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# Pial arteriolar vasomotion changes during cortical activation in rats

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**The oscillatory pattern of pial arterioles, i.e. vasomotion, has been described since early 1980s, but the impact of neural activation on such oscillations has never been formally examined. Sciatic nerve stimulation, a well characterized model for studying neurovascular coupling (NVC), leads to a neural activity-related increase of pial arteriolar diameter in the contralateral hindlimb somatosensory cortex. Exploiting such an experimental model, the aim of the present study was to explore vasomotion and its changes during NVC with a novel analytical approach. Indeed, to characterize oscillations, we evaluated the total spectral power in the range 0.02–2.00 Hz and subdivided this frequency interval into seven 50% overlapping frequency bands. Results indicated that only arterioles overlying the stimulated hindlimb cortex showed a significant increase of total power, unlike arterioles overlaying the whisker barrel cortex, used as control for the vascular response specificity. The total power increase was sustained mainly by marked increments in the low frequency range, with two peaks at 0.03 and 0.08 Hz, and by a wide increase in the high frequency range (0.60–2.00 Hz) in the averaged spectrum.**

**These activity-related spectral changes suggest: (i) that it is possible to assess the vascular responses by using total power; (ii) the existence of at least three distinct mechanisms involved in the control of NVC, two with a feedback frequency loop in the low frequency range and another one in the high range; (iii) a potential involvement of vasomotion in NVC. Moreover, these findings highlight the oscillatory nature of the mechanisms controlling NVC.**


## Introduction

In the brain, as well as in several other tissues, precapillary vessels exhibit slow spontaneous rhythmic diameter changes, which are responsible for the rhythmic oscillations of local microvascular blood flow (Hudetz, 1997a). This oscillatory activity, defined as vasomotion (VM), has also been detected in isolated vessels from the human (Gokina et al., 1996) and animal brain (Fujii et al., 1990). This basic phenomenon has been studied with a large number of techniques, such as intravital videomicroscopy (Auer and Gallhofer, 1981; Lefer et al., 1990; Ngai et al., 1995), laser Doppler flowmetry (LDF) (Hudetz et al., 1992; Jones et al., 2001; Morita-Tsuzuki et al., 1992), near infrared spectroscopy (NIRS) (Obrig et al., 2000; Schroeter et al., 2005), optical imaging spectroscopy (Mayhew et al., 1996), reflected and scattered light (Mayhew et al., 1996), transcranial Doppler (Lang et al., 1999), functional magnetic resonance imaging (fMRI) (Biswal et al., 1997; Cordes et al., 2000; Strangman et al., 2002), electrophysiology and cellular calcium imaging (Brown et al., 2002; Filosa et al., 2004). Even though several physiological factors and pharmacological probes are known to influence VM, the physiological role and the exact mechanisms underlying this multifactorial phenomenon are still not completely understood.

In the brain, VM has been suggested to be influenced by neurovascular coupling (NVC), defined as the local hemodynamic changes tied to modifications of neural activity (Brown et al., 2002; Filosa et al., 2004). *In vitro* approaches, by using simplified models, have shown VM suppression during increase of neural activity (Brown et al., 2002; Filosa et al., 2004). However, NVC and VM interplay is still unexplored throughout *in vivo* studies. On the other hand, the *in vivo* studies on this issue have been mainly directed to the removal of the "vascular artifacts" from the fMRI (Birn et al., 2006) and optical imaging spectroscopy (Mayhew et al., 1996) oscillatory signals to better identify NVC. The NVC is a complex phenomenon in which the tuning activity of local control mechanisms, that functionally link neurons, astrocytes, endothelial and smooth muscular cells, is interlaced with general control mechanisms, driven by vascular innervation. There is an increasing number of studies highlighting the importance of coupling between cerebral

*Abbreviations:* CBF, cerebral blood flow; HLC, hindlimb cortex; LDF, laser Doppler flowmetry; *M*, median; *m*, mean; $M_{Bc}$, median diameter of basal conditions; $M_{SPc}$, median of stimulus and poststimulus conditions diameter; NVC, neurovascular coupling; P1, P10, P90, P99, 1st, 10th, 90th and 99th percentiles; PB1–7, power content of the frequency band 1–7; $PB_{SPc}$, power spectral band in stimulus and poststimulus conditions; $PB_{Bc}$, power spectral band in basal conditions; PSD, power spectral density; se, standard error; SEPs, somatosensory evoked potentials; TP, total power; VM, vasomotion; WBC, whisker barrel cortex.

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parenchymal and vascular components. The impairment of this fine tuning could facilitate cerebral dysfunction or exacerbate neurological and neurovascular diseases such as alpha-synucleinopathies (Norris et al., 2004), Alzheimer disease, cerebral ischemia and hypertension-induced neuropathological effects (Girouard and Iadecola, 2006). Moreover, modifications of vascular oscillatory phenomena might reflect, or be a concurrent cause of, neurological diseases such as multiple sclerosis (Lowe et al., 2002) and cerebral microangiopathy (Schroeter et al., 2005).

Pial arterioles, as well as parenchymal ones, could play an important role in the regulation of the coupling between neuronal activity and the hemodynamic response. Indeed, it is well known that 50% of the overall cerebrovascular resistance is due to pial arterioles and larger extraparenchymal arteries. Such a regulatory role has been also suggested by the study of the hemodynamic oscillations both at rest (Biswal et al., 1997) and during neural activation (Ngai et al., 1995). Consequently, the intravital observation of pial arterioles could shed light on the control mechanisms acting during NVC and on VM changes caused by NVC. In particular, VM and its modifications after neural activation could provide new insights into the kinetics of biochemical and cellular pathways underlying NVC regulatory mechanisms.

The present study describes the time course of pial vascular responses to cortical somatosensory activation, by tracking pial arteriolar diameter before, during and after sciatic nerve stimulation. Moreover, according to other studies (Fujii et al., 1990; Lefer et al., 1990) on cerebral VM, a frequency spectral analysis was applied to study pial diameter oscillations. However, the proposed approach differs from previous reports since spectral analysis was performed during basal conditions and after the increase of neuronal activity associated to sciatic nerve stimulation. Therefore, the main goals of this study were to further characterize VM in basal conditions and to clarify the NVC influence on VM by using a novel analysis approach for assessing *in vivo* pial microcirculation reactivity after neural activation.

## Materials and methods

### *Animal preparation*

Adult male Wistar rats weighing 300–350 g ($n=32$) were used for this study.

Eight animals were studied for the anatomical identification of hindlimb cortex by means of somatosensory evoked potentials (SEPs) and for assessing the stimulation parameters (SEP group); 4 animals were used for sham-stimulation time controls (sham group); 20 animals were utilized for studying vascular response (vascular group).

All animals were anesthetized with α-cloralose (50 mg/kg, i.p.) (Merck and Co., NJ) and urethane (600 mg/kg, i.p.) (Fluka, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) for induction and with urethane alone (100 mg/kg, i.v., every hour) for maintenance. The rats were tracheotomized and mechanically ventilated. The left femoral artery was cannulated for arterial blood pressure recording and blood gases sampling. The left femoral vein was cannulated for drug administration. Throughout all experiments, mean arterial blood pressure, heart rate, respiratory $CO_2$ and blood gases values were recorded and maintained stable within physiological ranges. Rectal temperature was monitored and maintained at 37.0±0.5 °C with a custom made heating pad. An adequate level of anesthesia was maintained throughout the whole experimental session and vital signs (heart rate, blood pressure) were closely monitored. For cranial surgery and pial vessel observation, animals were secured in a stereotaxic frame. The skull was exposed by a longitudinal incision and the soft tissues were reflected laterally to the temporal crest. A cranial window (4×5 mm) was opened with a dental drill over the left parietal region to expose the hindlimb somatosensory cortex (1–2 mm caudally to the bregma and 2–3 mm laterally to the midline) and the whisker barrel cortex (2–4 mm caudal to the bregma and 4–8 mm lateral to the midline) (Ngai et al., 1988). To prevent overheating of the cerebral cortex during drilling, cold saline solution was suffused on the skull.

In the vascular and sham groups, dura mater was carefully removed and the exposed brain parenchyma was continuously suffused with artificial cerebrospinal fluid (aCFS) at 37 °C. The composition of the aCFS was: 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_47H_2O$, 1.0 mM $NaH_2PO_4$, 26.2 mM $NaHCO_3$, 2.5 mM $CaCl_2$ and 11 mM glucose (equilibrated with 10% $O_2$, 6% $CO_2$ and 84% $N_2$; pH 7.38±0.02) (Golanov et al., 1994).

All procedures were approved by the Ethical and Animal Care Committees of the University of Pisa.

### *Sciatic nerve stimulation*

For all experimental groups, the right sciatic nerve was carefully isolated and sectioned. The proximal end was placed in a bipolar ring electrode and bathed with warm mineral oil. With the exception of the sham group, the sciatic nerve was stimulated with constant current pulses of 2.0 ms, generated by a stimulus isolator unit (A365, World Precision Instruments, Sarasota, FL) triggered by a National Instruments board (PCI-6251, National Instruments, Austin, TX) via a dedicated software written by P. Orsini (Labview, National Instruments, Austin, TX), at 2.5 Hz for 10 s (Ngai et al., 1999). Intensity was set at 250 μA. Pilot experiments showed that blood pressure and heart rate were not affected by stimulation. To minimize variations in arteriolar dilation due to anesthetics or deterioration of the nerve, all experiments were performed within 4 h after the induction of anesthesia.

### *Somatosensory evoked potentials*

Somatosensory potentials evoked by the stimulation of the sciatic nerve were recorded with an $Ag/AgCl_2$ ball-tip electrode (100 μm diameter) lying on the dura mater over the hindlimb somatosensory cortex (Fig. 1). Recordings from the whisker barrel cortex during sciatic nerve stimulation were used as control. The reference electrode was inserted into the scalp muscle. SEPs were recorded with a microelectrode preamplifier (VF-180, BioLogic, France), amplified (gain 1 K) and filtered (bandpass 3–3000 Hz). For each animal, 5 train series of SEPs at 5 min interval were recorded. Sciatic nerve stimulation parameters were the same used for the vascular group.

### *Intravital microscopy and vessel diameter measurement*

After removal of the dura mater and injection of fluoresceine isothiocyanate bound to dextran of molecular weight 70 kDa (Sigma-Aldrich, St. Louis, MO) (Mayhan, 2000), pial vessels were visualized through a fluorescence microscope (Leitz Orthoplan, Wetzlar, Germany) fitted with a long working distance objective (×32, n.a. 0.4). Microvascular images were collected by a video camera (Dage-MTI 300 RC, Michigan City, IN) and recorded by a