Resting-state MRI detects donepezil effects on functional connectivity and maze learning in rodent brain

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Introduction

Donepezil is a clinically effective palliative treatment for improving cognitive functioning in patients with mild to moderate degrees of dementia [1]. It increases acetylcholine level in synapse by selectively inhibiting acetylcholinesterase [2]. Repeated administration of donepezil has also been found to reverse memory impairments in rodent models [3] and improve the performance of Morris watermaze in aged rats [4]. However, it is still unclear how cholinergic treatments alter or improve existing neural networks and neurophysiological mechanisms in specific regions of the brain. Recent studies using resting-state functional connectivity MRI (fcMRI) on dementia patients treated with donepezil showed increased connectivity in hippocampal [5] and prefrontal cortex [6], but how donepezil altered the brain connectivity in memory task is not clear. Previously we demonstrated that fcMRI could detect the plasticity of hippocampal network in rodents after maze learning [7]. Here, we assessed the effects of donepezil in the rodent brain before and after on-demand Atlantis watermaze learning.

Methods

This study was approved by the local Institutional Animal Care and Use Committee. **Animal preparation**: Male Wistar rats (350-400g) were divided into two groups: the Donepezil-treated group (n=10), and vehicle-treated group (n=10). Animals were dosed intraperitoneally at 0.5 mg/kg Donepezil or vehicle (0.9 % w/v saline) once a day for two weeks prior to the training and throughout the training period, 1 hour prior to the first trial. **Behavioral training:** animals learn to locate a hidden platform in the Atlantis watermaze (AWM) with spatial cues over four days (six trials/day) with increasing difficulty by gradually extending the dwell time required to trigger the platform. The latency, distance, and speed to reach the hidden platform for each rat were recorded. **MRI:** All rats were imaged at day 1 and day 7 after completion of the 4-day maze 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 9.4T scanner using spin-echo EPI with TR/TE=500/30 ms, 25.6x25.6 mm^2 FOV, 64x64 matrix size, and 6 slices with 1mm thickness and 0.1 mm gap. **Analysis:** fcMRI data were bandpass filtered at 0.01-0.1Hz, smoothed by 2.0x2.0 mm3 Gaussian filter, and registered to the Paxino's atlas for group analysis using FSL. Connectivity was analyzed by correlation with ROIs on left and right hippocampal CA3. One sample t-test was conducted to create group connectivity maps of the donepezil- and vehicle-treated rats ($p < 0.001$). Two sample t-tests were used to compare the donepezil versus vehicle groups ($p < 0.05$, uncorrected).

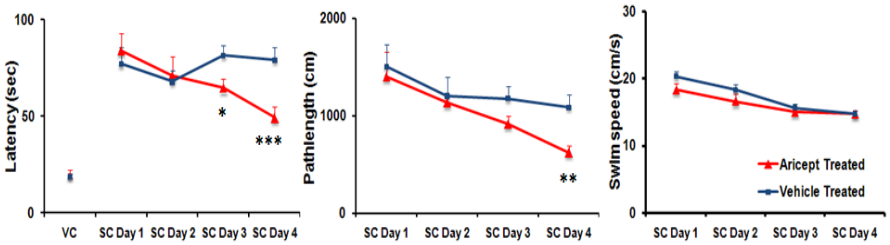

Fig. 1. Mean escape latencies, path length, and swim speed (±SEM) of the Donepezil- and Vehicle-treated group across 4 days of the AWM training.

Results: Donepezil administration significantly improved the AWM performance as manifested by a decrease in swimming time and path length taken to reach the platform with no change in speed compared to vehicles (Fig. 1). Fig. 2a shows that AWM training induced an increase in connectivity to regions known to be part of spatial memory networks such as the parietal cortex, habenula, medial thalamus, superior colliculus and contralateral hippocampus. Enhanced connectivity with the CA3 regions was detected after dosing with donepezil (Fig. 2b), Donepezil before training increased connectivity with the cortical areas dramatically, especially, the piriform cortex, caudate putamen, cingulate, sensorimotor, visual, substantia nigra, and hippocampus. After training with AWM, donepezil enhanced connectivity in subcortical areas such as the substantia nigra, hippocampal areas, habenula, and hypothalamus.

Discussion: Consistent with study in AD, donepezil treatment alone could increase connectivity in hippocampus and cortical areas. Furthermore, increased connectivity in brain networks associated with the hippocampal CA3 after donepezil treatment were observed, which reveals changes inherent to the memory processes. These results suggest that the alteration of the functional connectivity pattern after donepezil treatment is closely associated with cognitive improvement and therefore, changes in connectivity may serve as a marker to monitor effects of various drugs on treatment and therapy response. The capability to map functional changes in vivo using the same technique in both animal models and humans will facilitate our understanding of this important cognitive process and treatment effects.

References: [1]Cutuli D et al. Psychopharmacol 2008; 197 :661-73, [2] Haug KH et al. Neurochem Res 2005; 30:1511-20, [3] Bontempi B et al. Neuropsychopharmacol 2003; 28:1235-46, [4] Hernandez CM et al. J Pharmacol Exp Ther 2006; 16:679-94, [5] Goveas JS et al, JMRI 2011; 34:764–773, [6] Zaidel L et al. J Alzheimer's Dis 2012; 31:S221-6, [7] Nasrallah FA et al. Proc. ISMRM. 19 (2011).

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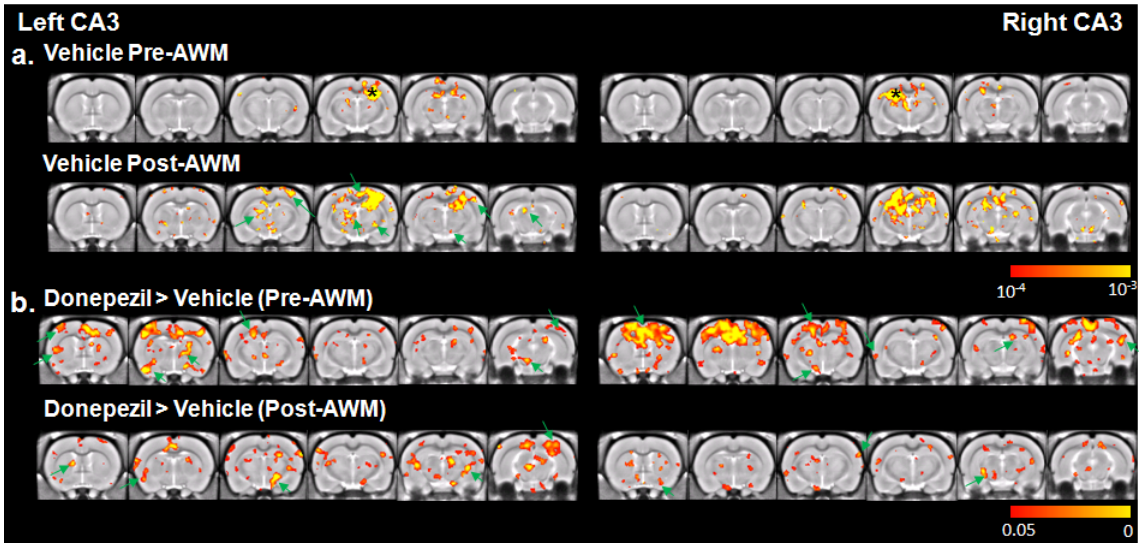

Fig. 2. (a) Group functional connectivity maps of rats treated with vehicle only before (top), and after (bottom) AWM training. Stars show the locations of seed ROIs.
(b) Difference in functional connectivity between donepezile and vehicle groups before (top) and after (bottom) AWM training. Green arrows indicate significant changes between groups.