Investigating the role of transcallosal projections in mediating neuroplasticity following injury in a rat using fMRI

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Introduction: FMRI studies in both human patients and animal models have demonstrated that lesions of the nervous system are followed by massive reorganization of cortical areas. Nevertheless, the underlying neuronal mechanisms that are involved in neuroplasticity and how they dictate the degree of recovery are not known. Currently there is no easy diagnostic clinical outcome predictor that would allow one to follow recovery and the effect of therapy. This hampers developments of new rehabilitation strategies for patients.

In stroke, amputees and multiple sclerosis patients, stimulation of the intact limb often leads to fMRI responses in ipsilateral cortical areas that are not normally activated (i.e. "inappropriate") [1-3]. In stroke patients, larger extent of "inappropriate" ipsilateral fMRI responses were correlated with decreased recovery [4], and in limb amputees, they were associated with an increase in suffering from phantom limb pain [1]. Similar to human studies, stimulation of the intact forepaw in denervated rats, 2 weeks following denervation procedure, resulted in increases in fMRI responses in the "inappropriate" ipsilateral somatosensory cortex. *In vivo* electrophysiology showed that the "inappropriate" ipsilateral fMRI responses reflect increases in inhibitory neuronal activity [5]. In addition, ablation of the healthy somatosensory representation (ipsilateral to the denervated limb) eliminated the fMRI responses in the deprived cortex [6]. Taken together, these studies suggest that the increases in the "inappropriate" ipsilateral fMRI responses are principally mediated through the inter-hemispheric, transcallosal pathways and may be negatively correlated to rehabilitation.

This study was designed to investigate the role of the transcallosal pathways in mediating neuroplasticity following injury in a rat model of sensory deprivation. We have determined the time course and the age-dependency of which the transcallosal projections effect cortical reorganization. These results promote the development of non-invasive diagnostic tools to assess cortical reorganization and monitor and quantify rehabilitation.

Materials and methods: Denervation procedure: Adult Sprague-Dawley rats (~100 g, n=12) were anesthetized with isoflurane, and the right radial, ulnar and median nerves were exposed and a 3 mm long cut (to prevent future regeneration) was made in each of the nerves. A similar forepaw denervation procedure was performed on 4 days old Sprague-Dawley pups (~9 g, n=10) under cryoanesthesia. **Animal preparation for fMRI:** Rats were initially anesthetized with Isoflurane. A bolus (0.05 mg/kg, S.C.) of dexodormitor (medetomidine) was given and isoflurane was discontinued. During imaging measurement anesthesia was maintained by a continuous infusion of dexodormitore (0.1 mg/kg). Two short stimulation electrodes were inserted in the intact forepaw. Respiration rate, PO_2 and heart rate were continuously monitored during experiment. Arterial blood gases were checked periodically. **Image acquisition**: All images were acquired using a Bruker 9.4 T animal dedicated scanner. A 1.1 cm diameter surface coil for was used to transmit and receive MR signal. A gradient-echo EPI sequence with a 128 × 128 matrix, TE=26 ms, TR=1000 ms, BW=200 kHz, FOV=1.92 × 1.92 cm, 120 repetition, and 3, 1-mm thick slices was used. **Stimulation Paradigm:** 3 mA, 300 µs pulse was repeated at 9 Hz. Stimulation paradigm consists of 40 scans during rest and 2X 20 scans during forepaw stimulation. **Data analysis:** FSL software was used for all data analysis. Independent component analysis (ICA) was performed for initial data exploration. Activation detection was performed using the general linear model. Z statistic results were cluster-size thresholded for effective significance of $p<0.05$.

Results: Time course of transcallosal neuroplasticity: In healthy rats, stimulation of one of the limbs results in fMRI responses in the contralateral somatosensory cortex. Figure 1 shows, that starting in the first week following the denervation procedure, stimulation of the intact forepaw resulted in both contralateral (healthy) and ipsilateral (deprived, "inappropriate") somatosensory cortical fMRI responses. This suggests that the transcallosal neuroplasticity involves the modification of existing neuronal pathways and does not necessarily require architectural modifications. However, the "inappropriate" ipsilateral fMRI responses attenuate 7 weeks following denervation procedure. This suggests that the transcallosal-mediated neuroplasticity is involved primarily in the initial phase of the reorganization of neuronal pathways following injury.

Age-dependency of transcallosal neuroplasticity: Rats underwent the denervation procedure during the critical period of brain development (the first week in rat's life). When the rats reached adulthood (9 weeks), fMRI measurements were performed. Figure 2 shows that stimulation of the intact forepaw resulted in fMRI responses only in the (healthy) contralateral somatosensory cortex. FMRI responses in the ipsilateral (deprived) somatosensory cortex were below detectability. However, minimal, but consistent across animals, "inappropriate" ipsilateral fMRI responses were detected in the motor cortex. Thus, it appears that the transcallosal projections affect neuroplasticity of motor cortical areas to some degree, when the injury occurs during the critical period.

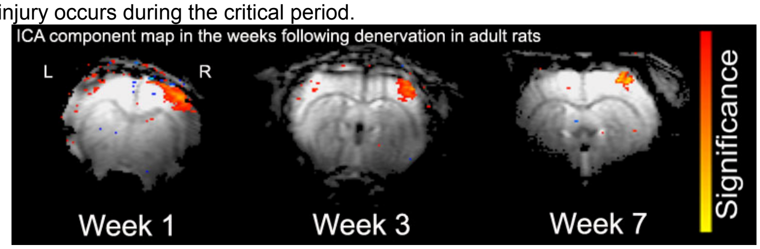

Figure 1 demonstrates bilateral fMRI responses in the healthy, contralateral (right) and the deprived, ipsilateral (left) somatosensory cortices following intact (left) forepaw stimulation in adult denervated rats in the first and the third week following injury. ICA extracted three to five components in each individual rat. One of the components was selected by visual inspection to be associated with the paradigm timing. The selected neuro-physiologically relevant component map is overlaid on the original EPI image.

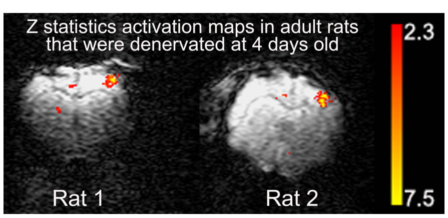

Figure 2 demonstrates that stimulation of the intact forepaw in rats that were denervated during their critical period, resulted mainly in fMRI responses in the healthy, contralateral (right) somatosensory cortex, although minimal fMRI responses are also detected in the deprived, ipsilateral (left) motor cortex.
Z statistic activation maps are overlaid on the original EPI image.

Discussion: Human and animal studies suggest the involvement of the transcallosal projection in shaping neuroplasticity following injury may be crucial in dictating the rehabilitation probability. The results demonstrate that when the nerve injury occurs during adulthood, transcallosal mediated neuroplasticity plays a significant role in modifying neuronal responses. The effect that the transcallosal projections have on somatosensory fMRI cortical responses appears immediately in the days following injury but decreases several weeks after. On the other hand, when the nerve injury occurs during the critical period, the transcallosal projections seem to affect ipsilateral motor cortical areas rather than somatosensory cortical areas. Although the transcallosal projections may play a role in mediating neuroplasticity during the critical period, the behavioral outcome of this neuroplasticity appears to be different compared to adults. Thus, the effect that the transcallosal projections have on neuroplasticity varies dependent on the age and the time following the injury and may introduce a critical consideration when choosing the right rehabilitation strategy.

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