# Functional connectivity MRI reveals memory networks after maze learning in rodents

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## Introduction

Memory employs a series of cognitive processes encompassing the acquisition of information and its subsequent consolidation, retention, and retrieval. Morris water maze has been widely used for studying spatial learning & memory and to evaluate memory impairment and treatment effects in rodent models [1, 2]. To understand how brain regions work together at each stage of memory, methods including cytochrome oxidase histochemistry, cFOS, and 2-deoxyglucose have been used to map functional changes after maze learning. However, these techniques are invasive and do not allow repeated measures. BOLD fMRI has been used to study brain activation under sensory stimulation in rodents, but probing cognitive function using this method is not possible due to physical restrictions of MRI and the use of anesthetics. Recent studies using functional connectivity MRI (fcMRI) have shown plasticity of the intrinsic brain network after intensive training in humans [3]. As this method does not require direct stimulation/task during scanning, we explored the potential to detect plasticity after learning longitudinally in anesthetized rodents.

## Methods

This study was approved by the local Institutional Animal Care and Use Committee. **Behavioral training:** Male Wistar rats (350-400g) were divided into two groups: the trained group (n=15), where animals learn to locate a hidden platform in the water maze with spatial cues, and the swim control group (n=12). Animals were habituated for 5 days before the training. The task consisted of 10 trials per day for 5 days. The latencies and distances to reach the hidden platform were recorded. **MRI:** All rats were imaged at day 1 and day 7 after training. Animals were first anesthetized with isoflurane (3%) after which medetomidine (Dormitor, Pfizer) was injected and isoflurane was turned off. A bolus of 0.05 mg/kg medetomidine was administered by i.p. and then sedation was maintained with 0.1 mg/kg/hr infusion rate. Resting-state fcMRI was acquired on a Varian 9.4T scanner using spin-echo echo-planar-imaging (EPI) with the following parameters: TR/TE=500/30 msec, 25.6x25.6 FOV, 64x64 matrix size, and 6 slices with 1mm thickness and 0.1 mm gap. **Analysis:** Correlation analysis was performed using an ROI based approach. The hippocampal CA3 region was chosen as a main hub for the functional network after maze training [4]. Correlation maps were thresholded at correlation >0.35 and cluster >4 pixels. Besides, correlation coefficients between the seed ROI and regions known to be involved in spatial learning were calculated, including anterior hippocampus (A hp), posterior dentate gyrus (DG p), entorhinal cortex (Ect), visual cortex (VC), cingulate cortex (CC), restrosplenial cortex (RSC), parietal cortex (PC), and anterodorsal thalamus (ADT) [4].

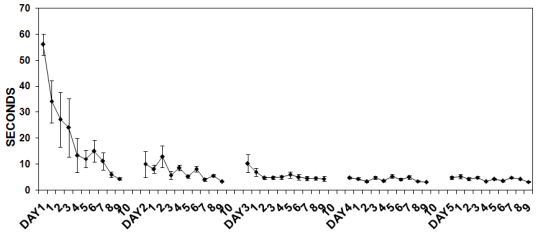


**Fig. 1.** Mean escape latencies (±S.D.) of the hidden-platform trained group across 5 days of the experiment.

## Results

Rats showed significant improvement in their performance as manifested by decreased swimming time (Fig 1). FcMRI of trained rat revealed extensive connectivity across all the brain especially in those regions related to spatial memory compared to swim control (Fig.2a). Functional connectivity was mostly maintained at day 7 in both groups. Significant increase of correlations were seen in the trained group in ADT, VC, RSC, A hp, DG p, and Ect, which were still significant even at day 7.

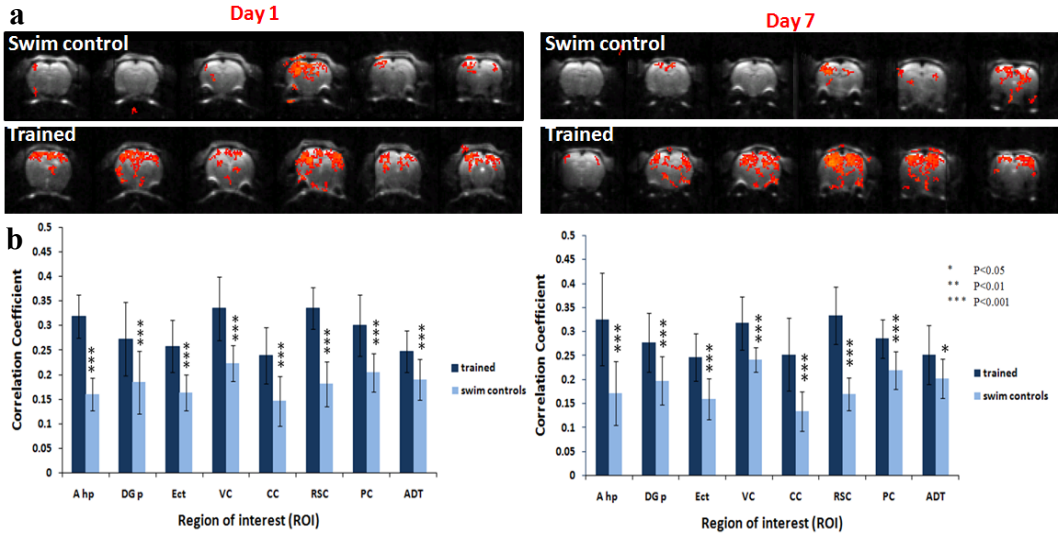


**Fig. 2.** (a) functional connectivity maps of a rat from each group at day 1and day 7 respectively. (b) correlation coefficients of different ROIs with respect to hippocampal CA3 at day 1 and day 7.

## Discussion

To our knowledge, this is the first demonstration that brain plasticity following a cognitive task can be detected in the anesthetized rat using fcMRI. Persistent and consistent brain networks after learning were observed, which mainly reveals changes associated with the hippocampal area that is inherent to the memory processes. The capability to map memory function in vivo using the same technique in both animal models and humans will facilitate our understanding of this important cognitive process, as well as its application for early detection of dementia, and evaluation of treatment. Additional analyses on how the brain connectivity relates to behavior and group analysis are in progress.

**References:** [1]Vorhees CV & Williams MT. Nat Protoc 2006; 1:848-858. [2]D'Hooge, R, De, Deyn PP. Brain Res Brain Res Rev 2001 ; 36 :60-90. [3] Lewis CM et al. PNAS 2009;106:17558-63. [4] Conejo et al. Neurobiology of Learning and Memory 2010 ; 93 : 362-371
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