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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 pyrithiamine-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.

Key words: amnesia, frontal cortex, hippocampus, reuniens nucleus memory, rat.

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 and Squire, 2006; Oscar-Berman, 2012; Brion et al., 2014). Thus, diencephalic or thalamic amnesia has been commonly referred as a ''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 and Harper, 2012). However, there is functional deactivation in other brain regions that contribute to the amnestic 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 pyrithiamine-induced thiamine deficiency (PTD) model of WKS (Savage et al., 2012). Thus, WKS patients and the PTD model display a breakdown of the functional integration of several regions within the limbic system (thalamus, HPC, cortex). However, using the PTD model, it has been demonstrated that if cholinergic tone is increased in the HPC (Roland et al., 2008) or frontal cortex (FC) (Savage, 2012) spatial memory can be restored. These results suggest that regions that are ''functionally'' (HPC, 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 HPC and PFC (Cassel et al., 2013). Projections from the HPC terminate in the medial and orbital PFC; however, reciprocal connections back to the HPC are

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Abbreviations: ACh, acetylcholine; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; FC, frontal cortex; HPC, hippocampus; ILM, intralaminar nuclei of the thalamus; IML, internal medullary laminar; PC, paracentral nucleus; PF, pair-fed; PFC, prefrontal cortex; Physo, physostigmine; PTD, pyrithiamine-induced thiamine deficiency; ReN, nucleus reuniens of the thalamus; Rh, rhomboid nucleus; WKS, Wernicke–Korsakoff syndrome.

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sparse (Vertes et al., 2007). Conversely, the PFC densely projects to the Re (Vertes, 2002; Hoover and Vertes, 2012) and the Re projects to the CA1 region of the HPC as well as the subiculum (Vertes et al., 2007; Prasad and Chudasama, 2013). 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 HPC; 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 and Mair, 2011; Hembrook et al., 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., Indianapolis, IN, USA) weighing between 250 and 275 g 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, USA) until the treatment begin. Rats were randomly divided into two treatment conditions: pair-fed (PF; control) and PTD (experimental).

Treatment

After 2 weeks of acclimation, all rats received 14–18 days of treatment. Regular lab chow (Lab Diet, St. Louis, MO, USA) with thiamine deficient food (Teklad Diets, Madison, WI, USA), as well as administering a daily intraperitoneal (i.p.) injection of thiamine (0.4 mg/kg, i.p. [Sigma–Aldrich, St. Louis, MO, USA]) for PF rats or pyrithiamine 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 h 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 and Mair, 1992). A second dose of thiamine hydrochloride (100 mg/kg, i.p.) was administered 24 h 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. Experimental procedures were conducted in accordance with the National Institute of Health (NIH) guide for the care and use of laboratory animals, and were approved by the Institutional Animal Care and Use Committee (IACUC) at the State University of New York at Binghamton. Care was taken to minimize animal suffering and the number of subjects used.

Surgery

After 2–3 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, USA) 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 and 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 (Paxinos and Watson, 2007; Hembrook et al., 2012).

In all surgeries, two self-tapping bone screws were positioned in the skull surrounding the guide cannulae. Dental acrylic (Lang Dental, Wheeling, IL, USA) was