BOLD and spiking activity

To the editor:

Viswanathan and Freeman[1] claim that oxygen concentration and, by inference, blood oxygen level–dependent (BOLD) functional magnetic resonance imaging (fMRI) reflect synaptic activity more than spiking activity. As this is a fundamental and controversial issue in fMRI research, this claim, if incorrect, may erroneously bias the interpretation of a large body of data.

The authors simultaneously recorded multi-unit activity (MUA), local field potentials (LFP) and tissue oxygen concentration in primary visual cortex of anesthetized cats stimulated with moving gratings. During high temporal-frequency stimulation, when thalamic inputs were active, but few cortical neurons responded, oxygen signals were observed without MUA. Therefore, the authors concluded that oxygen responses reflect synaptic inputs more than spiking. However, careful inspection of their results leads to the opposite conclusion and supports a tight coupling between oxygen signals and local cortical spiking.

Tissue oxygen responses show an initial decrease that is attributed to local oxygen consumption (negative peak) and a delayed increase that is attributed to more global changes in blood flow (positive peak). The authors showed that the initial negative peak was greater than zero during high-frequency stimulation (when spiking activity was absent), but it was in fact 80–90% smaller to high-frequency stimulation than to low-frequency stimulation (calculated from ref. 1, see red arrow in **Fig. 1**). Given that roughly the same thalamic input is expected on stimulation of either temporal frequency[2], we conclude that the initial negative oxygen response depended only slightly (10–20%) on thalamocortical synaptic activity and mostly (80–90%) on cortical spiking.

What about the more widespread delayed positive oxygen response? This component was evident during stimulation at high frequencies, but only in one of their two experiments that used large stimuli. A previous study demonstrated that delayed positive oxygen responses were associated with spiking outside the field of view of the electrode when using such large stimuli[3]. There is, therefore, a mismatch between the spatial extents of MUA (which involves the neurons closest to the electrode tip) and positive oxygen responses (which reflect a much larger neuronal population), making the comparison between the two measurements difficult to interpret. MUA measurements may also suffer from a sampling bias by failing to record spiking activity in particular types of neurons (small neurons or specific cortical layers). For example, neurons in layer 4 and adjacent area 18 that respond to higher frequencies[4,5] may have contributed to the residual LFP and delayed oxygen responses while being invisible to the MUA electrode.

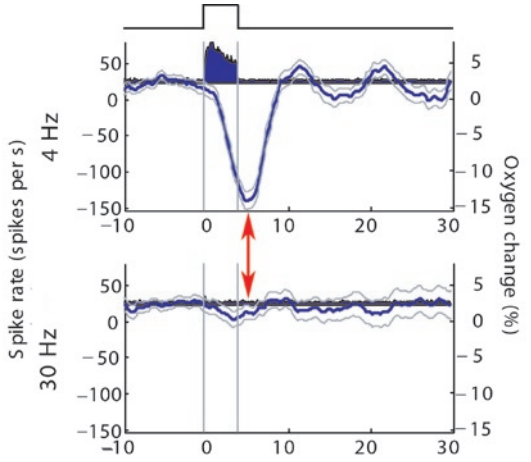

Figure 1 MUA and oxygen responses for low (top) and high (bottom) temporal frequencies (reprinted from Viswanathan and Freeman[1]).

The problem of deducing the population's state from a few recording sites is a general methodological concern in any attempt to compare spiking activity with LFP and vascular responses[6]. Spiking not detected by the electrode may be reflected in LFP and vascular responses, which sum activity over a larger population. An ostensible mismatch between the measured spiking activity and LFP or vascular responses may be a result of these biases even when the spiking, LFP and BOLD are well correlated[7].

We do not mean to suggest that vascular responses are driven directly by spiking, as if blood vessels are voltage sensitive. Indeed vascular responses are likely to be of synaptic origin[8]. In contrast to subcortical structures, however, cortical circuits are dominated by massive local connectivity in which most synaptic inputs originate from nearby neurons[9] and only a small minority of inputs originate from distant sites such as the thalamus. Thus, synaptic 'inputs' in cerebral cortex are mostly produced by local spiking of neighboring neurons, leading invariably to a tight coupling between synaptic and spiking activity, as well as oxygen responses[10].

The difficulty of Viswanathan and Freeman[1] in decoupling synaptic from spiking activity in the cortex is not surprising. The authors implicitly assumed a feedforward model of cortical processing, which is inaccurate. Whatever the mechanisms of neurovascular coupling are, the extent of decoupling between synaptic and spiking activity ultimately depends on the nature of cortical processing; that is, whether the cortical dynamics can be switched from a local recurrent mode to a strictly feedforward mode in which synaptic inputs to a cortical area and the targets of its spiking outputs are segregated. The Viswanathan and Freeman[1] study was designed to reveal such decoupling, but the results of their experiments argue against such segregation by showing that 80–90% of the local vascular response is coupled to local spiking activity.

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Viswanathan and Freeman reply:

The criticisms of Nir and colleagues are founded largely on pre-existing bias and a misleading interpretation of our results. Their primary argument concerns the decreased amplitude of tissue-oxygen responses to high compared with low temporal-frequency stimuli and is based on their estimated measurements from a single site[1]. However, tissue-oxygen responses show a great deal of variability between sites. For 10 of our 13 large stimulus sites, the initial dip was significant (*t*-test, $P < 0.05$; 9 sites with $P < 0.0005$). For both large and small stimuli (**Fig. 1**), the positive peak amplitudes for low versus high temporal frequencies remained unchanged. Large stimuli showed a significant (*t*-test, $P < 0.0005$) change in initial dip amplitude, but this difference was only weakly significant for small stimuli (*t*-test, $P < 0.05$).

Nir and colleagues then use their estimations to suggest relative contributions of spiking and synaptic activity to the tissue-oxygen response. However, it is absurd to insinuate a 1:1 relationship between the amounts of synaptic and spiking activity and their comparative effects on tissue oxygen. The relationship between multi-unit activity (MUA) and the tissue-oxygen initial dip is nonlinear[2]. They also misinterpret previous work from our laboratory[3]. This study did not include local field potential measurements, and therefore, does not preclude synaptic activity from eliciting the observed responses.

Our stimuli had 100% contrast. MUA responses to high- versus low-contrast stimuli are attenuated at high temporal frequencies[4] in both lateral geniculate nucleus (LGN) and striate cortex, suggesting subcortical mechanisms[5]. Analysis of LGN cells ($n = 113$) showed higher spike responses to low (2.5 Hz) versus high (38 Hz) temporal-frequency stimuli at high (45%) contrast (**Fig. 2**). Average spike rates for 2.5 Hz and 38 Hz were significantly different at 17.1 and 1.4 spikes per s, respectively (*t*-test, $P < 0.0005$). This decrease in MUA could lead to proportional decreases in thalamocortical synaptic activity, causing a decreased tissue-oxygen response at 30 Hz. As this occurs at the first stage of feedforward processing, the effects of subsequent intracortical modulation are irrelevant.

Nir and colleagues contend that MUA responses at 30 Hz could remain undetected. Of 316 area 17 cells that we have studied[6], none showed spiking responses to frequencies ≥20 Hz. Moreover, none of our sites displayed a transient increase (500 ms following stimulus onset) in MUA at 30 Hz (≥1.25 standard deviations of spontaneous firing rate). The high significance of our tissue-oxygen responses at 30 Hz ($P < 0.0005$) also suggests that they are driven by measured neural responses. Sites with ≤5 spikes per s rarely produce significant tissue-oxygen responses. It is also unlikely that area 18 is involved because it prefers low spatial frequencies[7]. Our smaller stimuli had high spatial frequencies.

It is established that, under selected conditions, the BOLD signal correlates well with average spike rate[8]. Indeed, simple sensory stimulation is ideal for examining proportional increases in local field potentials and MUA and their relation to the BOLD response. This distinction between spiking and synaptic activity is most crucial in awake preparations that are used to examine higher cognitive functions. Neuromodulation inherent to cognitive states such as attention depends on neurotransmitters, whose release into the extracellular space is not spatially specific[9,10]. This affects the balance between spiking and synaptic activity, potentially dissociating the two. Our basic stimulus procedure suggests that the BOLD signal is unlikely to reveal average spiking activity under more complex conditions.

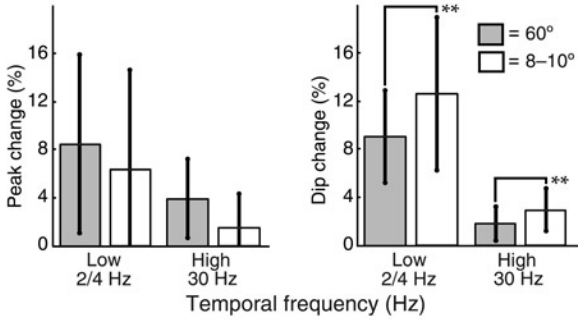

Figure 1 Comparison of tissue oxygen–signal amplitudes. For both large (60°, gray bars) and small (8–10°, white bars) stimuli, positive peak and initial dip signal amplitudes are shown in percent change from the 10-s prestimulus baseline. Responses are shown to low (2 or 4 Hz, based on peak MUA tuning) and high (30 Hz) temporal-frequency stimuli. Error bars denote s.d. ** significant difference (*t*-test, $P < 0.05$).

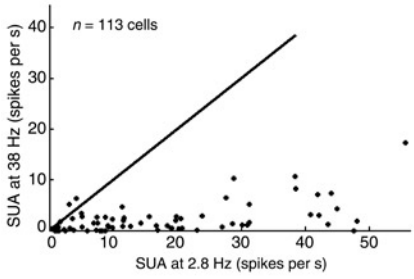

Figure 2 Scatter plot of single-unit spiking activity (SUA) in response to low (2.5 Hz) and high (38 Hz) temporal-frequency stimuli at 45% contrast. Each point represents a single LGN cell. Spatial frequencies are based on peak SUA tuning, and stimulus size equals receptive field size. The identity line (solid black) depicts the points at which the spike responses to both stimulus types are equal.

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