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Impaired, Spared, and Enhanced ACh Efflux Across the Hippocampus and Striatum in Diencephalic Amnesia is Dependent on Task Demands

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Abstract

Diencephalic amnesia manifests itself through a host of neurological and memory impairments. A commonly employed animal model of diencephalic amnesia, pyriethamine-induced thiamine deficiency (PTD), results in brain lesions and impairments similar in nature and distribution to those observed in humans with Wernicke-Korsakoff syndrome (WKS). In the current investigation, 2 separate experiments were conducted in which acetylcholine (ACh) efflux was assessed in the hippocampus and striatum of PTD-treated and pair-fed (PF) control male Sprague-Dawley rats. The goal was to determine under what behavioral conditions and in which brain structures ACh efflux was spared, impaired, or adaptively enhanced. In Experiment 1, rats were assessed on a spontaneous alternation task; in Experiment 2, rats were tested on a T-maze discrimination task that could be learned via a hippocampal- or striatal-based strategy. In Experiment 1, PTD-treated rats were impaired on the spontaneous alternation task and ACh efflux in the hippocampus during testing was significantly reduced, but spared in the striatum. In Experiment 2, PTD- and PF-treated rats did not differ in the number of trials to criterion, but PTD-treated rats demonstrated greater reliance upon egocentric cues to solve the task. Furthermore, ACh efflux in the striatum was greater during maze learning in the PTD-treated animals when compared to the PF animals. These results suggest that there is behavioral and systems level plasticity that can facilitate the use of alternative strategies to solve a task following diencephalic damage and WKS.

Keywords

diencephalon; microdialysis; acetylcholine; spontaneous alternation; spatial

Introduction

Diencephalic amnesia, associated with a deficiency of thiamine (vitamin B1), is manifest across a range of disorders, including malnourishment (Wernicke-Korsakoff syndrome [WKS]), ischemic infarcts, viral infections, and tumors. The anatomical damage observed in this disorder is predominantly localized to the midline thalamic nuclei, anterior thalamic nuclei, mammillary bodies, and the internal medullary lamina (Aggleton & Pearce, 2001; Langlais &

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Savage, 1995; Langlais, Zhang, & Savage, 1996; Mair, 1994; Troncoso, Johnston, Hess, Griffin, & Price, 1981). Furthermore, there is evidence of degeneration in key limbic system fiber tracts, such as the mammillothalamic tract and fornix (Langlais & Zhang, 1993; 1997), which may lead to potential downstream impairments in other regions of the brain (Jenkins, Dias, Amin, Brown, & Aggleton, 2002; Markowitsch, 1988; Markowitsch & Pritzel, 1985; Reed et al., 2003; Savage, Chang, & Gold, 2003; Vann & Aggleton, 2003; Warrington & Weiskrantz, 1982). The main features of diencephalic amnesia, including those seen in animal models, are severe anterograde learning impairments (Gold & Squire, 2006; Pires, Pereira, Oliveira-Silva, Franco, & Ribeiro, 2005) and long-lasting memory disturbances (Gold & Squire, 2006; Langlais & Savage, 1995; Mair, 1994; Reed et al., 2003). Specifically, human patients exhibit explicit memory deficits but spared implicit memory (Kopelman, 1995; Squire, 1981). Similarly, in animal models there is evidence of impaired performance on hippocampal-dependent spatial memory tasks (Langlais, Mandel, & Mair, 1992; Mair, Anderson, Langlais, & McEntee, 1985; Mair, Anderson, Langlais, & McEntee, 1988; Mair, Otto, Knoth, Rabchenuk, & Langlais, 1991; Robinson & Mair, 1992). However, performance on many simple discrimination tasks was spared following diencephalic amnesia (e.g., Langlais & Savage, 1995; Mair et al., 1988; Mumby, Mana, Pinel, Banks, & David, 1995).

Using the pyridoxamine-induced thiamine deficiency (PTD) animal model of WKS, our laboratory has demonstrated a functional impairment of the hippocampus as evidenced by blunted hippocampal acetylcholine (ACh) efflux when rats were tested on spontaneous alternation (Savage et al., 2003; Savage, Roland, & Klintsova, 2007) and nonmatching-to-position tasks (Roland & Savage, 2007). The observed deficits in behavior and hippocampal ACh efflux could be the result of a number of several factors. First, there is decreased choline acetyltransferase (ChAT) immunopositive cells in the medial septum/diagonal band (Pitkin & Savage, 2001; 2004; Savage et al., 2007) and fibers in the hippocampus (Nakagawasai, 2005; Nakagawasai, Tadano, Hozumi, Tan-No, Nijima, & Kisara, 2000). Second, damage to structures of the extended hippocampal pathway can lead to a “disconnection syndrome” (Aggleton & Brown, 1999). In the PTD model there is cell loss in the anterior thalamus and/or mammillary bodies (Langlais & Savage, 1995) as well as fornix and mammillothalamic tract abnormalities (Langlais & Zhang, 1997).

The chronic cholinergic deficit observed in the PTD model may be limited to the septohippocampal pathway (Savage et al., 2007) as there is evidence of spared striatal (Roland & Savage, 2007) and amygdalar (Langlais & Savage, 1995; Nakagawasai et al., 2000; Savage et al., 2007) ACh functioning following PTD treatment. Although hippocampal ACh efflux plays a key role in cognitive function, other task-dependent increases in ACh release have been reported in the striatum and amygdala of rats assessed on various tasks (Chang & Gold, 2003; Gold, 2003; McIntyre, Pal, Marriott, & Gold, 2002; Pych, Chang, Colon-Rivera, & Gold, 2005; Pych, Chang, Colon-Rivera, Haag, & Gold, 2005; Roland & Savage, 2007; Savage et al., 2007). Furthermore, evidence of both compensatory and competitive interactions between these structures during task acquisition suggests a more complex and interactive dynamic underpinning select spatial processes (Compton, 2004; McDonald & White, 1995; Packard & McGaugh, 1996). Specifically, the hippocampus has been purported to mediate spatial or place learning in which external cues are mapped onto location (Eichenbaum, Stewart, & Morris, 1991) whereas the striatum is thought to mediate response learning in which body-centered cues (i.e., left, right) are associated with external cues (Kesner, Bolland, & Dakis, 1993; Packard & McGaugh, 1996).

The current investigation used two behavioral paradigms to assess impaired, spared, and adaptive behavioral and neural plasticity following PTD treatment. Acetylcholine efflux was assessed in the hippocampus and striatum of male Sprague-Dawley rats in both experiments. In Experiment 1, rats were trained on a spontaneous alternation task that is sensitive to

diencephalic amnesia (e.g., Langlais & Savage, 1995; Roland & Savage, 2007; Savage et al., 2007) as well as changes in ACh efflux in the hippocampus (McIntyre et al., 2002; Ragozzino, Unick & Gold, 1996). During Experiment 2, rats were assessed on a T-maze discrimination task that has been shown to be solvable via two different navigational strategies: place and response (Chang & Gold, 2003; Gibson & Shettleworth, 2005; Pych et al., 2005). It was expected that striatal ACh efflux, representative of striatal functioning, would be spared or perhaps adaptive in nature, leading to behavioral plasticity following diencephalic damage.

Experiment 1

Methods

Subjects—Nineteen male Sprague-Dawley rats (3–4 months; 250–300 g) were used as subjects in this study. They were housed one per cage with unlimited access to water and Purina rodent chow in a colony room on a 12-hr light/12-hr dark cycle (onset at 7:00 am). All rats were food deprived the night before behavioral testing.

PTD treatment—Animals were first randomly assigned to one of the following treatments: (i) pair-fed (PF, $n = 10$), or (ii) pyriethamine-induced thiamine deficiency (PTD, $n = 9$). Subjects in the PTD treatment group were free-fed thiamine-deficient chow (Teklad Diets, Madison, WI) and received daily injections (0.25 mg/kg, i.p.) of pyriethamine HBr (Sigma-Aldrich, St. Louis, MO). On days 14–16 of treatment, animals developed signs of local tonic-clonic movement of the front and hind limbs, and generalized convulsions (seizures). Within 4 hr of seizure onset, PTD-treated animals were given an injection of thiamine HCl (100 mg/kg, i.p.) every 8 hr until seizure activity disappeared and the rat's regained upright posture. The PF animals were fed an amount of thiamine-deficient chow equivalent to the average amount consumed by the PTD group on the previous day of treatment and received daily injections of thiamine HCl (0.4 mg/kg, i.p.). After treatment, all subjects were placed on regular chow. Pre-treatment body weights were restored within 14 days following the termination of PTD- and PF-treatment.

Surgery—Sixteen days after recovering from PTD or PF treatments, stereotaxic surgery (David Kopf Instruments, USA) was performed on animals anesthetized with an i.p. injection (0.1 mL/kg) of a Ketamine (83.0 mg/kg)/Xylazine (17.0 mg/kg) mixture. Each subject was implanted chronically with two plastic guide cannulae (CMA/12 3 mm, Carnegie Medicine Associates, Chelmsford, MA). Stereotaxic coordinates were chosen based on the atlas of Paxinos and Watson (1986) and are similar to previous studies (see Chang & Gold, 2003; Savage et al., 2007). One cannula was lowered into the hippocampus (coordinates for A/P and M/L was based on bregma and D/V was based on dura; 5.0 mm A/P, 5.0 mm M/L, and 4.2 mm D/V) and the other guide cannula was lowered into the medial striatum (0.2 mm A/P, 3.5 mm M/L, and 3.7 mm D/V). One-half of the subjects in each group received guide cannula in the left hippocampus and right striatum, and the remaining animals received converse implantation. Immediately following surgery, the animals received a dose of Buprenorphine (0.3 mg/kg, i.p., Reckitt Benckiser, Richmond, VA) and Gentamicin (0.2 mg/kg, i.p., Abraxis, Schaumburg, IL), and were placed in a warm incubator until an upright posture was regained. Five days after surgery, animals were handled daily (5 min/day) for 5 days prior to behavioral testing.

Microdialysis and Maze Testing/HPLC—Ten days following surgery, two microdialysis probes (CMA/12, 3 mm) were inserted into the hippocampal and striatal guide cannulae and the animal was placed into the holding cage (41 cm $\times$ 30 cm $\times$ 35 cm) located in the testing room. The probes were connected to plastic tubing and driven by a microinfusion system. The dialysis probes were perfused continuously at a rate of 2.0 μ L/min with artificial CSF (in mM:

127.6 NaCl, 4.0 KCl, 1.3 CaCl₂, 0.9 MgCl₂, 0.9 NaH₂PO₄, 2.0 Na₂HPO₄, and 1.0 glucose; pH = 7.4), which contained the AChE inhibitor neostigmine (500 nM). After 60 min of stabilization, dialysis samples (sample volume 12 µL) were collected every 6 min for a period of 18 min in the holding cage to determine basal levels of ACh in awake rats. During the initial baseline phase, the animal was free to move about the holding cage. After 3 baseline samples were collected, the rat was gently picked up and placed on the center of the maze. The plus-maze used for behavioral testing was constructed of wood with clear Plexiglas sidewalls (12 cm high) and a painted black floor with 4 arms of equal distance (55 cm), elevated 80 cm above the floor. The maze testing room contained various extramaze cues (posters, doors, tables, etc.). The rat was allowed to transverse the maze freely for 18 min. The number and sequence of arms entered were recorded to determine alternation scores. An alternation was defined as the choice arm to the left or right of the rat when it reached the center area, not the arm straight ahead or a re-entry into the same arm. The percent alternation score was equal to the ratio of: (actual alternations/possible alternations) × 100.

Upon completion of 18 min of maze testing, the rat was transferred back to the holding cage for an additional 18 min. Dialysate samples were assayed for ACh using HPLC with electrochemical detection (Bio Analytic Systems, West Lafayette, IN). The system included an ion-exchange microbore analytical column, a microbore ACh/Ch immobilized enzyme reactor containing AChE and choline oxidase, and a peroxidase wired working electrode. The detection limit of this system was 10 femto mol. ACh peaks were quantified by comparison to peak heights of standard solutions (ACh [Sigma-Aldrich] at 20 and 100 nM) and corrected for *in vitro* recovery of the probe.

Tissue fixation and histological analysis—At the conclusion of behavioral testing, animals were anesthetized (0.5 mg/kg, i.p. Sleep-Away [26.0% sodium pentobarbital in 7.8% isopropyl alcohol and 20.7% propylene glycol solution], Fort Dodge, IA) and perfused transcardially using 0.9% saline solution and 4.0% phosphate-buffered paraformaldehyde. Their brains were removed, post-fixed in a 10% formalin solution for at least 72 hr, and transferred to a 30% sucrose solution. Coronal sections from the brains were cut (60 µm thick) on a vibratome from the level of the anterior commissure to the level of the posterior pontine tegmentum. Sections were collected into wells in sequential order and every fifth section was stained with cresyl violet for assessment of probe location. One PTD and 2 PF rats had microdialysis collection problems during behavioral testing and their data was discarded.

Results

Statistical Analyses—The mean percent of alternation, perseveration, and total number of arm entries during spontaneous alternation were analyzed with a one-factor (Group = PF vs. PTD) ANOVA. The neurochemical microdialysis/HPLC data was analyzed with a one-between (Group), two-within factors repeated measures (Phase [Baseline, Maze, After], Sample Time [Block 1, Block 2, Block 3]) ANOVA.

Histology—Figure 1-AB displays the intact thalamus of a PF rat (A) in relation to neuropathology in the midline thalamus observed in PTD-treated rats (B). Figure 1-CD displays representative cannulae placement in the hippocampus (C) and striatum (D).

Spontaneous alternation—As shown in Figure 2-AB, PTD-treated rats (n=8) had a significant decrease in spontaneous alternation rates relative to PF (n=8) control rats ($F[1,14] = 8.10, p < .02$). However, there were no group differences in the number of arms entered during maze training ($F[1,14] < 1$). The rate of perseveration (defined as a the number of re-entries into an arm or an immediate repeat of the last arm entered) was greater in the PTD-treated rats,

compared to the PF-treated rats (PF=7.29%±1.54 [SEM]; PTD=17.59%±4.31; $F[1,14]=5.07$, $p<.05$).

Acetylcholine efflux—Basal amounts of ACh in the hippocampus (PTD: 53.5±11.5 fento mol; PF: 39.7±10.2 fento mol) and striatum (PTD: 264±125.3 fento mol; PF: 253±52.9 fento mol) were not different as a function of Group (both F 's[1,14]<1).

Although hippocampal ACh efflux increased during behavioral testing relative to baseline levels in the holding cage in both PF and PTD rats ($F[2,28]>11.38$, $p<.01$; see Figure 3-A), there was a significant Group main effect ($F[1,14]=5.32$, $p<.05$) as well as a Group × Phase interaction ($F[2,14]=4.32$, $p<.05$). Figure 3-A shows that PTD-treated rats had significantly blunted ACh efflux (expressed as percent increase above baseline values) during maze testing, relative to PF rats ($F[1,14]=6.36$, $p<.05$). There was no significant main effect of Sample Time ($F<1$) and the remaining interactions were not significant (all F 's<1.7). There was a positive correlation between hippocampal ACh efflux and alternation score, but it was not significant ($r=.37$). The correlation was greater than that seen between the number of arms entered and hippocampal ACh efflux during maze training ($r=.008$).

As shown in Figure 3-B, there was also a significant rise in striatal ACh efflux during maze testing in both groups (main effect for Phase: $F[2,28]=11.37$, $p<.01$). However, the groups did not differ in the degree of rise in striatal ACh efflux (main effect of Group: $F<1$). Furthermore, there were no other significant main effects or interactions. Thus, in contrast to impaired hippocampal functioning, striatal ACh functioning was spared in the PTD model as measured on the spontaneous alternation test. The positive correlation between striatal ACh efflux and alternation score was not significant ($r=.29$). However, there was a nonsignificant trend for a negative correlation between the number of arms entered and striatal ACh efflux during maze testing ($r=-.40$).

Discussion

As expected, PTD-treated rats were impaired on the spontaneous alternation task relative to PF controls in the current experiment, a finding that replicates previous research (Mair et al., 1985; Roland & Savage, 2007; Savage et al., 2003; 2007). However, there was no difference in the number of arm entries across groups indicating that the observed behavioral impairment in the PTD animals was not due to a deficit in motor ability (see Savage et al., 2003). The PTD-treated rats did engaged in more preservative behavior and this likely contributed to the impaired alternation rates (see also Langlais & Savage, 1995).

In conjunction with the impaired spontaneous alternation performance, the PTD-treated rats demonstrated a significant reduction in hippocampal ACh efflux during maze testing relative to PF-treated controls. This impaired hippocampal ACh functioning replicates two previous studies from our laboratory (Savage et al, 2003; Savage et al, 2007). In all three studies the spontaneous alternation scores of PTD rats are about 33% lower than the scores of PF rats and the hippocampal ACh efflux of PTD rats during maze testing is only 50% of that of control rats. Increased levels of ACh efflux in the hippocampus and striatum have been proposed to indicate relative participation of these structures (McIntyre, Marriott, & Gold, 2003) and the above results parallel the observed behavioral deficits indicating that the hippocampus was dysfunctional during maze testing. However, there was a similar increase in striatal ACh efflux in both groups suggesting there was no striatal cholinergic dysfunction following PTD treatment. Thus, similar to the data obtained by Roland and Savage (2007) using a matching to position task, ACh functioning in the striatum appeared to be spared and functional during spontaneous alternation testing following PTD treatment. It should be noted that spontaneous alternation has been shown to rely heavily upon an intact and functional hippocampal system and that this task is sensitive to fluctuations of ACh in the hippocampus (McIntyre et al.,

2002). There was a lack of correlation between ACh efflux in either structure and alternation score, which conflicts with some previous data showing a correlation between hippocampal ACh efflux and alternation behavior (Savage et al, 2003, 2007). However, regression analyses are dramatically affected by sample size and under such conditions are sensitive to moderate variations in range (see Howell, 2002; McClure, 2005; McIntyre et al, 2002). As a consequence, the lack of correlation does not mean that hippocampal ACh efflux is not related to alternation behavior; rather it is not a direct predictor of such behavior.

Although Experiment 1 revealed maze-related activation in both structures during spontaneous alternation testing in normal (PF) rats, the overall increase in ACh was greater in the hippocampus than was observed in the striatum (115% in the hippocampus vs. 55% in the striatum). Given the present data, coupled with the fact that striatal ACh efflux is normal in PTD-treated rats, it appears that striatal ACh release plays a less significant role than hippocampal ACh efflux in the behavioral success on the spontaneous alternation task.

In Experiment 2, a task was chosen that could be equally solved via a hippocampal- or striatal-based strategy (see McIntyre et al., 2003). The goal of Experiment 2 was to determine whether under certain task demands, could the spared striatal ACh system become functional, leading to behavioral success in the PTD model.

Experiment 2

Methods

Subjects—Sixteen naïve male (2–3 months; 250–350 g) Sprague-Dawley rats (8 PTD; 8 PF) were used as subjects. Treatment was identical to that described in Experiment 1, except that approximately 10 days before behavioral testing (5 days post-surgery), animals were placed on food deprivation to attain 80% of their *ad libitum* body weight. Once running weight was attained, the animals began T-maze testing. Maze testing began 3 weeks after treatment and 15 days after surgery.

PTD treatment—The animals received identical PTD or PF treatment to those in the previous experiment (see Experiment 1).

Surgery—Surgical procedures were identical to those reported in Experiment 1 and are not reported here. Five days after surgery, food deprivation commenced and was continued for 10 days prior to maze testing. The rats were handled daily (5 min/day) for 5 days before maze testing and on the 9th day of food deprivation, the rats were habituated to the maze environment.

Maze habituation—A distinct plus-maze from Experiment 1, with one arm blocked off with an opaque guillotine door to create a T and a start box, was used for discrimination testing. The maze was constructed of wood and painted black with clear Plexiglas sidewalls (15 cm high). All arms were identical in length (65 cm) and the apparatus was elevated 80 cm above the floor. A manually operated transparent Plexiglas guillotine door separated the start box (19 cm × 12.5 cm × 15 cm) from the goal areas of the maze. The testing room was dimly lit and contained numerous extramaze cues (posters, doors, tables, etc). The reinforcers used were 1/3 of a Frosted Cheerio (General Mills, Minneapolis, MN). Beginning 3 days before testing began (7th day of food deprivation), the animals received 6 pieces of Frosted Cheerios in the home cage to reduce neophobia. Twenty-four hr before testing, each rat was transported to the running room and habituated. Habituation consisted of 10 min of exposure to the holding box and 5 min in each baited (6 pieces of Frosted Cheerios) goal arm (blocked off to prevent maze exploration). Immediately after maze habituation, microdialysis guide cannulae were assessed for patency by removal of the stylet.

Microdialysis and Maze Testing/HPLC—On the day of behavioral testing, the microdialysis probes were inserted into the hippocampal and striatal guide cannulae immediately prior to placement of the animal into the holding cage (same as Experiment 1) located in the testing room. After 60 min of stabilization in the holding box, dialysate samples (volume ~12 μ L) were collected every 6 min for a period of 18 min prior to the commencement of maze testing and served as baseline samples. After baseline samples were collected, rats were placed on the maze and ran 90 consecutive trials that were 1 min each. During maze testing, microdialysis samples collected in 6 min increments, producing a total of 15 ACh samples during testing.

On each training trial, the arm facing the start arm was blocked creating a T-maze. Rats were trained to enter one arm (left or right counterbalanced across subjects and group) to obtain the Frosted Cheerio reward located at the end of the goal arm. Each trial began with opening of the guillotine start box door and the rats were allotted 45 sec to choose an arm and consume the reward. Once the food was consumed, the guillotine door was lowered and the maze was rotated 90° so the start box returned to the same spatial location in the room. Between each trial, the rat remained in the start box for 15 seconds. Thus, the total time from the start of one trial to the next was 60 sec. The first probe trial occurred after reaching criterion (7 correct choices in 10 consecutive trials) and the remaining probe trials were conducted on trials 60 and 90. A probe trial consisted of rotating the maze 180° so that the start box was opposite from its original spatial location and both arms were baited. The rats were allowed to enter either arm to consume the food reward. If a rat entered the arm in the same spatial location of maze training, the behavior was termed a place solution. If, instead, a rat chose the arm in the opposite location of the trained spatial area, the behavior was labeled as a response solution. Three probe trials were conducted and upon completion of maze testing, the rats were transferred back to the holding cage for an additional 18 min. The T-maze discrimination task was completed in one session that occurred on a single day.

To test the reliability of the microdialysis probe, it was placed into a standard solution (100 nM concentration of ACh and Ch) to assess recovery rate. After collection, brain dialysis samples were analyzed using HPLC as reported in Experiment 1.

Tissue fixation and histological analysis—After the completion of spatial discrimination testing, all animals were euthanized and the brains examined histologically as previously described (see Experiment 1).

Results

Statistical analyses—Trials to criterion and rate of perseveration were analyzed with a one-factor (Group: PF vs. PTD) ANOVA. The strategy used (Place [allocentric] vs. Response [egocentric]) to solve the task during early (Probe Trial (PT) 24), middle (PT 60), and late (PT 90) maze testing was analyzed by Chi-square analysis that compared the PF (expected) to the PTD (observed) values. To determine changes in ACh efflux from baseline through maze testing, the neurochemical microdialysis/HPLC data was analyzed with a one-between (Group), one-within factors repeated measures (Phase [Baseline (B1-3), Maze (M1-3), M4-6, M7-9, M10-12, M13-15]) ANOVA for each structure (hippocampus and striatum). In order to determine the magnitude of change in ACh efflux during maze testing only, the data was further analyzed with a one-between (Group), one-within factors repeated measures (Sample Time [1–15]) ANOVA for each structure (hippocampus and striatum). One PTD rat had microdialysis collection problems during behavioral testing and the data was discarded. The final number of subjects was 8 in the PF group and 7 in the PTD group.

Histology—All remaining subjects had accurate probe placement in the hippocampus and striatum as represented in Figure 1-CD.

Behavioral testing—There was no difference in the number of trials to criterion between PF (21.6 mean; ± 2.85 S.E.) and PTD-treated (17.3 ± 2.83) rats ($F < 1$). The PF rats (18.125% ± 4.62 [SEM]) tended to make more perseverative errors (defined as the number of consecutive [3 in a row] entries into the incorrect non-rewarded arm prior to attaining criterion) than PTD-treated rats (5.0% ± 2.18 ; $F[1, 13] = 5.98$, $p < .05$).

Although more PTD than PF rats used a response strategy, the effect was only significant during the middle phase of maze training: PTD-treated rats (86%) exhibited greater usage of a response strategy when compared to PF (38%) animals ($\chi^2[1, N=15] = 6.2$, $p < .025$; see Figure 4). It should be noted that this same behavioral effect approached significance in the late phase of maze testing ($\chi^2[1, N=15] = 3.25$, $p < .07$).

Acetylcholine efflux—Basal amounts of ACh in the hippocampus (PTD: 162.7 ± 49.7 fento mol; PF: 161.9 ± 27.3 fento mol) and striatum (PTD: 207.2 ± 41.3 fento mol; PF: 188.3 ± 32.6 fento mol) were not different as a function of Group (both F 's $[1, 13] < 1$).

As shown in Figure 5-A, in the hippocampus there was a significant main effect of Phase ($F[17, 221] = 7.9$, $p < .01$): ACh levels increased during behavioral testing in both groups relative to baseline levels in the holding cage. When ACh efflux was assessed during maze testing only, the groups did not differ in the degree of rise in hippocampal ACh efflux (main effect of Group: $F < 1$). Furthermore, there was no effect of Sample Time ($F < 1.8$); the rise in hippocampal ACh efflux was not gradual as training progressed. Rather, hippocampal ACh efflux reached asymptote after sample time 2 and remained consistent through out testing.

In contrast to the hippocampus, there was a significant Group ($F[1, 13] = 5.54$, $p < .05$) as well as a Group $\times$ Phase $\times$ Sample Time interaction ($F[10, 130] = 1.99$, $p < .05$) for striatal ACh efflux. Analysis of ACh efflux in the striatum during maze testing only yielded an overall main effect of Group ($F[1, 13] = 5.52$, $p < .05$) and Sample Time ($F[14, 182] = 3.1$, $p < .001$), but no Group $\times$ Sample Time interaction ($F < 1$). Although all animals displayed a gradual increase in striatal ACh efflux across testing, PTD-treated rats displayed higher overall levels of ACh efflux.

Discussion

On the T-maze discrimination task, PTD-treated rats did not display a behavioral impairment when compared to the PF animals, as there was no difference in the number of trials required to reach criterion. However, the PTD rats tended to use a different strategy than the PF rats. On the later probe trials, PTD-treated rats demonstrated greater reliance upon a striatal-based response strategy, which was supported by the cholinergic neurochemical data. These results complement previous research demonstrating that dysfunction in the hippocampal system increases the use of response-based strategies (Packard & McGaugh, 1996). Conversely, the PF-treated rats demonstrated no preference for either a place or response strategy across probe trials. This finding supported the work of McIntyre et al. (2003), who reported no preference for strategy selection after rats learn the task. In contrast, other researchers have reported a consistent pattern of place strategy selection early in training followed by use of a response strategy with continued training (e.g., Chang & Gold, 2003). However, variations in behavioral patterns obtained on this task appear to reflect differences in the training environment, such as abundance of extramaze cues, lighting, and maze conditions (see Chang & Gold, 2003; McIntyre et al., 2003; Restle, 1957). In the present study, the experimental environment was dimly lit, which may have contributed to the probability of equally evoking activation of either strategy in normal (PF) rats. This seems logical given that striatal involvement has been proposed to increase under conditions of decreased illumination in the testing room (see Chang

& Gold, 2004). Thus, under our task conditions, there was not a bias for rats without brain damage (PF rats) to choose either a response or place strategy. However, under these conditions more PTD-treated rats used a striatal-based response strategy than a hippocampal-based place strategy.

Acetylcholine efflux in the hippocampus of both PF- and PTD-treated rats tested on the T-maze discrimination task increased from baseline, but remained at a constant level throughout testing. This neurochemical profile was also obtained by Chang and Gold (2003). The observed absence of a hippocampal ACh efflux deficit in the PTD-treated rats may be a consequence of a lack of relationship between ACh efflux and learning ability on this type of task. In a previous study, we found that PTD rats did not display impaired hippocampal ACh efflux early in training of a matching-to-position T-maze task. Rather, the deficit only appeared at the later time periods when the accuracy of PF rats was significantly better than PTD-treated rats (Roland & Savage, 2007).

It has been proposed that ACh efflux might serve better to indicate the degree of structural activation rather than the level of behavioral performance (Chang & Gold, 2003; McIntyre et al., 2002). Therefore, although there was significant ACh efflux in the hippocampus, just like there was significant ACh rise in the striatum during spontaneous alternation testing, the rise in either case did not lead to successful performance. Thus, in PTD-treated rats there is only dysfunctional hippocampal ACh efflux when the hippocampus is critical for successful performance on the task.

General Discussion

As seen in our previous studies (Savage et al, 2003; Savage et al, 2007), when tested on the spontaneous alternation task, PTD-treated rats were behaviorally impaired and displayed hippocampal dysfunction, as reflected by the reduction in ACh efflux. However, during spontaneous alternation testing the striatal ACh release was similar to the PF control rats, suggestive of spared function in the striatum. On this behavioral measure, striatal activation was not able to gain control of performance and lead to successful adaptation of performance in PTD rats. In contrast, on the T-maze discrimination task used in Experiment 2, which could be solved using either a place or response strategy, there was no behavioral impairment in the PTD-treated rats and no hippocampal ACh dysfunction. However, the PTD rats displayed enhanced striatal ACh efflux and tended to use a response strategy to a greater extent compared to PF rats.

The current investigation indicated that both task rules and environmental conditions could evoke different types of behavioral strategies that require certain brain region activation. When hippocampal functioning was critical for successful performance on a task, such as on a spontaneous alternation task or matching-to-position, there is both behavioral and neurochemical (ACh) impairment in PTD rats (Roland & Savage, 2007; Savage et al., 2003; 2007). However, past data from our laboratory (e.g., Roland & Savage, 2007) has been demonstrated that the striatal cholinergic system is unimpaired in the PTD-treated rat. Thus, in the current investigation the ACh efflux in the striatum was functionally increased only under conditions in which a response strategy could be effectively or selectively used to solve the task—such as the T-maze discrimination task. A similar phenomenon was demonstrated when matching-to-position task was modified so that it could be solved through reward contingencies rather than through the use of a spatial place strategy. This modification completely eliminated the PTD-induced learning impairment (Langlais & Savage, 1995) providing evidence of an amygdalar-based compensatory mechanism (Savage, Koch, & Ramirez, 2007) in the PTD model.

In summary, this study provides further evidence that task demands can evoke different neural substrates and that on such tasks learning and memory impairments (i.e., amnesia) can be mitigated through the use of alternative strategies and regional activation in brain damaged populations. Although in normal rats different neural systems (such as the hippocampus and striatum), and the behavioral strategies they evoke, can compete with one another to obstruct behavioral success (see Gold, 2003; White & McDonald, 2002), in brain damaged subjects a spared neural system could become adaptive under certain environmental conditions. Specifically, the current study along with other studies from our laboratory (e.g., Langlais & Savage, 1995; Roland & Savage, 2007; Savage et al., 2007), demonstrate that rats with PTD-induced diencephalic amnesia have intact cholinergic functioning in the amygdala and striatum. In addition, behavioral evidence suggests that these amygdalar and striatal systems can be utilized to solve some complex cognitive problems depending on the nature of the task (Savage & Langlais, 1995).

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References

- Aggleton JP, Brown MW. Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behavioral and Brain Sciences* 1999;22:425–489. [PubMed: 11301518]
- Aggleton JP, Pearce JM. Neural systems underlying episodic memory: Insights from animal research. *Philosophical Transactions Of The Royal Society Of London Series B, Biological Sciences* 2001;356:1467–1482.
- Chang Q, Gold PE. Switching memory systems during learning: Changes in patterns of brain acetylcholine release in the hippocampus and striatum in rats. *The Journal of Neuroscience* 2003;23:3001–3005. [PubMed: 12684487]
- Chang Q, Gold PE. Inactivation of dorsolateral striatum impairs acquisition of response learning in cue-deficient, but not cue-available, conditions. *Behavioral Neuroscience* 2004;118:383–388. [PubMed: 15113264]
- Compton DM. Behavioral strategy learning in rat: Effects of lesions of the dorsal striatum or dorsal hippocampus. *Behavioural Processes* 2004;67:335–342. [PubMed: 15518984]
- Eichenbaum H, Stewart C, Morris RGM. Hippocampal representation in place learning. *The Journal of Neuroscience* 1991;10:3531–3542. [PubMed: 2230943]
- Gibson BM, Shettleworth SJ. Place versus response learning revisited: Tests of blocking on the radial maze. *Behavioral Neuroscience* 2005;119:567–586. [PubMed: 15839803]
- Gold PE. Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiology of Learning and Memory* 2003;80:194–210. [PubMed: 14521863]
- Gold JJ, Squire LR. The anatomy of amnesia: Neurohistological analysis of three new cases. *Learning & Memory* 2006;13:699–710. [PubMed: 17101872]
- Howell, DC. *Statistical Methods for Psychology*. 5. London: Duxbury; 2002.
- Jenkins TA, Dias R, Amin E, Brown MW, Aggleton JP. Fos imaging reveals that lesions of the anterior thalamic nuclei produce widespread limbic hypoactivity in rats. *Neuroscience* 2002;22:5230–5238. [PubMed: 12077218]
- Kesner RP, Bolland BL, Dakis M. Memory for spatial locations, motor responses, and objects: Triple dissociation among the hippocampus, caudate nucleus, and extrastriate visual cortex. *Experimental Brain Research* 1993;93:462–470.
- Kopelman MD. The Korsakoff Syndrome. *British Journal of Psychiatry* 1995;166:154–173. [PubMed: 7728359]
- Langlais PJ, Mandel RJ, Mair RG. Diencephalic lesions, learning impairments, and intact retrograde memory following acute thiamine deficiency in the rat. *Behavioural Brain Research* 1992;48:177–185. [PubMed: 1616608]

- Langlais PJ, Savage LM. Thiamine deficiency in rats produces cognitive and memory deficits on spatial tasks which correlate with tissue loss in diencephalon, cortex and white matter. *Behavioral Brain Research* 1995;68:75–89.
- Langlais PJ, Zhang SX. Extracellular glutamate is increased in the thalamus during thiamine deficiency-induced lesions and is blocked by MK-801. *Journal of Neurochemistry* 1993;61:2175–2182. [PubMed: 8245970]
- Langlais PJ, Zhang SX. Cortical and subcortical white matter damage without Wernicke's encephalopathy after recovery from thiamine deficiency in the rat. *Alcoholism: Clinical and Experimental Research* 1997;68:75–89.
- Langlais PJ, Zhang SX, Savage LM. Neuropathology of thiamine deficiency: An update on the comparative analysis of human disorders and experimental models. *Metabolic Brain Disease* 1996;11:19–37. [PubMed: 8815388]
- Mair RG. On the role of thalamic pathology in diencephalic amnesia. *Reviews in Neuroscience* 1994;5:105–140.
- Mair RG, Anderson CD, Langlais PJ, McEntee WJ. Thiamine deficiency depletes cortical norepinephrine and impairs learning processes in the rat. *Brain Research* 1985;360:273–284. [PubMed: 4075172]
- Mair RG, Anderson CD, Langlais PJ, McEntee WJ. Behavioral impairments, brain lesions, and monoaminergic activity in the rat following recovery from a bout of thiamine deficiency. *Behavioral Brain Research* 1988;27:223–239.
- Mair RG, Otto TA, Knoth RL, Rabchenuk SA, Langlais PJ. Analysis of aversively conditioned learning and memory in rats recovered from pyriithiamine-induced thiamine deficiency. *Behavioral Neuroscience* 1991;105:351–359. [PubMed: 1907456]
- Markowitsch HJ. Diencephalic amnesia: A reorientation towards tracts? *Brain Research* 1988;472:351–370. [PubMed: 3066440]
- Markowitsch HJ, Pritzel M. The neuropathology of amnesia. *Progress in Neurobiology* 1985;25:189–287. [PubMed: 4089179]
- McClure P. Correlation Statistics: Review of the basic and some common pitfalls. *Journal of Hand Therapy* 2005;18:378–380. [PubMed: 16059861]
- McDonald RJ, White NM. Hippocampal and nonhippocampal contributions to place learning in rats. *Behavioral Neuroscience* 1995;109:579–593. [PubMed: 7576202]
- McIntyre CK, Marriott LK, Gold PE. Patterns of brain acetylcholine release predict individual differences in preferred learning strategies in rats. *Neurobiology of Learning and Memory* 2003;79:177–183. [PubMed: 12591225]
- McIntyre CK, Pal SN, Marriott LK, Gold PE. Competition between memory systems: acetylcholine release in the hippocampus correlates negatively with good performance on an amygdala-dependent task. *The Journal of Neuroscience* 2002;22:1171–1176. [PubMed: 11826146]
- Mumby DG, Mana MJ, Pinel JPJ, Banks K, David E. Pyriithiamine-induced thiamine deficiency impairs object recognition in rats. *Behavioral Neuroscience* 1995;109:1209–1214. [PubMed: 8748969]
- Nakagawasai O. Behavioral and neurochemical alterations following thiamine deficiency in rodents: Relationship to functions of cholinergic neurons. *The Pharmaceutical Society of Japan* 2005;125:549–554.
- Nakagawasai O, Tadano T, Hozumi S, Tan-No K, Nijima F, Kisara K. Immunohistochemical estimation of brain choline acetyltransferase and somatostatin related to the impairment of avoidance learning induced by thiamine deficiency. *Brain Research Bulletin* 2000;52:189–196. [PubMed: 10822160]
- Packard MG, McGaugh JL. Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiology of Learning and Memory* 1996;65:65–72. [PubMed: 8673408]
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. Academic Press; San Diego, CA: 1986.
- Pires RG, Pereira SRC, Oliveira-Silva IF, Franco GC, Ribeiro AM. Cholinergic parameters and the retrieval of learned and re-learned spatial information: A study using a model of Wernicke-Korsakoff syndrome. *Behavioral Brain Research* 2005;162:11–21.
- Pitkin SR, Savage LM. Aging potentiates acute neurological symptoms and brain pathology in pyriithiamine-induced thiamine deficient rats. *Behavioral Brain Research* 2001;119:167–177.

- Pitkin SR, Savage LM. Age-related vulnerability to diencephalic amnesia produced by thiamine deficiency: The role of time of insult. *Behavioral Brain Research* 2004;148:93–105.
- Pych JC, Chang Q, Colon-Rivera C, Gold PE. Acetylcholine release in hippocampus and striatum during testing on a rewarded spontaneous alternation task. *Neurobiology of Learning and Memory* 2005;84:93–101. [PubMed: 15950501]
- Pych JC, Chang Q, Colon-Rivera C, Haag R, Gold PE. Acetylcholine release in the hippocampus and striatum during place and response training. *Learning & Memory* 2005;12:564–572. [PubMed: 16322358]
- Ragozzino ME, Unick KE, Gold PE. Hippocampal acetylcholine release during memory testing in rats: Augmentation by glucose. *Proceedings of the National Academy of Sciences of the United States of America* 1996;93:4693–4698. [PubMed: 8643466]
- Reed LJ, Lasserson D, Marsden P, Stanhope N, Stevens T, Bello F, et al. FDG-PET findings in the Wernicke-Korsakoff syndrome. *Cortex* 2003;39:1027–1045. [PubMed: 14584565]
- Restle E. Discrimination of cues in mazes: a resolution of the “place-vs.-response” question. *Psychological Review* 1957;64:217–228. [PubMed: 13453606]
- Robinson JK, Mair RG. MK-801 prevents brain lesions and delayed-nonmatching-to-sample deficits produced by pyriithamine-induced encephalopathy in rats. *Behavioral Neuroscience* 1992;106:623–633. [PubMed: 1386989]
- Roland JJ, Savage LM. Blunted hippocampal, but not striatal, acetylcholine efflux parallels learning impairment in diencephalic-lesioned rats. *Neurobiology of Learning and Memory* 2007;87:123–132. [PubMed: 16978888]
- Savage LM, Chang Q, Gold PE. Diencephalic damage decreases hippocampal acetylcholine release during spontaneous alternation testing. *Learning & Memory* 2003;10:242–246. [PubMed: 12888541]
- Savage LM, Koch AD, Ramirez DR. Basolateral amygdala inactivation by muscimol, but not ERK/MAPK inhibition, impairs the use of reward expectancies during working memory. *European Journal of Neuroscience* 2007;26:3645–3651. [PubMed: 18052977]
- Savage LM, Roland J, Klintsova A. Selective septohippocampal-but not forebrain amygdalar-cholinergic dysfunction in diencephalic amnesia. *Brain Research* 2007;1139:210–219. [PubMed: 17289001]
- Squire LR. Two forms of diencephalic amnesia: An analysis of forgetting. *The Journal of Neuroscience* 1981;1:635–640. [PubMed: 7346571]
- Troncoso JC, Johnston MV, Hess KM, Griffin JW, Price DL. Model of Wernicke’s encephalopathy. *Archives of Neurology* 1981;38:350–354. [PubMed: 7236062]
- Vann SD, Aggleton JP. Evidence of a spatial encoding deficit in rats with lesions of the mammillary bodies or mammillothalamic tract. *The Journal of Neuroscience* 2003;23:3506–3514. [PubMed: 12716960]
- Warrington EK, Weiskrantz L. Amnesia: A disconnection syndrome? *Neuropsychologia* 1982;20:233–248. [PubMed: 7121792]
- White NM, McDonald RJ. Multiple Parallel Memory Systems in the Brain of the Rat. *Neurobiology of Learning and Memory* 2002;77:125–184. [PubMed: 11848717]

Figure 1. Cresyl violet stained sections demonstrating the thalamus of a PF rat (A) for comparison to a PTD-induced lesion of the thalamus (B). Cresyl violet stained sections showing acceptable placement of the microdialysis probe in the hippocampus (C) and striatum (D).

Figure 2.

Experiment 1: Behavioral data (Mean $\pm$ S.E.M.) from spontaneous alternation testing for PTD- and PF-treated rats. Panel A displays an alternation deficit in PTD-treated rats in relation to PF control rats. Panel B indicates the number of arms entered during maze training for both PTD- and PF-treated rats.

Figure 3.

Experiment 1: Profiles of ACh efflux (mean percent rise above baseline $\pm$ S.E.M. from the hippocampus (Panel A) and striatum (Panel B) of PTD- and PF-treated rats as a function of phase during spontaneous alternation assessment: Baseline, behavioral testing, and post-baseline sampling.

Figure 4.

Experiment 2: Behavioral data from the T-maze discrimination task for PTD- and PF-treated rats, which displays the percent strategy used during early (PT 24), middle (PT 60), and late (PT 90) maze testing.

Figure 5.

Experiment 2: Profiles of ACh efflux (Mean percent rise above baseline $\pm$ S.E.M. from the hippocampus (Panel A) and striatum (Panel B) of PTD- and PF-treated rats during baseline and T-maze discrimination testing.