Transcallosal Connectivity Changes in Rodent Visual Cortex Following Monocular Enucleation or Light Deprivation: An MEMRI Study

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INTRODUCTION: Visual cortex has been intensively studied for answering fundamental questions regarding cortical development and plasticity[1]. Binocularity is one of the most prominent characteristics of neurons in the visual cortex. The binocularity relies on both direct ipsilateral geniculocortical inputs and callosal projections from the contralateral cortex[2,3]. Understanding these underpinnings of binocularity plasticity may shed light on new therapies for amblyopia. Although neurons in rodent visual cortex responsible for each eye are not highly organized into alternate ocular dominant columns as in higher mammals, rodent visual cortex does show ocular dominance plasticity[4]. In this study, we hypothesize visual manipulation of left or right eye may exert different impact on right to left callosal transfter, which could be characterized by high resolution MEMRI utilizing manganese ion (Mn^{2+}) as an anatomical and functional neuronal marker, aiming to understand how the inputs from the two eyes interact for binocularity and how the geniculocortical pathway and callosal pathway were affected by monocular manipulation.

METHODS: **Animal Preparation:** 500mM, 100nl Mn^{2+} (pH=7.4) was injected slowly into the right visual cortex, at 6.5mm posterior and 5.5mm lateral to bregma, in 42 male Sprague Dawley rats (350-400g) divided into 4 groups: the normal group (n=12), the left eye enucleated (contralateral to the injection side, CME) group (n=9), the right eye enucleated (ipsialteral to the injection side, IME) group (n=10), and the right eye deprivation (IMD) group (n=11). Monocular enucleation (ME) and monocular deprivation (MD) were performed at 7 days prior to manganese injection by either removing one eye or suturing one eyelid[2,5]. Buprenorphine (0.1mg/kg) was given subcutaneously to both groups as analgesics for 3 days after surgery. MEMRI was performed at 24 hours after Mn^{2+} administration. **MRI Protocols:** All MRI experiments were performed on a 7T Bruker MRI scanner. During imaging, rats were anesthetized with isoflurane (3% for induction and 1.5% for maintenance) with respiration monitoring and were kept warm at 37°C. Enhancement due to Mn^{2+} transfer was visualized by acquiring Modified Driven-Equilibrium Fourier Transform (MDEFT) images with TR=4000ms, Echo TR/TE=12/4ms, TI=1100ms, FOV=32×32mm^2, matrix=256×256, slice thickness=0.5mm, number of slices=28, number of segments=4 and averages=8. **Data Analysis:** Regions of interests (ROIs) were manually placed in the left V1/V2 TZ, posterior corpus callosum (CC), right lateral geniculate nuclei (LGN), and right visual cortex by referencing to a rat brain atlas[5]. Signal intensities (SI) of these ROIs were measured in ImageJ. SI of the left V1/V2 TZ, posterior CC and right LGN were further normalized by that of the right posterior cortex, and used for statistical analysis (two-tailed Mann-Whitney test).

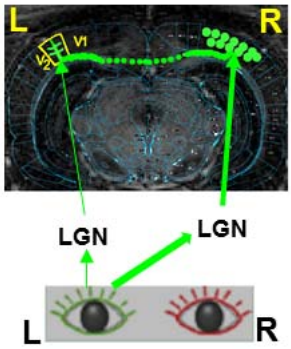

Fig. 1 Schematic retinal projection pathways to bilateral visual cortex.

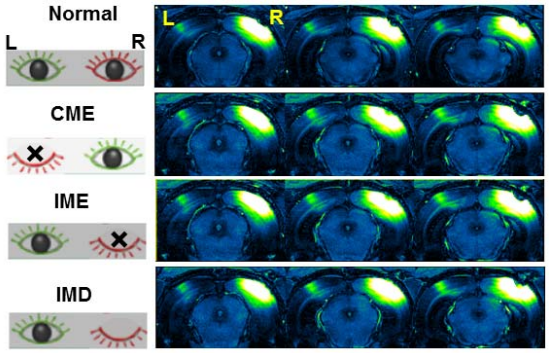

Fig. 2 Color-coded MDEFT images illustrating Mn^{2+} enhancements in the left hemisphere after locally injected into right cortex in normal rats, left eye enucleated rats (CME), right eye enucleated rats (IME) and right eyelid sutured rats (IMD).

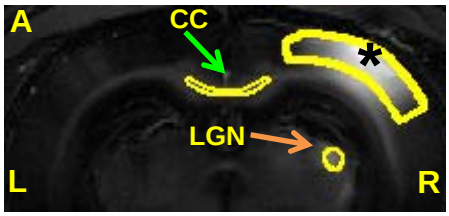

Fig. 3 ROI definitions in corpus callosum (CC) and lateral geniculate nucleus (LGN), and right visual cortex (star).

RESULTS: Fig. 1 shows the schematic visual pathway. Take the left eye as an example, the majority of its retinal axons (more than 95%) crossed at optic chiasm and projects to the left hemisphere via LGN, with the remaining axons projected to the left side. Callosal cells in the right hemisphere send axons via corpus callosum to the left side, and terminated collectively in a narrow stripe centered on the primary/secondary visual cortex (V1/V2) border, termed as V1/V2 transition zone (V1/V2 TZ)[6], as encircled in the yellow box. Thus the geniculocortical inputs and callosal projects both contribute to binocular vision of one hemisphere. Fig. 2 shows color-coded MDEFT images to demonstrate callosal projection pattern was preserved in monocularly manipulated rats. Fig. 3 illustrates ROIs in corpus callosum (CC), lateral geniculate nucleus (LGN) and right visual cortex (star) for statistical comparisons between different groups as shown in Fig. 4.

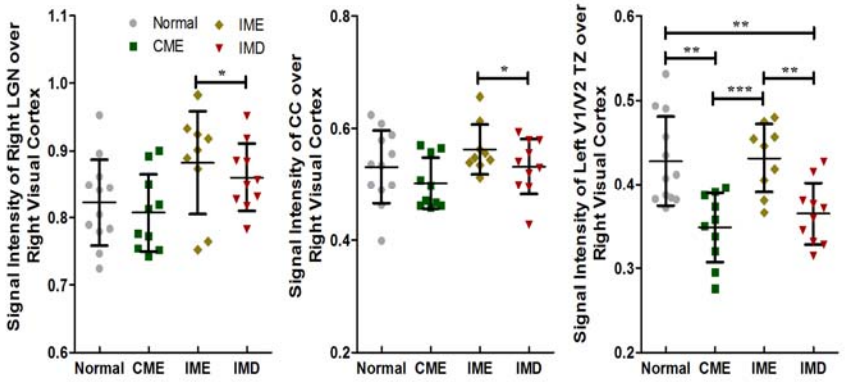

Fig. 4 Statistical comparisons of right lateral geniculate nucleus (LGN), corpus callosum (CC) and left V1/V2 transition zone (V1/V2 TZ) between different groups. *P<0.05, **P<0.01, ***P<0.001, two-tailed Mann-Whitney tests. Data were shown as mean ± SD. No statistical difference was observed where p value was not indicated.

DISCUSSIONS AND CONCLUSION: In CME rats, the cortical activity was significantly reduced due to loss of its dominant inputs, thus callosal transfter of information from this side to the contralateral side was reduced[2], resulting in a decreased Mn^{2+} transfer. In this study, we observed left V1/V2 TZ signal enhancement decrease following IMD and its increasing trend following IME. These results are consistent with the findings from eletrophysiological[7] and c-fos staining experiments[8]. In cats, callosal terminals preferentially connect with the contralateral domain in V1/V2 TZ, our results implies same rule may be applied in rodents. MD still permits residual visual stimulation through the sutured eyelid and retains spontaneous retinal activity, thus it is possible that left eyelid suture in adult rats in this study would produce no significant cortical responses to the right eye in the left cortex, while IME could induce slightly higher responses to contralateral vision. Furthermore, callosal terminals in left visual cortex following right eye enucleation may be recruited to make synaptic connections with neurons of other modalities or connect with neurons responsible for the left eye responses, which is already slightly upregulated. In conclusion, this study demonstrated MEMRI as a powerful tool for probing corpus callosum functions and plasticity with efficiency, sensitivity and specificity.

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