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Brief Communication

# Functional reorganization of the forepaw cortical representation immediately after thoracic spinal cord hemisection in rats

J.G. Yagüe, D. Humanes-Valera, J. Aguilar, G. Foffani *

*Hospital Nacional de Parapléjicos, SESCAM, Toledo, Spain*

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ABSTRACT

Spinal cord injury may produce long-term reorganization of cortical circuits. Little is known, however, about the early neurophysiological changes occurring immediately after injury. On the one hand, complete thoracic spinal cord transection of the spinal cord immediately decreases the level of cortical spontaneous activity and increases the cortical responses to stimuli delivered to the forepaw, above the level of the lesion. On the other hand, a thoracic spinal cord hemisection produces an immediate cortical hyperexcitability in response to preserved spinothalamic inputs from stimuli delivered to the hindpaw, below the level of the lesion. Here we show that a thoracic spinal cord hemisection also produces a bilateral increase of the responses evoked in the forepaw cortex by forepaw stimuli, associated with a bilateral decrease of cortical spontaneous activity. Importantly, the increased cortical forepaw responses are immediate in the cortex contralateral to the hemisection (significant within 30 min after injury), but they are progressive in the cortex ipsilateral to the hemisection (reaching significance only 2.5 h after injury). Conversely, the decreased cortical spontaneous activity is progressive both ipsilaterally and contralaterally to the hemisection (again reaching significance only 2.5 h after injury). In synthesis, the present work reports a functional reorganization of the forepaw cortical representation immediately after thoracic spinal cord hemisection, which is likely important to fully understand the mechanisms underlying long-term cortical reorganization after incomplete spinal cord injuries.

## Introduction

Spinal cord injury produces an immediate deafferentation of the cerebral cortex, which can lead to long-term reorganization of cortical maps in the adult brain (Bruehlmeier et al., 1998; Curt et al., 2002; Endo et al., 2007; Freund et al., 2013; Ghosh et al., 2009, 2010; Green et al., 1998; Jain et al., 1997, 2008; Tandon et al., 2009; Wall and Egger, 1971). Although cortical reorganization is important for functional recovery (Chen et al., 2012; Ghosh et al., 2009; Kaas et al., 2008; Kao et al., 2009), excessive or aberrant reorganization can lead to pathological situations, such as phantom limb sensations (Makin et al., 2013; Moore et al., 2000) or neuropathic pain (Gustin et al., 2012; Henderson et al., 2013; Wrigley et al., 2009).

In order to gain insights into the early pathophysiological mechanisms underlying long-term somatosensory alterations after spinal lesions, we recently investigated the immediate functional reorganization of the somatosensory cortex in two rat models of spinal cord injury. On the one hand, we observed that a complete thoracic transection of the spinal cord immediately changes the state of cortical spontaneous activity, slowing it down (Aguilar et al., 2010; Foffani et al., 2011), and increases the cortical responses to stimuli delivered above the level of the lesion (Humanes-Valera et al., 2013). On the other hand, we observed that a thoracic hemisection of the spinal cord produces an immediate cortical hyperexcitability in response to preserved spinothalamic inputs from stimuli delivered below the level of the lesion (Yague et al., 2011). Whether a thoracic hemisection also immediately affects the state of cortical spontaneous activity and/or alters the cortical responses to stimuli delivered above the level of the lesion remains unexplored.

To address this issue, we performed new analyses on the same animals used in our previous work (Yague et al., 2011), in which we bilaterally recorded local field potentials (LFPs) and multi-unit activity (MUA) in the primary somatosensory cortex before and immediately after a thoracic spinal cord hemisection in urethane-anesthetized rats. In our previous work we focused on the hindpaw representations of the somatosensory cortex (partly deafferented) in response to stimuli delivered to the hindpaws (below the level of the lesion). In the present work we focus on the forepaw representations of the somatosensory cortex in response to stimuli delivered to the forepaws (above the level of the lesion). We specifically studied the early bilateral changes in the spontaneous activity of the forepaw cortex as well as in the responses evoked by forepaw stimuli, within few hours after a thoracic hemisection of the spinal cord. To obtain a complete comparison

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* Corresponding author at: Finca La Peraleda s/n, 45071, Toledo, Spain. Fax: +34 925 247745.

*E-mail address:* gfoffani@sescam.jccm.es (G. Foffani).

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between the hemisection and full transection models, we also performed new analyses on an additional set of experiments from a previously published work (Aguilar et al., 2010).

## Materials and methods

The main data for this study come from a set of adult male Wistar rats used in a previous work on the thoracic hemisection model of spinal cord injury (Yague et al., 2011). We also performed new analyses on an additional set of rats used in a previous work on the thoracic transection model (Aguilar et al., 2010). For clarity, all the experimental procedures are briefly explained below. Experiments were performed following the rules of the International Council for Laboratory Animal Science, European Union regulation 86/609/EEC, and were approved by the Ethical Committee for Animal Research from the Hospital Nacional de Parapléjicos (Toledo, Spain).

### *Surgery*

Rats were anesthetized with intraperitoneal urethane (1.5 g/kg) and placed in a stereotaxic frame (SR-6R, Narishige Scientific Instruments, Tokyo, Japan). Lidocaine 2% was applied over the area of incision. The skin and muscle above the midline of the back were softly removed at thoracic level, and a laminectomy was performed on the thoracic vertebras 9–10 (T9–T10). Following the laminectomy, the skin of the head was removed from the top of the skull, the cisterna magna was opened and a bilateral craniotomy was performed over the primary somatosensory cortex (AP: 2 to −2; L: 1 to 5; atlas of Paxinos and Watson (1986)). The cortical surface was covered with artificial cerebrospinal fluid (ACSF) containing the following (in mM): 126 NaCl, 3 KCl, 1.25 $NaH_2PO_4$, 26 $NaHCO_3$, 1.3 $MgSO_4$ $7H_2O$, 10 dextrose, and 1 $CaCl_2$ $2H_2O$. The body temperature was automatically maintained constant with a heating pad at 37 °C. The level of anesthesia was monitored using the electro-corticogram and limb-withdrawal reflexes.

### *Electrophysiology*

We extracellularly recorded local field potentials and multi-unit activity using 5 MΩ tungsten electrodes (TM31A50KT of WPI, Inc Sarasota, FL, USA; impedance at 1000 Hz). The electrodes were stereotaxically placed bilaterally (in hemisected animals) or unilaterally (in transected animals) in the infragranular primary somatosensory cortex. In this work we specifically focused on the forepaw representation (antero-posterior: 0.5 mm; medio-lateral: 4 mm) following the coordinates of Chapin and Lin (1984). Beside the stereotactic coordinates, the exact antero-posterior and medio-lateral locations of the electrodes were adjusted with multiple penetrations (typically 1–3 penetrations per electrode) in order to optimize the physiological responses to the corresponding peripheral stimuli (maximum amplitude, minimal latency). To adjust the depth, we first identified layer 4, where response latencies are shortest, and then lowered the electrodes to the infragranular layers (depth: 1.1 to 1.8 mm). Once the electrodes were fixed in place, stimulation protocols were applied before spinal cord injury, as a control condition, as well as at three different times after the injury: 30 min (POST 1), 1.5 h (POST 2) and 2.5 h (POST 3). Animals never required additional anesthesia between the control protocol and the last protocol after the spinal cord injury.

All recordings were amplified and filtered (1 Hz to 3 kHz) using a modular system composed of a preamplifier, filter and amplifier (Neurolog, Digitimer Ltd, Welwyn Garden City, UK). Analog signals were converted into digital data at 20 kHz sampling rate and with 16-bit quantization using a CED Power 1401 (Cambridge Electronic Design, Cambridge, UK) controlled by Spike2 software (v6.09; Cambridge Electronic Design). Signals were stored in the hard disk of a PC for subsequent analysis.

### *Peripheral stimulation*

Electrical pulses were applied using bipolar needle electrodes located subcutaneously in the wrist of the forepaw, one pole in each side of the paw. The protocol consisted of a total of 100 stimuli with the duration of 1 ms and frequency of 0.5 Hz. Two different intensities were applied: low-intensity stimuli (0.5 mA) and high-intensity stimuli (5 mA). Low-intensity stimuli were intended to activate only a fraction of the available fibers, mainly low threshold primary fibers running through the lemniscal pathway, from the dorsal columns to the brainstem (Lilja et al., 2006; Yague et al., 2011). High-intensity stimuli were intended to activate the maximum number of fibers, including high-threshold primary fibers that make synapse in the dorsal horns of the spinal cord, in turn activating the spinothalamic tract of the paralemniscal pathway (Lilja et al., 2006; Yague et al., 2011).

### *Spinal cord injury*

Once we had recorded the spontaneous activity and evoked responses in intact animals, either a thoracic (T9–T10) spinal cord hemisection on the left side of the spinal cord or a thoracic spinal cord transection was performed using microscissors under microscope visualization. In our previous work on the hemisection model, this thoracic level of the injury allowed us to comparatively investigate the cortical effects of the deafferentation of the dorsal column (stimuli ipsilateral to the hemisection) vs the deafferentation of the spinothalamic tract (stimuli contralateral to the hemisection) in the hindpaw representation (Yague et al., 2011). This thoracic level does not produce a direct spinal deafferentation of forepaw inputs to the forepaw cortical representation, the region of interest in the present work.

### *Data analysis*

In this work we evaluated 15 of the 20 animals used in our previous work on the hemisection model (Yague et al., 2011), in which we had recordings in the forepaw cortex (left cortex: n = 11; right cortex: n = 13) with multi-unit activity of sufficiently high signal-to-noise ratio – with spikes exceeding the background activity by at least 7.5 standard deviations – both in control conditions and after hemisection. We also evaluated 11 of 22 animals used in our previous work on the transection model (Aguilar et al., 2010), in which not only signal-to-noise ratio requirements were satisfied, but also at least 3 full protocols were performed after thoracic deafferentation (our original work focused exclusively on the first protocol after deafferentation).

#### *Evoked responses*

Local field potential (LFP) responses were obtained by averaging across stimuli the raw signals recorded from the electrodes. LFP response amplitude was evaluated as the absolute value of the negative peak in the average response. Throughout the paper we only considered contralateral stimuli.

#### *Spontaneous activity*

Spontaneous activity was studied during 60 s periods immediately before the high-intensity stimulation protocols. In order to quantify the level of spontaneous cortical activity, we extracted the rectified multi-unit activity (rMUA) by band-pass filtering the raw signals at high frequencies (300–3000 Hz) and rectifying the resulting signal. The rMUA is a good measure of cortical state, as it correlates with the membrane potential of adjacent intracellularly recorded neurons (Hasenstaub et al., 2007). During slow-wave activity the rMUA amplitude displays a typical bimodal distribution, similar to the membrane potential of intracellularly recorded neurons (Hasenstaub et al., 2007). The overall level of spontaneous cortical activity was therefore assessed by the mean of the rMUA, which was the most sensitive measure of