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TIGHT NEUROVASCULAR COUPLING IN THE SPINAL CORD DURING NOCICEPTIVE STIMULATION IN INTACT AND SPINAL RATS

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Abstract—Functional magnetic resonance imaging (fMRI) is based on neurovascular coupling, which allows inferring neuronal activity from hemodynamic changes. Spinal fMRI has been used to examine pain processes, although spinal neurovascular coupling has never been investigated. In addition, fluctuations in mean arterial pressure (MAP) occur during nociceptive stimulation and this may affect neurovascular coupling. The objective of this study was to examine neurovascular coupling in the rat spinal cord during nociceptive stimulation while MAP was manipulated by cervical spinal transection, which prevents nociception-related MAP increases. Six male Wistar rats were anesthetized with isoflurane (1.2–1.5%). Local field potentials (LFP) and spinal cord blood flow (SCBF) were recorded concurrently in the lumbar enlargement, where activity was evoked by electrical stimulation of the sciatic nerve. In intact conditions, stimulation of graded intensity produced proportional changes in SCBF and LFP that were paralleled by similar changes in MAP. However, spinal transection almost abolished MAP changes ($p < 0.001$), while SCBF and LFP responses were not significantly affected ($p > 0.3$) and remained similarly coupled before and after spinal transection. This indicates that spinal hemodynamic changes reflect neuronal activity even when large fluctuations in MAP occur. This contrasts with results from previous studies on cerebral neurovascular coupling and suggests that spinal autoregulation might allow better adaptation to sudden MAP changes than cerebral autoregulation. Although assessment of the coupling between spinal neuronal activity and BOLD signal remains to be investigated, this study supports the use of spinal fMRI, based on the tight coupling between SCBF and LFP. © 2017 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: pain, fMRI, spinal cord injury, nociception, neurovascular coupling.

INTRODUCTION

Assessment of spinal cord functions in human is of critical importance to further our understanding of physiological and pathological spinal processes and to validate, improve and monitor clinical interventions or rehabilitation protocols. To date, this is mostly restricted to electrophysiological and neurological examinations, which are limited by indirect measures of spinal cord activity and functions.

In the past two decades, functional magnetic resonance imaging (fMRI) of the spinal cord was developed to provide a more direct assessment of spinal cord activity during sensory and motor tasks in human and animals (Yoshizawa et al., 1996; Cohen-Adad et al., 2009; Stroman et al., 2014; Wheeler-Kingshott et al., 2014; Kolesar et al., 2015; Martin et al., 2016). The most common fMRI methods rely on the blood oxygen-level-dependent (BOLD) signal (Ogawa et al., 1990), which was used to investigate nociceptive and pain-related processes in the spinal cord (Summers et al., 2010; Brooks et al., 2012; Kong et al., 2012; Sprenger et al., 2012; Geuter and Buchel, 2013; Dobek et al., 2014; Rempe et al., 2014; Bosma et al., 2015; Bosma and Stroman, 2015; Khan and Stroman, 2015; Sprenger et al., 2015; Bosma et al., 2016). However, BOLD signal relies on a tight coupling between neuronal activity and the associated hemodynamic changes, the so called neurovascular coupling. Indeed, BOLD signal is an indirect measure of neuronal activity (Logothetis and Wandell, 2004), which may be measured as multiunit activity or local field potentials (LFP), and it is associated with proportional changes in regional blood flow (Goense et al., 2012), both in the brain (CBF) and spinal cord (SCBF). To our knowledge, however, neurovascular coupling has never been examined in the spinal cord. Importantly, it was shown that in the primary somatosensory cortex of the rat, neurovascular coupling and cortical blood flow are altered during nociceptive processing due to fluctuations in mean arterial pressure (MAP) (Jeffrey-Gauthier et al., 2013; Uchida et al., 2016). Therefore,

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Abbreviations: BOLD, blood-oxygen-level dependent; fMRI, functional magnetic resonance imaging; LFP, local field potentials; MAP, mean arterial pressure; SCBF, spinal cord blood flow; SEM, standard error of the mean.

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neurovascular coupling of the spinal cord remains to be investigated, especially for the study of nociceptive and pain-related processes, in which systemic MAP may affect hemodynamic responses. Accordingly, it is recognized that autoregulation mechanisms cannot maintain constant SCBF at MAP values above 135 mmHg (Kobrine et al., 1976). Also, sudden MAP fluctuations as in the case of nociceptive or painful stimulation may not be compensated instantaneously by autoregulation. Therefore, systemic MAP fluctuations induced by nociceptive stimulation may bias the regional hemodynamic change associated with nociceptive activity in the spinal cord and may lead to erroneous measures.

The main objective of the present study was to examine the relationship between spinal neuronal activity (LFP) and SCBF responses in the lumbar spinal cord. In order to allow an invasive and direct measure of neurovascular coupling, LFP and SCBF were recorded in isoflurane-anesthetized rats that were subjected to electrical stimulation of the sciatic nerve. To manipulate systemic MAP and examine its effect on SCBF and neurovascular coupling, responses were compared before and after a complete transection of the spinal cord at upper cervical segment (C1). Spinal transection at this level interrupts sympathetic pathways and prevents MAP increases during nociceptive stimulation (Sato et al., 1997).

Based on previous studies on cerebral neurovascular coupling during nociceptive processing (Jeffrey-Gauthier et al., 2013), we hypothesized that the abolition of systemic MAP changes by spinalization would decrease the SCBF response evoked by sciatic nerve stimulation, while LFP amplitude would be unaffected. Accordingly, we expected a change in the neurovascular coupling after the C1 spinal transection. Based on previous studies on cerebral neurovascular coupling, we also anticipated that SCBF responses to high stimulus intensity in intact conditions would be more vulnerable to MAP changes.

EXPERIMENTAL PROCEDURE

Ethical approval

Experiments were performed on six male Wistar rats (body weight: 350–475 g; Charles River Laboratories, Saint-Constant, Québec, Canada). Animals were kept in the animal facilities of Université du Québec à Trois-Rivières, where a light–dark cycle of 14-h–10-h was maintained. All experimental procedures were approved by the Université du Québec à Trois-Rivières animal care committee, in accordance with the guidelines of the Canadian Council on Animal Care, and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP).

Animals and surgical procedures

All animals were in good health and showed robust responses to somatosensory stimuli. On the day of the experiment, surgical procedures were initiated after animals were deeply anesthetized with isoflurane (2.5%). In addition to stable systemic MAP, the depth of anesthesia was routinely confirmed during surgeries by the absence of withdrawal reflexes (paw pinching).

A schematic representation of the experimental set-up is shown in Fig. 1. Briefly, the right jugular vein was catheterized for intravenous injections and MAP was continuously recorded from the right carotid artery with an intra-arterial cannula connected to a pressure transducer (Harvard Apparatus, Holliston, MA, USA). Animals were artificially ventilated (SAR-830/P Ventilator, CWE Inc., Ardmore, PA, USA) using a tracheal cannula to maintain end-tidal CO_2 around 3.0% (CAPSTAR-100 Carbon dioxide analyzer, CWE Inc., Ardmore, PA, USA). Body temperature was monitored with a rectal probe (TCAT-2LV controller, Physitemp instruments Inc., USA) and was maintained at 37.5 ± 0.5 °C with a custom made temperature control system preventing electrophysiological artifacts. The animal's head was fixed in a stereotaxic frame (Model 900, Kopf Instruments, Tujunga, CA, USA) and two vertebral clamps (Model 986C, Kopf Instruments, Tujunga, CA, USA) were used to stabilize the spine. A laminectomy was made between T12 and L2 vertebral segments to expose the lumbar enlargement of the spinal cord, which receives sciatic nerve afferents. After a longitudinal incision of the dura matter, warm mineral oil was then applied on the spinal cord and was added

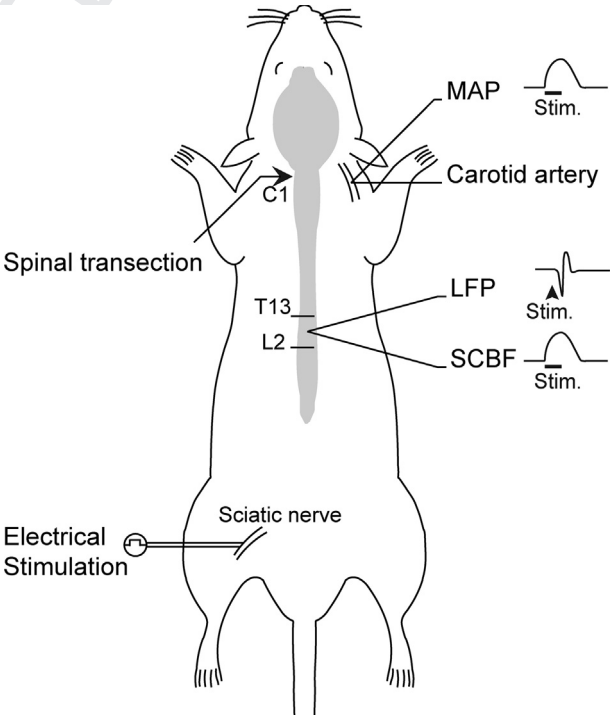

Fig. 1. Schematic representation of the experimental set-up. Mean arterial pressure was continuously recorded from the right carotid artery with an intra-arterial cannula connected to a pressure transducer. A laminectomy was made between T13 and L2 to expose the lumbar enlargement, from which local field potentials (LFP) and spinal cord blood flow (SCBF) were recorded concurrently. The left sciatic nerve was mounted on hook electrodes and was stimulated with constant current of graded intensity. MAP, SCBF and LFP were recorded before and after a spinal transection at C1.

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