Modeling the contribution of neuron-astrocyte cross talk to slow blood oxygenation level-dependent signal oscillations

Mauro DiNuzzo,1 Tommaso Gili,2,3 Bruno Maraviglia,1,4 and Federico Giove1,3

1Dipartimento di Fisica, Sapienza Università di Roma, Rome, Italy; 2Cardiff University Brain Research Imaging Centre, School of Psychology, Cardiff University, Cardiff, United Kingdom; 3Magnetic Resonance for Brain Investigation Laboratory, Museo Storico della Fisica e Centro di Studi e Ricerche “Enrico Fermi,” Rome; and 4Fondazione Santa Lucia IRCCS, Rome, Italy

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DiNuzzo M, Gili T, Maraviglia B, Giove F. Modeling the contribution of neuron-astrocyte cross talk to slow blood oxygenation level-dependent signal oscillations. J Neurophysiol 106: 3010–3018, 2011. First published September 14, 2011; doi:10.1152/jn.00416.2011.—A consistent and prominent feature of brain functional magnetic resonance imaging (fMRI) data is the presence of low-frequency (<0.1 Hz) fluctuations of the blood oxygenation level-dependent (BOLD) signal that are thought to reflect spontaneous neuronal activity. In this report we provide modeling evidence that cyclic physiological activation of astroglial cells produces similar BOLD oscillations through a mechanism mediated by intracellular Ca2+ signaling. Specifically, neurotransmission induces pulses of Ca2+ concentration in astrocytes, resulting in increased cerebral perfusion and neuroactive transmitter release by these cells (i.e., gliotransmission), which in turn stimulates neuronal activity. Noticeably, the level of neuron-astrocyte cross talk regulates the periodic behavior of the Ca2+ wave-induced BOLD fluctuations. Our results suggest that the spontaneous ongoing activity of neuroglial networks is a potential source of the observed slow fMRI signal oscillations.

astrocytes; calcium waves; brain resting state

RESTING OSCILLATORY PATTERNS in cortical activity are a common feature of the human brain (Buzsaki and Draguhn 2004). Recently, the interest in neuronal oscillations has been further renewed, if possible, due to the hypothesis that information processing in the brain is the result of the precise timing in the cycling activation of synchronized, resonant neurons through a series of opportune frequency windows (Uhlhaas et al. 2009). It is argued that this temporal coordination stems from the topology of neuronal networks (Brunel and Wang 2003; Buzsaki and Draguhn 2004). Nevertheless, the emergence of synchrony applies crucially to fast oscillations, whereas low-frequency oscillations are thought to be largely independent of the underlying network configuration (Brunel and Wang 2003; Mazzoni et al. 2008). Furthermore, slow periodic changes in cortical activity may be a feature of the input received by a neuronal population (Mazzoni et al. 2008) or may originate from metabolism-related mechanisms (Cunningham et al. 2006). Specifically, rhythmic variations in the metabolic state of neuronal and nonneuronal (astroglial) cells can have an important role in the balance between recurrent excitation and local inhibition, most likely via astrocytic Ca2+ transients (Lorincz et al. 2009).

Astrocytes are ubiquitous inhabitants of synaptic regions, where they are primarily involved in the clearance of neuroactive compounds from the synaptic cleft (Hertz et al. 2007). Not only do astrocytes express transporters for the uptake of transmitters, but they also have receptors for those substances (Perea and Araque 2002). Accordingly, astrocytes respond to neurotransmitters released at the synapse by generating elevations in intracellular Ca2+ concentration that have the potential to propagate as waves within the astrocytic syncytium via gap junctional communication (Cornell-Bell et al. 1990; Perea and Araque 2002; Schipke and Kettenmann 2004). Most importantly, the increase in astrocytic Ca2+ level mediates the release of transmitter molecules, including the excitatory neurotransmitter glutamate (Purpura et al. 1994), a mechanism termed gliotransmission. This means that astrocytes can signal across the brain and possibly engage cortical regions away from the site of initial activation (Fellin and Carmignoto 2004). Interestingly, astrocytic Ca2+ oscillations occur spontaneously as well, resulting in correlated activity of the associated neuronal network (Agudo et al. 2002). It should be realized that this does not imply that regular astrocytic Ca2+ oscillations set uniformity on neuronal firing patterns. On the contrary, theoretical studies indicate that robust neuronal synchronization is effectively broken by neuron-astrocyte interactions, which however promote increased levels of complexity in the periodic fluctuations of neuroglial network (see Allegrini et al. 2009).

Astrocytes are also in intimate contact with cerebral vasculature, and astrocytic processes (i.e., endfeet) are a primary locus of release of vasoactive compounds, thereby playing a pivotal role in the control of the vascular tone (Iadecola 2004; Rossi 2006). In particular, Ca2+ elevations in astrocytes initiate a metabolic cascade that participates in smooth muscle cells activation and subsequent response of adjacent arterioles (Bennett et al. 2008a). The fact that astrocytes are intermediary in synaptic transmission-mediated functional hyperemia is relevant for functional magnetic resonance (fMRI) studies (Wang et al. 2009), in particular those showing low-frequency fluctuations in the blood oxygenation level-dependent (BOLD) signal (Fox et al. 2005). Indeed, the range of frequencies of spontaneous astrocytic Ca2+ oscillations (0.003–0.1 Hz) (Lorincz et al. 2009) largely overlaps with that of the so-called resting state networks indicated by BOLD signal fluctuations observed in the absence of stimuli (Fox and Raichle 2007). Since the change in blood oxygenation induced by synaptic activity form the basis of fMRI (Logothetis et al. 2001), it is important to study how the BOLD signal can be influenced by astrocyte-neuron interactions and spontaneous astrocytic activation (Wang et al. 2009).
In the present article, we examine theoretically the contribution of astrocytes in the generation of the magnetic resonance signal changes in the absence of focal neuronal stimulation. Our modeling outcomes suggest a connection between the regional metabolic and vascular responses of cerebral tissue to astrocytic Ca\(^{2+}\) signaling and the alterations of physiological parameters that can be detected as slow BOLD oscillations.

**METHODS**

We have extended our previous kinetic models of functional brain metabolism (DiNuzzo et al. 2010a, 2010b) by including Ca\(^{2+}\) dynamics in astrocytes (Lavrentovich and Hemkin 2008) and Ca\(^{2+}\)-dependent astrocytic transmitter release (De Pitta et al. 2009; Di Garbo et al. 2007). The model incorporates the current biological understanding related to the signaling pathways underlying both the spontaneous and the glutamate-induced generation of Ca\(^{2+}\) oscillations in astrocytes (Fig. 1). In particular, synaptically released glutamate activates astrocytic metabotropic glutamate receptors (mGluRs), thereby initiating an intracellular cascade that stimulates the production of inositol trisphosphate (IP\(_3\)) by phospholipase C (PLC). Binding of IP\(_3\) to its receptors located on endoplasmic reticulum (ER) enhances the release of Ca\(^{2+}\) from internal stores, which in turn stimulates IP\(_3\) receptors. Cytosolic Ca\(^{2+}\) is rapidly extruded by the cell via both the plasma membrane Ca\(^{2+}\)-ATPase and Na\(^{+}\)/Ca\(^{2+}\) exchanger or is sequestered back into ER through sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase. Degradation of IP\(_3\) occurs via either phosphorylation by 3-kinase or hydrolysis by 5-phosphatase. Intracellular Ca\(^{2+}\) exhibits oscillating pulses depending on the rates of the relevant reactions, which constitute the so-called inositol cross-coupling (ICC) and CICR mechanisms (see Lavrentovich and Hemkin 2008).

In our previous theoretical accounts (DiNuzzo et al. 2010a, 2010b), we considered a compartmentalized model of cerebral metabolism by identifying the coupling between neuronal activity and cell energetics in the activation of Na\(^{+}\)/K\(^{-}\)-ATPase (sodium pump). The activity of the Na\(^{+}\)/K\(^{-}\)-ATPase, which consumes energy to restore ion gradients across neuronal and astrocytic plasma membrane, upregulates astrocytic glycolysis, glycolytic and oxidative metabolism in both cellular compartments, and ATP buffering systems of creatine and adenylyl kinases (Table 1, but see DiNuzzo et al. 2010a for a comprehensive description of the model; DiNuzzo 2010b). The link between the level of activation of neurons and astrocytes is represented by glutamatergic neuro- and gliotransmission mediated by ICC/CICR-mediated Ca\(^{2+}\) oscillations. It should be noted that the spreading of the wave in the volume of interest might alter the local profile of the Ca\(^{2+}\) transient within an individual cell. Intercellular Ca\(^{2+}\) waves typically propagate at a velocity of 10–60 μm/s, extending over distances ranging from 50 to 300 μm (Scemes and Giaume 2006; Tian et al. 2006). Assuming average values of 25 μm/s for speed and 150 μm for propagation radius, this gives a time scale of about 6 s, which is significantly less than the period of an individual Ca\(^{2+}\) oscillation (>30 s). Therefore, we assumed that although the shape of the propagating wave may broaden due to its spatial extent,
Table 1. Mass-balance equations of the unified model

<table>
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<tr>
<th>Model</th>
<th>Ref.</th>
<th>Variable</th>
<th>Balance equation</th>
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<tbody>
<tr>
<td>Astrocytic Ca(^{2+}) signalling</td>
<td>(Lavrentovich and Hemkin 2008)</td>
<td>[Ca(^{2+})_{astro}]</td>
<td>[\nu_{\text{Leak,astro}} - \nu_{\text{SERCA}} - \nu_{\text{NCX/PMCA}} + \nu_{\text{Leak,astro}} + \nu_{\text{CICR}}]</td>
</tr>
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<td>Neuronal and astrocytic</td>
<td>(Aubert and Costalat 2005; DePitta et al. 2009; DiNuzzo et al. 2010a)</td>
<td>[IP_{\alpha}]</td>
<td>[\nu_{\text{IP_{\alpha}}} - \nu_{\text{Pump}} + \nu_{\text{Stim}}]</td>
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<tr>
<td>electrical activity (neurotransmission)</td>
<td></td>
<td>[\nu_{\text{Leak,Na}} - 3\nu_{\text{Pump}} + \nu_{\text{Stim}}]</td>
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<td>Astrocyte to neuron</td>
<td>(Nadkarni and Jung 2004)</td>
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<td>feedback currents (gliotransmission)</td>
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<td>Cell functional</td>
<td>(Aubert and Costalat 2005; DiNuzzo et al. 2010a, 2010b)</td>
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<td>metabolism</td>
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<tr>
<td>Nutrient transport</td>
<td>(DiNuzzo et al. 2010a; Mangia et al. 2009; Simpson et al. 2007)</td>
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<td>Hemodynamics</td>
<td>(Aubert and Costalat 2005; Buxton et al. 1998)</td>
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The model altogether consists of the explicit reaction/transport rate equations (with relevant parameter values) corresponding to the terms appearing in the above mass-balance equations. The text describes the sole rate equations that constitute the links between different models. In particular, \(\nu_{\text{Pump}}\) and cerebral blood flow (CBF) appear only in the present model, whereas the novel expressions of \(\nu_{\text{Stim}}\) substituted those of our previous model simulating focal activation. For details about each individual model, see the corresponding references.

The effect of glutamate on astrocytes was modeled using a transfer function similar to that used by Di Garbo et al. (2007) for transmitter-evoked astrocytic Ca\(^{2+}\) response and that by used by DePitta et al. (2009) for glutamate-induced PLC activity. However, since our model does not take into account the glutamate concentration explicitly, we assumed that the extracellular concentration of the neurotransmitter is proportional to the neuronal Na\(^+\) level by recasting presynaptic glutamate release to postsynaptic Na\(^+\) influx [model parameter \(k_{\text{d}} = 0.3\) (mmol/l)/s\(^{-1}\), see below], in agreement with biophysical calculations (Attwell and Laughlin 2001). The lack of quantitative data for the mechanisms underlying the glutamate-induced activation of PLC pathway led us to conform, for simplicity, to the previously proposed (DePitta et al. 2009) Hill binding reaction scheme.
\[ v_{\text{IP3}}(\text{mGluR}) = v_p^{g-c} \frac{1}{1 + (K_s \left[ N_{a}^+ \right])^{-b_h}} \]

where \( v_p \) is the maximum velocity of PLC (Lavrentovich and Hemkin 2008) and the parameters \( K_s = 24 \text{ mmol/l} \) and \( b_h = 1.4 \) were chosen to reproduce the theoretical relationships between glutamate and IP3 levels obtained elsewhere (De Pitta et al. 2009). The term \( c_{\text{cross talk}} \) (which we constrained to the range 0 \( \leq c_{\text{cross talk}} \leq 1 \)) represents the neuron-astrocyte mutual coupling constant. The Na\(^+\) influx into astrocytes after glutamate reuptake was similarly calculated as

\[ v_{\text{sim}} = k_c c_{\text{cross talk}}[\ln \left( \frac{[\text{Ca}^{2+}]_{\text{astrocyte}}}{[\text{Ca}^{2+}]_{\text{resting}}} - 196.69 \right)] \ln \left( \frac{[\text{Ca}^{2+}]_{\text{astrocyte}}}{[\text{Ca}^{2+}]_{\text{resting}}} - 196.69 \right) \]

where \([Na^+]_c\) is the neuronal Na\(^+\) concentration and the rate constant \( k_c = 0.1 \text{ (mmol/l)}^{-1} \text{ s}^{-1} \) was set to obtain a neuronal vs. astrocytic activation ratio of 3:1 (DiNuzzo et al. 2010a).

The Ca\(^{2+}\)-dependent activation of astrocytes was assumed to follow the experimentally derived relation between the astrocytic Ca\(^{2+}\) concentration and the feedback synaptic currents on neurons (Nadkarni and Jung 2004). Specifically, the overall neuronal Na\(^+\) influx due to cross talk was described by the expression

\[ BOLD = CBV_0 OEF_0 \left[ 7 \left( 1 - \frac{dH_b}{dH_b_0} \right) + 2 \left( 1 - \frac{dH_b CBV_0}{dH_b_0 CBV_0} \right) \right] + \frac{2 - \frac{0.2}{OEF_0} \left( 1 - \frac{CBV_0}{CBV_0} \right)}{dH_b_0} \]

where \( CBV_0 \) and \( H_b_0 \) (see Table 1 for the relevant balance equations) are the resting cerebral blood volume and deoxyhemoglobin concentration, \( OEF_0 \) is the basal oxygen extraction fraction, and the constant values are estimated for a magnetic field of 1.5T (see Aubert and Costalat 2002). Finally, the cerebral metabolic rate of oxygen is simply given as

\[ \text{CMRO2} = -\frac{d}{dt}[O_{2,c}] \]

Model simulations were performed using Berkeley Madonna version 8 (Department of Molecular and Cellular Biology, University of California, Berkeley, CA; http://www.berkeleymadonna.com) by semi-implicit fourth-order Runge-Kutta numerical integration (Rosenbrock algorithm for stiff systems).

RESULTS

The basal leakage of Ca\(^{2+}\) into astrocytes during resting conditions (i.e., without external input stimulation) results in the generation of spontaneous Ca\(^{2+}\) oscillations (Fig. 2) with frequency, amplitude, and concentration profiles similar to previous theoretical outcomes (see for example De Pitta et al.
We found that the rise in neuronal excitatory feedback on astrocytes, i.e., in the presence of cross talk, produces increasing frequency of Ca$^{2+}$ pulses in astrocytes, consistent with what has been commonly reported (Aguado et al. 2002; De Pitta et al. 2009; Lavrentovich and Hemkin 2008; Parri et al. 2001).

First, we studied the effect of astrocytic Ca$^{2+}$ signaling on brain metabolism and hemodynamics in the absence of cross talk between astrocytes and neurons (i.e., neglecting gliotransmission) (Fig. 3). The increase in [Ca$_{u}^{2+}$] produces a corresponding rise in CBF (Fig. 3A), CBV (Fig. 3B), and CMRO$_2$ (Fig. 3C). These changes in hemodynamic and metabolic variables affect the concentration of deoxyhemoglobin in the blood (not shown), resulting in BOLD fMRI signal changes of $\sim$1% (Fig. 3D). To obtain quantitative data about the impact of astrocytic Ca$^{2+}$ transients on BOLD oscillations, we varied the contribution of Ca$^{2+}$-dependent regulation of vasculature by astrocytes. The amplitude of an individual BOLD oscillation is a function of the CBF response, which depends on astrocytes via Ca$^{2+}$ signaling. We found that if astrocytic Ca$^{2+}$ contribution to the observed CBF response decreases from 36% to 0%, the relevant changes in the BOLD signal are rapidly and significantly reduced (Fig. 3D). These results indicate that without Ca$^{2+}$-induced gliotransmission, the spontaneous Ca$^{2+}$ dynamics in astrocytes generates detectable BOLD fluctuations provided that CBF regulation is substantially Ca$^{2+}$ dependent.

Figure 4 shows the modeling outcomes when we take into account the excitatory feedback of astrocytes on neurons (i.e., gliotransmission). Note that this means that neuron-to-astrocyte stimulation also changes, because there is mutual cross talk between the two cell types. In these conditions, the changes in Ca$^{2+}$ oscillations produce minor alterations in hemodynamic parameters, thus producing CBF and CBV responses similar to the previous simulations (Fig. 4, A and B; compare with Fig. 3, A and B). However, the variation in CMRO$_2$ in the presence of astrocyte-neuron cross talk is significantly different from that in the absence of gliotransmission (compare Figs. 3C and 4C), reflecting augmented metabolic rate. Notably, the rise in CMRO$_2$ stays elevated independently of the blood flow response. The fact that the relative contribution of the metabolic vs. vascular component increases in the presence of astrocyte-neuron cross talk affects both the shape and the amplitude of the BOLD signal (Fig. 4D). Remarkably, we found that the BOLD response when CBF is not regulated by astrocytic Ca$^{2+}$ (i.e., the large BOLD undershoot in Fig. 4D) accounts for most of the signal. These results indicate that when astrocytes respond to neuronal activity, the Ca$^{2+}$-mediated BOLD oscillations are detectable even if CBF regulation is not Ca$^{2+}$ dependent.

We next examined how the frequency of Ca$^{2+}$-induced BOLD fluctuations is modulated by the neuron-astrocyte cross talk and the level of external input stimulation (Fig. 5). When only cross talk or input is considered, an increase of the corresponding variable is accompanied by an increase in the frequency of BOLD signal oscillations. However, when both external and mutual neuronal/astrocytic stimulation are high, these oscillations slow down until they are abolished over a
given threshold, the latter finding being compatible with the fact that for high astrocytic stimulation, the intracellular Ca\(^{2+}\) oscillations vanish (see Lavrentovich and Hemkin 2008). This suggests that the frequency modulation of slow BOLD fluctuations by external stimulation depends on the concurrent level of astrocytic coupling to the stimulated neurons. It should be noted that in the presence of cross talk, an external input Na\(^+\) influx as low as 0.3 mmol·l\(^{-1}\)·s\(^{-1}\) (corresponding to \(v_{\text{input}} = 1\)) is sufficient to abolish the periodic regime of BOLD oscillations. This is to be compared with the level of Na\(^+\) influx estimated during focal neuronal activity, which is in the order of 0.5 mmol·l\(^{-1}\)·s\(^{-1}\) after habituation establishes but much higher at stimulus onset (Aubert and Costalat 2005; DiNuzzo et al. 2010a), suggesting that a significant neuron-astrocyte cross talk is not compatible with the occurrence of BOLD oscillations during focal brain activity.

We also investigated the consequence of adding noise to the Ca\(^{2+}\) passive leakage from extracellular space into astrocytes (\(j_{\text{Leak, Ca}}\); see Table 1). The fact that the intracellular Ca\(^{2+}\) concentration varies stochastically, besides resembling more physiological conditions, has indeed been suggested to be an important mechanism for both the initiation and the dynamics of the Ca\(^{2+}\) pulses (Aguado et al. 2002; Jung et al. 1998; Lavrentovich and Hemkin 2008; Postnov et al. 2009). We constrained the noise level to be low enough to preserve the causality of the signal (i.e., Ca\(^{2+}\) waves occurring in response to neuronal activity), as previously suggested (Postnov et al. 2009). We found that the introduction of a noise source affects the BOLD signal oscillations in the presence of input but not at rest (Fig. 6). This indicates that the periodic regime of Ca\(^{2+}\)-induced BOLD oscillations quickly subsides when the level of external input stimulation rises.
DISCUSSION

To study the contribution of neuron-astrocyte functional and metabolic partnership to the slow BOLD fMRI signal oscillations, we coupled previous kinetic modeling studies by including the ability of astrocytes to respond to neuronal activity with the generation of intracellular Ca$^{2+}$/H$^{11001}$ waves, which in turn exercise feedback on neurons via gliotransmission. In the context of fMRI, astrocytic Ca$^{2+}$/H$^{11001}$ signaling is all-important, because a substantial component underlying functional hyperemia is mediated by the action of vasoactive compounds released by astrocytes in response to Ca$^{2+}$ (Bennett et al. 2008a, 2008b; Iadecola 2004; Rossi 2006). We found that the energy demand associated with Ca$^{2+}$/H$^{11001}$ signaling and the Ca$^{2+}$/H$^{11001}$-dependent changes in hemodynamic parameters can generate detectable BOLD oscillations. We also found that BOLD changes due to neuron-astrocyte cross talk are associated with a substantial CMRO$_2$ response. Interestingly, periodic variations in cortical oxidative metabolism have been observed in animal studies (Vern et al. 1988). This does not imply that the resting brain activity (i.e., in the absence of stimuli) is purely a vascular/metabolic effect. Indeed, although these oscillations have a clear metabolic component (Fukunaga et al. 2008), it is likely that they support a functional role as well. For example, the intracellular Ca$^{2+}$ oscillations have been correlated with the activity of the multifunctional Ca$^{2+}$/calmodulin-dependent protein kinase (CaM kinase II), an enzyme that regulates the activity of many cellular targets involved in neuronal plasticity, gene expression, and neurotransmission (Dupont and Goldbeter 1998).

We found that the frequency of astrocytic Ca$^{2+}$ oscillations is dependent on the level of stimulation, confirming that astrocytes are accurate detectors of neuronal activity (Schipke and Kettenmann 2004). The bidirectional communication between neurons and astrocytes indeed suggests that astrocytes are involved in the neural integration of information (Agulhon et al. 2008; Lorincz et al. 2009; Perea and Araque 2002; Scemes and Giaume 2006; Schipke and Kettenmann 2004). However, our simulations showed that the oscillatory pattern of Ca$^{2+}$ concentration follows/encodes neuronal activity only at basal and low-stimulation conditions. Interestingly, the presence of neuron-astrocyte excitatory feedback shows that for increasing input level, the frequency of astrocytic Ca$^{2+}$ waves decreases and the fluctuations progressively lose periodicity up to disappearance (see Figs. 4 and 5).

The theoretical dependency of Ca$^{2+}$ wave-induced BOLD fluctuations on the level of activity, which we report in this study, is in agreement with experimental findings obtained in human visual cortex showing that the cyclic spontaneous fMRI activity in the frequency range 0.01–0.05 Hz is largely modulated by behavioral state (i.e., eyes closed vs. eyes open) (Anderson 2008; Bianciardi et al. 2009). We found that the range of frequencies that are effectively modulated by neuron-astrocyte coupling (0.01 to 0.04 Hz, see Fig. 4) match those reported in the primary visual cortex in the absence of input from lateral geniculate nucleus (Bianciardi et al. 2009). Furthermore, we observed that the transition from no input (e.g., eyes closed) to activation (e.g., eyes open, fixation, or visual stimulation) is accompanied by a reduction in the amplitude.

Fig. 6. Effect of noise on the periodicity of Ca$^{2+}$-induced BOLD oscillations. Noise dependence of BOLD signal oscillations during basal and moderately stimulated conditions ($V_{input} = 0$ or $1$, respectively, and with $c_{cross talk} = 1$). At rest, Ca$^{2+}$ pulses (A) are not affected by the presence of noise (B). On the contrary, Ca$^{2+}$ pulses during stimulation (C) are highly noise sensitive (D). In the presence of a noise source, the BOLD oscillations remain strongly time-locked without external stimulation and turn aperiodic with increasing input.
and periodicity of the BOLD oscillations (Fig. 5), consistent with experimental evidence (Anderson 2008; Bianciardi et al. 2009).

The major limitation of this theoretical study is the reliability of the scaling between a cell-based process like intracellular Ca\textsuperscript{2+} waves and the changes of BOLD signal that are measured in voxel-sized volumes. One might ask how a mechanism originating at the level of individual astrocytes can be synchronized to produce macroscopic effects that are meaningful at the voxel scale. This is especially important in the modeling context, since synchronization of oscillations is one of the open problems in computational neuroscience. However, as we pointed out in the Introduction, these kinds of oscillations are likely to be network independent, and as such they possibly cannot be explained as an emergent property of the system. The available experimental evidence indicates that spontaneous Ca\textsuperscript{2+} signals in astrocytes have discrete, nonrandom origin sites and that they occur in the same location with overwhelming recurrence (see Hoogland et al. 2009 and references therein). Moreover, astrocytic Ca\textsuperscript{2+} signaling elicited by physiological stimulation in vivo is restricted to small, short-range networks of responding cells, and it is unlikely that a long-range Ca\textsuperscript{2+} wave synchronization occurs through signaling between individual astrocytes (Agulhon et al. 2008). Accordingly, the periodicity that we report in this study applies to independent clusters of few astrocytes with no link to the synchronization of interconnected neuroglial units. Some experimental evidence suggests that the activation of a few cells can already produce macroscopic effects. For example, the BOLD signal is still measured at a resolution of 110 \times 110 \times 125 \textmu m in rat olfactory bulb in response to the activation of a single glomerulus (Kida et al. 2002). Interestingly the “only” \approx 20 astrocytes associated with one individual olfactory glomerulus (Nawroth et al. 2007) regulate local blood flow via a mGluR-dependent, Ca\textsuperscript{2+}-mediated mechanism (Petzold et al. 2008). Therefore, synchronization would be not necessary to detect the effect of small, independent clusters of activated astrocytes on macroscopic variables. Accordingly, theoretical modeling indicates that only a few astrocytes are sufficient to produce a measurable change in the BOLD signal (Bennett et al. 2008a, 2008b). Further experimental research is needed to establish the vascular (e.g., CBV) and metabolic (e.g., CMRO\textsubscript{2}) contribution of single astrocytes on voxel-sized volumes.

On the basis of the present study and available literature, we can thus hypothesize two different mechanisms for astrocytic Ca\textsuperscript{2+} wave-induced BOLD signal oscillation in the neocortex: a purely intracortical mechanism, in which the Ca\textsuperscript{2+} transients propagate locally through the astrocytic syncytium, and a subcortical mechanism, in which the periodic fluctuations of Ca\textsuperscript{2+} level in cortical astrocytes result from remote brain structures. Although both mechanisms could be operational at the same time, the above-mentioned arguments suggest that the contribution of astrocytic Ca\textsuperscript{2+} waves to the resting-state synchronization that is measured by functional connectivity BOLD fMRI experiments might have a subcortical (e.g., thalamic) origin. A role for cyclic activity of the thalamus in maintaining the basal excitatory tone of the cortex was previously suggested (Lorincz et al. 2009), consistent with the idea that thalamic nuclei could modulate the stand-by excitation of cortical areas based on the input received (Feige et al. 2005; Goldman et al. 2002). Our results predict that the periodic activation of neurons and astrocytes underlying astrocytic Ca\textsuperscript{2+} waves should vanish for increasing stimulation level. This would configure either a reduction of intrinsic spontaneous excitation during the processing of incoming external input stimuli or an enhancement of slow oscillatory patterns during resting conditions (i.e., in the absence of “disturbing” focal stimulation) due to, for example, long-term information management.

It should be noted that the present model does not include several astrocytic Ca\textsuperscript{2+}-mediated mechanisms acting on neuronal excitability. In particular, glutamatergic and purinergic neurotransmission can be affected by a number of paracrine interactions mediated by molecules such as ATP, cyclic ADP-ribose, D-serine, and homocysteic acid, all neuroactive compounds that are released by astrocytes after intracellular Ca\textsuperscript{2+} signal or participate in the propagation of Ca\textsuperscript{2+} waves through the astrocytic syncytium (Baranano et al. 2001; Perea and Araque 2002; Schipke and Kettenmann 2004). Because these signals can regulate synaptic transmission “at distance,” they have the potential to modulate the frequency and the synchronization of the slow BOLD oscillations across the volume of interest in a manner that cannot be predicted by the present theoretical study. Moreover, it is likely that more than a unique resting state exists depending on the specific brain area and behavioral state, possibly with different dependencies on the level of stimulation. Further refinement of theoretical modeling might help in characterizing the modulation of amplitude and frequency of Ca\textsuperscript{2+}-induced BOLD oscillations, which notwithstanding requires the experimental estimation of the number and size of astrocytic networks involved in a Ca\textsuperscript{2+} wave. In conclusion, our modeling outcomes suggest that the metabolic and vascular effects induced by increased Ca\textsuperscript{2+} concentration in astrocytes might at least concur to shape the complex pattern of slow BOLD fMRI signal changes.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.D.N. and F.G. conception and design of research; M.D.N. performed experiments; M.D.N. analyzed data; M.D.N., T.G., and F.G. interpreted results of experiments; M.D.N. prepared figures; M.D.N. drafted manuscript; M.D.N., T.G., B.M., and F.G. edited and revised manuscript; M.D.N., T.G., B.M., and F.G. approved final version of manuscript.

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