Imaging Motor Function in Vivo Using Manganese-Enhanced MRI

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Introduction:

Invasive assessment of motor function before symptoms occur has long been an attractive issue for investigators. Traditional fMRI does work to certain extent for human subjects, but in terms of various animal models the modality suddenly gets pale. It is hard to have animal conscious and also control its movement in the scanner at the same time. Passive motor task is far from physiological status. Muscle electrophysiological exam provides little information on the central nervous system. Manganese-enhanced MRI (MEMRI), which is able to reveal neuronal activity that happened outside of scanner and even quite a long period of time ago, directs to a prospective way to evaluate motor function of animals in vivo[1,2]. Here mice with $MnCl_2$ were let run actively and their motor cortex activation was analyzed later with MEMRI.

Materials and Methods:

Ten male ICR mice (12-16 week, 28-32g) received three bolus intraperitoneal (IP) injections of 50 mM $MnCl_2$/saline solution within 50 minutes, reaching final dose of 126 mg/kg and followed by 0.5 ml saline injection to minimize dehydration effect. Mice were let run on a home-built motor controlled mouse treadmill at 5h, 12h, and 20h post-$MnCl_2$ injection. To determine the best time for motor stimulation, different two mice were let run at each time point for a total of 1.5 hours in a recursive pattern of 10-min run and 3-min break. Speed at each point was adjusted to maximum that both mice can afford. Another two mice also ran at 12h point at previous speed and in the same pattern but for three hours with 30-min rest after the first 1.5h run. The rest two mice were left with only $MnCl_2$ injection but no run at all to behave as control group. With Biospec 9.4T/31 cm scanner and mouse gradient and birdcage RF coils (Bruker, Germany), all animals underwent MRI scans under IP 60mg/kg pentobarbital anesthesia at 24h after $MnCl_2$ administration. T1-weighted MRI parameters were as follows: multi-slice spin-echo sequence TR/ TE= 400/ 8 ms; FOV=2x2 cm^2; Matrix size= 128x128; Slice thickness= 0.5 mm, 30 continuous slices, NEX=4, 4 repetitions. Animal ECG, respiratory, and anal temperature were monitored and respiratory gating was performed during every scan. A water bath warming pad was used to maintain animal core temperature within 36±1℃ range. AFNI software was used for image processing. ROIs were drawn in 3-4 slices according to mouse brain atlas[3] for primary and secondary motor cortex (M1, M2 respectively). SNR of both motor cortexes was normalized to area of corresponding ROI and compared among different time point groups as well as the control group with student t-test. Correlation between time of run and normalized pixel-wise enhancement for those 4 mice in 12h group was statistically analyzed.

Results:

The maximum speeds that mice could afford in group 5h, 12h, and 20h were about 4 cm/s, 11 cm/s, and 15 cm/s respectively. T1-weighted images (Fig 2) of animals in all groups showed great contrast in the brain. The normalized SNR (nSNR) for both motor cortexes in 12h was significantly higher than those in the other groups (all $P< 0.05$). NSNR in 20h group was also significantly higher than that in control ($P< 0.05$). No significant difference was found between nSNR in 5h and control groups (Fig 1). At same speed for all four animals in the 12h group, a rough correlation ($r= 0.846$) was found between the exercise time and pixel-wise enhancement of each ROI when control group was taken as baseline image source.

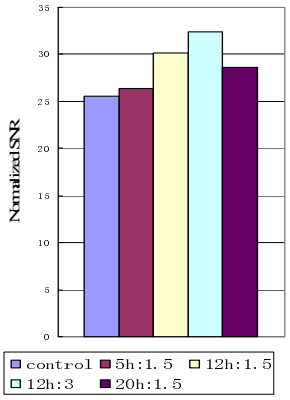

Fig 1 ROI area Normalized SNR in different groups

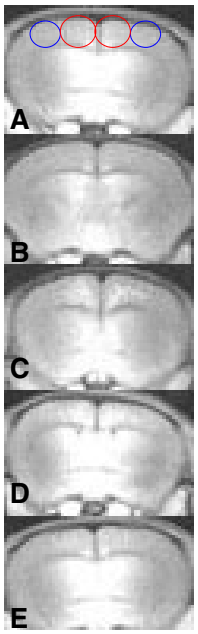

Figure 2 T1-weighted images of M1 (red circle) and M2 (blue circle) motor cortexes at 24h after $MnCl_2$ injection: control group (A); 5h group (B); 12h group with 1.5h run (C); 12h run with 3h run (D); 20h group (E). C, D, E but not B show enhancement in ROI compared with A. The observed enhancement is most significant in D and least prominent in E.

Discussion:

In our study, conscious motor stimulation of run did result in mouse motor cortex activation in MEMRI. Even with medium speed but same amount of time, motor stimulation occurred at 12h post-$MnCl_2$ injection ended up with maximum activation, therefore seemed to be the best timing among all three time points. This is different from usual timing of sensory stimulation for previous studies of MEMRI. From our other study, the maximum manganese uptake interval for gray matter should be 4-8 h after $MnCl_2$ injection based on dynamic R1 relaxivity changes (data not shown here). The reason would be for 5h group, manganese neural inhibitory effect still dominated, and mice were reluctant to run even to move, resulting in a low affordable speed. The minor stimulation later turned out to be hard to detect with MRI. Image acquisition was taken at 24h to prevent the wash-out effect of manganese thereafter. For 20h group, although the stimulation intensity (speed of run) was high, manganese uptake was slow and time as well as available amount for manganese to transport to cortexes before imaging was limited. For four mice in 12h group, the time of run was only roughly correlated with pixel-wise motor cortex enhancement. The confounding factors could be the limited number of control group mice; stimulation-irrelevant neuronal activities; non-linear correlation between signal enhancement and local manganese concentration. Future study could apply MEMRI to disease animal models or developing animals to test whether same amount of motor stimulation would recruit different amount or even different types of motor neurons for animals with different health-conditions or in different developing stages. In conclusion, our results suggested that MEMRI may become a quantitative way to evaluate motor function for animals in vivo.

References:

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