1. Viswanathan, A. & Freeman, R.D. *Nat. Neurosci.* **10**, 1308–1312 (2007).
2. Bonin, V., Mante, V. & Carandini, M. *J. Neurosci.* **25**, 10844–10856 (2005).
3. Thompson, J.K., Peterson, M.R. & Freeman, R.D. *J. Neurosci.* **25**, 9046–9058 (2005).
4. Hawken, M.J., Shapley, R.M. & Grosof, D.H. *Vis. Neurosci.* **13**, 477–492 (1996).
5. Li, B., Peterson, M.R., Thompson, J.K., Duong, T. & Freeman, R.D. *J. Neurophysiol.* **94**, 1645–1650 (2005).
6. Logothetis, N.K., Pauls, J., Augath, M., Trinath, T. & Oeltermann, A. *Nature* **412**, 150–157 (2001).
7. Nir, Y. *et al. Curr. Biol.* **17**, 1275–1285 (2007).
8. Iadecola, C. & Nedergaard, M. *Nat. Neurosci.* **10**, 1369–1376 (2007).
9. Amir, Y., Harel, M. & Malach, R. *J. Comp. Neurol.* **334**, 19–46 (1993).
10. Heeger, D.J. & Ress, D. *Nat. Rev. Neurosci.* **3**, 142–151 (2002).

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.

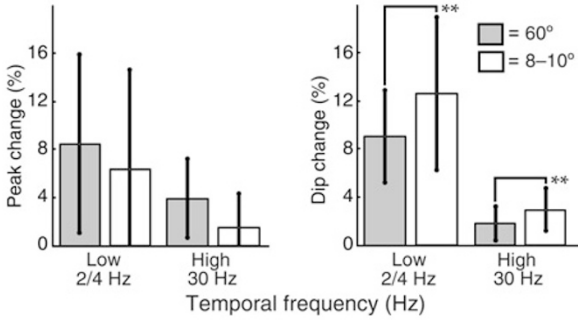

Figure 1 Comparison of tissue oxygen–signal amplitudes. For both large (60°, gray bars) and small (8–10°, white bars) stimuli, positive peak and initial dip signal amplitudes are shown in percent change from the 10-s prestimulus baseline. Responses are shown to low (2 or 4 Hz, based on peak MUA tuning)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

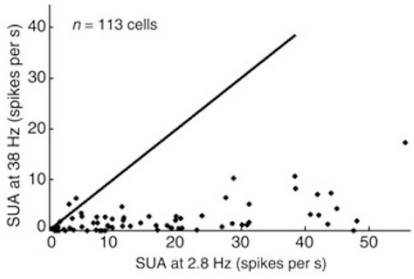

Figure 2 Scatter plot of single-unit spiking activity (SUA) in response to low (2.5 Hz) and high (38 Hz) temporal-frequency stimuli at 45% contrast. Each point represents a single LGN cell. Spatial frequencies are based on peak SUA tuning, and stimulus size equals receptive field size. The identity line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

Ahalya Viswanathan[1] & Ralph D Freeman[1,2]

[1]Group in Vision Science, School of Optometry, [2]Helen Wills Neurosciences Institute, 360 Minor Hall, University of California, Berkeley, Berkeley, California 94720-2020, USA.
e-mail: freeman@neurovision.berkeley.edu

1. Viswanathan, A. & Freeman, R.D. *Nat. Neurosci.* **10**, 1308–1312 (2007).
2. Li, B. & Freeman, R.D. *J. Neurosci.* **27**, 10223–10229 (2007).
3. Thompson, J.K., Peterson, M.R. & Freeman, R.D. *J. Neurosci.* **25**, 9046–9058 (2005).
4. Saul, A.B. & Humphrey, A.L. *J. Neurophysiol.* **64**, 206–224 (1990).
5. Alitto, H.J. & Usrey, W.M. *J. Neurophysiol.* **91**, 2797–2808 (2004).
6. Li, B., Peterson, M.R., Thompson, J.K., Duong, T.Q. & Freeman, R.D. *J. Neurophysiol.* **94**, 1645–1650 (2005).
7. Movshon, J.A., Thompson, I.D. & Tolhurst, D.J. *J. Physiol. (Lond.)* **283**, 101–120 (1978).
8. Logothetis, N.K., Pauls, J., Augath, M., Trinath, T. & Oeltermann, A. *Nature* **412**, 150–157 (2001).
9. Buracas, G.T. & Boynton, G.M. *J. Neurosci.* **27**, 93–97 (2007).
10. Hasselmo, M.E. *Behav. Brain Res.* **67**, 1–27 (1995).