Please cite this article in press as: Uchida S et al. Systemic blood pressure alters cortical blood flow and neurovascular coupling during nociceptive processing in the primary somatosensory cortex of the rat. Neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.12.014

Neuroscience xxx (2016) xxx–xxx

SYSTEMIC BLOOD PRESSURE ALTERS CORTICAL BLOOD FLOW AND NEUROVASCULAR COUPLING DURING NOCICEPTIVE PROCESSING IN THE PRIMARY SOMATOSENSORY CORTEX OF THE RAT

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Abstract—Inference on nociceptive and pain-related processes from functional magnetic resonance imaging is made with the assumption that the coupling of neuronal activity and cerebral hemodynamic changes is stable. However, since nociceptive stimulation is associated with increases in systemic arterial pressure, it is essential to determine whether this coupling remains the same during different levels of nociception and pain. The main objective of the present study was to compare the amplitude of local field potentials (LFP) and cerebral blood flow (CBF) changes in the primary somatosensory cortex during nociceptive electrical stimulation of the contralateral or ipsilateral forepaw in isoflurane-anesthetized rats, while manipulating mean arterial pressure (MAP). MAP changes induced by nociceptive stimulation were manipulated by transecting the spinal cord at the upper thoracic segments (T1–T2), which interrupts sympathetic pathways and prevents nociception-related MAP increases, while sensory pathways between the forepaws and the brain remain intact. Intensity-dependent increases in MAP and CBF were observed and these effects were abolished or significantly decreased after spinal transection ($p < 0.001$ and $p < 0.05$, respectively). In contrast, the intensity-dependent changes in LFP amplitude were decreased for the contralateral stimulation but increased for the ipsilateral stimulation after spinal transection ($p < 0.05$). Thus, neurovascular coupling was altered differently by stimulus-induced MAP changes, depending on stimulus intensity and location. This demonstrates that CBF changes evoked by nociceptive processing do not always match neuronal activity, which may lead to inaccurate estimation of neuronal activity from hemodynamic changes. These results have important implications for neuroimaging of nociceptive and pain-related processes.

Key words: pain, nociception, blood pressure, cerebral blood flow, neurovascular coupling, local field potentials.

INTRODUCTION

Several brain imaging techniques allow the investigation of brain function based on neurovascular coupling, i.e. the relationship between neuronal activity and the associated hemodynamic changes (cerebral blood flow, CBF, volume and oxygenation). For instance, functional magnetic resonance imaging (fMRI), which is based on changes in blood oxygen level-dependent (BOLD) signal, has been used extensively to investigate nociceptive and pain-related processes. These studies have provided evidence of a brain network that is commonly activated during acute pain (Apkarian et al., 2005; Duerden and Albanese, 2013) and a neurological pain signature that allows predicting acute experimental pain (Wager et al., 2013).

Notwithstanding, inference on nociceptive and pain-related processes from fMRI is made with the assumption that neurovascular coupling is stable in physiological conditions. Because nociceptive stimulation induces cerebral hemodynamic changes hardly separable from the BOLD signal related to neuronal activity (Erdos et al., 2003; Jeffrey-Gauthier et al., 2013), it is essential to examine the relationship between neuronal activity and cerebral hemodynamic changes during nociception and pain and to determine the conditions in which it is stable or altered. To date, however, this has been largely overlooked.

In a previous study, a strong link was reported between systemic mean arterial pressure (MAP) changes and neurovascular coupling in the primary somatosensory cortex (SI) of the rat during nociceptive stimulation of the hindpaw (Jeffrey-Gauthier et al., 2013). In this study, it was proposed that the alteration

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Abbreviations: BOLD, blood oxygen level dependent; CBF, cerebral blood flow; fMRI, functional magnetic resonance imaging; LFP, local field potentials; MAP, mean arterial pressure.

http://dx.doi.org/10.1016/j.neuroscience.2016.12.014

of neurovascular coupling leaded to an overestimation of the response to nociception, due to the influence of MAP on cortical blood flow (CBF), which increased in parallel with very similar temporal characteristics. Considering the impact that this may have on neuroimaging of pain and pain modulation mechanisms, it is critical to conduct a systematic investigation in which MAP is controlled or manipulated.

The main objective of the present study was to compare neuronal activity (local field potentials – LFP) and CBF responses in the primary somatosensory cortex (SI) of the rat induced by nociceptive stimulation of the forepaw, between a control condition and after a complete transection of the spinal cord at the upper thoracic segments (T1–T2). Spinal transection at this level interrupts sympathetic pathways and prevents MAP increases, while sensory pathways between the forepaw and the brain remain intact (Adachi et al., 1990; Uchida et al., 2000). We hypothesized that abolition of MAP changes would decrease the CBF response evoked by forepaw stimulation, while LFP amplitude would be unaffected or increased (Aguilar et al., 2010; Bazley et al., 2012; Alonso-Calvino et al., 2016). Therefore, we expected a change in the neurovascular coupling after spinal transection. We also anticipated that CBF responses to high stimulus intensity in intact conditions would be more vulnerable to MAP changes due to intensity-dependent effects (Jeffrey-Gauthier et al., 2013), while these effects should be abolished after spinal transection.

In previous studies on CBF, in spite of the well-known lateralization of nociceptive systems and the greater neuronal response in the hemisphere contralateral to nociceptive stimulation, it was not clear that the CBF response was lateralized and how it was coupled to neuronal activity (Adachi et al., 1990; Uchida et al., 2000; Uchida and Kagitani, 2015). Therefore, the second objective of this study was to examine CBF and LFP responses during nociceptive stimulation, with the hypothesis that contralateral responses should be greater than ipsilateral ones.

The present findings indicate that neurovascular coupling is altered differently by stimulus-induced MAP changes, depending on stimulus intensity and location. This demonstrates that CBF changes evoked by nociceptive processing do not always match neuronal activity, which leads to inaccurate estimation of neuronal activity from hemodynamic changes. These results have important implications for neuroimaging of nociceptive and pain-related processes.

EXPERIMENTAL PROCEDURES

Animals and surgical procedures

Experiments were performed on nine male Wistar rats (body weight: 300–500 g, Laboratoires Charles River, Saint-Constant, Québec, Canada) that were subjected to electrical stimuli in intact conditions and after a complete transection of the spinal cord. The 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, were 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). All animals were in good health and showed robust responses to somatosensory stimuli.

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 the surgeries by the absence of withdrawal reflexes (paw pinching). The right femoral vein was catheterized for intravenous injections. MAP was continuously recorded from a cannula inserted into the right femoral artery and 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, and the end-tidal CO_2 level was continuously monitored (CAPSTAR-100 Carbon dioxide analyzer, CWE Inc., Ardmore, PA, USA) and kept constant around 3.0% by controlling respiratory rate and tidal volume. 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 artefacts in electrophysiological recordings. Rats were placed in a stereotaxic frame (Model 900, Kopf Instruments, Tujunga, CA, USA). A craniotomy was made over the frontoparietal cortex at the following coordinates: anteroposteriorly from bregma and mediolaterally from the midline suture (A–P: 4 to −2 mm; L: 1–5 mm). This window included the forepaw representation in the right SI for electrophysiological and CBF recordings, as defined using the Paxinos and Watsons stereotaxic atlas (Paxinos and Watson, 1986). Warm paraffin oil was then applied on the brain and was added during the experiment as needed. Before the experiment began, the level of anesthesia was decreased to 1.2–1.5% of isoflurane. After confirming that the level of anesthesia was adequate to prevent paw withdrawal evoked by pinching, the experimental protocol began and lasted approximately 2 h.

After the first part of the experiment was completed (intact condition, *see Experimental protocol*), the level of anesthesia was increased to 2.5% and the spinal cord was transected between the 1st and 2nd thoracic (T1–2) level. Spinalization was performed in order to prevent MAP changes induced by electrical stimulation. With this procedure, we take advantage of the segmental organization of the sympathetic nervous system by interrupting the pathways between the brain and sympathetic preganglionic neurons regulating cardiovascular function (see Fig. 1). This prevents MAP changes while the cervical spinal cord remains intact and can still transmit sensory information to the brain when either forepaw is stimulated. After transection of the spinal cord, a bolus of 1 ml of Ficoll 4% (Sigma–Aldrich, Ontario, Canada) was injected intravenously.

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