Manganese-enhanced MRI of Perilesion Cortex in Subchronic Focal Brain Ischemia

K. C. Chan[1,2], and E. X. Wu[1,2]

[1]Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Hong Kong SAR, China, People's Republic of, [2]Department of Electrical and Electronic Engineering, The University of Hong Kong, Hong Kong SAR, China, People's Republic of

INTRODUCTION: Rescuing the ischemic penumbra from infarction is the mainstay of acute stroke therapy (1). Yet, to date, little is known about the subchronic/chronic events of recovery mechanisms in the brains, which are equally important in determining and improving the functional consequences of brain lesions. Our previous Mn-enhanced MRI (MEMRI) study had demonstrated the transient brain changes in the early recovery period after focal brain ischemia (2). In this study, MEMRI was employed to investigate into the late changes in Mn^{2+} enhancement in subchronic focal brain ischemia, with emphases on the temporal evolutions in different subregions of the perilesion cortex.

MATERIALS AND METHODS: Animal Preparation: Adult Sprague-Dawley male rats (200-250 g, N=18) were prepared and were divided into 4 groups. The first 3 groups (n=4 each) were subjected to focal cortical photothrombotic lesions (PCI) in the center of motor cortex of one side of the brain using the rose bengal techniques (3), while the other animals in Group 4 (n=6) were untreated and served as control. Two and 6 days after surgery, animals in Groups 1 and 2 were respectively administered with an intraperitoneal injection of $MnCl_2$ solution (45mg/kg, 100mM), while Group 3 received no Mn^{2+} injection. MRI was performed to the ischemic animals at 3, 7, 14, 21 and 28 days after ischemia. For the normal rats in Group 4, MEMRI was performed before, and at 1, 5 and 12 days after Mn^{2+} injection to compare with the ischemic groups at the corresponding time points with and without Mn^{2+} administration.

MRI Protocols: All MRI measurements were acquired utilizing a 7 T Bruker scanner. Under inhaled isoflurane anaesthesia (3% induction and 1.5% maintenance), animals were kept warm under circulating water at 37°C and were imaged using a receive-only surface coil. 2D T1-weighted RARE sequence was acquired with FOV = 3.2 x 3.2 cm^2, matrix resolution = 256 x 256, slice thickness = 1 mm, number of slices = 10, TR/TE = 400/7.5 ms, RARE factor = 4 and NEX = 16; T2WI was performed under the same dimensions with TR/TE = 4200/65 ms, RARE factor = 12 and NEX = 2; Signal intensities (SI) in the ipsilesional cortex, including the ischemic core, ventral rim, lateral rim, and parietal cortex were obtained using ImageJ v1.42q, and were normalized with the SI in the contralesional cortex (Fig. 1, bottom left). The SI in the ipsilesional dorsal thalamus was normalized with the contralesional dorsal thalamus. Two-tailed paired t-tests were compared between ipsilesional cortical/subcortical regions and the contralesional cortex/thalamus in the same groups. Results were considered significant when $p<0.05$.

Histology: After MR examinations at Day 7, 2 rats from Groups 1 and 3 each were sacrificed for histology, another 2 rats from Groups 2 and 3 at Day 14, and another 2 from Groups 1 and 2 at Day 28. The rats were transcardially perfused with 4% paraformaldehyde. The brains were then removed, cut into 10 µm sections, and immunostained for H&E, glial fibrillary acidic protein (GFAP), manganese superoxide dismutase (MnSOD) and glutamine synthetase (GS), which are markers for gliosis, oxidative stress and glutamate excitotoxicity, respectively.

RESULTS AND DISCUSSION: As shown in Figures 1 and 2, Mn injection to the ischemic animals at Days 2 or 6 led to a transient increase in SI in the lateral rims (Fig. 1, solid arrows) surrounding the hypointense ischemic core at Days 3 and 7 ($p<0.05$) in consistency with our previous study (2). Interestingly, in the ventral rim, a delayed enhancement was found at Day 14 (Fig. 1, open arrows) ($p<0.05$), which remained observable at later time points. Significant T1W enhancement was observed in the lateral rim at Day 7 without Mn injection, yet no apparent enhancement was found in the ventral rim at Day 14 (Fig. 1) ($p>0.05$). In Figure 3, the T1W hyperintensity appeared to colocalize with the enhanced GFAP, MnSOD and GS expression at the lateral rim in Group 1 at Day 7 (open arrows), while only mild expression was observed in the entire rim in Group 3 and the ventral rim in Group 1. Delayed Mn enhancement was also found in the thalamus in Group 2, which peaked at Day 21 (Fig. 1, arrowheads). No significant difference was found between contralateral sides of the cortex and thalamus in the normal group at any time points ($p>0.05$).

CONCLUSION: Previous studies suggested that after acute stroke, the rescued penumbra may be affected by selective neuronal loss and microglial activation, which may hinder functional recovery (1). Our results on the Mn detection of the differential temporal changes in the perilesion cortex may provide a new tool for *in vivo* monitoring of the evolutionary changes of salvageable tissues and hence represent potential new therapeutic targets for improving the functional consequences after stroke.

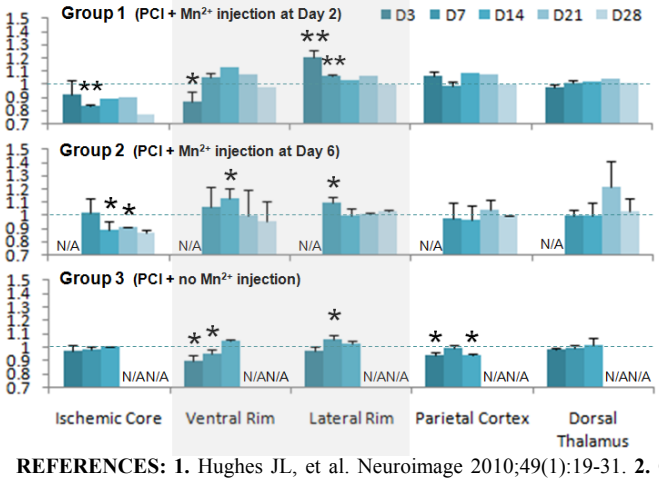

Fig. 2: Comparison of signal intensities in the ipsilesional cortical/subcortical regions to the contralesional cortex/thalamus. Note the significant enhancement of the ventral and lateral rims at different times after stroke (Two-tailed paired t-tests, *p<0.05; **p<0.01)

REFERENCES: 1. Hughes JL, et al. Neuroimage 2010;49(1):19-31. **2.** Chan KC, et al. Proc Intl Soc Mag Reson Med 2008;16:532. **3.** Bidmon HJ, et al. Stroke 1998;29(1):203-210;

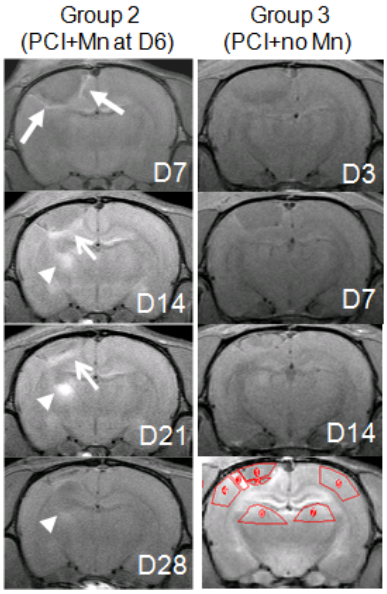

Fig 1: Typical T1WIs at the center of ischemic core at different time points after photothrombosis with (Group 2) or without (Group 3) Mn injection at Day 6. Image at bottom left shows the regions of interest definitions for quantification.

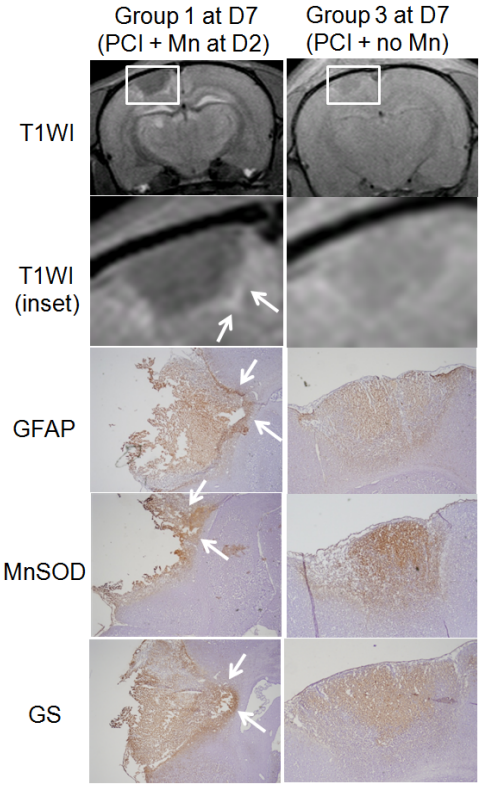

Fig 3: Typical T1WIs and immunohistochemical stains (counterstained with hematoxylin) at Day 7 with (Group 1) or without Mn injection at Day 2.