

Functional Magnetic Resonance Imaging (fMRI) of Neurotrophin-3 (NT3) treated Spinal Cord Injured Rats

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Introduction

Cortical reorganization, both in humans and animals, following spinal cord injury (SCI) has been demonstrated [1-3]. The use of neurotrophic factors, such as Neurotrophin-3 (NT3) has shown considerable potential in axonal sprouting and rescuing axons at risk following SCI [4] resulting in partial restoration of functional activity. NT3 is also expected to modulate the cortical reorganization in SCI. In these studies we used functional magnetic resonance imaging (fMRI) for visualizing the ongoing plastic changes in the brain following SCI with and without NT3 treatment.

Materials and Methods

The experimental protocol included 30 Sprague Dawley rats in the weight range of 300 -350 g. They were divided into 5 groups, six of each: uninjured (normal controls), saline treated injured (injured controls) at 4 and 8 weeks post- injury (PI), NT3 treated injured group at 4 and 8 weeks PI. The spinal cord was injured under controlled conditions at the T7 level using an-house-designed and fabricated injury device [5]. Animals were initially anesthetized using isoflurane. After positioning the animal in the scanner, isoflurane was discontinued and a single bolus of chloralose was injected ip (100 mg/kg) for fMRI scans. The body temperature was maintained at 37 °C. Heart rate, respiration, and rectal temperature were continuously monitored throughout the MRI scan. MR images were acquired at 7T on a 70/30 Bruker scanner. High resolution RARE images of the brain were acquired for spatial normalization and for superimposing the functional activation. The paradigm for the fMRI was electrical stimulation of the forepaw using a block design. The stimulation and all the timings were controlled by an in house designed unit based on a microprocessor. A multislice, single-shot spin echo EPI sequence with TR/TE of 3000/50 ms was used for acquiring the fMRI data. Software for real time fMRI analysis was developed and implemented on the scanner to ascertain the proper working of the stimulation paradigm. Detailed fMRI analysis was performed using the Statistical Parameter Mapping (SPM) [6] and in-house developed software. For group comparison, initially the rat brains were transformed to the Paxinos and Watson co-ordinate system using the digital histology atlas [7]. The number of activated voxels and the center of mass on the registered rat brain were computed. Automated cluster analysis was performed to determine the center of mass activation based on anatomical locations. Statistical analysis was performed using the ANOVA + Fisher PLSD and factorial measurements for detecting the temporal changes and treatment effect.

Results

Robust, contralateral response to the forepaw stimulation was observed in all normal controls. At both 4 and 8 weeks PI, saline treated rats showed an increasing trend in activation in comparison with the normals ($p < 0.01$) with the activation often extending to the sub-cortical structures and secondary sensorimotor cortex. In NT3 treated rats, almost all the structures in the brain, including thalamus, hippocampus and the caudate putamen have exhibited high degree of activation (**Figure 1**). Significant differences were detected between the activation volumes of the normals and the NT3 treated rats ($p < 0.01$) and between the saline and NT3 treated rats ($p < 0.01$). These results are summarized in **Figures 1** and **2**. Activation clusters were separated using an automated procedure as shown in **Figure.1** and the center of mass values of activation was determined for each anatomical structure. The center of mass $R \{R = \sqrt{x^2 + y^2 + z^2}\}$ of the contralateral somatosensory cortex which is normally responsible for left forepaw stimulation differed significantly in the injured groups in comparison to the normals ($p < 0.01$). The BBB scores that are mainly determined by the motor activity did not show any significant differences with treatment.

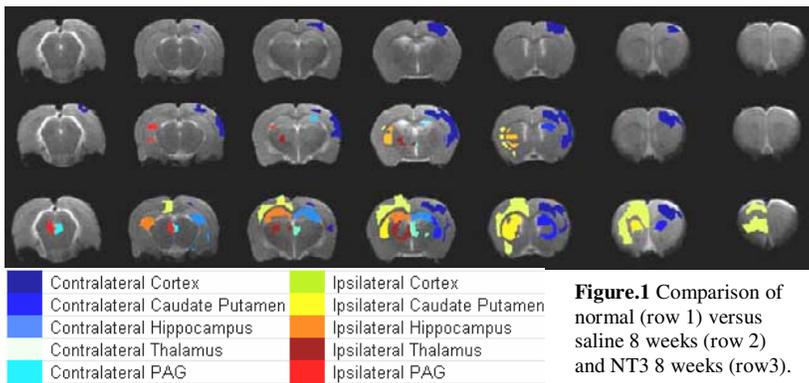


Figure.1 Comparison of normal (row 1) versus saline 8 weeks (row 2) and NT3 8 weeks (row3).

Discussion

The observed extensive activation pattern that extended to areas which were normally devoid of fMRI signals following SCI and treatment is an evidence of cortical plasticity in SCI. The number of structures that showed activation increased substantially in the treated groups relative to the saline treated groups at both the time points. We believe that these are the first reports that quantitate both the shift and extent of activation in SCI. These measures can be used for quantitating the cortical plasticity that could be used in objectively evaluating the efficacy of treatment. The absence of improvement in the BBB scores suggests that the effect of NT3 is perhaps more directed towards improvement in the sensory behavior.

References

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