Simultaneous Functional Magnetic Resonance Imaging of the Rat Spinal Cord and Brain

K. Majcher[1], B. Tomanek[2,3], A. Jasinski[1], T. Foniok[2], P. W. Stroman[4], D. Kirk[3], U. I. Tuor[2,3], G. Hess[5]

[1]Institute of Nuclear Physics, Polish Academy of Sciences, Krakow, Malopolska, Poland, [2]Institute for Biodiagnostics, National Research Council of Canada, Calgary, Alberta, Canada, [3]Dept of Neurosciences, University of Calgary, Experimental Imaging Centre, Calgary, Alberta, Canada, [4]Dept of Diagnostic Radiology, Queen's University, Kingston, Ontario, Canada, [5]Institute of Pharmacology, Polish Academy of Sciences and Jagiellonian University, Krakow, Malopolska, Poland

Introduction

Functional MR Imaging (fMRI) of the brain has been widely used in the recent years [1] while spinal fMRI has been introduced for human studies in 2001 [2] and more recently for the study of animals [3]. There is a need for a non-invasive technique allowing observation of both brain and spinal neuronal activity elicited by the same stimulus, both for clinical assessment and for research of the Central Nervous System (CNS) function. Simultaneous brain and spinal fMRI is a non-invasive technique for the complete study of neuronal activity of the interconnected regions within the CNS. As indicated by electrophysiological data in the case of complete spinal cord injury, there may be instances where there is no activity elicited by a stimulus in the brain but does occur only caudal to a spinal cord injury, yet there is currently no tool to investigate this *in vivo*.

Materials and Methods

Animal preparation: Five Wistar rats were used. The animals were anesthetized with isoflurane. The rectal temperature was monitored and maintained at 37 ± 0.5˚C. Animals were intubated and ventilated (ventilation volume 3-4 ml; BP: PO_2 100-120 mm Hg, PCO_2 35-45 mm Hg). Bupivacaine was administered into the cannulation wound site before closure. Anesthesia was gradually switched from isoflurane to α-chloralose (30 mg/ml, 80 mg/kg) administered intravenously over approximately 20 min at the initial dose of 80 mg/kg and maintained at 20mg/kg, administered every 45 min. Following the completion of the imaging experiment, rats were immediately euthanasied with pentobarbital (120mg/kg, i.v.). Experimental setup: A 9.4T/21cm horizontal bore magnet was used. The animals were placed supine in the 5 × 7cm volume rf coil with the brain and cervical lower spine within the homogenous B_1 field of the rf coil allowing slice positioning in the areas of expected neuronal activation. Experiment design: Functional images were acquired from the brain and spinal cord simultaneously using the same pulse sequence and the same imaging parameters in both regions. Two axial slices were positioned within the brain in somatosensory cortex and five axial slices within the spinal cord. A multislice, single-shot FSE sequence was used (TE =3ms, TE_{eff} = 43.7ms, TR=7sec, FOV=2×2 cm, matrix size 64×64, 4 averages, slice th 2 mm, 0.5 mm gap in the spine and no gap in the brain). 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 31 time points with continuous 5 rest and stimulation periods (5 off – 6 on – 7 off - 7 on – 6 off). The total acquisition time of the one stimulation experiment was 14.4 min. Five stimulation experiments were performed with each animal with 5 min break period, allowing comparison of the results between animals and between single stimulation experiments within the same animal. For electrical stimulation (6 mA, 0.3 ms pulse length, 3 Hz) two small needle electrodes were placed subcutaneously and taped.

Results and Discussion

Functional images of appropriate quality were obtained from four animals. The amplitude of changes in image intensity was approximately 3% and followed the stimulation paradigm ($p \leq 0.001$). Electrical stimulation of the forepaw resulted in consequent activation within gray matter of the spinal cord and somatosensory cortex in brain. The sites of activity were localized mainly in the dorsal horn of the spinal cord. An example of simultaneous spinal and brain fMRI obtained from one animal is shown (Fig. 1A-F) at the level of C8-C6, where transduced signal reaches gray matter neurons and then crosses the cord to the opposite side within the ventral horn. Neuronal activity was detected within the somatosensory cortex corresponding to the forelimb (S1FL), contralaterally to the stimulated forelimb, mainly in the slices positioned –1 mm from Bregma. The fMR images of the spinal cord were superimposed on anatomical images at five different levels of the cervical spinal cord corresponding to T2/T1, T1/C8, C7, C6 and C5 cervical levels (Fig. 1 A-E) respectively. The brain fMRI (Fig. 1F) shows an activation of the somatosensory cortex. The intensity changes of the activated voxels within either spinal cord (Fig. 2A) or brain (Fig. 2B) follow the stimulation paradigm ($p \leq 0.001$) for each animal. These results demonstrate that the technique developed is able to assess simultaneously functional activation in the spinal cord and brain of the rat. This technique will provide a valuable tool to understand the interaction of functional pathways in normal conditions and the effects of injury and treatment.

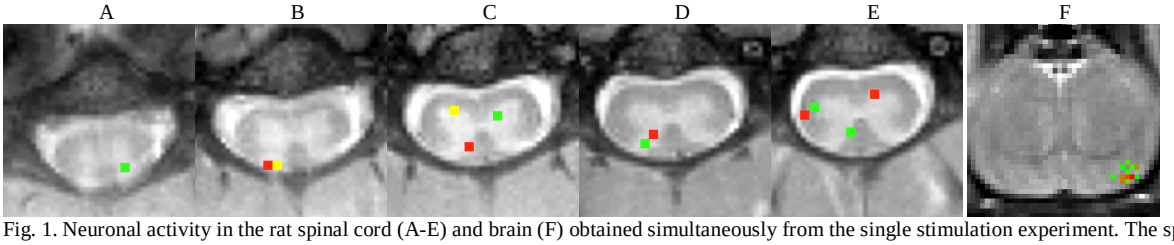

Fig. 1. Neuronal activity in the rat spinal cord (A-E) and brain (F) obtained simultaneously from the single stimulation experiment. The spinal images are superimposed on anatomical images at five different levels of the cervical spinal cord: T2/T1, T1/C8, C7, C6 and C5 cervical levels from left to right (A-E) respectively. The ventral surface is at the top, dorsal surface at the bottom. The color used to indicate the active voxels corresponds to the level of the correlation to the paradigm: red corresponds to the highest, yellow -medium and green the lowest correlation coefficient respectively.

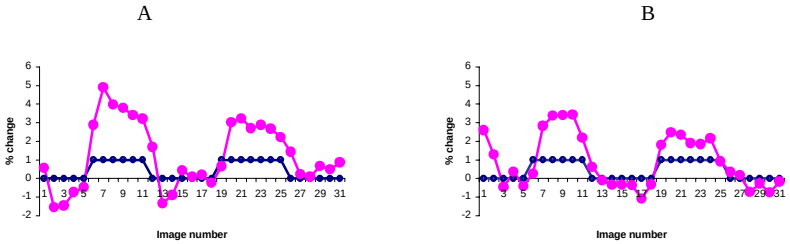

Fig.2. Time course corresponding to the activity shown in Fig 1: the blue line indicates the stimulation paradigm, and the red line indicates the actual time courses for spinal cord (A) and brain (B).

References

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