MEMRI detects neuronal activity and connectivity in hypothalamic neural circuit.

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Purpose: Hypothalamic neural network of energy regulationis crucial in understanding central dysfunction in obesity. Manganese-Enhanced MRI (MEMRI) has been used to detect brain connectivity and activity *in vivo*[1, 2]. With its superior resolution, it has been shown to differentiatedistinct patterns of activation in hypothalamic nuclei in the mouse brain responding to different physiological conditions such as fasting[3] or peptide signals like ghrelin[4] and leptin[5]. Since Mn2+ entrance to hypothalamus relies on the leaky BBB near the arcuate nucleus (ARC), the MEMRI signal depends on various factors affecting Mn2+ uptake and transport, such as, neuronal activity, density of projections, distance from the primary neuron, Mn2+ diffusion, etc. To what extent can MEMRI reflect neuronal activity and connectivity is still uncertain. Here we evaluated whether MEMRI signal in different hypothalamic nuclei represents Ca2+ activity by Ca2+ channel blockade, axonal transport by microtubule disruption, and overall neural activity by c-Fos expression.

Methods: Animal experiments were approved by the instutional animal care and use committee (BRC, ASTAR, Singapore). Four sets of experiments were performed on C57BL/6 (male, 3-5 months)mice. Firstly, measuring MEMRI signal (MnCl2, 5ml/Kg, 62.3 mM, i.v.) under four kinds of physiological manipulations: non-fasted, fasted, and with injection of vehicle or leptin. Secondly, we used calcium channel blocker, diltiazem (i.c.v.,5 µl/100µg), to determine the role of calcium influx in non-fasted mice.Thirdly, we used microtubule disrupter, colchicine (i.c.v, 5µl/20µg), to confirm the axonal transport of Mn2+ to secondary neurons in non-fasted mice. Lastly, we performed immunofluorescence staining of c-fos and dapi as marker of neuronal activation under the four fasting/leptin manipulations. MRI was conducted on a 9.4T MRI (Agilent) using a volume coil for transmission and a surface loop coil for reception. T_1-weighted MRI was acquired using 3D MPRAGE with TR/TE/TI=8/2.55/1000ms ,resolution= 75×75×400 µm3 and 3min/time frame for 189 mins.

Results: Firstly, we compared MEMRI signal intensity (SI) time-course in ARC in fasted vs non-fasted, where increased SI in fasted compared to non-fasted condition (Fig. 1A). By injecting leptin in both conditions, decrease SI in fasted condition and increase in non-fasted condition were seen. Secondly, SI in ARC, DMH (dorsomedial nucleus) and PVN (paraventricular nucleus) were decreased by Ca blockade, but not in VMH (ventromedial nucleus) (Fig. 1B). SI time-to-rise with respect to ARC was delayed in PVN and DMH after microtubule disruption (Fig. 1C). Lastly, immunofluorescence staining of c-fos (Fig. 1D) showed that ratio of c-fos/Dapi is highly correlated with the MEMRI plateau SI (Fig. 1E).

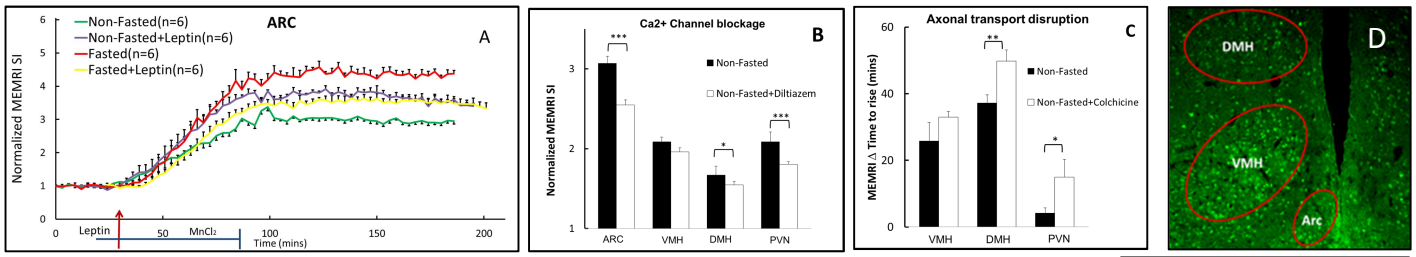
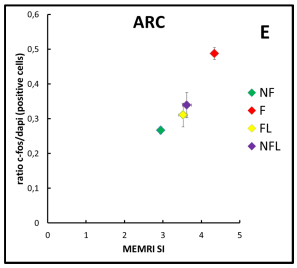


Fig.1 Timecourse of T1-weighted signal intensity in the ARC C57BL/6 (A), Normalized MEMRI SI in non-fasted diltiazem treated mice (B), MEMRI ΔTime to rise in non-fasted colchicine treated group (C), Immunofluorescence staining of c-fos ARC, VMH and DNH (D); Correlation between MEMRI SI and ratio c-fos/Dapi in non-fasted(NF), fasted(F), fasted+leptin(FL), non-fasted+leptin(NFL)(E).



Discussion: Increased MEMRI SI in fasted condition can be explained by firing of neurons i.e. higher activity of voltage-gated Ca2+ channels and as a results increase of Mn2+ entry and accumulation. Therefore, MEMRI can be used to access hypothalamic neuronal activity in ARC. However, Ca2+ channels are not the only route for Mn2+ to enter the neuron. Largely decreased signal after diltiazem treatment indicated that calcium influx may be the main route in ARC and PVN but not in DMH or VMH. Though, partial attenuation of the signal could be due to the selected channels being blocked. The delay of Mn2+ uptake under colchicine treatment confirmed microtubule-based axonal transport of Mn2+ into DMH and PVN, which could be related to density of neuronal projections and distance from primary neuron. According to the literature[6] PVN and DMH have abundant neuronal projections, but not VMH, which are consistent with longer MEMRI signal delay in PVN and DMH. Lastly, the good correlation between c-fos/dapi and MEMRI SI confirms the neuronal basis because expression of c-fos is an indirect marker of neuronal activity during action potential firing. Number of c-fos expressed neurons is higher in fasted condition compared to non-fasted one, which can support the hypothesis that MEMRI SI reflects neuronal activation.

Conclusion: We have confirmed that MEMRI can be used for mapping certain but not all activated neuronal pathway in hypothalamus. The understanding on how MEMRI is reflective of neuronal activation and connectivity can improve the interpretation of hypothalamic neurocircuit network in responding to nutritional and hormonal signals using MEMRI.

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