Manganese-enhanced MRI Detection of Neural Compensatory Changes after Neonatal Monocular Enucleation

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INTRODUCTION:

Monocular enucleation (ME) is a useful experimental model for studying the mechanisms of neural plasticity [1]. Previous studies suggested that ME, when performed during an early postnatal period (up to 15 days after birth), initiates not only neurodegeneration in both dorsal lateral geniculate nucleus (DLG) and superior colliculi (SC) in the deafferented side, but also a series of adaptive reactions in the visual (and other sensory) system(s) which tend to compensate for the lost sensory capacity [2, 3]. However, to further study the morphological and physiological changes after ME quantitatively and longitudinally, we need noninvasive measurements with high spatial resolution. Manganese ion (Mn^{2+}), which can be taken by biological cells via voltage gated calcium channels, has been introduced as a valuable cellular contrast agent for tracing neuronal pathways, for the enhancement of neural architecture and brain function [4]. Several previous studies have reported the use of manganese-enhanced MRI (MEMRI) to detect longitudinal neurodegeneration such as brain ischemia [5, 6]. In this study, we aim to map the change of rat visual system after postnatal ME using MEMRI.

METHODS:

Animal Preparation: Male Sprague-Dawley rats (N = 4) were used in the present study. Right monocular enucleation was performed at postnatal day (P) 10 under inhaled isoflurane anaesthesia through an incision in the conjunctiva followed by sectioning of the extraocular muscles and the optic nerve. The eyeball was removed and the empty socket was filled with oxidized regenerated cellulose Surgicel® (Johnson & Johnson). Three weeks after injury, a 100 mM solution of $MnCl_2$ in isotonic saline (0.9% NaCl in water) was injected at a dose of 60 mg/kg body weight intraperitoneally 24 hours before imaging.

MRI Protocols: All MRI experiments were performed on a Bruker PharmaScan 7 T scanner using a 72-mm birdcage transmit-only RF coil with an actively decoupled receive-only quadrature surface coil. During the MRI scan, mice were anaesthetized with isoflurane (3% induction and 1.5% maintenance) with respiratory monitoring and kept warm under circulating water at 37 ºC. T1-weighted images were collected with a RARE sequence using FOV = 32×32 mm, matrix resolution = 256×256, slice thickness = 0.5 mm, number of slices = 10, TR/TE = 420/7.5 ms, RARE factor = 4 and NEX = 64; T2-weighted images were acquired using the same voxel dimensions and slice geometry with TR/TE = 4200/38.7ms, RARE factor = 8 and NEX = 6.

Data Analysis: Images were analyzed using ImageJ (NIH) and ROIs were manually defined according to rat brain atlas. Color-coded maps were computed from T1WIs for visualization and quantification. Before running statistical analysis, averaged signal intensity (SI) over ROIs was normalized by SI from the thalamus in the ipsilateral side of the ROIs. Mann-Whitney test was performed between contralateral sides of normalized signal intensity measurements with $p < 0.05$ considered as statistically significant.

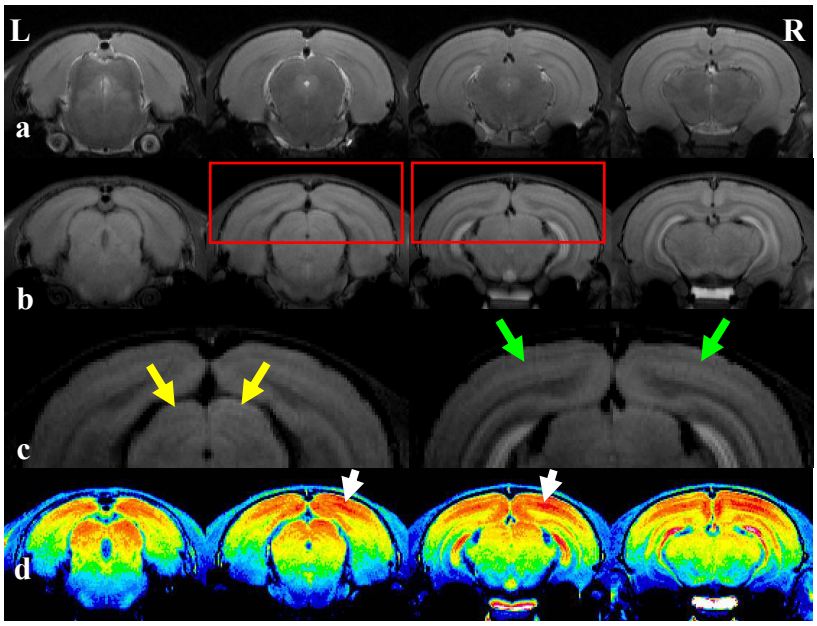

Fig.1 T2WIs (a) and T1WIs (b) of rat brain 3 weeks after neonatal ME. Magnified images (c) of T1WIs (red boxes) show cortical and subcortical structure. Note the smaller size of left SC (yellow arrows) and better enhancement of the right V1 (green arrows). Color-coded T1W images (d) give direct visualization of the Mn deposition in rat brain. Concentrated Mn-enhanced pattern is observed in V1M (white arrows).

RESULTS AND DISCUSSION:

While there is no severe morphological asymmetry in visual cortex and SC found in the T2W anatomical images (Fig.1 [a]) 3 weeks after postnatal ME, significantly smaller size ($p<0.05$, Fig.2) of left SC (contralateral to the enucleated eye) compared to the normal SC was observed in Mn-enhanced T1W images of every animal (Fig.1 [b, c], yellow arrows). This considerable reduction in the volume of the visual relay nuclei after neonatal unilateral eye removal is partly due to the loss of optic nerve terminals, but there is also a pronounced neuronal cell death [7]. With the Mn-induced signal intensity enhancement, clear laminar structure of SC (Fig.1 [c, d], yellow arrows) and primary visual cortex (V1) (Fig.1 [c, d], green arrows) can be detected. (I) The Mn uptake by collicular neurons between the normal and the deafferented sides is remarkably different ($p<0.05$, Fig.3), especially in the superficial layers of SC. Because extirpation of one eye destroys afferents to SC, resulting in severe impairment by the degeneration of collicular synapses[1]. (II) V1 ipsilateral to the enucleated eye, especially monocular area (V1M) (Fig.1 [d], white arrows), has slightly higher Mn-induced enhancement than V1 of the deafferented side ($p=0.1$). Since more Mn uptake could be due to hyper cellular density or hyper cellular activity, this moderately better enhancement correlates well with the previous study reporting that an increased density of neuronal somata was found in V1M ipsilateral to the remaining eye [1]. But the difference is not as significant as SC, which may be due to that an early loss of sensory driven activity could lead to massive functional reorganization in the cortex. The unilaterally enucleated animals still receive other sensory inputs and the visual cortex may get functionally re-specified and devoted to auditory and somatosensory processing [3]. Work is currently underway to further investigate this visual pathway reorganization. In conclusion, the experimental finding of our study showed that using *in vivo* MEMRI, impaired SC with high spatial resolution revealing the laminar structure. Moreover, enhancement of monocular area of V1 can be observed after neonatal monocular enucleation noninvasively. The current study supports the validity of MEMRI approach in exploration of neural plasticity and the adaptive and compensatory modifications within the brain following neonatal monocular enucleation.

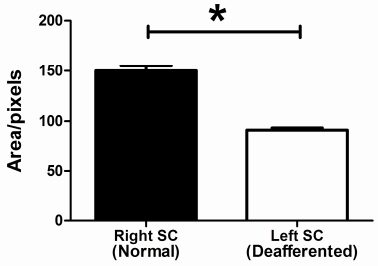

Fig.2 Mean ± SD illustrates the area of left and right SC which is measured by ROIs covering the superficial layers of SC. The right SC is significantly larger than the right one. Mann-Whitney test between the two sides (N=4), *$p<0.05$.

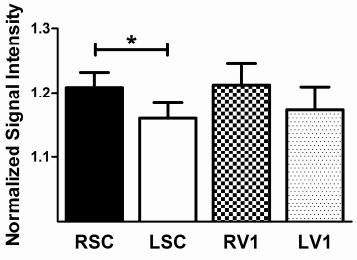

Fig.3 Comparison of T1WI signal intensity (SI) of SC (RSC: right SC; LSC: left SC) and V1 (RV1: right V1; LV1: left V1) which is normalized by the averaged SI of ipsilateral thalamus. Both SC and V1 contralateral to the remaining eye have significantly better Mn enhancement after neonatal ME. Mann-Whitney test between the two sides (N=4), *$p<0.05$.

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