Optogenetics-guided Cortical Plasticity Following Forepaw Denervation

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Introduction: Although 20 million Americans suffer from peripheral nerve injury there are still only a limited number of strategies to promote recovery. Despite refined surgical techniques, adult patients generally experience persisting sensory dysfunction and pain problems [1]. Functional MRI studies have demonstrated that lesions of the nervous system are followed by massive reorganization of cortical areas. Both human patient and animal model studies have shown that stimulation of the intact limb often leads to fMRI responses in ipsilateral cortical areas that are not normally activated (i.e. "inappropriate") [2-4]. Recent studies [5, 6] suggest that the "inappropriate" ipsilateral fMRI responses originate from cortical-cortical inhibition mediated through the transcallosal pathways and may be negatively correlated to rehabilitation. The goal of this study was to decrease the cortical inhibition through the transcallosal pathway in a rat model of sensory deprivation. For that purpose we used optical-genetic (optogenetics) manipulations [7] that offer precise and transient control of neuronal firing rate. We engineered the excitatory neurons in rat primary somatosensory cortex (S1) to express halorhodopsin (eNpHR) (light-sensitive chloride pump) using direct neuronal infection with lentivirus. Thus, in the presence of light, the chloride pumps open and trigger neuronal hyperpolarization i.e. decreases in neuronal firing rate. The neuronal activity of the healthy cortex was optogeneticly manipulated, and the functional responses of the deprived, "inappropriate" brain regions were evaluated by multimodal *in vivo* measurements from single cell to the whole brain network. Here we demonstrate for the first time the capability of optogenetics manipulation to "guide" the cortical plasticity in order to promote rehabilitation.

Materials and Methods: The halorhodopsin plasmid (Lenti-CaMKIIa-eNpHR-EYFP-WPRE) was injected into the right lateral ventricle of 3 day old rat pups. Complete denervation of the right forepaw was performed in 7 weeks old rats (denervated n=16, control n=16). **Animal preparation:** Rats were initially anesthetized with isofluorane. A craniotomy was performed above the right and left S1. Anesthesia was then maintained by a continuous infusion of dexdomitor (0.1 mg/kg). Respiration rate, PO2 and heart rate were continuously monitored during the experiment. **Forepaw stimulation:** Two mA and 300 μs pulses were repeated at 3 Hz and 9Hz. **Light activation of halorhodopsin (eNpHR):** The end of the optic fiber (400 μm in diameter) coupled to a yellow laser with 594nm wavelength was placed directly over the right exposed S1. The light was turned on during forepaw stimulation. **Functional MRI:** Images were acquired using a Bruker 9.4 T animal scanner. Rats were placed in a custom-built MRI holder equipped with a dedicated device for positioning the light source coupled MRI compatible optic fiber. A custom-built 1.1 cm diameter surface coil was used to transmit and receive MR signal. A gradient-echo EPI sequence with a 128 × 128 matrix, TE=21 ms, TR=1000 ms, BW=250 kHz, FOV=1.92 × 1.92 cm, and 3, 1-mm thick slices was used. Forty scans were collected during rest and 2 × 20 scans were collected during forepaw and eNpHR stimulation. FSL software was used for all data analysis [8]. Activation detection was performed using the general linear model (GLM). Z statistic results were cluster-size thresholded for effective significance of p<0.05. **Electrophysiology:** Tungsten electrodes were lowered into both right and left S1 in 150 μm increments. Single unit and local field potentials (LFP) were collected simultaneously and analyzed using Spike2 sofware. **Optical imaging:** Cerebral blood flow (CBF) responses from both right and left S1 were measured by laser speckle imaging (LSI). Red laser with wavelength 632nm was used to provide coherent illumination. Z-test analysis results were cluster-size thresholded for effective significance of p<0.05. **Immunostaining:** All rats were sacrificed and perfused at the end of the measurements. Free floating 50 μm thick brain sections were immunostained with antibodies targeted towards YFP and CaMKII to confirm eNpHR expression in excitatory neurons.

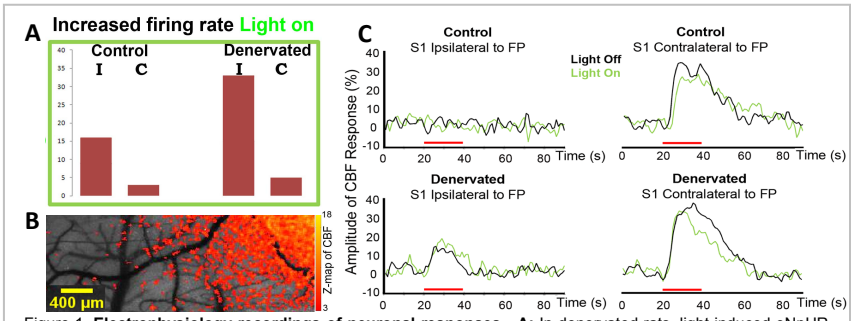

Figure 1. **Electrophysiology recordings of neuronal responses - A:** In denervated rats, light induced eNpHR activation resulted in increased neuronal firing rate in the deprived, ipsilateral S1 during intact FP stimulation. (I: ipsilateral S1, C: contralateral S1) **Optical imaging of CBF responses - B:** An example of LSI CBF responses Z-map (p<0.05) forepaw (FP) stimulation. **C:** In denervated rats, light induced eNpHR activation resulted in increased CBF responses in the deprived, ipsilateral S1 during intact FP stimulation. Red bars represent FP stimulation. Red bars represent FP stimulation.

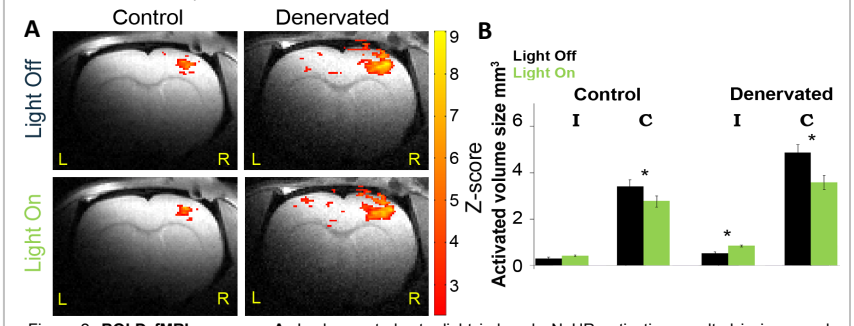

Figure 2. **BOLD fMRI response-A:** In denervated rats, light induced eNpHR activation resulted in increased BOLD responses in the deprived, ipsilateral S1 during intact FP stimulation. Z-map (p<0.05) is overlaid on RARE anatomical images. **B:** Group analysis of the BOLD fMRI results of the light induced eNpHR activation. *=p<0.05, n=10. (I: ipsilateral S1, C: contralateral S1)

Results: **Single unit responses:** More than 200 single neurons were recorded and sorted. In denervated rats, eNpHR activation of the healthy, contralateral S1 resulted in increased firing rate of neurons located within the deprived, ipsilateral S1 during stimulation of the left, intact forepaw (Figure 1A) (p<0.05). **CBF responses:** In denervated rats, eNpHR activation of the healthy, contralateral S1 resulted in increased CBF responses of the deprived, ipsilateral S1 during stimulation of the left, intact forepaw (Figure 1B, C) (p<0.05) **BOLD fMRI responses:** Stimulation of the left, intact forepaw in denervated rats resulted in increased BOLD responses both in healthy, contralateral and deprived ipsilateral S1 cortices (p<0.05). Consistent with electrophysiology and optical imaging measurements, in denervated rats, eNpHR activation of the healthy, contralateral S1 resulted in increases in BOLD responses of the deprived, ipsilateral S1 during stimulation of the left, intact forepaw (Figure 2A,B) (p<0.05).

Discussion: Electrophysiology, optical imaging and fMRI results show that the increased inhibitory activity usually observed in the denervated rats' deprived cortex, can be reversed by optogenetically manipulating the neuronal activity of the healthy cortex. Human and animal studies suggest the involvement of the transcallosal projection in shaping neuroplasticity with increased cortical inhibition following injury may be crucial in dictating the rehabilitation probability. Thus, our findings demonstrate that in the denervated rat, the transcallosal communication can be manipulated in a manner that could potentially promote recovery. This offers a novel therapeutic strategy to facilitate rehabilitation.

References:

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