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THE ROLE OF VENTRAL MIDLINE THALAMUS IN CHOLINERGIC-BASED RECOVERY IN THE AMNESTIC RAT

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Abstract

The thalamus is a critical node for several pathways involved in learning and memory. Damage to the thalamus by trauma, disease or malnourishment can impact the effectiveness of the prefrontal cortex (PFC) and hippocampus (HPC) and lead to a profound amnesia state. Using the pyridoxamine-induced thiamine deficiency (PTD) rat model of human Wernicke-Korsakoff syndrome, we tested the hypothesis that co-infusion of the acetylcholinesterase inhibitor physostigmine across the PFC and HPC would recover spatial alternation performance in PTD rats. When cholinergic tone was increased by dual injections across the PFC-HPC, spontaneous alternation performance in PTD rats was recovered. In addition, we tested a second hypothesis that two ventral midline thalamic nuclei, the rhomboid nucleus and nucleus reuniens (Rh-Re), form a critical node needed for the recovery of function observed when cholinergic tone was increased across the PFC and HPC. By using the GABA_A agonist muscimol to temporarily deactivate the Rh-Re the recovery of alternation behavior obtained in the PTD model by cholinergic stimulation across the PFC-HPC was blocked. In control pair-fed (PF) rats, inactivation of the Rh-Re impaired spontaneous alternation. However, when inactivation of the Rh-Re co-occurred with physostigmine infusions across the PFC-HPC, PF rats had normal performance. These results further demonstrate that the Rh-Re is critical in facilitating interactions between the HPC and PFC, but other redundant pathways also exist.

Introduction

The thalamus is a critical node in several neural circuits involved in learning and memory. Damage to distinct thalamic regions can lead to severe memory impairments. In humans, damage to the thalamus after trauma, stroke or thiamine deficiency leads to profound anterograde amnesia that resembles temporal lobe amnesia and prefrontal cortical dysfunction (Gold & Squire, 2006; Brion, Pitel, Beaunieux & Maurage, 2014; Oscar-Berman, 2012). Thus, diencephalic or thalamic amnesia has been commonly referred as a

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“disconnection syndrome” (Nahum et al, 2014). However, beyond serving as connection nodes, key thalamic nuclei have independent mnemonic functions and directly influence the effectiveness of other memory-related brain structures (Aggleton, 2014).

Wernicke-Korsakoff Syndrome (WKS) is classified as a type of diencephalic amnesia as the critical diagnostic neuropathology is anterior thalamic and medial mammillary body tissue loss (Kril & Harper, 2012). However, there is functional deactivation in other brain regions that contribute to the amnesic state. Functional imaging studies using WKS patients have revealed hypoactivation of the hippocampus (HPC) and medial temporal lobe during encoding and recognition, whereas during retrieval there is a reduction of activity in the ventrolateral prefrontal cortex (PFC; Caulo et al, 2005; Nahum et al, 2014). This hypofunctioning of the PFC and HPC, in addition to extensive anterior and midline thalamic pathology and shrinkage of the mammillary bodies, is also observed in the pyridoxamine-induced thiamine deficiency (PTD) model of WKS (Savage, Hall & Resende, 2012). Thus, WKS patients and the PTD model display a breakdown of the functional integration of several regions within the limbic system (thalamus, hippocampus, cortex). However, using the PTD model, it has been demonstrated that if cholinergic tone is increased in the HPC (Roland et al., 2008) or FC (Savage, 2012) spatial memory can be restored. These results suggest that regions that are “functionally” (hippocampus, frontal cortex), rather than “structurally” (thalamus, mammillary bodies) lesioned are critical targets for neurochemical modulation that can recover cognitive performance.

The ventral midline nuclei of the thalamus, specifically the rhomboid nucleus and reuniens of the thalamus (Rh-Re), have emerged as a critical region influencing mnemonic processes dependent on the cooperation between hippocampus and prefrontal cortex (Cassel et al, 2013). Projections from the HPC terminate in the medial and orbital PFC; however, reciprocal connections back to the HPC are sparse (Vertes, Hoover, Szigeti-Buck & Leranthe, 2007). Conversely, the prefrontal cortex densely projects to the Re (Vertes, 2002; Hoover & Vertes, 2012) and the Re projects to the CA1 region of the HPC as well as the subiculum (Prasad & Chudasama, 2013; Vertes et al., 2007). Several brain stem regions (raphe nuclei, reticular formation, the lateral dorsal tegmental nucleus, substantia nigra) as well as cortical regions (infralimbic, prelimbic, anterior cingulate, somatosensory and motor cortices) innervate the Rh (Vertes, 2002; 2006). Similar to the Re, the Rh projects to the regions of the frontal and parahippocampal cortices as well as the hippocampus; however, there are also dense projections to nucleus accumbens, and the basolateral amygdala nucleus (Van Der Werf et al., 2002). Thus, neuroanatomically, the Rh-Re is positioned to serve as a critical link between the PFC and the HPC. Behavioral data further suggests that the Rh-Re is needed for newly-learned information to be transferred between, and manipulated by, the PFC and HPC (Hembrook & Mair, 2011; Hembrook, Onos & Mair, 2012; Loureiro et al., 2012).

In the current set of experiments, we hypothesized that circuit-level cholinergic stimulation across the PFC and HPC would restore memory deficits observed in PTD rats. We also assumed that this recovery of function is not due to unilateral, single structure activation of either the PFC or HPC, but rather a return of activation within the PFC-HPC cortex circuit. Given the recent research that indicates that the represents a critical link between the PFC

and HPC, as well as the possibility that the Rh-Re modulates the flow of information between the two structures (Vertes et al., 2007), we hypothesized that inactivating the Rh-Re would block the recovery of function induced by drug-induced enhancement of cholinergic tone across the PFC and HPC in the PTD model. Such affirmative results would provide further evidence that the Rh-Re is the crucial communicative node linking the PFC and HPC in the memory circuit, even in cases of diencephalic amnesia.

Experimental Procedures

Subjects

Subjects were male Sprague-Dawley rats (Harlan Laboratories, Inc.) weighing between 250 and 275 grams at the start of the experiment. Rats were pair-housed until surgery, then single-housed in plastic cages measuring 25.5 cm wide, 47 cm long, and 21 cm high in a temperature controlled room with a 12-h light/dark cycle (lights on at 7:00 a.m.). Rats had ad libitum access to water and normal rat chow (Purina, Gray Summit, MO) until the treatment begin. Rats were randomly divided into two treatment conditions: pair-fed (PF; control) and pyriethamine-induced thiamine deficiency (PTD; experimental).

Treatment

After two weeks of acclimation, all rats received 14–18 days of treatment. Regular lab chow (Lab Diet, St. Louis, MO) with thiamine deficient food (Teklad Diets, Madison, WI), as well as administering a daily intraperitoneal (i.p.) injection of thiamine (0.4 mg/kg, i.p. [Sigma-Aldrich, St. Louis, MO]) for PF rats or pyriethamine for PTD rats (0.25 mg/kg, Sigma-Aldrich.). As treatment continued, PTD rats started to display neurological signs of anorexia, ataxia, loss of righting reflex, and eventually opisthotonus activity. Approximately 4 hours and 15 min after the onset of opisthotonus, PTD-rats were administered a reversal injection of thiamine hydrochloride (100 mg/kg, i.p.). The seizure-like activity that occurs with PTD treatment is a marker of glutamate excitotoxicity that subsides shortly after the administration of thiamine (Robinson & Mair, 1992). A second dose of thiamine hydrochloride (100 mg/kg, i.p.) was administered 24 hours later to PTD rats to ensure survival and recovery. The control PF group had food reduced to match the weight of the PTD group. After PTD rats were reversed with thiamine both PTD- and PF-treated rats were given ad libitum access to regular rat chow.

Surgery

After 2 to 3 weeks of free-feed recovery, each rat underwent stereotaxic surgery to implant cannulae into any of three locations: PFC, HPC, and Rh-Re. Prior to surgery, animals were anesthetized with an i.p. injection (1.0 mL/kg) of a ketamine (83 mg/kg) / xylazine (17 mg/kg) mixture. After subjects were nonresponsive to a tail-pinch, they were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA).

In Experiment 1a, rats had two guide cannulae (26 gauge, Plastics One, Roanoke, VA) implanted, aimed at both the PFC and HPC. Half of the rats (randomly determined) had the cannulae implanted in the same hemisphere (unilateral) and the other rats had the cannulae implant in different hemispheres (bilateral). The stereotaxic coordinates (Paxinos & Watson,

2007) were 2.7 mm anterior to bregma, ± 0.7 mm lateral to the midline, and 3.0 mm below dura for the PFC, and 5.3 mm posterior to bregma, ± 5.1 mm lateral to the midline and 4.2 mm below dura for the HPC. In Experiment 1b, rats had a single guide cannula implanted into either the PFC or the HPC in one hemisphere. The same coordinates used in Experiment 1a were used in Experiment 1b.

Similar to Experiment 1a, rats in Experiment 2 had two cannulae implanted bilaterally across the PFC and HPC, plus an additional third cannula aimed at the Rh-Re (26 gauge), implanted at a 26° angle. The stereotaxic coordinates for Rh-Re were 2.2 mm posterior to bregma, ± 3.5 mm lateral to the midline, and 8.0 mm below dura (Hembrook, Onos & Mair, 2012; Paxinos & Watson, 2007).

In all surgeries, two self-tapping bone screws were positioned in the skull surrounding the guide cannulae. Dental acrylic (Lang Dental, Wheeling, IL) was used to secure the guide cannula in place atop the skull. Immediately after surgery, all subjects were placed in a warm incubator until they regained an upright posture. All subjects received the analgesic buprenorphine (0.3 ml sc, Buprenorphine hydrochloride, Hospira Inc, Lake Forest, IL) directly after surgery and another injection 24 h post-surgery. Rats were allowed to recover for 7–10 days, followed by a handling period of 4–7 days. After their recovery period, all rats were mildly food-restricted for a few days (90–95% of their free-feed weight). All rats had free access to water throughout the study. Behavioral training began directly after the handling period.

Apparatus

A four-arm plus maze was used for spontaneous alternation. This task was chosen because acetylcholine efflux in the HPC and FC correlates with performance (Anzalone et al., 2010) and lesions of the HPC and PFC disruption performance on this task (Lalonde, 2002). The plus maze had wooden-floors that were painted black and clear plastic-sided arms that measured 44 cm long, 12 cm wide, and the height of the maze walls was 14.5 cm. The maze was elevated 75 cm above the floor in a room with several extra-maze cues.

Microinjections and behavioral testing

Ten minutes prior to behavioral testing, rats were gently restrained and received a series of infusions (0.5 μ L), counterbalanced across days, of artificial cerebrospinal fluid [aCSF; 127.6 mM NaCl, 4 mM KCl, 1.3 mM CaCl₂ dihydrate, 1.0 mM glucose, 0.9 mM MgCl₂, 0.9 mM NaH₂PO₄, and 2 mM Na₂HPO₄, brought to pH 7.0] or the physostigmine doses (see doses dependent on region below), and in Exp. 2 the additional drug muscimol (0.285 pg/ μ L) was infused. All infusions were delivered via a 10 μ L glass syringe (Hamilton, Reno, NV, USA) connected to polyethylene tubing (PE-50, Plastics One) that was attached to a microinjection needle (26-gauge, Plastics One) that extended 0.5 mm beyond the tip of the cannula.

The injection process was timed to take 2 min/site and the needles were left in situ for an additional 1 min, after which rats were given 3 additional minutes in their home cage to allow for adequate diffusion. Each subject was behaviorally tested under all dose conditions

with the order of drug delivery counterbalanced. The order of drug delivery was semi-random, as subjects across groups were matched for dose.

The volume (0.5 μ L) and regional concentrations of physostigmine that were effective in enhancing learning in rats were selected from the literature (Chang & Gold, 2004; Herremans, Hijzen & Olivier, 1997). For Experiment 1a, three counterbalanced dose conditions were used: aCSF, low dose physostigmine, and high dose physostigmine. The low doses of physostigmine were 1.0 μ g/ μ L for the PFC and 20.0 ng/ μ L for the HPC, whereas the high doses were 2.0 μ g/ μ L for the PFC and 40.0 ng/ μ L for the HPC.

In Experiment 1b, we wanted to determine whether unilateral cholinergic stimulation of either the PFC or HPC alone would recover alternation performance in PTD rats. To accomplish this, we used aCSF and the most effective regional doses of physostigmine from Exp 1.a : 40.0 ng/ μ L in the HPC and 2.0 μ g/ μ L in the PFC.

The goal of Experiment 2 was to determine whether deactivating the ReN with muscimol would block the effectiveness of physostigmine delivered across the PFC and HPC. Thus, all rats received 0.5 μ L of either aCSF or physostigmine in their HPC (40 ng/ μ L) and PFC (2 μ g/ μ L) in conjunction with 0.5 μ L of either aCSF or muscimol (0.285 pg/ μ L) in the Rh-Re. The volume and dose of muscimol infused into the ReN was referenced from Hembrook et al. (2012).

Following infusions, the rat was placed back in the holding cage for 3 minutes. Immediately afterward, the rat was picked up and placed on the center of the maze. The rat was allowed to traverse the maze freely for an 18-min period and the number and sequence of arms entered were recorded to determine spontaneous alternation scores. An alternation score was determined by recording the number of different arms (minimum = 1, maximum =4) entered during a four-choice sequence, dividing the number of different arms entered by 4, and multiplied by 100. Upon completion of 18 min of maze testing, rats were transferred back to their home cage and returned to the colony room.

Tissue fixation and histological analysis

At the conclusion of behavioral testing, animals were anesthetized with 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 h, and transferred to a 30% sucrose solution. Coronal sections from the brains were cut (40 μ m thick) on a sliding microtome (Sm2000r; Leica Instruments, Wetzlar, Germany) 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 PTD-induced lesions and probe location.

In order to assess infusion spread, two cohorts of rats' brains (N=10) were subjected to fluorescent muscimol infusion (Life Technologies Corporation, Eugene, OR) prior to perfusion. Ten minutes prior to perfusion, 0.5 μ L injection of fluorescent muscimol was

administered into each cannulated brain region – the HPC, PFC and ReN. In very dim light, after reaching the posterior depth of each cannulation site during slicing, every other slice containing visible signs of cannulation was mounted with an antifade reagent (ProLong Gold with DAPI [Life Technologies Corporation, Eugene, OR]).

Statistical Analysis

In Experiment 1a, the mean percent of alternation and total number of arm entries during spontaneous alternation were analyzed with a two between-subjects factor (Group [PF vs. PTD]; Hemisphere [unilateral=the injection sites of the PFC and HPC were in the same hemisphere], bilateral= the injection sites of the PFC and HPC were in the opposite hemisphere]) repeated measure (within-subjects factor: Dose [aCSF, Low, High]) analysis of variance (ANOVA). A similar mixed model design was used for Experiment 1b, but Hemisphere was replaced by the variable of Structure (PFC or HPC). Additionally, a mixed model ANOVA was used in Experiment 2, with the between-subjects factor of Group and within-subject factor of Dose Condition (aCSF/aCSF, physostigmine/aCSF, aCSF/muscimol, physostigmine/muscimol). All statistical analyses used a significance level of 0.05.

Results

Histology

Across all three experiments a total of 108 rats received treatment of either PTD or PF control treatment. After cresyl violet staining and histological analysis of tissue, it was determined that a total of 22 rats had at least one misplaced cannula (23% miss rate) and were not included in the data analysis. Thus, the final number of rats included was 83. Across the individual experiments, the final number of subjects in each condition was as follows: Experiment 1a: 16 PF [8 contralateral, 8 ipsilateral], 16 PTD [8 contralateral, 8 ipsilateral]; Experiment 1b: 16 HPC [8 PF, 8 PTD], 16 PFC [8 PF, 8 PTD]; Experiment 2: 9 PF and 10 PTD rats.

Thalamic pathology

The PTD-treated rats in all three experiments had neuronal loss in the following nuclei (see Figure 1 for examples of PF [A,C,E] and PTD [B,D,F]): Anteroventral ventrolateral (AVVL) and gelatinosus (G). Neurons in the internal medullary lamina (IML) region of PTD-treated rats were more sparse, but not absent, with the exception that in some animals there was a loss of neurons in the paracentral (PC) and posterior (Po) thalamic nuclei. Thus, the majority of PTD-treated animals (over 65%) fit the classification of IML-spared (see Langlais, Mandel & Mair, 1992). However, the rhomboid nucleus and nucleus reunions were relatively intact. Mammillary body damage was not evaluated, but it has consistently been shown that there is greater than 50% loss of neurons in the medial mammillary bodies (Langlais, Zhang & Savage, 1996). Although the PTD model also causes brain stem pathology, the brain stem cell loss was not evaluated in the current study as previous work suggests that extensive brain stem damage occurs following a more severe PTD treatment paradigm (6–8 hrs after the appearance of opisthotonus activity) than used in the current study (see Langlais, Zhang & Savage, 1996).

Infusion distance

In all regions, fluorescent muscimol spread ranged from about 0.15–0.25 μm laterally and about 0.20 μm in the ventral direction (See Figure 2). However, it should be noted that the molecular weight of fluorescent muscimol is more than the weight of the standard muscimol and thus the reported spread could be an underestimation. Given the size of the PFC and HPC, it is unlikely that there was drug spread into other regions, but the midline thalamic nuclei are smaller, and there could have been some spread into nearby regions such as the lateral gelatinosus nuclei.

Experiment 1a: Circuit-level activation is required for memory recovery in PTD rats

—The hypothesis that increasing cholinergic tone across the PFC and the HPC would increase spontaneous alternation behavior in PTD rats, but not PF rats, was supported (see Figure 3-A; Group X Dose interaction, $F[1, 56]=5.63$, $p<.001$). However, the expectation that the bi-lateral cholinergic stimulation across the PFC-HPC would be more effective than co-stimulation within a single hemisphere was not supported ($F[1,28]=1.10$, $p>0.30$). Regardless of whether the co-infusion of physostigmine across the PFC and HPC occurred in the same or opposite hemispheres, PTD rats showed an improvement on spontaneous alternation scores as the dose of physostigmine increased ($F[2,30]=13.50$; $p<0.01$). In contrast, in the PF Group, increasing the dose of physostigmine in both the PFC-HPC, within or across hemispheres, did not significantly change alternation scores ($F[2,30]=3.08$; $p=0.06$), although there was a trend for the low dose condition to decrease alternation scores relative to the high dose condition.

Based on our previous studies, we did not expect either the PTD treatment or the doses of physostigmine within the PFC-HPC to alter activity level on the maze. In line with these predictions, PTD and PF rats entered an equivalent number of arms during testing session ($F<1$; $p>0.30$) and increasing the dose of physostigmine across the PFC-HPC did not alter this measure of activity ($F<1.72$; $p>0.15$). Overall, across group and dose conditions, the mean number of arms entered during the 18-min testing session was 27 (mean)+3.74 (SEM; see Figure 3.B)

Experiment 1b: Unilateral cholinergic stimulation of a single structure does not recover memory performance

—To determine whether the recovery observed in Experiment 1.a was due co-stimulation across the PFC and HPC, rather than an increase in cholinergic tone in one unilateral site, we conducted Experiment 1.b. The most effective dose of physostigmine from Experiment 1.a, compared to aCSF, was infused into either the PFC or the HPC in one hemisphere. It was found that PF rats has higher alternation scores than PTD rats (main effect of Group, $F[1,14]=15.18$; $p<0.01$). Although the single infusion of physostigmine did not increase alternation performance, there was a significant effect of Dose ($F[2,28]=3.51$; $p<0.05$): Regardless of Group, rats performed best under aCSF, relative to the high dose physostigmine infused into a single hemisphere of the PFC or HPC. There was no Group x Dose interaction ($F<1$). In summary, a unilateral infusion of physostigmine into either the PFC or the HPC, at a dose that was effective at improving alternation performance in PTD rats when bilaterally infused into either structure (Roland et al., 2008; Savage, 2012), or co-infused into both structures across hemispheres (Experiment 1.a), did

not improve alternation scores (see Figure 4.A). Furthermore, unilateral infusion of a high dose of physostigmine into either the HPC or PFC did not affect the number of arms entered ($p>0.15$) and the groups did not differ in the number of arms entered ($p>0.15$, see Figure 4.B).

Experiment 2: The ReN is a vital mediator of PFC-HPC cholinergic-based recovery in PTD rats—

The goal of Experiment 2 was to replicate the effects of Experiment 1.a, as well as test the hypothesis that if the ReN was inactivated, the recovery of behavior seen in PTD rats when physostigmine was infused across the PFC and HPC would be blocked. Similar to Experiment 1.a there was significant interaction of Group x Drug ($F[3,51]=5.03$; $p<0.01$; see Figure 5-A), meaning that the PF and PTD rats responded differently to the drug treatments. To access this interaction, contrasts were conducted to determine how each treatment condition (Group: PF vs. PTD) responded to the drugs conditions.

Similar to Experiments 1.a and 1.b, in the non-drug condition (aCSF [PFC-HPC]/aCSF [Rh-Re]), the PF mean alternation score was significantly higher than the PTD alternation score ($p<0.01$). Replicating the main finding of Experiment 1.a, in the physostigmine (PFC-HPC)/aCSF (Rh-Re) drug condition, there was no significant difference between PF and PTD performance ($p>0.5$), due to the improved performance of PTD rats ($p<0.05$). Conversely, in the aCSF (PFC-HPC)/muscimol (Rh-Re) dose condition, there was no significant difference between PF and PTD performance ($p>0.35$), due to the worsened performance of PF rats (relative to the PF aCSF [PFC-HPC] /aCSF [Rh-Re] condition; $p<0.05$).

The critical test was whether alternation performance would change in both treatment groups when physostigmine was infused across the PFC-HPC and muscimol was co-infused into the Rh-Re. First, the significant difference between the PF and PTD groups re-emerged under this dose condition ($p<0.01$). This was due to the fact that in PTD rats the co-infusion of muscimol into the ReN blocked the enhancing effects of the physostigmine infusion across the PFC-HPC (mean difference of 13.14%, $p<0.05$). Second, in PF rats the impairing effects of muscimol infused into the ReN were counteracted by the co-infusion of physostigmine across the PFC-HPC (mean difference 18.19%, $p<0.01$). Thus, the effects of deactivating the Rh-Re while increasing cholinergic tone in the HPC-PFC were different as a function of diencephalic pathology.

As in Experiments 1.a and 1.b, there were no significant main effects of Group or Dose on the number of arms entered during the testing session ($p>0.50$), and no interaction of Group x Dose ($p>0.15$). Thus, changes in alternation performance were not due to changes in activity on the maze.

Discussion

Thalamic and hypothalamic damage created by thiamine deficiency disrupts hippocampal and frontal cortical functioning, producing a behavioral profile of impaired spatial mapping and working memory dysfunction. Despite the diencephalic damage in the model, behavioral restoration is still obtainable through the modulation of non-diencephalic regions.

This series of studies provided evidence that if cholinergic tone is increased conjointly across the PFC and HPC, by co-infusing regionally effective doses of physostigmine, the traditional spatial memory deficit observed on spontaneous alternation is eliminated in the PTD model. This was predicted given previous work demonstrating that acetylcholine levels in the HPC or FC/PFC are suppressed when PTD rats are engaging in spatial behavior (Anzalone et al., 2010, Savage et al., 2012). A second important finding was that the Rh-Re is a critical and necessary region for the effectiveness of the PFC-HPC cholinergic-based recovery in the PTD model, but it is not necessary in intact PF rats. Although inactivation of the Rh-Re with muscimol impaired spontaneous alternation behavior in control rats (PF group), that deficit was recovered when cholinergic tone was increased across both the PFC-HPC.

Literature implicating the Rh-Re in memory processes (Hembrook & Mair, 2011; Hembrook et al., 2012) and its connectivity to both the HPC and PFC (Vertes, 2002, 2006; Vertes et al., 2007), contributed to our third hypothesis that inactivating the ReN would block the effectiveness of increasing cholinergic receptor activation across the PFC and HPC in the PTD model. This is precisely what was found, demonstrating that in the PTD model, the Rh-Re is a critical region required for restoration of effective HPC-PFC communication. Using both pharmacological activation to increase cholinergic tone in the HPC and PFC, along with inhibition the Rh-Re with muscimol, a new venue of evidence was found that Rh-Re is a key mediating structure for memory information being processed by the HPC and PFC.

Another interesting finding is that while we expected bilateral infusions of physostigmine across the HPC-PFC to produce significant behavioral recovery in PTD rats, we did not hypothesize that a unilateral co-infusion of physostigmine across the HPC-PFC would lead to recovery as well. However, the Re can promulgate signals bilaterally, thus still ensuring circuit-level activation (Dolleman-Van der Weel et al., 1997; Van der Werf et al., 2002; Vertes et al., 2007). Importantly, Experiment 1.b found that unilateral stimulation of either the HPC or the PFC alone did not rescue memory performance. This is an important finding for the notion that activation must occur at the circuit-level for recovery.

Although PF rats were not initially affected by cholinergic co-activation of the HPC-PFC circuit, they demonstrated a different behavioral profile when contrasted to the amnesic PTD rats, after Rh-Re deactivation. Similar to other studies using intact rats (Hembrook & Mair, 2011; Hembrook et al., 2012), deactivation of the Rh-Re impaired memory performance of PF rats. The novel finding was that the PF group was not impaired on spontaneous alternation when deactivation of the Rh-Re was paired with bilateral co-infusion of physostigmine across the HPC and PFC. Thus, when cholinergic tone is increased across the HPC and PFC, a fully functioning Rh-Re complex is no longer critical for spatial working memory in the intact rat. Therefore, there must be parallel pathways that can compensate for an inactive Rh-Re when there is cholinergic stimulation of the PFC-HPC circuit. Furthermore, given the memory impairment of PTD rats when the Rh-Re is inactivated at the same time that the PFC-HPC circuit is infused with physostigmine, the alternative pathways are likely to be dysfunctional in the PTD model.

Compensation for a dysfunctional ReN in intact rats may be mediated through the interactions across other thalamic circuits such as the anterior thalamus-mammillary body-HPC circuit and/or the intralaminar nuclei-PFC circuit, which are both damaged by thiamine deficiency. The HPC, via the fornix, sends projections to the anterior thalamus and mammillary bodies and indirectly through the mamillothalamic tract (Groenewegen & Witter, 2004). The anterior thalamic nuclei project directly to the hippocampal formation via the cingulum bundle. Furthermore, the anterior thalamic nuclei have an indirect route to the hippocampal formation via the retrosplenial cortex, a route that is reciprocal. In addition, the anterior nuclei, as a complex, project to the medial PFC (Aggleton et al., 2010). Furthermore, projections from several rostral and lateral intralaminar nuclei target the medial and orbital areas of the PFC, and agranular insular areas also receive input from the HPC (Price, 1995; Groenewegen & Witter, 2004). A non-thalamic parallel pathway that could also compensate for HPC- Rf/Re -PFC interactions is the parahippocampal-hippocampal-prefrontal cortex pathway. The perirhinal cortex is reciprocally connected with the ventromedial PFC (Delatour & Witter, 2002) and, via entorhinal cortex, to the HPC (van Strien et al., 2009; Witter et al., 2000). Thus, there are several alternative pathways may enable information flow between the HPC-PFC when the Rh-Re is deactivated.

In conclusion, we discovered that circuit-level bilateral cholinergic activation of the PFC and HPC recovers memory functioning in the PTD model, but that unilateral-single-structure activation of either the PFC or HPC is insufficient for such a recovery. Also, we provided additional evidence that the Rh-Re is a pivotal region for transmission of memory information between the PFC-HPC circuit. The first set of data supporting the importance of the Rh-Re is provided by the fact that the recovery of spatial memory after cholinergic activation of the PFC-HPC in the amnesic rats is eliminated when the Rh-Re is deactivated with muscimol. Second, similar to other research (Cholvin et al., 2013; Hembrook et al., 2012), we revealed that inactivation of the Rh-Re disrupts working memory performance in normal rats. However, in intact rats, when inactivation of the Rh-Re co-occurs with cholinergic activation of the PFC-HPC regions, successful spatial behavior is sustained. Thus, there are other parallel pathways, potentially involving other thalamic regions or the entorhinal cortex, that have the ability to mediate communications between the PFC and HPC in a manner similar to the Rh-Re. This evidence of parallel pathways reveals future avenues for memory circuit research.

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List of Abbreviations

Ach	acetylcholine
aCSF	artificial cerebrospinal fluid
AD	anterodorsal nucleus
AVDM	anteroventral dorsal medial nucleus

ANOVA	analysis of variance
AVVL	anteroventral ventrolateral nucleus
CL	centrolateral nucleus
CM	central medial nucleus
FC	Frontal Cortex
HPC	hippocampus
ILM	intralaminar nuclei of the thalamus
MD	medial dorsal nucleus
PC	paracentral nucleus
Physo	physostigmine
PF	pair-fed
PfN	parafascicular nucleus of the thalamus
PFC	prefrontal cortex
Po	posterior thalamic nucleus
PTD	pyrithiamine-induced thiamine deficiency
ReN	nucleus reuniens of the thalamus
Rh	rhomboid nucleus
WKS	Wernicke-Korsakoff Syndrome

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Highlights

- Anterior and midline thalamic damage impairs alternation behaviors.
- Increasing cholinergic tone across the prefrontal cortex and hippocampus recovers spatial behaviors.
- Inactivation of the ventral thalamus blocks the efficacy of cholinergic stimulation.

Figure 1.

Neuronal specific nuclear protein (NeuN) stained slides comparing the thalamus of PF and PTD rats (from uncannulated rats). The top row reveals the anterior thalamic nuclei in both PF (A) and PTD (B) rats. In PTD rats there is selective cell loss (see arrows) in the anteroventral ventrolateral (AVVL) with relative sparing of the anteroventral dorsal medial (AVDM) and anterodorsal nuclei (AD). The middle row (C=PF and D=PTD images are of ventral midline thalamic structures. As in previous papers using a similar model, there is a decrease in neurons in the gelatinosus nuclei (G) and relative sparing in the rhomboid

nucleus (Rh) and the nucleus reuniens (ReN). The last row (E=PF and F=PTD) consists of the dorsal midline thalamic nuclei. In this series of experiments the majority of PTD rats had mild cell loss in some intralaminar nuclei (central medial [CM], centrolateral [CL] nuclei) and the medial dorsal (MD) nucleus. Significant cell loss was seen in paracentral (PC) and posterior thalamic (Po) nuclei (see arrows).

Figure 2.

Cannula placement within the prefrontal cortex (A-PFC), hippocampus (C- HPC), and nucleus reuniens (E- Rh-Re). Coordinates (in mm) in A, C, and E are from Paxinos and Watson (2007) and relative to bregma. Each dot corresponds to the location of the cannula as identified under a microscope (5X). Panels B (PFC), D (HPC) and F (Rh-Re) are photomicrographs showing a representative location/diffusion radius of fluorescent muscimol injections. White lines delineate the boundaries of the PFC (B), the HPC (D) and the midline thalamus (F).

A. Dual HPC-PFC infusion: Spontaneous Alternation Data

B. Dual HPC-PFC infusion: Activity Data

Figure 3.

Panel A. The significant effect of unilateral and bilateral (collapsed due to non significance) physostigmine infusion into the HPC and PFC. The low dose was 1.0 $\mu\text{g}/\mu\text{L}$ for the PFC and 20.0 $\text{ng}/\mu\text{L}$ for the HPC, and high dose was 2.0 $\mu\text{g}/\mu\text{L}$ for the PFC and 40.0 $\text{ng}/\mu\text{L}$ for the HPC. PF rats experienced no change in spontaneous alternation performance, whereas PTD rats experienced a dose-dependent recovery of function. Panel B. The PF and PTD groups did not display any differences as a function of dose in activity as measured by the mean number of arms entered during the testing session.

ns = no significant difference; * = $p < 0.05$ for PF>PTD; Λ = $p < 0.05$ for drug dose increasing performance

Figure 4.

Panel A. Infusions of physostigmine into the a single unilateral site within the HPC (40.0 ng/ μ L) or PFC (2.0 μ g/ μ L) in either PF or PTD rats did not change spontaneous alternation behavior relative to an infusion of artificial cerebrospinal fluid (aCSF). Regardless of drug condition, PF rats had significantly higher alternation scores than PTD rats. Panel B. PF and PTD groups did not display any differences in activity as a function of drug condition as measured by the mean number of arms entered during the testing session.

A: Contralateral stimulation with/without ReN Blockade Spontaneous Alternation

B: Contralateral stimulation with/without ReN Blockade Activity Data

Figure 5.

Panel A. Changes in spontaneous alternation performance as a function of increasing cholinergic tone contralaterally across the HPC and PFC and inactivation of the midline Rh-Re. The first set of bar graphs (aCSF/aCSF) reveals that when aCSF was infused into the HPC and PFC, as well as the Rh-Re, we see the traditional working memory impairment in PTD rats (black bars), relative to the PF rats (white bars). Similar to Exp 1.A, when physostigmine was infused across the PFC-HPC, and the Rh-Re received aCSF (Physo/aCSF), the working memory impairment in the PTD group was eliminated (second set of bar graphs). When both the PFC-HPC received aCSF, in combination with inactivation of the

Rh-Re with muscimol (aCSF/Musc), the performance of the PF rats drops to the level of the PTD rats. A key finding was that when physostigmine was infused across the PFC-HPC, and the Rh-Re was inactivated (Physo/Musc), the traditional recovery seen in PTD rats after cholinergic stimulation of the PFC-HPC was blocked. In contrast, inactivation of the Rh-Re while the PFC-HPC was infused with physostigmine, blocks the impairing effects of muscimol infusion into the Rh-Re in PF rats. Physostigmine dose was 2.0 $\mu\text{g}/\mu\text{L}$ for the PFC and 40.0 $\text{ng}/\mu\text{L}$ for the HPC, and muscimol dose was 0.285 $\text{pg}/\mu\text{L}$. Panel B. The PF and PTD groups did not display any differences in activity as a function of drug condition as measured by the mean number of arms entered during the testing session.

ns = no significant difference; * = $p < 0.05$ for PF > PTD; v = $p < 0.05$ for drug decreasing performance; Λ = $p < 0.05$ for drug increasing performance