Determination of Laminar Specific Tracing of Somatosensory Pathways Using Manganese Enhanced MRI

J. Tucciarone[1], G. Pelled[1], K. Chuang[1], and A. P. Koretsky[1]

[1]Laboratory of Functional and Molecular Imaging, National Institutes of Health, Bethesda, Maryland, United States

Introduction: The anatomical and functional architecture of the somatosensory cortex has been studied extensively using neuronal tracing and electrophysiological methods. There is evidence that functional changes associated with learning and plasticity take place in a laminar specific manner. However, the ability to image *in vivo* the structural dynamics of the laminar changes remains a challenge. The primary and the most dominant afferent input from the peripheral nerves to the somatosensory cortex originate in the thalamus. Eighty-five percent of these projections terminate in lamina 4 and this information is further processed in the other cortical laminae. Another major input to the somatosensory cortex arises from the opposite hemisphere where 20% of cortical neurons project from one hemisphere to the other (into laminae 3 and 5), via the corpus callosum[1]. To date information about lamina specific connections comes from classical neuronal tracers that rely on histology.

Manganese Enhanced MRI (MEMRI) utilizes the paramagnetic manganese ion to track neuronal pathways.[2] A number of neuronal pathways in a number of animal models have been mapped with MEMRI tract tracing. Only one report has used MEMRI tract tracing to measure callosal connections involved in interhemispheric communication.[3] Furthermore there is only one report that manganese transport by neuronal afferents from the thalamus to the cortex in rats can be visualized by MRI in a laminar specific manner.[4] MEMRI used to trace lamina specific neuronal connections in somatosensory pathways could be applied to assess changes in connections that may occur with learning, plasticity and pathological states such as stroke, paralysis or amputation. Therefore, the goal of this work was to determine if neural track tracing using MEMRI could distinguish between these two major afferent pathways by revealing specific cortical input layers

Methods:

Animal Procedure*:** 7 adult males Sprague-Dawley rats (140-200g) were scanned pretreatment, immediately post stereotaxic injection of 1µl of 60mM aqueous $MnCl_2$ into the right somatosensory cortex (S1) forepaw area, and 6, 12, and 24hrs post injection. For the thalamus, 200nl of the same solution was stereotacticly injected into the left thalamus of 2 rats and imaged pre, post, 6 and 12hrs after injection. ***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. 2D T_1 mapping images were obtained using the Look-Locker method in order to gather more absolute, quantitative T_1 measurements (TR=12s, TE=2.5ms, α=20°, τ= 500ms, N=20).[5] 22 coronal slices with FOV=2.56 x 2.56cm, matrix 128x128, thickness=1.0 mm, and gap 0.1 mm were used to cover the whole brain at 200µm in plane resolution. For higher resolution images (100 µm) of thalamic transport to layer 4, a standard multi-slice spin echo T_1 weighted sequence was used with a TR/TE of 500/8.9ms, FOV=2.56 x 2.56cm, matrix 256x256, thickness=1.0 mm, and gap 0.1 mm. ***Data Processing***: For T_1 maps the T_1 of each pixel was calculated using a custom written program that accounts for the dependence of actual T_1 on the flip angle.[5] Group statistical maps were created by paired t-test in each voxel ($p<0.05$). Region of Interest (ROI) analysis was performed on the 2D LL and T_1W data using Image J. According to the rat brain atlas, linear ROIs perpendicular to the surface of the cortex, throughout S1, were selected to get averaged T_1 values from T_1 maps, at various cortical depths, at different time points. From these values, the change in relaxation rate due to manganese was calculated using the expression: $\Delta R_1 = 1/T_{1exp} - 1/T_{1control}$. Paired student *t*-tests ($p<.05$) were used to compare averaged ΔR_1 values in these regions between rats with manganese at different time points post injection with the pre-injection, baseline values.

Results:

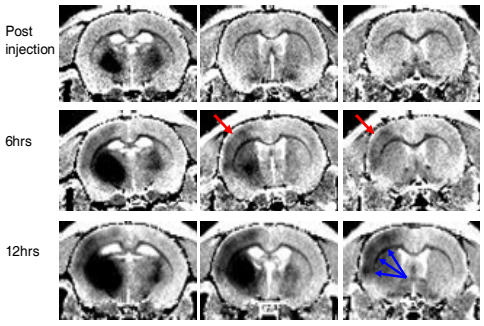

Figure 1: T_1 maps after thalamic injection

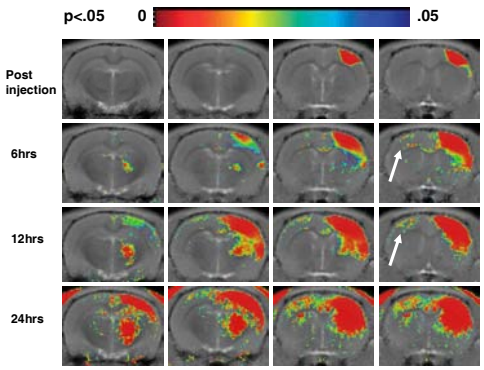

Figure 3: Probability maps compared to controls for reductions in T_1 after injection and 6, 12 and 24 hours post injection into S1

T_1 maps after thalamic injection indicated T_1 reductions in lamina 4 of the somatosensory cortex (Fig. 1 red arrows), which can be seen most clearly at 6 hours post injection. At 12 hours, separate, potential, representational areas of the cortex could be seen (blue arrows). T_1W images (Fig. 2 top) allowed for a more detailed look of enhanced lamina 4 attributed to thalamic transport, with twice the resolution (100µm) in a time comparable to the T_1mapping sequence. From these images, ROIs through the cortex revealed greater signal enhancement about laminae 4 and 5 at six hours post injection (Fig. 2 bottom).

Group average probability maps for a change in T_1 obtained from quantitative T_1mapping data is shown in Figure 3. After direct S1 injections, significant reductions in T_1 can be seen in the opposite hemisphere, homotopic to the injection site (Fig. 3 white arrows). Subcortical regions such as thalamus also had significant changes in T_1. T_1 maps were more sensitive at detecting the small T_1 changes associated with manganese transport than traditional T_1W sequences (data not shown). Figure 4 shows the change in R_1 at 6, 12, and 24 hours in S1 in the opposite hemisphere as the injection site. Laminae 2-5 had significant changes in R_1 as compared to baseline, with larger changes in R_1 throughout the cortical thickness at 24 hours.

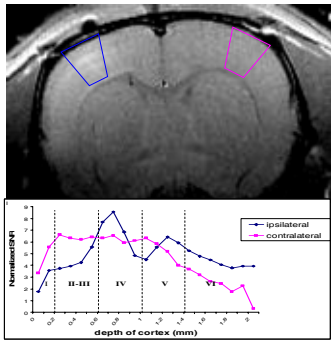

Figure 2: T_1W image of S1 6hrs after left thalamic injection (top), normalized SNR of the ROI through various cortical depths of S1 contralateral and ipsilateral to the injection site (bottom)

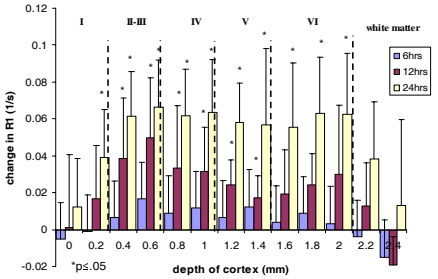

Figure 4: ROIs at various depths of the S1forepaw area within the cortex contralateral to the injection site at 6, 12, and 24 hour post injection

Discussion: Both T_1 mapping and T_1W protocols could detect the T_1 reductions specific to lamina 4 after thalamic injections of manganese. These results confirm that MEMRI has the sensitivity to reveal laminar specific inputs in the thalamocortical network. Additionally, T_1 mapping has the sensitivity to detect small changes in R_1 associated with manganese transport between hemispheres of homologous representational areas in the somatosensory cortex. There was no significant difference in laminar enhancement for this pathway at the time points studied. It may be that imaging between six and twelve hours will reveal laminar specific effects. Subcortical regions ipsilateral to the injection site including the striatum, thalamus, and the secondary somatosensory cortex also exhibited T_1 reductions. These results demonstrate lamina specific neuronal track tracing of manganese ion, along the thalamo-cortical pathway and cortical interhemispheric communication associated with somatosensory afferents.

References: (1) Wise and Jones. *J. of Comp. Neurol.* 1978; 178: 187-208 (2) Pautler, RG. *NMR Biomed* 2004; 117:595-601 (3) Leergard TB, et al. *Neuroimage* 2003; 20: 1691-1600 (4) Silva A, et al. *Cerebral Cortex* (submitted) (5) Chuang KH, Koretsky AP. *Magn Reson Med* 2006; 55: 604-611