Diazepam Administration During Acute Thiamine Deficiency Attenuates Subsequent Neuropathology and Spatial-Memory Deficits in Rats

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ABSTRACT

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Pyrithiamine-induced thiamine deficiency (PTD) in rats causes deficits on a wide variety of spatial-memory tasks. PTD-induced memory deficits are thought to result from damage in areas of the diencephalon that are selectively vulnerable to the metabolic disruption of thiamine deficiency. However, a high incidence of seizures during the late stages of PTD treatment complicates this interpretation, as these seizures may contribute to the neuropathological and behavioural outcome of PTD. The present experiment assessed spatial allocentric working-memory in recovered PTD rats. Two groups of rats received PTD treatment; one group received diazepam (5mg/kg) upon the first sign of overt seizures in order to attenuate them until treatment reversal (Group PTD+DZ), and the other group received no diazepam (Group PTD). Following recovery, the rats were trained on a delayed matching-to-place (DMTP) task in a water maze, and tested with retention delays of 4, 30, and 120 seconds. Both experimental groups displayed DMTP acquisition deficits, and PTD rats were subsequently impaired at all retention delays. By contrast, the PTD+DZ rats were impaired only at the 120-sec delay. Moreover, thalamic neuropathology was attenuated in the PTD+DZ rats. Although further studies will be needed to determine the mechanism by which diazepam exerts its protective effects in this model, the present study suggests that PTD-induced neuropathology and memory deficits can be attenuated by treatments that reduce seizure activity.

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This thesis is dedicated to the memory of my grandfather, Mr. Jack Kofsky.

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Korsakoff's amnesia (KA) is the most common form of human amnesia (Langlais, 1992) and is observed primarily, though not exclusively, in chronic alcoholics. Patients who suffer from KA experience a loss of memories that were formed prior to the onset of the disease (retrograde amnesia) as well as an inability to form certain kinds of new long-term memories (anterograde amnesia). Short-term memory, intellect and cognition are relatively spared. KA has been linked to neuropathology in the diencephalon for more than a century (Squire, 1987). It is well established that the lesions observed in KA patients are caused by an acute thiamine deficiency that is brought on by a poor diet as well as the inhibiting effects of alcohol on thiamine utilization within the body (Victor, Adams & Collins, 1989).

The pyrithiamine-induced thiamine deficiency (PTD) rat model of KA (Troncoso et al., 1981) involves the dietary restriction of thiamine in combination with daily injections of pyrithiamine, a thiamine antagonist that inhibits the active form of thiamine within the brain. Recovered PTD rats exhibit a pattern of neuropathology that is similar to the neuropathology seen in KA. Both PTD rats and KA patients sustain pathology, indicated by neuronal thinning, tissue cavitation and gliosis, to structures in the diencephalon, including the thalamus and the mammillary bodies (Troncoso et al., 1981). Further, there is evidence of reduced neuronal density and atrophy in the periacqueductal grey, the brainstem, the basal forebrain and the frontal cortex in both PTD rats and in KA patients (Langlais, Zhang & Savage, 1996).

PTD also produces behavioural deficits that are similar to the deficits displayed in human KA. For example, PTD rats and KA patients exhibit deficits in delayed-alternation and delayed-response tasks, both of which assess spatial memory (Kopelman, 1995; Langlais, 1992).

PTD-induced thiamine deficiency is therefore a good model of KA because it produces neuropathology and behavioural deficits that are similar to those observed in KA.

Moreover, the PTD model demonstrates that thiamine deficiency, which is strongly implicated as the primary etiological factor in KA, causes neuropathology and behavioural deficits in experimental animals.

However, PTD is a poor model of KA for several reasons: First, KA patients, but not PTD rats are exposed to the chronic effects of alcohol toxicity. Second, KA patients, but not recovered PTD rats, display severe retrograde amnesia. These considerations are discussed in more detail below.

Another important difference between KA and PTD is that PTD rats are exposed to a high incidence of severe tonic-clonic convulsions during the late stages of PTD. Repeated seizures can directly cause alterations in brain activity and neuropathology, and can also result in behavioural and memory deficits (Lynch, Rutecki & Sutula, 1996). Therefore, it is possible that the cognitive and pathological outcome of PTD results from a combination of primary and secondary pathogenic events. Thiamine deficiency causes primary pathology, which produces severe seizures in the PTD rat. The repeated seizures may then cause secondary pathology.

There were two main purposes of this study. The first was to assess spatial allocentric working-memory in recovered PTD rats that did not receive any training prior to treatment. Allocentric spatial memory refers to the storage of the spatial layout of external cues within the environment, thus enabling the rat to form a "cognitive map" which can be used flexibly by the animal to navigate efficiently in the environment (O'Keefe & Nadel, 1978). Working-memory refers to the memory for trial-specific information. Spatial allocentric working-memory was assessed by a delayed matching-to-place task (DMTP) in a water maze. Second, this study addressed the idea that the occurrence of seizures during the late stages of PTD contributes to the neuropathological and behavioural outcome of the treatment. Rats were exposed to identical bouts of thiamine deficiency, but some of the rats received an anticonvulsant (diazepam) in the late stages of the treatment in order to reduce

the occurrence of seizures. All of the PTD rats were monitored for seizures throughout the late stages of PTD. It was hypothesized that if the exposure to repeated seizures during the late stages of PTD causes pathology beyond that directly resulting from thiamine deficiency, then DMTP deficits and neuropathology would be attenuated in the PTD rats that received diazepam. Further, because PTD rats may experience covert, subconvulsive seizures as well as overt seizures, some of the rats were implanted with recording electrodes prior to PTD treatment. These rats underwent the same procedures (i.e., PTD treatment with or without diazepam and behavioural testing) as did the nonimplanted rats. Electroencephalographic (EEG) activity was monitored in these rats throughout PTD treatment.

The data that are reported in this thesis indicate that recovered PTD rats have an impairment in spatial allocentric working-memory. Moreover, the present results suggest that some of the behavioural deficits and neuropathology that are observed in recovered PTD rats may be caused by a high incidence of seizures during the late stages of PTD treatment rather than by thiamine deficiency, *per se*. All of the PTD rats were impaired on the DMTP task. However, DMTP deficits were attenuated in the PTD rats that received diazepam during the late stages of PTD. Finally, diazepam attenuated the neuropathology that is typically observed in PTD rats.

The remainder of this introduction consists of three main sections. The first main section addresses the etiology of KA, as well as the neuropathological and cognitive consequences of the disease. The second main section focuses on the behavioural and neuropathological outcome of PTD. The third main section addresses the need for an evaluation of the role of seizures in the PTD model of KA.

1. Korsakoff's Amnesia in Humans

Korsakoff's syndrome was characterized by Korsakoff in 1887, when he published several articles noting a link between chronic alcoholism and severe memory disturbances (cited in Kopelman, 1995). Korsakoff noted that the memory disturbances were commonly preceded by reduced levels of awareness, the presence of an agitated and confused state as well as disturbances in gait. Wernicke, who was also studying chronic alcoholics, described these symptoms in 1881 and related them to pathological changes in the diencephalon. This pattern of neuropathology and clinical symptoms is now known as Wernicke's encephalopathy.

The similarity between the clinical symptoms observed in Wernicke's encephalopathy and Korsakoff's syndrome was noted by Gudden in 1896 (cited in Mair, Warrington & Weiskrantz, 1979). Moreover, the neuropathology in Wernicke's encephalopathy and Korsakoff's syndrome is almost identical (Kopelman, 1995). Wernicke's encephalopathy and Korsakoff's syndrome are now considered as consisting of two stages along the same disease continuum. Wernicke's disease is referred to when the early neurological symptoms are present but the patient does not display any memory deficits. The patient is said to be suffering from Korsakoff's amnesia (KA) when the earlier neurological symptoms have waned and the patient displays clear indications of a persistent amnesic syndrome. It is important to note that Wernicke's encephalopathy is reversible, whereas KA is not.

1.1 *Memory Deficits Associated with KA*

Although the cognitive deficits that are displayed by patients with KA were first described by Korsakoff in 1887, associations between chronic alcoholism and memory disturbances were noted as early as the late 17th century (Kopelman, 1995). Korsakoff noted that his patients repeatedly asked the same questions, would read the same pages of a

book over and over again and were unable to remember people that they had met during the illness. All of these memory disturbances are examples of anterograde amnesia because they reflect an inability to form new long-term memory. Moreover, Korsakoff's patients also displayed signs of retrograde amnesia; they were often unable to recall events that had occurred up to 30 years prior to the onset of the illness. Korsakoff noted that many remaining cognitive abilities were intact. His patients were alert and responsive, were able to reason, to make witty remarks and were even able to play chess (cited in Kopelman, 1995).

Recent evidence has demonstrated a wide range of memory impairments in human KA. They display deficits on tasks that examine the memory for temporal order, spatial location and modality of information (Kopelman, 1995). A case study of a man called E.A. (Mair et al., 1979) illustrates the severity of memory impairments and the extent of cognitive abnormalities that are commonly observed in KA patients. E.A. experienced a five- to seven-year history of excessive drinking and inadequate diet before undergoing a sudden onset of confusion and memory loss. He was unable to learn the spatial layout of the hospital to which he was admitted and was unable to recognize the medical staff. E.A. displayed several indications of anterograde amnesia on both verbal and visual forced-choice memory tasks. E.A.'s retrograde memory was assessed by his ability to report events from his own life, as well as his ability to identify well known faces and past public events. He displayed severe retrograde amnesia that extended up to 30 years prior to the onset of his illness, but he could readily recall events from his childhood. This is consistent with evidence that KA patients often display a temporal gradient to their retrograde amnesia; they recall events from the distant past more readily than events that occurred closer to the onset of the illness (Albert, Butters & Levin, 1979).

Repeated neuropsychological testing revealed that many of E.A.'s remaining cognitive abilities were intact. E.A. had a forward digit span of eight items, indicating

normal short-term memory. Moreover, E.A.'s scores on measures of verbal and performance IQ were about average, although his verbal IQ began to deteriorate eight years later (Mair et al., 1979).

Consistent with the initial clinical symptoms described by Korsakoff in 1887, KA patients often display neurological symptoms prior to the onset of amnesia. KA patients may display signs of disturbances in gait (ataxia), disturbances of ocular motility (ophthalmoplegia) as well as a reduced level of awareness which precede any indications of amnesia (Troncoso et al., 1981). However, these features of Wernicke's encephalopathy do not invariably precede the memory disturbances. Also, Wernicke features may be present for an extended length of time without any indications of memory loss (Kopelman, 1995).

1.2 *Neuropathology of KA*

Postmortem analyses of the brains of KA patients have revealed that the most commonly affected structures are in the diencephalon, including the mammillary bodies and the thalamus (Langlais, Zhang & Savage, 1996). Although nuclei throughout the thalamus are affected in KA, the medial dorsal and anterior nuclei appear to be the most reliably and severely affected. There is evidence of necrosis, demyelination, gliosis and neuronal thinning in both of these nuclei (Kopelman, 1995). The brains of KA patients also show a pronounced enlargement of the third ventricle (Jacobson & Lishman, 1990), presumably due to the atrophy of the surrounding tissue. Limbic-diencephalic fiber pathways, including the mammillo-thalamic tract (MMT) and the internal medullary lamina (IML), are also pathologic in the brains of KA patients (Kopelman, 1995; Langlais, 1992). The neuropathology is not restricted to the diencephalon. Signs of hemorrhaging, necrosis, demyelination and gliosis have been observed throughout the brains of KA patients.

Several structures within the brainstem and the basal forebrain are also affected in KA (Langlais, et al., 1996). Moreover, cortical atrophy is also observed (Mair et al., 1979).

The fact that there is widespread neuropathology in KA raises the question as to which aspect of the neuropathology is responsible for the memory deficits displayed by KA patients. It is widely assumed that the critical lesion sites for memory disorder in KA are located in the diencephalon, and include the medial dorsal and anterior thalamic nuclei, the MMT and the mammillary bodies (Victor et al., 1989). These sites usually have some evidence of sparing in patients who are diagnosed as suffering from Wernicke's encephalopathy, but who do not display any indications of amnesia (Kopelman, 1995). Further, memory impairments are often observed in patients with diencephalic tumours and thalamic infarcts that have produced lesions in these sites (Langlais, 1992).

1.3 *Etiology of KA*

There are several converging lines of evidence that thiamine deficiency is the primary etiological factor in the development of the neuropathological and behavioural deficits observed in KA. First, KA is most commonly seen in chronic alcoholics. The chronic alcoholic is typically malnourished, which leads to a deficiency in thiamine and other essential nutrients. Thiamine deficiency is implicated in the development of KA because ethanol interacts with thiamine uptake, storage and utilization in peripheral and central sites (Butterworth, 1995), thus attenuating the absorption and utilization of available thiamine. Second, experimental thiamine deficiency in rats (Troncoso et al., 1981; Langlais et al., 1992), cats (Irle & Markowitsch, 1982) and rhesus monkeys (Witt & Goldman-Rakic, 1983) results in neuropathology and behavioural deficits that are similar to those displayed by human KA patients. Third, some of the cognitive abnormalities that are present in KA are responsive to thiamine therapy. Patients who are treated with large doses of thiamine display improvements in ocular responsiveness (ophthalmoplegia) as well as

improvements to the global confusion state (Wood & Currie, 1995). The memory loss that is displayed in KA is somewhat responsive to thiamine therapy; statistically, 26% of KA patients who receive thiamine therapy do not exhibit any improvement in their memory disturbances, 53% display a slight to mild improvement over time, and 21% exhibit an almost complete recovery (Victor et al., 1989). The KA patients that display the greatest recovery are able to recall past events, and are able to retain new information. However, they retain no information of events that occurred during the illness. A fourth line of evidence suggesting that thiamine deprivation is the primary etiological factor in KA comes from the presence of KA in patients with beriberi, a disease that is caused by thiamine deficiency (Kopelman, 1995). The precise mechanism by which thiamine deficiency produces neuropathology is not known. However, as discussed below, one possible explanation involves glutamate excitotoxicity (Langlais, 1995).

However, the role of thiamine deficiency in the etiology of KA is complicated by the fact that KA is observed primarily in chronic alcoholics. This is an important consideration because some of the neuropathology and behavioural deficits that are seen in KA may be directly related to the effects of alcohol toxicity (Wood & Currie, 1995). Further, alcoholics often have other indirect problems that may contribute to the neuropathological outcome of KA, such as cirrhosis of the liver, head injuries from repeatedly falling down, epilepsy and cerebrovascular disease (Krill, 1995). Nevertheless, reports of KA in non-alcoholic patients (e.g., Butterworth et al., 1991) suggest that alcohol plays a secondary role in the etiology of KA (Victor et al., 1989). The fact that alcohol inhibits the absorption and utilization of available thiamine (Butterworth, 1995), suggests that exposure to chronic alcohol accelerates the progression of KA.

2. The PTD Model of KA

The PTD animal model of Wernicke-Korsakoff's syndrome demonstrates that thiamine deficiency is sufficient to cause neuropathology and behavioural deficits in experimental animals that are similar to those seen in KA patients. The PTD model produces thiamine deficiency in rats by exposing them to a thiamine-free diet in conjunction with daily injections of pyrithiamine, a thiamine antagonist (Troncoso et al., 1981). PTD produces a reliable pattern of neurological sequelae: food intake begins to decline on approximately day 7 of the treatment. This decline in food intake continues until the rats become completely aphagic (usually by day 9). Also by day 9, their grooming habits have deteriorated and they are inactive. After 11-12 days on the PTD treatment, rats display signs of ataxia, piloerection and dystonic posturing of the tail and body. This is followed by the loss of righting reflexes (day 12-13). Within hours, the rats display sensory-evoked seizures and then spontaneous generalized seizures. In behavioural studies, the treatment is typically reversed after the rats have displayed spontaneous seizures for 3-5 hours (e.g. Savage & Langlais, 1995; Langlais & Savage, 1995; Langlais & Zhang, 1997). The treatment is reversed by the administration of a large dose of thiamine. The seizures typically stop within 1-3 hours of reversal, at which point normal chow is returned to the rats.

2.1 *Neuropathology Produced by PTD*

The pattern and extent of neuropathology that is produced by PTD depends on treatment duration. PTD treatments that are not reversed until the rat has experienced several hours of generalized seizures typically produce neuropathology similar to that seen in human KA. The treatment produces symmetrical lesions in the thalamus, the mammillary bodies, the pontine tegmentum and periacqueductal grey (Langlais, Zhang & Savage, 1996). Within the thalamus, most of the nuclei that are damaged in human KA are also

damaged in recovered PTD rats. These include thalamic intralaminar nuclei (paracentral, central medial, anteromedial, interanteromedial, rhomboideus and central lateral nuclei), the midline, ventral portion of the mediodorsal nucleus, and the posterior nuclear group and parafiscular nucleus (Langlais, 1992). The PTD treatment also produces enlarged third ventricles, presumably due to the atrophy of the surrounding tissue. The IML is typically damaged in 70% of recovered PTD rats (Langlais, 1992).

As is the case in human KA, PTD rats consistently display lesions in brainstem and basal forebrain that are similar to KA neuropathology (Langlais et al., 1996). There is also evidence of cortical white matter loss and cortical shrinkage in PTD rats that resembles the damage seen in human KA (Langlais & Zhang, 1995). Similar neuropathology has been reported in studies that have used rhesus monkeys as subjects (e.g.,: Witt et al., 1983). Examination of the brains of recovered PTD rats has not revealed any pathological changes in several areas that are believed to play a role in memory functioning. These include the hippocampus, the medial septum and the amygdala (Langlais, 1992).

2.2 *Memory Deficits Produced by PTD*

Recovered PTD rats display memory impairments that are similar to the memory disturbances displayed by human KA patients. Most striking are the deficits that PTD rats display on tests that assess anterograde memory.

PTD rats display object-recognition deficits. Mumby et al. (1995) assessed object-recognition in PTD rats by training them on a delayed nonmatching-to-sample (DNMS) task. The DNMS task makes use of an elevated runway that has two goal areas that each contain two recessed food wells. Objects are placed over the food wells, and the rats are trained to displace the objects in order to receive a reward. Each DNMS trial includes a sample phase and a choice phase. On the sample phase, the rat is presented with only one object, and receives a reward after it has displaced the object. On the choice phase, the rat is

presented with two objects. One of the two objects was used on the sample phase, while the second is novel to the rat. On the choice phase the rat is rewarded only if it displaces the novel object. This is an object-recognition task because the rat must remember which of the two objects it has already seen on the sample phase. Two different objects are used in the subsequent trial. The delay between the sample phase and the choice phase can be varied in order to manipulate the memory demands. Mumby et al. (1995) trained rats on the DNMS procedure and then exposed them to PTD. Following recovery, the PTD rats required more trials to re-master DNMS at a 4 second retention delay, and performed more poorly than control rats at longer retention delays of 15, 30, 60, and 120 seconds. Similar results were reported for rats that were not pretrained prior to PTD (Mumby et al., 1995). The DNMS deficits displayed by the PTD rats in the Mumby et al. study are consistent with evidence of DNMS impairments in human KA patients (Oscar-Berman & Bonner, 1989).

PTD rats display a mild learning impairment on an allocentric spatial memory task (Langlais, Mandel & Mair, 1992). In the water maze (Morris, 1981), rats are placed into a pool of water and are permitted to locate a hidden platform that is submerged just below the surface of the water. The rats cannot see the platform because the water is made opaque. On the fixed-platform version of this task, the hidden platform is always in the same location within the pool and the rats are released into the pool at four different locations.

Langlais et al. (1992) trained PTD rats and pair-fed controls to locate a hidden platform in the Morris water maze. Upon histological examination of the brains of the PTD rats, these authors noted that two of the eleven PTD rats did not sustain detectable lesions within the regions of the internal medullary lamina (IML), a site that is typically pathologic in PTD rats (Langlais, 1995). Therefore, Langlais et al. (1992) analyzed the behavioural data from these two rats separately. The data revealed that all of the rats had similar latencies to locate the platform on the initial days of testing. However, unlike the control rats and PTD rats with sparing of the IML, PTD rats with lesions that included the IML

failed to display shorter latencies on subsequent days of testing. PTD rats with lesions of the IML did eventually acquire the task, as there were no significant differences between the rats by the tenth day of testing. Rats typically confine their swimming to the perimeter of the pool during initial sessions of swim maze testing, but learn to search for the hidden platform throughout the swim maze during subsequent sessions. However, in the Langlais et al. (1992) study, the PTD rats with IML lesions spent more time swimming around the perimeter of the pool in later sessions than did the control rats and the PTD rats with sparing of the IML. Therefore, it is not clear whether the PTD rats with IML lesions were experiencing a difficulty in retaining the location of the hidden platform or whether they had difficulty in switching search strategies. The latter possibility suggests that a non-mnemonic cognitive abnormality may have resulted in the perseveration of the thigmotaxic search strategy.

The delayed nonmatching-to-position procedure is a second way to assess spatial memory in PTD rats. This is typically carried out in a T-Maze, an apparatus consisting of two goal boxes that can be blocked by guillotine doors and that are placed perpendicularly to a central start area. As with the DNMS task described above, each trial in the delayed nonmatching-to-position procedure includes a sample phase and a choice phase. On the sample phase, the rat is placed into the apparatus in the start area and permitted to access one of the two goal boxes to retrieve a reward. On the choice phase, the rats has access to both goal areas. The rat receives a reward only if it enters the goal area opposite from the sample phase. The delay between the sample phase and the choice phase can be varied in order to manipulate the memory demands.

PTD rats made significantly more errors during acquisition of the delayed nonmatching-to-position task than did control rats, and fewer of the PTD rats were able to reach a criterion of correct responses, which consisted of 13 correct responses in 15 trials on 2 consecutive days (Mair et al., 1988). These data suggest an impairment in spatial

memory. However, the PTD rats in the Mair et al. study were eventually able to perform at an accuracy level of 80%, indicating that they were able to use spatial information to some extent.

Langlais and Savage (1995) also trained PTD rats on the delayed nonmatching-to-position task. Consistent with the findings of Mair et al. (1988), PTD rats were able to acquire this task, but they did so at a slower rate than did the controls. Langlais and Savage (1995) then increased the delay between the sample and choice phases and found that the longer delays did not differentially affect the PTD rats; the performance of all of the rats decreased equally as the delay was lengthened.

Following testing on the delayed nonmatching-to-position task, Langlais and Savage (1995) trained the rats on a matching-to-position task. In this case, the rats were reinforced on the choice phase only if they selected the same goal area in which they received reinforcement on the sample phase. As with the nonmatching procedure, the PTD rats required significantly more trials to reach criterion on the delayed matching-to-position task and made more errors throughout acquisition. However, the PTD rats were affected by the longer delays in the case of the delayed matching-to-position task. PTD rats made more errors than did control rats when the delay between the sample phase and the choice phase was increased to 90 seconds. Human amnesic patients have similar difficulties retaining spatial information over long delays (Langlais, 1992), suggesting that functions of the diencephalic structures damaged in KA are important for the maintenance of short-term, trial specific memory (i.e., working-memory).

The delayed non-matching and matching-to-position tasks that are referred to above (Langlais & Savage, 1995; Mair et al., 1988) are examples of spatial memory tasks that can be solved using either allocentric or egocentric spatial information. The tasks can be solved using allocentric spatial information if the rat retains information concerning the location of the goal areas relative to external extramaze cues (e.g., enter the goal area that is facing the

door). Contrarily, the tasks can be solved using egocentric spatial information because the rat can perform correctly simply by using a fixed-navigational path from the start area to the goal area (e.g., enter the left goal area). The fixed platform version of the Morris water maze is an allocentric spatial memory task if the rat is released into the pool at different locations, thus preventing the rat from learning that a specific path will always lead it to the hidden platform. Instead, the rat must retain the location of the hidden platform relative to extramaze cues. However, this is also a reference-memory task because the platform is always in the same location within the swim maze; there is no critical information that is trial specific. This distinction between tasks that assess different kinds of spatial memory is important because the nature of the task that is used may determine whether a spatial memory impairment is observed.

Unlike KA patients, there is no evidence of retrograde memory deficits in recovered PTD rats. Rats that were pretrained to locate a hidden platform in the Morris water maze retained the location of the hidden platform when tested following recovery from the PTD treatment (Langlais et al., 1992).

Recovered PTD rats also display intact retrograde memory on tasks that assess nonspatial memory. Mumby, Cameli and Glenn (1997) assessed retrograde memory for object-discrimination by training rats to displace objects in an elevated runway. On each trial, the rat was presented with the same two objects and was rewarded only if it displaced the object that was designated as S+. The primary dependent measure in this task was the percentage of correct choices (i.e., displacing S+) the rat made during each session of trials. Mumby et al. trained rats on three object discrimination problems, one at three different time-points prior to PTD treatment. When tested following recovery, PTD rats displayed normal retention of all three object-discrimination problems.

Another task that has been used to assess retrograde memory deficits in PTD rats is the passive avoidance task. Langlais and Savage (1995) assessed passive avoidance

through the use of an apparatus with two compartments. The rats were placed into one of the compartments and received a mild footshock if they entered the second compartment. The dependent measure was the latency for the rat to re-enter the second (shocked) compartment. The rats received two trials of passive avoidance and were then exposed to the PTD treatment. Following recovery, the PTD rats displayed retention of the passive avoidance task; the PTD rats were not more likely to enter the previously shocked compartment than were the control rats.

It is not clear why PTD rats do not exhibit evidence of retrograde amnesia whereas KA patients do. However, this discrepancy may be due to the fact that the type of information that human KA patients fail to recall is different from the type of information that must be retained by recovered PTD rats. Retrograde amnesia in KA is typically assessed by asking the patient to verbally recant events that are recalled from his or her own life. In contrast, assessing retrograde amnesia in PTD rats requires pretraining the rats on a particular task prior to exposing them to PTD and then assessing their retention of this one particular task. Moreover, except for the study by Mumby et al. (1997), the interval between the point at which the rat acquires a task and the onset of the PTD treatment has not been varied within individual studies. Therefore, it is possible that systematically varying the learning-to-lesion interval may detect retrograde amnesia in PTD rats. However, another possible explanation as to why KA patients exhibit evidence of retrograde amnesia whereas PTD rats do not, involves the direct exposure to the toxic effects of alcohol in KA, but not in PTD. Further, as stated above, KA patients are also exposed to several indirect effects of chronic alcoholism that may contribute to the neuropathological outcome of KA.

2.3 *Possible Mechanism of PTD Induced Neuropathology*

The precise mechanism by which thiamine deprivation causes neuropathology, and why certain brain sites are more vulnerable to thiamine deficiency than others is not known. One theoretical model involves the combination of alterations in energy metabolism with the presence of glutamate excitotoxicity (Robinson & Mair, 1992; Langlais, 1995).

Glutamate is an excitatory amino acid neurotransmitter that when present in an overabundance causes neural degeneration. It is implicated in PTD induced neuropathology because increased extracellular levels of glutamate have been found in those structures that are the most sensitive to thiamine deficiency, including the medial dorsal thalamic nuclei (Langlais & Zhang, 1993). Moreover, PTD-induced neuropathology is attenuated by the administration of MK-801, a glutamate antagonist (Langlais & Mair, 1990; Robinson & Mair, 1992).

The glutamate excitotoxicity model of PTD-induced neuropathology proposes that thiamine deficiency results in diencephalic neuropathology in the following manner: First, pyrithiamine inhibits brain thiamine pyrophosphokinase, which in turn reduces the level of thiamine pyrophosphate (TPP), the active form of thiamine within the brain (Langlais, 1995). TPP acts as a coenzyme for α-ketoglutarate dehydrogenase (α-KGDH), an enzyme involved in maintenance of cerebral energy metabolism (Butterworth, 1995). This reduction in cerebral energy metabolism results in reduced adenosine triphosphate (ATP). The decline in ATP results in defective repolarization following a glutamatergic input, resulting in enhanced receptor response to extracellular glutamate. This in turn results in an increased influx of Na^+ and Ca^{++}, which triggers neuronal edema and cell death (Langlais, 1995). According to this model, structures in the diencephalon are more vulnerable to thiamine deficiency because these structures are constantly receiving glutamatergic excitatory inputs from ascending sensory pathways. Moreover, a higher energy demand in the thalamus and other areas that are damaged in PTD would explain why these areas are

more vulnerable to the pathogenic effects of PTD (Langlais, 1995). However, this possibility remains to be verified.

3. *The Role of Seizures in the PTD Model*

The presence of generalized seizures during the late stages of the PTD treatment raises the possibility that some of the neuropathology and behavioural impairments that are displayed by recovered PTD rats may be attributable to the pathogenic effects of seizures rather than to the thiamine deficiency *per se*. This hypothesis was suggested by Mumby et al. (1996) in the context of ischemia-induced object-recognition deficits. Mumby et al. demonstrated that transient forebrain ischemia produced neuropathology that was limited to certain parts of the hippocampus and resulted in severe DNMS deficits. Contrarily, hippocampal ablation produced mild DNMS deficits. Mumby et al. hypothesized that postischemic seizures, presumably originating in the hippocampus, produce extrahippocampal neuropathology in structures that are important for object recognition. This hypothesis was supported by the fact that mild DNMS deficits were observed in rats that were subjected to ischemia followed one hour later by hippocampal ablation, while severe DNMS deficits were observed in rats that were subjected to ischemia followed by a sham surgery. Mumby et al. therefore differentiated between the primary pathogenic effects of transient forebrain ischemia and the secondary pathogenic effects of postischemic seizures.

The preceding hypothesis suggests that it is possible that there are primary and secondary pathologic events during PTD. The thiamine deprivation causes primary neuropathology through the metabolic disruption outlined above, and this evokes a seizure-prone state. The repeated seizures may cause secondary neuropathology. This is also an important consideration in human KA. Epileptic seizures of the grand mal type are

observed in 15% of chronic alcoholics (Avdaloff, 1979). Moreover, severe generalized seizures are also exhibited during withdrawal from alcohol (Carlson, 1991).

Repeated generalized seizures can cause alterations in brain activity and can also result in neuropathology (Lynch et al., 1996). In the kindling model of epilepsy, repeated high frequency stimulation of the hippocampus provokes severe generalized seizures in rats. Regionally specific cell death in the kindling model occurs after as few as three generalized seizures (Cavazos, Das & Sutula, 1994). Repeated generalized seizures can also result in behavioural and memory deficits. Kindled rats display spatial memory deficits when assessed one month following the last evoked seizure, and the severity of the deficit is correlated to the number of evoked seizures (Sutula et al., 1995).

Whether the seizures that are displayed during the late stages of PTD are causing some of the neuropathology and memory deficits is not clear. There are three main reasons for this. First, there is inconsistency in the reporting of seizures during the PTD treatment. Some studies report that seizures were observed, but not in any quantifiable manner. Further, many studies report neither the presence nor the absence of seizures. Second, there is great variability as to when the PTD treatment is reversed. Usually the treatment is reversed from 3-5 hours following the onset of generalized seizures (e.g., Langlais & Savage, 1995). However, several studies have reversed the treatment at or prior to the onset of seizures (e.g., Mair et al., 1988). PTD rats that are reversed earlier generally exhibit less neuropathology and less severe memory impairments (Langlais & Zhang, 1997). Rats that are reversed earlier also exhibit less seizures. However, it is difficult to assess the role of seizures in these rats because they are also exposed to a shorter duration of thiamine deficiency. The third reason why it is difficult to assess the role of seizures in the PTD model is that rats in the late stages of the treatment may experience covert, subconvulsive seizures that will not be detected by casual observations of the rat.

One way to assess whether seizures contribute to the neuropathology and memory deficits that are exhibited by rats following PTD treatment would be to expose rats to an equivalent duration of thiamine deficiency while attenuating seizures in some, but not all of the rats. Further, the occurrence of seizures should be quantified during the late stages of PTD as well as immediately following reversal. In order to address the possibility that subconvulsive seizures may influence the outcome of the PTD treatment, EEG activity should be monitored in PTD rats. This would allow comparisons to be made between rats that had similar bouts of thiamine deficiency but quantifiably different exposure to generalized seizures.

4. General Purpose

There were two main purposes of this study. First, by testing PTD rats on the delayed matching-to-place (DMTP) task in the Morris water maze this study assessed spatial allocentric working-memory in recovered PTD rats. As mentioned above, the delayed non-matching and matching-to-position tasks (e.g., Langlais & Savage, 1995; Mair et al., 1988) are examples of spatial working-memory tasks that can be solved using either allocentric or egocentric spatial information. The fixed platform version of the Morris water maze is an allocentric spatial memory task. However, this task does not assess trial specific information (i.e., working-memory).

The DMTP task assesses both allocentric spatial memory and working-memory. This task is similar to the delayed matching-to-position task that was described previously. Each trial consists of two swims. On the first swim, the rat is placed into the pool and permitted to locate the hidden platform. On the second swim, the rat is placed into the pool at the same location and the platform is located in the same position within the pool as it was on the first swim. Rats that retain information about the position of the platform from the first swim should be able to locate the platform more quickly on the second swim. The

location of the platform as well as where the rat is placed into the pool are different on each successive trial. The delay between the first and second swims can be varied in order to manipulate the memory demands of the task. The primary dependent measure is the latency for the rat to locate the hidden platform. The use of multiple platform locations in the DMTP procedure ensures that the rat must retain trial-specific information concerning the location of the platform relative to extramaze cues in order to locate the platform more quickly on the second swim of each trial.

Mumby et al. (1997) pretrained rats on the DMTP task prior to exposing them to PTD. Following recovery, PTD rats were impaired at a 300 second retention delay, but not at retention delays of 4 or 60 seconds. The present study was the first to assess the performance of PTD rats that have not received any DMTP training prior to PTD treatment.

The second main purpose of this study was to address the idea that a high incidence of seizures during the late stages of PTD contributes to the neuropathological and behavioural outcome of the treatment. The view that the neuropathology and memory deficits in recovered PTD rats are a consequence of the primary effects of the thiamine deprivation and the secondary effects of repeated seizures predicts that attenuating the seizures will attenuate the neuropathology and memory deficits. This thesis evaluated the role of seizures in the PTD model of KA in the following manner: One group of rats was exposed to PTD and was not reversed until three hours following the onset of seizures (group PTD). A second group of rats received a prophylactic (diazepam) in order to attenuate seizures during the late stages of the treatment (group PTD+DZ). Each of the PTD+DZ rats was yoked to a PTD rat such that the treatment was initiated and reversed at the same time for the two rats. The treatment was reversed three hours following the first observation of overt seizures in the PTD rat. A third group of rats served as a control group, and did not undergo the PTD treatment. These rats received a daily injection of saline and injections of diazepam at the same time that the rats in group PTD+DZ did in

order to compare the two groups of PTD rats to rats that have not sustained brain damage but have undergone similar procedures (group SAL+DZ). The occurrence of seizures was carefully monitored in all of the rats during the late stages of the PTD treatment as well as for several hours following reversal.

To address the possibility that PTD rats may experience covert, subconvulsive seizures as well as overt seizures, some of the rats were implanted with recording electrodes in each hippocampus prior to undergoing the PTD treatment. The hippocampus was chosen because it is not typically damaged in recovered PTD rats and because it is an epileptogenic structure (Johnston & Brown, 1984). These rats underwent the same procedures (i.e., PTD treatment with or without diazepam and behavioural testing) as did the nonimplanted rats. EEG activity was monitored throughout the PTD treatment and for a period of 5 days following recovery.

It was hypothesized that if the exposure to repeated seizures during the late stages of PTD causes pathology that is secondary to the primary effects of thiamine deficiency, then DMTP deficits and neuropathology would be attenuated in the PTD rats that received diazepam because these rats would not be exposed to repeated seizures. Conversely, rats that underwent the treatment in the absence of diazepam would experience both the primary effects of the thiamine deficiency as well as the secondary effects of the seizures and would therefore exhibit additional neuropathology and behavioural deficits.

METHOD

Subjects

The subjects consisted of 57 male Long-Evans rats (Charles River, St. Constant, QC) that weighed between 300-325g at the start of the experiment. All of the rats were experimentally naive and were individually housed in hanging wire mesh cages in a colony room under a 12:12 h light:dark cycle with light onset at 8:00 am. The rats had free access

to water throughout the experiment and received a daily ration of approximately 30g of rat chow. All behavioural testing occurred in the rat's light phase and commenced 14 days following reversal of the PTD treatment.

Apparatuses

Behavioural testing was carried out in a Morris water maze (Morris, 1981), which consisted of a galvanized circular tank that was 1.37m in diameter and 46cm deep. The tank was filled with water (maintained at 24° C) until the water level was 10cm below the top of the tank. The rats were required to swim to a platform (10cm in width, 10cm in length and 28cm in height) that was placed into the pool. The top of the platform was located 1.5cm below the surface of the water. The platform was hidden from the rats by making the water opaque through the addition of a nontoxic white paint (Reeves & Poole, Toronto, ON). The rat's swim path was recorded by a video tracking system that included a VP118 supertracker (HVS Image Limited, Hampton, U.K.), a Panasonic video camera (model WV-BL200), a Panasonic video monitor (model WV-BM900), and an IBM PC 486DX computer.

HVSWater for Windows (HVS Image Ltd., Hampton, U.K.) software was used to analyze each rat's performance on every swim. All behavioural testing was carried out in the same room. There were posters affixed to the walls in order to provide extramaze cues. A black curtain was used to hide the testing room from the rats during intertrial intervals and during retention delays. A radio was set at a moderate volume in order to mask sounds coming from outside the testing room.

All EEG recording occurred in a chamber that was 55cm wide, 40cm long and 42cm high. The electrode assembly in the head of the rat was attached to a commutator (Josef Biela Engineering, Irvine, CA) which was mounted on top of the recording chamber. The output was filtered (low end cut off = 1 Hz; high end cut off = 5 KHz; model

number NL126; Digitimer Ltd., Hertfordshire, U.K.) and amplified (gain = 1000X; model number NL106, Digitimer Ltd.) before it was digitized by an A/D board (DataWave Technologies, model number 20B, Longview, CO) and sent to an IBM PC 486DX computer. The Experminenter's Workbench software package (DataWave Technologies, Longview, CO) was used to control the sampling rate, display and recording of all electrophysiological output.

The implanted rats were videotaped during the late stage of PTD with a Panasonic camera (model number PV-900-K) and a Panasonic VCR (model number AG2350P). To correlate EEG activity with seizures, a Panasonic time-date generator (model number WJ810) that was accurate to 0.01 seconds was synchronized with the time-stamp on the EEG record; the time-date generator inserted a time-stamp onto the video recording of the seizures.

Procedure

PTD Treatment

A total of 45 rats were placed on the PTD treatment; 30 rats were assigned to group PTD and 15 rats were assigned to group PTD+DZ. Six PTD rats and three PTD+DZ rats were implanted with recording electrodes. Twelve rats were assigned to a control group and received daily injections of saline and two injections of diazepam (group SAL+DZ).

The PTD treatment consisted of daily injections of pyrithiamine hydrobromide (0.25mg/kg, i.p.; Sigma, St. Louis, MO), delivered at a volume of 0.1 ml/100g of body weight. The rats' diet was restricted to a thiamine-deficient chow (ICN Biomedicals, Aurora, OH), to which they had *ad libitum* access for the duration of PTD treatment.

Diazepam was administered to the rats in group PTD+DZ following the first observation of the loss of the righting reflex (i.e., the rat was lying on its side and not attempting to return to an upright position). When one of the two rats in a yoked pair

displayed the loss of the righting reflex, the two rats were randomly assigned either to group PTD or to group PTD+DZ, and the rat that was assigned to group PTD+DZ was administered diazepam (5 mg/kg, i.p.; Hoffmann-La Roche, Mississauga, ON) at a volume of 0.1 ml/100g of body weight. A second injection of diazepam was administered to each of the rats in group PTD+DZ six hours following the first injection. The rats in group PTD+DZ typically received the first injection of diazepam three hours prior to the termination of PTD treatment.

PTD was continued until 3 hours after the rat in group PTD exhibited a generalized seizure (typically treatment day 14). The treatment was then reversed for both of the rats by the administration of a large dose of thiamine (100 mg/kg, i.p.; Sigma, St. Louis, MO), delivered at a volume of 0.4 ml/100g of body weight. The rats then had ad libitum access to regular rat chow for one week, after which the rats received a daily ration of 30g of regular chow.

Each rat in group SAL+DZ received a daily injection of 0.9% saline (i.p., Abbott, Montreal, QC) at a volume of 0.1 ml/100g of body weight and two injections of diazepam, at the same concentration and volume as the rats in group PTD+DZ on treatment day 14 for the PTD and PTD+DZ rats. These rats were not fed the thiamine-deficient chow, rather, they were given a daily ration of 30g of regular chow throughout the experiment.

In order to quantify overt seizure activity, the rats in groups PTD and PTD+DZ, including the implanted rats, were under careful observation during the late stage of PTD. At the first observation of behavioural immobility (typically day 12 of treatment), the rats were observed at two hour intervals for a period of five minutes per observation. From the first observation of the loss of the righting reflex (typically day 13 of treatment), seizures were quantified by observing the rats for a period of ten minutes per hour until five hours following PTD reversal. Seizures were quantified by the number of seizures observed during the ten minute period, as well as the duration of each observed seizure. The

behavioural characteristics of each seizure were also classified as 1) automatisms (chewing, twitching of the vibrissae); 2) bilateral forelimb clonus; 3) full tonic-clonic seizures.

EEG Assessment

In order to observe EEG activity during and following PTD, bipolar recording electrodes were bilaterally implanted into the hippocampus for a total of nine rats. One electrode was implanted into the CA1 field of the hippocampus and the second electrode was implanted into the contralateral dentate gyrus.

Each rat was given an injection of atropine sulfate (1.0 mg/kg, s.c.) 25 minutes prior to surgery. The rat was anaesthetized with sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Cambridge, ON; 65 mg/kg, i.p.) and placed in a stereotaxic apparatus (Kopf Instruments, Tajunga, CA) with the incisor bar set at -3.3mm relative to bregma. The scalp was then incised and retracted and one hole was drilled on each side of the skull at 3.6mm posterior and 1.8mm lateral to bregma. A bipolar recording electrode, that was constructed of stainless steel, teflon-coated wire (diameter=125 microns; A & M Systems Inc., Everett, WA) and male gold-plated amphenol pins (A & M Systems Inc.) was then lowered 2.8mm relative to bregma when CA1 was targeted and 4.1mm relative to bregma when the dentate gyrus was targeted.

A ground electrode, constructed of bare wire and soldered to a screw, was then anchored into the left frontal skull plate and an indifferent recording electrode, constructed of teflon-coated wire and soldered to a screw, was embedded in the interparietal skull plate. The male pins from both recording electrodes, the ground electrode and the indifferent electrode were then attached to a McIntyre connecter socket (Carleton University, Ottawa, ON). The socket was cemented into place with dental acrylic (HCC Hygenic Corp., St. Catherines, ON) and the rat was allowed to recover for a period of two weeks.

Each implanted rat received a one hour recording session every day throughout PTD. The rat was placed into the recording chamber and allowed to roam freely while its EEG was continuously sampled (1024 Hz). All EEG output was saved to disk for later observation.

All implanted rats were monitored for the first signs of PTD-induced ataxia, which was typically observed on Day 12 of PTD. Once ataxia was observed, the rat was taken out of its home cage and left in the recording chamber, so that a continuous record of its EEG could be saved to disk for the duration of PTD. The treatment was reversed for all of the implanted rats in the same manner as it was for the nonimplanted members of their respective groups, and implanted rats in group PTD+DZ received diazepam as did the nonimplanted rats in group PTD+DZ. EEG activity was continuously recorded for each rat for a period of 24 hours following the reversal of the treatment. One hour of EEG was recorded every day thereafter for a period of five days.

The baseline EEG that was recorded during the initial days of PTD was later compared to the EEG that was recorded during the late stage of PTD and immediately following the reversal of the treatment. The EEG activity was monitored for signs of seizures. These included brief (less than 100ms) EEG spikes as well as signs of prolonged seizure activity. The latter was defined as an increase in EEG amplitude by at least 100% for a duration of longer than 1 second.

Delayed Matching-To-Place

The rats were transported to the testing room in groups of four to six rats and received one testing session per day, approximately 5 days per week. The rats were always placed into the water at one of four release points that were located around the perimeter of the pool. There was an equal distance between the four release points and each one corresponded to a cardinal compass location (E,S,W,N). Each rat was tested individually and was placed in a wire mesh cage behind a black curtain during intertrial intervals.

Pretraining: In order to train the rats to search for a hidden platform and also to help reduce their tendency to circle the perimeter of the pool, the first three sessions consisted of fixed-platform trials wherein the platform was always in the centre of the north-west quadrant of the swim maze. A trial began when a rat was placed into the pool at one of the four release points and ended when the rat placed three of its paws on the platform or when 60 seconds had elapsed. If the rat did not locate the hidden platform within 60 seconds then the rat was placed onto the platform by the experimenter. The rat remained on the platform for 10 seconds, after which it was removed by the experimenter and returned to its cage behind the curtain. At this point, the next rat was placed into the pool. There were eight fixed-platform trials per session. Each of the four release positions were used twice during the eight trials in the same order for every rat. The order of the release positions was determined pseudorandomly, with the constraint that all four of the locations were used twice during the eight trials.

The procedure was altered in the fourth session: for the first four trials, the platform was hidden in the same location that had been used during the first three sessions. However, the platform was moved to a new location for the next four trials. Two novel platform locations were used in the fifth session; each location was used for four consecutive trials.

DMTP acquisition: Each DMTP trial consisted of paired-swims. On the first swim, the rat was placed into the pool at one of the four release points and permitted to locate the hidden platform. If the rat did not locate the platform within 60 seconds then it was guided to the platform by the experimenter. The rat remained on the platform for 10 seconds before it was removed by the experimenter. On the second swim, the rat was placed into the pool at the same release point and the platform was hidden in the same location that had been used on the first swim. The retention delay between the first and second swims was 4 seconds throughout DMTP acquisition. During the 4 second retention delay, the rat was

held in an opaque cage by the experimenter. There were 4 trials of DMTP per session (i.e., eight swims), and each of the four cardinal locations was used as a starting point for one trial (i.e., two swims) in a randomly determined order. During the first session of DMTP acquisition, the platform remained hidden in one target location for all 4 trials. During the second session of DMTP acquisition, a novel platform location was used for the first 2 trials and a second novel platform location was used for the last two trials. Commencing from the third session of DMTP acquisition, a different platform location was used for every trial. That is, the platform was moved to a different location every time a trial began (i.e., for every first swim). A total of ten different platform locations were used (see Figure 1). All of the platform locations were used equally throughout testing, and each platform location was paired with each of the four cardinal release points.

Following ten sessions of moving-platform DMTP trials, the rat's second-swim escape latencies were analyzed in order to observe whether they had attained an asymptotic level of performance. A rat was considered to be performing at asymptote when the following conditions were met: 1) all of the second swim escape latencies were less than the maximum of 60 seconds for three consecutive sessions, 2) the difference between the mean second-swim escape latencies for two consecutive sessions was less than five seconds and 3) obtained p values were greater than 0.20 when paired t-tests were performed comparing the mean second-swim escape latencies from three consecutive sessions. A rat that did not meet the criteria for asymptotic performance within the 10 sessions received up to three additional sessions, after which it proceeded to the next stage of testing.

DMTP training at longer retention delays: The next stage of testing involved the introduction of longer retention delays between the first and second swims. The rats received three sessions of 4 trials of DMTP with a 30 second delay, followed by three

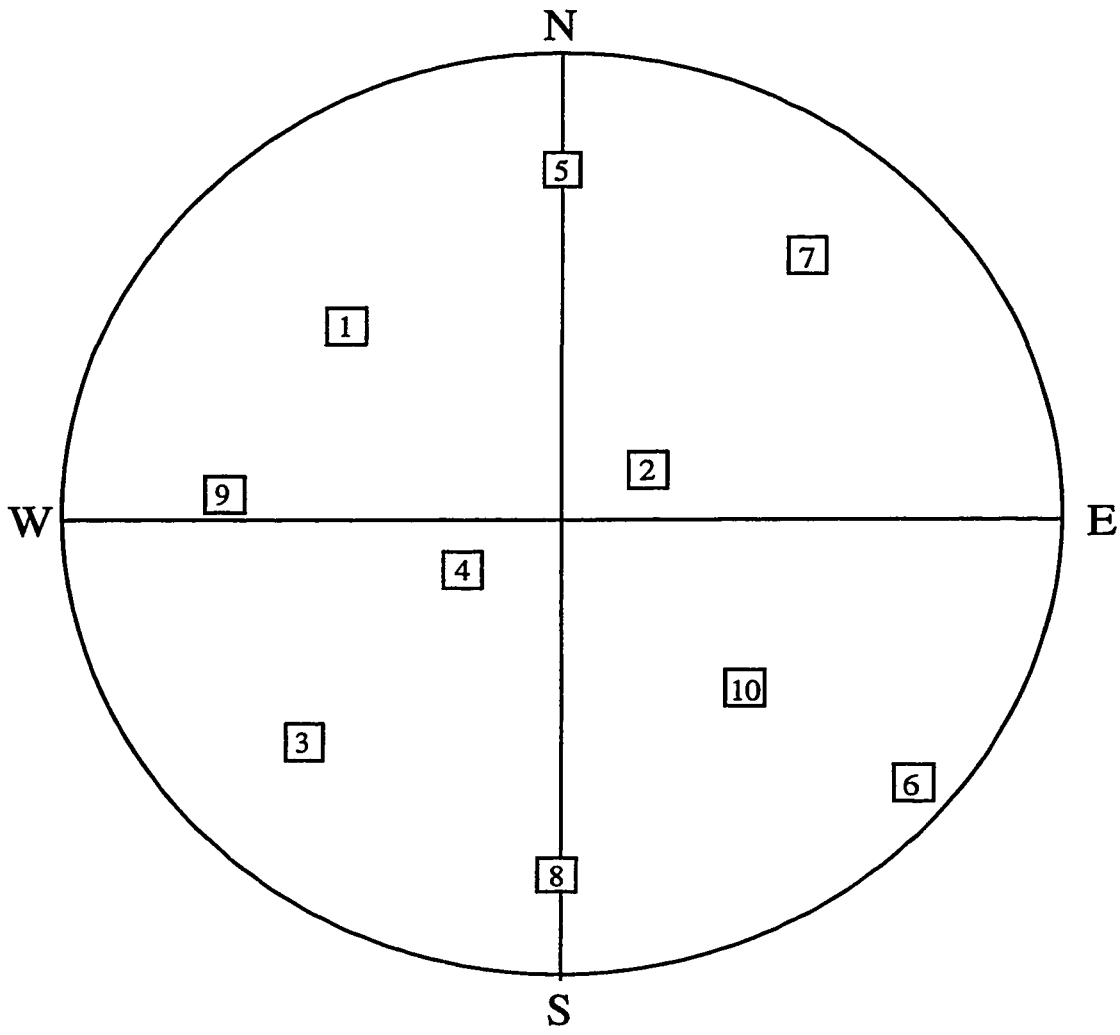

Figure 1. A schematic diagram showing the 10 different platform locations used during training and testing on the DMTP task in the water maze.

sessions of DMTP with a 120 second retention delay. During the 30 and 120 second retention delays, the rat was placed in an opaque cage behind a black curtain.

DMTP with mixed-delay sessions: The next stage of testing consisted of mixed-delay sessions. Each rat received 6 trials of DMTP per session, two trials at each of the three delays (4, 30, 120 seconds). There were six sessions of mixed-delay testing, resulting in 12 trials at each of the three delays. Each of the three delay conditions occurred in a randomly determined order on each of the six mixed-delay testing sessions. Also, all of the 10 platform locations were used during each of the three delay conditions.

Visible-platform DMTP trials: One visible-platform session was conducted on the day following the last mixed-delay session. The platform was made visible by the addition of black tape around its perimeter and also by raising it to 2cm above the surface of the pool. The session began with four fixed-platform trials wherein the platform was placed at the centre of the pool. This was followed by four trials of DMTP. The platform was moved to a new location for each of the four DMTP trials. The delay between the first and second swims was 4 seconds.

Histology

Following behavioural testing, the rats received an overdose of sodium pentobarbital (100 mg/kg, i.p.) and were perfused transcardially with 0.9% saline and 10% formol-saline. The brains were removed, stored in a formol-saline solution, and cut on a vibratome at a thickness of 30μm. To verify the extent and location of thalamic neuropathology, every fourth section throughout the thalamus (from 0.92mm to 6.04mm posterior to bregma) was saved. Sections were mounted on gel-coated slides and stained with cresyl-violet.

Performance Measures and Statistical Analyses

The primary dependent measure was the rat's latency (seconds) to locate the hidden platform (i.e., when the rat has placed three of its paws on the platform). Other dependent

measures included the rat's mean swim speed (cm/second), and the rat's mean proximity to the platform (cm). The latter measure was defined as the average distance between the rat and the hidden platform from the start of each swim until the rat has located the hidden platform. All of these measures were provided by the HVSWater for Windows software.

Mixed-factorial analyses of variance were conducted in order to analyze first and second swim escape latencies, mean proximity to platform and mean swim speeds. The analyses were conducted with group (PTD, PTD+DZ, SAL+DZ) as the between-subjects factor and retention delay condition (4, 30, 120 sec) as the within-subjects factor. The results of each analysis are tabulated in Appendix A. Post Hoc analyses were computed for each of the groups at each delay condition for all of the above measures. A significance level of .05 was used for all statistical tests.

Finally, in order to determine whether seizures affect DMTP performance in PTD rats, correlations were performed using the total duration of observed seizures as one factor and mean second-swim escape latency during mixed-delay sessions as the second factor. Only the rats from group PTD were included in these correlations.

RESULTS

Effects of PTD Treatments

General Progression of PTD

All of the rats from groups PTD and PTD+DZ progressed through the typical neurological sequelae. Food intake declined to the point that the rats were completely aphagic by the 10th Day of PTD treatment. The rats then displayed signs of muscular incoordination, piloerection and dystonic posturing of the tail and body (Day 12). The onset of the loss of righting reflexes typically occurred on Day 13 or Day 14 of PTD. However, several rats displayed this neurological symptom on Day 15 and one rat did not display the loss of the righting reflex until Day 18.

Group PTD

Each rat was observed for 10 minutes of every hour from the onset of the loss of righting reflexes until several hours following treatment reversal. Prior to treatment reversal, sensory-evoked and spontaneous tonic-clonic seizures were observed in all of the rats in group PTD. Typically, one seizure was observed in each 10 minute period. The total number of individual seizures that were observed for each rat ranged from 1 to 6, with a mean of 3.25 seizures per rat. The mean total amount of time that each rat was observed in a seizure was 82.67 seconds (range = 12 to 225 seconds). The mean duration of each individual seizure was 25.43 seconds (range = 10 to 60 seconds). All of the rats displayed multiple automatisms and bilateral forelimb clonus.

The administration of thiamine triggered seizures in many of the PTD rats, presumably because of the handling they received at the time. There were far fewer observed seizures following reversal and the duration of each seizure was shorter. There were no observed seizures in 23 rats following reversal. One hour following reversal, four of the remaining rats exhibited one seizure each, while one rat exhibited three seizures. Two rats exhibited one seizure each two hours following reversal. No tonic-clonic seizures were observed in any of the PTD rats at any subsequent point.

The administration of thiamine had a dramatic effect on most of the PTD-treated rats. Several of the rats from group PTD ate within a few hours following reversal. These rats were also able to right themselves and were fairly mobile within 24 hours of reversal. However, many of the rats in group PTD displayed clear neurological symptoms several days following reversal. These included an inability to recover self-feeding behaviour, and thigmotaxic behaviour within their cages. Any rat that was unable to self-feed was manually fed a mixture of normal chow and water (i.e., wet mash). Most of the recovered PTD rats made clear attempts to ingest their food, to the point that recovered PTD rats were frequently observed with their snouts immersed in the wet mash. However, many of these

rats were unable to ingest the food. A total of 24 nonimplanted rats and 6 implanted rats were assigned to group PTD. Of these, 14 nonimplanted rats and 4 implanted rats displayed normal feeding behaviour within 48 hours. These rats were allowed to recover for 2 weeks and then received behavioural testing. A total of 4 nonimplanted PTD rats and 2 implanted PTD rats died within a few days of the treatment reversal, and 6 PTD rats failed to recover feeding behaviour after seven days following reversal and were removed from the study and euthanized. The present post-PTD survival rate is consistent with data reported by various researchers (e.g., Langlais & Savage, 1995; Mair et al., 1985).

Group PTD+DZ

The administration of diazepam to the rats in group PTD+DZ had a dramatic effect on susceptibility to seizures. Seizures were observed in only 5 of the 15 PTD+DZ rats. One seizure was observed prior to or immediately following the first administration of diazepam in each of these five rats. This is most likely due to the handling that the rats received during the injection. The mean total amount of time that each rat was observed in a seizure was 7.9 seconds (range = 0 to 21 seconds). A planned comparison based on the total amount of time that each rat was observed in a seizure revealed that the PTD+DZ rats were exposed to significantly fewer seizures than were the PTD rats ($t(41) = 5.51$, $p < .01$). The few seizures that were observed in group PTD+DZ had a mean duration of 15.8 seconds (range = 10 to 19 seconds). No seizures were observed in any of the PTD+DZ rats one hour or more subsequent to the first injection of diazepam.

The administration of diazepam to the PTD+DZ rats typically had a sedative effect. However, several of the PTD+DZ rats displayed an increased responsiveness and mobility following diazepam administration.

Almost all of the rats in group PTD+DZ were able to self-feed within a few hours following reversal, and most of the PTD+DZ rats were able to right themselves and were fairly mobile within 24 hours of reversal. However, 2 of the 12 nonimplanted rats and 2 of

the 3 implanted rats that were assigned to group PTD+DZ failed to recover the ability to self-feed and were removed from the study and euthanized.

Histological Results

PTD rats that sustain damage to the internal medullary lamina (IML) and associated intralaminar thalamic nuclei, typically display more severe deficits on spatial memory tasks compared to PTD rats with sparing of the IML (e.g., Langlais et al., 1992). In order to determine whether this was true in the present study, all of the rats from group PTD and group PTD+DZ were characterized as either IML-lesioned or IML-spared. Rats with evidence of neuropathology in the IML and associated intralaminar thalamic nuclei (i.e., central medial (CM), paracentral (PC), ventrolateral (VL), parafiscular (PF) and centrolateral (CL)) were characterized as IML-lesioned. Rats without evidence of neuropathology in these nuclei were characterized as IML-spared. As discussed below, all of the PTD rats were characterized as IML-lesioned. Contrarily, only half of the PTD+DZ rats were characterized as IML-lesioned. Figures 2, 3 and 4 display representative midline thalamic nuclei pathology in the anterior, middle, and posterior thalamus for the rats from group PTD, as well as for PTD+DZ rats with IML lesions and PTD+DZ rats with IML sparing. Representative sections from control rats (group SAL+DZ) are also displayed.

All of the rats from group PTD that provided behavioural data sustained bilateral and symmetrical tissue cavitation and intense gliosis throughout the anterior-posterior extent of midline thalamic nuclei. The most consistent damage was to the IML and associated intralaminar thalamic nuclei (CM, PC, VL, PF and CL), the ventral part of the mediodorsal (MD) nucleus and the ventral posteromedial (VPM) nucleus. All of these rats were therefore characterized as IML-lesioned.

The dorsal 3rd ventricle was enlarged in the anterior thalamus, indicating tissue loss in the anterior paraventricular (PVA), paratenial (PT), medial habenular (MHb) and the

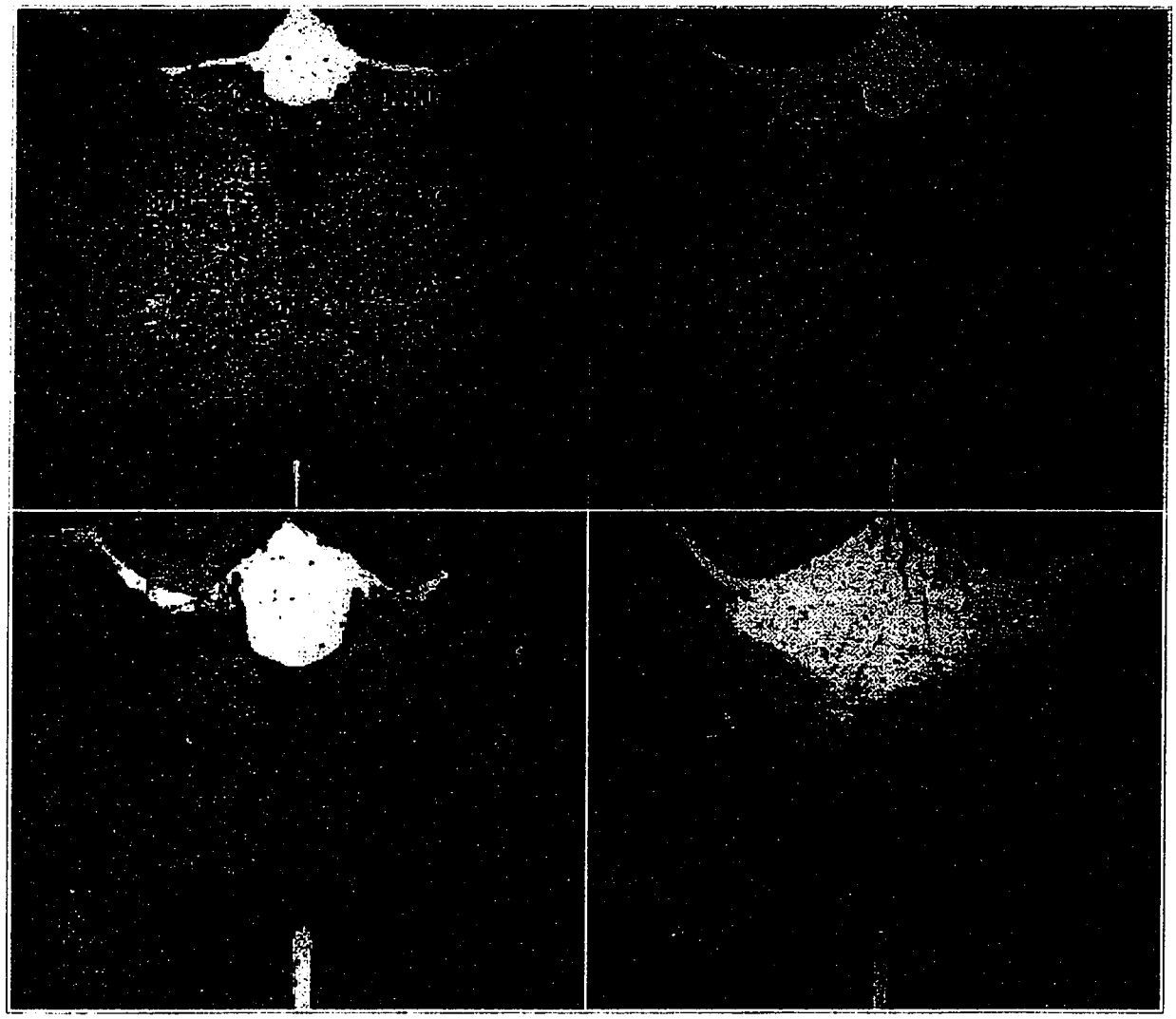

Figure 2. Representative digitized images of cresyl-violet-stained coronal sections (30 μm thick) of the anterior thalamus (2.12 mm posterior to bregma) from a SAL+DZ rat (A), a PTD+DZ rat with IML sparing (B), a PTD+DZ rat with IML damage (C), and a PTD rat (D). Gliotic scarring and neuronal loss (as indicated by the area between the arrows) is present in the PTD+DZ rat with IML damage (C) and the PTD rat (D). There is also evidence of tissue cavitation in the PTD rat (D). Moreover, the 3rd ventricle is enlarged in B and C, as indicated by the decreased distance between the floor of the dorsal 3rd ventricle and the roof of the 3rd venticle. Abbreviations: MD, medial dorsal nucleus; PC, paracentral nucleus; CM, central medial nucleus; IML, internal medullary lamina; PVA, anterior paraventricular nucleus; LHb, lateral habenular nucleus; AV, anteroventral nucleus; AD, anterodorsal nucleus.

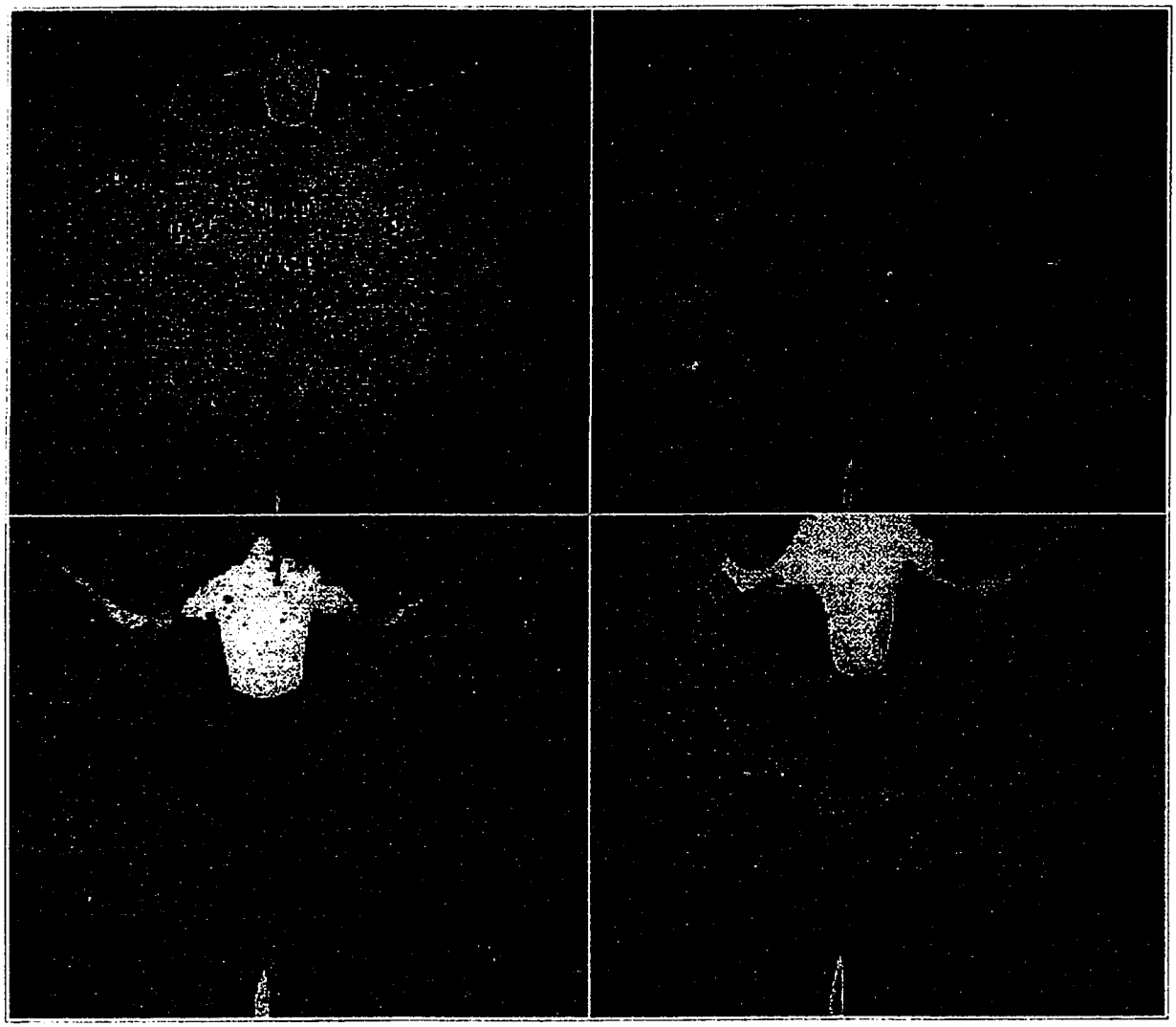

Figure 3. Representative digitized images of cresyl-violet-stained coronal sections (30 μm thick) of the thalamus (2.56 mm posterior to bregma) from a SAL+DZ rat (A), a PTD+DZ rat with IML sparing (B), a PTD+DZ rat with IML damage (C), and a PTD rat (D). Gross pathology, indicated by moderate neuronal loss and gliotic scarring is present in the PTD+DZ rat with IML damage (C; the area between the arrows). Gross tissue cavitation (see arrowhead) and gliosis (arrows) are present in the PTD rat (D). The 3rd ventricle is enlarged in C and D. Abbreviations: PC, paracentral nucleus; CM, central medial nucleus; IMD, interomedial dorsal nucleus; VL, ventral lateral nucleus; Po, posterior nucleus; Rh, rhomboid nucleus.

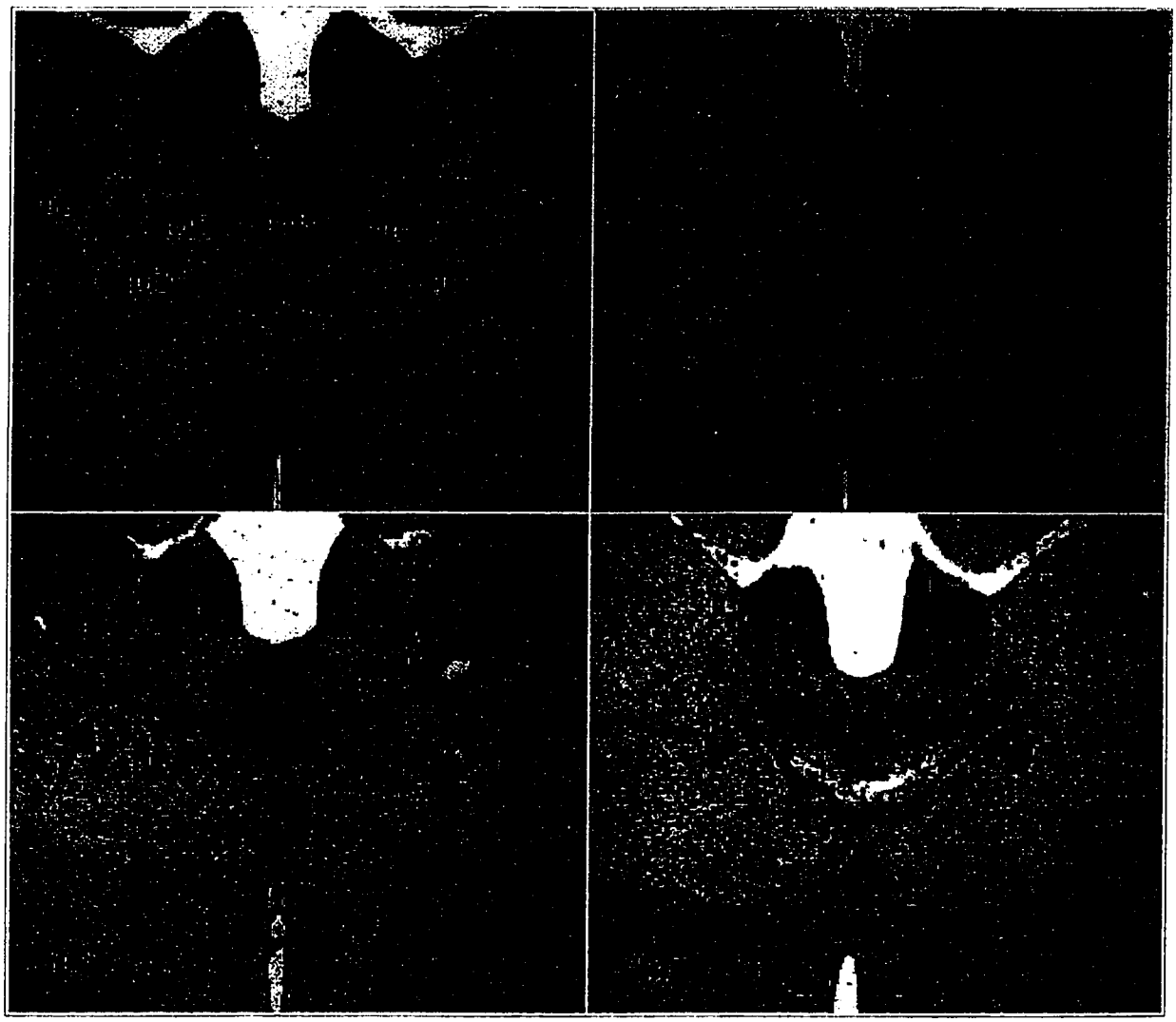

Figure 4. Representative digitized images of cresyl-violet-stained coronal sections (30 μm thick) of the posterior thalamus (3.6 mm posterior to bregma) from a SAL+DZ rat (A), a PTD+DZ rat with IML sparing (B), a PTD+DZ rat with IML damage (C), and a PTD rat (D). Mild gliosis is present in the PTD+DZ rat with IML sparing (B; see arrows). Gross pathology, represented by intense gliotic scarring is present in the PTD+DZ rat with IML damage (C; the area between the arrows). Gross pathology in the PTD rat (D) is indicated by gliotic scarring (arrows), and tissue cavitation (arrowhead). The 3rd ventricle is enlarged in C and D. Abbreviations: PC, paracentral nucleus; CM, central medial nucleus; IMD, interomedial dorsal nucleus; MD, medial dorsal nucleus; Po, posterior nucleus; CL, central lateral nucleus; PVP, posterior paraventricular nucleus.

anterodorsal (AD) thalamic nuclei. The posterior (Po) nucleus was frequently pathologic in the posterior thalamus. All of the rats from group PTD also sustained moderate gliosis and neuronal loss to the medial mammillary bodies (MB).

All of the nuclei that were affected in the brains of the rats from group PTD that contributed behavioural data were also affected in the brains of the rats from group PTD that failed to recover from the treatment. However, the PTD rats that did not recover from the treatment sustained pathology that affected lateral and ventral nuclei that were unaffected in the former. These include the reuniens (Re), the rhomboid (Rh), the posterior paraventricular (PVP), the ventral posterolateral (VPL), the ventromedial (VM) and the gelatinous (G) thalamic nuclei. Further, gross pathology continued into more posterior areas of the thalamus, as evident by tissue cavitation throughout the entire Po, VPL and VPM nuclei. The 3rd ventricle was greatly enlarged throughout the thalamus.

Of the ten PTD+DZ rats that contributed behavioural data, 5 sustained neuropathology that was similar to the neuropathology sustained by the PTD rats that contributed behavioural data. This includes tissue cavitation and gliosis to the IML and associated intralaminar thalamic nuclei. For the purpose of behavioural comparisons, these rats were characterized as PTD+DZ: IML-lesioned. Compared to the rats from group PTD, there was less evidence of gross pathology in more lateral thalamic nuclei (e.g., VPM). However, there was evidence of moderate neuronal loss in the Po in the brains of the PTD+DZ: IML-lesioned rats.

The remaining PTD+DZ rats exhibited mild CM gliosis, but sparing of the IML and all of the remaining intralaminar thalamic nuclei. For the purposes of behavioural comparisons, these rats were characterized as PTD+DZ: IML-spared. There was also evidence of Po tissue cavitation and lateral MD gliosis in the PTD+DZ: IML-spared rats.

The MB was pathologic in one of the five PTD+DZ: IML-spared rats and three of the five PTD+DZ: IML-lesioned rats. Within the rats from group PTD+DZ, mild seizures were observed in two of the IML-lesioned rats and one of the IML-spared rats.

Electrophysiological Results

Six implanted rats were assigned to group PTD and three implanted rats were assigned to group PTD+DZ. During PTD treatment however, the assembly that was attached to the rat's head (i.e., the headcap) became dislodged for three of the PTD rats and one PTD+DZ rat. These rats could no longer provide EEG data. However, these rats were not removed from the study; rather, PTD treatment was continued for these rats as per the members of their respective groups.

A second problem with the interpretation of the EEG data concerns their reliability. Several of the rats displayed frequent large amplitude EEG spikes during baseline EEG. Moreover, in another rat there were no detectable signs of seizures in the EEG, despite the fact that the rat was displaying severe tonic-clonic convulsions. The EEG data from these rats were deemed unreliable.

However, the present study does provide preliminary evidence of subconvulsive seizures during the late stages of thiamine deficiency in PTD rats and of much less in PTD+DZ rats. Figure 5a displays five continuous seconds of baseline (i.e., prior to PTD treatment) EEG that was recorded for one of the implanted PTD rats. During the late stages of PTD (i.e., subsequent to the loss of righting reflexes), this rat displayed frequent indications of seizures in its EEG. As displayed in Figure 5b, the seizures were characterized by high frequency EEG that had twice the amplitude of baseline EEG. Moreover, large amplitude spikes occurred within each EEG seizure. The duration of these seizures was 13 to 48 seconds. Further, seizures with these characteristics were displayed at a remarkable rate throughout the late stages of PTD. For example, during one thirty

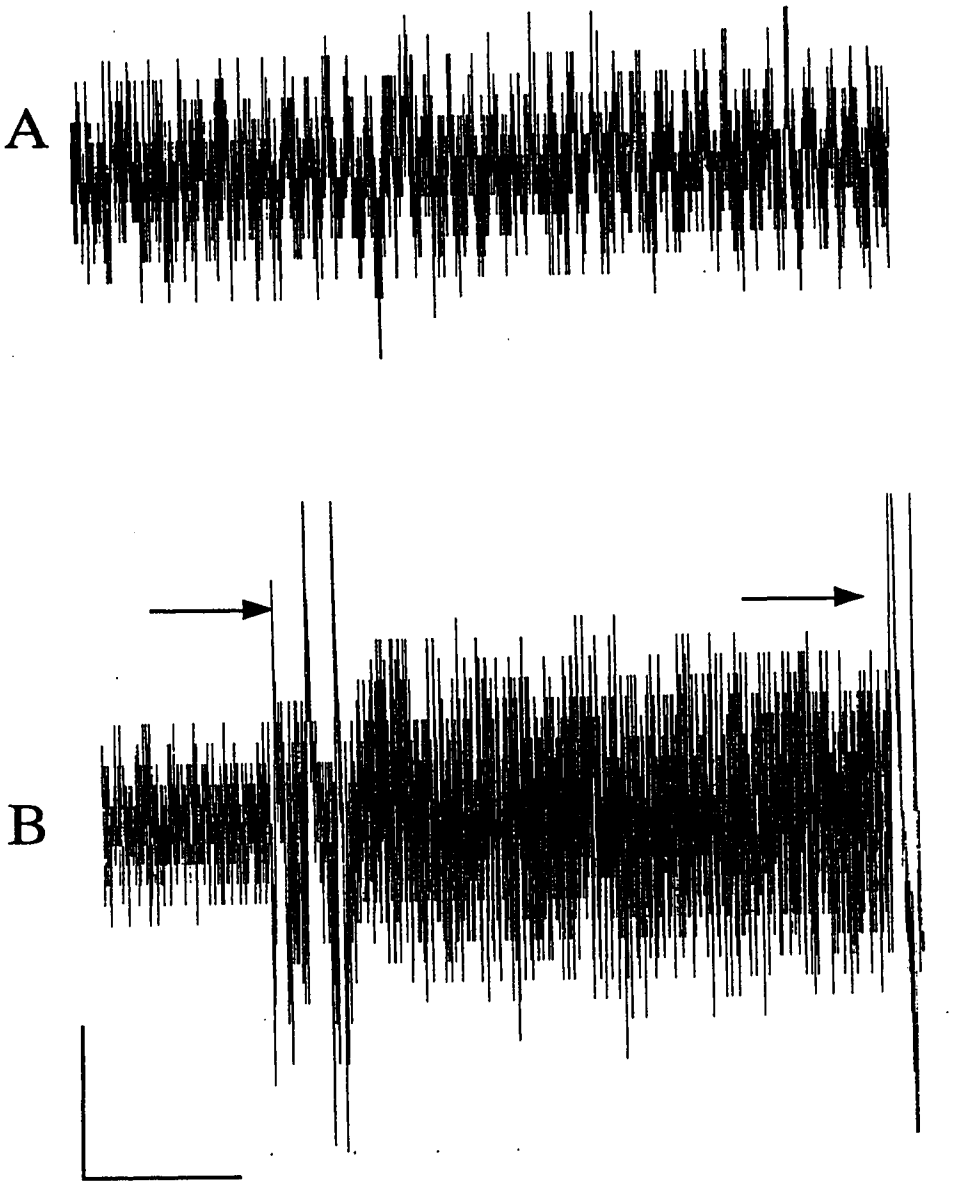

Figure 5. Five seconds of continuous EEG recorded from a PTD rat prior to PTD treatment (A) and during the late stages of PTD (B). The first arrow indicates the onset of a seizure in B. The seizures were characterized by an increase in EEG frequency and amplitude as well as frequent large amplitude spikes (see arrows). This seizure, which had a duration of 24 seconds, was not accompanied by any overt behavioural seizures, and therefore suggests that PTD rats experience subconvulsive seizures. The horizontal bar represents 1 sec and the vertical bar represents 10mV.

minute period there were five of these seizures, each of which had a duration of at least 20 seconds. Only one of these EEG seizures occurred at the same time as tonic-clonic convulsions. During the remaining EEG seizures, the rat was immobile and displayed no outward indications of behavioural seizures. Therefore, this PTD rat has provided preliminary evidence of subconvulsive seizures during the late stages of PTD. There were no subconvulsive seizures observed for this rat at any time subsequent to one hour following treatment reversal. There were no indications of subconvulsive seizures at any time in the one PTD+DZ rat that provided reliable EEG. Rather, this rat's EEG was stable throughout the late stages of PTD.

Behavioural Results

Fixed-platform testing

The rats that provided behavioural data included 18 from group PTD (14 nonimplanted and 4 implanted), 13 from group PTD+DZ (10 nonimplanted and 3 implanted) and 12 from group SAL+DZ. Many of the rats from both group PTD and group PTD+DZ spent the first few trials of the fixed-platform task with their swimming confined to the perimeter of the pool. Most of the rats quickly abandoned this thigmotaxic behaviour and eventually acquired the fixed-platform task. However, 9 nonimplanted rats and 1 implanted rat from group PTD, and 1 nonimplanted and 2 implanted rats from group PTD+DZ consistently displayed this thigmotaxic behaviour on every trial throughout the first five fixed-platform sessions. These rats were removed from the study and did not receive any further behavioural testing. All of the behavioural data that are reported below are therefore based on the 5 nonimplanted and 3 implanted rats from group PTD (n=8), the 9 nonimplanted and 1 implanted rat from group PTD+DZ (n=10) and all of the rats from group SAL+DZ (n=12) that completed behavioural testing. Unless indicated, group PTD+DZ includes rats that were characterized both as IML-lesioned as well as IML-spared.

Figure 6 shows the mean escape latencies for each group over the first three sessions of fixed-platform testing. An analysis of variance (ANOVA) revealed a significant main effect of Group ($F[2,27] = 6.36$, $p < .01$). Post hoc analyses revealed that rats in groups PTD and PTD+DZ had significantly longer latencies than did rats in group SAL+DZ (both $ps < .01$). However, there was no significant difference between the PTD and the PTD+DZ rats ($p > .05$).

DMTP acquisition

The mean second-swim escape latencies for each group over the 10 days of DMTP acquisition are displayed in blocks of two sessions in Figure 7. An ANOVA revealed a significant main effect of Group ($F[2,27] = 12.01$, $p < .01$). Post hoc analyses revealed that rats in group PTD and PTD+DZ had significantly longer latencies than did rats in group SAL+DZ (both $ps < .01$). Moreover, the PTD rats tended to have longer latencies than did the rats in group PTD+DZ ($p = .055$).

The rats were not aware of the location of the hidden platform on the first swim of each trial. As a result, there should be no group differences between the first-swim latencies. To verify this, a factorial ANOVA was conducted using the mean first-swim escape latencies. The analysis revealed a main effect of Group ($F[2,27] = 3.38]$, $p < .05$). Post hoc analyses revealed that overall, the PTD rats had longer first-swim escape latencies than the SAL+DZ rats ($p < .05$). Group PTD+DZ had first-swim escape latencies that were not significantly different from either group PTD or from group SAL+DZ (both $ps > .05$).

The fact that the PTD rats had longer first-swim escape latencies than the SAL+DZ rats makes it difficult to interpret the differences in second-swim escape latencies that were revealed between the three groups of rats. In order to further evaluate the differences in second-swim escape latencies, a savings ratio was computed for every trial of DMTP acquisition for each rat. The savings ratio, which was defined as the total swim time (i.e., first-swim escape latency + second-swim escape latency) divided by the second-swim

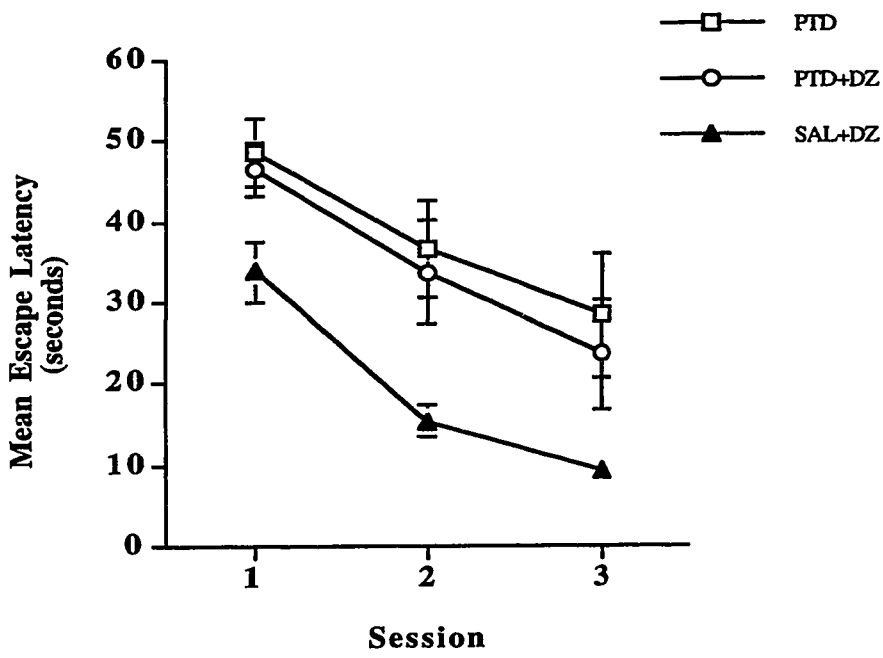

Figure 6. Mean escape latency for the three groups of rats during three sessions of fixed-platform trials. Error bars represent standard errors of means.

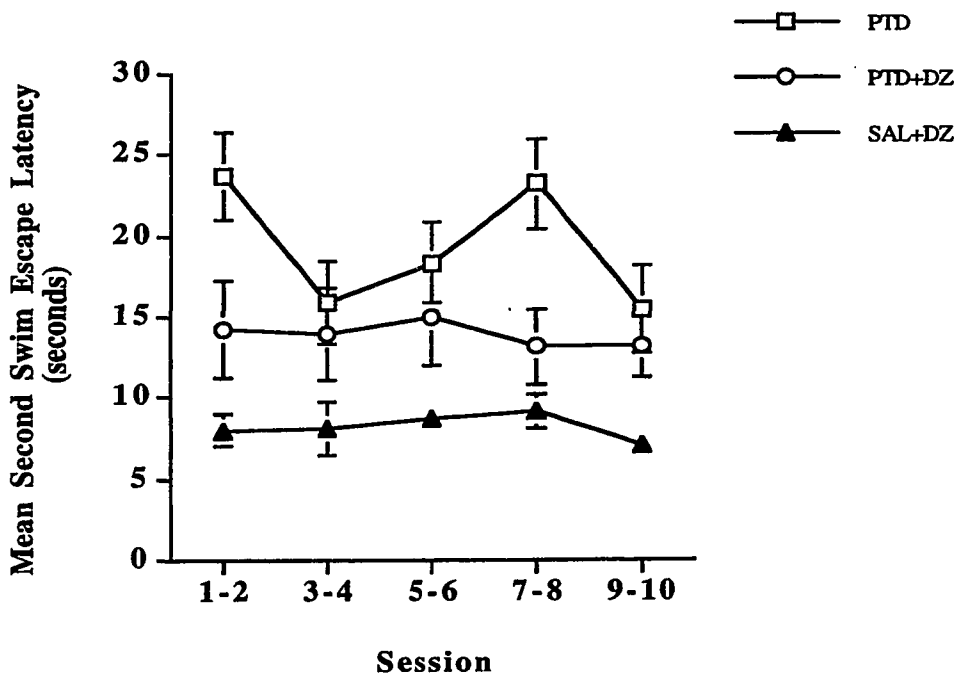

Figure 7. Mean second-swim escape latency during DMTP acquisition. The data are presented in blocks of two sessions each. Error bars represent standard errors of means.

escape latency, reflects the duration of the second-swim escape latency relative to the total swim time. The savings ratio therefore reflects the amount of savings from the first swim to the second swim. A savings ratio of 0.5 would indicate that the second-swim escape latency is half the length of the total swim time (i.e., there is no savings from the first to the second swim). The savings ratio would decrease as the magnitude of the difference in escape latency between the first and second swim increases (i.e., the savings from the first to the second swim are increasing).

The mean savings ratios for the three groups of rats over the 10 days of DMTP acquisition are displayed in blocks of two sessions in Figure 8. An ANOVA revealed a significant main effect of Group ($F[2,27] = 10.22$, $p < .01$). Post hoc analyses revealed that rats in group PTD and PTD+DZ had significantly less savings than did rats in group SAL+DZ (both $ps < .05$). Moreover, the PTD rats tended to have less savings than did the rats in group PTD+DZ ($p = .08$).

In summary, the analyses of the DMTP acquisition data have revealed the following: 1) compared to the rats from group SAL+DZ, both the PTD and the PTD+DZ rats had longer second-swim escape latencies throughout acquisition of DMTP, 2) PTD rats tended to have longer second-swim escape latencies when compared to PTD+DZ rats, 3) the first-swim escape latencies displayed by the rats from group PTD were longer than those displayed by both the PTD+DZ rats and the SAL+DZ rats, 4) compared to the rats from group SAL+DZ, both the PTD and PTD+DZ rats displayed less savings from the first to the second swim and 5) PTD rats tended to display less savings than PTD+DZ rats.

DMTP training at longer retention delays

A mixed-factorial ANOVA was conducted in order to evaluate the performance of the PTD and PTD+DZ rats during DMTP training at longer retention delays. Group served as the between-subjects factor, while Delay served as the within-subjects factor. The data consisted of the mean second-swim escape latencies over the last three sessions of DMTP

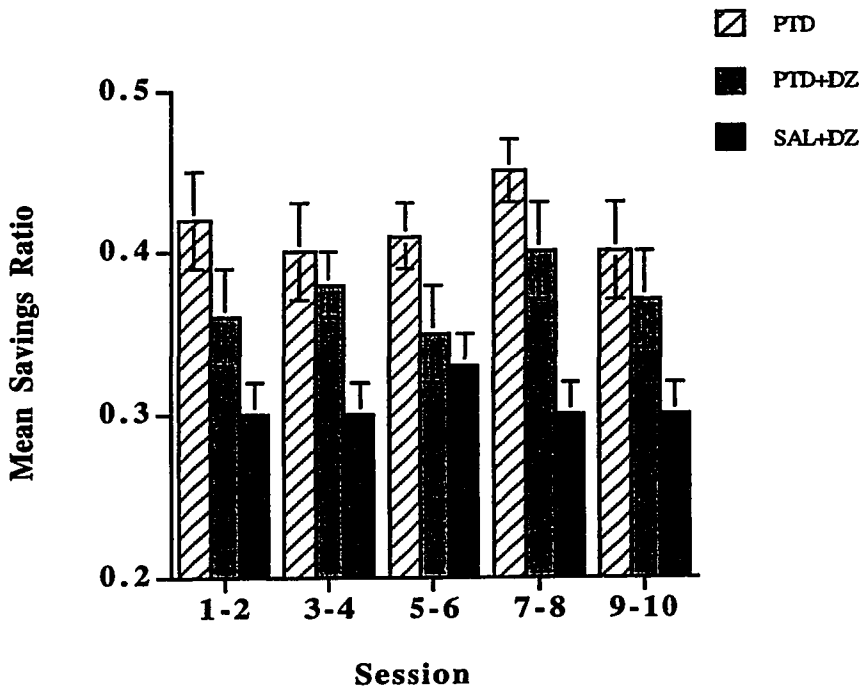

Figure 8. Mean savings ratios during DMTP acquisition. A savings ratio that is closer to 0 indicates increased savings from the first to the second swim. A savings ratio of 0.5 represents little or no savings. Error bars represent standard errors of means.

with the 4 second retention delay and the mean second-swim escape latencies during the three sessions of 30 second retention delay trials and the three sessions of 120 second retention delay trials. The ANOVA revealed a significant effect of Group ($F[2,27] = 13.03$, $p < .01$). Post hoc analyses revealed that overall, both the PTD and the PTD+DZ rats had longer latencies than did the control rats ($ps < .05$). Further, the rats in group PTD had longer latencies than the rats in group PTD+DZ ($p < .01$). There was no significant effect of Delay ($F[2,27] = 1.43$, $p > .05$) and no interaction between Group and Delay ($F[4,27] < 1$).

In summary, compared to the rats from group SAL+DZ, PTD and PTD+DZ rats had longer second-swim escape latencies during DMTP training at longer retention delays. Further, PTD rats had longer second-swim latencies than did the PTD+DZ rats. There was no significant effect of delay.

DMTP with mixed-delay sessions

The mean second-swim escape latencies for each group over the six mixed-delay sessions are presented in Figure 9. A mixed-factorial ANOVA revealed significant main effects of Group ($F[2,27] = 11.02$, $p < .01$) and Delay ($F[2,27] = 4.80$, $p < .05$). However, the interaction between Group and Delay was not significant ($F[4,27] = 1.92$, $p > .05$). Post hoc analyses across the three delay conditions revealed that both the PTD and the PTD+DZ rats required more time to locate the hidden platform (both $ps < .01$) than did the rats in group SAL+DZ. Further, there was a tendency for PTD rats to have longer latencies than PTD+DZ rats ($p = .07$).

Post hoc analyses were conducted within each of the three delay conditions in order to determine whether there were any delay-dependent effects in the mixed-delay sessions. Delay-dependent effects were not observed in the PTD rats. These rats had significantly longer latencies than the control rats at all three retention delays (all $ps < .05$). However, rats in group PTD+DZ were differentially affected by the longer delays. PTD+DZ rats

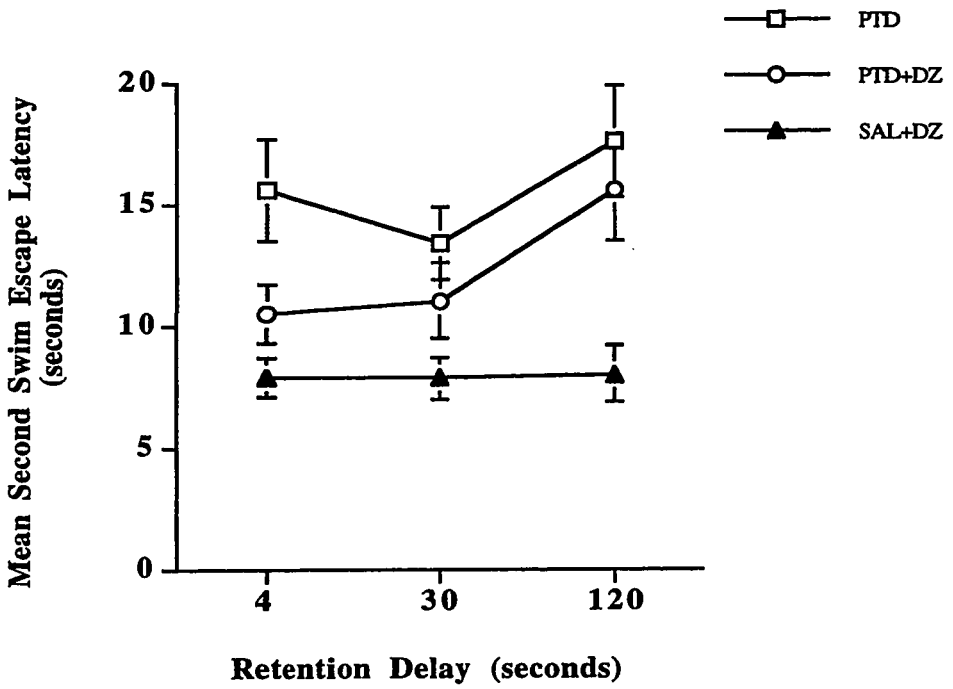

Figure 9. Mean second-swim escape latency during mixed-delay sessions of DMTP. Error bars represent standard errors of means.

required more time than the SAL+DZ rats to locate the hidden platform during the 120 second delay ($p < .01$), but not during either of the shorter delays (both $ps > .05$). Comparisons between the PTD rats and the PTD+DZ rats revealed that the PTD rats had longer latencies than the PTD+DZ rats at the 4 second delay ($p < .05$), but not at either of the longer delays (both $ps > .05$).

In order to determine whether there were group differences in the first-swim latencies, a mixed-factorial ANOVA was conducted. Group was the between-subjects factor and Delay was the within-subjects factor. The analysis revealed a main effect of Group ($F[2,27] = 5.06$], $p < .05$), but neither Delay ($F[2,27] < 1$) nor the interaction between Group and Delay ($F[4,27] < 1$) were significant. Post hoc analyses revealed that overall, the PTD rats had longer first-swim escape latencies than the control rats ($p < .05$). Group PTD+DZ had first-swim escape latencies that were not significantly different from either group PTD or group SAL+DZ (both $ps > .05$).

The savings ratios between the first and second swims during the mixed-delay sessions are presented for the three groups of rats in Figure 10. In order to evaluate the savings between the first and second swims during the mixed-delay sessions, a mixed-factorial ANOVA was conducted in which Group served as the between-subjects factor and Delay served as the within-subjects factor. The ANOVA revealed a significant effect of Group ($F[2,27] = 10.12$, $p < .01$). Post hoc analyses revealed that overall, both the PTD and the PTD+DZ rats had less savings from the first to the second swim than did the SAL+DZ rats (both $ps < .01$). There was no significant difference between the savings ratios of the rats from group PTD and group PTD+DZ ($p > .05$). There was no significant effect of Delay ($F[2,27] = 2.32$, $p > .05$) and no interaction between Group and Delay ($F[4,27] < 1$).

Post hoc analyses were conducted within each of the three delay conditions in order to determine whether the savings ratios were affected in a delay-dependent manner in the

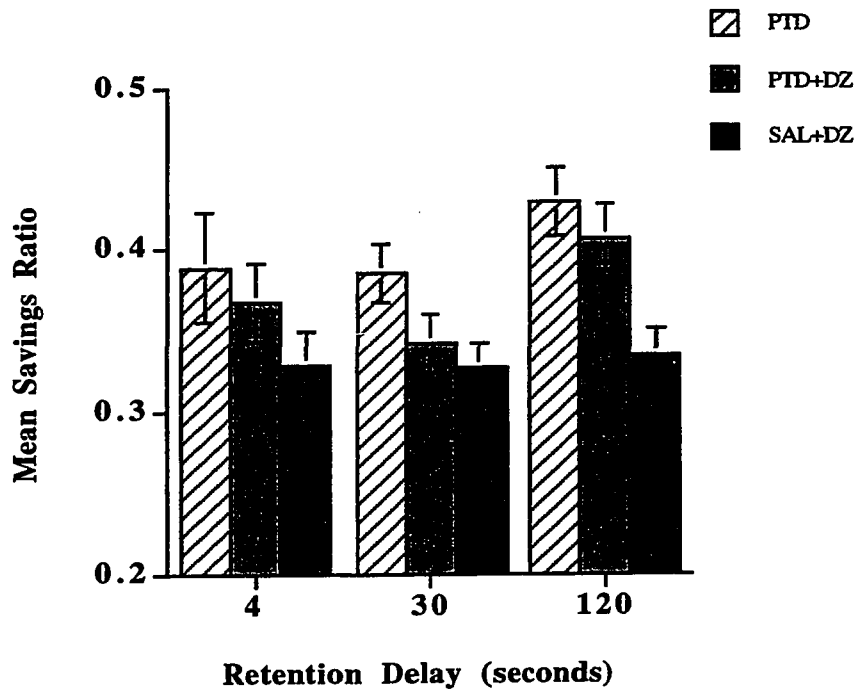

Figure 10. Mean savings ratios during mixed-delay sessions of DMTP. The savings ratio approaches 0 as the savings from the first to the second swim increase. A savings ratio of 0.5 represents little or no savings. Error bars represent standard errors of means.

mixed-delay sessions. Delay-dependent effects were observed in the PTD rats. These rats had significantly less savings than the control rats at the 30 and the 120 second delays (both $ps < .05$), but not at the 4 second delay ($p > .05$). Delay-dependent effects were also observed in group PTD+DZ. There was a significant difference between group PTD+DZ and group SAL+DZ at the 120 second delay ($p < .05$), but not at either of the two shorter delays (both $ps > .05$). There were no significant differences between group PTD and group PTD+DZ at any of the three delays (all $ps > .05$).

Another explanation as to why there were differences between the first-swim escape latencies would be that the rats in groups PTD and PTD+DZ were not swimming as quickly as the rats in group SAL+DZ. In order to verify whether group differences in second-swim escape latencies were influenced by swimming speed, a mixed-factorial ANOVA was conducted using the mean swimming speed during the second swim as the dependent variable. Group was the between-subjects factor and Delay was the within-subjects factor. The analysis revealed that there were no significant differences in swim speed between the groups and that swim speed was not affected by retention delay (both $Fs < 1$; see Figure 11).

Throughout the second swims of mixed-delay sessions, it was observed that the control rats appeared to spend a greater percentage of time swimming in close proximity to the hidden platform than the PTD and PTD+DZ rats. Figure 12 shows the mean second-swim proximity to platform for the three groups across retention delay condition. A mixed-factorial ANOVA using the same variables described for the swim speed analysis revealed a significant effect of Group ($F[2,27] = 11.27$, $p < .01$) and a significant effect of Delay ($F[2,27] = 26.44$, $p < .01$). Moreover, the interaction between Group and Delay was also significant ($F[4,27] = 2.76$, $p < .05$). Post hoc analyses revealed that the rats in group PTD had a greater mean proximity to platform than both the rats in group PTD+DZ and the rats in group SAL+DZ (both $ps < .01$). The rats in group PTD+DZ also tended to have a

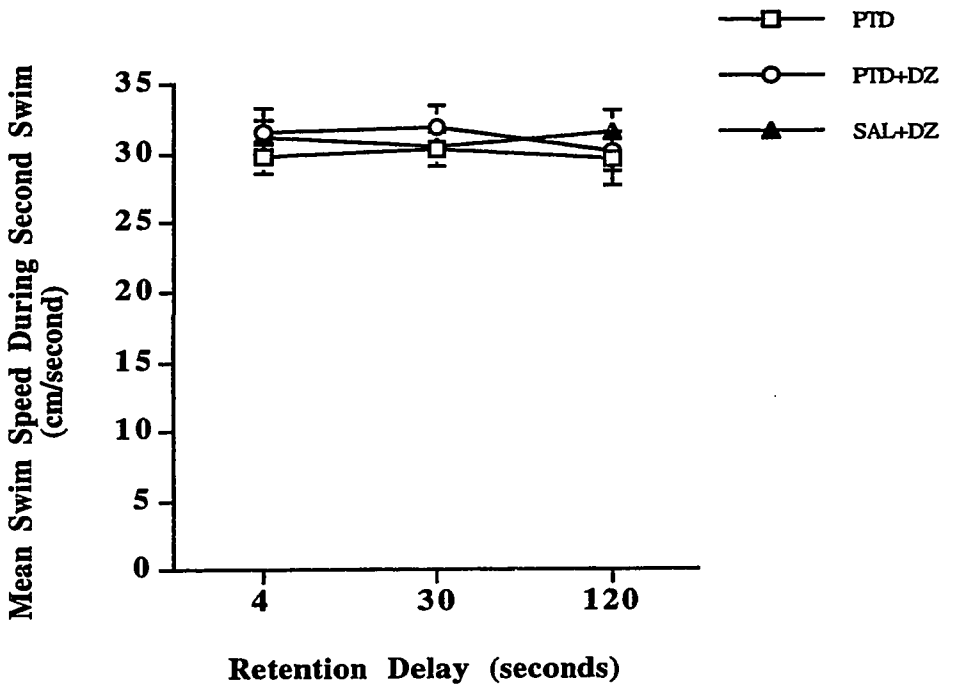

Figure 11. Mean swim speed during the second swim of DMTP mixed-delay sessions. Error bars represent standard errors of means.

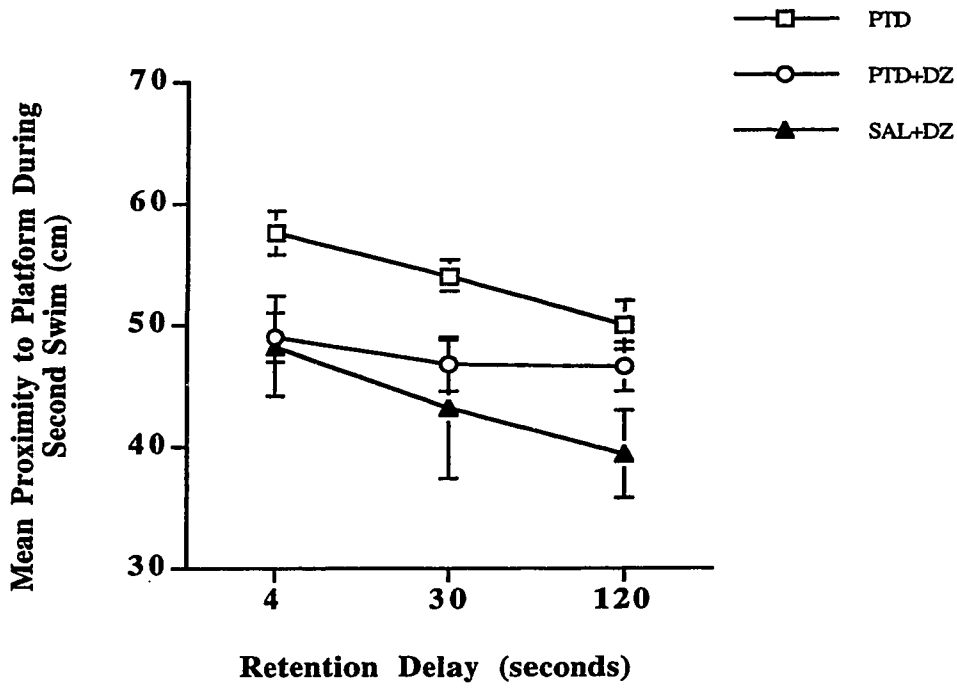

Figure 12. Mean proximity to platform during the second swim of DMTP mixed-delay sessions. Error bars represent standard errors of means.

greater mean proximity to platform than the rats in group SAL+DZ ($p = .06$). Post hoc analyses within each delay condition revealed that the PTD rats always spent more time further away from the hidden platform than did the control rats (all $ps < .01$). However, the mean proximity to platform was affected in a delay-dependent manner for the rats in group PTD+DZ. The mean second-swim proximity to platform for the PTD+DZ rats differed from the SAL+DZ rats at the 120 second delay ($p < .01$), but not at either of the two shorter delays (both $ps > .05$). Comparisons between the two groups of PTD-treated rats revealed that the rats in group PTD+DZ swam in closer proximity to the hidden platform than the PTD rats at the 4 and 30 second delays (both $ps < .05$), but not at the 120 second delay ($p > .05$).

In summary, the analyses of the DMTP mixed-delay sessions have revealed the following: 1) PTD rats had longer second-swim escape latencies than SAL+DZ rats at all three delays, whereas the longer delays exerted a delay-dependent effect in the PTD+DZ rats; compared to group SAL+DZ, PTD+DZ rats had longer second-swim escape latencies at the 120 second delay but not at either of the shorter delays, 2) compared to rats from group PTD+DZ, rats from group PTD had longer second-swim escape latencies at the 4 second delay but not at either of the longer delays, 3) the savings ratios analyses revealed delay-dependent effects in group PTD+DZ and in group PTD; compared to group SAL+DZ, group PTD displayed similar savings at the 4 second delay, but not at either of the longer delays; group PTD+DZ displayed savings that were similar to those displayed by group SAL+DZ at the 4 and 30 second delays, but less savings at the 120 second delay, 4) the analyses of the mean second-swim proximity to platform data revealed delay-dependent effects in group PTD+DZ but not in group PTD; rats in group SAL+DZ swam in closer proximity to the hidden platform than rats in group PTD at all three delays; compared to group PTD+DZ, rats in group SAL+DZ swam in closer proximity to the platform at the 120 second delay, but not at either of the shorter delays, 5) compared to rats from group

SAL+DZ, PTD rats had longer overall first-swim escape latencies and 6) there were no differences in swimming speeds between any two groups of rats.

Visible fixed-platform trials

A factorial ANOVA was conducted in order to determine whether there were any differences in escape latency between the three groups of rats across the four visible fixed-platform trials. The analysis revealed that there were no differences between any two groups of rats (all $ps > .05$).

Visible-platform DMTP trials

Figure 13 displays the mean first and second swim escape latencies for the three groups of rats on the four visible-platform DMTP trials. A mixed-factorial ANOVA using Group as the between-subjects factor and Swim (first versus second) as the within-subjects factor revealed main effects of Group ($F[2,27] = 4.19$, $p < .05$) and Swim ($F[1,27] = 14.04$, $p < .01$). The interaction between Group and Swim was not significant ($F < 1$). Post hoc analyses revealed that PTD rats had longer escape latencies than the rats in group SAL+DZ on both the first and the second swims (both $ps < .05$). There were no significant differences between group PTD+DZ and group SAL+DZ, or between group PTD and group PTD+DZ on either swim (all $ps > .05$).

Figure 14 displays the mean second-swim escape latencies for the three groups of rats on each of the four visible-platform DMTP trials. Factorial ANOVAs and post hoc analyses using Group as the between-subjects factor were conducted at each of the four trials. The analyses revealed that on the first trial, rats in group PTD had significantly longer latencies than did rats in group SAL+DZ ($p < .05$), and on the third trial rats in group PTD+DZ had significantly longer latencies than did rats in group SAL+DZ ($p < .05$). There were no other significant differences between any two groups of rats during any of the four trials (all $ps > .05$).

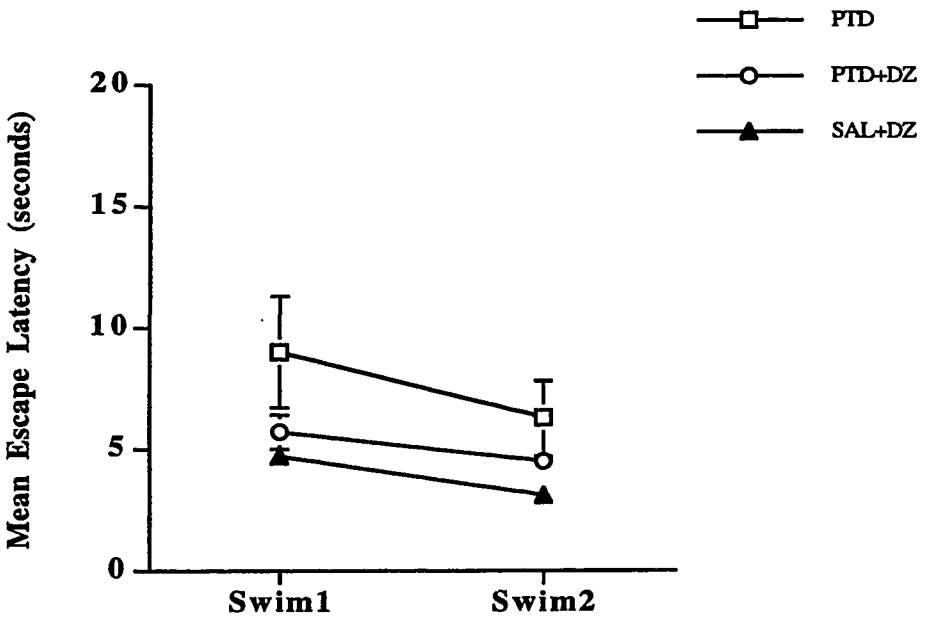

Figure 13. Mean first and second-swim escape latencies across the four visible-platform DMTP trials. Error bars represent standard errors of means.

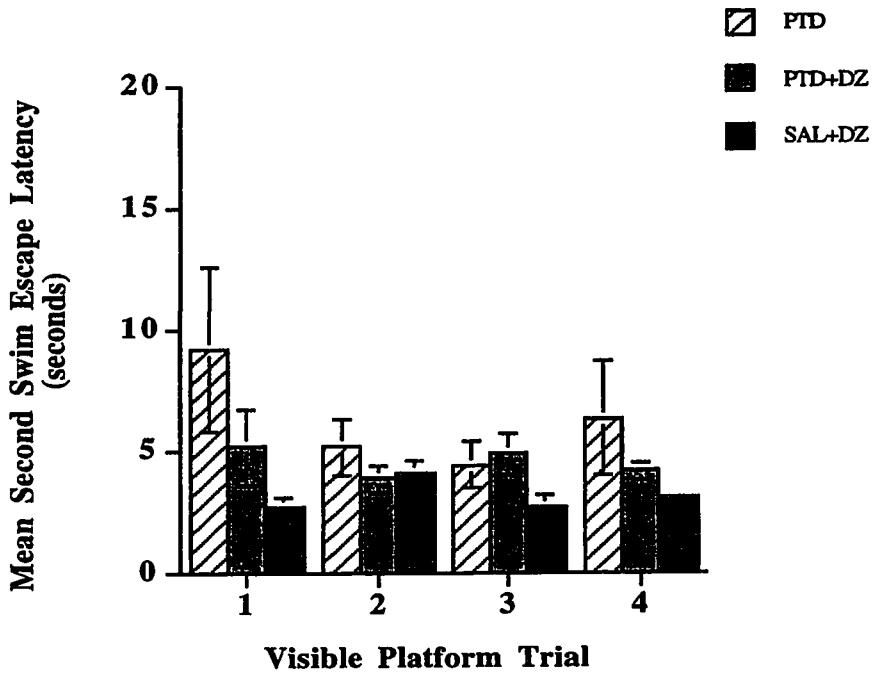

Figure 14. Mean second swim escape latencies during each of the four visible-platform DMTP trials. Error bars represent standard errors of means.

PTD+DZ rats: IML-lesioned versus IML-spared

It was important to determine whether PTD+DZ rats with IML lesions were displaying a more severe memory deficit than the PTD+DZ rats with IML sparing. Therefore, a mixed-factorial ANOVA was conducted on the second-swim escape latencies during mixed-delay sessions in which the data from PTD+DZ rats with IML lesions and PTD+DZ rats with sparing of the IML were analyzed separately. Both groups of PTD+DZ rats were compared to the rats from group PTD (all of which were classified as IML-lesioned) and to the rats from group SAL+DZ. Group served as the between-subjects factor and Delay as the within-subjects factor.

Figure 15 shows the second-swim escape latencies across the three delay conditions with the PTD+DZ rats divided into those with IML lesions and those with IML sparing. The mixed-factorial ANOVA revealed significant main effects of Group ($F[3,26] = 7.78$, $p < .01$) and Delay ($F[2,26] = 5.07$, $p < .01$). Further, the interaction between Group and Delay approached significance ($F[6,26] = 2.18$, $p = .058$). Post hoc analyses across the three delay conditions revealed that the PTD rats required more time to locate the hidden platform relative to the PTD+DZ rats with sparing of the IML ($p < .05$). Further, PTD+DZ rats with IML lesions had longer latencies than the control rats ($p < .01$). There was no significant difference between PTD rats and PTD+DZ rats with IML lesions ($p > .05$), nor between the PTD+DZ rats with sparing of the IML and control rats ($p > .05$).

Post hoc analyses within each of the three delays revealed that there were no significant differences between PTD+DZ rats with IML lesions and PTD+DZ rats with sparing of the IML (all $ps > .05$). However, while the PTD+DZ rats with IML sparing did not significantly differ from the control rats at any of the three delays (all $ps > .05$), PTD+DZ rats with IML lesions had longer escape latencies than control rats at both the 30 and the 120 second delays (both $ps > .05$). Compared to the rats from group PTD, the PTD+DZ rats with IML lesions had shorter escape latencies at the 4 second delay ($p < .05$),

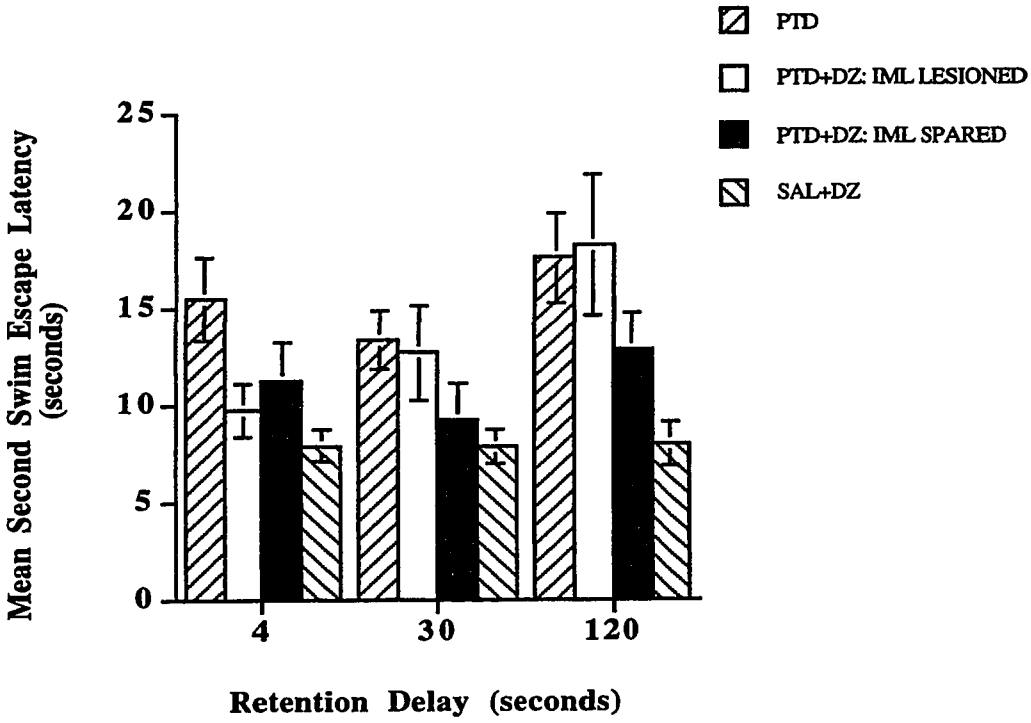

Figure 15. Mean second-swim escape latencies during mixed-delay sessions of DMTP with group PTD+DZ separated into rats with IML lesions and IML sparing. Error bars represent standard errors of means.

but not at either of the longer delays (both ps > .05). PTD+DZ rats with IML sparing tended to have shorter escape latencies than the PTD rats at the 4 second delay ($p = .07$) as well as at the 30 second delay ($p = .08$). There was no significant difference between the rats from group PTD and the PTD+DZ rats with IML sparing at the 120 second delay ($p > .05$).

In summary, the analyses of the IML-lesioned versus IML-spared PTD+DZ rats have revealed the following: 1) there were no significant differences between the PTD+DZ rats with IML sparing and the control rats at any of the delays, while PTD+DZ rats with IML lesions had significantly longer escape latencies than control rats at both the 30 and the 120 second delays and 2) compared to the rats from group PTD, PTD+DZ rats with IML sparing had shorter latencies at both the 4 and the 30 second delays, while PTD+DZ rats with IML lesions had shorter escape latencies than the rats from group PTD only at the 4 second delay. In other words, PTD+DZ rats with IML lesions were more similar to PTD rats and those with IML sparing were more similar to controls.

Seizure duration

Finally, the relationship between the total duration of observed seizures and second-swim escape latencies for the rats in group PTD was determined. The mean second-swim escape latencies during mixed-delay sessions were not significantly correlated with the total duration of observed seizures in the rats from group PTD. The correlation between mean second-swim escape latency and total duration of observed seizures was 0.36 at the 4 sec delay, -0.13 at the 30 second delay and -0.10 at the 120 second delay. None of these correlations were significant (critical r = 0.66).

DISCUSSION

The results presented in this study provide evidence of impaired allocentric spatial working-memory in recovered PTD rats. Further, the present data demonstrate that the

administration of diazepam during the late stages of PTD attenuates subsequent neuropathology and spatial memory deficits.

The longer second-swim escape latencies displayed by PTD rats indicate that they were impaired throughout acquisition of the DMTP task, as well as at all of the delays during mixed-delay sessions (i.e., 4, 30, 120 seconds). These findings are consistent with previous evidence of PTD-induced spatial memory deficits (e.g., Mair et al., 1988; Langlais & Savage, 1995). However, Mumby et al. (1997) reported that PTD rats were impaired on DMTP at a delay of 300 seconds, but performed normally at delays of 4 and 60 seconds. Procedural differences between the present study and the study by Mumby et al. most likely account for this inconsistency. The rats in the Mumby et al. study acquired the DMTP task prior to undergoing PTD, while none of the rats in the present study received any training prior to PTD. Further, PTD treatment was terminated for the rats in the Mumby et al. study upon the first observation of seizures. PTD treatment was not reversed in the present study until three hours following the first observation of seizures. PTD rats that are reversed earlier generally exhibit less severe memory impairments (Langlais & Zhang, 1997).

The present findings are also consistent with evidence of spatial memory impairments in KA. For example, Oscar-Berman et al. (1982) demonstrated that KA patients are impaired on spatial delayed response and delayed alternation tasks. Moreover, KA patients are also impaired on a task that assesses their ability to retain the spatial location of words on a computer monitor (Mayes, 1991).

The finding that PTD rats were impaired during the fixed-platform sessions that they received prior to DMTP is consistent with previous evidence of fixed-platform impairments in PTD rats (Langlais et al., 1992). The fact that PTD rats were impaired on both DMTP and fixed-platform sessions indicates that PTD produces allocentric spatial memory deficits on tasks that require both working and reference-memory.

In the present study, the rats were not aware of the location of the hidden platform on the first swim of each trial of DMTP. As a result, there should be no significant differences between PTD rats and control rats on the first swim. However, longer first-swim escape latencies were displayed by PTD rats. This makes it difficult to interpret the differences that were found between control rats and PTD rats on the second swim, and suggests a non-mnemonic impairment in the PTD rats. Moreover, the fact that compared to controls, PTD rats displayed longer escape latencies on both swims during DMTP trials in which the platform was made visible also indicates a non-mnemonic impairment.

However, there are several findings in the present study that suggest that PTD rats were indeed experiencing a memory impairment. First, longer escape latencies were not caused by differences in swimming speed. Second, evidence of a mnemonic impairment in PTD rats stems from the delay-dependency of the savings ratio effects. That is, compared to control rats, PTD rats displayed less savings at delays of 30 and 120 seconds, but equivalent savings at the 4 second delay. A third reason to suggest that PTD rats were experiencing a memory impairment involves the rat's proximity to the hidden platform during the second swim. Compared to PTD rats, control rats swam in closer proximity to the hidden platform during all three delays.

The differences that were found between PTD rats and control rats on the visible-platform DMTP trials are more difficult to interpret. It must be noted, though, that there were no differences in escape latencies between the groups on fixed-platform trials when the platform was made visible. Moreover, post hoc analyses revealed that PTD rats displayed longer second-swim escape latencies on only one of the four visible-platform DMTP trials.

The present findings suggest a PTD-induced allocentric spatial memory impairment. However, non-mnemonic impairments may have influenced the data to some extent. One possible non-mnemonic explanation that can account for the longer first-swim latencies in

PTD rats, as well as longer visible-platform latencies, involves the rats' initial reaction to being placed into the pool. PTD rats often confined their swimming to the perimeter of the pool for the first few seconds of a swim. This thigmotaxic behaviour was rarely observed in control rats.

The second main finding of this experiment was that the neuropathological and behavioural outcome of PTD were attenuated by a treatment that reduced seizure activity. This is consistent with the idea that some of the neuropathology and memory impairments that are observed in recovered PTD rats are a result of a high incidence of seizures, and not the pathogenic effects of thiamine deficiency *per se*. Moreover, although KA patients are vulnerable to seizures during alcohol withdrawal, they do not experience repeated generalized convulsions like those that are observed during the late stages of PTD. Therefore, the present results suggest that the high incidence of seizures during the late stages of PTD present a problem for this model.

The administration of diazepam during the late stages of PTD had three main effects: First, rats from group PTD+DZ were far less likely to display observable seizures. In fact, no seizures were observed in any of the PTD+DZ rats at any time subsequent to one hour after the first injection of diazepam. Further, there were no indications of subconvulsive seizures in the one PTD+DZ rat that provided reliable EEG data.

Second, compared to PTD rats, PTD+DZ rats sustained less thalamic pathology. This was true regardless of whether the PTD+DZ rats were characterized as IML-lesioned or IML-spared. Compared to group PTD, PTD+DZ rats with IML lesions sustained less pathology in lateral thalamic nuclei. However, diazepam did not eliminate thalamic pathology, as there was evidence of moderate gliosis in the CM, MD, and Po thalamic nuclei in PTD+DZ rats with IML sparing. PTD+DZ rats were also far more likely to recover from the treatment, presumably because of differences in PTD-induced neuropathology.

The third effect of diazepam administration was that PTD-induced DMTP deficits were attenuated. Compared to group PTD, group PTD+DZ had shorter escape latencies and displayed more savings during acquisition of DMTP. Moreover, during mixed-delay sessions, PTD+DZ rats had shorter second-swim escape latencies at the 4 second delay, and spent more time in closer proximity to the hidden platform during the 4 and the 30 second delays. However, although group PTD+DZ displayed less of a DMTP deficit than group PTD, they were still impaired relative to controls. The memory deficit displayed by group PTD and by group PTD+DZ was similar during the fixed-platform trials; both groups of rats were equally impaired relative to controls. Further, unlike group PTD, there were delay-dependent effects for group PTD+DZ during the mixed-delay sessions. Compared to controls, PTD+DZ rats had longer second-swim escape latencies at the longest delay, but not at either of the two shorter delays. It is unlikely that these differences can be attributed to non-mnemonic impairments for the PTD+DZ rats, as there was no difference in first-swim escape latencies between controls and PTD+DZ rats. Moreover, PTD+DZ rats displayed longer second-swim escape latencies on only one of the four visible-platform DMTP trials.

The present results are consistent with evidence that damage to the IML and associated intralaminar thalamic nuclei produces PTD-induced spatial memory impairments (e.g., Langlais et al., 1992). In the present study, PTD-induced memory impairments were attenuated in PTD+DZ rats with sparing of the IML. There were no significant differences in second-swim escape latencies between the PTD+DZ rats with IML sparing and the control rats at any of the three delays. Conversely, PTD+DZ rats with IML lesions had significantly longer escape latencies than control rats at both the 30 and the 120 second delays. Compared to group PTD, PTD+DZ rats with IML sparing had shorter latencies at both the 4 and the 30 second delays, while PTD+DZ rats with IML lesions had shorter escape latencies only at the 4 second delay. The present results therefore suggest that the

attenuation of PTD-induced memory deficits displayed by group PTD+DZ resulted from sparing of the IML and associated intralaminar thalamic nuclei in half of the PTD+DZ rats. The IML is part of the limbic-diencephalic fiber pathway, which projects from the amygdala to the thalamus. The fact that this pathway is thought to be involved in selective attention and the enhancement of cortical processing (Langlais, 1992) might explain why PTD rats with IML lesions are more impaired relative to PTD rats with IML sparing.

Group PTD and group PTD+DZ were exposed to an equivalent duration of thiamine deficiency. However, group PTD+DZ, but not group PTD, received diazepam during the late stages of PTD. Therefore, the present findings clearly indicate that the administration of diazepam during the late stages of PTD attenuates seizure susceptibility as well as PTD-induced neuropathology, and DMTP deficits. This correlation between the attenuation of seizures and the attenuation of neuropathology and behavioural deficits in PTD rats is consistent with the hypothesis that PTD-induced neuropathology and memory deficits normally observed in studies in which treatment reversal comes hours after the onset of seizures are a result of the primary pathogenic effects of thiamine deficiency and the secondary pathogenic effects of seizures.

However, the finding that the total duration of observed seizures was not significantly correlated with second-swim escape latencies during mixed-delay DMTP trials for group PTD makes it difficult to interpret the pathogenic effects of seizures in PTD. Seizures were quantified in PTD rats by observing them for a period of ten minutes per hour. It is possible that more frequent or longer periods of observation would have produced a more accurate picture of the incidence of seizures during the late stages of PTD. Moreover, in the present study there were only eight PTD rats that provided behavioural data. It is possible that a larger sample size may have revealed a correlation between seizure duration and second-swim escape latencies. Further, it is unfortunate that the EEG data did not prove to be more reliable. Nevertheless, this study has provided preliminary evidence

that PTD rats experience subconvulsive seizures during the late stages of PTD. Further studies will be needed to provide a better characterization of subconvulsive seizures during PTD in order to determine whether subconvulsive seizures contribute to the neuropathological and cognitive outcome of experimental thiamine deficiency.

The precise mechanism of the pathogenesis of cell death during acute thiamine deficiency is not known. However, the glutamate excitotoxicity model of PTD-induced neuropathology proposes that thiamine deficiency causes diencephalic neuropathology through a reduction in cerebral energy metabolism that results in defective repolarization following glutamatergic input. This results in an excessive influx of Na^+ and Ca^{++}, which triggers neuronal edema and cell death (Langlais, 1995). Moreover, this process is exacerbated by a sharp increase in the levels of thalamic extracellular glutamate during the late stages of PTD (Langlais & Zhang, 1993).

Future research is needed to determine the mechanism by which diazepam exerted its protective effects in the present study. One possibility is that diazepam inhibits the pathogenic effects of thiamine deficiency by inhibiting the effects of glutamate excitotoxicity. Diazepam acts as an anticonvulsant by potentiating the effects of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). GABA acts as an inhibitory neurotransmitter because it opens Cl^- channels, thus increasing the influx of Cl^- into the cell (Snyder, 1986). This makes it more difficult to fire the cell due to hyperpolarization of the cell membrane. Diazepam potentiates the inhibitory effects of GABA by permitting lower concentrations of GABA to open Cl^- channels (Alberts et al., 1992), thus making it harder for the excitatory neurotransmitter glutamate to fire the cell. Glutamatergic and gabaergic neurons are both present in the thalamus (Coté & Crutcher, 1991), therefore, it is possible that in the present study, diazepam attenuated the pathogenic effects of thiamine deficiency by inhibiting the effects of glutamate excitotoxicity.

One of the purposes of the present study was to assess the idea that there are primary and secondary pathogenic events during PTD. This hypothesis suggests that the primary consequence of thiamine deficiency is thalamic pathology, which is caused by the pathogenic process that is outlined above. Moreover, this hypothesis suggests that seizures, which are a direct result of the primary consequences of thiamine deficiency, cause secondary neuropathology. The precise mechanism by which PTD produces behavioural seizures is not known. Further, assuming that seizures do indeed cause secondary neuropathology, it is not clear how they might do so. However, there are reciprocal projections between the motor cortex and the thalamus (Coté & Crutcher, 1991). Therefore, it is possible that glutamate excitotoxicity in the thalamus causes behavioural seizures as a result of thalamic input into the motor cortex. Similarly, behavioural seizures may cause secondary thalamic neuropathology as a result of input from the motor cortex to the thalamus. This idea is supported by the fact that the ventral posterolateral thalamic nucleus, which receives glutamatergic input from cortex, has been shown to degenerate in response to cortical seizure activity (Collins & Olney, 1982). Moreover, there is a direct projection from the motor cortex to the central medial thalamic nucleus (Coté & Crutcher, 1991), a structure that in the present study, was particularly vulnerable to the pathogenic effects of thiamine deficiency.

Glutamate-induced excitotoxicity is also implicated in seizure-induced neuropathology (Lynch et al., 1996). Therefore, it is possible that diazepam attenuated the outcome of PTD by inhibiting the pathogenic effects of seizures as well as the pathogenic effects of thiamine deficiency. As a result, the findings from the present study do not permit the secondary pathogenic effects of seizures to be unequivocally dissociated from the primary pathogenic effects of thiamine deficiency. That is, it is not clear whether PTD+DZ rats were protected by diazepam because it protected against the secondary

pathogenic effects of seizures, or rather, because diazepam attenuated the primary pathogenic effects of thiamine deficiency.

As stated above, glutamate excitotoxicity is implicated in the pathogenic processes of both acute thiamine deficiency, as well as seizures. Therefore, it is conceivable that there is an interaction between the pathogenic effects of thiamine deficiency and the pathogenic effects of seizures. However, it is possible that seizures are an indication of severe thalamic pathology, rather than a causal event. That is, it is possible that seizures are observed in PTD rats once sufficient thalamic neuropathology has occurred, and that the seizures in and of themselves do not cause additional neuropathology.

Nevertheless, the present findings indicate that PTD rats that have experienced seizures are more likely to sustain gross thalamic pathology and more severe memory deficits than are PTD rats that have experienced an equivalent duration of thiamine deficiency but little or no exposure to seizures. This is consistent with the idea that there is an interaction between these two pathogenic factors.

In humans, diazepam is typically used as an anti-anxiety agent (Snyder, 1986). Therefore, it is possible that in addition to its direct anticonvulsant effects, diazepam attenuated the neuropathological and behavioural outcome of PTD in the present study by decreasing seizure susceptibility through a reduction in anxiety. A similar argument that could be put forth is that the administration of diazepam to the PTD+DZ rats reduced their level of anxiety during behavioural testing. However, this possibility is unlikely because the PTD+DZ rats received diazepam two weeks prior to the commencement of behavioural testing.

In conclusion, the findings that are reported in this thesis demonstrate an impairment in spatial allocentric working-memory in recovered PTD rats that are not pretrained prior to treatment. The functions of the IML are implicated as a critical requirement for normal performance on memory tasks that assess this type of memory.

Moreover, the present study suggests that the occurrence of seizures during the late stages of PTD contributes to the neuropathological and behavioural outcome of the treatment. Further studies are needed to determine the precise mechanism by which diazepam exerts its protective effects in the PTD model. Nevertheless, the present study demonstrates that PTD-induced neuropathology and memory deficits can be attenuated by a treatment that reduces seizure activity.

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APPENDIX A

Analysis of Variance Source Tables

Table 1

Analysis of Variance for the Mean Escape Latencies During the Three Sessions of Fixed-Platform Testing

Source	SS	df	MS	F	P
Between Subjects					
Group	6007.09	2	3003.54	6.36	.00
Error	12742.30	27	471.93		
Within Groups					
Day of Testing	7993.69	2	3996.84	44.98	.00
Group X Day	145.66	4	36.41	0.41	.80
Error	4797.43	54	88.84		

Table 2

Analysis of Variance for the Mean Second-Swim Escape Latencies During DMTP Acquisition

Source	SS	df	MS	F	P
Between Subjects					
Group	6131.45	2	3065.72	12.00	.00
Error	6893.07	27	255.29		
Within Groups					
Day of Testing	831.28	9	92.36	2.25	.01
Group X Day	1059.73	18	58.87	1.43	.11
Error	9976.94	243	41.05		

Table 3

Analysis of Variance for the Mean First-Swim Escape Latencies During DMTP Acquisition

Source	SS	df	MS	F	P
Between Subjects					
Group	1134.61	2	567.30	3.38	.04
Error	4525.42	27	167.60		
Within Groups					
Day of Testing	2518.57	9	279.84	3.75	.00
Group X Day	1511.83	18	83.99	1.12	.32
Error	18130.47	243	74.61		

Table 4

Analysis of Variance for the Mean Savings Ratios During DMTP Acquisition

Source	SS	df	MS	F	P
Between Subjects					
Group	0.60	2	0.30	10.22	.00
Error	0.79	27	0.02		
Within Groups					
Day of Testing	0.10	9	0.01	1.32	.22
Group X Day	0.14	18	0.00	0.87	.60
Error	2.15	243	0.00		

Table 5

Analysis of Variance for the Mean Second-Swim Escape Latencies During DMTP Training at Longer Retention Delays.

Source	SS	df	MS	F	P
Between Subjects					
Group	1570.61	2	785.30	13.03	.00
Error	1626.70	27	60.24		
Within Groups					
Delay	27.84	2	13.92	1.43	.24
Group X Delay	32.79	4	8.19	0.84	.50
Error	523.15	54	9.68		

Table 6

Analysis of Variance for the Mean Second-Swim Escape Latencies During Mixed-Delay Sessions of DMTP.

Source	SS	df	MS	F	P
Between Subjects					
Group	865.92	2	432.96	11.02	.00
Error	1060.24	27	39.26		
Within Groups					
Delay	127.39	2	63.69	4.80	.01
Group X Delay	101.87	4	25.47	1.92	.11
Error	715.20	54	13.24		

Table 7

Analysis of Variance for the Mean First-Swim Escape Latencies During Mixed-Delay Sessions of DMTP.

Source	SS	df	MS	F	P
Between Subjects					
Group	500.30	2	250.15	5.06	.01
Error	1334.28	27	49.41		
Within Groups					
Delay	30.91	2	15.45	0.62	.53
Group X Delay	81.57	4	20.39	0.82	.51
Error	1332.90	54	24.68		

Table 8

Analysis of Variance for the Mean Savings Ratios During Mixed-Delay Sessions of DMTP.

Source	SS	df	MS	F	P
Between Subjects					
Group	0.07	2	0.03	10.12	.00
Error	0.10	27	0.00		
Within Groups					
Delay	0.02	2	0.01	2.32	.10
Group X Delay	0.01	4	0.00	0.54	.70
Error	0.24	54	0.00		

Table 9

Analysis of Variance for the Mean Swim Speed During the Second-Swim of Mixed-Delay Sessions of DMTP.

Source	SS	df	MS	F	P
Between Subjects					
Group	29.47	2	14.73	0.26	.76
Error	1477.30	27	54.71		
Within Groups					
Delay	2.46	2	1.23	0.32	.72
Group X Delay	22.08	4	5.52	1.44	.23
Error	206.94	54	3.83		

Table 10

Analysis of Variance for the Mean Proximity to Platform During the Second-Swim of Mixed-Delay Sessions of DMTP.

Source	SS	df	MS	F	P
Between Subjects					
Group	1510.26	2	755.13	11.27	.00
Error	1808.19	27	66.97		
Within Groups					
Delay	608.69	2	304.34	26.44	.00
Group X Delay	127.19	4	31.79	2.76	.03
Error	621.45	54	11.50		

Table 11

Analysis of Variance for the Mean Escape Latencies During the Visible Fixed-Platform Trials.

Source	SS	df	MS	F	P
Between Subjects					
Group	0.17	2	0.08	0.00	.99
Error	2105.64	27	77.98		

Table 12

Analysis of Variance for the Mean First and Second-Swim Escape Latencies During the Visible-Platform Session of DMTP.

Source	SS	df	MS	F	P
Between Subjects					
Group	133.98	2	66.99	4.19	.02
Error	431.37	27	15.97		
Within Groups					
Swim	46.23	1	46.23	14.04	.00
Group X Swim	5.66	2	2.83	0.86	.43
Error	88.89	27	3.29		

Table 13

Analysis of Variance for the Mean Second-Swim Escape Latencies During the Four Visible-Platform DMTP Trials.

Source	SS	df	MS	F	P
Between Subjects					
Group	188.30	2	94.15	3.99	.03
Error	636.09	27	23.55		
Within Groups					
Trial	29.28	3	9.76	0.94	.42
Group X Trial	103.43	6	17.24	1.66	.14
Error	840.64	81	10.37		

Table 14

Analysis of Variance for the Mean Second-Swim Escape Latencies During Mixed-Delay Sessions of DMTP With Group PTD+DZ Separated into Rats With IML Lesions and IML Sparing.

Source	SS	df	MS	F	P
Between Subjects					
Group	911.20	3	303.73	7.78	.00
Error	1014.96	26	39.03		
Within Groups					
Delay	127.39	2	63.69	5.07	.00
Group X Delay	164.67	6	27.44	2.18	.05
Error	652.41	52	12.54		