The Effect of Inactivation of the Nucleus Reuniens on Spatial Working Memory

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Introduction

- The Nucleus Reuniens (RE) of the ventral midline thalamus has extensive reciprocal innervations with the medial prefrontal cortex (mPFC) and the hippocampus (HIP) in the rat brain (Hoover & Vertes, 2012). Both structures are essential for encoding, retrieval, and delayed spatial working memory (Churchwell & Kesner, 2011).
- Working memory refers to the holding in mind of task-relevant information for use in goal-directed behavior (Baddeley, 1986).
- The RE is believed to play a vital role in facilitating cognitive function and affective behavior and has been shown to be critical for a variety of working memory tasks (Vertes et al., 2015).
- Here, we examined the role of RE in increasing working memory demand in a delayed spatial alternation T maze task.
- We hypothesized impairments would be greater for randomized than fixed delays at both 30s and 120s following RE activation.

Methods

Subjects: 7 Long-Evans rats (repeated measures, within subjects design).

Surgical Procedures: Rats were anesthetized using isoflurane (4-5% induction, 1.5-3% maintenance). Guide cannula were implanted at the following stereotaxic coordinates targeting RE: *-2.1 AP* from *Bregma; +2.0 L* at an angle of 15 degrees & *6.0 V.* Following a one week post-surgical recovery period, rats were put on a food-restricted diet, maintaining their body weight at 85% of their free-feeding weight for the entire duration of the experiment.

Behavioral Testing: Rats were trained on a delayed alternation task, using an elevated T-maze. Each testing day consisted of 10 trials, each with a choice and a sample run. Food wells containing a chocolate pellet (BioServ, CA, USA) reward were located at the end of each goal arm. During the sample run, each goal arm was baited with a reward and the rat was allowed to choose either arm to receive the pellet. After returning to the start arena, between each trial, rats were presented with a pseudo-randomized or fixed delay period of 30s, or 120s. During the choice run, only the previously non-visited goal arm contained a food reward. For a trial to be considered correct, the rat must choose a path during the sample run and then alternate pathways during the choice run. If the rat selects the incorrect goal arm during the choice run, they are to repeat the trial until the correctly baited arm is visited and the reward is successfully received. After reaching 80% correct performance, rats were infused over several testing days with muscimol, a GABA-A receptor agonist and saline on separate testing days. Afterwards, rats were given infusion-free days until they regained their asymptotic performance.

Histology: At the conclusion of behavioral testing, rats were deeply anesthetized with 250mg/kg of Euthasol and intra-cardially perfused with 4% paraformaldehyde. Brains were then extracted; coronally cut into 50 um slices; mounted onto chrome-alum gelatin-coated slides; and Nissl-stained with Cresyl Violet to assess cannula placement.

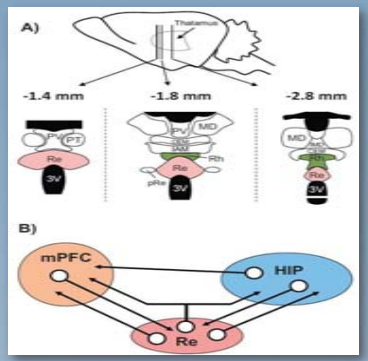
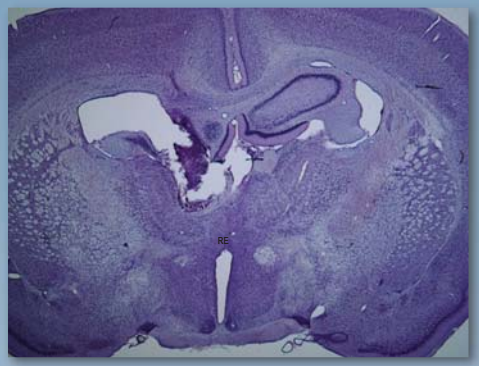
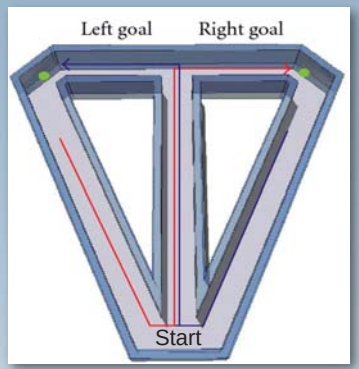


Figure taken with permission from Cassel et al (2013). This schematic diagram of the midline thalamus illustrates the location of nucleus reuniens (RE) and circuit diagram of the innervations between the medial prefrontal cortex (mPFC), RE, and the hippocampus (HIP).



Photomicrograph of cresyl violet-stained coronal section through the midline thalamus for a representative case showing guide cannula placement above mid rostrocaudal level of RE.



Adapted from Lipton (2008). Actual Behavior Apparatus: T-maze plan. Dimensions in cm: central stem 116L x 10W; goal arms (each) 56.6L x 10W; return arms (each) 112L x 10W. Wall height for maze 10 cm.

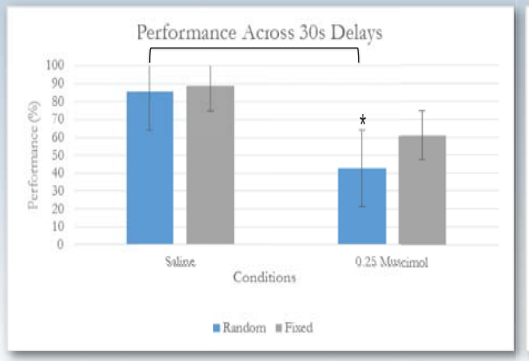


Figure 1. Differences between fixed and randomized 30s Delays. Bars represent SE.

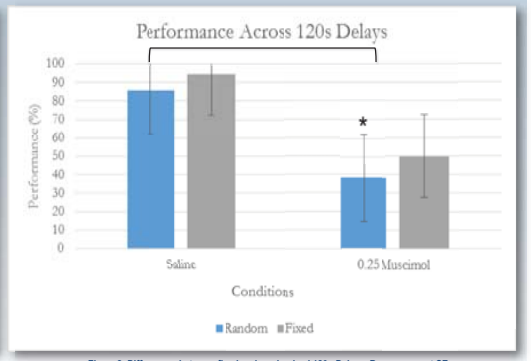


Figure 2. Differences between fixed and randomized 120s Delays. Bars represent SE.

Results

- A paired samples t-test was conducted to compare performance across delay periods, between 30s fixed vs random delays and 120s fixed vs random delays.
- There was a significant difference in performance between saline 30-second random delay trials (M= 85.71, SD= 17.82) and 0.250 mg/ml muscimol 30-second random delay trials [M=42.85, SD=25.19; t(6)= 3.58, p< 0.05].
- There was a significant difference in performance between saline 120-second random delay trials (M= 85.71, SD= 26.23) and 0.250 mg/ml muscimol 120-second random delay trials [M= 38.09, SD= 22.99; t(6)= 3.33, p<0.05].
- Muscimol reduced performance at 30s and 120s fixed delays compared to saline fixed delays. Fixed delay ratios are not significant, nevertheless, a performance at fixed delays seemed higher than at random delays for both delay periods (30s, 120s).

Discussion

- In support of our hypothesis, the selective inactivation of RE of the ventral midline thalamus using muscimol caused working memory performance deficits in the T-maze spatial alternation task across random and fixed delays (30s & 120s).
- To compare, saline infusions in RE had no effect on performance across any delay period.
- Although no significant difference was found between randomized delay trials (30s, 120s) and fixed delay trials (30s, 120s), we believe this was due to the small sample (fixed delays N=2) collected thus far. However this provides preliminary findings of differences among delay ratios. Further investigation will give a better view of the effect that randomized and fixed delays have on memory performance.
- Working memory tasks require both the mPFC and the HIP. Studies have found that the HIP is recruited in a delay-dependent manner, whereas mPFC is independent of delay restraints, only necessary for spatial navigation in T-maze tasks (For review, see Dudchenko, 2004).
- Delayed spatial alternation impairments after inactivation of RE suggests that RE plays an important role as a modulator of information exchange between mPFC and HIP.

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