Detection of Thalamocortical Inputs of the Rat Whisker Barrel Field Using Manganese Enhanced MRI

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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 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. Furthermore, it has been well established that lamina 4 of the rodent somatosensory cortex contains cell dense areas that create a "map" of inputs from the body's periphery. In particular individual barrel columns of the mouse and rat whisker barrel field, which receive thalamic inputs from the ventroposterior nucleus of the thalamus, are known to be functional cellular units for individual whisker vibrissa.[1] To date information about layer 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 pathways from a number of brain regions in a number of animal models have been mapped with MEMRI track tracing. 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.[3] MEMRI used to trace layer 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. Therefore, the goal of this work was to determine if neural track tracing using manganese enhanced MRI (MEMRI) can distinguish individual thalamocortical inputs to whisker barrels of the barrel field cortex.

Methods:

Animal Procedure*:** 5 adult male Sprague-Dawley rats (140-200g) were scanned immediately post stereotaxic injection of 50nl of 60mM aqueous $MnCl_2$ into the left posterior nucleus of the thalamus, and then were imaged again 3-5 hrs post 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. A standard multi-slice spin echo T_1 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 performed on the T1W data using Image J. Linear ROIs parallel to the lateral border of the cortex were drawn through the enhanced cortical region of axial slices at a cortical depth of .8mm, corresponding to the depth of layer 4 of the rat frontal cortex. The same linear ROIs were drawn in a homotopic area of untreated, contralateral cortex. The signal intensity profiles of there ROIs were normalized to the standard deviation of noise in a region outside of the head of the corresponding slice. From these normalized signal intensity profiles, peak to trough analysis was performed by calculating the difference between local maxima and local minima of profiles acquired from the treated and untreated hemispheres. The two groups of normalized peak to trough differences were summed and averaged. A Paired student *t*-test ($p<.001$) was used to compare the peak to valley difference between the treated and untreated hemisphere groups.

Results:

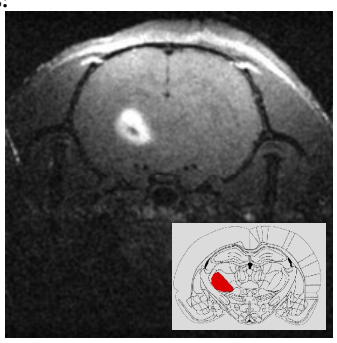

Figure 1: T1W after thalamic injection

The brain template of Figure 1 (lower right) shows the anatomical position of the ventroposterior thalamic nucleus (in red). The T1W image immediately post injection, shows an enhanced region of tissue contrast with good correspondence to the position of this thalamic area. The enhanced region of layer 4 was narrowly defined to the rat barrel field representational area (red arrows) as is seen in coronal slices acquired with an MP-RAGE sequence in Figure 2.

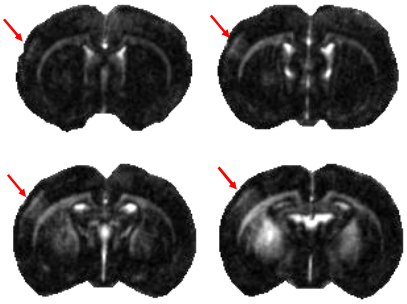

Figure 2: Consecutive T1W coronal images 4hrs after left thalamic indicating a narrow band of enhancement through the barrel field cortex

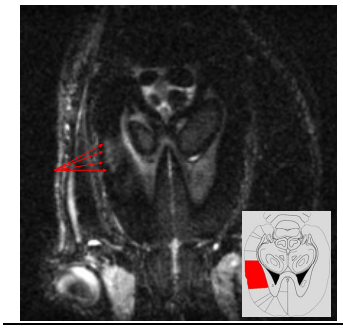

Figure 3: Axial view of enhancement profile through an area corresponding to the rat barrel field cortex

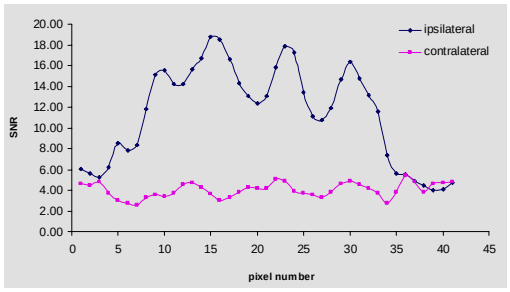

Figure 4: Signal intensity profile through barrel field of brain hemispheres ipsilateral and contralateral to injected thalamic nucleus

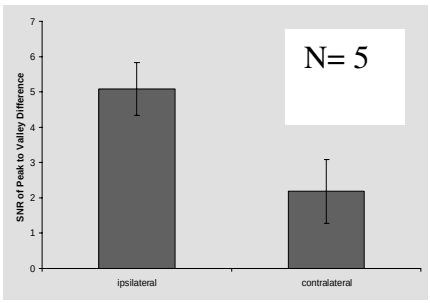

Figure 5: Normalized peak to trough difference from intensity plots of the barrel cortex of brain hemispheres ipsilateral and contralateral to injected thalamus

Axial views of the rat brain show well defined circular regions of contrast within 4-5 well defined rows as seen in Figure 3 (red arrows). These circular regions extended at a depth corresponding to layer 4. According to anatomical standards such as the Paxinos and Watson rat brain atlas[4] these regions of contrast were narrowly located within the whisker barrel field cortex and are putatively assigned to be whisker barrel columns. The signal intensity profile through this enhanced region (Figure 4) has significantly ($p<.001$) larger differences in signal between peaks and troughs of signal enhancement when compared to the untreated, contralateral cortex (Figure 5).

Discussion: T1W protocols could detect the T1 enhancement specific to layer 4, three to five hours after thalamic injections of manganese. These results confirm that MEMRI has the sensitivity to reveal laminar specific inputs in the thalamocortical network. Additionally, the T1 enhancement had a periodic structure consistent with the size and location of whisker barrels. The 4-5 putative whisker barrel columns seen in axially oriented slices would correspond to the 5 rows of the whisker barrel field seen in the rat. Further work is required to histologically and/or electrophysiologically verify that the enhanced regions represent whisker barrels. Additionally, deconvolution of potential contrast due to vascular are needed to establish that these circular regions of contrast are not the product of a combination of venous contrast and manganese enhanced contrast within the cortical area. This technique has potential applications to investigate anatomical changes in the whisker barrel cortex during plasticity in individual rodents.

References: (1) Welker, C. *J. Comp. Neurol.* 1976. 166: 173-190. (2) Pautler, RG. *NMR Biomed* 2004; 117:595-601 (3) Silva A, et al. *Journ of Neuro Methods.* 2007 (in press) (4) Paxinos G and Watson C. *The Rat Brain In Stereotaxic Coordinates.* 5th edition. 2005. Elsevier Academic Press.