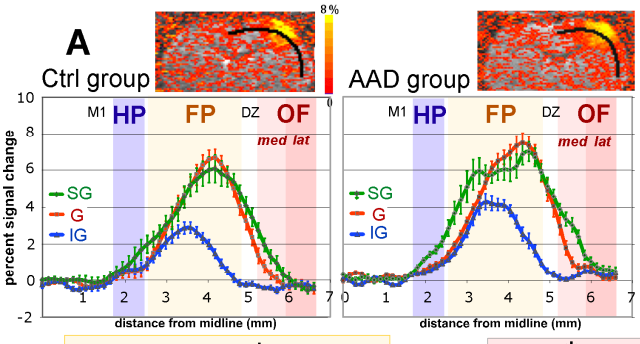
Detecting Acute Cortical Layer-Specific Plasticity in Rat Model using High Field fMRI, Part 2- a non-thresholded, raw data analysis study

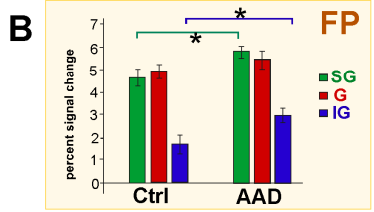
A. Petiet[1], and C. W-H. WU[1]
[1]NeuroSpin / CEA, Gif Sur Yvette, Île-de-France, France

Introduction: Most fMRI analyses apply thresholding processes to generate statistical-derived activation maps. This thresholding procedure removes all voxels below an arbitrary level of activation from the analysis, which might cut off many meaningful voxels and therefore might not be suitable for detecting subtle changes. In addition, the statistical-derived map only allow revealing the spatial extents of regions that reject null-hypothesis, and therefore not suitable for quantifying results nor comparing differences between conditions. In the accompanying paper, we employed multiple-scans averaging approach (average of 16 scans in each animal) from high resolution fMRI data collected from the high field, to greatly improve signal to noise ratio and to increase the spatial and temporal stability of the BOLD signals. Together with conventional thresholding processing, we were able to reliably detect small increases in the spatial extent of activated cortical maps (>1 mm) and found an immediate expansion of cortical territory devoted for forepaw (FP) following trigeminal denervation. The present study aims to further analyze the non-thresholded raw data in order to examine (a) whether there is subtle, sub-threshold changes in the regions that normally undetectable after the statistical thresholding processes? (b) is the magnitude of the signal intensity changes varies spatially as a function of cortical depth? and (c) if there are layer-specific differences in the magnitude of expressing cortical plasticity?

Methods: Data were previously acquired from 11.7T MRI scanner using blocked designed spin-echo BOLD-fMRI paradigm, as described in our accompanying paper. With our paradigm, averaged ~8 % maximum raw signal intensity change at the hot spots of activation foci is detected in response to the forepaw electrical stimulation. Total 23 adult Sprague-Dawley rats (350-450g) were assigned into 3 groups and underwent fMRI scans: the denervated group (AAD, 3 facial nerve cuts 1-2 hrs prior imaging, n=10), the sham group (facial nerves exposed with a skin incision, no denervation, 1-2 hrs prior to imaging, n=5) and the normal group (no surgery, n=8). The control (Ctrl, n=13) group combined the normal and the sham groups when no significant differences were found between the two. Upon completion of the fMRI data collection, images were averaged on voxel by voxel basis for each animal. This multi-scans averaged data was then used to calculate the raw percent signal changes. For every animal, the best brain slice that has maximum activation was chosen for group data analysis. Raw percent signal changes of one best activated slice were sampled along the lines (300 or 450 µm width) drawn parallel to the cortical surface of S1, to create the spatial profiles of the percent signal changes in three major layer compartments: the infragranular (IG), the granular (G) and the supragranular (SG) layers. The positions of each layer compartments were determined by their distances below the brain surface according to histology and brain atlas. Special caution was taken to avoid sampling data from the larger blood vessels that are usually located on the cortical surface. All layers were drawn from the cortical midline (0 mm) and extending laterally (up to 6.6 mm) parallel to the brain surface along the mediolateral dimension, covering the hindpaw (HP), the forepaw (FP) and the orofacial (OF) cortical subdivisions of S1 (see Fig.A top MRI images). The borders of cytoarchitectonically-defined subdivisions were delineated from an additional 5 animals from which their brains were perfused and brain slices were stained for Nissl or cytochrome oxidase. Data analyses were performed using IDL, STIMULATE, and ImageJ programs.

Results: **(1)** Magnitude of signal intensity change expresses differently based on its laminar positions. In the control condition, the highest peak magnitude (7-8% raw percent signal change) is in either G layer or SG layer, although these two layers are not significantly different. IG layer has the lowest peak magnitude, about half of that in G or SG layer (Fig.A left panel). (**2**) Electrical forepaw stimulation evokes the largest signal intensity increase in the FP subdivion of S1, as well as a much smaller signal intensity increase (about 1/3 - ¼ of the peak value, or < 2% raw percent signal change) in neighboring HP and OF subdivions of S1 cortex, for about 1 mm away from the borders, in all three layers (Fig.A, left panel). (3) Despite of being relatively small, SG layer has the highest magnitude of signal change in both the HP and OF cortex. **(3)** Immediately after denervation, increased signal intensity change is readily detected in the FP and OF cortices. In the FP cortex, the biggest increase is found in IG layers (~40% of increase in peak magnitude), followed by SG layers (~15% of increase in peak magnitude) (Fig.A right panel). As a result, IG layer exhibits the largest increase in the averaged signal intensity (75%), and SG has much milder increase (25%) (Fig. B). **(4)** Within the OF cortex, about 1 mm away from the FP-OF border (i.e. OF_{med}), significant elevation in magnitude is found in both SG and IG layers, but not in G layer (Fig.A right panel; Fig.C right panel). Further away in the OF cortex (i.e. OF_{lat}), all layers exhibited significantly increases after denervation. Surprisingly we found the BOLD signal changes from negative to positive values (Fig.A; Fig.C mid panel). **(5)** Although the magnitude of these elevated activation in OF_{lat} is extremely weak, about 0.3-0.6% raw percent signal change or 4-5% of the peak magnitude, they are evidently detectable in our non-thresholded data processes (Fig.A right panel; Fig.C mid panel). Often these subtle changes can be easily neglected or removed during the statistical thresholding processes. **(6)** By contrast, in HP cortex, denervation does not change the magnitude of activation in all layers (Fig.C right panel).





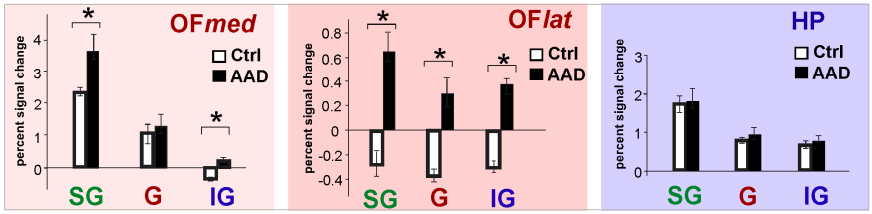


Fig.A. (Top) Two representavie fMRI images showing signal intensity change maps from the raw non-thresholded image data (left: normal control, right: AAD). The G layer compartment is outlined in black lines. **(Bottom)** Averaged group data showing spatial profile plots in Ctrl group (left) and AAD group (right). The spatial profiles display the percentage of signal intensity changes as a function of the distance starting from the midline. The major subdivions of S1 are highlighted with different background colors. The borders between subdivisions are delineated by the averaged locations of cytoarchitectonically-defined borders. Note that a dramatic increase of signal change can be easily detected in IG layer of FP cortex following denervation. Also a smaller increase is found in all layers of the medial region of OF cortex, and an even smaller but evident increase in the lateral region of OF cortex. **Fig.B.** Within FP cortex, the averaged signal intensity has largest increases in IG layer, followed by SG layer. **Fig.C.** In the control condition, detectable positive signal change are already present in nearly the full extent of medial region of OF cortex **(left)** and HF cortex **(right).** However denervation elevates the magnitude of these signal changes in OF cortex but not HF cortex. Interestingly, In the most lateral region of OF cortex where the weak negative BOLD signals are normally present, denervation rapidly shifts these negative values to become positive **(middle)**. These sub-threshold changes might be ignored or cut-off during statistical processing, but is evidently detectable in the raw data set with our experimental settings.

Conclusions: Analyzing raw data allows quantitatively measurement of BOLD signal changes, and therefore enable comparing the magnitude of changes. Denervation rapidly induces location-specific and laminar-specific cortical changes. These subtle but detectable BOLD changes indicate that the functional reorganization of the cortex is laminar-specific, and may reflect the intrinsic interplay of microcircuitry in the somatosensory cortex.

Acknowledgements All this work was supported by an ANR grant #NT09-462818. **References** (1) Silva and Koretsky, PNAS 99(23):15182-15187 (2002); (2) Goloshevsky et al., BrainRes, 1195:67-76 (2008);(3) Herkenham, Science 207:532-535 (1980).