as a "2-4-6 procedure" in which each animal was returned to its cage for 1 minute after the 2nd, 4th and 6th reinforcements of every session.

Before placing each individual animal on the maze, the apparatus was cleaned of any residue from previous trials, each of the eight reinforcement wells was baited with water (0.2 ml), and control wells, located underneath each arm, directly beneath each reinforcement well were completely filled. The subject was placed in the center of the maze facing the juncture between Arms 1 and 2 both at the initiation of a session and following each of the delays. Again, we found the use of a consistent starting point necessary to prevent the use of adjacent arm strategies to complete the task. Placement of all four paws in an arm constituted an entry. Correct choices (an entry with drinking), and errors (a revisit to an arm in which reinforcement has previously been obtained) were recorded. Following surgery, reacquisition to the same criterion was measured.

SSR. Upon completion of the RAM task, all animals underwent SSR training similar to that described previously by Mair and Knoth (1991). In the present study, animals received two sessions of dipper training followed by shuttle training. During dipper training, the choice port in which reinforcement was available was darkened, while lights remained lit in the start port as well as the choice port in which reinforcement was not available. Reinforcement (0.1 ml of tap water) was delivered on an FI-6 s schedule in response to licks at the drinking spouts located in the choice end ports. Reinforcements (5 per side) were alternately delivered until the animals reached a criterion of 20 reinforcements per side within 20 minutes on two separate sessions.

Shuttle training was done in two phases (7 sessions each). In Phase 1, subjects were given 3 reinforcements on one side and then alternated to the other until they achieved a criterion of 15 reinforcements per side in 20 minutes on two separate sessions. Each 3:3 session began with the right port light off. Once 3 reinforcements were delivered on the right side, both choice port lights turned on while the start port light went off. A poke in the start port turned that light on and the left choice port off, indicating the availability of reinforcement.

Once an animal reached criterion on the 3:3 task, it was transferred to a 1:1 task. In this phase, the procedure remained essentially the same with the exception that only one reinforcement was delivered per side prior to a nose poke in the start end. Animals remained on 1:1 shuttle training until they achieved a final criterion of 15 reinforcements per side in 20 minutes on five separate sessions.

Upon completion of dipper and shuttle training, animals began training on the SSR task. A "side preference" was determined for each individual subject by running 7 trials with reinforcement available in either choice port. The side receiving the fewest responses was designated "non-preferred" and subsequently became the S+ for the initial learning problem. Each session began with the choice lights on and start light off. Individual trials were initiated with a nose poke in the start port

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which lit the start port while turning both choice lights off. When the animal licked the spout in the S+ choice port, reinforcement was delivered, a correct choice was recorded, and the trial terminated. When an animal licked the spout on the S- side, the chamber buzzer sounded, an error was registered and the trial was terminated immediately without reinforcement. At the end of the trial, the choice lights were once again turned on and the start light turned off. Training consisted of daily sessions of 60 trials until animals reached a criterion of 10 consecutive correct choices on 2 consecutive days or 2 sets of 10 consecutive correct choices in one session. Two strings of 10 correct choices terminated a given session. Once an animal reached criterion, the side reinforced was reversed on the following day. The total numbers of correct choices and errors per session were recorded for each animal.

Histological Analyses

Upon completion of behavioral training and testing, animals were sacrificed under deep anesthesia (100 mg/kg Ketamine and 10 mg/kg Rompun IM) via transcardiac perfusion with physiological saline followed by 10% neutral buffered formalin. Tissue was frozen, sectioned in the coronal plane at 30 μm, mounted onto gelatinized slides at 150μm intervals, and stained with cresyl violet.

Results and Discussion

All Analyses of Variance (ANOVA) were done in accordance with Kirk's (1982) three step testing procedure for randomized block designs. According to Kirk, in the event an obtained F value is declared not significant following a comparison with the critical value of F for $(p-1)$ and $(n-1)(p-1)$ degrees of freedom, the analysis should be terminated and F declared not significant. However, when an obtained F statistic is declared significant, a Geisser-Greenhouse conservative F test should also be done. Last, in the event the obtained F does not exceed the critical value in the Geisser-Greenhouse test, an adjusted F test may be done. All significant values reported in this

text were tested against Geisser-Greenhouse critical values. No adjusted F tests were required.

All Post Hoc comparisons were done by the Newman-Keuls method. Although this method is susceptible to Type I error, Glass and Hopkins (1984) assert that its utilization of alphas for each null hypothesis tested avoids the overconservatism of Tukey tests which are dependent upon a single critical value.

Finally, behavior on the three spatial tasks described here was correlated with lesion size in the frontal groups. Correlations were not done for L-IMLs due to the consistent size and placement of their lesions.

Behavioral Assessments

RAM. Fifteen sessions of the 2-4-6 RAM procedure were run postoperatively. The first eight choices of each session were then analyzed. Animals with lesions of the L-IML demonstrated severe and consistent deficits postoperatively. Impairments in both frontal groups were transient.

Figure 1 represents the number of correct choices made in the first eight selections of each session. While controls performed at criterion levels on Day 1 of postoperative testing, all three experimental groups exhibited a drop in performance immediately following surgery. The L-IML group demonstrated the most extreme deficit in postsurgical performance, never exceeding 72% correct. None of the subjects in this group achie:.ed criterion levels of performance following surgery. While the thalamic animals demonstrated a persistent impairment, this was not true of the frontal groups. Nine of the ten MWs eventually relearned the task to criterion. Finally, although RSs showed an extreme drop in performance on Day 1, performance was comparable to that of the other frontal group by Day 2, and 8 of 10 animals achieved criterion by the final session of retraining.

The behavioral findings were verified by a two factor analysis of variance demonstrating significant differences overall in RAM performance between treatment

groups, $F_{(3, 36)}$ = 48.410, p < .0001, and across sessions, $F_{(14, 504)}$ = 7.276, p < .0001. In addition, the interaction between these two factors was significant, $F_{(42, 504)}$ = 3.057, p < .0001. Post hoc analyses (a = .01) revealed that animals with lesions of the L-IML performed significantly worse than all other groups. Although the frontal groups differed significantly from controls overall, these differences disappeared when analyses were restricted to the final five days of testing where the performance of all groups had stabilized. In this case, significant differences still emerged between groups, $F_{(3,36)}$ = 32.899, p < .0001; but not across session, $F_{(4, 144)}$ = .955, p = .4302; and the interaction between these two factors was no longer significant, $F_{(12, 144)}$ = .606, p = .8236. Post hoc analyses (a = .01) confirmed that only L-IMLs differed significantly from the other groups on the last days of postoperative testing.

A question arose as to whether there were any measurable differences in performance on those choices made immediately prior to the one minute delay (numbers 2, 4 , 6 and 8 of each session) and those made immediately after the one minute delay (number 3, 5, and 7), i.e. those that truly test working memory. One factor ANOVAs, again restricted to the final five days of testing, demonstrate significant differences between treatment groups on both the "nonworking", F $(3, 36)$ = 9.203, p < .0001, and "working" choices, $F_{(3,36)}$ = 17.103, p <.0001. However, the interaction between these factors was not significant, $F_{(3,36)}$ = 2.356, p = .0880. Post hoc analyses (a = .01) revealed that L-IMLs performed significantly worse than all other groups on both "working" and "nonworking" selections, and that the frontal groups neither differed from each other nor from controls in either case.

The performance of the RS group on Day 1 of postoperative testing suggested a need for further analysis. Group means (see Table 1) indicate that L-IMLs were the fastest overall to make their choices, followed by controls, and the RS and MW groups respectively. Daily means further indicate that the RS group initially was very slow as compared to the others, but exhibited the greatest change over time. A two factor

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ANOVA confirms these observations by demonstrating that there were no significant differences overall in running times between treatment groups, $F_{(3, 36)} = 1.504$, $p = .230$. However, speed did differ significantly across session, $F_{(14, 504)} = 10.429$, $p < .0001$, with the session X group interaction also being significant, $F_{(42, 504)} = 2.673$, $p < .0001$.

What would account for the initial slowness and subsequent change seen in the RS animals?. Anecdotal accounts of postoperative behavior led us to investigate the possibility that "entries without drinking", and not physical slowness, were the critical factor. Entries without drinking are defined as occurring when an animal enters an arm to the point of making physical contact with the well while failing to obtain a water reinforcement. This behavior was observed postoperatively in a sufficient number of the surgical animals to warrant recording of the frequency of its occurrence. It should also be noted that this behavior occurred in the absence of any specific difficulties in consuming water. None of the subjects failed to drink while in their home cages postoperatively.

Figure 2 demonstrates the degree to which this behavior was found to occur in RSs as compared to the other treatment groups. While it was found to a small degree in MWs, the behavior was strikingly apparent in the RS group on Session 1 of postoperative testing. By Session 2 the behavior began to diminish, reaching negligible levels by Session 4 of testing. A two factor ANOVA demonstrated significant differences between treatment groups, $F_{(3, 36)} = 18.669$, $p < .0001$; across sessions, $F_{(14, 504)} = 14.121$, $p < .0001$; and again a significant session X group interaction, $F_{(42, 504)} = 9.280$, $p < .0001$. Post hoc analyses ($a = .01$) further confirmed that animals with lesions of the RS engaged in significantly more entries without drinking than did the other treatment groups, and that the other groups did not differ from one another overall.

The results of the RAM study therefore demonstrate significantly greater impairments following lesions of the L-IML than following lesions of either of the two cortical MDn projection areas. These findings are consistent with those recently

reported by Kivlihan (1992), who observed that L-IMLs are also significantly more impaired on this same task than are animals with lesions restricted to the MDn. Although the frontal groups showed some degree of impairment in the early stages of retraining, the extreme drop in RS performance on Session 1 was largely accounted for by "entries without drinking", and the deficits in both MWs and RSs virtually disappeared by the end of testing.

SSR. Three subjects (one each in the L-IML, RS and control groups) failed to complete the SSR task. Once again the L-IML group demonstrated impairments, however all animals reached criteria for initial learning and the seven subsequent reversals within 30 postoperative sessions (range = 9 - 30, median = 10).

A one factor ANOVA demonstrated a significant difference between groups in the number of trials to complete initial learning: $F_{(3,33)} = 4.173$, $p = .0130$. Post hoc analyses revealed that L-IMLs differed significantly from all other groups at the .05 level. A two factor ANOVA analyzing the number of trials to criterion across reversals was again significant between groups: $F_{(3,33)} = 28.546$, $p < .0001$, and across reversals: $F_{(6,198)} = 15.374$, $p < .0001$, but the interaction of these factors was not significant: $F_{(18, 198)} = .596$, $p = .9001$. Post hoc analyses revealed that L-IMLs differed significantly from all other groups at the .01 level.

The performance of experimental and control groups was also measured in terms of errors to criterion. L-IML animals committed more errors on the initial discrimination problem and throughout the reversals than any of the other groups (see Figure 3). Despite the greater numbers of errors committed by the L-IMLs, all animals in every group reached criterion on all problems. In addition, all groups eventually demonstrated positive transfer between problems. Given the ability of L-IMLs to learn the spatial discrimination problem and eventually complete all reversals, the question arose whether rates of improvement in L-IMLs might be similar to those of the other groups. Rates of improvement are illustrated in Figure 4 which expresses mean errors

to criterion on each reversal as a proportion of mean errors to criterion committed during initial learning. Although the rate of improvement was slow initially in the thalamic group, on R5 - R7 performance approximated that of the other three groups.

These observations were confirmed by a two factor ANOVA demonstrating significant differences in SSR performance between treatment groups, $F_{(3, 33)} = 33.470$, $p < .0001$. Post hoc analyses (a = .01) confirmed that animals with lesions of the L-IML performed significantly worse than the other treatment groups, while the frontal and control groups did not differ from one another overall. A significant main effect was also found for reversals, $F_{(7, 231)} = 10.670$, $p < .0001$ confirming that performance improved across problems. However, the interaction of these two factors was not significant, $F_{(21, 231)} = 1.074$, $p = .377$, indicating that rates of improvement were in fact similar across all groups.

The performance deficits observed in L-IMLs in the present study are comparable to those seen previously in post thiamine deficient animals. When tested on this same SSR task, PTDs committed more errors to criterion than did controls during initial learning and during the reversal phase (Mair et al., 1991). Like L-IMLs, PTD animals also learned the initial two-choice spatial discrimination and eventually demonstrated positive transfer across reversals. The present findings therefore indicate that lesions of the L-IML produce SSR deficits that are qualitatively similar to those observed in the PTD model.

The pattern of results across RAM and SSR studies demonstrates impairments in the performance of L-IMLs on two tests of learning and memory based on spatial cues. Lesions of the two prefrontal target areas of the MDn of thalamus produced neither consistent nor comparable deficits to those seen in animals with lesions of the L-IML. Therefore, Experiment 1 presents evidence that lesions of the MW and the RS alone cannot account for the deficits observed in animals with lesions of the L-IML.

<u>Lesion Analyses</u>

     <u>Histology.</u> Figures 5a and 5b are photomicrographs of lesions representative of each of the three surgical groups. The cresyl violet stained brain sections were examined under light microscope and lesions drawn onto templates via camera lucida. Two drawings were made relative to Bregma (as designated in the stereotaxic atlas of Paxinos and Watson, 1982) representing the anterior and posterior lesion dimensions for each animal. Drawings were done at 10X magnification. Compensations were made for alterations in shape due to collapsed tissue. For the frontal groups, the anterior section was drawn at +4.0mm from Bregma. Posterior sections were drawn +2.6mm from Bregma. For L-IMLs, the anterior section was drawn at -1.80 from Bregma, the posterior at -3.80 from Bregma. Figures 6 and 7 show the minimum and maximum lesions for MW and RS groups. In these cases, drawings were projected from our templates onto comparable templates taken from the Paxinos and Watson atlas (1982).

     Lesion means for the two frontal groups are listed in Table 2. The criterion for the measurement of cortical lesion extent was as follows: where cortex was spared through Layer IV, it was considered intact; where there was evidence of lesion or severe gliosis in Layer IV, the deep layers were considered destroyed. Measures reported here represent the length of each lesion in millimeters, with the medial wall and rhinal sulcus subdivided according to Zilles' (1985) descriptions. Included in the MW are: frontal area 2, cingulate areas 1 & 3, and the infralimbic area. Included in the RS are dorsal portions of the agranular insular cortex (designated simply as area "AI" on the Watson and Paxinos templates), and the lateral, ventrolateral, ventral and medial orbital areas. No subject was eliminated from the analyses on histological grounds.

     A comparison of the lesion sizes of the three frontal animals failing to achieve criterion performance on the RAM task with their group measures revealed that all had lesions that were less than one standard deviation from their group means. In MW-161 the anterior measure was 6.36mm and the posterior measure was 7.60mm. In RS-113

the anterior measure was 7.79mm and the posterior measure was 6.17mm. Finally, in RS-154 the anterior and posterior measures were 8.17mm and 5.70mm respectively.

Of the three animals failing to acquire the SSR task, neither the L-IML nor the RS animals advanced beyond shuttle training. L-IML lesions were very consistent in size and location, and the lesion in L-IML-136 was uniform with that of its group. The lesion in RS-105, at 6.84mm in the anterior RS and 5.94mm in the posterior RS, was less than one standard deviation from the RS group mean. Finally, the control subject in question did acquire the initial discrimination problem. However, during the reversal phase C-142 consistently retrieved shavings from the pan located beneath the operant chamber floor and utilized them to permanently break the photocell beam in the start chamber, allowing this animal to obtain reinforcement without meeting the procedural demands of the task.

Pathophysiology

RAM. Correlations were done between behavior and lesion size. Table 3a presents an analysis of performance in relation to MW lesion size. Performance was analyzed both across all postoperative sessions, as well as across the last 5 days where performance of all groups had stabilized. No significant correlations were found between size of lesion and RAM performance in the MW group.

Table 3b represents an analysis of performance in relation to RS lesion size. In this case, a marginally significant relationship was found between the extent of anterior MW encroachment and total RAM performance. Restricting analyses to Days 1 - 3 of RAM for the RS group (Table 3b) revealed a significant relationship between extent of anterior MW encroachment and Day 1 performance only. In addition, significant relationships were found between: a) anterior MW encroachment and entries without drinking; b) extent of anterior RS lesion and entries without drinking; and c) extent of posterior RS lesion and entries without drinking.

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The RAM correlation analyses are exploratory in nature, and must be evaluated very carefully in light of successful relearning by both frontal groups. In addition, group n's are too small to allow for anything other than the suggestion of behavioral trends. The pattern of relationships between lesion size, initial RAM performance and entries without drinking in the RS group suggests that lesion severity in this area of PFC might influence RAM performance. However, two issues should be considered with respect to the anomalous entries without drinking in these animals. First, although the correlation between the frequency of this behavior and extent of anterior medial wall encroachment in rhinal sulcal animals appears to be strong, the relationship does not hold with respect to the size of anterior MW lesion in the medial wall group. Second, one cannot ignore either the anatomic relationship between primary olfactory cortex and RS (Kretteck and Price, 1977b; Price, 1985) or the proximity of gustatory cortex to the posterior RS (Zilles, 1985). Thus a potential explanation for the early emergence and swift disappearance of this behavior relates to the disruption of either olfactory or gustatory projections in or near the area of the rhinal sulcus. It may be that the sensory qualities of the water reinforcer were somehow altered, and that the behavior subsided once a significant amount was consumed postoperatively.

SSR. Table 4 presents an analysis of SSR performance in relation to MW and RS lesion size. Again, these exploratory analyses must be evaluated very carefully in light of the successful acquisition and reversal performance of this task by both frontal groups. Extent of MW lesion did not correlate significantly with performance on the SSR task. However, correlations between lesion size in both the anterior and posterior portions of the RS and SSR performance were significant, indicating that perhaps a larger lesion would have produced measurable impairments in this group.

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Experiment 2

Subjects

Thirty male Long Evans rats, also obtained from the Charles River Laboratories served as subjects. Housing and animal care were identical to that listed above.

Apparatus

Animals underwent training and testing in the same Lafayette 8500 automated chambers housed in sound isolating boxes as used in the SSR task.

Treatment

Surgical procedures were identical to those described for Experiment 1, with the exception of the omission of a L-IML group. The thalamic group was not included in this study given the extensive assessments of L-IML performance done previously in our laboratory on SDNMTS tasks.

Procedure

All animals received 2 sessions of dipper training, 3 sessions of 3:3 shuttle training, and 4 sessions of 1:1 shuttle training as described in Experiment 1. They were subsequently pretrained on a DNMTS task similar to that used by Mair et al. (1992). Each DNMTS trial involved two runs, a sample and a choice run. During sample runs, a reinforcer was delivered in response to a lick in the choice end port that was darkened. During choice runs, both choice ports were darkened and a reinforcer delivered in response to a nosepoke in the choice port that had not been associated with reinforcement on the preceding sample run. An error during a choice run terminated a trial immediately without delivery of reinforcement. Errors were signalled by the activation of a buzzer for a 3.0 s duration. DNMTS trials were repeated following errors in order to avoid any development of side preferences. Finally, the side reinforced on sample runs was varied following correct responses according to an irregular but balanced schedule. This procedure ensured that there was an equivalent probability

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that the side reinforced on each successive sample run either remained the same or was alternated.

Animals were trained on 20 DNMTS trials at a retention interval of 0.1 s during their first preoperative session. They were then trained on 50 trials per session at the same interval for 600 trials. This was followed by training on 300 trials at a 1.5 s interval. Finally, the animals received a minimum of 750 DNMTS trials at a 3.0 s interval. On each day of surgery, subjects were ranked according to their performance (percent correct) across the preceding 150 trials. The top performers were then selected in blocks of 3 for random assignment to surgical treatments. This design kept treatment groups balanced while providing additional training for animals needing it prior to surgical treatment. The maximum number of trials given any animal during pretraining was 1200.

Following a 12-14 day recovery period, animals were tested at the same 3.0 second delay across 16 postoperative sessions for a total of 800 trials. Response measures included: total correct responses and total errors per session. In addition, measures of response latency and accuracy as a function of latency were obtained by recording the total numbers of fast correct and fast errors (i.e. correct and incorrect responses made within 3.0 s of the end of the retention interval) per session. Finally, the total number of correct choices made following a correct response (noncorrection trials) and the total number of correct choices made following an error (correction trials) were also recorded. These final two measures monitored the effects of the correction procedure.

Histological Analyses

HRP. Upon completion of behavioral training and testing, a subset of animals was selected for an additional treatment prior to being sacrificed. Horseradish peroxidase (HRP) was introduced bilaterally into the MDn of these animals. The peroxidase enzyme, which is absorbed by axon terminals at the injection site, travels

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up axons to their point of origin where it is taken up into the somal lysosomes. Following sacrifice, when the brain tissue is reacted cell bodies containing transported HRP darken to the point of visibility under magnification (cf. Jones & Hartman, 1978; Mesulam, 1978). In the present study, implants of crystalline wheat germ agglutinate-horseradish peroxidase (WGA-HRP) were used so that both anterograde transport (as evidenced by visible axons) and retrograde transport (as evidenced by filling of cell bodies) might be observed. The purpose of this experiment was to determine the extent to which prefrontal cortical lesions found to disrupt DNMTS performance destroy neural connections with the MDn.

The WGA-HRP was delivered to the MDn by glass micropipettes prepared in accordance with the method described by Mori et al. (1980). Micropipettes were formed by pulling glass capillaries (1.0 mm diameter) to form tips approximately 50 μm in diameter. The micropipette tips were then dipped in melted paraffin and filled via capillary action to a point above the surface level of the paraffin bath. After being left at room temperature for 5 - 10 minutes, the tips were subsequently dipped in ethyl ether for 5 minutes or until microscopic inspection revealed that paraffin within the tip area was dissolved. This procedure produced an empty cavity capped with a plug separating the tip from the remaining length of micropipette. Micropipettes were then left overnight to allow for the evaporation of any remaining ether.

To load the micropipettes, crystalline WGA-HRP was placed onto a Kimwipe (paper) platform, and positioned under a microscope from which it was packed into each of the tips. Each micropipette was carefully examined to ensure that the tip cavity was completely filled (approximate quantity: 0.15mg).

A subset of 11 animals (4 MW, 4 RS, and 3 control) was selected for treatment. All prefrontal animals selected for this procedure presented clear evidence of postoperative DNMTS deficits. Each of the frontal groups was represented by two animals demonstrating mean performance and two animals demonstrating

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performance well below the group mean. At the time of surgery animals weighed 450 - 550 grams.

Surgery was performed under clean conditions. Animals were anesthetized with a combined IM dose of Ketamine (60 mg/kg) and Rompun (6 mg/kg) and mounted onto a Kopf stereotaxic instrument with incisors 3.3 mm below the interaural line. The scalp was sectioned along the midline and retracted. Stainless steel microscrews were mounted onto the skull approximately 2.5 mm on either side from the midline. An opening was then made over the midline with a dental burr. The micropipettes were lowered bilaterally 1.0mm off the midline at AP 6.2/DV 4.6. They were then fixed into place with cranioplastic cement used in sufficient quantity to encapsulate not only the micropipettes but also the screws. This formed a cement cap which rendered the micropipettes immovable. Once the cranioplastic cement had hardened, the exposed portions of the micropipettes were cut, the skull sutured, and the wound bathed in Povodine Solution.

Body temperature was maintained by wrapping the animals in small blankets both during surgery and during recovery in their home cages. Micropipettes were left in place for a period of 36 hours. This was found to be the optimal length of time for successful transport.

Animals were sacrificed under deep anesthesia (100 mg/kg Ketamine and 10 mg/kg Pompun IM) by transcardiac perfusion beginning with saline at room temperature, followed by 10% buffered (pH 7.4) formalin at room temperature (30 minutes) and finally 10% sucrose in pH 7.4 phosphate buffer cooled to 4° C (30 minutes). Following perfusion, the brain was extracted, stored in the sucrose buffer solution, and kept at 4° C for 16 hours. The tissue was then frozen, sectioned in the coronal plane at 30µm, and stored in pH 7.4 phosphate buffer kept at 4° C. One set of sections were mounted immediately onto gelatinized slides at 150 µm intervals and allowed to dry at room temperature for a maximum of 3 hours before being reacted. A second set of

sections from each animal was stored in 5% formalin solution for mounting and staining with cresyl violet at a later date. Thus cresyl stained sections were available for all animals in Experiment 2.

The tissue was reacted in a modified version of the procedure devised by Mesulam (1978) in which tetramethyl benzidine (TMB) serves as the chromogen. Slides were rinsed for 3 minutes in distilled water. They were then transferred to a prereaction soak comprised of two different solutions designated as Solutions A and B, which were mixed in the reaction vessel immediately prior to introducing the tissue. To Solution A (277.5 ml of distilled water, 15.0 ml of pH 3.3 acetate buffer, 100 mg of sodium nitroferricyanide) was added a total of 7.5 ml of Solution B (20.0 mg of TMB per every 10.0 ml of absolute ethanol). After 30 minutes, the enzymatic reaction was initiated by adding 12.0 ml of 0.3% hydrogen peroxide to the prereaction solution. The slides were then periodically agitated during a 15 minute reaction period. The tissue was subsequently transferred to a postreaction solution (15.0 ml of pH 3.3 acetate buffer and 285 ml distilled water) for 10 minutes.

The reacted sections were left to dry at room temperature overnight. They were then counterstained by soaking for 3 minutes in a neutral red solution (40 ml of pH 4.8 acetate buffer per L of 1.0% aqueous neutral red solution - filtered). The slides were then dipped for 15 s in distilled water, differentiated in graded alcohols (15 s each in 70%, 95% and absolute ethanol), and finally cleared in xylene before coverslipping with Permount.

The slides were examined under dark field to verify the existence and location of WGA-HRP activity. The criterion for a successful WGA-HRP implant was bilateral filling centered within the MDn. Drawings were done via camera lucida (10X magnification) onto templates, with the anterior section drawn at +4.0 from Bregma and the posterior section drawn at +2.6.

Cresyl Violet. The remaining animals were sacrificed under deep anesthesia

(100 mg/kg Ketamine and 10 mg/kg Rompun IM) via transcardiac perfusion with

physiological saline followed by 10% neutral buffered formalin. Again, tissue was

frozen and sectioned in the coronal plane at 30 μm, mounted onto slides at 150 μm

intervals, and stained with cresyl violet. Tissue was examined under light field, and

lesions drawn via camera lucida (10X magnification) at +4.0 and +2.6 from Bregma.

Results and Discussion

Behavioral Assessments

Analyses of the final three days of preoperative training confirmed that

performance was comparable across surgical groups. Table 5 reports the mean number

of correct responses for each of the three surgical cohorts. On the final 3 days of

pretraining all groups performed at 86% correct or better. A two factor ANOVA

confirmed that there were no significant differences between groups: $F_{(2, 27)} = 0.232$, p =

0.7948, or across the 3 days: $F_{(2,54)} = 0.050$, p = 0.9509. The interaction between these

two factors was also not significant: $F_{(4,54)} = 0.649$, p = 0.6303.

Both frontal groups demonstrated DNMTS impairments postoperatively. While

MWs showed severe deficits, RSs eventually performed comparably to controls. Figure

8 illustrates performance on the final 3 days of pretraining and across 16 sessions of

postoperative testing. Controls performed at 80% correct on the first postoperative

session and maintained performance between 80 - 92% correct throughout testing. The

MWs dropped to nearly chance performance levels (55% correct) and never exceeded

70% correct following surgery. Although RSs demonstrated an immediate drop to 70%

correct, by the final day of testing, performance (88% correct) was equivalent to that of

the control group. Mean performance for all three groups throughout postoperative

testing is presented in Table 6.

An analysis (two factor ANOVA) of the number of correct DNMTS responses

revealed significant postoperative differences between groups: $F_{(2, 27)} = 18.890$, p <

.0001 and across sessions: $F_{(15, 405)}$ = 4.646, p < .0001. However, the group X session interaction was not significant: $F_{(30, 405)}$ = 0.824, p = 0.7347, indicating that all groups showed some measure of improvement over time. Post hoc analyses revealed that both frontal groups differed significantly from controls at the .05 level, however only MWs differed from the others at the .01 level of significance. When analyses were restricted to the last five days of testing, significant differences in performance still emerged between groups: $F_{(2, 27)}$ = 12.990, p < .0001; but not across session, $F_{(4, 108)}$ = 1.434, p = .2277; and once again the interaction between these two factors was not significant, $F_{(8, 108)}$ = 1.312, p = .2453. Post hoc analyses (a = .01) verified that once DNMTS performance had stabilized, MWs continued to differ significantly from the other groups, while RSs performed comparably to controls.

Earlier investigations have found that PTDs make significantly fewer DNMTS responses than controls within 3.0 s of the end of each retention interval (Knoth & Mair, 1991). These same studies also showed that response accuracy generally decreases at latencies longer than 3.0 s. To determine whether or not this tendency to respond slowly is responsible for the DNMTS deficits observed in both PTDs and L-IMLs, all subsequent studies have included measures of: a) response speed, and b) accuracy of responses made at latencies less than 3.0 s. Our laboratory has demonstrated that L-IMLs exhibit the same tendency to make fewer fast responses (Mair & Lacourse, 1992). However, fast responses made by PTDs and L-IMLs have also been found to be less accurate (Kivlihan, 1992; Mair & Lacourse, 1992; Mair et al., 1992; Robinson & Mair, 1992), indicating that increases in response latency cannot account entirely for the DNMTS performance deficits observed in these two subject populations.

The analyses of response latency and response accuracy as a function of latency in the present study were complicated by an equipment failure not discovered until after the completion of testing. A counter responsible for recording the number of fast correct responses made in chamber #1 failed early in postoperative testing, providing

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inaccurate data. Animals were not assigned to any particular box, and thus by virtue of this daily random assignment the range of information lost was 1 - 8 sessions of data per animal. Therefore, erroneous data for each individual animal were eliminated on a session by session basis leaving an average of 10 sessions of accurate data per group (controls = 11, MWs = 10, RSs = 10) available for analysis.

In Experiment 2, the MW group was both slower to respond and less accurate postoperatively. One factor ANOVAs revealed significant differences between groups in the number of choice run responses made within 3.0 s of the termination of the retention interval: F: (2, 27) = 36.782, p < .0001. Differences also emerged in the accuracy of fast responses: F(2, 27) = 11.121, p = .0003. Post hoc analyses (a = .01) confirmed that MWs, like PTDs and L-IMLs tested previously, made significantly fewer responses at short latencies, and that this group made fewer correct choices when responses were made within 3.0 s of the end of the retention interval. Since the accuracy deficit is present when long latency responses are eliminated from analysis, it would be difficult to argue that increases in response latency account for the MW deficit.

Previous studies in our laboratory have also demonstrated that PTDs and L-IMLs do not repeat errors during correction trials with any greater frequency than controls (Kivlihan, 1992; Mair et al., 1992; Robinson & Mair, 1992). Similarly, all groups in the present study tended to perform poorly on these repeated trials. A three factor (treatment vs. trial type and session) ANOVA revealed significant differences between trial types (correction vs. noncorrection): F (1, 25) = 49.788, p < .0001, but the interaction between treatment and trial type was not significant: F (2, 25) = 0.439, p = .6496. Thus the DNMTS deficits observed in the frontal groups also cannot be accounted for by an abnormal repetition of errors during correction trials. Restricting analyses to the noncorrection trials provides further support for this argument by indicating that the frontal groups tended to make more errors as compared to controls on these trials. In this case, a two factor ANOVA demonstrated significant differences

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in the number of correct responses between groups: $F_{(2, 27)} = 17.473$, $p < .0001$, and across sessions: $F_{(15, 405)} = 5.616$, $p < .0001$, with the interaction between these two factors not being significant: $F_{(30, 405)} = 0.960$, $p = .5294$. Post hoc analyses revealed that the frontal groups differed from controls as well as from one another at the .05 level, but that only MWs differed from the other groups at the .01 level of significance.

Similar to PTDs with pathology involving the IML (Knoth & Mair, 1991; Robinson & Mair, 1992), and animals with radiofrequency lesions of lateral portions of the IML (Mair & Lacourse, 1992), MWs in the present study exhibited a DNMTS deficit characterized by a decrease in both the frequency and accuracy of choices made at short latencies. Animals with RS lesions, on the other hand, did not demonstrate these same changes and eventually performed at levels comparable to those of controls. The behavioral findings therefore suggest that lesions of the medial wall of prefrontal cortex produce DNMTS deficits that are qualitatively similar to those observed following pathology of the medial thalamus.

Although RS animals showed deficits postoperatively, they were neither as impaired as MWs, nor did they demonstrate the changes in response latency and accuracy that were observed in MWs. Leonard (1969) has noted that the restriction of lesions to the RS is difficult given the relative inaccessibility of this area of cortex. Thus a potential explanation for RS deficits on DNMTS was that these deficits resulted from encroachment into MW. It was expected that lesion analyses would clarify this issue.

Lesion Analyses

Histology and Pathophysiology. Once again, two drawings were made via camera lucida relative to Bregma (as designated in the stereotaxic atlas of Paxinos and Watson, 1982) representing the anterior and posterior lesion dimensions for each animal. Drawings of an anterior section (+4.0 from Bregma) and a posterior section (+2.6 from Bregma) were done at 10X magnification. Compensations were made for

alterations in shape due to collapsed tissue. Figures 9 and 10 show the minimum and maximum lesions for MW and RS groups. Once again, drawings were projected from our templates onto comparable templates taken from the Paxinos and Watson atlas (1982).

Lesion means are listed on Table 2. The criterion for the measurement of cortical lesion extent was identical to that used in Experiment 1. No subject was eliminated from the analyses on histological grounds.

Correlations between lesion size and behavior are presented in Table 7. In the MW group, the size of the anterior MW lesion was negatively related to performance. In the RS group, negative correlations emerged between performance and both the extent of posterior MW encroachment and posterior RS lesion. The findings not only favor the participation of the MW in the execution of DNMTS, but also suggest a possible role for the RS in performance of this task.

Scatter plots were done comparing prefrontal lesion size across experiments. Figures 11 and 12 compare the extent of anterior and posterior medial wall lesions in the two MW groups. Figures 13 and 14 compare the extent of anterior and posterior rhinal sulcus lesions in the two RS groups. All animals in Experiments 1 and 2 had bilateral lesions producing damage in the intended target areas. Lesions were relatively homogeneous across experiments. To determine if there were differences in prefrontal lesion extent across studies, t - tests were done . No significant differences were found. The results of these analyses are presented in Table 8.

WGA-HRP. Figure 15 is a photomicrograph demonstrating typical cases of MW and RS filling following WGA-HRP implants into the MDn. In general, MDn projection areas in prefrontal cortex were readily distinguished by filled cells in deep cortical layers (V and VI) and axons terminating diffusely in layer IV, a pattern characteristic of cortex reciprocally connected with a specific thalamic nucleus. For the purposes of the present analyses, areas of WGA-HRP activity were identified according to the cortical subdivisions outlined in Zilles' (1985) atlas. Again, Zilles subdivides the anterior MW

into frontal area 2 (Fr2), cingulate areas 1 and 3 (Cg1 & Cg3). The posterior aspect is further subdivided into Fr2, Cg1, Cg3, and the infralimbic area (IL). The anterior RS includes the dorsal portions of the agranular insular cortex (AID), lateral orbital (LO), ventrolateral orbital (VLO), and medial/ventral orbital (MO/VO) areas. Finally, the posterior aspect includes the AID, LO and VLO.

Two experimental animals were eliminated from the analysis, leaving 3 successful cases per group. Case RS-224 was eliminated on the basis of an unsuccessful implant as evidenced by unilateral WGA-HRP activity in the MDn. Although the implant appeared successful in MW-201, the frontal cortical tissue in this animal contained an overabundance of artifact. The remaining 9 animals are reviewed on a case by case basis. The general findings are presented in Table 9 and further detailed in the text that follows..

Control group: Following the introduction of WGA-HRP into the MDn of thalamus, all control animals demonstrated activity in the form of visible cells (retrograde transport) and axons (anterograde transport) in the MW and RS subdivisions of prefrontal cortex.

1. Case C-11: The anterior section (+4.0 from Bregma) was characterized by cells filled in Cg1 and Cg3 of the medial wall. Also, axons were visible in the AID and LO areas of the rhinal sulcus. In the posterior section (+2.6 from Bregma) cells were filled in the AID and LO. Axons also appeared in the LO.

2. Case C-53: The anterior section of tissue showed large numbers of cells filled in area Cg3, with some cells also appearing in area Fr2 of the medial wall. Additionally, cells were found in the AID, LO and MO/VO areas of the rhinal sulcus. Finally, axons were visible in the LO and VLO of the RS. The posterior section was characterized by cellular activity in Cg3, the LO, VLO. Axons were also found in area LO.

3. Case C-164: In the anterior section sparse cellular activity was found in Cg3. However, axons were also readily visible in area Cg3 of the medial wall, and areas AID,

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LO, VLO and MO/VO of the rhinal sulcus. In the posterior section, cells were found in Cg1, Cg3, LO and VLO. Axons were also visible in the LO and VLO.

Medial Wall Group: All MW animals demonstrated that pathways from MDn to RS remained intact following lesions to the MW. Figure 16 (Case MW-251) shows a typical pattern of intact projections between the RS and MDn.

1. Case MW-226: The anterior section of this animal showed visible axons in the MO/VO, VLO, LO and a small amount of crossover into the AID of the rhinal sulcus. Axons were also visible in portions of Cg3 spared by the medial wall lesion. Cells and axons were also visible in the posterior AID, LO, and VLO areas of the RS. In addition, evidence of cellular activity was found in the posterior Cg3 and IL areas spared by the MW lesion.

2. Case MW-245: The anterior sections in this case revealed cells filled in the LO and VLO of the RS. Sparse axons were also found in the AID. Finally, some sparse cells appeared in area Fr2 of the MW. The posterior section of this animal showed both cells and axons in the AID, LO, and VLO. Once again, cells also appeared in Fr2.

3. Case MW-251: In this case, cells were found in the anterior AID and LO; axons appeared in the AID and LO with some crossover into the VLO of the RS. Cells were also visible in Fr2. In the posterior section, cells and axons were found in the AID, LO and VLO.

Rhinal Sulcus Group: All RS cases demonstrated intact MDn projections to the MW and to RS areas spared by the lesion. Figure 17 (Case RS-243) shows a typical pattern of intact projections between the MW and MDn.

1. Case RS-236: No visible WGA-HRP activity was found in the anterior section of tissue. Axons were visible in the posterior Cg1, Cg3 and IL of the MW. Some sparse cells were also found in AID spared by the lesion.

2. Case RS-243: In this animal, cells were found throughout the anterior subdivisions of the MW. In addition, cells and axons appeared in the MO/VO, VLO and

LO spared by the lesion. In the posterior section of tissue, cells and axons appeared in the Fr2, Cg1, Cg3, and IL areas. Finally, some cells and axons appeared in the VLO spared by the lesion.

3. Case RS-246: The anterior tissue (+4.0 from Bregma) was damaged during sectioning. The posterior section of tissue revealed both cellular and axonal activity in areas Cg1, Cg3 and IL of the MW. Once again, cells were found in the LO and VLO areas of the RS spared by the lesion.

The WGA-HRP analyses therefore provide additional evidence confirming that lesions in the present investigations succeeded in destroying most of the intended targets in the MW and RS groups. The apparent relationship between postoperative performance and extent of lesion in the MW group supports the argument that this area of prefrontal cortex plays a critical role in spatial DNMTS performance, a finding that is strikingly consistent with the primate literature (cf. Fuster, 1989). The source of the RS deficits, however, is left open to interpretation. The smaller magnitude of impairment as compared to MWs may be attributable to two different factors. On the one hand, the observed deficits may be due to the encroachment into the MW in this group of animals. However, the results of the WGA-HRP study indicate that neither PFC lesion disrupted projections from the MDn to the other prefrontal target area. On the other hand, it is possible that posterior portions of the RS are also somehow involved in the execution of this task. Recent findings in our laboratory indicate that this possibility is not unfounded. Zhang (1992) has just demonstrated that lesions of the anterior half of the L-IML denervate PFC in both MW and RS areas, whereas lesions of the posterior half demonstrate a selective denervation of the dorsal portion of the agranular insular cortex. In addition, in a comparison of anterior vs. posterior vs. complete lesions of the L-IML, Mair et al. (1992) reported that only the complete lesion disrupted DNMTS performance. These findings, combined with the behavioral

findings reported here, leave open the possibility of a more pronounced DNMTS deficit in animals with combined lesions of the MW and RS.

GENERAL DISCUSSION

Findings

In recent years our laboratory has presented evidence of learning and memory impairments in rodents with medial thalamic pathology. The purpose of the present set of experiments was to further investigate the nature of these deficits.

In Experiment 1, rodents with lesions of lateral portions of the thalamic internal medullary lamina demonstrated severe and consistent deficits on a pretrained radial arm maze task. Subsequently, this group of animals exhibited difficulty with the acquisition and multiple reversals of a two-choice spatial discrimination problem. The behavioral deficits of the L-IML group not only corroborate recent findings (Kivlihan, 1992) of significant RAM impairments following stereotaxic lesions of this area, but they are also consistent with earlier findings of SSR, NMTS and DNMTS deficits in post thiamine deficient animals (Knoth & Mair, 1991, Robinson & Mair, 1991)). These results, taken together with multiple reports from our laboratory of DNMTS deficits following lesions of the L-IML (Kivlihan, 1992; Mair & Lacourse, 1992; Mair et al., 1991), demonstrate global learning and memory impairments following medial thalamic lesions.

Rodents with lesions of either the medial wall or rhinal sulcal areas of prefrontal cortex, on the other hand, were not impaired on the RAM and SSR tasks. Both groups eventually demonstrated stable, criterion level performance on the radial arm maze comparable to that of controls. Neither group presented evidence of discrimination or reversal deficits. In Experiment 2, however, impairments were observed in both prefrontal groups, with MWs demonstrating DNMTS deficits of a similar magnitude to that observed previously in our laboratory in animals with

lesions of the L-IML (Kivlihan, 1992; Mair & Lacourse, 1992; Mair et al., 1991). Taken together, the results of Experiments 1 and 2 demonstrate more selective impairments with lesions of prefrontal cortex than of the L-IML.

The Findings in Relation to the Literature

The RAM has previously been used to assess impairments in spatial memory following lesions of the medial thalamus (Kessler et al., 1982; Stokes & Best, 1988), as well as lesions involving, but not exclusive to the prefrontal areas studied here (Kesner et al., 1987; Kolb, 1985). The present findings are consistent with earlier reports of transient RAM deficits in both medial frontal (Kesner et al., 1987; Kolb et al., 1983) and orbital frontal animals (Kolb et al., 1983).

In rodents with MDn lesions, deficits have been described as subtle, with impairments emerging only following long (1 hr) imposed delays or testing in impoverished environments. These findings have prompted Kessler et al. (1982) and Stokes and Best (1985, 1988) to assert the importance of task difficulty in the demonstration of deficits in these animals. This argument does not hold with respect to findings in our laboratory. Both the present investigation and Kivlihan's recent study provide evidence of substantial L-IML deficits in tests conducted in cue rich environments. In addition, the delays in these studies were only of a 1 minute duration following the 2nd, 4th and 6th reinforcements on the maze. Finally, Kivlihan's study demonstrates that animals with lesions of the MDn are not as impaired on the RAM as are animals with lesions of the L-IML. This finding relates directly to the investigations done by Kessler et al., and Stokes and Best. The lesions in these studies were not only restricted to the MDn, but were also limited both in size and in AP extent.

The combined results from our laboratory therefore indicate that consistent and severe RAM deficits occur following more extensive lesions of the medial thalamus. In addition, the present results provide a direct comparison of thalamic and cortical

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lesions, a comparison demonstrating that impairments seen in MWs and RSs are small as compared to L-IMLs.

The SSR task was selected for a number of reasons. First, an analysis of the behavior of animals with stereotaxic lesions of thalamic and frontal areas known to be affected in the PTD model (cf. Knoth & Mair, 1991 and Mair et al., 1989) had yet to be done in our laboratory. Second, studies have demonstrated multisensory impairments on discrimination learning and reversal tasks in PTD animals (Mair et al., 1991) and following lesions of MDn (Eichenbaum et al., 1980; Kolb, 1977; Slotnick and Kaneko, 1981; Staubli et al., 1987). However, clear measures of spatial discrimination performance following lesions of the MDn projection areas in PFC have remained elusive. The present results demonstrate that the L-IML group alone presents a pattern of deficits similar to that seen in post thiamine deficient animals. Destruction of either of the major MDn cortical projection sites fails to produce deficits.

Although the L-IMLs required a greater number of trials to reach criterion on each of the problems, they demonstrated clear evidence of learning and of improvement over time. Oscar-Berman and Zola-Morgan (1980a, 1980b) have demonstrated that Korsakoff patients also require more trials to acquire spatial and visual discriminations, however they do learn to perform these tasks to criterion and present evidence of positive transfer on spatial serial reversals. Thus the pattern of behavior observed in L-IMLs is not only similar to that seen in the PTD model, but is also reminiscent of that seen in human Korsakoff patients.

One of the possible outcomes considered prior to initiating the present set of experiments was that MW impairments might be restricted to either RAM or DNMTS or both, and that RS deficits might be restricted to the SSR task. Such findings would have been consistent with Eichenbaum et al.'s (1983) reports of a dissociation in deficits in which rodents with lesions of the MW were selectively impaired on the the performance

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of a spatial delayed alternation task, whereas those with lesions of the RS were selectively impaired on an olfactory discrimination task. The results of Experiments 1 and 2 provide no evidence of such a dissociation.

In their paper, Eichenbaum et al. also characterize the observed deficits in both groups as "task-specific perseveration". The issue of perseveration is one that cuts across several literatures, and one that has received a great deal of attention. Whereas Mishkin (1964) has argued that delayed response impairments observed following frontal lesions may be accounted for by the perseveration of central sets, Goldman-Rakic (1987a) has asserted that perseverative behavior is a consequence of the disruption of representational memory. The question therefore arises whether or not the MW and RS deficits observed in Experiment 2 are better attributed to perseveration than to some form of memory impairment. Insofar as the correction procedure may be taken as a measure of the tendency to repeat responses, the present study presents no evidence of perseveration in either of the prefrontal groups.

The Nature of the L-IML Deficits

The similarity of performance between L-IMLs in the present study and PTDs, and in particular the performance of both these groups of animals on SSR tasks, is directly relevant to the interpretation of the nature of their deficits. Both subject populations made significantly more errors during initial learning as well as during the subsequent reversal phase than controls. However, L-IMLs and PTD animals also eventually succeed at learning all the spatial discrimination problems and present evidence of positive transfer across reversal problems comparable to controls. This finding is of importance when attempting to characterize the type of memory deficit observed in these animals.

Several types of memory have been described in the literature. Of these types, two sets of memory distinctions appear to be of potential relevance here. On the one hand, it may be argued medial thalamic pathology spares the ability to respond to

stimuli that are physically present (eg. the SSR task), while disrupting the ability to respond on the basis of stimuli that are not physically present (eg. the DNMTS task) at the time of response. In other words, dispositional memory remains intact while representational memory is impaired. This dichotomy appears to provide a reasonable description of the impairments observed in both PTDs and L-IMLs, and it lends itself to comparisons across literatures. Goldman-Rakic (1987a & 1987b), for example, invokes the concept of representational memory when analyzing impairments observed in primates on delayed conditional response tasks following prefrontal cortical lesions. However, problems arise with the use of these concepts. Although the representational component of this memory dichotomy provides an adequate explanation for the disruption of performance on delayed conditional response tasks, the dispositional component cannot easily account for the positive transfer demonstrated by both PTDs and L-IMLs in the course of serial reversal learning. The capacity for transfer implies more than a preserved ability to associate stimuli with reinforcement. If this were the only mechanism preserved in these animals, performance on the SSR task would resemble that on a series of novel problems. The savings observed in both PTDs and L-IMLs suggests that these animals are still able to learn something about the task, eg. a strategy, that is carried over from one problem to the next.

For these reasons, Mair and colleagues (1991) have forgone these distinctions, preferring to employ the terms "working" and "reference" memory as described by Honig (1978). In tests of working memory, stimulus information must be updated on a trial by trial basis. In DNMTS, for example, a subject must be able to disregard the memory of stimuli from preceding trials and respond based only on the port reinforced in the preceding sample. Conversely, in tests of reference memory, the strategy of responding is the same on every trial. In other words, responding is dependent upon the long-term maintenance of rules that can be applied across different instances.

The findings that both PTD and L-IML animals eventually learn to respond consistently in SSR tasks are taken as evidence that reference memory remains intact in these animals. On the other hand, the RAM impairments in L-IMLs, and DNMTS impairments in both PTDs and L-IMLs can be taken as an indication of impaired of working memory.

The Nature of the PFC Deficit

Whereas L-IML deficits have been found to be widespread, the present results demonstrate the relatively selective nature of the impairments observed following lesions of prefrontal cortex. The performances of the MW and RS groups in this study did not account for the full complement of deficits observed following lesions of the L-IML. However, MWs are more impaired on DNMTS than animals with lesions restricted to the MDn (Kivlihan, 1992) and as impaired as animals with lesions of the L-IML (Mair & Lacourse, 1992; Mair et al. 1992). One potential explanation for the observation of DNMTS deficits in several subject populations tested in our laboratory is that this task is generally more sensitive to lesion effects than the RAM. Yet, the performance of animals with lesions of the fornix runs counter to this argument. Fornix animals demonstrate RAM impairments that are equivalent to those seen in L-IMLs, however DNMTS deficits are small as compared to those of the L-IMLs (Kivlihan, 1992; Mair & Lacourse, 1992). These findings lend themselves to arguments in the literature that delayed response tasks, including DNMTS tasks such as the one used here, are attuned to selective impairments in memory (cf. Fuster, 1989). The question therefore arises as to whether or not the memory deficits observed in MWs and L-IMLs are the same.

Are MWs demonstrating a working memory impairment? On the one hand, the success of MWs on the RAM task is not consistent with a working memory deficit. However, the RAM and DNMTS are two very different tasks that tap into different types of working memory. Whereas both are dependent upon a form of "temporary memory"

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for information critical to task performance, the RAM task makes demands on the memory for lists of spatial locations within a given trial, while the DNMTS task makes demands on the ability to make temporal distinctions between critical events across trials. Thus the highly selective impairments found here are consistent with Squire's (1987) conceptualization of this area of PFC as possessing working memory capacities that lend themselves to the temporo-spatial organization of behavior.

When viewing the DNMTS performance of the MW group, one is tempted to speak of functional homologies between the rodent PFC and that of primates. However, for every consistency in behavior, the combined results of Experiments 1 and 2 also provide several inconsistencies. First, neither prefrontal group exhibited the perseverative tendencies observed in primates with prefrontal lesions (Fuster, 1989; Goldman-Rakic, 1987a; Mishkin, 1964). Second, RSs also demonstrated DNMTS deficits which may or may not be accounted for by lesion encroachment into the MW. Third, neither group demonstrated the reversal deficits that have been reported following both dorsolateral and orbitofrontal lesions in primates (Mishkin, 1964). Yet, the present DNMTS findings do not diverge entirely from the primate literature. There are some important similarities between the DNMTS task used here and the delayed response and delayed alternation tasks on which primates with dorsolateral prefrontal lesions (cf. Goldman-Rakic, 1987a), and both human frontal patients and Korsakoff patients are impaired (Freedman & Oscar-Berman, 1986; Oscar-Berman et al., 1982). In all three tasks, correct choices are predicated upon critical events occurring in the recent past. In addition, correct choices are contingent upon the ability to distinguish the critical event in a particular trial from those that have occurred previously. Therefore the underlying factor in the successful completion of all three tasks is the ability to make temporal distinctions. In this respect, the deficits observed here are consistent with findings in the primate and human literatures, and they lend support to the role of the PFC in the temporal organization of behavior.

The Neurobiology of the PTD Model of Amnesia

The present findings are pertinent to the development of a clearer

understanding of the neural basis of the learning and memory deficits seen in post

thiamine deficient animals. Rodents having undergone pyrithiamine induced

thiamine deficiency have demonstrated learning and memory impairments on several

different tasks including delayed conditional discriminations and serial reversals

based upon spatial cues (Knoth & Mair, 1991; Robinson & Mair, 1992). The observed

memory impairments are qualitatively similar to those seen in Korsakoff patients.

Subsequent histopathological analyses have consistently revealed medial

thalamic lesions extending beyond the MDn to include lateral portions of the IML. In

fact, animals with sparing of the IML exhibit smaller SSR and DNMTS deficits than

those with more extensive medial thalamic pathology (Knoth & Mair, 1991; Mair et al.,

1991). Comparisons of performance following either MDn or L-IML lesions have added

to the PTD findings by demonstrating that although MDn animals are impaired on the

RAM and DNMTS, those with lesions of L-IML demonstrated significantly greater

impairments (Kivlihan, 1992). Therefore, one purpose of the present set of experiments

was to further investigate the behavioral deficits of animals with lesions of the L-IML.

The findings confirm previous observations of RAM and DNMTS deficits, and extend

these observations by demonstrating SSR deficits in L-IMLs that are qualitatively

similar to those seen in PTD animals.

Fink-Heimer analyses of fiber degeneration in both PTD animals (Mair et al.,

1989) and those with lesions of the L-IML (Zhang, 1992) have also demonstrated diffuse

denervation of prefrontal cortex in these animals. More specifically, Layer IV of much

of neocortex is denervated in PTDs, whereas the L-IML lesion selectively denervates

Layer IV in frontal cortex and Layer I across all areas of neocortex. These findings are

consistent with suggestions in the human literature that the deficits observed in

Korsakoff patients reflect associated frontal pathology (Goldman-Rakic, 1987a & b;

Freedman & Oscar-Berman, 1986). Therefore, the second purpose of the present experiments was to venture into the rodent PFC in an attempt to determine whether the behavioral deficits observed in L-IMLs could be accounted for by lesions of the two major projection areas of the MDn. The present findings indicate that destruction of MDn projection areas in frontal cortex can account for some, but not all the effects of post thiamine deficiency or L-IML lesions. Therefore the destruction of either nonspecific projections to Layer I, other cortical projections, or other subcortical pathways must also contribute to the PTD and L-IML deficits.

In conclusion, the present set of experiments both replicate and extend previous findings in our laboratory. In doing so, they provide further insight into the PTD model of amnesia.

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APPENDIX

Table 1

Mean Time to Complete First 8 RAM Responses

Treatment	Mean (sec.)	Standard Error (sec.)
Medial Wall	223.28	8.71
Rhinal Sulcus	214.55	9.71
L-IML	170.68	8.04
Control	189.73	4.44

Table 2

Frontal Group Lesion Measures: Experiments 1 and 2

Treatment	Lesion Extent	Median	Mean	Standard Deviation
Experiment 1 Medial Wall	Ant. Medial Wall	7.17	7.25	1.11
	Post. Medial Wall	7.72	8.09	2.25
	Ant. Rhinal Sulcus	0.00	1.50	2.69
	Post. Rhinal Sulcus	N/A	N/A	N/A
Experiment 1 Rhinal Sulcus	Ant. Medial Wall	3.09	3.72	2.78
	Post. Medial Wall	0.00	0.14	0.45
	Ant. Rhinal Sulcus	7.93	7.68	1.38
	Post. Rhinal Sulcus	5.82	6.53	1.60
Experiment 2 Medial Wall	Ant. Medial Wall	6.65	6.46	1.01
	Post. Medial Wall	9.73	9.52	1.82
	Ant. Rhinal Sulcus	2.66	2.86	2.04
	Post. Rhinal Sulcus	N/A	N/A	N/A
Experiment 2 Rhinal Sulcus	Ant. Medial Wall	4.46	5.03	2.52
	Post. Medial Wall	0.00	0.28	0.90
	Ant. Rhinal Sulcus	7.32	7.15	1.31
	Post. Rhinal Sulcus	7.88	7.72	1.99

Table 3a

Correlations Between RAM Performance and Lesion Size: Medial Wall Group (r-values)

	Anterior Medial Wall	Anterior Rhinal Sulcus	Posterior Medial Wall	Posterior Rhinal Sulcus
RAM (15 Sessions)	0.56	-0.29	-0.19	N/A
RAM (Last 5 Sessions)	0.41	-0.40	-0.01	N/A
Entries Without Drinking (Session 1 Only)	0.12	-0.18	0.15	N/A

Table 3b

Correlations Between RAM Performance and Lesion Size: Rhinal Sulcus Group (r-values)

	Anterior Medial Wall	Anterior Rhinal Sulcus	Posterior Medial Wall	Posterior Rhinal Sulcus
RAM (15 Sessions)	-0.56 †	-0.21	0.08	-0.07
RAM (Last 5 Sessions)	-0.14	0.14	0.36	0.22
RAM (Session 1 Only)	-0.92 ††	-0.47	-0.40	-0.51
RAM (Session 2 Only)	-0.28	-0.27	0.07	0.28
RAM (Session 3 Only)	0.16	-0.09	0.19	0.34
Entries Without Drinking (Session 1 Only)	0.72 ††	0.57 †	0.26	0.64 †

†   Significant at .05
††   Significant at .01
Note. All tests are one tail.

Table 4

Correlations Between SSR Performance and Lesion Size (r-values)

Treatment	Anterior Medial Wall	Anterior Rhinal Sulcus	Posterior Medial Wall	Posterior Rhinal Sulcus
Medial Wall (SSR)	-0.35	-0.12	0.08	N/A
Rhinal Sulcus (SSR)	-0.43	-0.61 †	-0.20	-0.59 †

†   Significant at .05
Note.   All tests are one tail.

Table 5

Mean Number of Correct DNMTS Choices Across Final 3 Days of Pretraining

Treatment	Mean	Standard Error
Medial Wall	43.67	0.68
Rhinal Sulcal	43.90	0.46
Control	44.17	0.57

Table 6

Mean Number of Correct DNMTS Choices Across 16 Postoperative Sessions

Treatment	Mean	Standard Error
Medial Wall	32.26	0.67
Rhinal Sulcal	39.16	0.46
Control	43.69	0.33

Table 7

Correlations Between DNMTS Performance and Lesion Size (r-values)

Treatment	Anterior Medial Wall	Anterior Rhinal Sulcus	Posterior Medial Wall	Posterior Rhinal Sulcus
Medial Wall (DNMTS)	-0.72 †	-0.28	-0.27	N/A
Rhinal Sulcus (DNMTS)	-0.50	0.07	-0.67 †	-0.71 †

†   Significant at .05
Note.   All tests are one tail.

Table 8

Comparisons of Prefrontal Lesion Size Across Experiments 1 and 2 (t-tests)

	Comparison Between Medial Wall Groups		Comparison Between Rhinal Sulcus Groups	
Ant. Medial Wall	t(18) = 1.68	p = 0.11	t(17) = -1.08	p = 0.30
Post. Medial Wall	t(18) = -1.56	p = 0.14	t(18) = -0.45	p = 0.66
Ant. Rhinal Sulcus	t(18) = -1.28	p = 0.22	t(17) = 0.86	p = 0.40
Post. Rhinal Sulcus	N/A	N/A	t(18) = -1.48	p = 0.16

Note. All tests are two tail.

Table 9

WGA-HRP Activity

| | Anterior | | | | | | | Posterior | | | | | | |
| | Medial Wall | | | Rhinal Sulcus | | | | Medial Wall | | | | Rhinal Sulcus | | |
	Cg3	Cgl	Fr2	AID	LO	VLO	MO VO	IL	Cg3	Cgl	Fr2	AID	LO	VLO
C-11	•	•		★	★							•	• ★	
C-53	•		•	•	• ★	★	•		•				• ★	•
C-164	• ★			★	★	★	★		•	•			• ★	• ★
MW-226	★			• ★	• ★	• ★	★	•	•			• ★	• ★	• ★
MW-245			•	★	•	•					•	• ★	• ★	• ★
MW-251			•	• ★	• ★	★						• ★	• ★	• ★
RS-236								★	★	★		•		
RS-243	•	•	•		• ★	• ★	• ★	• ★	• ★	• ★	• ★			• ★
RS-246	--------------------N/A--------------------							• ★	• ★	• ★			•	•

•   Filled cells
★   Visible axons

Figure 1. Radial Arm Maze Performance

Legend:
- Medial Wall
- Rhinal Sulcus
- L-IML
- Control

Y-axis: Number Correct (first 8 choices)
X-axis: Daily Postoperative Sessions
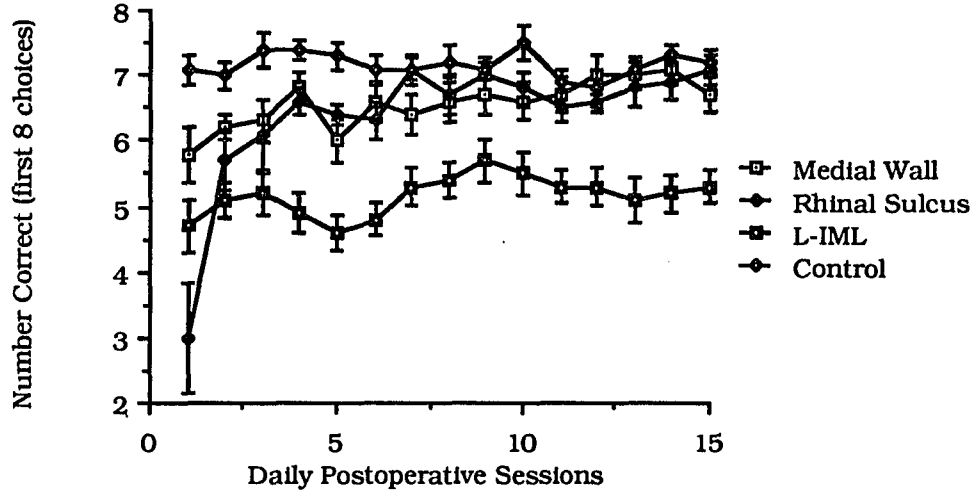
Figure 2. Entries without Drinking
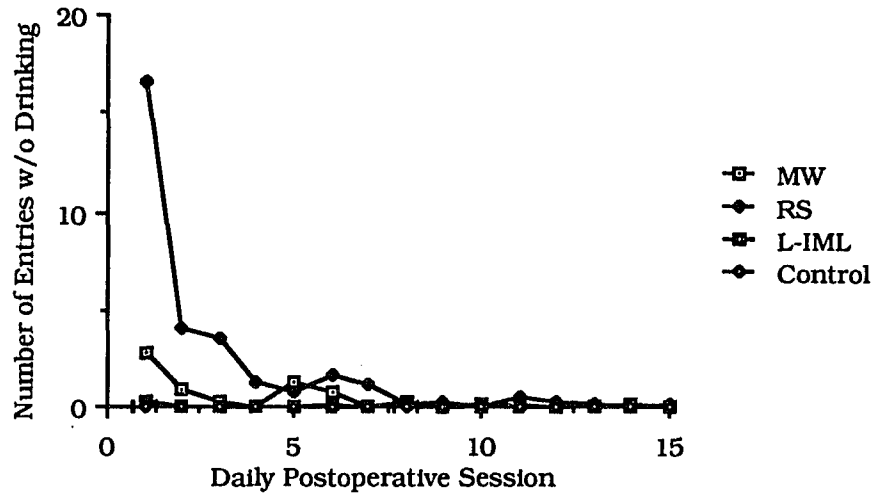
Figure 3. Serial Reversal Learning

Figure 4. Rates of Improvement

	Slope
MW	-0.2111
RS	-0.2704
L-IML	-0.1643
Controls	-0.1904
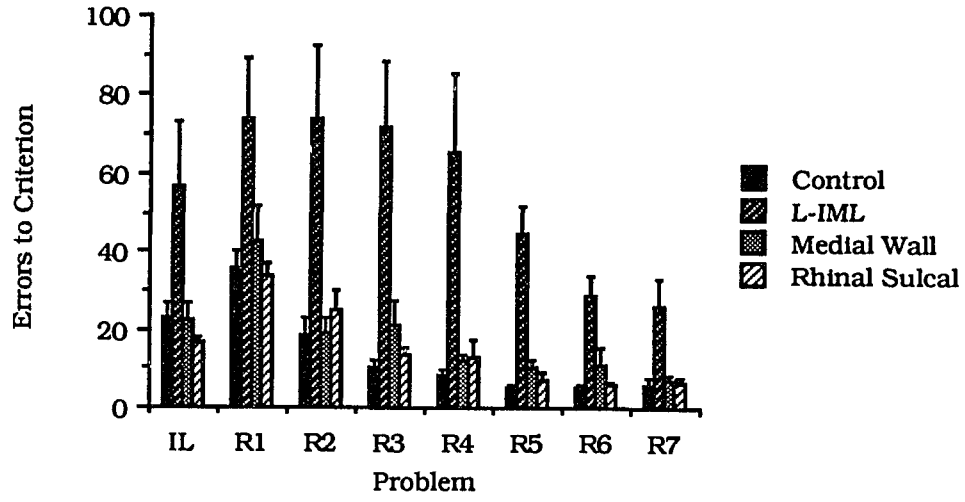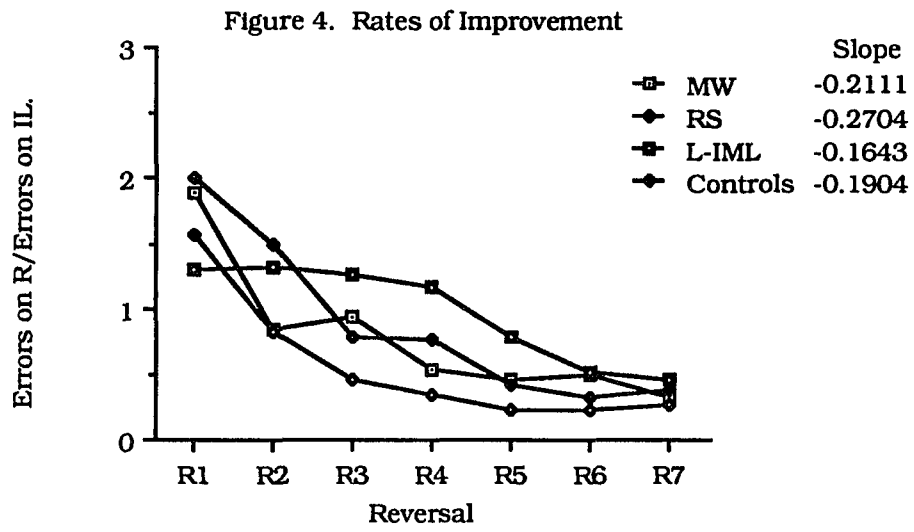
Figure 5a. Photomicrograph of Representative MW and RS Lesions
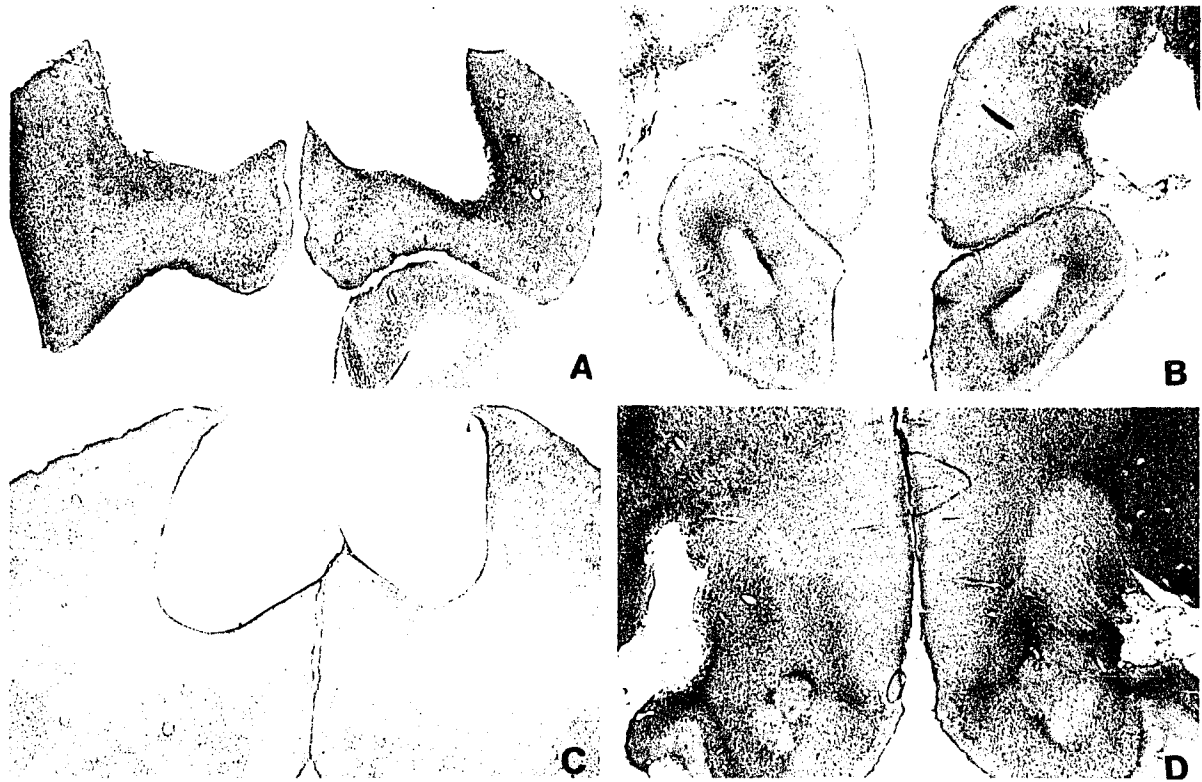
Figure 5b. Photomicrograph of Representative L-IML Lesions
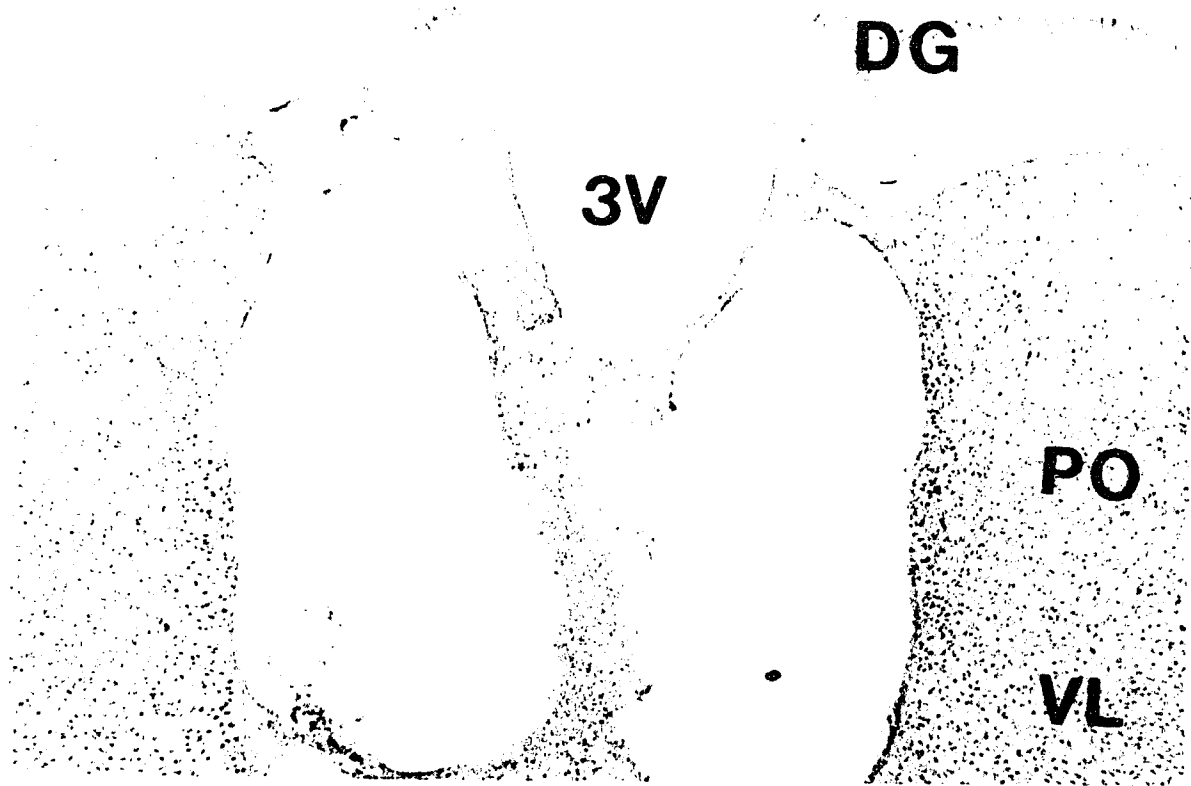
Figure 6. Minimum and Maximum MW Lesions: Experiment 1
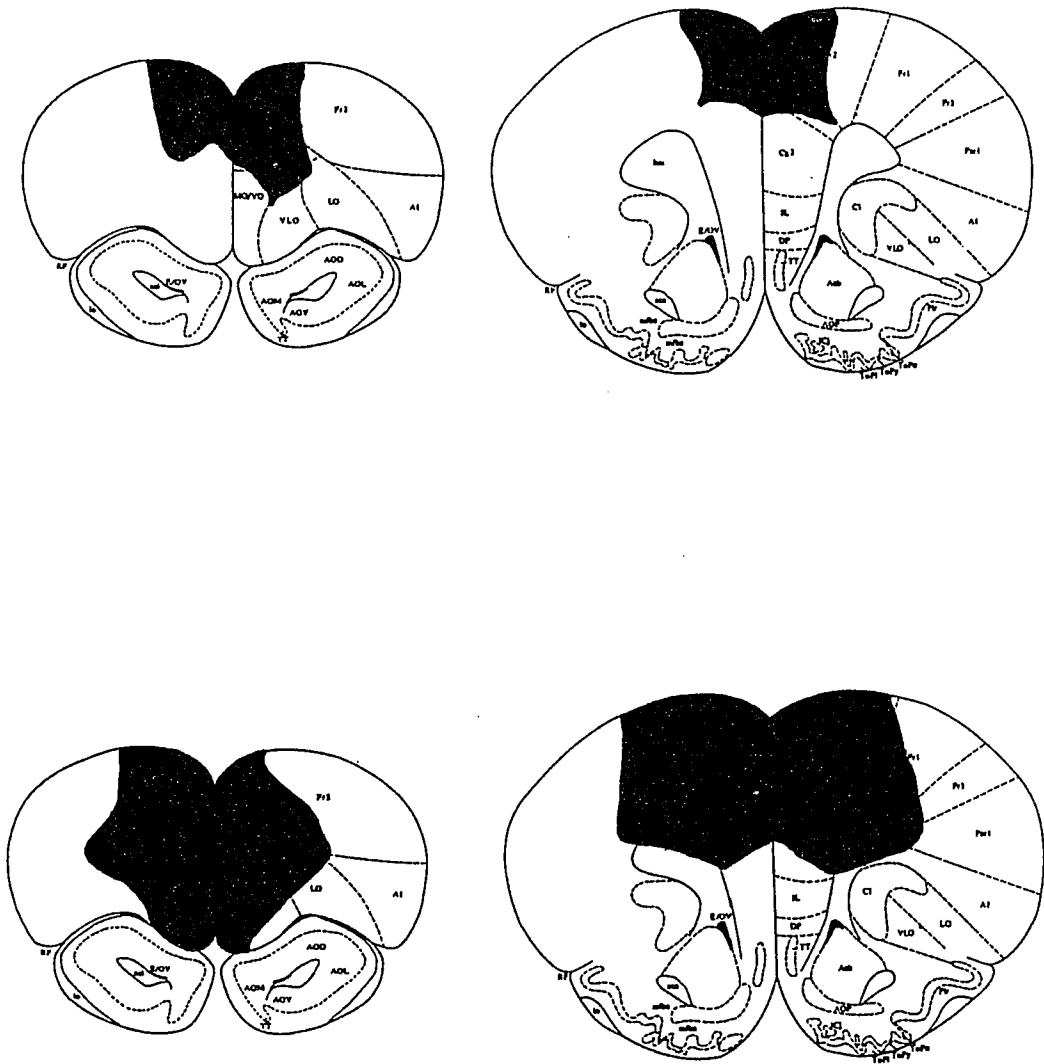
Figure 7. Minimum and Maximum RS Lesions: Experiment 1

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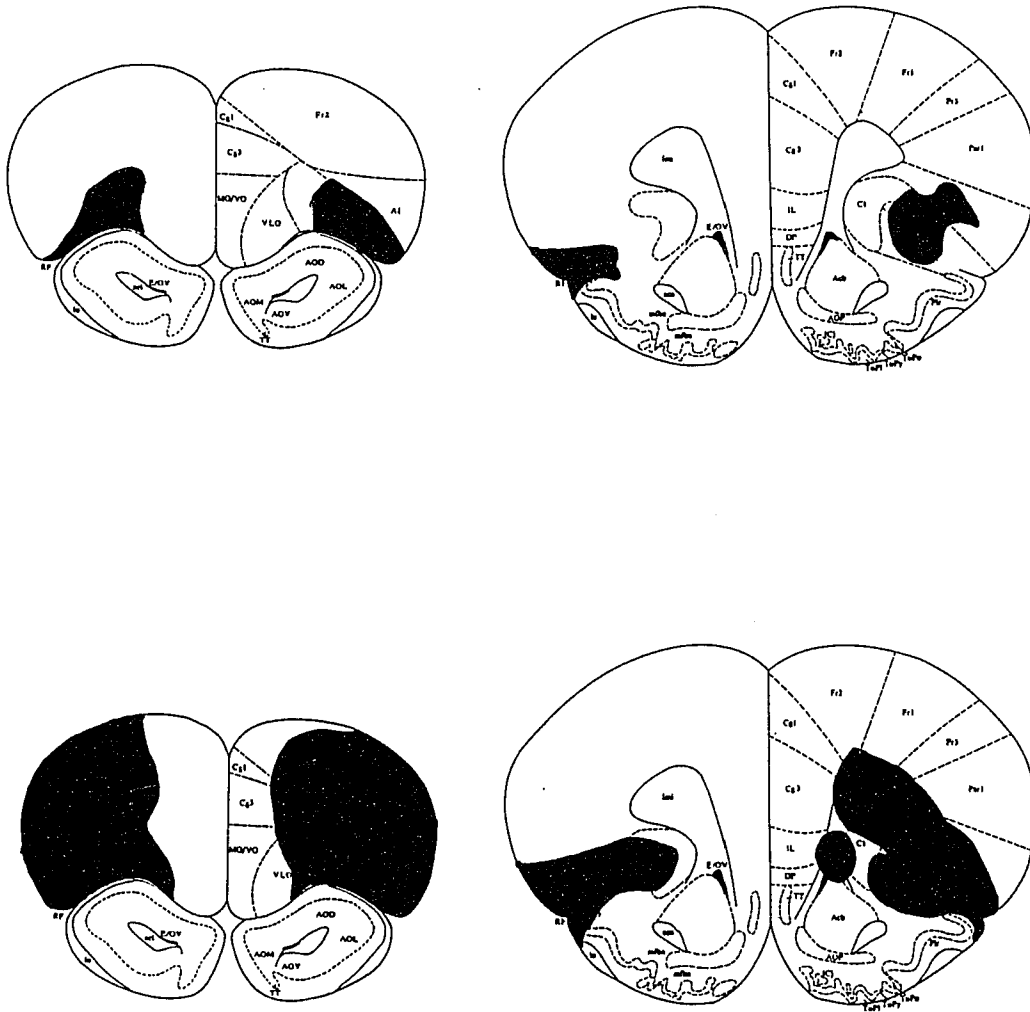
Figure 8. DNMTS Performance
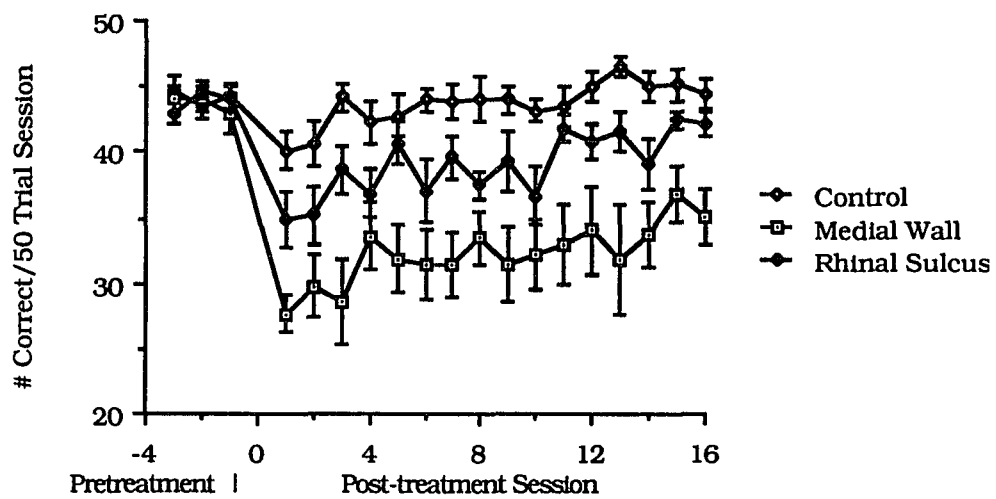
Figure 9. Minimum and Maximum MW Lesions: Experiment 2

Figure 10. Minimum and Maximum RS Lesions: Experiment 2
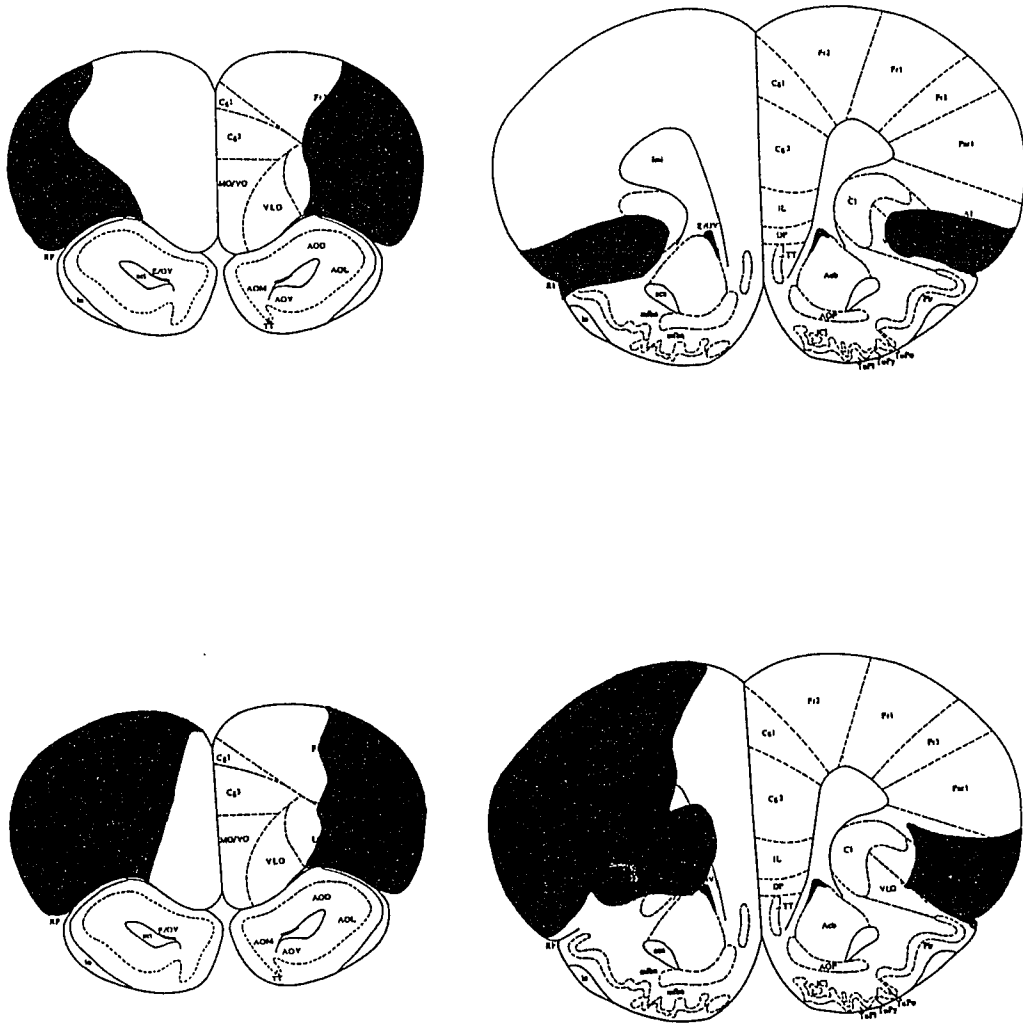
Figure 11. Comparison of Anterior Medial Wall Lesions in Experiments 1 & 2
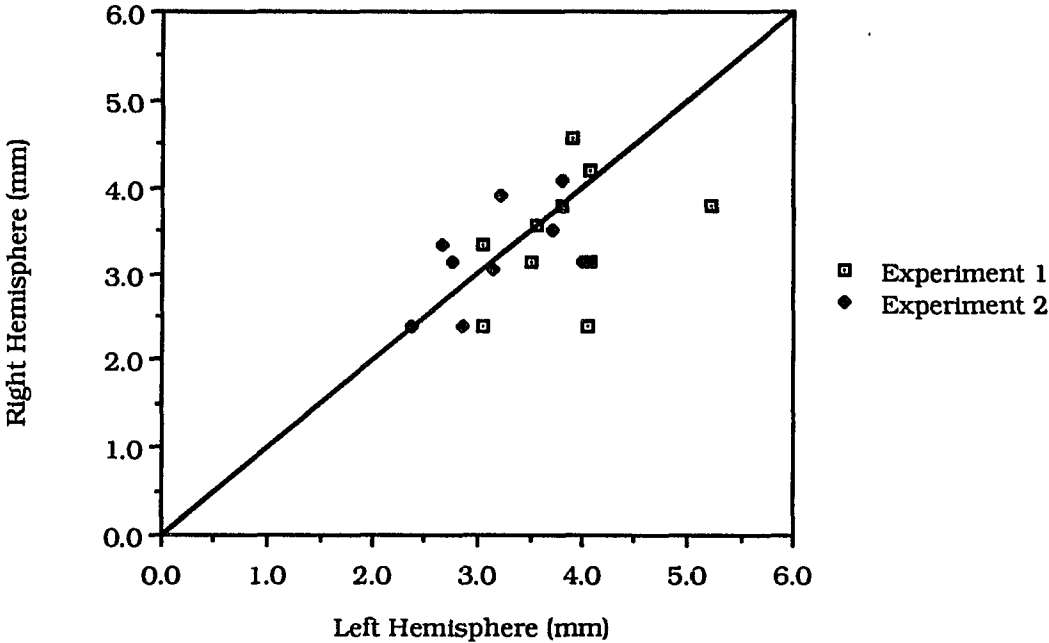
Figure 12. Comparison of Posterior Medial Wall Lesions in Experiments 1 & 2
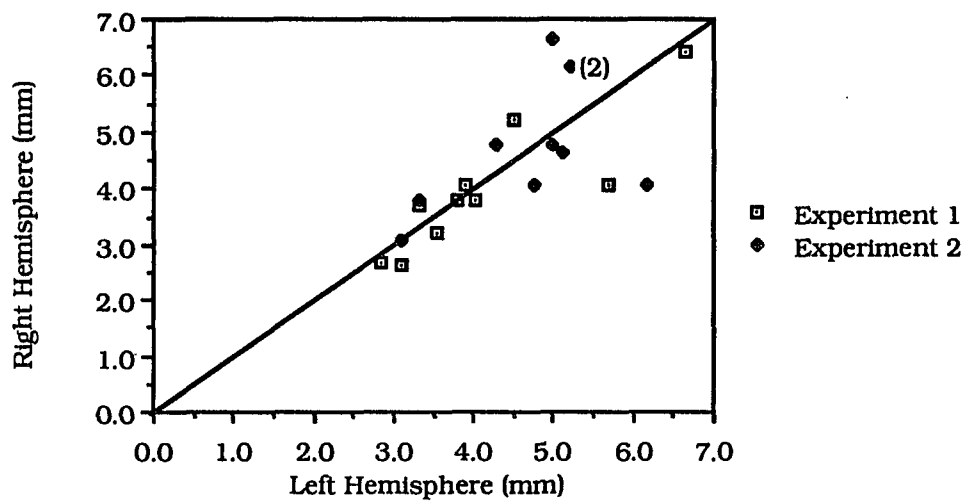
Figure 13: Comparison of Anterior Rhinal Sulcus Lesions in Experiments 1 & 2
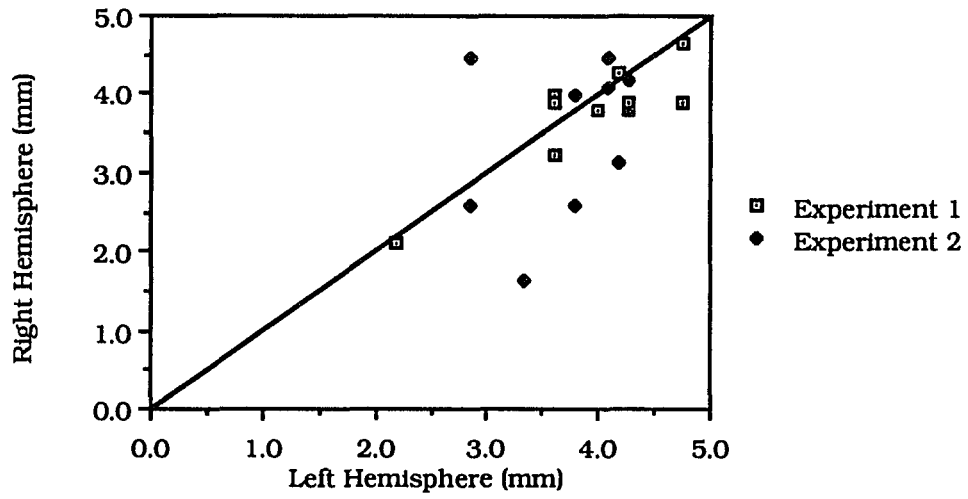
Note: For Experiment 2: n = 9 subjects.

Figure 14. Comparison of Posterior Rhinal Sulcus Lesions in Experiments 1 & 2
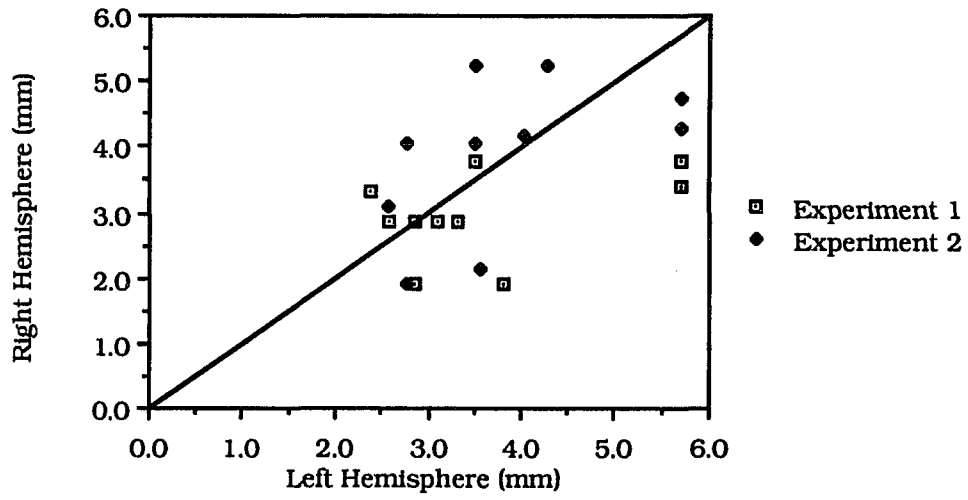
Figure 15. Photomicrograph of Typical Cases of MW and RS Filling Following WGA-HRP Implants into the MDn.
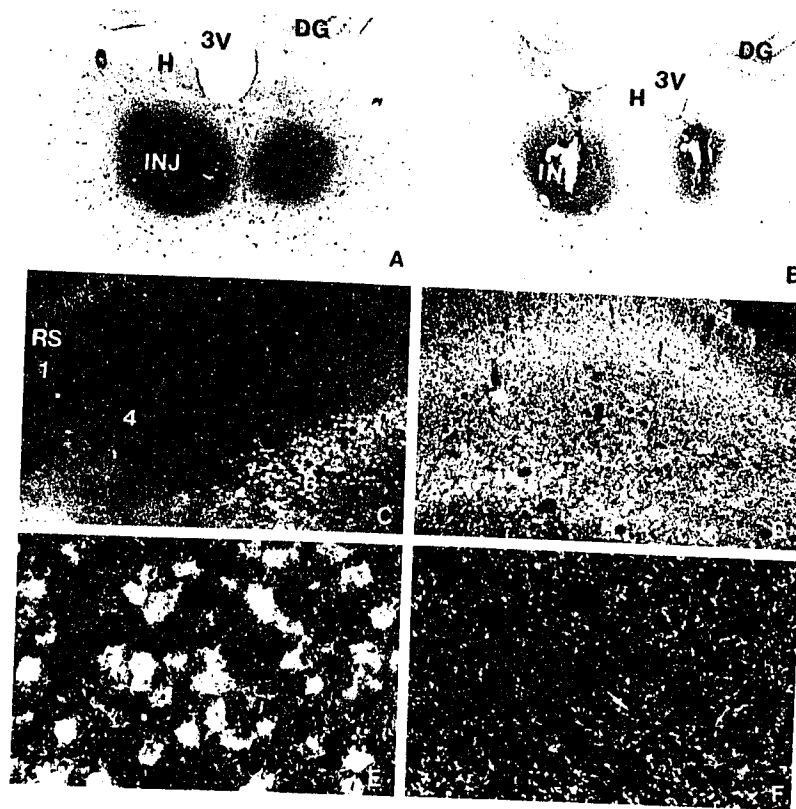
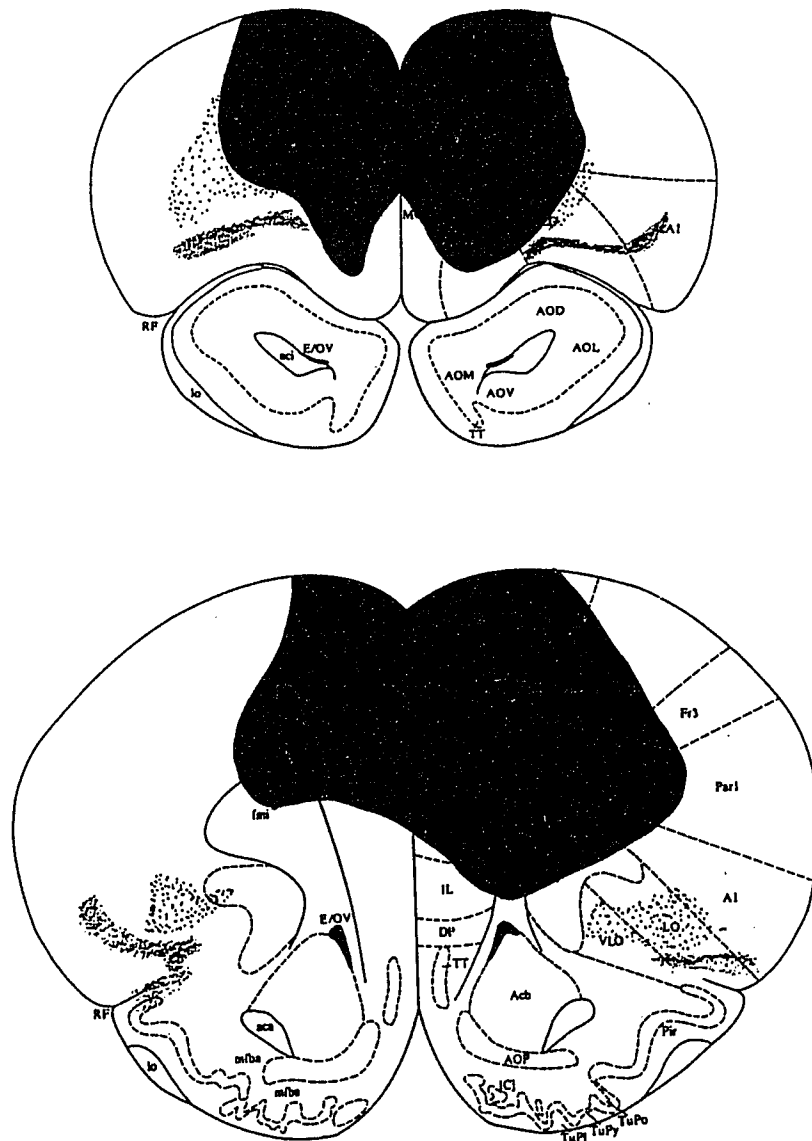Figure 16.   Intact RS Projections in an Animal with a MW Lesion

Figure 17. Intact MW Projections in an Animal with a RS Lesion

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