INTRODUCTION: Intracerebral hemorrhage (ICH) is a common type of stroke. It often produces severe motor function deficits in survivors, which is closely related to secondary consequences of contusion (CST) injury. Wallerian degeneration (WD) of CST after stroke has been characterized in humans by diffusion tensor imaging (DTI). However, such observations were largely preliminary due to large intersubject variations. Rodent models of ICH have greatly promoted the understanding of histopathology underlying brain injury after ICH and have been employed widely for exploring therapeutic strategies. This study aims to observe WD of pyramidal tract (PY) after ICH, an important part of CST, longitudinally in a well-controlled rodent model by T2-weighted imaging and DTI as well as histological evaluations.

METHODS: Animal Preparation: Fourteen female Sprague-Dawley (SD) rats (~15 weeks; 320g-340g) were infused with 0.28U collagenase (Type IV, Sigma) in 1.4 μL heparinized saline (0.125μL/min) into the right striatum. Three rats were sacrificed for histology at day 3, day 7 and day 42 after surgery. Six animals were used for normal control. MRI Protocols: All MRI experiments were performed on a 7T Bruker MRI scanner at 3 to 4 hours (D0), day 1, 3, 7, 14, 28, 42, 90 and 120 (D1, 3, 7, 14, 28, 42, 90, 120) after ICH. The animals were kept warm with circulating water at 37°C after anesthesia with intraperitoneal isoflurane. T2-weighted images (T2Ws) were acquired with TR=4200ms, TE=38.9 ms, FOV=30×30mm, matrix=128×128, slice thickness=1.0mm and NEX=2. Diffusion-weighted images (DWIs) were acquired with a SE 4-shot EPI with 30 diffusion gradient directions and 5 b-values of 0-1000s/mm². The diffusion tensor was estimated using DTIStudio. ROIs in the PY were first established on T2Ws and corresponding diffusion maps at the last time point, and then copied to the other time points (Fig. 1). Ratios of T2 signal intensities between the right (ipsilateral) and left (contralateral) side of right and left hemispheres were plotted on single animal basis. The signal difference between right and left sides on FA, λ, and trace maps were compared by two-tailed paired t-test at each time point, after it was confirmed that the left PY of ICH rats showed no significant difference along time as compared with normal control. One-way ANOVA was performed for comparison of the right and left difference across different time points.

RESULTS: Typical T2Ws of one normal rat and one ICH rat were shown in Fig.1. Hypointense T2W signal were observed in the right PY at D7 and D42 (green arrows), while hyperintense T2W signal was found at D120 after ICH (red arrow). As shown by the regions of interest (ROI) analysis, rT2S1 scattered around 1.0 at D3 in 12 out of 14 rats, then became lower than 1.0 in 13 rats at D7, followed by increase of normalization until D42. Afterwards, rT2S1 continued to increase till D120, the end of the experiment. As for diffusion metrics as shown in Fig. 2, right PY showed significant decrease of FA, λ, and trace at D3, followed by increase of λ and trace at D7, as compared with the left side (two-tailed paired t-test). One-way ANOVA analysis detected a further decrease of FA and increase of λ and trace in ipsilateral PY at D28, as compared with that at D3. Afterwards, FA decrease and λ increase persisted and became more obvious at D120.

DISCUSSIONS AND CONCLUSION: Our results demonstrated that WD of PY started within D3 after experimental ICH, which was first detected with DTI with characteristic decrease of FA and λ, followed by λ increase within D7. As shown in the previous DTI studies, the decrease of FA and λ could be related with disorganization of axonal structure identified by SMI-31 staining at D3, while the increase of λ was related with myelin loss identified by increased myelin debris at D7. While T2 signals of injured PY failed to show signal abnormality at D3, and underwent a pseudo-normalization between D42 and D90, DTI could detect such injury within D3 and provide further proof of injury deterioration along time. To conclude, DTI can serve as a reliable tool for detecting WD in early phase and for longitudinal monitoring of WD with much better accuracy than T2-weighted imaging. In line with previous study, histology: Paraffin sections were prepared from rat brain samples for staining for myelin with Luxol blue staining (LBS), and for intact axons using immunohistochemical staining for phosphorylated neurofilaments (SMI-31).