Functional Magnetic Resonance Imaging of the Rat Spinal Cord After Peripheral Nerve Injury

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Introduction

Spinal cord injuries (SCI) cause motor and/or sensory disabilities. Clinicians have investigated a number of different strategies to restrict damage for example by stimulating nerve growth, axonal regeneration or blocking secondary excitotoxic events. However there is no effective method of treatment and there is also a lack of a reliable method for the assessment of function within the spinal cord following SCI. Spinal cord fMRI was previously proposed as a tool for the evaluation of CNS function in the study of healthy spinal cord function [1,2]. In this study we introduce spinal cord fMRI (spinal fMRI) for the evaluation of spinal cord function in an animal model of graded peripheral nerve injury [3]. In this model, input to the spinal cord was reduced in a graded manner and fMRI activation to hindpaw stimulation was used to investigate whether such graded restrictions in sensory input provide a a good model of sensory deprivation for evaluating the usefulness of spinal fMRI.

Materials and Methods

Animal preparation: Five Wistar rats were used. All anesthesia and monitoring procedures were performed as described previously [1,2]. Experiments were performed on each rat in three steps: (1) a control stimulation experiment, (2) L3 nerve transection followed by (3) L4 nerve transection. At each step spinal fMRI was carried out. Nerve sections were performed on the stimulated side via a ventral approach [4] external to the bone of the spinal column at a distance of about 4 mm from its entrance to the spinal cord. Following the completion of the last imaging session, rats were immediately euthanasied with pentobarbital (120mg/kg, i.v.).Experimental Setup: A 9.4T/21cm horizontal bore magnet (Magnex, UK) with an Avance console (Bruker, Germany) was used. The animals were placed supine in a 5 × 7cm volume rf coil with the lumbar spinal cord within the homogenous B_1 field allowing slice positioning in the areas of expected neuronal activation. Experiment design: Functional images were acquired from the spinal cord. Five axial slices were positioned within the spinal cord at the level of L6, L5, L4, L3, and L2 spinal cord nerves. A multislice, single-shot FSE sequence was used (TE =3ms, TE_{eff} = 43.7ms, TR=7sec, FOV=2×2 cm, matrix size 64×64, 2 averages, slice thickness 2 mm, 0.5 mm gap. Data acquisition was gated with the respiratory cycle. Anatomical T_2-weighted FSE images of the spinal cord were also acquired. Stimulation paradigm: The stimulation paradigm consisted of 62 time points with continuous 5 rest and stimulation periods (10 off – 12 on – 14 off - 14on – 12 off). Data were analyzed using EvIdent (IBD, Winnipeg) program. The total acquisition time of one stimulation experiment was 10 min. For electrical stimulation (6 mA, 0.3 ms pulse length, 3 Hz) two small needle electrodes were placed subcutaneously in the right hindpaw.

Results and Conclusions

Functional images of appropriate quality were obtained from four animals. The amplitude of signal intensity changes was approximately 3% and followed the stimulation paradigm ($p \leq 0.001$). Electrical stimulation of the hindpaw resulted in activation within gray matter of the spinal cord. The sites of activation were localized mainly ipsilaterally in the dorsal horn of the spinal cord. An example of fMRI activation obtained from one animal is shown in Fig. 1 demonstrating activation at the spinal cord levels of L6-L2, where processing of inputs from branches of the femoral nerve occurs within gray matter neurons in the ventral horn. The fMR images of the spinal cord were superimposed on anatomical images at five different levels of the lumbar spinal cord corresponding to L6, L5, L4, L3 and L2 lumbar spinal cord levels (Fig. 1) respectively. A dependence of the number of active voxels on the degree of gradually reduced sensory input was observed. This demonstrate that spinal cord fMRI in animal model of peripheral nerve injury allows the functional assessment of different grades of injury, and may be a useful tool for monitoring peripheral nerve regeneration processes and spinal cord repair strategies as well.

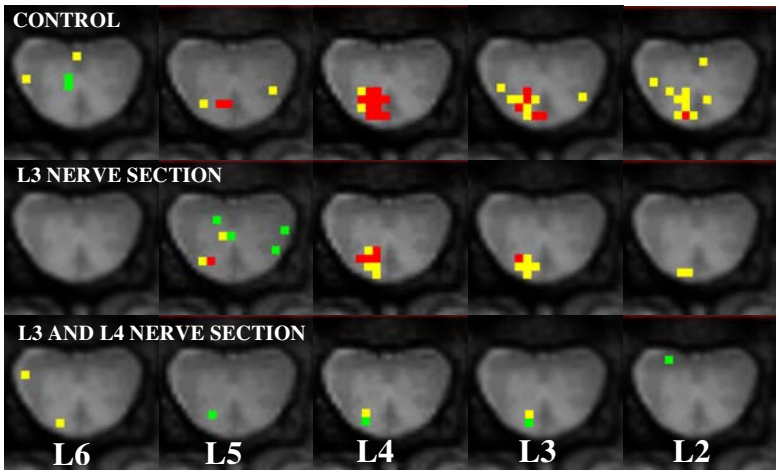

FIG. 1. Rat spinal fMRI of five slices at different levels of the lumbar spinal cord (L6 to L2 respectively): control (first row); L3 nerve transected (second row); L3 and L4 nerve transected (third row). The spinal cord images are superimposed on anatomical images. The ventral surface is at the top, dorsal surface at the bottom. The color corresponds to the level of correlation to the paradigm: red corresponds to the highest, yellow -medium and green the lowest correlation coefficient threshold (all within $p \leq 0.001$).

References

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