Mapping the plasticity of the striatonigral pathway in a Transgenic Mice Model using MEMRI

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Introduction: The dopaminergic pathway from striatum to substantial nigra (SN) plays a critical role on the control of motor function in some neurodegenerative diseases such as Huntington's Disease(HD) and Parkinson's Disease(PD). There exist two striatal efferent projections to the SN, one is the direct striatonigral pathway with D1 receptor (D1R)-positive striatofugal fibers, the other is the indirect D2 dopamine receptor (D2R)-positive striatopallidal pathway via polysynaptic connections in the globus pallidus(GP) and subthalamic nucleus(STh). We have created a novel transgenic mouse model with the loss of striatal D1R- positive receptor to mimic the human Parkinson-plus syndromes and HD [1]. In this study, Manganese Enhanced Magnetic Resonance Imaging (MEMRI) was used to reveal the plasticity of dual pathways of basal ganglia circuits due to the D1R- positive depletion.

Materials and Methods: Seven MT (24-26 weeks old, 24-38 g) and seven littermate control (WT) mice (24-26 weeks, 24-51 g) were used. All procedures involving live animals conformed to the Australian NHMRC code of practice. Manganese Chloride ($MnCl_2$) solution (300 nl, 50mM; Sigma-Aldrich) was injected using a picospritzer into the caudate putamen, according to the coordinates of Paxinos and Franklin [2], 1 mm rostral to bregma, 1.7 mm lateral of the midline and 3.0 mm below the level of the skull. MRI scans were performed at 6hours, 12hours and 24 hours after the administration of Manganese on a Bruker Biospin 4.7T animal MRI scanner. The mice were anaesthetized with a 1-1.5% isoflurane-oxygen mixture (flow rate 1.0-1.5 liter/minute via a nose cone) and respiratory rate was monitored during the scan. A volume coil (inner diameter 72mm) was used for excitation and a custommade surface coil (diameter 15mm) was used for receiving. 3D T1 weighted images were acquired using SNAP sequence with TR/TE =15/ 4 ms, flip angle =25; FOV=15x15x15 mm^3; Matrix dimensions = 256x256x64; spatial resolution= 58x58x234um; Number of averages = 16 . 3D MEMRI data was processed using Paravision3.0.1 software (Bruker Biospin, Ettlingen, Germany). The axial slices on breg 0.2mm, -0.2mm, 2.0mm, and -3.1mm were chosen to quantitatively evaluate the manganese deposit on for CPu, GP , STh and SN separately. Region of interests (ROIs) were manually drawn in Mn2+ enhanced side and contralateral non-enhanced brain structures. The mean intensities were measured in all the ROIs. The ratio of the two symmetrical ROIs represented the concentration of manganese.

Results: It is clearly showing in Figure 1. signal intensity of SN in mutant is much lower than control at six hours after manganese injection. Also the area of manganese deposit in the SN is much smaller in the mutant, which reveals the atrophy of the SN. Figure 2. demonstrates the intensity changes over time in the SN and STh. The intensities of STh in knockout group are significant larger than in the control group ($p<0.02$) at 12 hours after the manganese injection and less intensity in SN ($P<0.02$) as well. Another interesting finding is, compared to the control, much more manganese deposited in the interpedunucular nucleus (IP) in the transgenic model (shown in figure 1).

Discussion and conclusion: Our results confirmed the existing efferent projections of the striatum with the previous studies using MEMRI[3]. In this novel transgenic model, histology results showed the low level expression of D1R- positive and about 50% increase in D2R expression in the striatum[1]. The lower signal in SN was due to the loss of the D1R- positive and the direct pathway decreased its function. An increased signal in the striatopallidal indirect pathway, which is related to D2R, could be reflecting compensation of loss of D1R-positive depletion. Our findings demonstrated MEMRI is a potential approach in vivo to study the development and plasticity of neural connections in neurodegenerative transgenic disease mouse models.

Reference: [1] Gantois I. *PNAS* submitted (2006). [2] Paxinos G Academic Press (2001). [3] Pautler R G. *Magn Reson Med.* **50**: 33-39 (2003).

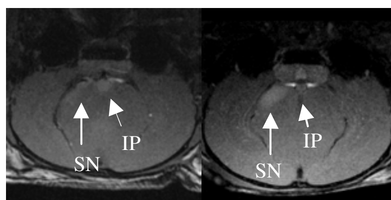

Figure 1: Typical mice brain coronal images of SN and IP of the transgenic mouse (left) and wild-type mouse (right) at six hours after administration of Mn.

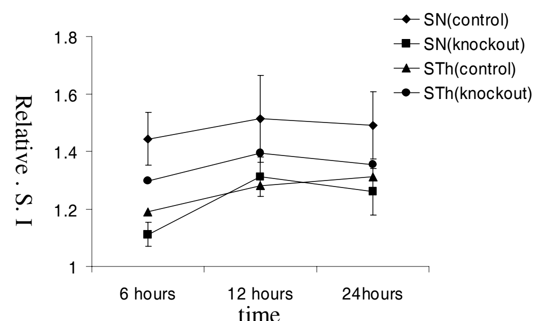

Figure 2: the relative signal (mean±std) changes in SN and STh along the time in transgenic group and control group. The intensity of SN in knockout mice is significant lower than the control at each time point.($P < 0.02$).