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Neurovascular coupling during nociceptive processing in the primary somatosensory cortex of the rat

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ABSTRACT

Neuroimaging methods such as functional magnetic resonance imaging (fMRI) have been used extensively to investigate pain-related cerebral mechanisms. However, these methods rely on a tight coupling of neuronal activity to hemodynamic changes. Because pain may be associated with hemodynamic changes unrelated to local neuronal activity (eg, increased mean arterial pressure [MAP]), it is essential to determine whether the neurovascular coupling is maintained during nociceptive processing. In this study, local field potentials (LFP) and cortical blood flow (CBF) changes evoked by electrical stimulation of the left hind paw were recorded concomitantly in the right primary somatosensory cortex (SI) in 15 rats. LFP, CBF, and MAP changes were examined in response to stimulus intensities ranging from 3 to 30 mA. In addition, LFP, CBF, and MAP changes evoked by a 10-mA stimulation were examined during immersion of the tail in non-nociceptive or nociceptive hot water (counter-stimulation). SI neurovascular coupling was altered for stimuli of nociceptive intensities ($P < 0.001$). This alteration was intensity-dependent and was strongly associated with MAP changes ($r = 0.98$, $P < 0.001$). However, when the stimulus intensity was kept constant, SI neurovascular coupling was not significantly affected by nociceptive counter-stimulation ($P = 0.4$), which similarly affected the amplitude of shock-evoked LFP and CBF changes. It remains to be determined whether such neurovascular uncoupling occurs in humans, and whether it also affects other regions usually activated by painful stimuli. These results should be taken into account for accurate interpretation of fMRI studies that involve nociceptive stimuli associated with MAP changes.

1. Introduction

Functional magnetic resonance imaging (fMRI) has been used extensively to investigate pain-related processes. These studies have provided evidence of a brain network that is commonly activated during acute pain, including the primary somatosensory cortex (SI) [3]. Nevertheless, accurate inferences from the fMRI signal rely on tight coupling of neuronal activity with the associated hemodynamic changes, which underlie the blood oxygen level-dependent (BOLD) signal. Because nociceptive stimulation may be associated with cortical hemodynamic changes unrelated to local neuronal activity [11], it is essential to determine whether neurovascular coupling is maintained during nociceptive processing.

Among pain-related areas, SI is of particular interest. Its roles in nociceptive processing and in the sensory-discriminative aspect of pain have been clearly established in animal and human studies [3,8,22,43,46]. In addition, SI shows a precise somatotopic organisation and it is activated by both nociceptive and non-nociceptive stimuli [21]. Although several studies indicate that the coupling of neuronal activity to hemodynamic changes in SI is relatively constant in many conditions, numerous reports have shown some discrepancies.

For instance, electrical stimulation at intensities ranging from 0.6 to 1.4 mA and at frequencies ranging from 3 to 5 Hz is associated with proportional neuronal and hemodynamic changes when applied to the whisker pad [15,27,40], the infraorbital nerve [31], the sciatic nerve [30], and the fore and hind paws [16,40,41]. However, a number of studies observed nonlinear

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coupling with intensities and frequencies below or above this range [1,2,15,17,31]. Altogether, these studies indicate that the neurovascular coupling in SI of the rat during non-nociceptive stimulation is affected by stimulus parameters. It is presumed that further nonlinearities are induced during nociceptive processing, but this has never been investigated. Indeed, although the brain shows remarkable vascular autoregulation in a wide range of mean arterial pressure (MAP) [14,18–20,34,37], sudden blood pressure changes induced by nociceptive stimulation may affect the neurovascular coupling [4].

Another condition that may induce dissociation between neuronal activity and cortical blood flow (CBF) changes in SI is counter-stimulation. Counter-stimulation involves the application of 2 competing stimuli on different regions of the body. Stimuli can be nociceptive or non-nociceptive, but it has been demonstrated that only nociceptive counter-stimulation may produce inhibitory effects [23,47]. In the anaesthetised rat, when 2 nociceptive stimuli are applied, the strongest stimulus decreases the activity of wide-dynamic-range (WDR) neurons induced by the competing stimulus, through descending pathways [23,24]. In humans, this mechanism is thought to partly underlie the decrease of SI activity measured with fMRI in similar conditions, although this effect also involves other cerebral processes [33]. However, SI is also activated by nociceptive counter-stimulation, which may affect the CBF response and the neurovascular coupling.

The main objective of the present study was to examine the neurovascular coupling in SI during nociceptive electrical stimulation of the hind paw and during counter-stimulation. We hypothesised that, in spite of the vascular autoregulation, SI neurovascular coupling would be altered during nociceptive processing associated with MAP changes.

2. Materials and methods

2.1. Animals and surgical procedures

Experiments were performed on 15 male Wistar rats (body weight 350–500 g; Laboratoires Charles River, Saint-Constant, QC, Canada). The animals were kept in our facilities, where a light-dark cycle of 14 hours–10 hours 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. All animals were in good health and showed robust responses to somatosensory stimuli on different body regions.

Surgical procedures were initiated after animals were deeply anaesthetised with a urethane bolus (1.2 g kg^{-1} intraperitoneal). The right jugular vein was catheterised and additional urethane doses were administered intravenously as needed (10% of initial dose) to maintain an adequate and stable level of anaesthesia throughout the experiment. In addition to stable systemic MAP, the depth of anaesthesia was routinely confirmed by the absence of withdrawal reflexes (paw pinching). MAP was continuously recorded from a cannula inserted into the right axillary 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 analyser; CWE Inc) and kept constant between 3.5% and 4% by controlling respiratory rate and tidal volume. Body temperature was monitored with a rectal probe and was maintained at 37.5 ± 0.5 °C with the help of a temperature control system (TCAT-2LV controller; Physitemp Instruments Inc, Clifton, NJ, USA). Rats were placed in a stereotaxic frame (Model 900; David Kopf Instruments, Tujunga, CA, USA). A craniotomy was performed over SI, which was defined using the Paxinos and Watson stereotaxic coordinates: anteroposteriorly from bregma and mediolaterally from the midline suture (A–P: 1 to −4 mm; L: 1 to 5 mm) [32]. Warm paraffin oil was then applied on the brain and was added during the experiment as needed. Experimental procedures lasted approximately 16 hours. At the end of the experiment, the deeply anaesthetised rat was perfused with saline and paraformaldehyde (4%) through the left cardiac ventricle.

2.2. Local field potential recordings

Using a hydraulic micromanipulator (Model 640, David Kopf Instruments), a glass microelectrode filled with 3 M KCl (impedance 1–2 MΩ) was inserted at a depth of approximately 600 μm below the cortical surface of SI, at the coordinates of the hind paw representation (A–P: −1.1 mm, L: 2.8 mm) [32]. This depth was chosen in accordance with the approximate location of layer IV because this layer receives thalamocortical input and shows somatosensory local field potentials (LFP) of greater amplitude [45]. To ensure that the recording site was exclusively responsive to hind paw stimulation, multiunit activity was recorded (band-pass: 300–3000 Hz; amplification: 10,000×, amplifier: Model P511K; Grass Medical Instruments, Quincy, MA, USA) during tactile stimulation and pinching. Multiunit activity was visualised on an oscilloscope and monitored with a loudspeaker. The recording site was selected only if the stimulation of the 4th and 5th digit produced robust and stable multiunit responses while stimulation of the tail produced no response. The LFP signal was amplified (10,000×, amplifier: Model P511K amplifier, Grass Medical Instruments), filtered (band-pass 1-300 Hz), and recorded (sampling rate: 5 kHz; Power 1401 acquisition system, Cambridge Electronic Design, Cambridge, UK) for offline analysis.

2.3. Cortical blood flow recordings

A laser-Doppler probe (Micro-needle probe TSD145; Biopac Systems, Goleta, CA, USA) was placed on the cortical surface of SI, as close as possible to the microelectrode without achieving contact. The probe was carefully positioned to avoid large vessels. CBF was measured with a time constant of 1 second and was processed with a DC remove (30 seconds) function (Spike2, Cambridge Electronic Design). The signal was recorded (sampling rate: 100 Hz; Power 1401 acquisition system, Cambridge Electronic Design) for offline analyses.

2.4. Somatosensory stimuli

Electrical stimulation of the hind paw (10-second trains of 1-ms pulses at 5 Hz with an intertrain interval of 55 seconds) was delivered by a constant-current stimulator (Model DS7, Digitimer Ltd, Welwyn Garden City, UK) triggered by a computer-controlled sequencer (Power 1401 acquisition system, Cambridge Electronic Design), using a pair of subdermal needle electrodes inserted on the lateral aspect of the 4th and 5th digits (Model E2, Grass Medical Instruments). These stimulus parameters were selected because they elicited robust LFP and CBF changes in pilot experiments, consistent with previous reports on neurovascular coupling in SI [16,30]. It should be noted that electrical stimulation does not allow a selective activation of specific fibre groups. However, MAP increases associated with hind paw electrical stimulation depend on fibre groups III and IV in the rat, and most reports on MAP changes induced by somatic afferent stimulation have demonstrated that group I and II afferent fibres are ineffective to produce