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From neuron to BOLD: new connections

Peter A. Bandettini and Leslie G. Ungerleider

A recent paper in *Nature* directly compares fMRI with simultaneously recorded neural activity in the monkey, yielding new insights into the interpretation of BOLD contrast.

Only ten years ago, the first papers appeared on functional magnetic resonance imaging (fMRI) using blood oxygenation level dependent (BOLD) contrast. The explosive world-wide growth of fMRI as a tool for non-invasively visualizing dynamic, localized processes in the human brain that followed reflects the promise that the technique will open up new avenues of research in cognitive neuroscience. The physical basis of BOLD contrast is oxygenation-dependent magnetic susceptibility of hemoglobin. Deoxyhemoglobin is paramagnetic, causing slightly attenuated signal intensity in MRI image voxels containing deoxygenated blood. During brain activation, localized increases in blood flow increase blood oxygenation and consequently reduce deoxyhemoglobin, causing the MRI signal to increase. It is therefore assumed that these localized increases in BOLD contrast reflect increases in neuronal activity.

Two primary questions remain about the interpretation of fMRI signals: the quantitative relationship between neural activity and BOLD contrast, and the biological mechanism underlying this relationship. The MRI signal in activated regions begins to increase approximately 2 seconds after neural activity begins, and plateaus in the 'on' state after about 7 to 10 seconds, remaining elevated while the activity continues. When activity ends, the signal returns to baseline after about 8 to 11 seconds. Transient signal changes are also described, including a 'pre-undershoot' (reduced BOLD signal within the first two seconds of activity) and a more commonly observed 'post-undershoot' (reduced signal for 10 to 40 seconds after activity ends). Despite extensive modeling, the biological basis and heterogeneity of BOLD signal dynamics and magnitude remain unclear, primarily because they reflect the interplay of many uncharacterized variables, including neural activity, metabolism, blood volume, blood flow and subsequent oxygenation changes. The most significant steps toward understanding the relationship between neural activity and BOLD contrast have come from direct, simultaneous and spatially registered measurement of these variables. Several papers have shown an essentially linear relationship between non-simultaneous measures of neuronal activity and hemodynamic changes in monkeys[1], and simultaneous measures in rats[2–4], but the recent paper in *Nature* by Logothetis *et al.*[5] should be considered a landmark because it is the most comprehensive, detailed and definitive set of comparisons yet made.

Logothetis *et al.* examined how well the BOLD signal correlated with neuronal activity simultaneously recorded within monkey primary visual cortex. Stimulus-driven unit activity and the local field potential (LFP) were recorded through a microelectrode while the anesthetized monkey was visually stimulated with a rotating checkerboard pattern. Unit activity represents the firing, or action potentials, of single or multiple neurons recorded near the electrode tip (within about 100 μm for single units and 200 μm for multi-units). In contrast to this fast 'spiking' activity, which represents the transmitted output of one or a few neurons, the LFP is a relatively slow oscillatory electrical wave, resembling an EEG recorded from the scalp. However, the spatial resolution of the EEG is very coarse, whereas the LFP reflects aggregate activity from a population of neurons located within a few millimeters of the electrode tip. This activity is thought to be a weighted sum of the membrane potentials generated from the population, with neurons closer to the electrode tip making the greatest contribution. Although changes in membrane potentials (both excitatory and inhibitory) mainly reflect synaptic activity localized to dendrites and soma, action potentials within the neuronal population may also contribute to the LFP.

Logothetis *et al.* distinguished unit activity from the LFP by filtering the broad band of activity into high-frequency and low-frequency components: 300–1500 Hz for units and 40–130 Hz (the so-called gamma range) for the LFP. They then correlated the time courses of activity and BOLD responses. (Because single-unit activity and multi-unit activity were highly correlated, the main comparisons were correlations between BOLD and multi-unit activity versus BOLD and LFP.) Both LFP and multi-unit activity correlated with the BOLD response, but the better predictor was the LFP. This was probably because about one quarter of the multi-unit responses were transient, return-

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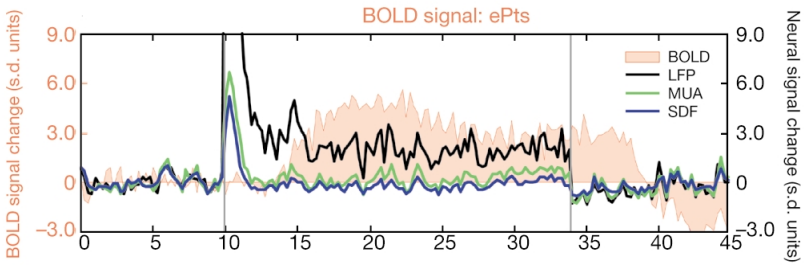

Fig. 1. Simultaneous neural and hemodynamic recordings from a cortical site showing transient neural response to a pulse stimulus of 24 seconds. Both single- and multi-unit responses adapt a couple of seconds after stimulus onset, with LFP remaining the only signal correlated with the BOLD response. SDF, spike-density function (see text); ePts, electrode ROI—positive time series. Reprinted by permission from *Nature* **412**, 150–157, copyright 2001 Macmillan Magazines Ltd.

ing to baseline levels within a few seconds of visual stimulation. By contrast, LFP responses, like BOLD, remained elevated for the duration of visual stimulation, and hence were more consistently coupled with the BOLD response (**Fig. 1**). Further, a more formal analysis of the estimated BOLD response, as predicted by both LFP and multi-unit activity, indicated that LFP gave a significantly better estimate. This is probably because synaptic activity consumes more energy, an important determinant of the BOLD response magnitude, than does the transmission of action potentials. So, the good news is that BOLD is related to neuronal activity. The bad news is that apparently one cannot assume a close relationship between BOLD and the kind of signals (single- and multi-unit activity) typically recorded through microelectrodes in physiological studies.

The LFP time courses in the Logothetis *et al.* paper also clarify a previously unresolved observation regarding the dynamic 'nonlinearity' of BOLD contrast. Boynton *et al.*[6] first demonstrated that with brief (< 3 second) stimuli, the magnitude of the BOLD signal was larger than expected from a linear system, assuming that the neuronal input was constant for all stimulus durations. Since then, several papers have demonstrated this same effect (most recently, ref. 7; **Fig. 2**). A central question in the interpretation of dynamic BOLD contrast has been what causes these 'nonlinearities'; are there nonlinearities in hemodynamics or neuronal input? Mathematical models of hemodynamic changes show that such nonlinear BOLD signal behavior is possible[8] without invoking nonlinearities caused by transiently high neuronal activity in the first three seconds. On the other hand, Boynton *et al.* suggested that the source of the observed BOLD nonlinearities could be transiently high neuronal activity, but evidence for this explanation was lacking. The neuronal activity necessary to create the hemodynamic changes observed with increasing stimulus duration has been estimated (**Fig. 2**, bottom), assuming a linear relationship between neural activity and measured BOLD signal. The hemodynamically derived high neural activity during the first three seconds is strikingly similar to the LFP recordings of Logothetis *et al.*[5] The degree of resemblance between these direct physiological recordings and Fig. 2 provides strong support for the idea that these hemodynamic 'nonlinearities' are primarily due to transiently high neuronal activity during the first three seconds of stimulation. The BOLD signal for brief responses thus seems to be more faithful to the underlying neural activity than might have been expected.

Other discrepancies remain, however. Several fMRI studies have reported BOLD increases that are larger than would have been expected from single-unit recordings in awake, behaving monkeys. Two examples, in which the imaging and physiological tasks are very similar, come immediately to mind. In studies of spatial attention, covertly directing attention to a particular location and waiting for a target to appear there increases the BOLD response in human extrastriate visual areas V2 and V4 about 35–50% as much as target presentation[9], an order of magnitude larger than the increase in single-unit activity measured in homologous visual areas during a similar expectation period[10].

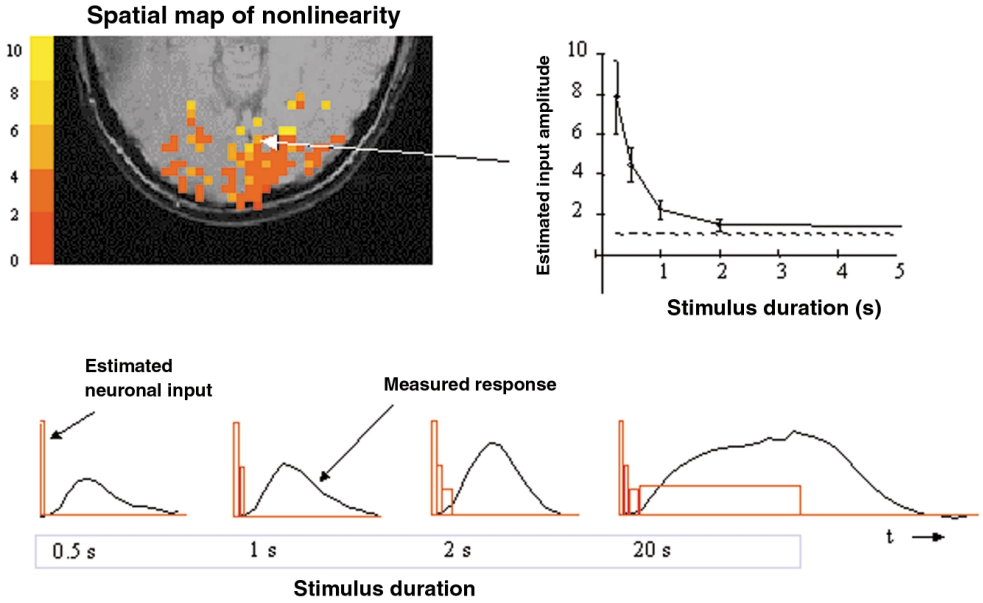

Fig. 2. The BOLD response has larger amplitudes than expected from a linear response to a constant input across stimulus durations. Top, spatial variation of the nonlinearity. The amplitude of the fMRI response relative to a linear response with constant neuronal input is shown for one voxel. Bottom, estimated neuronal activity (red), derived completely from the measured BOLD responses, with the assumption of a direct linear relationship between neuronal activity and BOLD contrast for different stimulus durations, shows a striking resemblance to the time course of LFP in Logothetis *et al.*[5] Adapted from ref. 7.

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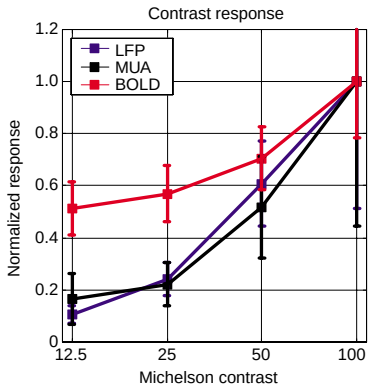

Fig. 3. Normalized response amplitude of LFP, multi-unit activity and BOLD against contrast. Data from five sessions with a pulse duration of 12.5 seconds. Reprinted by permission from *Nature* **412**, 150–157, copyright 2001 Macmillan Magazines Ltd.

Similarly, in binocular rivalry experiments, in which disparate visual stimuli are presented simultaneously to the two eyes and the subject experiences an alternating percept of one and then the other, BOLD signal modulation in human visual cortex (V1, V2 and V4) evoked during the rivalry condition is about half as strong as the response to physically alternating the two visual stimuli[11], or twice as large as the modulation measured in single units in homologous monkey visual areas[12]. The most obvious explanation for these mismatches between BOLD and single-unit activity would be that BOLD is driven by the LFP rather than unit activity and, in such cases, the LFP reflects mainly subthreshold processes that fail to drive the units.

There is, however, an alternative explanation, which relates to the experiments by Logothetis *et al.* on stimulus contrast. The LFP, multi-unit activity and BOLD were all found to decrease as a function of reduced stimulus contrast, although at differing rates. Importantly, at low levels of contrast (12.5%), the BOLD response is about 50% of its maximum amplitude, whereas the LFP and multi-unit activity both drop to about 10–15% of their maximum amplitude (**Fig. 3**). These findings suggest that at low levels of neuronal activity, the LFP is not necessarily a better predictor of BOLD than multi-unit activity, and BOLD signal changes will be overestimated relative to both the LFP and multi-unit activity. This is exactly the situation in the spatial attention studies described above, where there was a very large BOLD response but the neuronal activity measured during the expectation period consisted of small, though significant, increases in baseline, spontaneous unit firing. Although it seems that at higher levels of neuronal activity, mismatches between BOLD and unit activity become less of a problem, they may still come into play and explain, at least in part, the greater-than-expected BOLD response in the binocular rivalry experiment as well. Thus, until we are able to characterize the precise relationship between BOLD and neuronal activity at all levels of activation, or better yet, understand the neural vascular coupling mechanism governing this relationship, interpretation of BOLD signal in this context remains limited.

Overall, the work of Logothetis *et al.*[5] is outstanding not only for the depth and sophistication with which it addresses some pressing questions about BOLD contrast, but also for the clarity with which it brings unanswered questions to bear. It may be prudent, however, to make a few cautionary remarks in closing. First, the results were obtained in anesthetized monkeys, and therefore it will be important to determine how well the results hold up in the awake, behaving animal. Second, although the LFP was a significantly better predictor of the BOLD response than multi-unit activity, the difference in predictability was not large. On average, the LFP accounted for only 7.6% more of the variance in the BOLD response compared to multi-unit activity. Third, it may be an overstatement to conclude, as the authors did, that because the LFP correlated more closely than multi-unit activity with the BOLD response, BOLD signals "reflect the input and intracortical processing of a given area rather than its spiking output". As mentioned earlier, although the LFP reflects mainly summated dendritic and somatic currents arising from synaptic activity, action potentials can also contribute to the LFP, depending on their phase and decay time and the distance of the spiking neurons from the electrode tip. Therefore, the LFP reflects more than just input and intracortical processes. Conversely, even though multi-unit activity is, by definition, spike activity, it may not, in the strictest sense, reflect the output of an area. Indeed, it has been estimated that about 80% of cortical axons terminate intrinsically on local neurons rather than on distant neurons in other cortical regions[13]. Thus, both the LFP and multi-unit activity likely reflect mainly the activity in local cortical circuits. Finally, it may be valuable to consider that the better coupling of LFP, compared to multi-unit activity, with the BOLD signal reflects a difference not between LFP and multi-unit activity *per se* but between gamma frequency oscillations (the frequency band examined in the LFP) and other frequency components. If, as suggested by others, the gamma frequency band holds special importance in both perceptual binding and attention[14,15], then one might predict a good correlation between the gamma component of multi-unit activity and BOLD. Indeed, it has been proposed that rhythmic firing of neurons in this frequency band is metabolically expensive. These comments notwithstanding, Logothetis *et al.* have made a significant step in elucidating the neural origins of the BOLD signal. Studies to come will build on the seminal findings reported in this paper.

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