Detecting Acute Cortical Plasticity in Rats using High Field fMRI, Part 1- fMRI Maps and Cytoarchitectonic Boundaries

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Introduction: The brain is capable of changes throughout life, allowing it to be continuously modified by learning experience or rehabilitation therapies. This ability is known as brain plasticity. In the somatosensory cortex, different body parts are organized at different locations to form a somatosensory map. It is believed that sensory experiences, as a result of changes in the incoming activities from the peripheral nerves, play an essential role in determining cortical maps. It is well documented that years after traumatic body injuries in which neuronal activities coming from the injured body parts were eliminated, massive reorganization in the somatosensory map can occur so that the deprived cortical region is invaded by the intact neighboring regions. Furthermore, this reorganized cortical map is accompanied by large-scale anatomical rewiring and cytoarchitectonic alterations (refs 1-5). Despite a mountain of evidence that massive reorganization occurs in both the physiological and the anatomical domains following long-term injuries, it remains unclear what happens to the somatosensory map immediately after injury and within the time frame before anatomical rewiring takes place. To better understand the acute effect on the cortical map of deprivation of input activities, we employed fMRI in the denervated rat model to examine any possible changes in the somatosensory maps 1-15 hours after denervation, a time frame in which no known anatomical rewiring nor cytoarchitectonic alteration occurs. To quantitatively determine the boundaries of the fMRI map, averaging of multitple fMRI scans was used in order to improve the spatial and temporal stability of the BOLD signals. Finally, the borders of fMRI maps were correlated with histologically stained brain sections to determine their positions relative to the anatomical boundaries.

Materials and Methods: A total of 28 adult Sprague-Dawly rats were used for the study. 23 rats were divided into three groups and underwent fMRI experiments : denervation (n=10), control non-operated (n=8), and control sham-operated (n=5). In the denervation group, three branches of the trigeminal nerve innervating the face (i.e. mandibular, ophthalmic, and maxillary divisions) were cut, lidocaine was applied to the cut nerve endings, and skin was sutured. In the sham-operated group, only a skin incision was made and sutured. Animals underwent isoflurane anesthesia for surgical procedures. After surgery, the rats were given an i.v. bolus of 80 mg/kg α-chloralose, and isoflurane was discontinued. Anesthesia was maintained with a constant α-chloralose infusion and i.v. injections of pancuronium bromide to maintain anesthesia and to prevent movement during the fMRI experiments. End-tidal CO_2, rectal temperature, tidal pressure of ventilation, heart rate, and arterial blood pressure were continuously monitored. Arterial blood gas levels were checked periodically, and corrections were made by adjusting respiratory volume or administering sodium bicarbonate. First fMRI scans were performed 1-2 hr post surgery, and continued for 7-14 hrs. Two needle electrodes were inserted just under the skin of a forepaw to deliver electrical stimulation (3-4 mA, 0.3 ms pulse repeated at 3Hz, block design, 30 s on epoch alternating with 30 s off period). fMRI images were acquired on an 11.7 T/31-cm horizontal bore magnet (Magnex Scientific), interfaced to an AVANCE console (Bruker BioSpin) and equipped with a 9-cm gradient set. An in-house made 15x20-mm-diameter receiving surface coil and a 90-mm-diameter birdcage transmitter coil were used to improve RF homogeneity and sensitivity. A multi-slice spin-echo EPI sequence was acquired to obtain fMRI image from S1 with in-plane resolution of 150 x 150 um pixel size, by using the following parameters: TR/TE=1500/46 ms; matrix size=128 x 128; 5 slices, slice thickness =1 mm, FOV=19.2 x 19.2. An average of 16.7±2.6 (mean±SE) fMRI scans were collected in each animal. Image reconstruction and analyses were performed using the ParaVision (Bruker Medical GmbH), STIMULATE (Univ of Minnesota, MN) and ImageJ (NIH, MD) programs. The averaged data from multiple scans collected in each animal was used to calculate the t-maps of the BOLD signal ($p<0.05$) in units of percent signal change. Only groups of at least 4 activated pixels were considered. The number of pixels above threshold from five brain slices was used to determine the full extent of the cortical forepaw representation. An additional 5 animals were sacrificed, their brains were perfused, and the standard histological procedures were carried out to stain for Nissl and cytochrome oxidase (CO) in order to reveal the locations of cytoarchitectonic boundaries between areas and the subdivisions within S1.

Results: Fig.1 (A) Averaging multiple fMRI scans significantly improves the signal-to-noise ratio and sharpens the anatomical contrast of the EPI image, thus enabling more precise analysis of the derived fMRI map. **(B)** The shape of the fMRI spatial profile also depends on the number of scans used for averaging. With our experimental settings, about 15 averaged scans is sufficient to generate a very stable activation spatial profile, so that the borders of functional maps can be precisely defined. **Fig.2** The denervation group has significantly more activated pixels than either control group, indicating that denervation of the facial nerves results in immediate expansion of forepaw representation**. Fig.3** In both non-operated and sham control groups, the stronger (i.e. hot spots) fMRI activation is situated within the cytoarchitectonically-defined S1 forepaw subdivision, while the weaker component extends into the neighboring representations. By contrast, the denervated group exhibits a wider extent of stronger activation that extends beyond the cytoarchitectonically-defined forepaw subdivision, and also has weaker activation that extends further into neighboring representations of S1, with spatial extent larger than in either control groups.

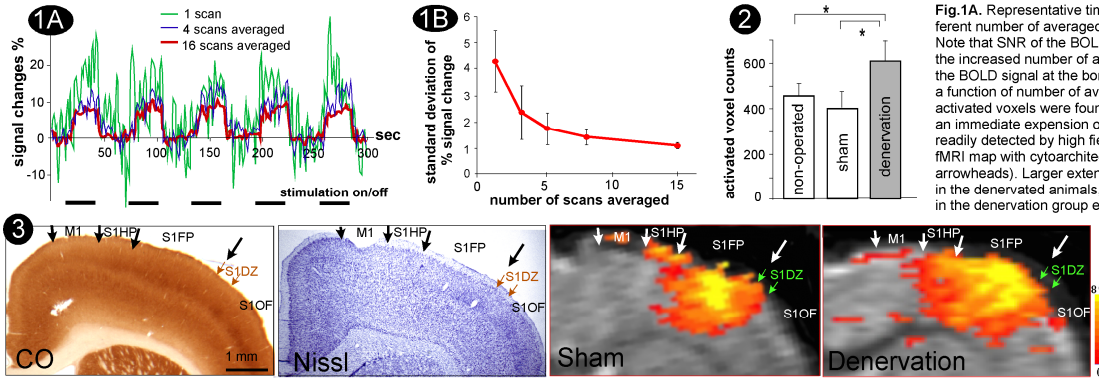

Fig.1A. Representative time course of the fMRI signals with different number of averaged scans taking from the same animal. Note that SNR of the BOLD response significantly improves with the increased number of averages. **Fig.1B.** Standard deviation of the BOLD signal at the border of activation region is expressed as a function of number of averaged scans. **Fig.2.** Significantly more activated voxels were found in the denervated group, indicating an immediate expension of the forepaw representation that is readily detected by high field MRI. **Fig.3.** Correlate borders of fMRI map with cytoarchitectonic boundaries (indicated by arrowheads). Larger extent of fMRI activation is conistently found in the denervated animals, Moreover, the hot spot fMRI activation in the denervation group extends well-beyond the cytoarchitectonically-defined forepaw subdivision of S1, and weaker activation has also further expanded into neighboring subdivisions that normally will not be activated in the control conditions. Activated fMRI map were overlaid on EPI images. S1HP: hindpaw subdivision of S1; S1FP: forepaw subdivison of S1; S1DZ: dysgranular zone of S1; S1OF:orofacial subdivison of S1.

Conclusions (1) High field, multiple-scans averaging allow reliable determination of the spatial extent of fMRI activation, and thus enable detection of acute cortical plasticity. (2) An immediate expansion of cortical forepaw representation was readily detected within few hours after trigeminal denervation, suggesting that the cortical sensory map is dynamically maintained. (2) Consistent with our previous observations (ref 6), the stronger component of fMRI activation is situated within the subdivisions of S1 forepaw region, while the weaker component extends well into the neighboring representations and may represent neural communication between subdivisions. This is also consistent with previous neurophysiological reports that neural representations are not completely confined by the underlying anatomy (Refs 7-8). (3) Such immediate cortical plasticity likely triggers anatomical rewiring and cytoarchitectonic alterations that are often observed at much later stages, and may underlie many sensory and motor dysfunctions and neurological disorders such as phantom limb perceptions.

References (1) Merzenich e at al., Neurosci.1983:639-665, (2) Pons et al., Science, 1991:1857-60, (3) Florence et al., Science, 1998, (4) Wu and Kaas, J Neurosci,1999: 7679-97, (5) Wu and Kaas, Somatosen Mot Res. 2002: :153-63. (6) Goloshevsky et al., 2007. *ENC. Florida.* (7) Chapin and Lin, J Comp Neurol1984:199-213; (8) Chapin et al., J.Comp Neurol1987:326-346