Increased Interneuron Activity is Associated with Ipsilateral fMRI Activation Following Forepaw Denervation

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Introduction

Peripheral injury that affects the afferent input to the primary somatosensory cortex (SI) or injury within SI can lead to short- and long-term plastic changes in both contralateral and ipsilateral SI (inter-hemispheric plasticity) (1-2). This effect is believed to be mediated via the corpus callosum. Callosal projections from one cortex synapse on both pyramidal cells and interneurons in the contralateral cortex, however, under normal conditions, callosal input results primarily in an inhibitory effect. Moreover, there is evidence that demonstrates that plasticity changes within SI occur in a laminar specific manner. Therefore, the goal of this work was to study the effect of peripheral nerve deafferentation on interhemispheric laminar communication.

Recently we showed that following a complete denervation of one of the rats' forepaw or hindpaw, sensory stimulation of the healthy forepaw or hindpaw, respectively, resulted with fMRI activation of both the contralateral (healthy) and ipsilateral (deprived) SI, two weeks after the nerve cut procedure. In order to study the laminar dynamics that underlie this phenomenon, we carried out high resolution fMRI experiments in addition to recording single unit activity and local field potentials (LFP) from both the healthy and the deprived cortex in the forepaw denervation and sham-operated rat model.

FMRI results demonstrate that the BOLD profile across the deprived cortical layers is different as compared to the contralateral, healthy SI and to the contralateral SI in sham-operated rats. In addition, single unit recordings show that there are more neurons in the deprived SI that respond to ipsilateral (healthy) forepaw stimulation, and that the vast majority of them are inhibitory interneurons. Thus, the fMRI activation detected in the deprived SI under peripheral denervation is associated with over-activation of inhibitory interneurons in the deeper cortical layers.

Methods

Forepaw denervation: 24 Sprague-Dawley Rats (70-90 g) were anesthetized with 2% Isoflurane, and the right radial, ulnar and median nerves were exposed and a 3 mm long cut was made. For the sham-operated control group, the nerves were exposed but were not truncated. FMRI and electrophysiology measurements were performed 2-3 weeks following this procedure. **Animal preparation for fMRI:** Rats were initially anesthetized with 2% Isoflurane and were orally intubated and placed on a mechanical ventilator. The femoral artery and vein were catheterized for sampling blood and infusing drugs. Two short stimulation electrodes were inserted in each forepaw. After surgery, anesthesia was maintained with a constant α-chloralose infusion. End-tidal CO_2, rectal temperature, tidal pressure of ventilation, heart rate, arterial blood pressure and arterial blood gases were monitored during the experiment. **Image acquisition**: All images were acquired with an 11.7 T / 31 cm horizontal bore magnet (Magnex), interfaced to an AVANCE console (Bruker).A 1.2 cm diameter surface coil for was used to receive MR signal. A gradient-echo EPI sequence with a 128 × 128 matrix, TE 30 ms, TR 750 msec, BW 200 kHz, FOV 1.92 × 1.92 cm, 380 repetition, and 3, 1-mm thick slices was used. **Stimulation Paradigm:** 2 mA, 300 μs pulse was repeated at 3 Hz. For fMRI experiments the paradigm consists of 40 scans during rest, 10 scans during forepaw stimulation, which was repeated 4 times. For electrophysiology experiments 200 pulses at 3 Hz were repeated once for each forepaw. **Data analysis:** For fMRI group statisticscal analysis, all images were spatially normalized to the brain of a control rat using 2D affine transform. The group *t*-score maps were calculated using a fixed-effects analysis and threshold at $p<0.0001$. For individual subject analysis, the mean number of pixels and the mean standard error were analyzed using STIMULATE (University of Minnesota) with cross-correlation threshold above 0.15. **Animal preparation for electrophysiology:** Rats were anesthetized with urethane and placed in a stereotaxic frame. A craniotomy was performed above the right and the left SI. A glass electrode with 4MΩ impedance filled with 2% Pontamine Sky Blue dye in 2 M NaCl was lowered into the cortex in a 100 μm increments. **Electrophysiology:** Extracellular action potentials recordings was amplified and monitored on digital oscilloscopes and audio monitors. Spikes and LFP were band pass filtered at 250-5000 Hz and 0.1-100 Hz, respectively. Discriminated signals were collected on a PC with a CED interface and Spike2 data acquisition and analysis software. LFPs were sampled at 1000 Hz and spikes at 25 KHz.

Results and Discussion:

Figure 1 shows group t-maps of high-resolution fMRI data that demonstrates inter-cortical plasticity after peripheral nerve damage. In the sham-operated rats (n=7), stimulation of one forepaw resulted solely with contralateral SI activation (78 ± 14 pixels). No ipsilateral fMRI activation was observed (2 ± 1 pixels). In contrast, when the healthy forepaw was stimulated, both contralateral (153 ± 32 pixels) and ipsilateral (55 ± 13 pixels) SI exhibited fMRI activation in the forepaw denervated rats (n=7). Note that as a result of forepaw stimulation, fMRI activation in the contralateral SI in the forepaw denervated rats is twice as large compared to sham-operated rats. Consistent with previous works, analysis of the laminar activation across the cortex of both sham-operated and forepaw denervated rats revealed that within the contralateral SI to the stimulated forepaw, the upper cortical layers through layer 4 exhibit the greatest percentile BOLD intensity changes. However, in the ipsilateral SI of the denervated rats, the percentile BOLD signal change was uniform across all cortical layers, in particular the ratio of signal from layer 4 as compared to deep layer 5 or 6 was much reduced on the ipsalateral side suggesting that the primary input to the deprived cortex does not arise from thalamic projections that synapse in layer 4.

LFP are believed to represent integrated synaptic activity originating from large pyramidal cells, and were analyzed using the Current Source Density (CSD) method. Using this analysis, one can identify the initiation and the time course of the LFP across the cortical layers. Forepaw stimulation resulted with similar laminar pattern in the contralaetral SI in both groups. CSD maps of the ipsilateral SI were also similar in both groups but were 10 fold lower in magnitude as compared to the contralateral SI. These findings also support our hypothesis that the BOLD activation observed in the deprived SI does not originate from thalamic excitation of large pyramidal neurons in layer 4. 88 single neurons were identified from both groups, mostly in layers 4-6 which were used for single cell analysis. 35 of the 88 single neurons responded robustly to forepaw stimulation. Only 4 neurons responded to ipsilateral stimulation in the sham-operated rats (n=4), and based on their action potential width (3), 3 of them could be classified as pyramidal cells. In contrast, 9 neurons were found to respond to ipsilateral stimulation in the deprived cortex of the forepaw denervated rats (n=4), and 8 of them could be classified as inhibitory interneurons. These results are shown in **Figure 2**.

Both the high-resolution fMRI and the electrophysiology findings suggest that the BOLD activation in the deprived cortex represents altered cortical dynamics as compared to the normal cortical dynamics due to thalamic inputs into contralateral cortex. Moreover, both methods demonstrate that not only the deprived SI exhibits long-term plastic changes, but also the contralateral healthy SI in the forepaw denervated rats. These results demonstrate that high-resolution fMRI can distinguish alternations in cortical laminar dynamics, and that fMRI activation can be directly related to inhibitory neuronal activity in the peripheral denervation model.

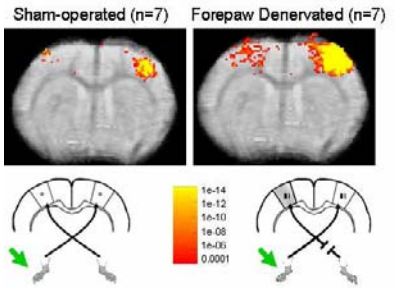
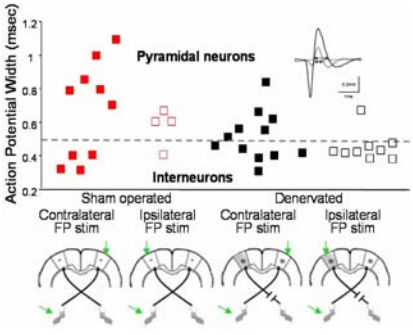


Figure 1. Group t-test maps overlaid on the EPI template image for the sham-operated rats (n=7) and forepaw denervated rats (n=7). The experimental procedure is shown on the bottom.

Figure 2. The neuronal population that responded to forepaw stimulation. Based on the action potential width pyramidal cells can be distinguished from interneurons. Illustration of the experimental paradigm is presented in the lower row.



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