Strengthening of Thalamocortical Synapses at Layer IV in the Juvenile Whisker Barrel Measured by MRI and Electrophysiology

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Introduction Studies of the rodent barrel cortex have shown that the critical period of thalamocortical plasticity ends in the first week after birth[1]. Barrel cortical plasticity occurred after this critical period is usually considered to occur in corticocortical connections. In this study, plasticity associated with unilateral denervation of the infraorbital (IO) nerve was studied in the protected whisker cortex in juvenile rats. Previously, plasticity after unilateral denervation of forepaw, hindpaw and IO has been shown to lead to detection of increased bilateral cortical fMRI responses to stimulation of the protected side in rats [2,3]. Here, we focused on determining whether there were changes in the contralateral thalamocortical pathway to the good whisker pad that could explain the increased cortical fMRI. First, stronger BOLD response was detected in the contralateral barrel cortex of rats with unilateral infraorbital denervation (IO rats) in comparison to sham rats. The relation between thalamus and cortical fMRI was consistent with a strengthening of the thalamocortical input [2]. To further analyze the underlying circuit changes, Manganese-enhanced MRI (MEMRI) was applied to map the contralateral thalamocortical connection between the ventral posteromedial/posterior nucleus of thalamus (VPM/PO) and barrel cortex. MEMRI can be used to trace the antegrade neuronal projections into layers 4/5 of the barrel cortex [3]. The synaptic strength of thalamocortical projection can be estimated by measuring the amount of Mn transported from VPM/PO to the Layer 4-5 of the barrel cortex. Mn-enhanced signal in the Layer 4-5 of IO rats was significantly higher than that of the sham rats. This result indicated a strengthened thalamocortical projection toward the contralateral barrel cortex of the good whisker pad in IO rats. This layer-specific thalamocortical plasticity changes led us to studying the underlying synaptic mechanism with *in vitro* slice electro-physiology. Synaptic responses of Layer 4 stellate cells, the major cortical neurons receiving thalamocortical inputs, were recorded after stimulation of VPM thalamic projection fibers. The increased amplitude of Sr-induced miniature excitatory postsynaptic current (EPSC) in IO rats indicated a postsynaptic modulation on the strengthened thalamocortical inputs. summary, by combing multi-modal MRI imaging methods and electrophysiology strengthening of the thalamocortical synapse was demonstrated in the whisker barrel system of juvenile rats.

Methods Unilateral infraorbital denervation and sham surgeries were performed on rats at postnatal 4 weeks. MRI imaging and electrophysiological recordings were done at postnatal 6-7 weeks. BOLD-fMRI was performed on 18 rats anesthetized with α-chloralose. MEMRI were done on 20 rats. Detailed procedures for imaging and animal preparation for BOLD-fMRI and MEMRI were similar to those previously described [4, 5]. Briefly, all images were acquired with an 11.7T/31cm horizontal bore magnet (Magnex, Abingdon, UK), interfaced to an AVANCE III console (Bruker, Billerica, MA) and equipped with a 12 cm gradient set. A custom-built, 9 cm diameter transmitter coil was used for transmit and a custom-built surface coil was used for receive employing a transmit/receive decoupling device. A single-shot 3D gradient-echo, EPI sequence was used for the fMRI studies (matrix 64 x 64 x 32, TE 16ms, TR 1.5s, isotropic resolution, 300μm). A sub-skin electrical stimulation with 2.5 mA, 300μs pulses, 3Hz was delivered to forepaw (FP) and whisker pads in a block design (30s on/off). MPRAGE (TI, 1s) was used to examine Mn-tracing (matrix 192x192x16, in plane 100μm, thickness, 500μm). AFNI software was used for fMRI image processing. For group analysis, custom C++ scripts were developed to register MRI images to rat brain atlas to define brain ROIs. Student's t test (two-tail) was used for statistical analysis.

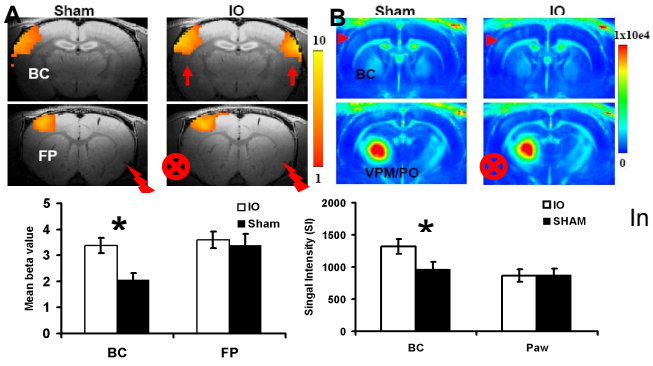

Fig. 1. A. BOLD-fMRI mapping the barrel cortex (BC) and forepaw (FP) representative S1area of Sham and IO rats. The mean BOLD signal in the contralateral BC was significantly higher in IO than sham rats (*, p=0.003, n=9), but not in the FP S1. B. MEMRI tracing the thalamocortical projections from VPM/PO to the BC. Color-coded MPRAGE images were averaged from 10 IO and sham rats. Layer IV of the BC was highlighted by red arrowhead, the injection site was in VPM/PO (lower panel). ROI group analysis showed a higher Mn signal in the IV layer of the BC of IO rats than sham rats (*, p=0.04, n=10).

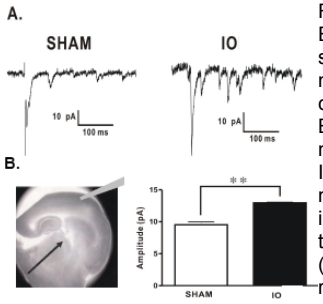

Fig. 2. Sr-induced miniature EPSC recorded from Layer 4 stellate cells. A illustrates the mEPSC recorded in stellate cells of IO and Sham rats. B. Comparison of the mEPSC amplitude between IO and Sham rats (sham, n=7, IO, n=5, p<0.004). The inset showed the location of the stimulation electrode (black arrow) and the recording electrode.

Results Fig1A shows averaged 2D fMRI β-maps overlaid on anatomical MRI images across the barrel cortex (BC) and the forepaw S1 (FP) cortex of IO and Sham rats. Bilateral activation in the BC was observed in IO rats (contra-BC left; ipsi-BC, right). Group analysis of the mean β value in contralateral BC ROI showed a significantly increased β value in the IO rats up to 65% compare to only 6% increase in the contralateral FP ROI. In addition, little difference (less than 5%) was observed in the contralateral VPM/PO (data not shown)[2]. This result indicated a specific plasticity changes in the contralateral BC of IO rats. Next, we locally administrated Mn into the VPM/PO of the contralateral thalamus (50mM, 200nl). Detectable Mn signal in the Layer 4-5 first appears 5 h after Mn injection. In IO rats, Mn-enhanced MRI signal in the Layers 4-5 of the BC was significantly higher than that of sham rats with 37% signal increase, but no difference was detected in the layer 4-5 of FP/HP S1 cortex(less than 2%). This result indicated an increased thalamocortical synaptic strength along the functional ascending pathway of IO rats. Finally, we extracted the rat brain to record the synaptic response in Layer 4 stellate cells. Among multiple electrophysiological tests, we presented the Sr-induced miniature EPSC, showing significantly increased amplitude of mEPSC in IO rats. These results indicated that the thalamocortical synapse can strengthen after unilateral IO in the BC of juvenile rats well past the critical period for the barrel thalamocortical plasticity. **Ref**:[1] Daw et al, MCN. 34, 293-502 (2007) [2] Yu et al., ISMRM, 4137 (2010) [3] Pelled et al. PNAS, 106 :114-9 (2009) [4] Yu X. et al., NI, 49 :1667-76 (2010). [5]Tucciarone et al. NI 44 : 923-31 (2009)