Spikes versus BOLD: what does neuroimaging tell us about neuronal activity?

David J. Heeger, Alex C. Huk, Wilson S. Geisler and Duane G. Albrecht

By demonstrating that fMRI responses in human MT+ increase linearly with motion coherence and comparing these responses with slopes of single-neuron firing rates in monkey MT, a new paper provides the best evidence so far that fMRI responses are proportional to firing rates.

Functional magnetic resonance imaging (fMRI) is a noninvasive technique for measuring changes in cerebral blood flow and oxygenation that reflect the underlying neuronal activity. This new technology offers a powerful method for exploring the neuronal basis of human cognition, perception, and behavior, but its ultimate success will depend to a large extent on the relationship between the fMRI signal (the blood-oxygenation level dependent or BOLD signal) and the underlying spiking activity of neurons. Despite the many fMRI papers appearing in top journals, we still have only a rudimentary understanding of this relationship. Although it is known that the fMRI signal is triggered by oxygen depletion due to the metabolic demands of increased neuronal activity, the details of this process are only partially understood[1–3]. In this issue of *Nature Neuroscience*, Rees *et al.*[4] have taken a major step forward in establishing a quantitative link between neuronal responses and fMRI signals; their findings offer the most compelling support to date for the hypothesis that fMRI responses are directly proportional to average neuronal firing rates.

In a typical fMRI experiment, one collects a time-series of images while a stimulus or cognitive task is systematically varied. If the stimulus or task variations evoke a large enough change in metabolic demand in a certain brain region, then the image intensity in that region will modulate over time about its mean intensity value. From these changes, which are typically less than 5%, researchers hope to infer something about the changes in underlying neuronal activity.

The vascular source of the fMRI signal places important limits on the usefulness of the technique. At best, the fMRI signal (at each image position and temporal frame) would be proportional to the local firing rate, averaged over a small region of cortex and a short period of time. If that were true, then it would allow us to make direct inferences about firing rates from fMRI data. There is some indirect empirical support for a proportional relationship between average firing rates of neurons and the fMRI signal[5], and two recent studies have demonstrated a qualitative match between fMRI measurements in human visual cortex and multi-unit electrode recordings in homologous areas of the macaque monkey cortex[6,7]. However, the fMRI signal might reflect not only the firing rates of the local neuronal population but also subthreshold activity, for example, simultaneous excitation and inhibition that would not result in an action potential but which would nevertheless deplete blood oxygen. If that were true, the interpretation of fMRI data would be confounded.

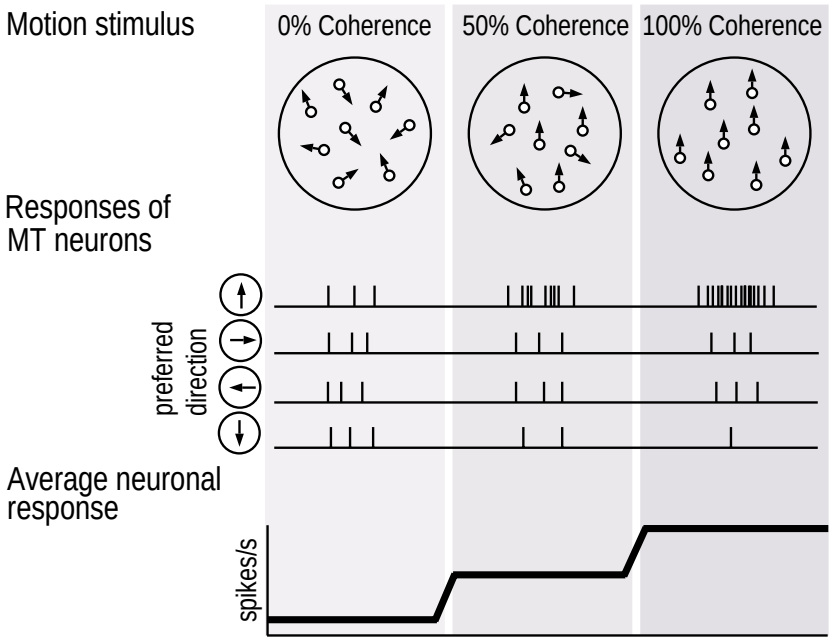

Fig. 1. Average firing rate in monkey MT increases linearly with motion coherence. Top, coherent motion stimuli. Middle, responses of four hypothetical MT neurons. At 0% coherence (column 1), all 4 neurons respond with the same baseline firing rate. At 50% and 100% coherence (columns 2 and 3), the MT neuron that prefers upward motion increases its firing rate, the responses of neurons with rightward and leftward preferred directions do not change their firing rates appreciably, and the neuron that prefers downward motion decreases its firing rate slightly. Bottom, average neuronal activity increases linearly with motion coherence.

David Heeger and Alex Huk are at the Department of Psychology, Stanford University, Stanford, California 94305, USA. Wilson Geisler and Duane Albrecht are at the Department of Psychology, University of Texas, Austin, Texas 78712, USA.
e-mail: heeger@stanford.edu

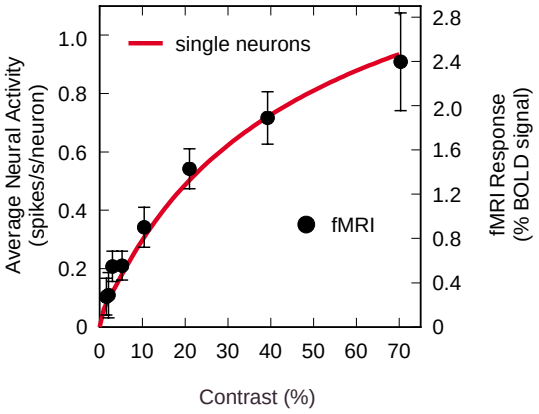

Fig. 2. fMRI responses in human V1 are proportional to average firing rates in monkey V1. Data points, human V1 activity as a function of stimulus contrast, averaged across subjects and averaged across two different stimulus patterns (adapted from ref. 10). Error bars, ± 1 s.e.m. Red curve, average firing rate in monkey V1, as a function of contrast, estimated from a large database of microelectrode recordings (333 neurons)[11]. The ordinates were scaled to obtain the best match between the fMRI responses and the average firing rates. The average firing rates were determined in the following fashion. Responses as a function of contrast were measured using drifting sine wave gratings of the optimal spatiotemporal frequency, orientation and direction of motion. The responses for each neuron were fitted with a descriptive function, and the smooth curves were summed across the population. After subtracting the total maintained activity and dividing by the number of neurons, the average response was then scaled, based upon the measured distributions of selectivity of V1 neurons for spatial frequency, temporal frequency, orientation and direction of motion[11]. The average response for the contrast-reversing plaid patterns used in ref. 10 was determined by adjusting for the differences in spatiotemporal contrast between sine waves and contrast-reversing plaids; this adjustment contributed another scaling factor, but had almost no effect on the shape of the average contrast response. The average maximum response of V1 neurons in our sample for optimal drifting sine waves is approximately 45 spikes per second. The relatively low average response rates in Fig. 2 are the result of the high degree of selectivity of V1 neurons. A contrast-reversing plaid pattern of a given spatial frequency and orientation produces a response in only a small minority of V1 neurons. Therefore, given that most of the cells are not responding, the average spike rate per neuron for the population as a whole is rather low.

Rees and colleagues measured fMRI responses in human MT+ (the MT complex, also known as V5), a motion-responsive cortical region that is widely thought to be homologous to monkey cortical areas MT (also known as V5) and MST. Neurons in macaque MT are direction selective; they increase their firing rates when a stimulus moves in a certain 'preferred' direction. Their responses are suppressed when the same stimulus moves opposite to the preferred direction. Motion in directions orthogonal to this axis has little effect on the firing rate. Across the population of neurons in MT, different neurons have different preferred directions, so that different directions of motion stimulate different subpopulations of neurons.

MT neurons are also sensitive to the strength, or coherence, of the motion signal. Using a stimulus that consists of a field of moving dots, motion coherence can be varied parametrically from 0% (each dot moves in a random direction) to 100% (all dots move in the same direction). A motion coherence of 50% means that 50% of the dots move in the same direction while the other 50% move in random directions (Fig. 1, top).

The responses of MT neurons to such stochastic motion stimuli have been extensively characterized[8,9]. Figure 1 (middle) shows the responses of four hypothetical MT neurons, each with a different preferred motion direction, as a function of motion coherence in the upward direction. Across the full range of motion coherences (from 0% to 100% coherence), the responses of the upward-preferring neuron increase linearly with motion coherence, the responses of the rightward- and leftward-preferring neurons do not change appreciably, and the responses of the downward-preferring neuron decrease linearly. Crucially, the slope with which the upward neuron's responses increase is steeper than the slope with which the downward neuron's responses decrease. Because of this asymmetry, the average neuronal response across these four hypothetical neurons increases linearly with coherence (Fig. 1, bottom).

Rees *et al.* reasoned that if the fMRI signal were proportional to average firing rate, then it too would increase linearly with motion coherence. They then measured fMRI responses in human MT+ across a range of motion coherences and confirmed that the fMRI responses increased linearly with motion coherence. Technically, they compared a linear fit to the data with higher-order polynomial fits and found that whereas the linear fit accounted for a statistically significant proportion of the variance in the data, the higher-order polynomials did not fit significantly better. The reader might be slightly disappointed, however, that the graphs in Figs. 4 and 5 of their paper show only the best-fit 3rd-order polynomials, not the actual measurements of cortical activity.

Because of the simple proportional relationship observed, Rees *et al.* were able to compare the slopes of the average single neuron's firing rates with their fMRI data. Assuming that monkey MT and human MT+ are truly homologous and that firing rates are similar in the two species, they estimated that a change in the fMRI signal of 1% corresponds to a change in average firing rate of 9 spikes/second per neuron.

Motivated by this report, we performed a similar analysis on some of our previously reported measurements of the activity in human and monkey primary visual cortex (V1). In our fMRI experiments, we measured human V1 activity as a function of stimulus contrast for a particular stimulus pattern[10]. In our electrophysiological experiments, we measured the responses of macaque V1 neurons to stimuli of varying contrasts, orientations, directions and spatiotemporal frequencies[11]. From this large database of individual V1 neurons, we estimated the average firing rate in monkey V1, as a function of contrast, for the specific stimulus pattern that had been used in the fMRI experiments.

Our results, like those of Rees *et al.*, imply a proportional relationship between fMRI response and average firing rate. The red curve in Fig. 2 represents the average neuronal activity (left ordinate), and the data points represent the fMRI measurements (right ordinate). As is evident in the figure, the two data sets are strikingly similar; both increase steeply at low contrasts and then saturate (level off) at high contrasts. Assuming again that humans and monkeys show comparable firing rates in V1, we estimate the constant of proportionality to be approximately 0.4 spikes/second per neuron for each 1% change in the fMRI signal (see legend). Thus, in two different cortical areas (V1 and MT), using different stimulus conditions, different behavioral protocols and different data analysis methods, both we and Rees *et al.* found that fMRI responses are proportional to average firing rates (although with different proportionality constants, 0.4 versus 9).

There are many possible explanations for the discrepancy between the two estimates of the proportionality constant, including differences in the methodology as well as actual differences in the physiology between the two cortical areas. It is not likely that the differences in the fMRI signals are due to differences in the neuronal firing rates. The response ranges of the cortical neurons in the two areas are quite similar: the average maximum firing rates are 40 spikes/second for MT[8] and 45 spikes/second for V1[11], and the strongest stimuli used in the two studies (100% coherence and 100% contrast respectively) would be expected to evoke these maximum rates. The difference in the proportionality constant may be related to the fact that the ranges of the fMRI signal measured in the two laboratories differ by a factor of ten: 0.25 % in MT+ for 100% coherence and 2.4% in V1 for 70% contrast. Regardless of what the final explanation may turn out to be, the important finding that the fMRI signal and the neuronal spike rate appear to be linearly related.

To fully understand why the fMRI signal is proportional to average firing rate will require a great deal of further research, but the explanation might be based on the following observations. First, it is reasonable to assume that the cerebral blood flow response is linked to metabolic demand. Second, synaptic activity is responsible for nearly all of the energy metabolism in the cortex, both for neurotransmitter recycling[1] and for repolarizing the dendritic membranes[12]. Third, synaptic activity is of course highly correlated with the firing rates of the presynaptic neurons. Finally, the majority of synapses (both excitatory and inhibitory) can be traced to a local network of connections originating in the nearby cortical neighborhood, leaving only a small minority of inputs from more remote cortical and subcortical structures[13,14]. Taken together, these observations imply that there should be a functional relationship between average firing rates and fMRI responses. The results reported by Rees *et al*., as well as our own results (Fig. 2), suggest that this relationship is linear: an increase in average firing rate causes a proportional increase in local synaptic activity, which causes a proportional increase in metabolic demand, which causes a proportional change in the vascular response.

One might think that synaptic inhibition would complicate matters because, for example, the energy required to recycle inhibitory neurotransmitter might be canceled out by the decreased activity in the inhibited postsynaptic cells. We would argue, however, that this is unlikely for several reasons. First, we have observed a qualitative match between fMRI measurements and average firing rates even under conditions that seem to involve strong inhibitory interactions[6]. Second, because excitatory and inhibitory neurons are tightly interconnected in the local cortical circuits, it is unlikely that one could observe a large increase in inhibition without a concomitant increase in excitation. After all, for an inhibitory neuron to increase its firing rate, it must be receiving more excitatory input, and most of the excitatory input comes from the local cortical neighborhood[13,14]. Hence, excitation and inhibition are likely to be balanced so that the firing rates of excitatory and inhibitory neurons will increase and decrease in unison[15]. To what extent these assumptions hold true for other brain regions is an open question, and it will be important to find out whether the linear relationship between spike rates and fMRI signals applies to subcortical structures.

Functional MRI offers an unprecedented opportunity to study the neuronal underpinings of human behavior. (There is something particularly captivating about coming into the lab each day to literally watch your own brain at work.) The full promise of this technique will not be realized, however, unless fMRI measurements can be specifically related to conventional electrophysiological measurements of neuronal activity. Rees *et al*. have taken a major step forward in bridging this gap, allowing neuroscientists to move beyond colored spots on pictures of brains toward quantitative measurements of neuronal activity in meaningful units of spikes/second per neuron.

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Growth of the NMDA receptor industrial complex

Morgan Sheng and Sang Hyoung Lee

Grant ***et. al.*** use mass spectroscopy to identify new components of the NMDA receptor-associated protein complex, revealing a more complex postsynaptic organization than previously thought.

The NMDA subtype of glutamate receptor is critical in excitatory synaptic transmission and activity-dependent synaptic plasticity. Neurobiologists have been trying to unravel the molecular mechanisms of NMDA receptor signaling for many years, most recently by analyzing the specific intracellular signaling proteins that are physically associated with NMDA receptors[1]. In this issue of *Nature Neuroscience*, Grant and colleagues[2] have combined the latest mass spectrometry (MS) techniques with large-scale immunoblotting (looking at 106 candi-

The authors are in the Department of Neurobiology and Howard Hughes Medical Institute, Massachusetts General Hospital and Harvard Medical School, 50 Blossom Street, Boston, Massachusetts 02114, USA.
e–mail: sheng@helix.mgh.harvard.edu; sanglee@helix.mgh.harvard.edu