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Blocking GABA-A receptors in the medial septum enhances hippocampal acetylcholine release and behavior in a rat model of diencephalic amnesia

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ABSTRACT

Wernicke–Korsakoff syndrome (WKS), a form of diencephalic amnesia caused by thiamine deficiency, results in severe anterograde memory loss. Pyrithiamine-induced thiamine deficiency (PTD), an animal model of WKS, produces cholinergic abnormalities including decreased functional hippocampal acetylcholine (ACh) release and poor spatial memory. Increasing hippocampal ACh levels has increased performance in PTD animals. Intraseptal bicuculline ($GABA_A$ antagonist) augments hippocampal ACh release in normal animals and we found it (0.50 μg/μl and 0.75 μg/μl) also increased in-vivo hippocampal ACh release in PTD animals. However, the 0.75 μg/μl dose produced a greater change in hippocampal ACh release in control animals. The 0.50 μg/μl dose of bicuculline was then selected to determine if it could enhance spontaneous alternation performance in PTD animals. This dose of bicuculline significantly increased hippocampal ACh levels above baseline in both PTD and control rats and resulted in complete behavioral recovery in PTD animals, without altering performance in control rats. This suggests that balancing ACh–GABA interactions in the septohippocampal circuit may be an effective therapeutic approach in certain amnestic syndromes.

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1. Introduction

Wernicke–Korsakoff Syndrome (WKS), most often diagnosed in patients with chronic alcoholism, also is seen in disorders in which malnourishment – specifically thiamine deficiency– is a component (AIDS, chronic feeding tube, cancer, etc.). WKS has an acute and chronic phase that often culminates in severe explicit memory deficits (Harper and Kril, 1986; Kopelman, 1995; Squire, 1981). The animal model of this disorder, known as pyrithiamine-induced thiamine deficiency (PTD), produces damage in both the mammillary bodies and several thalamic nuclei (anterior, medial dorsal, intralaminar, internal medullary lamina) that are interconnected with the limbic system (Langlais and Savage, 1995; Langlais et al., 1996; Mair, 1994; Victor et al., 1971).

Beyond damage to diencephalic structures, a number of studies have demonstrated dysfunction in the septohippocampal pathway. Both WKS patients (Reed et al., 2003; Sullivan and Marsh, 2003) and PTD-treated animals (Langlais and Zhang, 1997) display extensive degeneration in both the mammillothalamic tract and the fornix which interconnect with the septum and hippocampus (Aggleton and Brown, 1999). In addition, PTD-treated rats display a 25–30% loss of choline acetyltransferase (ChAT)-positive neurons in the medial septum/diagonal band (MS/DB) (Pitkin and Savage, 2004, 2001; Savage et al., 2007). Furthermore, our laboratory has demonstrated that PTD animals have a selective decrease in hippocampal ACh efflux that parallels their poor performance (Roland and Savage, 2007). Similar results have been found when cortical ACh release was stimulated using potassium: Rats fed on a thiamine deficient (TD) diet alone had significantly less hippocampal and frontal cortex ACh release, compared to saline injected controls (Pires et al., 2005). Together these results demonstrate a septohippocampal cholinergic dysfunction that occurs after thiamine deficiency. Drugs that enhance cholinergic activity, such as acetylcholinesterase (AChE) inhibitors have been shown to improve the cognitive functioning in WKS and animal models of the disorder (Nakagawasai et al., 2000) — as well as increase hippocampal ACh release (Roland et al., 2008). It has been suggested that AChE inhibitors, such as physostigmine, are having their cognitive enhancing effects in the medial septal region of the basal forebrain (Mulder et al., 2005; Wu et al., 2003b).

However, the medial septum contains not only cholinergic, but also GABAergic and glutamatergic neurons that have local and diffuse projections (Manseau et al., 2005; Wainer et al., 1985). The interactions of the cholinergic and GABAergic populations in the MS/DB are important for modulating hippocampal-dependent learning and memory performance. The MS/DB cholinergic and GABAergic neurons contain both muscarinic and $GABA_A$ receptors (Gao et al., 1995; Van der Zee and Luiten, 1994), therefore medial septal infusions of cholinergic and GABAergic agonists/antagonists will have an effect on both receptor systems. In turn, hippocampal activation is modulated by both of these MS/DB neurotransmitter systems. The presence of a muscarinic agonist in the medial septal region decreases the activity of the hippocampal GABAergic interneurons *in vitro* (Alreja et al., 2000b);

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increasing overall activity of the hippocampal pyramidal neurons through disinhibitory mechanisms (Toth et al., 1997).

The role of $GABA_A$ receptor activation in the MS on the modulation of hippocampal activity and behavior has begun to be elucidated. Medial septal infusions of a $GABA_A$ antagonist increase hippocampal ACh release, whereas a $GABA_A$ agonist decreases hippocampal ACh release (Moor et al., 1998a,b). Furthermore, there is some evidence that the behavioral effects of activation of MS $GABA_A$ receptors are further modulated by the action of GABA in the hippocampus. When animals were given septal muscimol ($GABA_A$ agonist) combined with hippocampal bicuculline ($GABA_A$ antagonist), the memory impairing effects normally produced by septal muscimol were attenuated (Krebs-Kraft et al., 2007). However, this study does not rule out the possibility that MS $GABA_A$ receptor activation also influences cholinergic projection neurons.

The MS/DB cholinergic neurons supply an excitatory drive to the MS/DB GABAergic neurons (Alreja et al., 2000b); therefore a loss of cholinergic neurons, as occurs in PTD-treatment, could reduce activity in both the cholinergic and GABAergic septohippocampal pathways. Within the PTD model, studies have focused primarily on the cholinergic system; however, long-term changes in the GABAergic system have been reported. Reductions in midline-thalamic GABA levels have been seen up to 9 weeks after recovery from PTD-treatment (Langlais et al., 1988). However, the septohippocampal GABAergic system has not been examined for neuronal damage after PTD-treatment. Given the role of septohippocampal GABA in cognitive processes and the interaction between septohippocampal ACh and GABA, a change in GABAergic functioning following recovery from PTD treatment could be contributing to the learning and memory impairments associated with this model.

Therefore, in Experiment 1 a dose–response curve was conducted using two different doses (0.5 μg/μl and 0.75 μg/μl) of the $GABA_A$ antagonist bicuculline that have been shown to increase hippocampal ACh levels in normal animals (Chang et al., 2006). Increasing functional hippocampal ACh levels in PTD animals has been shown to increase spatial memory performance (Roland et al., 2008). Previous work has demonstrated that either i.c.v. or intrahippocampal bicuculline administration increases memory retention on a passive avoidance task (Zarrindast et al., 2002; Zarrindast et al., 2004). If bicuculline increased hippocampal ACh levels, it was expected that it would also increase behavioral performance in PTD-treated animals. In Experiment 2, bicuculline was infused into the medial septum while in-vivo microdialysis was performed for ACh in the ventral hippocampus and animals were tested on spontaneous alternation. Our main aim of these experiments was to determine if antagonizing septal GABA-A receptors to enhance hippocampal ACh efflux would recover spatial performance in PTD rats.

2. Experimental procedures

2.1. Subjects

A total of 32 adult (3–4 months old) male Sprague–Dawley rats were used for these experiments (12 for Exp. 1 and 20 for Exp. 2; Harlan, Indianapolis, IN). All animals were maintained throughout the experiments in a 12 h light/dark cycle and housed in standard laboratory Plexiglas cages (dimensions: 30×45×18 cm). Animals were pair housed until surgery, after which they were single housed. All behavioral and microdialysis testing was performed during the light cycle. All procedures involving rats were approved by the Binghamton University Institutional Animal Care and Use Committee (IACUC).

2.2. PTD treatment

At the onset of the treatment phase, both PTD (Exp 1: $n=6$; Exp 2: $n=10$) and pair-fed (PF) animals (Exp 1: $n=6$; Exp 2: $n=10$) had ad libitum access to water and thiamine-deficient chow (Harlan-Teklad Mills, WI). PTD treatment consisted of daily injections of pyrithiamine HBr (0.25 mg/kg, i.p. Sigma Chem. Corp., MO). Pyrithiamine is a thiamine antagonist that inhibits thiamine pyrophosphokinase, the enzyme that catalyzes the production of the coenzyme form of thiamine (Butterworth and Heroux, 1989). Within 14–16 days of treatment, PTD subjects developed symptoms of anorexia, ataxia, loss of righting reflexes, and eventually display 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 and Savage, 1995). The administration of thiamine and return to normal chow completely reversed the acute symptoms within 8 h and animals typically were fully recovered within 24 h. The PF control rats were fed daily with thiamine deficient chow equal to the average amount eaten by the PTD-treatment group (to mimic the anorexia effects) and were given daily injections of thiamine HCl (.04 mg/kg i.p.). All rats were given two 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 a solution of sodium pentobarbital and isopropyl solution (Sleepaway, Fort Dodge Laboratories, Fort Dodge IA, 1 mg/kg, i.p.).

2.3. Stereotaxic surgery

Three weeks after PTD/PF treatment and one week prior to microdialysis, all subjects were anesthetized with a ketamine (8.25 ml)/xylazine (1.75 ml) mixture (50 mg/kg i.p.) in preparation for stereotaxic surgery. All animals were implanted with a microdialysis guide cannula (CMA/11) in the hippocampus and a drug cannula that is 5.0 mm in length (22 gauge; Plastics One, Roanoke, VA) in the medial septum. Within each group, subjects were matched in terms of hippocampal microdialysis cannula placement (right vs. left hemisphere). Stereotaxic coordinates were obtained from the atlas of Paxinos and Watson (1986) and are based on previous studies (see Chang and Gold, 2003). The coordinates for the hippocampus microdialysis cannula were: Anterior–Posterior (AP): −5.1 mm; Medial–Lateral (ML): 5.0 mm; Dorsal–Ventral (DV): 4.2 mm. The coordinates for the medial septum drug cannula were: AP: +0.30 mm; ML: 0; DV: 5.0 mm. Acrylic cement and 2 skull screws were used to hold the cannulae in place. Immediately after surgery, subjects were placed in a warm incubator until they regained an upright posture. All subjects received a subcutaneous injection of the analgesic buprenorphine (0.1ml/100 g) directly after surgery and another injection 24 h later. All subjects were allowed to recover for 1 week with ad libitum access to food and water.

2.4. High performance liquid chromatography (HPLC)

Acetylcholine 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 horseradish peroxidase coated in the surface of a 3 mm glassy carbon working electrode), and a low dispersion injected value with a 10 μl polyetheretheketone loop. The mobile phase is a 50 mM dibasic potassium phosphate buffer (pH=8.5) containing Kathon (BAS). The mobile phase was delivered at a rate of 140 μl/min by a PM-91 pump (BAS). The detection level was about 10 fmol. ACh standards (5 μl of 20 and 100 nM ACh+Ch) were injected before and after samples to verify detection stability.

2.5. Histology

After the completion of testing, all 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 be frozen and sliced (60 μm) with a sliding microtome from the anterior commissure to the posterior pontine tegmentum. Sections were evaluated for diencephalic damage and cannula location using cresyl violet staining. All animals in both Experiments 1 and 2 had accurate medial septal and hippocampal cannula placement, therefore all animals were included in final data analysis.

2.6. Experimental design

2.6.1. Experiment 1. The effects of intraseptal bicuculline administration on hippocampal acetylcholine release in PTD and PF animals

Experiment 1 used a total of 12 animals (PF = 6; PTD = 6); all rats survived the PTD/PF-treatment and were given three weeks to recover and return to a normal free-feeding weight. Following recovery from PTD treatment and cannula surgery, all animals received saline preceding the two doses of drug in a single in-vivo microdialysis session. All animals received both drug doses with a 2-hour window between active dose deliveries.

In-vivo microdialysis and pharmacological challenge: Animals received intraseptal infusions of saline (1 μl) and bicuculline (0.50 μg/μl and 0.75 μg/μl) during a single session of in vivo microdialysis. Within each group of 6 rats (PF and PTD), after an infusion of saline, 3 rats received the low dose (0.5 μg/μl) of bicuculline first and the other 3 rats received the high dose (0.75 μg/μl) of bicuculline first. This was done to control for an effect of drug dose. Bicuculline doses were based on a study showing that in normal animals, similar doses of bicuculline in the septum increased hippocampal ACh levels without any adverse side effects (Chang et al., 2006). During the single microdialysis session, samples from the posterior ventral hippocampus were collected and later analyzed for ACh level. Subjects were transported to the testing room and placed in a microdialysis holding cage [acrylic cage (30 cm × 40 cm, depth 35 cm) with wood shavings at the bottom]. The microdialysis probe (CMA/11, 3 mm) was connected to a microinfusion system and perfused continuously at a rate of 2 μl/min with artificial cerebrospinal fluid (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) plus neostigmine bromide (500 nM) brought to pH 7.0. Following an initial period of habituation to the cage (60 min), dialysate samples (volume ~ 30 μl) were collected every 15 min for a period of 30 min to determine basal levels of ACh in awake rats. Changes in ACh levels caused by the drug were later determined as a change from baseline samples. After 30 min of baseline collection, an infusion needle (Plastics One, 15 mm) was inserted into the septal drug cannula and 1 μl of saline was infused over 1 min. The infusion needle extended 1 mm beyond the drug cannula and was always left in place for an additional 2 min to allow for diffusion of drug into the site. After the saline infusion, samples continued to be collected for an additional 30 min (total of 2 samples). After which 1 μl of bicuculline (either 0.5 μg or 0.75 μg) was infused into the medial septum. The first drug phase lasted a total of 90 min (6-samples). Ninety minutes after the first dose of bicuculline, 1 μl of the second dose of bicuculline was infused. The second drug phase was also 90 min. Following the second drug phase, 2–15 minute post-baseline samples were collected. Half of the animals within each group (PF and PTD) received the low dose of bicuculline first while the other half received the high dose of bicuculline first. To test the reliability of the microdialysis probe, it was placed into a standard solution (100 nM concentration of ACh and choline) to assess recovery rate (RR). The minimal amount of ACh that can be detected in the system was 10 fmol. After collection, brain dialysis samples were frozen for later analysis using HPLC.

2.6.2. Experiment 2. The effects of intraseptal bicuculline administration on spontaneous alternation behavior in PF and PTD-treated rats

Experiment 2 used a total of 20 animals (PF = 10; PTD = 10); all rats survived the PTD/PF-treatment and were given three weeks to recover and return to a normal free-feeding weight. Prior to microdialysis and behavioral testing, every animal was handled for 4 days, 10 min each to reduce anxiety on the maze as well as food restricted overnight to increase maze exploration.

In-vivo microdialysis, drug infusions and behavioral testing: During the two days of in-vivo microdialysis and behavioral testing, samples from the posterior ventral hippocampus were collected and later analyzed for ACh levels. Subjects were transported to the same testing room and holding cage as Experiment 1. The microdialysis probe (CMA/11, 3 mm) was connected to a microinfusion system and perfused continuously at a rate of 2 μl/min with aCSF. Following an initial period of habituation to the cage (60 min), dialysate samples (volume ~ 12 μl) were collected every 6 min for a period of 18 min to determine basal levels of ACh in awake rats. After the 18 min of baseline sample collection, the drug infusion needle (Plastics One, 15 mm) was inserted into the drug cannula and infused over 1 min with 1 μl of either saline or bicuculline (0.5 μg/μl). The drug infusion phase was a total of 18 min (3 samples). Within each group of 10 rats (PF and PTD), 5 rats received the saline infusion on the first day and bicuculline (0.5 μg/μl) on the second session; the remaining 5 rats had the counterbalanced sequence (bicuculline on session 1, saline on session 2).

Eighteen minutes after the start of infusion, the animal was placed on the plus-maze to perform non-rewarded spontaneous alternation for 18 min. The plus maze used for behavioral testing was made of wood with clear Plexiglas sidewalls (12 cm high) and a painted black floor with four arms of equal distance (55 cm) and was elevated 80 cm from the floor. The rat was allowed to transverse the maze freely for an 18 minute period, the number and sequence of arms entered were recorded to determine alternation scores. The percent alternation score was equal to the ratio of: (actual alternation / possible alternations) × 100. The maze and post-baseline phases consisted of 3–6 minute samples for a total of 18 min each.

To test the reliability of the microdialysis probe, after the post baseline phase, it was placed into a standard solution (100 nM concentration of ACh and choline) to assess recovery rate (RR). After collection, brain dialysis samples were frozen for later analysis using HPLC.

3. Results

3.1. Experiment 1

Fig. 1AB displays PTD thalamic neuropathology compared to a PF brain. Representative drug cannula placement in the medial septum and microdialysis cannula placement in the ventral hippocampus are shown in Fig. 2AB. Before ACh levels were analyzed, it was confirmed that there was no overall order effect in terms of drug dose (low dose first vs. high dose first) on ACh release (F [1, 10] < 1).

3.1.1. Hippocampal ACh levels

A single-factor (Group: PF vs. PTD) ANOVA revealed that basal amounts of ACh in the hippocampus were not different as a function of Group (PF = 34.67 ± 14.3 fmol; PTD = 56.04 ± 22.9 fmol; F [1, 10] < 1). A mixed design of one between-subjects (Group), one within-subjects (Phase: Baseline, Saline) factor ANOVA that compared ACh efflux during baseline and after saline infusion revealed that there was a non-significant rise above baseline (20%) in ACh levels after saline infusion across both groups. Therefore, the drug rise was calculated as a percent

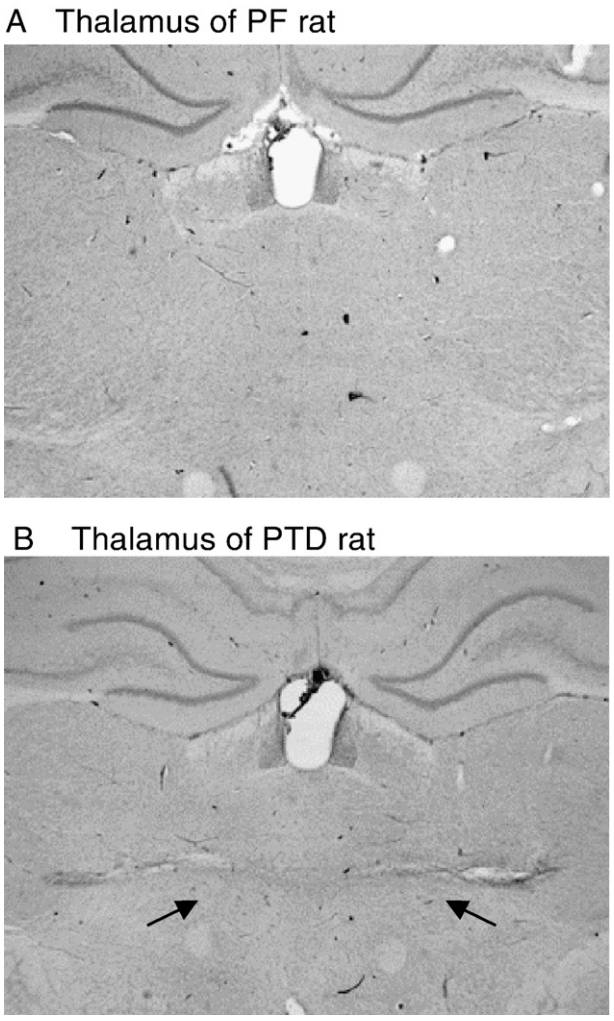

Fig. 1. Cresyl violet stained sections illustrating the intact thalamus of a PF control (A) and a midline thalamic lesion produced by PTD-treatment (B). The arrows indicate the prototypical PTD-induced lesion.

rise above saline to account for the effect of the injection alone. The mixed design of one between-subjects (Group), two within-subjects (Dose [0.5 μg/μl, 0.75 μg/μl]; Sample time [D1–D6]) factorial ANOVA revealed a significant main effect for Sample time (F [5, 50] = 6.81; p<.0001) and a significant interaction of Group × Dose (F[1,10] = 5.47; p<.05). To determine the locus of this interaction, post-hoc Fisher's LSD t-tests contrasting the Group difference collapsed across Sample time were conducted at each dose. As shown in Fig. 3, the groups did not differ in their ACh efflux levels to the challenge of 0.50 μg/μl of bicuculline (main effect of Group: t [10]<1). However, when subjects were administered the high dose, the rise in the PF rats, relative to the PTD rats, was greater t [10] = 2.77, p<.05). Given that the low dose (0.50 μg/μl) of bicuculline created an equal and significant rise of ACh in both PF and PTD animals, this was the only dose behaviorally tested in Experiment 2.

3.2. Experiment 2

Two PF and two PTD animals had misplaced cannula placement and were therefore not included in the final data analysis (final subject totals: PF = 8; PTD = 8). Once again, an analysis of order effects revealed that counterbalancing the drug condition across sessions did not affect alternation rates, number of arms entered or ACh levels in either group (all p's>.17).

3.2.1. Behavioral performance

Fig. 4A displays a significant difference in percent alternation between PF and PTD-treated rats under saline, with bicuculline resulting in behavioral recovery in PTD rats. A two-factor ANOVA (2×2: Group: PF, PTD; Drug: Saline, Bicuculline) revealed a significant effect of Group (PF vs. PTD: F [1, 14] = 4.89; p<.05) and a significant effect of Drug (F [1, 14] = 5.58; p<.05), but no interaction between Group and Drug (F [1, 14] = 2.78). The Group effect was primarily due to the PF rats alternating at a significantly higher rate than the PTD rats during the saline condition (F [1, 14] = 9.78; p<.01), as there was no significant difference between the Groups in the bicuculline condition (F [1, 14]<1). The main effect of Drug was a result of bicuculline infusion into the septum increasing alternation rates in both groups; however, follow-up analyses using a one within-subjects factor (Drug: Saline, 0.5 μg/μl) ANOVA for each Group condition revealed that the effect of the drug was significant in the PTD rats (17.38%; F [1, 7] = 5.21, p = .05), but not in the PF rats (3%, F [1, 7]<1).

As shown in Fig. 4B, a two-factor (Group, Drug) ANOVA demonstrated that there were no differences in the number of arms

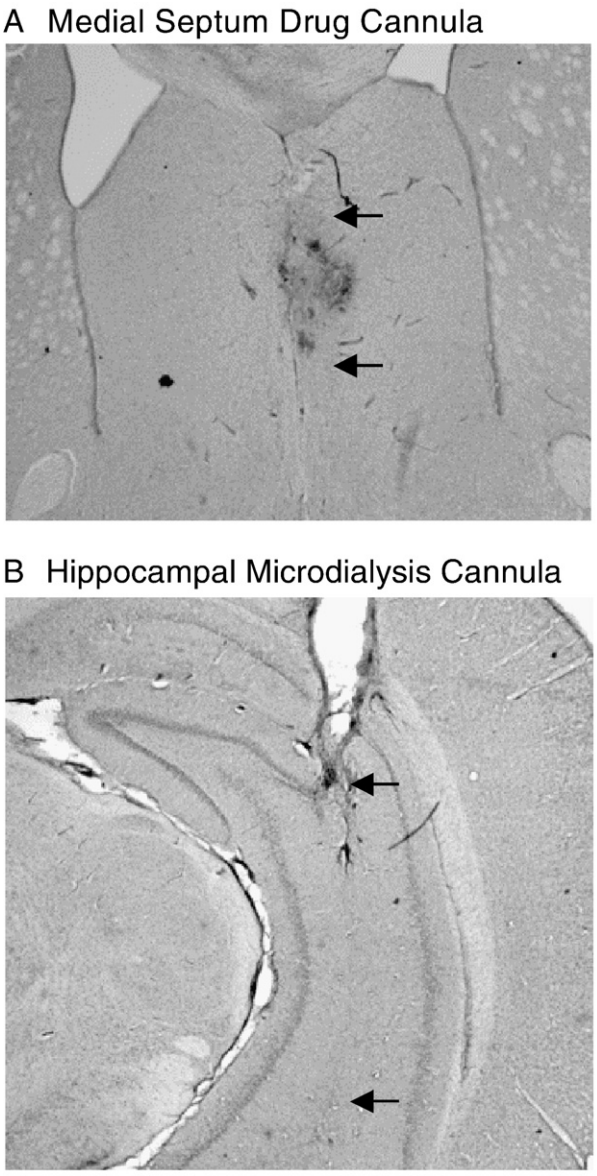

Fig. 2. Cresyl violet stained section showing acceptable probe placement for the medial septal drug cannula (A) and the hippocampal microdialysis cannula (B). The arrows indicate the regions of infusion (A) or occupied by the microdialysis cannula (B).

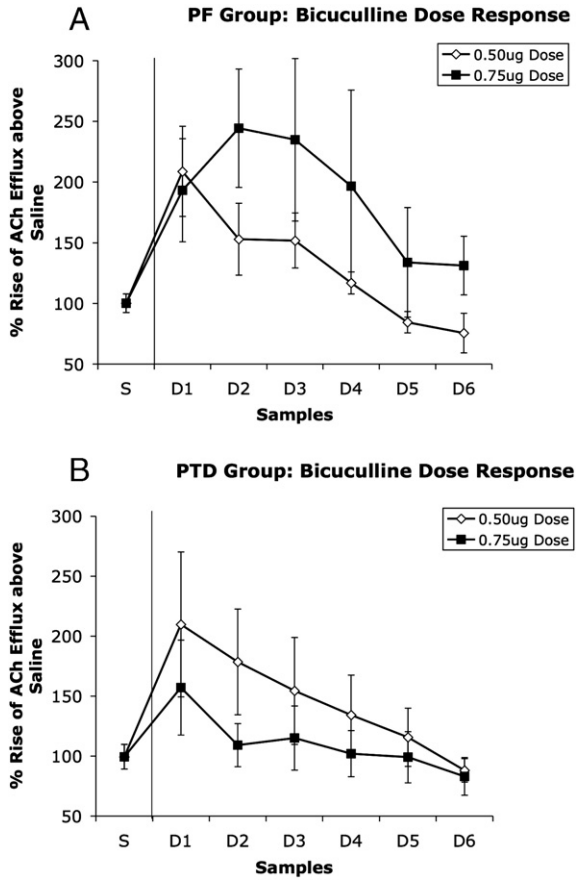

Fig. 3. Experiment 1: Bicuculline dose–response curve for both the PF (A) and PTD (B) animals. Rise in ACh release (mean ± SEM) after bicuculline infusion was calculated as the percent rise above the ACh release caused by saline infusion. Saline infusion occurred at time point S and bicuculline infusion occurred at time point D1. Each sample (S, D1–D6) represents a 15-min time bin of microdialysis collection.

entered as a function of Group (F [1, 14] = 1.23; p = .29), Drug (F [1, 14] < 1) or the interaction of Group × Drug (F [1, 14] < 1).

3.2.2. Hippocampal ACh levels

Differences in basal amounts of ACh in the hippocampus (Saline: PF = 19.63 ± 5.7, PTD = 22.42 ± 6.2 fmol; Bicuculline: PF = 19.2 ± 4.1, PTD = 23.29 ± 4.7 fmol) were assessed with a one-factor (Group) ANOVA. This analysis revealed that there were no differences in basal ACh levels between the Groups (F [1, 10] < 1]). A mixed statistical model of one between-subjects (PF vs. PTD), three within-subjects [Drug (Saline, Bicuculline); Phase (Baseline, Infusion, Maze, After); Sample (One, Two, Three)] factorial ANOVA revealed a significant main effect for Phase (F [3, 42] = 16.32; p < .0001), a significant Drug × Phase interaction (F [3, 42] = 4.37; p < .01) and a significant Phase × Sample interaction (F [6, 84] = 2.76; p < .05). The overall main effect of Group was not significant and it did not interact with any variables.

Follow-up investigations of the Drug × Phase interaction were conducted using one between-subject (Group), one between-subject (Saline, Drug) factorial ANOVAs for each Phase. These analyses revealed that relative to the saline condition, intraseptal infusion of 0.50 μg/μl of bicuculline increased hippocampal ACh efflux in both PF and PTD animals during the infusion phase and during maze testing (both Fs [1, 15] > 5.0, p's < .05), but not in the post-baseline phase (F [1, 15] < 1).

Planned comparisons were conducted to assess whether the groups displayed differential changes in hippocampal ACh efflux to the drug challenge itself or to how the drug changed the ACh profile during behavior. Changes in ACh efflux were assessed separately in each group using a one within-subjects (Phase) factorial ANOVA. This analysis revealed that in the saline condition the PF rats had a significant rise above baseline in ACh levels during the Drug infusion phase (F [1, 7] = 7.35; p < .05) and Maze testing phase (see Fig. 5A; F [1, 7] = 7.01; p < .05). However, after saline infusion PTD-treated rats only had a significant rise in ACh levels during the Maze testing phase (F [1, 7] = 8.91; p < .05; Fig. 5B).

Intraseptal bicuculline administration increased hippocampal ACh efflux during Drug infusion (both Fs [1, 7] > 12.70; p's < .01]) and Maze testing (both Fs [1, 7] = 12.30; p's < .01), relative to baseline ACh levels, in both PF and PTD rats. However, relative to the effect of the saline infusion, as assessed by one within-subjects factor ANOVAs for each group, the effect of increased ACh efflux during the bicuculline infusion phase was not significant in the PF rats (F [1, 7] = 1.21), but was in the PTD rats (F [1, 7] = 6.34; p < .05). This effect was a result of the increased reactivity of the PF rats to the saline infusion.

4. Discussion

The results from Experiment 1 demonstrated that intraseptal bicuculline administration increases hippocampal ACh levels in both PF and PTD animals. The doses and volume of bicuculline chosen for this study were based on a previous study in normal animals (Chang et al., 2006). In the PTD animals the low dose of bicuculline increased ACh levels by 167% ± 33.5, whereas the high dose of bicuculline

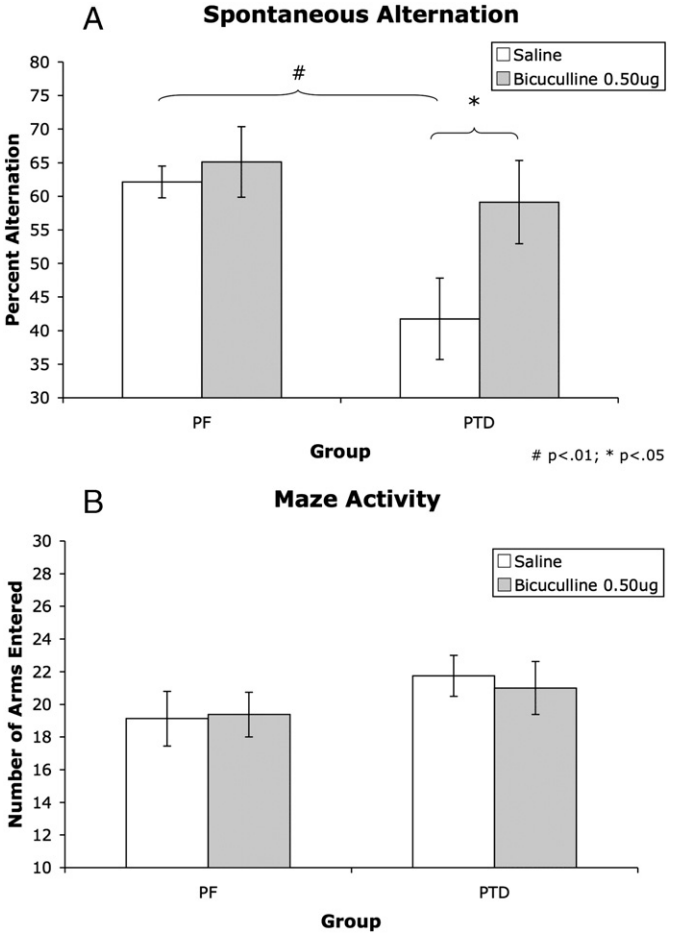

Fig. 4. Experiment 2: Behavioral data (mean ± SEM) from spontaneous alternation testing displaying the percent alternation (A) and activity on the maze (B) after both saline and bicuculline administration in PF and PTD rats. Panel A displays the significant difference between PTD and PF rats in alternation behavior after saline infusion. Although intraseptal bicuculline infusion enhanced alternation rates, the effect was only significant in PTD animals. Panel B shows that maze activity did not change as a function of drug in either group.

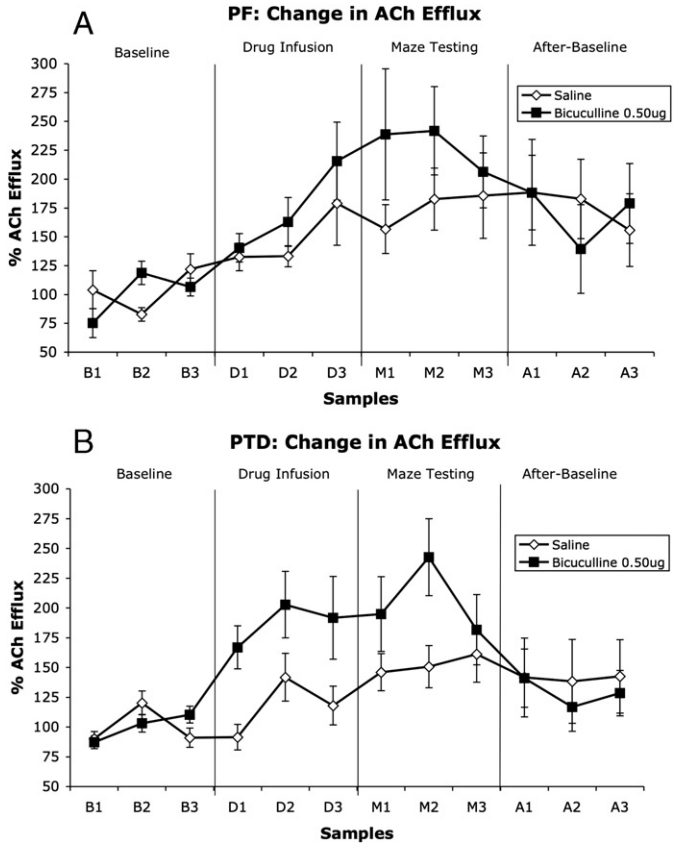

Fig. 5. Experiment 2: Profiles of hippocampal ACh release (mean percent rise above baseline ± SEM) after saline and bicuculline infusions in PF (A) and PTD (B) animals shown in 6-minute sample bins during baseline, drug infusion, maze testing and after-baseline phases. Under saline, PF rats had a significant rise above baseline in ACh levels during the drug infusion phase and maze testing phase (A), while PTD-treated rats only had a significant rise in ACh levels during the maze testing phase (B). After bicuculline administration, both PF (A) and PTD (B) animals showed a significant rise above baseline during the drug infusion and maze testing phases.

increased ACh levels by 131% ± 21.6. However, in PF rats the low dose resulted in an increase of 177% ± 20.5 and the high dose resulted in an increase of 225% ± 51.8. There was less of a hippocampal ACh response to the intraseptal administration of the high dose (0.75 μg/μl) of bicuculline in the PTD rats. Previous studies have shown that PTD animals can react differently to pharmacological manipulations than control animals (Nakagawasai et al., 2001; Savage et al., 1999). The 0.5 μg/μl dose of bicuculline produced comparable changes in ACh efflux in both the groups and was therefore chosen for behavioral analysis. This suggests that antagonizing the $GABA_A$ receptor could be an effective way to modulate ACh function and potentially lead to some behavioral recovery in amnestic subjects.

This is exactly what Experiment 2 illustrated: Intraseptal bicuculline led to complete behavioral recovery on the spontaneous alternation task without altering activity level on the maze. After bicuculline infusion, PTD animals alternated at levels comparable to saline-treated PF animals. Similar to Experiment 1, there was a comparable increase in hippocampal ACh levels in both groups in response to bicuculline administration. Although bicuculline infusion increased alternation performance in both groups, the percent of enhancement was only significant in the PTD group (PTD = 17%; PF = 3%). It should be noted that further increases in hippocampal ACh levels do not always lead to better performance. In normal rats increased hippocampal ACh efflux by an acetylcholinesterase inhibitor can actually lead to impaired behavior (Roland et al., 2008). Thus, there does appear to be an optimal range of cholinergic activity for optimal behavioral and hippocampal function.

Studies that have previously administered bicuculline into the medial septum of normal animals have produced mixed results. Post-training intraseptal bicuculline has been shown to increase memory retention in a passive avoidance task (Farr et al., 1999; Zarrindast et al., 2002) as well as performance in a stimulus–response task (Messier et al., 1999). However, others using young-normal rats have found that intraseptal bicuculline given either pre- or post-training can disrupt spatial working memory (Chrobak and Napier, 1992, 1991). Studies using various doses of intraseptal oxotremorine (Bunce et al., 2003), intraseptal carbachol (Bunce et al., 2004), intraseptal tacrine (Sabolek et al., 2005), and intrahippocampal physostigmine (Roland et al., 2008) have all found dose-dependent decreases in spatial memory performance in normal rats. In the current study, intraseptal bicuculline increased performance in cognitively impaired animals on a spontaneous alternation task — but had little effect in control PF rats. This difference in results may be due to the compromised integrity of the MS/DB cholinergic neurons within the PTD animal as well as task difficulty. Increasing the interval between trials on alternation can be a more sensitive assay for cognitive screening (Deacon and Rawlins, 2006).

Earlier reports have suggested that endogenous GABA could be inhibiting the MS/DB cholinergic neurons and affecting hippocampal ACh release by acting on the $GABA_A$ receptor located on the cholinergic neurons (Degroot and Parent, 2001). PTD-treated animals display a degeneration of MS/DB cholinergic neurons (Pitkin and Savage, 2004, 2001; Savage et al., 2007) as well as a decrease in functional hippocampal ACh release (Roland et al., 2008; Roland and Savage, 2007; Savage et al., 2003), which could contribute to a reduction in hippocampal pyramidal cell activity.

In the present study, both saline and bicuculline increased hippocampal ACh release. However, factors such as handling can evoke significant ACh efflux (see Nilsson et al., 1990) and the handling during the saline injection procedure could have caused a rise in ACh levels in both groups. Thus, the ACh response to the saline infusion procedure may have masked the traditional efflux differences seen between PTD and PF rats during behavioral testing. However, the rise in ACh induced by either behavioral testing or bicuculline infusion was greater than that produced by the saline injection. Bicuculline produces antagonism of the GABAergic neurons as well as an increase in ACh release in cholinergic neurons (Moor et al., 1998b); together these mechanisms could be beneficial in balancing the septo-hippocampal system within the PTD animal.

Wernicke–Korsakoff's syndrome is still a prevalent disease in many parts of the world and is widely under diagnosed in both adults and children (Harper, 2006; Sechi and Serra, 2007). The neuropsychiatric symptoms associated with WKS such as ocular abnormalities, changes in mental state and ataxia are usually treated with doses of thiamine when the patient is diagnosed. Nevertheless, treatment with thiamine alone is not effective at treating the anterograde amnesia related to WKS (Thomson and Marshall, 2006). Acetylcholinesterase inhibitors have been used with partial success for the treatment of the cognitive impairment associated with WKS (Angunawela and Barker, 2001; Cochrane et al., 2005; Luykx et al., 2008). Although bicuculline itself may not be the appropriate therapeutic tool, drugs influencing the GABAergic–cholinergic interactions in the septohippocampal pathway could be beneficial for the treatment of amnestic disorders.

Studies have demonstrated that increasing septohippocampal GABAergic pathway activity ultimately results in increased hippocampal pyramidal cell activity (Manns et al., 2001; Wu et al., 2003a). Therefore, drugs that increase the activity of the GABAergic septo-hippocampal pathway either alone or in combination with an AChE inhibitor could be more successful than an AChE inhibitor alone. In the past, $GABA_A$ antagonists specific for the benzodiazepine binding site have improved cognition but have high pro-convulsant side effects (Duka et al., 1996; Little et al., 1984; Venault et al., 1986). Recently a new class of benzodiazepine inverse agonists specific for the α5 GABA

receptor subunit has been shown to improve cognitive function with little to no pro-convulsant effects (Ballard et al., 2009 in press; Quirk et al., 1996; Sternfeld et al., 2004). These drugs are currently being investigated in humans and could be beneficial in WKS patients (Nutt et al., 2007). Overall, the involvement of the septohippocampal GABAergic neurons in the pathology of PTD-treatment and WKS requires further investigation to possibly determine more efficient treatment strategies.

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