MRI CHARACTERIZATION OF SECONDARY DEGENERATION IN IPSILATERAL SUBSTANTIA NIGRA FOLLOWING EXPERIMENTAL INTRACEREBRAL HEMORRHAGE

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INTRODUCTION: Secondary neurodegeneration in substantia nigra (SN) has been well recognized in ischemic strokes[1]. Similarly, intracerebral hemorrhage (ICH) was also observed to cause some functional deficits due to neuropathological changes in remote areas from the hemorrhage[2]. We hypothesized multi-parametric MRI could be employed to explore such injury after ICH and characterize its evolution, and provide better understanding of the underlying neuropathological changes.

METHODS: ***Animal Preparation:*** 10 female Sprague-Dawley (SD) rats (~15wks; 320g~340g) were stereotactically infused with 0.28U collagenase (Type IV, C5138, Sigma) in 1.4 μL heparinized saline (0.125μL/min) in the right basal ganglia[2]. MnCl2 solution (3.6ml/kg, 100mM in ddH_2O) was administrated by intraperitoneal injection at week 2 (wk2, n=7), week 6 (wk6, n=5) and 3 month (mon3, n=3) after the surgical insult. Three age-matched female rats were used as normal control. 2 rats were sacrificed after MnCl2 administration at each time point for histology. ***MRI Protocols:*** All MRI experiments were performed on a 7T Bruker MRI scanner. The animals were kept warm with circulating water at 37°C while anaesthetized with inhaled isoflurane. 2D T_1-weighted images (T_1WIs) were acquired immediately before and after Mn^{2+} administration, with TR/TE=400/8ms, FOV=30×30 mm^2, matrix=256×256, slice thickness=1.0mm, RARE factor=4 and NEX=28. Multi-echo SE (MESE) images were acquired with TR=3000ms, TEs=18, 36, 54, 72, 90, 108, 126 and 144ms, FOV=30×30mm^2, matrix=128×128, slice thickness=1.0mm and NEX=1. Diffusion-weighted images (DWIs) were acquired at 3 to 4 hours, day 1, 3, 7, 14, 28, 42 after ICH, with a SE 4-shot EPI with 30 diffusion gradient directions and 5 b_0 with TR/TE=3750/32ms, δ/Δ = 5/17 ms, resolution=273×273×1000μm^3, b=1000s/mm^2 and NEX=3. ***Data Analysis:*** R_2 (=1/T_2) maps were computed from MESE images for the measurement of T_2 relaxation time. Fractional anisotropy (FA), axial diffusivity ($\lambda_{//}$), radial diffusivity ($\lambda_\perp$) and mean diffusivity (MD) maps were generated from DWIs using DTIStudio. ROIs were manually drawn over the ipsilateral and contralateral SN regions, as shown in Fig. 1. T_2 values, T_1W signal intensities and DTI metrics were subsequently measured within the ROIs in ImageJ. Two-tailed paired t-test was performed to compare MRI parameters in ipsi- and contra-lesional SN at each time point.

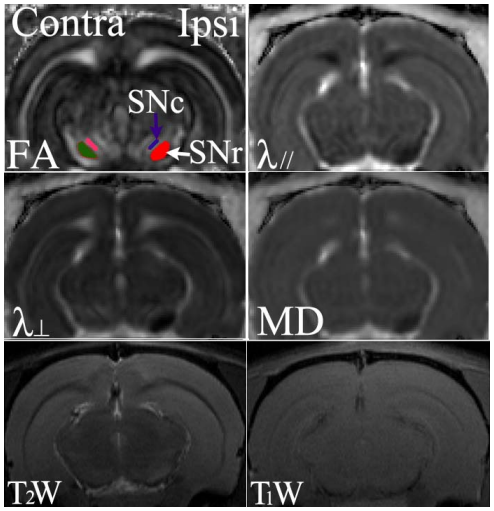

Fig. 1 Typical diffusion maps and anatomical images of one ICH rat at day 3 after the surgical insult. SN was delineated into *pars reticulata* (SNr) and *pars compacta* (SNc) on FA maps.

RESULTS: Fig. 1 shows typical diffusivity maps and anatomical images of SN at day 3 after ICH. Ipsilesional SN, which was delineated into *pars reticulata* (SNr) and *pars compacta* (SNc), showed hypointensity on all diffusivity maps, hyperintensity on T_2W images and isointensity on T_1W images, as compared with the contralesional side. T_2 values of ipsilateral SN (76.84±5.66 ms) was also significantly higher than that of contralateral SN (53.50±2.98 ms) (n = 4, p = 0.002). Fig. 2 shows the comparison of diffusivities between ipsi- and contra-lesional SN (abbreviated as Ipsi SN and Contra SN, respectively) at multiple time points after ICH. Ipsilesional SNr showed increase in $\lambda_\perp$ and MD at day 1, followed by a decrease in $\lambda_{//}$ as detected at day 3. Note that all the diffusivities showed a sharp drop at day 3 and gradually recovered to the contralesional level after day 14. Ipsilesional SNc showed a transient increase in $\lambda_{//}$, $\lambda_\perp$ and MD at day 1 after ICH. Fig. 3 shows the changes of the ratio of T_1W signal intensities (T_1W SI) of ipsilesional SN over contralesional SN, which was normalized by that of pituitary in the corresponding hemispheres, before and after Mn2+ administration. Significant decrease of the ratio was detected at wk2 after systemic Mn^{2+} administration, as compared with that before Mn^{2+} administration (1.10±0.04 versus 1.07±0.07, n=7, p<0.024).

DISCUSSIONS AND CONCLUSION: The results of this study demonstrated that secondary injury of ipsilesional SN would occur after ICH. Such injury was first detected by DTI at day 1 after ICH, with increased diffusivities in both SNr and SNc. Then the injured SNr showed a dramatic decrease in diffusivity at day 3, with a significant increase in T_2 values. These changes might mainly result from cytotoxic edema, which was confirmed by histology (slightly enlarged volume and lighter eosin stain in cellular plasma). As shown in Fig. 3, T_1W signal intensities in the ipsilesional SN gradually increased over time, which might due to manganese and calcium deposition after cellular injury[3]. Macrophage/microglia infiltration was previously observed within 3 days in experimental ICH rats[2], which would increase the T_1WI contrast in manganese-enhanced MRI (MEMRI)[4]. However, T_1W signal intensity of ipsilesional SN in this study was less enhanced by MEMRI than that of the contralesional side at day 14, implying a marked decrease in cellular activity[3] due to predominant neuronal cell death. Furthermore, the increase in T_1W signal intensity was retained at month 3 and further enhanced by systemic Mn^{2+} administration, suggesting progressive injury and reactive gliosis[4] in ipsilesional SN at the chronic phase of ICH. Interestingly, immunohistochemistry at 3 month after ICH detected an increase in glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS), as well as neuronal nuclei, in the ipsilesional SN (images not shown). This increase in neuronal nuclei could suggest reactive neuroregeneration in the ipsilesional SN and could also be related to the T_1W signal intensity enhancement. In conclusion, this study characterized secondary neurodegeneration and its evolution in ipsilesional SN using multi-parametric MRI. Such degeneration might be detected by DTI at day 1 after hemorrhage, which is much earlier than that in ischemic strokes[5]. These results suggested that DTI could be a valuable tool for early diagnosis of SN injury induced by ICH, and hence it could promote timely treatment for the patients.

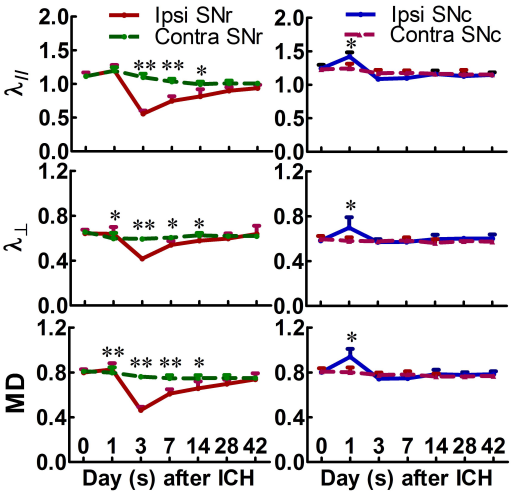

Fig. 2 Comparison of diffusivity (Mean±SD,μm/mm^2) between ipsi- and contra-lesional SN at multiple time points after ICH. Two-tailed paired t-tests were performed at each time point (n=5, *p<0.05, **p<0.01).

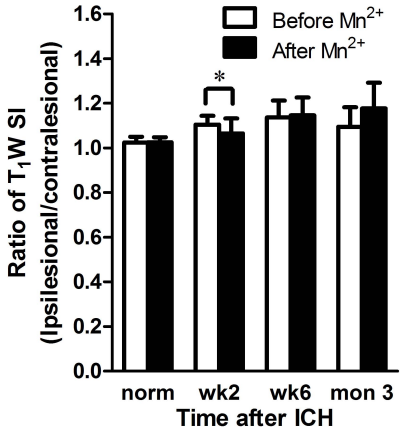

Fig. 3 Comparison of the ratio of T_1-weighted signal intensity (T_1W SI) (ipsilesional over contralesional SN) before and after Mn^{2+} administration at wk2, wk6 and 3 month after ICH. Two-tailed paired t-tests were performed at each time point (*p<0.05).

REFERENCES: [1] Nakane M, et al. Stroke.1992; 23: 328-332. [2] Imamura N, et al. Neurosci Res. 2003; 46: 289-298. [3] Yang J, et al. Magn Reson Med 2008;59:1329-1339. [4] Kaiwai Y, et al. NeuroImage 2010; 49: 3122-3131. [5] Tamura A, et al. Brain Res. 1990;51:615-618. [6] Nakajima M, et al. Inter Med 2010;49: 65-68.