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Diversity of neural–hemodynamic relationships associated with differences in cortical processing during bilateral somatosensory activation in rats

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ABSTRACT

The neural–hemodynamic relationships may vary depending on cortical processing patterns. To investigate how cortical hemodynamics reflects neural activity involving different cortical processing patterns, we delivered electrical stimulation pulses to rat hindpaws, unilaterally or bilaterally, and simultaneously measured electrophysiological (local field potential, LFP < 100 Hz; multiunit activity, MUA > 300 Hz) and optical intrinsic signals associated with changes in cerebral blood volume (CBV). Unilateral stimulation evoked neural and optical signals in bilateral primary somatosensory cortices. Ipsilateral optical responses indicating an increased CBV exhibited a peak magnitude of ~30% and mediocaudal shifts relative to contralateral responses. Correlation analyses revealed different scale factors between contralateral and ipsilateral responses in LFP–MUA and LFP–CBV relationships. Bilateral stimulation at varying time intervals evoked hemodynamic responses that were strongly suppressed at 40-ms intervals. This suppression quantitatively reflected suppressed LFP responses to contralateral testing stimulation and not linear summation, with slowly fluctuating LFP responses to ipsilateral conditioning stimulation. Consequently, in the *overall responses* to bilateral stimulation, CBV-related responses were more linearly correlated with MUA than with LFPs. When extracting *high-frequency* components (> 30 Hz) from LFPs, we found similar scale factors between contralateral and ipsilateral responses in LFP–MUA and LFP–CBV relationships, resulting in significant linear relationships among these components, MUA, and cortical hemodynamics in *overall responses* to bilateral stimulation. The dependence of LFP–MUA–hemodynamic relationships on cortical processing patterns and the LFP temporal/spectral structure is important for interpreting hemodynamic signals in complex functional paradigms driving diverse cortical processing.

Introduction

Humans and other mammals continuously obtain information on relationships between themselves and their environment through the five major sensory systems. These various types of informative signals are processed sequentially and in parallel using top-down guidance based on the ongoing behavioral state (Lamme and Roelfsema, 2000; Nicolelis, 2005). They are selected or integrated for planning and executing the actions of daily life. At the microscopic level, these signals converge on cells in the cortical layers through different neural pathways from subcortical regions and other cortical areas and layers. The signals interact with one another and are enhanced or suppressed by local excitatory and inhibitory networks, proceeding through successively higher levels of hierarchical processing.

This study was motivated by the following question: *What can be known about the neural activity underlying functional integration by using noninvasive neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), which are based on the measurement of activity-related hemodynamic changes?* To address this question, the types of cortical activities contributing to these hemodynamic changes must first be understood. Recent studies have demonstrated that dendritic processing of excitatory synaptic inputs correlates more closely with hemodynamic changes than the generation of spikes (Lauritzen, 2005; Mathiesen et al., 1998; Thomsen et al., 2004). Since local field potentials (LFPs) are currently the best *in vivo* indicator of synaptic activity, several research groups have tested a hypothesized linear relationship between LFPs and hemodynamic changes by manipulating the intensity, duration, number and interval (or frequency) of simple repetitive stimulation applied to a primary

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receptive field or a part of a neural circuit (Devor et al., 2003; Hewson-Stoate et al., 2005; Jones et al., 2004; Masamoto et al., 2007; Nemoto et al., 2004; Ngai et al., 1999; Nielsen and Lauritzen, 2001; Sheth et al., 2004b; Ureshi et al., 2004). The researchers found both linear and nonlinear neural–hemodynamic relationships. Furthermore, recent studies using natural stimuli have revealed that fMRI signals are tightly correlated with *high-frequency* LFP components, such as the gamma frequency band in the visual and auditory cortex (Kayser et al., 2004; Logothetis et al., 2001; Mukamel et al., 2005; Nir et al., 2007, 2008; Privman et al., 2007).

In this study, we focused on multifaceted neural activity rather than functional imaging signals, and examined the aspects of neural activity associated with hemodynamic changes during stimulation paradigms that activate different cortical areas and in which neural signals interact with each other. We employed simple functional paradigms consisting of unilateral and bilateral somatosensory stimulation. In general, neural activity evoked by unilateral somatosensory stimulation generates electromagnetic fields not only in contralateral primary and secondary somatosensory cortices (S1/S2) and parietal association areas, but also in ipsilateral somatosensory cortices (Allison et al., 1989). However, significant differences are observed in cortical processing between contralateral and ipsilateral S1, regarding the involvement of afferent neural pathways, the activation of cortical subareas, layers and neuronal subpopulations, and the balance of excitatory and inhibitory neurotransmission (Akers and Killackey, 1978; Armstrong-James and George, 1988; Berwick et al., 2004; Conti and Manzoni, 1994; Lipton et al., 2006; Olavarria et al., 1984; Petreanu et al., 2007; Shuler et al., 2001; Wise and Jones, 1976). Neural network activity generated by such different cortical processing should have different impacts on recording electrodes and should evoke distinct hemodynamic responses through the release of different vasoactive mediators from pyramidal cells, GABA (gamma-aminobutyric acid) interneurons and astrocytes (Cauli and Hamel, 2010; Enager et al., 2009; Iadecola, 2004; Kleinfeld et al., 2011; Lauritzen, 2005). These differences logically imply that neural–hemodynamic relationships will differ between contralateral and ipsilateral responses. In fact, hemodynamic responses recorded in ipsilateral S1 vary considerably, unlike those recorded in contralateral S1, from positive (Lipton et al., 2006; Nemoto et al., 1999b; Nihashi et al., 2005; Pelled et al., 2009) to negative (Alonso et al., 2008; Boorman et al., 2010; Devor et al., 2008; Hlushchuk and Hari, 2006), depending on the experimental conditions. A rational interpretation is required to reconcile these diverse observations. Here we simultaneously measured broadband extracellular field potentials and optical intrinsic signals (OIS) associated with changes in cerebral blood volume (CBV) and oxygenation in the rat S1, while delivering electrical pulses to the hindpaws, unilaterally or bilaterally. We analyzed the field potential signals using frequency band filtering (LFP<100 Hz; multiunit activity, MUA>300 Hz) to estimate synaptic and *population* spiking activities. With these methods, we examined neural activity, as reflected in hemodynamic changes, due to functional integration mediated by different processing patterns. With unilateral stimulation, significant differences were observed in the activated subareas and LFP–MUA–hemodynamic relationships between contralateral and ipsilateral responses. With bilateral stimulation, LFP, MUA and hemodynamic signals were strongly suppressed at 40-ms intervals, while being loosely coupled to each other (Ogawa et al., 2000). However, when analyzing *overall responses*, including ipsilateral responses, hemodynamic responses accurately reflected MUA, rather than LFP. This unexpected result can be explained by the examination of sub-band LFP components, which revealed that LFPs in all frequency ranges were not always strongly correlated with MUA (Liu and Newsome, 2006) or hemodynamic responses (Mukamel et al., 2005; Niessing et al., 2005) across different cortical processing patterns.

Materials and methods

Animal preparation

Ten adult male Sprague–Dawley rats (350–550 g, Japan SLC Inc., Hamamatsu, Japan) were initially anesthetized with isoflurane (4% during induction, 1.5–2.5% during surgery). The femoral vein and tail artery were cannulated for intravenous drug administration, blood pressure monitoring and blood gas analysis. Following tracheotomy, the animals were immobilized with pancuronium bromide (2 mg kg^{-1} h^{-1}) and artificially ventilated with 60% N_2 and 40% O_2. End-tidal PCO_2 was monitored using a CO_2 analyzer (CapStar-100, CWE Inc., Ardmore, PA, USA), and ventilation was adjusted to maintain the end-tidal PCO_2 within 40 ± 5 mmHg. Anesthesia was maintained with continuous infusion of α-chloralose (25–35 mg kg^{-1} h^{-1}) and the level of anesthesia was adjusted to maintain a constant arterial blood pressure. Rectal temperature was stabilized at 37 °C using a heating pad. Rats were placed in a stereotaxic frame (Narishige Scientific Instrument Lab., Tokyo, Japan), and lidocaine was applied to the incision sites for local anesthesia. The skull was exposed, and the parietal bone was thinned (5×7 mm) bilaterally over the sensorimotor cortex using a dental drill under continuous cooling with physiological saline. The experimental procedures were approved by the Animal Research Committee of the Tokyo Institute of Psychiatry.

Stimulation protocols

We delivered electrical stimuli (0.8 mA intensity, 1-ms duration for each pulse) using a pair of needle electrodes with the anode and cathode inserted under the skin of the ankle and plantar regions, respectively. To investigate neural–hemodynamic relationships, we simultaneously measured electrophysiological signals in the right somatosensory cortex and OIS in the bilateral cortical hemispheres, while delivering stimulus pulses to the contralateral (left) or ipsilateral (right) hindpaw, or both. Stimulation protocols were as follows.

Protocol 1 (unilateral stimulation to the left or right hindpaw)

A stimulus train (10 pulses with an interstimulus interval [ISI] of 200 ms) was delivered to the left or right hindpaw in order to elicit hemodynamic changes that could be measured with a high contrast-to-noise ratio even in the cortical hemisphere ipsilateral to the stimulation. This trial was alternately repeated 10 times on each side with an inter-trial interval of 30 s. The data from the 20 trials was recorded in 10 min.

Protocol 2 (bilateral stimulation and variable time interval)

We modulated neural interactions by varying the time interval between contralateral and ipsilateral stimulations. A testing stimulus train (TS train; 10 pulses to the contralateral hindpaw with a 200-ms ISI) was delivered after a conditioning stimulus train (CS train; same pulse parameters were used for the ipsilateral hindpaw) while varying the time interval between stimuli (80, 60, 40, 20 and 0 ms), or without a CS train, in a single trial. In this bilateral stimulation paradigm, trials performed for these six stimulus conditions were alternated in a certain sequence assigned to individual rats so that fluctuations in the basal state and the responsiveness of brain activity and microcirculation (Suppl. Fig. 3s; Jones et al., 2008; Martin et al., 2006) would have a similar impact on each stimulus condition. The stimulus condition sequence was repeated 10 times with an inter-trial interval of 30 s, and the dataset from the 60 trials was recorded in 30 min (Suppl. Fig. 3s). In eight rats, different stimulation parameters (4 pulses, 300-ms ISI) were subsequently used for the TS and CS trains (*Protocol 2s*), and whether similar findings were generally applicable to neural–hemodynamic relationships was assessed.

These stimulation protocols were automatically executed using two stimulators (ISO-flex; A.M.P.I., Jerusalem, Israel) triggered by a