Mapping nociceptive pathways using activity-dependent manganese-enhanced MRI

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Introduction

Manganese enhancement magnetic resonance imaging (MEMRI) provide a powerful tool to investigate neural circuits in vivo. Manganese ion (Mn^{2+}) is a Ca^{2+} analog. Manganese ions enter cells via voltage gated calcium channels, transported along microtubules, and is then released at the synaptic cleft. It can cross synapses in the neural circuits. Manganese ion is also paramagnetic and can be detected by MR system. MEMRI uses a combination of these characteristics of manganese ions to provide functional information and in identified neural connections.

The objective of the present study was to use MEMRI to study medial pain pathway. Mn ions were deposited in the medidorsal and intralaminar nuclei. Noxious stimulation was applied to the forepaw and Mn transportation patterns to the forebrain were compared with innocuous stimulation control and morphine-treated control.

Materials and Methods

Animal Preparation: Adult Long Evans rats (National Laboratory Animal Center, Taiwan) weight (250-350g) were used for this study. All rats were initially anesthetized with 3 % isoflurane in a 1/1 Oxygen/Air mixture and injected with atropine (20 μg/kg) to avoid excessive amount of salivation. The rats were anesthetized by intraperitioneal (i.p.) injection of urethane (1.3 g/kg) and α-chloralose (80 mg/kg).

$MnCl_2$ injection and forepaw stimulation: A 120 mM $MnCl_2$ was iontophoretically injected with 5uA, 5 s on, 5 s off current for 15 min into the mediodorsal nucleus of the thalamus (Bregma= -2.5 mm, lateral=1 mm, and depth=5 mm). Leave the glass electrode in place for 10 min to prevent a leakage of $MnCl_2$. All the animals were taken forepaw stimulation experiment before MRI scans. Forepaw stimulation were delivered by inserting two needle electrodes between two digits of the forepaw. The electrical stimulation was delivered with 5 mA (noxious) current with a pulse width of 2 ms and a frequency of 0.17Hz.

MRI protocol and acquisitions: The animal experiments were performed in a 3T Bruker BioSpec (Bruker Biospec, Karlshruhe, Germany) scanner with a 30-cm bore. Radio frequency pulses were transmitted by quadrature volume coil and received by a quadrature surface coil. After tuning and shimming, the T2 images were acquired using Bruker implementation of a multi-slice spin echo sequence. The coronal, horizontal and sagittal images were taken with a Field Of View (FOV) of 40 mm, a gradient echo time (TE) of 60 ms, a repetition time (TR) of 3000 ms, and matrix size of 256×256, consisting of 16 coronal slices of 1-mm thickness. The T1W images were performed at the same position of the T2 images. A multi-slice spin echo sequence was used to obtain T1W images (TR=500 ms, TE=13 ms, FOV=40 mm, matrix size= 256×256).

Results

Fig.1, Manganese transport $MnCl_2$ injections into medial dorsal thalamus nucleus of rats. Three experimental group of the averaged T1 weighted images (upper column) and color maps (low column) was shown 5 hours after injection, manganese was detected ipsilaterally throughout the anterior cingulate cortex (ACC) and medial caudate-putamen (CPu). For the volume of interest (VOI) analysis stereotactic coordinates from a standard atlas (bottom panel) were used (Paxion and Watson, 2007). To draw polygon VOIs in ACC (yellow), CPu (green) and Insular cortex(orange).

Fig. 2, Volume of interest (VOI) analysis of ACC, CPu and Insular in the control, noxious and morphine treated group. The averaged T1 weighted signal intensity in the ACC and CPu were significantly higher in the noxious group (*P<0.05; **P<0.001, t-test) associated to the other groups. The effect of morphine injection on the T1 weighted signal intensity of the ACC and CPu. A morphine treated group, the T1 weighted signal intensity greatly decreased in ACC. The levels of T1 weighted signal intensity no difference between morphine treated and innocuous group. There also have effect the T1 weighted signal intensity of the CPu, but still higher than control group. No significant differences were found between the control group and innocuous group. There was no significant differences in insular cortex region within four experimental groups.

Summary

Mn ions transportation was activity dependent. Noxious stimulation enhance Mn transportation from medial thalamus to anterior cingulate cortex and caudate-putamen. Morphine pretreatment prevented nociception dependent Mn transportation in the anterior cingulated cortex.

Conclusion

Anterior cingulate cortex and caudate-putamen are important areas in medial pain pathway.

Acknowledgements

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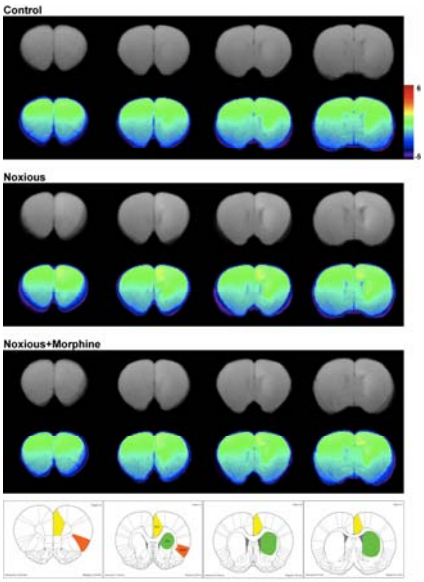

Fig.1, Four consecutive slices of averaged manganese enhanced T1W images in three experimental group. The averaged T1 weighted images (up column) and color maps (low column) was showed. manganese enhancement was observed in right ACC and CPu. A stereotactic coordinates from a atlas (bottom column) were used to place polygon VOI in ACC (yellow), CPu (green) and Insular cortex (orange).

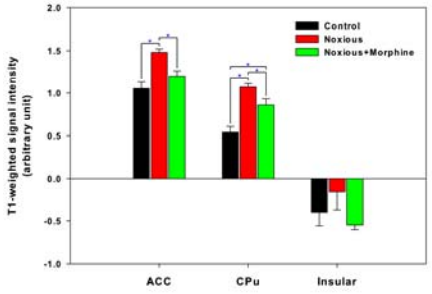

Fig. 2, Volume of interest analysis of ACC, CPu and Insular in the control, noxious and morphine treated group. A signal intensities calculated from T1W images, and *t*-test was used to supply the statistical evidence. Comparing the Mn2+ enhancement between control, noxious stimulation and morphine treated group. The images intensity in the ACC and CPu were significantly higher in the noxious group (*P<0.05) associated to the other groups.