MEMRI reveals neuronal changes in specific hippocampal substructures following sleep deprivation

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INTRODUCTION: Sleep is a vital biological process in mammalian brains. The sleeping process can be categorized into two major types, namely rapid eye movement (REM) sleep and non-REM (NREM) sleep. NREM sleep is important for energy conservation and recuperation while REM sleep is closely related to learning, memory and emotional regulation (1). Hence, the lack of sleep not only compromises alertness and cognitive performance, but also induces adverse effects in memory and emotional behaviors (2). Presently, studies related to sleep deprivation have focused on the cognitive ability and task performance of the subjects (3, 4). Meanwhile, electrophysiological recording techniques were employed in some studies to probe the sleep deprivation induced brain activity changes (5). In this study, we aim to employ high resolution in vivo MEMRI to probe the neuronal changes in the rat brain following 72 hours of REM sleep deprivation (REM-SD).

MATERIALS AND METHODS: Animal Preparation: Sprague Dawley rats (N=12, 8 weeks old, 350-380g) were divided into two groups. Group 1 (N=6): Sleep deprivation group; Group 2 (N=6): Normal control without sleep deprivation. All animals received IP injection of $MnCl_2$ solution (30mg/kg) daily for three consecutive days, immediately after the pre-scan. MRI scans were then performed on the animals 72 hours after the pre-scans to obtain the post enhancement images. Animals in Group 2 had the same day/night schedule as Group 1 only without sleep deprivation. **Sleep Deprivation** (6)**:** A 72-hour REM-SD was employed using the multiple small platform technique. Five platforms with each 6 cm diameter were placed in the middle of a water tank. Platforms were spaced 9 cm apart so that rats could easily move between them but could not lie across any two. Food and water were available ad libitum. All treatments lasted 72 hour under a 12 hour day/night cycle. Video monitoring was performed throughout REM-SD and was used for later behavioral validation. **MRI Protocol:** All images were acquired using a 7T Bruker MRI scanner. During MRI scan, rats were anaesthetized with isoflurane with respiratory monitoring. T1WIs were acquired with a RARE sequence using FOV = 3.0x3.0mm, MTX = 256x256, slice thickness = 0.8mm, TR/TE = 460/7.5ms, RARE factor = 4, and NEX = 40. T2WIs were acquired using the same voxel dimension and slice geometry with TR/TE = 4200/39ms, RARE factor = 8, and NEX = 2. **Data Analysis:** Each pre and post Mn T1WI datasets was first normalized by the average signal in the entire brain. All pre and post deprivation T1WIs were then co-registered together with reference to their respective T2WIs using SPM5. A percentage change map revealing the difference between the two groups was computed using the co-registered image sets for visualization and quantification of the signal intensity differences. ROIs were manually defined according to the rat brain atlas. Mann-Whitney test was performed between the two groups.

RESULTS: Fig.1 illustrates the design of the experiment. In this study, fractionated Mn injection (7) was employed and the injection dosage was chosen to minimize the toxicity effects on the animals while allowing sufficient T1 contrast enhancement. Fig.2 shows the averaged post-Mn T1WIs of the two groups of animals. The percentage change map revealed that the hippocampus was enhanced less in the SD animals. In particular, the dentate gyrus (DG) sub-region of the hippocampus showed the most pronounced difference. The cortical region was also observed to have low Mn uptake in SD animals. Fig.3 shows the statistical comparisons, confirming the Mn enhancement differences in hippocampus ($p<0.01$), especially the DG sub-region ($p<0.001$).

DISCUSSION AND CONCLUSION: The major finding of this study is the significant difference in post-Mn T1WIs signal intensity in the hippocampus between SD and control group. This neuronal change in SD animals as inferred from the decreased Mn uptake may arise from neuronal loss and synaptic remodeling in different substructures of the hippocampus. Studies have related these changes to impaired hippocampal-dependent learning (6, 8, 9). The DG in the hippocampus showed the most significant signal difference. This may be due to the reduced cell proliferation in the DG as a result of reduced REM sleep (10). In addition, DG is known to be one of the key sites for neurogenesis in the adult mammalian brain (11). It is thus plausible that the inhibited hippocampal neurogenesis and reduced cell survival may contribute to the prominent neuronal changes in the DG, rendering this specific hippocampal sub-region more susceptible to SD (9, 12, 13). Lastly, the slight decrease in cortical Mn uptake that reflected reduced neuronal activity may be a consequence of the prolonged period of wakefulness (14). Looking ahead, complementary MEMRI studies involving longer sleep deprivation period or concerning the Mn uptake pattern following recovery sleep can be performed to further shed light on the neuronal alterations induced by sleep deprivation. In summary, the result of this study reveals the sleep deprivation induced neuronal changes in specific hippocampal substructures.

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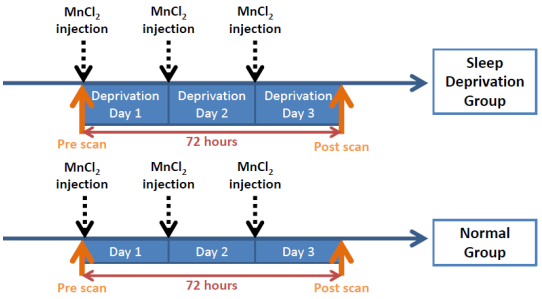

Fig.1 The experimental design. Manganese (Mn) injection was carried out in a fractionated manner with a daily dose of 30mg/kg for three consecutive days. Post injection scans were carried out 3 days after the induction.

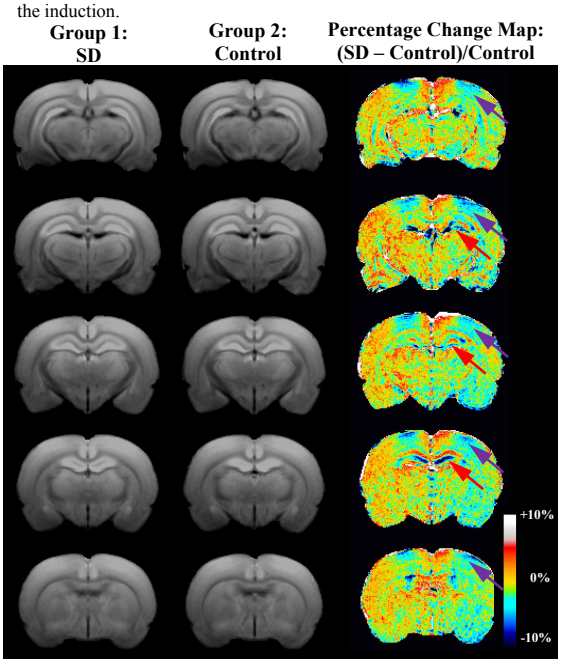

Fig.2. Averaged post-Mn T1WIs from Group 1 (sleep deprivation - SD) and Group 2 (control) with the percentage change map showing the percentage signal differences between them. Reduced Mn uptake was generally observed in the hippocampal (red arrows) and cortical regions (purple arrows) in SD animals. Note the most pronounced signal change in the DG region.

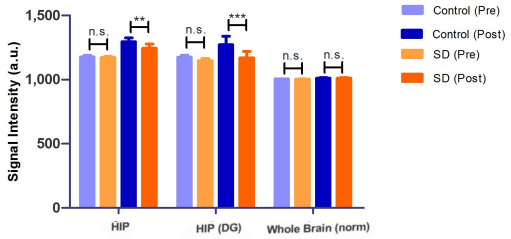

Fig.3. T1WIs signal intensity changes (mean ± standard deviation) before and after Mn injection were compared between the two groups in the entire hippocampus (HIP), DG and whole brain. **$p<0.01$, ***$p<0.001$. No significant signal intensity changes were seen in the whole brain due to the global intensity normalization procedure.