Changes in Callosal and Thalamic Connectivity Following Peripheral Nerve Damage to the Rodent Forepaw Detected with Manganese Enhanced MRI

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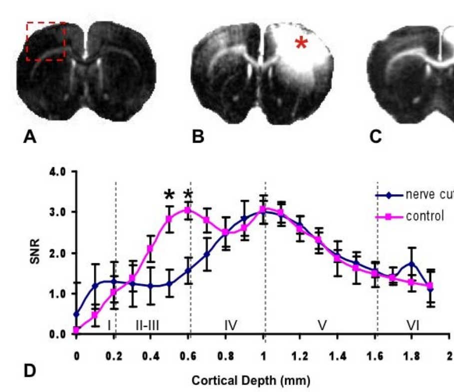
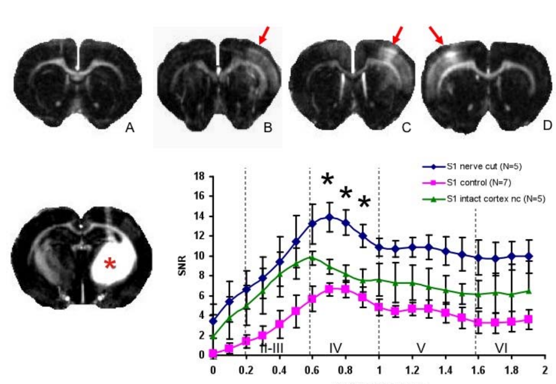
Introduction: Sensory deprivation results in cortical reorganization that will ultimately affect the degree of functional recovery. Previously it has been shown that peripheral nerve injury to the rat forepaw will lead to massive reorganization of both affected (deprived) and healthy cortices.[1] Preliminary evidence indicates that the healthy cortex has upregulated neural activity and the neural activity to the deprived cortex is due to increased interneuron activity. The modifications associated with this type of cortical organization should be laminae specific. It is challenging to determine the precise changes in neuronal network activity, which mediates changes in activity of the deprived and healthy cortex using electrophysiological techniques. Previously it has been demonstrated that Manganese Enhanced MRI (MEMRI) is capable of resolving laminar specific connections of the somatosensory cortex to the contralateral somatosensory cortex through the corpus callosum (via lamina 3 and 5), and from the thalamus to lamina 4 of the ipsilateral cortex.[2] Since MEMRI has the ability to provide specific information about neuronal connections from cortex to cortex and thalamus to cortex at the resolution of individual laminar levels *in vivo*[2], it provides the unique ability to track the laminar specific changes that occur during cortical reorganization following injury. The purpose of this work was to determine if MEMRI neural tracing could detect changes in the laminar functional architecture of cortical-cortical and thalamo-cortical somatosensory pathways following complete nerve deafferentation of the rat forepaw.

Methods: ***Animal Procedure*:** 33 adult male Sprague-Dawley rats (140-200g) were used in this study. 18 rats underwent surgeries where their right radial, median and ulnar nerves of the right forepaw were completely severed. Two weeks after dennervation they were split into 3 groups: one group received a 50nl injection of 60mM aqueous $MnCl_2$ into the right posterior thalamic nucleus and one group was injected the same into the left posterior thalamic nucleus. Both groups were imaged 3-5 hours post injection. The third group was injected with 1ul into the right forepaw area of the primary somatosensory cortex and imaged 24 hours post injection. Two groups served as controls for the thalamic and cortical injections with the same procedure mentioned above but without nerve deafferentation. ***MRI:*** Images were acquired on an 11.7T/31cm horizontal magnet (Magnex) interfaced to a Bruker Avance console (Bruker) using a volume transmit coil and circular surface receive coil. A standard multi-slice spin echo T1 weighted sequence was used to evaluate the success of the injection site. A TR/TE of 500/8.9ms,FOV=2.56 x 2.56cm, matrix 256x256, thickness= 0.5 mm, and gap 0.6 mm was used to acquire 16 coronal slices. For data analysis 3D T1 weighted images were obtained using an MP-RAGE sequence. 64 slices were acquired with a FOV=2.56x2.56cm, matrix 256x256, thickness=.1mm, TR= 4000ms, Echo TR/TE = 15ms/5 ms, TI= 1000ms, number of segments=4, Averages=4. ***Data Processing*:** Region of interest (ROI) analysis was done on T1W data using ImageJ. 10-15 linear ROIs perpendicular to the surface of the forepaw somatosensory cortex, through its thickness, were selected to get T_1 values from T_1 weighted images, at various cortical depths, which were then averaged. The averaged signal intensity profiles of the ROIs were normalized to the standard deviation of noise in a region outside of the head of the corresponding slice. Averaged profiles for each animal were grouped, summed, and averaged. A multiple comparisons ANOVA was done to compare groups of SNR profiles and for comparing within groups at differing cortical depths. Post-hoc multiple comparisons using an unpaired two-tailed Student t-test with bonferonni correction were done to assess the statistical significance of MRI signal changes of unique cortical depths to the remaining cortical depth measurements within an experimental group. Values are reported graphically as ± s.d. A p value < 0.05 was considered significant.

Results: **Figure1 (**top *right)*: Image A shows a coronal slice without injection and with the region of interest (ROI) indicated by a red box, which represents the forepaw somatosensory cortex. Image B shows a slice from a control animal injected into the right hemisphere (indicated by red asterix) with contralateral transport to the ROI, image C shows a slice from a nerve deafferentated animal with noticeable reductions in contralateral transport. Plot D shows the signal enhancement profile in the somatosensory cortex contralateral to injection site as a function of cortical depth for right median, radial and ulnar nerve cut animals (n=7) and control animals (n=8). Consistent with previous results, two peaks of enhancement can be detected due to tracing into laminae 3 and 5.[2] A significant reduction in signal peak can be observed in the nerve cut animals at a cortical depth corresponding to lamina 3 (indicated by black asterix, p <.05).

Figure 2 (*bottom):* Image A demonstrates T1W image of an animal without injection, and image B shows a cortical enhancement pattern (indicated by red arrow) in control animals (n=7) after injection into the right posterior thalamic nucleus (injection site seen by red asterix in brain slice at the lower left). Image C shows cortical enhancement in animals with right nerve deafferentation after injection into the right posterior thalamic nucleus (n=5). Figure D shows cortical enhancement in animals with right forepaw nerve cut (n=5) after injection into the left posterior thalamic nucleus. The plot in Figure 2 shows the signal from the forepaw somatosensory cortex ipsilateral to either right posterior thalamic injection or left posterior thalamic injection as a function of cortical depth. The enhancement profile of the somatosensory cortex corresponding to somatosensory inputs from the nerve cut (blue plot) shows greater than a 2 fold increase in signal enhancement due to manganese compared to control (pink) at every cortical level. There was also a significant increase in signal enhancement in lamina 2-3 of the cortex corresponding to somatosensory inputs from the intact forepaw (green plot) compared to control (pink).





Discussion: Previously it has been demonstrated that cortical reorganization observed following complete peripheral nerve injury affects functional MRI responses both in healthy and deprived cortices. In order to study the neuronal basis of this bilateral reorganization we used laminar specific MEMRI neural tracing. The results demonstrate that both cortical-cortical and thalamo-cortical pathways are involved in this reorganization. First, reduced manganese transport was found in lamina 3 of the deprived cortex after injection of manganese to the healthy cortex predicting that input into this layer has decreased. Second, increases in T1 enhancement in the healthy cortex were found after injecting manganese into the healthy thalamus. There was increased tracing throughout the cortex which was highest at the lamina 3/4 interface. Thus, both cortical-cortical and thalamic-cortical neuronal pathways are affected by the injury, and contribute to the deprived somatosensory reorganization. Currently we are applying immunostaining techniques in order to identify the specific neuronal populations that undergo changes in their activity following injury. These results will be correlated with MEMRI findings, and will aid to elucidate the neuronal mechanisms and pathways that are involved in cortical reorganization.

References: **(1)** Pelled G. et al. *Neuroimage*. (2007). 34(3);1220-1226. **(2)**Tucciarone J. et al. *Neuroimage* (2008) [epub ahead]. **(3)** Wise and Jones. (1978) *J. of Comp. Neurol.*178: 187-208