# Detection of gray matter damage in neonatal rat model of mild hypoxic-ischemic insult by manganese-enhanced MRI

**J. Yang[1,2], and E. X. Wu[1,2]**

[1]Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Pokfulam, Hong Kong, [2]Department of Electrical and Electronic Engineering, The University of Hong Kong, Pokfulam, Hong Kong

## INTRODUCTION

Periventricular leukomalacia (PVL) is a predominant form of cerebral injury observed in pre-term infants and is a major cause of cerebral palsy associated with developmental deficits in motor, sensory, visual or cognitive functions in later life. Although gray matter injury like cortical neuronal necrosis is not a prominent feature of PVL, but the deficits mentioned above suggest cerebral cortical neuronal dysfunction [1]. In particular, subplate neurons, which are the first cortical neurons to mature, are selectively vulnerable to early hypoxic-ischemic brain injury. Timing of subplate neuron death determines the resulting defect in thalamocortical development [2]. Therefore, in vivo diagnosis of such gray matter injury at an early stage is necessary for developing optimal clinical management and treatment strategies. Recent MRI studies of mild hypoxic-ischemic (H-I) injury in neonatal rats demonstrated certain transient changes in periventricular white matter in $T_1$-weighted images (T1WI), $T_2$-weighted images (T2WI), ADC, and cerebral perfusion imaging in acute and sub-acute phase. In these studies, these transient changes in MRI images normalized more quickly in the cortical gray matter (within 48h) than in the white matter despite of the histological changes [3]. Divalent manganese ion ($Mn^{2+}$) has been used as a contrast agent for tracing neuronal pathways and study of ischemic neural tissues [4]. This study aims to use manganese-enhanced MRI (MEMRI) to investigate the progression and permanence of the gray matter injuries in a neonatal rat model of relatively selective white matter injury induced by mild H-I insult.

## MATERIALS and METHOD

*Animal Preparation*:

Mild hypoxia-ischemia was induced in neonatal rats (Sprague-Dawley, 7-days old, 12-16g, n=16) by unilateral carotid artery occlusion plus exposure to hypoxia for 1 hour at ambient temperatures of 34°C [5]. The models of selective white matter damage were successfully produced in 14 neonatal rats. They were divided into four groups according to the time between $MnCl_2$ injection and H-I insult (Fig.1). Group 1 - $MnCl_2$ injection in acute phase; Group 2 - $MnCl_2$ injection in subacute phase; Group 3 - $MnCl_2$ injection in later phase; Control group – H-I insult without $MnCl_2$ injection. In this study, an isotonic $MnCl_2$ solution (0.1 mol/L, 87.5mg/kg) was administrated by intraperitoneal injection.

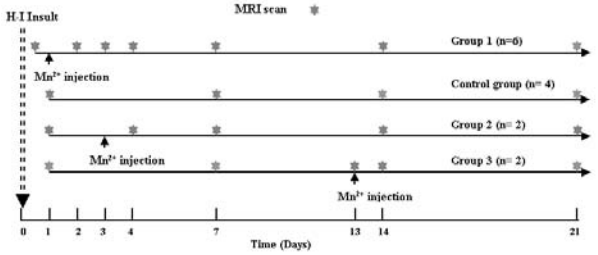


**Figure 1** Schedule of H-I insults, MnCl2 injections, and MRI scans.

*MRI protocols:*

All MRI scans were performed on a PharmaScan 70/16 7 T scanner (Bruker, Germany) using a 23mm mouse brain coil (for the rat below 3 weeks old) and 38mm rat brain coil (for the rat above 3 weeks old). Axial images were obtained in the same location (FOV = 2.5cm, slice thickness = 0.5mm, matrix = 256×256, 20 slices). 2D T1WI were obtained by RARE sequence with the following parameters: TR = 500ms, TE = 7ms, NEX=6 or 12. T2WI was acquired using a Turbo RARE sequence with TR =6000ms, TE = 60/200ms, NEX=2 or 4. SE EPI based diffusion weighted imaging was also performed with TR/TE=3000/28 and b value of 1000 s/mm$^2$, and ADC maps calculated.

## RESULTS and DISCUSSION

Transient changes in T2WI and ADC map: During the 1st day after H-I insult in all animals with and without $Mn^{2+}$ injection, the ipsilateral gray and white matter showed hyperintense zones in T2WI, more pronounced in cerebral cortex than in periventricular white matter (Fig. 2a). In ADC map, cortical gray matter showed a corresponding ADC decrease (yellow arrow in Fig. 2b) while the white matter exhibited an ADC increase (green arrow in Fig. 2b), indicating intracellular and extracellular edema, respectively [3]. Seven days after H-I insult, these changes in T2WI and ADC map were not observed in either gray or white matter regions in all the control animals (i.e., no $Mn^{2+}$ injection) (Figs. 2c and 2d). Therefore, the cortical gray matter injuries in this type of mild H-I insult were transient and could not be observed on T2WI and ADC map in later phase.

Enhancement of gray matter injuries by MEMRI: Among all animals in Group 1 (n=6), gray matter injury areas after day 3 were observed to be hyperintense in T1WI (red arrow in Fig, 3) and hypointense in the corresponding T2WI (yellow arrow in Fig. 3), likely caused by local Mn accumulation. These Mn induced changes remained clearly visible up to day 21. In Group 2 (n=2), gray matter injury enhancement was found 1 day after $Mn^{2+}$ injection (i.e., day 4 after the H-I insult) and persisted up to day 21. Similar late-phase Mn enhancement was observed in Group 3 (n=2) where $Mn^{2+}$ injection was made 13 days after the H-I insult (see Fig. 4). Overall, these Mn enhancements of gray matter injuries were found mostly in ipsilateral cortical and/or subcortical areas (10/10), basal ganglion (4/10), but not in areas of white matter injuries. Note that Mn enhancement or hypointensity in T2WI was pronounced (Fig. 3). This may partly arise from the increased local accumulation of Mn in the forms of mitochondrial Mn-superoxide dismutase (Mn-SOD, associated with oxidative stress [6]) and glutamine synthetase (GS, associated with NMDA receptors [6, 7]) in presence of increased Mn availability due to $MnCl_2$ injection.

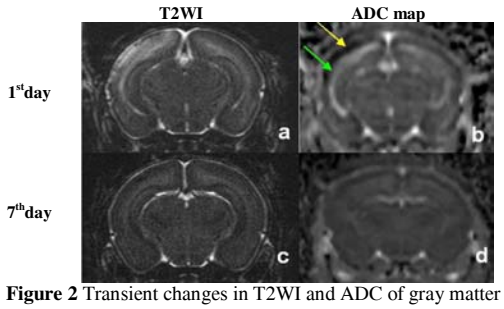


**Figure 2** Transient changes in T2WI and ADC of gray matter injuries in early phase of mild H-I insult.

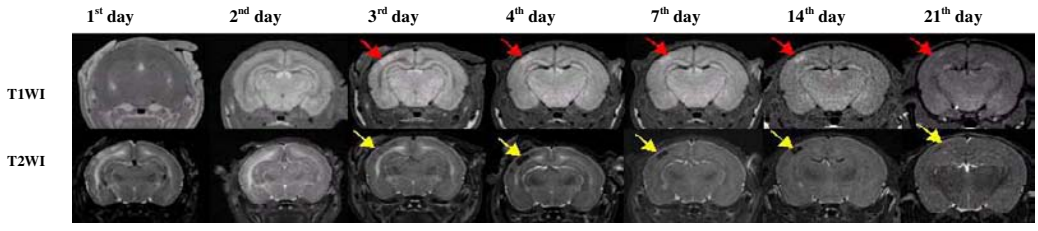


**Figure 3** Typical Mn enhancement of gray matter injuries over time. $MnCl_2$ was injected 1 day after H-I insult.

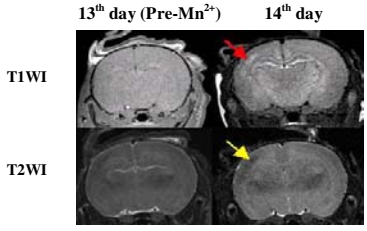


**Figure 4** Mn enhancement of gray matter injury. $MnCl_2$ was injected 13 days after H-I insult.

## CONCLUSION

The transient changes associated with gray matter injuries in the neonatal model of mild H-I insult were shown to be enhanced during middle and later phases by systemic $Mn^{2+}$ administration. These Mn enhancements persisted up to 21 days after H-I insult. Such Mn enhancement paradigm may be potentially useful in detecting the gray matter injuries in late phase of neonatal models of selective white matter injuries (i.e., PVL) that are not visible in the conventional T2WI, T1WI and DWI.

## REFREENCES

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