

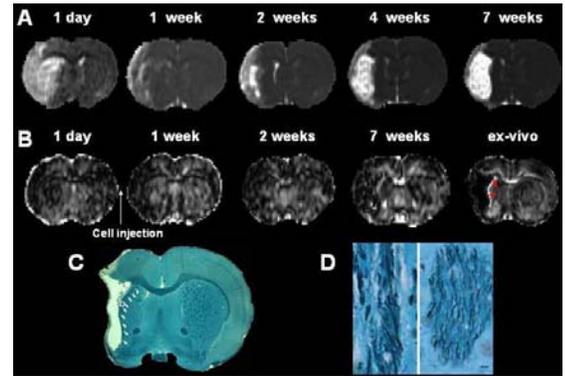
## MRI Detection of White Matter Remodeling after Neural Progenitor Cell Treatment of Stroke

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**INTRODUCTION:** Neural progenitor cell (NPC) treatment of stroke has demonstrated the ability to promote brain remodeling and recovery<sup>1</sup>. Although the mechanism of cell based treatment of stroke has been more focused on angiogenesis and neurogenesis<sup>1</sup>, white matter reorganization has started to demonstrate its importance in functional recovery after stroke<sup>2</sup>. In current study, we evaluated the effects of NPC treatment of stroke on white matter reorganization using MRI. We demonstrate that MRI fractional anisotropy (FA) identifies tissue with white matter reorganization, and fiber tracking of axonal projection from fiber tracking algorithms of diffusion tensor imaging detects the changes of axonal orientation in the ischemic boundary region after stroke.

**MATERIALS AND METHODS:** Neural progenitor cells (NPCs) isolated from human fetal brain tissue were labeled by superparamagnetic particles, ferumoxides, with transfection agent, protamine sulfate<sup>3</sup>. Male Wistar rats (n=18) were subjected to 3 h of middle cerebral artery occlusion and sacrificed at 5-7 weeks without (n=7) and with NPC treatment (n=11) at 48 h after ischemia. MRI measurements were performed one day, and weekly for 5-7 consecutive weeks after stroke. Rats were sacrificed after the last MRI measurements. MRI measurements were performed with a 7 T, 20 cm bore, Magnex superconducting magnet equipped with a 20 G/cm, 12 cm bore gradient insert. To measure migration and localization of labeled cells, three dimensional gradient echo MR images were obtained. T<sub>1</sub>, T<sub>1sat</sub> (T<sub>1</sub> in the presence of an off-resonance irradiation of the macromolecules of brain), T<sub>2</sub>, and FA were used to characterize biophysical changes of white matter reorganization after stroke. T<sub>1sat</sub> was measured using Look-Locker (L-L) sequence with saturation pulse<sup>4</sup>. To detect superparamagnetic labeled NPCs in the host brain and white matter reorganization, brain sections were stained for iron using Prussian blue reaction, and for white matter reorganization using Bielschowsky (axons, black) and Luxol fast blue (myelination, blue) immunoreactive staining. The ischemic damaged areas were determined by using the threshold T<sub>2</sub> value of mean + 2 standard deviations from T<sub>2</sub> value measured in the contralateral hemisphere on T<sub>2</sub> maps after stroke. The ischemic recovery regions were identified by subtracting the ischemic core areas obtained 5 weeks after cell injection from the ischemic area in T<sub>2</sub> maps obtained 24 h after stroke. The relative changes of MR measurements in ischemic core and recovery ROIs (ischemia/contralateral ischemia) were used to detect the regional and group differences.



**Fig. 1** The evolution changes in T<sub>2</sub> (A) and FA (B) maps after NPC treatment and corresponding Bielschowsky Silver and Luxol fast blue stained coronal section (C, D) from the same rat. The left image in D is a higher magnified image from the box area in panel C and the corresponding contralateral area (right image in D). The bar in D = 10  $\mu$ m.

**RESULTS:** MRI measurements revealed that grafted NPCs selectively migrated towards the ischemic boundary regions. Although some labeled cells detected in or nearby the white matter reorganized areas, the overall distribution of labeled cells were not located in the hyperintense regions in FA maps. White matter reorganization, confirmed by an increase in axons (black in C and D) and myelination (blue in C and D), was coincident with increases of FA ( $p < 0.01$  from 2 to 5 weeks for treated group and from 3 to 5 weeks for control group after stroke, and coincident with decreases of relative T<sub>1</sub>, T<sub>2</sub>, T<sub>1sat</sub> ( $p < 0.05$  from 3 to 5 weeks) in the ischemic recovery regions compared to that in the ischemic core region in both treated and control groups. The treated group appears early and large increase in FA ( $p < 0.01$ ) in the ischemic recovery regions compared with control group at 5 weeks after stroke. However, there was no significant differences in T<sub>1</sub>, T<sub>2</sub>, and T<sub>1sat</sub> between treated and control groups in both ischemic core and recovery ROIs. Also, the Bielschowsky and Luxol fast blue immunoreactive staining showed that axonal projections emanating from individual parenchymal neurons exhibited an overall orientation parallel to lesion areas after stroke. The fiber tracking maps derived from diffusion tensor imaging revealed similar orientation patterns as the immunohistological results. White matter reorganization after NPC treatment of stroke is predominantly located in the extended area of the corpus callosum in ipsilateral striatum.

**DISCUSSION:** Our data suggest that MRI can detect white matter reorganization after NPC treatment of stroke. White matter reorganization after NPC treatment of stroke is dominantly located in the extended area of corpus callosum in striatum. FA differentiated white matter reorganized brain tissue from other ischemic damaged tissues. T<sub>1</sub>, T<sub>2</sub>, and T<sub>1sat</sub> provide complementary information to characterize status of ischemic tissue with and without brain remodeling. The NPCs may be involved in the activation of endogenous restorative mechanisms of white matter recovery by the induction of oligodendrocyte progenitor cells or by the release and expression of neurotrophic factors where not be located the same location of the labeled neural progenitor cells<sup>5</sup>. Of these MRI methods, DTI related parameters appear to be the most useful MR measurements which identify the location and area of white matter reorganization.

### REFERENCES:

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