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Blunted Hippocampal, but not Striatal, Acetylcholine Efflux Parallels Learning Impairment in Diencephalic-Lesioned Rats

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Abstract

A rodent model of diencephalic amnesia, pyriethamine-induced thiamine deficiency (PTD), was used to investigate the dynamic role of hippocampal and striatal acetylcholine (ACh) efflux across acquisition of a nonmatching-to-position (NMTP) T-maze task. Changes in ACh efflux were measured in rats at different time points in the acquisition curve of the task (early= day 1, middle = day 5, and late = day 10). Overall, the control group had higher accuracy scores than the PTD group in the latter sessions of NMTP training. During the three microdialysis sampling points, all animals displayed significant increases in ACh efflux in both hippocampus and striatum, while performing the task. However, on day 10, the PTD group showed a significant behavioral impairment that paralleled their blunted hippocampal—but not striatal—ACh efflux during maze training. The results support selective diencephalic–hippocampal dysfunction in the PTD model. This diencephalic–hippocampal interaction appears to be critical for successful episodic and spatial learning/memory.

Keywords

Hippocampus; Striatum; Microdialysis; Acetylcholine; Learning; Rat

1. Introduction

In both the human disease of Wernicke–Korsakoff's syndrome (WKS) and the animal model of this disorder [pyriethamine-induced thiamine deficiency (PTD)] the long-term cognitive impairment is associated with damage to the midline thalamus and mammillary bodies caused by thiamine deficiency (Kopelman, 1995;Langlais & Savage, 1995;Mair, 1994;Victor, Adams, & Collins, 1971). However, the cognitive impairment and sparing of function observed in WKS is related to the hippocampal-dependent nature of the assessment task. WKS patients display the classical dissociation between explicit and implicit memory systems: explicit memory is impaired, whereas implicit memory remains intact (Kopelman, 1995;Squire, 1981). Memory and learning deficits associated with PTD have also been related to hippocampal-dependent spatial tasks (Langlais, Mandel, & Mair, 1992;Langlais & Savage, 1995;Mair, Anderson, Langlais, & McEntee, 1985;Mair, Knoth, Rabchenuk, & Langlais, 1991a;Mair, Langlais, Otto, Knoth, & Rabchenuk, 1991b;Savage & Langlais, 1995;Savage, Pitkin, & Knitowski, 1999).

Despite the lack of lesions or cell loss to the hippocampus in WKS (Reed et al., 2003) or PTD (Langlais, Zhang, & Savage, 1996), there is evidence of global limbic system dysfunction in

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this disorder and the animal model. Degeneration is reported in key limbic system fiber tracts such as the mammillothalamic tract and fornix in WKS and PTD (Langlais & Zhang, 1997; Sullivan & Marsh, 2003). Thus, many components of the “extended hippocampal system” are affected in WKS and after PTD treatment. From a clinical standpoint, it is debated whether there is a functional distinction between diencephalic and temporal lobe amnesia (Aggleton & Brown, 1999; Caulo et al., 2005). This is supported by human imaging studies as well as electrophysiological, gene expression, and neurochemical data from animal models that suggest that diencephalic damage has a profound effect on hippocampal functioning (Jenkins, Dias, Amin, Brown, & Aggleton, 2002; Reed et al., 2003; Savage, Chang, & Gold, 2003; Vertes, Hoover, & Viana Di Prisco, 2004).

The animal models of WKS have demonstrated that there are also cholinergic abnormalities associated with thiamine deficiency (TD) that may play a key role in the protracted amnesic syndrome. Rats exposed to TD show a 30% loss of choline acetyltransferase (ChAT) positive neurons in the basal forebrain (Pitkin & Savage, 2001, 2004) and reduced fluorescence intensity of ChAT Wbers in the cortex, hippocampus, and thalamus (Nakagawasai et al., 2000). In addition, in PTD-treated rats there is also reduced hippocampal ACh efflux during spontaneous alternation (Savage et al., 2003). Furthermore, chronic physostigmine administration in rats exposed to TD has been shown to reverse the impairment on an avoidance-learning task if started at an early phase of treatment (Nakagawasai et al., 2000). A limited number of case studies have revealed that WKS patients show improved cognitive abilities when given acetylcholinesterase inhibitors (Angunawela & Barker, 2001; Cochrane, Cochrane, Jauhar, & Ashton, 2005). These neurochemical as well as pharmacological data point towards the cholinergic system being involved in the learning and memory problems associated with thiamine deficiency and more generally diencephalic amnesia.

Acetylcholine (ACh) efflux has been shown to increase in a number of memory related structures during behavioral testing—dependent on the demands of the task (Gold, 2004). There is a dramatic rise in hippocampal ACh while rats are transversing a T-maze or a radial arm maze—that in some instances is related to the degree of learning (Fadda, Cocco, & Stancampiano, 2000; Fadda, Melis, & Stancampiano, 1996). In addition to the hippocampus, ACh efflux in the striatum and amygdala can also be used to assess amount of neural activation during maze learning (Chang & Gold, 2003a; McIntyre, Marriott, & Gold, 2003; McIntyre, Pal, Marriott, & Gold, 2002; Stancampiano, Cocco, Cugusi, Sarais, & Fadda, 1999). ACh release in the hippocampus and striatum predicts which system and thus which strategy (place or response) a given rat will use to solve a T-maze discrimination task (Chang & Gold, 2003a; McIntyre et al., 2003).

Recently, our laboratory has used similar microdialysis techniques to examine system level dysfunction in PTD rats. We demonstrated that during a single session of spontaneous alternation PTD-treated rats had significantly lower levels of hippocampal ACh efflux than pair-fed controls that correlated with poor alternation performance (Savage et al., 2003). The present study uses a T-maze task to assess the dynamic role of ACh efflux across the hippocampus and striatum in PTD and PF rats at three time points (early, middle, late) while acquiring the NMTP rule. This will create a more complete picture of the role of ACh efflux during both behavioral success and impairment. Our goal is to determine if ACh efflux changes across these two structures is related to the episodic spatial learning impairment observed in diencephalic amnesia produced by thiamine deficiency.

2. Methods

2.1. Subjects

Sixty male (2–3 months; 250–350 g) Sprague–Dawley rats were initially exposed to treatment regimes (see below), but technical or recovery difficulties reduced the number used in the data analysis to 41. Thirteen animals had probe problems [problems with flow in one of the two structures during behavior testing ($n = 10$) or misplaced cannulae ($n = 3$)] and six animals did not recover from PTD treatment. Of the Wnal 41 subjects, 21 recovered from PTD treatment and 20 served as pair-fed (PF) controls. All subjects were pair-housed until cannulae surgery, after which they were single housed. All subjects lived in a temperature controlled (68°F) vivarium that had a 12-h light/12-h dark cycle (onset at 06:00 h; offset at 18:00 h).

2.2. PTD treatment

At the onset of the treatment phase, PTD and PF animals had ad libitum access to water and thiamine-deficient chow (Harlan-Teklad Mills, WI). Once PTD animals showed signs of weight loss (day 12–13), PF animals were placed on a food deprivation regimen to mimic the weight loss in the PTD animals. Subjects in the PTD treatment group received daily injections of pyriethamine HBr (0.25 mg/kg, i.p.; Sigma Chem. Corp. MO) and were fed thiamine deficient chow. Within 14–16 days of treatment PTD subjects developed symptoms of anorexia, ataxia, loss of righting reflexes, and eventually displayed seizure activity. Animals were monitored bi-hourly for these neurological changes starting on day 13. Within 4–4.5 h of the appearance of seizure activity, all PTD-treated rats were reversed with a large dose of thiamine (100 mg/kg). The described protocol produces reliable diencephalic damage (Langlais & Savage, 1995). The administration of thiamine and return to normal chow usually completely reverses the acute symptoms within 8 h. The majority of animals were completely recovered within 24 h of reversal. The pair-fed control subjects were fed daily with thiamine deficient chow equal to the average amount eaten by the PTD-treatment group (to mimic the anorexia effects) and given daily injections of thiamine HCl (0.04 mg/kg, i.p.).

2.3. Recovery

All subjects were given three weeks to recover and return to a normal free-feeding weight. Weight was monitored daily and any animals that did not show signs of recovery, such as failure to gain weight, were euthanized with Sleep Away (1 mg/kg, i.p.; Fort Dodge Animal Health, IA).

2.4. Cannula surgery

After this recovery period, all subjects were chronically implanted with bilateral cannulae (CMA/12). One week prior to behavioral testing, all subjects were anesthetized with a Ketamine (8.25 ml)/Xylazine (1.75 ml) mixture (50 mg/kg, i.p.) immediately before stereotaxic surgery. Stereotaxic coordinates were chosen from the atlas of Paxinos and Watson (1986) and are based on previous studies (see Chang & Gold, 2003a). The coordinates for the hippocampus were: Anterior–Posterior (AP): 4.7 mm; Medial–Lateral (ML): 4.5 mm; Dorsal–Ventral (DV): 4.0 mm. The coordinates for the medial striatum were: AP: 0.3 mm; ML: 4.3 mm; DV: 3.7 mm. A 31-gauge stylet was used to close the cannulae. Acrylic cement and 2 skull screws were used to hold the guide cannulae in place. One-half of the subjects had cannulae in the left hippocampus and the right striatum, and one-half had the converse implantation. Immediately after surgery, subjects were placed in a warm incubator until they regained an upright posture. All subjects were allowed to recover for 1 week with ad libitum access to food and water. The following week, one week prior to behavioral testing, all subjects were placed on food deprivation such that subjects were 85% of their ad libitum food weight.

2.5. Behavioral testing

A plus-maze was used with one stem of the maze blocked off to create a T for nonmatching-to-position (NMTP). The maze was made of wood with clear Plexiglas sidewalls (12 cm high) and a painted black floor with the two goal arms of equal distance (55 cm) and the start arm a length of 66 cm. The maze was elevated 80 cm from the floor. Manually operated transparent Plexiglas guillotine doors separated the start box (SB) and the two goal boxes (GB) from the choice area. The reinforcers were 45 mg food pellets (Noyes, New Brunswick, NJ) that were delivered on a tray inserted through a slot in the correct GB.

Prior to testing rats were handled for 3 days, 10 min each. Subjects were habituated and trained to eat on the maze in 3 days. Each habituation session was at least 10 min in length and progressed until the rat ate the pellets that were used for a reward. After pre-training, testing consisted of 10 days each containing 12 trails (see Langlais & Savage, 1995; Savage & Langlais, 1995). Each trail of NMTP consists of a forced run followed immediately by a choice run. On the forced run trials, the subjects were placed in the SB and the door to the SB was raised allowing the subject to enter the choice area, in which one arm was blocked, and the other remained open. Once the subject entered the forced GB, the door was lowered and the reinforcer inserted. The choice run was identical to the forced run except both goal boxes were open. If the subject entered the opposite GB (nonmatching) as the preceding forced run, the door was lowered and the subject received the reward. If the subject entered the same GB as the previous forced run (matching), the door was lowered and there was no reinforcer. Each trail was approximately 2 min with an ITI of 30–60 s. The delay between the end of the forced run and the start of the choice run was approximately 4.5 s. The GB containing the reinforcer was pre-determined using a random schedule. All subjects were trained for 10 days, regardless of when microdialysis occurred.

2.6. In vivo microdialysis

Although all subjects were trained for 10 days of NMTP, each subjects underwent microdialysis procedures for only one session: either day 1 (PF: $n = 7$; PTD: $n = 7$), 5 (PF: $n = 7$; PTD: $n = 7$), or 10 (PF: $n = 6$; PTD: $n = 7$). All subjects were randomly assigned to one of the three microdialysis groups prior to behavioral testing. During the microdialysis session, ACh samples from the posterior ventral hippocampus and medial striatum were collected. Subjects were transported to the testing room and placed in a microdialysis holding cage [acrylic cage (30 cm $\times$ 40 cm, depth 35 cm) with wood shavings at the bottom]. The probes were connected to a microinfusion system and perfused continuously at a rate of 2 μ l/min with aCSF (127.6 mM NaCl, 4 mM KCl, 1.3 mM CaCl_2 dihydrate, 1.0 mM glucose, 0.9 mM MgCl_2 , 0.9 mM NaH_2PO_4 , and 2 mM Na_2HPO_4 , brought to pH 7.0) and neostigminebromide (500 nM). The neostigmine bromide was added to obtain consistent measurable levels of behaviorally relevant changes in ACh efflux (see Chang, Savage, & Gold, 2006). There was a 60 min period of habituation to the cage. After 60 min of habituation to the probe, dialysate samples (volume $\sim$ 12 μ l) were collected every 6 min for a period of 24 min prior to the maze task. This pre-maze phase was used to determine basal levels of ACh in awake rats. During the NMTP task, dialysate samples were collected every 6 min for 24 min. To test the reliability of the probe it was put into a standard solution (100 nM concentration of ACh and choline) to assess recovery rate. After collection, brain dialysis samples were frozen for later analysis using high performance liquid chromatography (HPLC).

2.7. Measurement of ACh output

ACh output was assayed by HPLC (Epison, BAS, West Lafayette, IN) along with an enzyme reactor. The assay system includes an ion-exchange microbore analytical column (BAS, MR-8904), a microbore ACh/choline immobilized enzyme reactor containing acetylcholinesterase and choline oxidase (BAS MF-8903), an auxiliary electrode with a radical

flow electrochemical thin-layer cell and 13 mm thin layer gasket, a wired enzyme electrode kit (a redox polymer film containing horse-radish peroxidase coated in the surface of a 3 mm glassy carbon working electrode), and a low dispersion injected value with a 10 μ l polytheretheketone loop. The mobile phase is a 50 mM dibasic potassium phosphate buffer (pH 8.5) containing ProClin (BAS, CF-2150). The mobile phase was delivered at a rate of 140 μ l/min by a PM-91 pump (BAS). The detection level was about 10 fmol. ACh standards (5 μ l of 20 and 100 nM of ACh + Ch) were injected before and after samples to verify detection stability.

2.8. Histology

After the completion of testing, animals were deeply anesthetized with Sleep Away (1 mg/kg, i.p.) and sacrificed. The brains were removed and placed in a 10% formalin solution for 1 week followed by emersion in a 30% sucrose solution for an additional week. Brains were then frozen and sliced (40 μ m) with a cryostat from the anterior commissure to the posterior pontine tegmentum. Every fifth section was stained with cresyl violet. Sections were evaluated for diencephalic damage and cannulae location. Only animals with accurate cannula placement were included in the data analysis.

In addition, we quantitatively determined the degree of thalamic tissue loss induced by PTD treatment by measuring the intraventricular distance (IVD) at the midline. The IVD (in mm) was measured from the roof of the ventral third ventricle to the floor of the dorsal third ventricle using J-image (NIH, Bethesda, MD) on a Nikon Eclipse E400 microscope attached to a Mackintosh G5 computer at interaural site 6.44 mm (midline thalamus at the first appearance of the habenula) according to the atlas of Paxinos and Watson (1986).

3. Results

3.1. Histology

3.1.1. Diencephalic pathology—Fifteen of the 21 PTD-rats had an extensive gliotic scar in the midline region (see Fig. 1), but all PTD-rats displayed loss of thalamic mass. PTD-rats (mean = 1.69 mm; SE = .05) had a significantly reduced IVD measure ($F(1, 39) = 12.23, p < .01$) relative to PF subjects (2.01 mm $\pm$.07). However, there were no significant differences in IVD measurements in the three groups of PTD-treated rats as a function of microdialysis day ($F(2, 18) < 1$). Thus, the three PTD groups had equivalent neuropathology to the diencephalon.

3.1.2. Probe placement—Only subjects with correct probe placement in both the hippocampus and striatum were included in the analysis (see Fig. 2). As stated in Section 2, three subjects had misplaced hippocampal cannulae and were dropped from the study.

3.2. Behavioral testing

Statistical analyses of group differences in choice accuracy (% correct) were conducted on (A) the overall learning curve of all rats in both groups and (B) on the specific subjects (both PF and PTD) that received microdialysis for that given day. A one-between subject (Group), one within subject (days) repeated measures ANOVA comparing the learning curves of PF vs. PTD-treated rats (see Fig. 3) revealed a significant overall group difference ($F(1,39)=16.58, p<.01$). There was a significant main effect of day ($F(9,351)=4.34, p<.01$), as all rats performed better as sessions progressed. There was also a significant Group $\times$ Day interaction ($F(9,351)=1.96, p<.05$). The PF animals outperformed the PTD animals across the later sessions.

Three separate simple *t* test comparisons of accuracy performance between the PF and PTD rats were conducted for each session that microdialysis took place (days 1, 5, and 10). Only rats that received microdialysis on a given day were included in the contrast for that day. On

day 1 ($t(12)=1.36$) and day 5 ($t(12)<1$) of training there were no significant differences in choice accuracy between the PTD and PF rats. However, on day 10 the PF rats had a higher accuracy score than the PTD rats ($t(11)=2.54$, $p<.05$).

3.3. Microdialysis and HPLC

For each session of microdialysis sampling, separate a one-between subjects (Group), two-within subjects [Phase (baseline, maze), Sample time (1–4)] repeated measures ANOVA was conducted for ACh efflux in the hippocampus and the striatum. To assess a relationship between ACh efflux and behavior, simple regression analyses relating choice accuracy to ACh efflux in the hippocampus and the striatum were conducted for PF and PTD rats. The ratio of hippocampus/striatum ACh release has been used as a measure of system preference during learning (Chang & Gold, 2003b; McIntyre et al., 2003; Pych, Chang, Colon-Rivera, & Gold, 2005a); thus, the ratio of hippocampal to striatal percent increase of maze ACh was calculated for all three microdialysis days.

3.3.1. Hippocampus—As shown in Fig. 4 (left column), ACh efflux in the hippocampus significantly increased above baseline during NMTP maze testing on day 1 ($F(1, 12)=14.25$, $p<.05$), day 5 ($F(1, 12)=10.98$, $p<.01$), and day 10 ($F(1, 11)=54.94$, $p<.001$) in all rats—regardless of treatment condition. There was no main effect of Group on either day 1 or day 5 (both F 's <1.23). In addition, on days 1 and 5 there were no significant interactions (Group $\times$ Phase, both F 's <1 ; Group $\times$ Sample, both F 's <1.4 ; Group $\times$ Phase $\times$ Sample, F 's <1). However, on day 10 the main effect of Group was significant ($F(1, 11)=5.48$, $p<.05$), as was the Group $\times$ Phase interaction ($F(1, 11)=5.81$, $p<.05$) and the Group $\times$ Sample interaction ($F(1, 11)=3.76$, $p<.05$). The 3-way interaction (Group $\times$ Phase $\times$ Sample) approached, but did not reach, significance on day 10 ($F(1, 11)=2.51$, $p=.07$). Simple contrasts revealed that on day 10 the PF group had a greater hippocampal ACh efflux during the last three samples of maze training than the PTD group (all t 's (11) >2.8 , p 's $<.05$). Table 1 shows the median ACh efflux in fmol's in the hippocampus for both the baseline and maze phases for all subjects in both groups.

3.3.2. Striatum—As also shown in Fig. 4 (right column), ACh efflux in the striatum of all subjects significantly increased above baseline during NMTP testing on day 1 ($F(1, 12)=34.75$, $p<.01$), day 5 ($F(1, 12)=25.36$, $p<.01$), and day 10 ($F(1, 11)=26.47$, $p<.01$). The main effect of Group was not significant on any day (all F 's <2.6). There were no significant interactions between Group $\times$ Phase (all F 's <1.2) and Group $\times$ Sample (all F 's <2.40) on any of the days. Furthermore, the 3-way interaction of Group, Phase, and Sample was not significant on any of the days (all F 's <1.0). Thus, although there was a rise in striatal ACh efflux during behavioral testing, there were no group differences in striatal ACh efflux at any time point. Table 2 shows the median ACh efflux in fmol's in the striatum for both the baseline and maze phases in both groups.

3.3.3. Correlation between behavior and ACh efflux—A regression analysis revealed that in the PF rats there were trends for both hippocampal ($r=.42$, $p=.064$) and striatal ($r=.43$, $p=.059$) ACh efflux to be correlated with choice accuracy (see Fig. 5). In contrast, there were no trends in the PTD rats for ACh efflux in either the hippocampus ($r=.09$) or striatum ($r=.02$) to be related to choice accuracy. The lack of correlative trend between ACh efflux and behavior in the PTD group is related to the restriction of range in choice accuracy given the lack of behavioral improvement across sessions.

3.3.4. Hippocampus/striatum ratio—The ratio of percent increase of maze ACh in the hippocampus/striatum (H/S ratio) was calculated for all three microdialysis sessions. On day 1, the mean H/S ratio for the PF group was 1.26 ($\pm.28$) and for the PTD group it was 1.01 ($\pm.15$). On day 5 the mean H/S ratio for the PF group was 1.66 ($\pm.41$) and for the PTD group it was

1.44 ($\pm$.27). On day 10 the mean H/S ratio for the PF group was 1.30 ($\pm$.21) and for the PTD group it was 1.17 ($\pm$.16). The PTD and PF groups did not differ significantly on this measure on any of the microdialysis days (all t 's<1).

4. Discussion

The present study replicated previous findings that PTD-treatment decreases thalamic mass and impairs learning of a NMTP rule (Knoth & Mair, 1991;Langlais & Savage, 1995;Robinson & Mair, 1992). The microdialysis/HPLC data further suggest selective hippocampal system dysfunction after PTD treatment on a task that involves spatial and episodic processing. Although there was a rise in hippocampal ACh efflux during maze training in PF and PTD rats, we found that the PTD rats had a blunted hippocampal ACh efflux late in maze training that paralleled their impaired behavioral performance. These results further support data that in the PTD model there is dysfunction in the extended hippocampal system (Nakagawasai et al., 2000;Pires et al., 2001;Pires, Pereira, Oliveira-Silva, Franco, & Ribeiro, 2005;Savage et al., 2003). The diencephalic–hippocampal interaction appears to be critical for episodic and spatial memory (Jenkins, Amin, Brown, & Aggleton, 2006). In the current study we also evaluated striatal ACh efflux during maze testing: there was a comparable rise in striatal ACh efflux during maze testing in both groups through out the course of NMTP training. However, unlike the hippocampus, PTD-rats appear to have striatal ACh function within the normal range on this type of task.

We expected that blunted hippocampal ACh efflux would parallel behavioral impairment in the PTD-treated rats. There was no difference in behavioral performance between PTD and PF rats until day 8 of training. During this stage when neither group had determined a successful strategy, there were no significant differences between PF and PTD-treated rats in ACh efflux in either the hippocampus or striatum. The first microdialysis sampling session after the appearance of behavioral impairment was day 10. A blunted hippocampal ACh efflux complimented this behavioral impairment. The protracted impairment on acquisition of NMTP has been previously documented in both appetitive and aversive paradigms: PF rats have been shown to perform similarly to PTD-treated rats during the initial sessions of NMTP training: However, from days 6–10 the PF rats begin to display an accelerating acquisition curve that has not been reported in PTD rats (Langlais & Savage, 1995;Mair et al., 1991b;Savage & Langlais, 1995). During this phase of the learning curve it is assumed that the PF rats use a more hippocampal-based strategy.

In vivo measures of hippocampal ACh efflux (Savage et al., 2003) and ex vivo measures of hippocampal acetylcholinesterase (AChE) activity (Pires et al., 2001,2005) have been shown to be dysfunctional after thiamine deficiency. In addition, there is a loss of choline acetyltransferase (ChAT) positive cells in the medial septal/ diagonal band of PTD rats (Pitkin & Savage, 2001,2004) and thiamine deficiency also decreases ChAT fiber density in the hippocampus (Nakagawasai et al., 2000,2004). These studies suggest that the behavioral impairment in the PTD animals is due, at least partly, to an altered cholinergic functioning in the hippocampus and related structures.

Other animal models have revealed that when the hippocampus is altered by neurodegeneration (Barnes, Nadel, & Honig, 1980) or by drug (Chang & Gold, 2003a) there has been facilitation for the use of striatal-based response strategies. Past behavioral studies have suggested that PTD-rats are able to learn simple S-R discriminations (Langlais et al., 1992;Mair, Anderson, Langlais, & McEntee, 1988;Mair et al., 1991a,1991b) that can be striatal-based (McDonald & White, 1993). Although the PTD rats displayed abnormal hippocampal functioning during maze training, there was no appearance of recovery of function as indicated by a switch to striatal mechanisms. Unlike the PF rats (see below), the PTD rats tested at different time points

did not show any consistent pattern of ACh release in the hippocampus (146, 193, and 166% above baseline) or the striatum (157, 143, and 156% above baseline) as a function of sessions, but their performance change was neither linear nor large (54, 64, and 60%).

The lack of behavioral recovery and switch to a striatal-based strategy could be due to the type of task we used in this study. Previous research using a delayed alternation task demonstrated that rats start out typically using a place strategy early in maze training and later switch to a response strategy (Packard, 1999) and this is related to elevated ACh levels in the hippocampus and striatum, respectively (Chang & Gold, 2003b; Pych et al., 2005a; Pych, Chang, Colon-Rivera, Haag, & Gold, 2005b). The NMTP task is different from spontaneous and delayed alternation in that the to-be-remembered place changes in a random fashion from trial-to-trial. In contrast, correct performance on alternation tasks is directly dependent on the last place visited or previous response made by the rat. In the NMTP task a response strategy could be used within a trial (from forced to free choice phases: last response was left, now turn right), but would be ineffective across trials. This difference in within and across trial strategies could explain why the hippocampus and striatum are both activated in the NMTP task. There was a trend for percent rise of ACh release at the different time periods of microdialysis during maze learning to change as a function of choice accuracy across sessions (66, 72, and 80%) in PF animals in both the hippocampus (175, 207, and 230% above baseline) and striatum (154, 133, and 192% above baseline), suggest that both brain regions contribute to successful performance on this task. Furthermore, there were no significant changes in the hippocampus/striatum ratio across sessions or groups. That data and the correlative trend for increased ACh efflux as a function of phase (baseline, maze) in the PF group suggests that successful performance on the NMTP task requires activation of both the hippocampus and striatum. Although there are instances when it appears that the hippocampus and striatum compete with each other to regulate learning (McDonald & White, 1993; Packard & Teather, 1998), there is also evidence that these two structures can complement each other during learning.

Recent data suggest that when extra maze cues are available during learning an alternation task, ACh release in the hippocampus and striatum may complement the efforts of each other rather than compete for control over learning strategies (Pych et al., 2005b). Thus, the nature of the rule to be learned and the intra/extra maze contexts play a large role in whether brain structures compete or compliment one another as measured by ACh efflux (Chang & Gold, 2004; Pych et al., 2005b). It appears that both the hippocampus and the striatum are initially needed in the acquisition of spatial location or distance and direction from both external and internal cues (DeCoteau, Hoang, Huff, Stone, & Kesner, 2004; Pych et al., 2005b). Similar to performance on a rewarded alternation task in a cue rich environment (Pych et al., 2005a), our data with PF rats demonstrates that both the hippocampus and striatum can participate together in integrating information when rats are learning a direction (left/right) discrimination in a T-maze NMTP task. However, it appears that in PTD-treated rats the hippocampus is not fully activated during the critical stages of learning and the striatum is not able to compensate—at least in such severe cases of PTD on this type of task.

We found no significant deficit in striatal ACh efflux in our PTD-treated rats; they showed a comparable rise during maze training as the PF controls. The intralaminar thalamic nuclei have eVerent and afferent connections with the striatum; these connections have been shown to be important in the control of arousal and attention (Mair, Koch, Newman, Howard, & Burk, 2002). Lesions of the IML have been shown to affect striatal-dependent tasks such as the serial reaction time (SRT) task (Burk & Mair, 2001). The SRT task involves the performing of “habit-like” behavioral responses to exterior stimuli. IML lesions also transiently decreased performance on an attentional task but when a retention interval was introduced to increase working memory demands, the IML lesioned animals showed significant impairment throughout training (Newman & Burk, 2005). As mentioned previously, a response or striatal-

based memory strategy alone would not be sufficient in learning the NMTP task. Therefore this task would not be a good measure of severe striatal dysfunction in the PTD model. Before ruling out that there are no differences in striatal functioning between PTD and PF rats, a task that is more dependent on striatal based functioning should be assessed in conjunction with in vivo microdialysis. It is possible that under circumstances when task demands solely necessitate striatal processing, PTD-treated rats may then display abnormal ACh efflux in the striatum. However, that possibility remains to be tested.

In summary, although both the hippocampus and the striatum are activated in both groups during NMTP training; only hippocampal ACh efflux was dysfunctional at the time when impaired performance on learning a NMTP task was also documented in PTD-treated rats. This data support a selective behaviorally induced hippocampal cholinergic dysfunction in diencephalic amnesia. A question that remains is what causes this selective hippocampal cholinergic dysfunction in the PTD model: lesions to nuclei and degeneration of fiber tracts in the extended thalamic-hippocampal circuit or loss of cholinergic neurons in the basal forebrain? ACh has been shown to be important for the maintenance of theta rhythm within the extended hippocampal circuit (Meeter, Murre, & Talamini, 2004). Lesions to the thalamus and the mammillary bodies impact the electrophysiological regulation of the hippocampus (Kirk & Mackay, 2003; Leranth, Carpi, Buzsaki, & Kiss, 1999; Vertes et al., 2004), supporting the idea that lesions of the diencephalon impair hippocampal functioning during behaviorally relevant states. However, cholinergic input from the MS/DB is important for normal hippocampal functioning (Hasselmo, Hay, Ilyn, & Gorchetnikov, 2002; Sotty et al., 2003) and thiamine deficiency leads to ChAT-positive cell loss in the MS/DB (Pitkin & Savage, 2001, 2004) and a loss of fibers in the hippocampus (Nakagawasai et al., 2000). ACh neurons are particularly vulnerable to neuroinflammation and neurodegeneration (Hartig et al., 2002; Wenk & Willard, 1998) and such states are produced during thiamine deficiency (Todd & Butterworth, 1999). Thus, the hippocampal cholinergic dysfunction associated with the PTD model could be a factor of lesions in key diencephalic nuclei (anterior thalamus, mammillary bodies) and/or a loss of MS/DB cholinergic cells. Determining the mechanism of this selective dysfunction will aid in the understanding and development of recovery of function in diencephalic amnesia.

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Fig 1. Cresyl violet stained sections showing acceptable probe placement in the hippocampus (A) and the striatum (B).

- A** Thalamus of a PTD rat. Arrows are pointing to the midline thalamic lesion.

- B** Thalamus of a PF rat.

Fig 2.
Cresyl violet stained sections demonstrating the prototypical PTD-induced lesion of the thalamus (A) and the thalamus of a PF-rat for comparison (B).

Fig 3. Mean choice accuracy (% correct $\pm$ SEM) for 10 sessions of NMTP training for PTD-treated and PF rats. The arrows represent the sessions at which microdialysis sampling of acetylcholine (ACh) efflux occurred.

Fig 4.

Mean ($\pm$ SEM) percentage of baseline acetylcholine (ACh) efflux in hippocampus (left column) and striatum (right column) before and during of NMTP training on the maze in both the PTD-treated and PF control groups on days 1, 5, and 10. The asterisks represent a significant difference in ACh efflux during maze testing between the PF and PTD-treated rats.

Fig 5.

Correlative relationship between of ACh efflux and choice accuracy in the hippocampus (left column) and striatum (right column) as a function of Group [PF (top), PTD (bottom)]. Trend lines on each graph represents the overall combined regression analysis for all three microdialysis sampling points (days 1, 5, and 10).

Table 1

Hippocampal ACh efflux; median fmol and interquartile range (IQR: 25%, 75%) for baseline and maze phases

Days	PF		PTD	
	Baseline	Maze	Baseline	Maze
1	13.9 (11.2, 16)	21.9 (19.5, 23.7)	27.4 (7.4, 36.8)	34.7 (11.7, 64)
5	32.8 (19.5, 44.4)	59.6 (25.8, 80)	28.5 (10.1, 44.4)	51.6 (20.7, 71.1)
10	25.8 (14.2, 32)	60.5 (40, 67.6)	19.5 (15.3, 20.4)	32 (31.8, 40)

Table 2

Striatal ACh efflux; median fmol and IQR (25%, 75%) for baseline and maze phases

Days	PF		PTD	
	Baseline	Maze	Baseline	Maze
1	47.9 (16.4, 52.1)	73.5 (24.9, 85.9)	95.7 (32.5, 185.1)	128 (44.4, 213.5)
5	65.8 (24, 76.5)	72.9 (31.3, 85.4)	39.6 (24.9, 41.8)	55.3 (34.3, 66.7)
10	44.1 (11.5, 50.8)	100.5 (21.1, 151.2)	44.4 (33.8, 122.7)	76.5 (54.3, 154.8)