Published in final edited form as:

Neuroscience. 2012 June 28; 213: 112–121. doi:10.1016/j.neuroscience.2012.04.007.

Spatial summation of neurometabolic coupling in the central visual pathway

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Abstract

Noninvasive neural imaging has become an important tool in both applied and theoretical applications. The hemodynamic properties that are measured in functional magnetic resonance imaging (fMRI), for example, are generally used to infer neuronal characteristics. In an attempt to provide empirical data to connect the hemodynamic measurements with neural function, we have conducted previous studies in which neural activity and tissue oxygen metabolic functions are determined together in co-localized regions of the central visual pathway. A basic question in this procedure is whether oxygen responses are coupled linearly in space and time with neural activity. We have previously examined temporal factors, and in the current study, spatial characteristics are addressed. We have recorded from neurons in the lateral geniculate nucleus (LGN) and striate cortex in anesthetized cats. In both structures, there is a classical receptive field (CRF) within which a neuron can be activated. There is also a region outside the CRF from which stimulation cannot activate the cell directly but can influence the response elicited from the CRF. In this investigation we have used several specific spatial stimulus patterns presented to either the CRF or the surrounding region or to both areas together in order to determine spatial response patterns. Within the CRF, we find that neural and metabolic responses sum in a nonlinear fashion but changes in these two measurements are closely coupled. For stimuli that extend beyond the CRF, neural activity is generally reduced while oxygen response exhibits uncoupled changes.

Keywords

tissue oxygen; neural activity; spatial summation; visual stimulus

Introduction

The rapidly increasing use of noninvasive neural imaging techniques as a central procedure in a wide range of basic and applied applications requires appropriate interpretation of the measurements that are made. The main current technique, functional magnetic resonance imaging (fMRI) involves estimates of changes in hemodynamic activity from which neural function is implied. The changes in hemodynamic events involve various metabolic processes that underlie neural activity. We have conducted studies intended to elucidate some of the basic relationships between the neural and metabolic functions that are involved. For this purpose we have recorded from single and multiple cells and have used a

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co-localized sensor to determine changes in tissue oxygen levels during visual stimulation. The measured changes in oxygen concentration are presumed to follow energy demands from activated neural activity. We assume that the rules of the neurometabolic coupling that we determine are directly applicable to interpretation of noninvasive imaging procedures.

As cortical neurons are activated by a stimulus, a hemodynamic response is produced. This process includes local increases in oxygen metabolism, cerebral blood flow and cerebral blood volume, which in combination, determines the blood oxygenation level-dependent (BOLD) response function in fMRI. The BOLD signal is complex, with a brief initial dip that presumably reflects an increase in deoxyhemoglobin concentration from oxygen consumption by activated neurons, followed by a prolonged positive peak that results from an influx of oxygenated hemoglobin with increased blood flow. This is generally followed by an undershoot that may be accounted for by a reduced cerebral blood flow and a slow return of steady-state cerebral oxygen consumption and cerebral blood volume (see reviews, (Brown et al., 2007; Kim and Ogawa, 2012)). The positive BOLD signal has been widely used to probe brain function. The initial dip, however, is relatively small and unreliable, and is often not readily measurable (Buxton, 2001). However, it has been observed in both optical imaging and fMRI studies (Malonek and Grinvald, 1996; Kim et al., 2000). We have obtained analogous data in previous work by use of a dual sensor housed in a double micro-capillary tube by which simultaneous measurements of neural and tissue oxygen responses may be made in co-localized regions in the central visual pathway. Similar to the BOLD signal, tissue oxygen response generally exhibits a small initial dip followed by a large positive peak. The initial dip presumably reflects an increase in oxygen consumption by activated neurons, and the positive peak reflects an arising of oxygen concentration from activity-dependent increase in blood flow (Thompson et al., 2003, 2004, 2005; Li and Freeman, 2007, 2010, 2011). We have found that the initial dip of tissue oxygen response is approximately linear with stimulus duration in the lateral geniculate nucleus (LGN). However, the positive peak exhibits a nonlinear property in temporal integration (Li and Freeman, 2007). The positive peak is the measurement of choice in the blood oxygen level dependent (BOLD) signals that are used in fMRI. Our previous results are consistent with other observations of nonlinear temporal summation in noninvasive neural imaging studies (Boynton et al., 1996; Robson et al., 1998; Vazquez and Noll, 1998; Liu and Gao, 2000; Birn et al., 2001; Miller et al., 2001; Soltysik et al., 2004; Gu et al., 2005).

In the current study, to evaluate spatial rules of neural and metabolic responses, we have made measurements of local changes in tissue oxygen and neural activity that follow activation with visual stimuli. We have made use of the fact that there is both a classical receptive field (CRF) and a region outside the CRF, which has influence on the neural response to a visual stimulus within the CRF but cannot activate a neuron directly (Freeman et al., 2001). We have differentially activated these two regions using specifically tailored visual stimuli. In both LGN and primary visual cortex, our results show that for visual stimuli within the CRF, neural and oxygen responses sum nonlinearly, but their coupling is largely linear. For large stimuli which exceed the boundaries of the CRF, however, this linear relationship breaks down, i.e., tissue oxygen signals are not coupled with neural activity.

Experimental procedures

Physiological preparation

Our general physiological procedures have been described in previous publications (Thompson et al., 2003, 2004, 2005; Li and Freeman, 2007, 2010, 2011). All procedures were conducted in accordance with guidelines by NIH and by the Animal Care and Use Committee at the University of California, Berkeley. We obtained data from 21 mature cats

(2.5 – 4.2 Kg) used for different experimental protocols. Animals were monitored by veterinary staff. At the start of each experiment, an animal was anesthetized with 3% isoflurane. After a few minutes, anesthesia level was reduced to 2–2.5 % as adjusted individually for each animal. Following catheter placement in all four legs, isoflurane was stopped and anesthesia was maintained with intravenous infusion of pentothal sodium, starting at an infusion rate of about 6.0 mg/(kg·h) and fentanyl at a rate of 10.0 µg/(kg·h). During surgery, bolus injections of pentothal (10 mg/ml) were given as required. A tracheal cannula was positioned and the animal was artificially ventilated with a mixture of 25% O₂ and 75% N₂O. Expired CO₂ was maintained at 32–38 mmHg and body temperature was kept at around 38°C. A craniotomy was performed over area 17 at H-C P4 L2 or over LGN at A6L9. Relevant dura was then resected, and agar, then wax, was used to cover the aperture which formed a closed chamber. Following surgery, infusion of fentanyl was discontinued and pentothal was gradually reduced to a level required for steady-state anesthesia, as determined individually for each animal (generally 1–2 mg/(kg·h)). General muscle relaxation was then induced to prevent eye movements with pancuronium bromide (0.2 mg/(kg·h)). Lactated Ringer with 5% dextrose was intravenously infused at a rate of 4 ml/(kg·h). EEG, ECG, expired CO₂, and intra-tracheal pressure, were monitored throughout each experiment, which typically lasted for 4 days. An overdose of pentobarbital sodium was given to the animal at the end of each experiment.

Visual stimulus

Visual stimuli consisted of drifting sinusoidal gratings of varying parameters. Prior to detailed measurements at a recording site, preliminary estimations were made to determine approximately preferred orientation, spatial frequency, and temporal frequency, as well as the size and position of the CRF for each eye. We then obtained tuning functions to quantitatively determine the parameters that were later used in experimental protocols. Visual stimuli were presented simultaneously on two CRT monitors. Refresh rate of the monitors was 85 Hz. We used a 100% contrast level for recordings from LGN. Neurons in visual cortex are more susceptible to contrast adaptation, so we used a 50% contrast level for measurements in area 17. In all cases, mean screen luminance was 45 cd/m². Each grating stimulus was presented for 4 seconds. In general, visual stimuli were presented monoptically to the dominant eye while the other eye viewed a blank screen with the same mean luminance. Visual stimulus interval values were randomly varied from 30 to 44 seconds to avoid synchrony with spontaneous oscillations in the baseline oxygen signal which are believed to be relevant to regional cerebral microcirculation (Mayhew et al., 1996). Stimulus conditions were interleaved randomly and sequences were repeated in multiple trials (16 to 64).

Recording and analysis procedures

Tissue oxygen responses were measured with a Clark style (Fatt, 1976) polarographic oxygen sensor (Unisense, Aarhus, Denmark). Neural activity was recorded simultaneously with a platinum microelectrode enclosed in a double-barreled glass micropipette along with the oxygen sensor. The tip of the combined sensing system is around 30 µm. The spherical sensitivity region of the oxygen sensor is roughly 60 µm in diameter (Thompson *et al.*, 2003). The sensing unit was controlled and advanced via a micro-manipulator. For recordings from the LGN, electrode penetrations were made vertically from H-C coordinates A6L9. For visual cortex, penetrations were made along the medial bank of the postlateral gyrus from H-C coordinates P4L2 at an approximate angle of 10° medial and 20° anterior. The oxygen sensor was connected to a high impedance picoammeter. Sampling rate for tissue oxygen signals was 10 Hz. Impedance of the neural electrode was 0.2–1.0 M at 1 kHz in 0.9% saline at 38°C. Neural signals were amplified and filtered to generate extracellular multiple unit activity (MUA, 0.25–8 kHz), which was sampled at rates of 25 kHz. Local

field potential signals are not included in the analysis because they are generally weak and noisy in the LGN (Rasch et al., 2009; Li and Freeman, 2010).

Oxygen signals were averaged across multiple trials. Baseline levels 10 seconds prior to stimulus onset were subtracted from average oxygen signals. Oxygen responses were normalized by the mean oxygen levels in order to obtain percentages of change (Thompson et al., 2003). Spike rates 10 seconds prior to stimulus onset are defined as spontaneous activity which was subtracted from MUA.

Recording sites with significant tissue oxygen and neural responses to at least one stimulus condition were included for data analysis. Oxygen responses were evaluated to determine if there was a statistically significant change in the initial dip or subsequent positive peak compared with that of the baseline activity ($p < 0.05$, t-test). Similarly, MUA was considered significant if it was different from spontaneous activity ($p < 0.05$, t-test). The Wilcoxon Signed Rank Test (signrank.m, Matlab function) was used to compare responses to paired stimuli with those of individual components that were summed together. Error estimates are in the form of standard errors of the mean (SEM), unless otherwise noted.

Results

Spatial integration of neural and metabolic responses in the LGN

Neural responses in the LGN exhibit different characteristics of spatial integration in the CRF and its surround (Jones et al., 2000; Alitto and Usrey, 2008). We have utilized these differences to assess the spatial relation between metabolic responses and accompanying neural activity. The CRF of an LGN neuron typically has a concentric center-surround organization with excitatory and inhibitory opposite polarities (Kuffler, 1953; Hubel and Wiesel, 1961; Cai et al., 1997; Carandini, 2004). Based on this organization, we use grating stimuli of five different spatial configurations to estimate the degree of linearity of spatial summation as illustrated in Figure 1: a left semicircle (Fig. 1A), a right semicircle (Fig. 1B), a full circle (Fig. 1C), a full-field stimulus with a blank circular patch over the CRF (Fig. 1D), and a full-field stimulus (Fig. 1E). The three stimulus patterns in Fig. 1A–C have the same diameter, which matches the size of the CRF determined by use of a size tuning function for each recording site. The summation of the stimuli in Fig. 1A and Fig. 1B is equal to the stimulus in Fig. 1C. Similarly, the summation of the stimuli in Fig. 1C and Fig. 1D equals the stimulus in Fig. 1E. Tissue oxygen and MUA responses to these stimuli are shown in left and right columns, respectively, for a representative recording site in the LGN. The data are averaged across 32 trials. Consistent with our previous studies in the LGN (Thompson et al., 2004; Li and Freeman, 2007), tissue oxygen responses to small-size stimuli within the CRF are generally monophasically negative (Fig. 1A–C). The stimulus covering the surround of the CRF elicits a monophasic positive oxygen response (Fig. 1D) whereas the full-field stimulus (Fig. 1E) evokes a biphasic oxygen response with a small initial dip followed by a large positive peak. Substantial MUA responses were elicited by all stimuli except the one with a blank mask on the CRF. Different spatial summation characteristics of MUA are shown for stimuli within the CRF (Fig. 1A–C), and across the CRF and its surround (Fig. 1C–E). The full-circle stimulus (Fig. 1C) elicited a stronger MUA than each semi-circle stimulus (Fig. 1A, B). The full-field stimulus (Fig. 1E), however, elicited a weaker MUA than the full-circle stimulus (Fig. 1C), reflecting the influence of surround suppression.

We evaluate linearity of spatial summation of neural and oxygen responses within the CRF, and across the CRF and its surround as shown in Fig. 1C and E, respectively. For spatial summation within the CRF, we identify measured responses (Fig. 1C, black curves) as those to the full-circle stimulus; and composite responses (Fig. 1C, gray curves) are taken as the

sums of those to the two semi-circular stimuli individually. We find that nonlinear spatial summation is evident in both neural and tissue oxygen responses. The composite oxygen response exhibits a significantly larger initial dip than the measured (Fig. 1C, left column, $4.1 \pm 0.5\%$ vs. $2.9 \pm 0.4\%$, $p = 0.02$); and the composite MUA (mean value over the stimulus duration) is also significantly stronger than the measured (Fig. 1C, right column, 52.9 ± 6.1 vs. 42.6 ± 3.9 spikes/s, $p = 3.3 \times 10^{-3}$). Similarly, for spatial summation across the CRF and its surround, we define responses to the full-field stimulus as the measured (black curves), and the sums of responses to the full-circle stimulus and the full-field stimulus with a blank mask as the composite responses (gray curves). As shown in Fig. 1E, the composite oxygen response exhibits a slightly larger initial dip than the measured ($1.9 \pm 0.2\%$ vs. $1.3 \pm 0.1\%$, $p = 0.04$) but their positive peaks are similar ($4.8 \pm 0.3\%$ vs. $4.6 \pm 0.4\%$, $p = 0.5$) (Fig. 1E, left column). The composite MUA appears to be stronger than the measured (43.2 ± 3.8 vs. 26.6 ± 2.2 spikes/s, $p = 1.5 \times 10^{-6}$) (Fig. 1E, right column), which is evident because MUA to the full-circle stimulus alone (CRF) is much stronger than that to the full-field stimulus (CRF plus surround).

The nonlinear spatial integration described above is corroborated by results across our population of recording sites. Figs. 2A–B illustrates measured and composite oxygen and neural responses within the CRF for 32 recording sites in the LGN. On average, the composite oxygen response exhibits a larger initial dip than the measured (Fig. 2A, $8.8 \pm 1.0\%$ vs. $7.2 \pm 0.9\%$, $p = 2.4 \times 10^{-5}$) although some points fall on the linear equity ($y=x$) line. Similarly, composite MUA responses appear to be significantly stronger than the measured ones (Fig. 2B, 97.1 ± 7.3 vs. 77.7 ± 5.8 spikes/s, $p = 1.4 \times 10^{-6}$). We previously showed that neurometabolic coupling is largely linear in time within the CRF of the LGN (Li and Freeman, 2007). Here, we use the same approach to evaluate spatial linearity. Fig. 2C–D illustrates average measured and composite oxygen responses and MUA histograms within the CRF. To further examine these data, we use an impulse response function derived as follows:

$$R(t)=H(t) \otimes S(t) \quad (1)$$

where $R(t)$ and $H(t)$ represent tissue oxygen response, and the impulse response function, respectively. $S(t)$ represents estimated MUA (dashed gray lines in Fig. 2C–D) obtained by fitting MUA histograms with an exponential function:

$$S(t)=c+b \times \exp (-t/n) \quad (2)$$

where $S(t)$ represents estimated MUA, and c , b , and n are free parameters (Soltysik et al., 2004; Li and Freeman, 2007). Fig. 2E compares the measured (black curve) and composite (gray curve) impulse response functions deconvolved from the average oxygen and neural responses in Fig. 2C–D. We calculate the coefficient of determination (R^2) between the measured and composite impulse functions to quantify the linearity of neurometabolic coupling. The R^2 ranges from 0 to 1.0, where a value of 1 represents complete linearity. As shown in Fig. 2E, the measured and composite impulse response functions are nearly overlapped ($R^2 = 1.0$), indicating linear spatial summation of neurometabolic coupling within the CRF.

To determine the spatial summation of neurometabolic coupling across the CRF and its surround, we have compared measured and composite oxygen and neural responses over a population of recording sites in the LGN ($n = 58$) as illustrated in Fig. 3. Consistent with the example site in Fig. 1, the composite oxygen response tends to exhibit a larger initial dip (Fig. 3A, $4.1 \pm 0.6\%$ and $2.2 \pm 0.3\%$ respectively, $p = 2.7 \times 10^{-7}$) but a similar positive peak (Fig. 3B, $7.7 \pm 1.0\%$ and $7.8 \pm 0.9\%$ respectively, $p = 0.8$) compared to the measured

response. The composite MUA is also significantly stronger than the measured value (Fig. 3C, 80.2 ± 5.7 spikes/s and 47.2 ± 3.7 spikes/s respectively, $p = 3.5 \times 10^{-11}$). Note that nonlinear spatial summation occurs in both neural response and initial dip but not in positive peak of the oxygen response. To assess the spatial linearity of neurometabolic coupling, we obtain impulse response functions based on the average measured and composite oxygen and neural responses in Fig. 3D–E. We find that the measured impulse response function deviates substantially from the composite (Fig. 3F, $R^2 = 0.65$), indicating a nonlinear spatial summation of neurometabolic coupling across the CRF and its surround. Note here that the initial dips of the two impulse response functions match closely in amplitude. This suggests that the initial dip may better reflect neural response than the positive peak of oxygen response in the LGN when the stimulus size is larger than the CRF.

As we have shown in Fig. 1D, a stimulus that covers the surround of a recording site generally elicits a monophasic positive oxygen response. To examine this response in more detail, we divided the stimulus into left and right halves and measured tissue oxygen and neural responses to the full pattern and each half. Results are shown in Fig. 4. Black curves represent measured tissue oxygen and neural responses (left and right columns, respectively) to the full-field stimulus with a blank mask circular patch over the CRF (Fig. 4A), the left half (Fig. 4B) and the right half (Fig. 4C) for the same recording site as shown in Fig. 1. The gray curves in Fig. 4A represent composite oxygen and neural responses which are taken as the sums of those to the two stimuli in Fig. 4B and 4C. It is clear that both the positive oxygen response and the neural activity demonstrate nonlinear spatial summation. Compared to the measured responses, the composite ones show larger positive peaks ($9.07 \pm 0.87\%$ and $5.81 \pm 0.64\%$ respectively, $p=0.03$) and stronger neural responses (6.1 ± 0.2 and 2.9 ± 0.1 spikes/s respectively, $p = 4.8 \times 10^{-8}$). These results are consistent across the population of recording sites ($n=32$). Average amplitudes of the composite and measured positive oxygen responses are $14.9 \pm 1.9\%$ and $11.0 \pm 1.3\%$ respectively ($p = 9.4 \times 10^{-6}$). Average composite and measured neural responses are 4.0 ± 0.6 and 3.1 ± 0.5 spikes/s respectively ($p = 4.5 \times 10^{-3}$). Because all three stimulus patterns were presented outside the CRF, the neural responses shown in Fig. 4 suggest that the CRFs for some recording sites were underestimated (Cavanaugh et al., 2002). Note that the positive oxygen response presumably results from an increase in cerebral blood flow, and an increase in oxygenated hemoglobin with blood flow leads to an enhanced fMRI BOLD signal. Therefore, nonlinear spatial summation of the positive oxygen response suggests that this may also apply to the BOLD signal.

Spatial integration of neural and metabolic responses in area 17

The spatial summation properties of neurometabolic coupling in the LGN, described above, apply to concentric center-surround RF organization. At the next stage of the central visual pathway, the striate cortex, RFs are generally elongated and functional organization is more complex (Hubel and Wiesel, 1962). To compare cortical cells with those in LGN regarding properties of spatial summation of neural and metabolic responses, we have conducted similar experiments in striate cortex.

Neuronal responses in primary visual cortex have different characteristics of spatial integration in the CRF and in regions beyond this area (for a review, see (Freeman et al., 2001)). We have utilized these differences to assess spatial linearity of metabolic response and its coupling with neural activity. Five stimulus patterns are used for this experiment: a small circular grating patch, a larger annular patch, a large circular patch, a full-field stimulus with a blank circular center, and a full-field stimulus as depicted in Fig. 5A–E. For each recording site, the diameter of the outer circle in Fig. 5B, the circular patch in Fig. 5C, and the blank circular patch in Fig. 5D are all the size of the CRF, which is determined by prior size-tuning tests (Walker et al., 2000). The diameter of the small circular stimulus in

Fig. 5A, and that of the inner circle of the annular stimulus in Fig. 5B are set to be 50% of the CRF. The composite addition of the stimuli in Fig. 5A and B is equivalent to the stimulus in Fig. 5C. Similarly, stimuli in Fig. 5C, D are non-overlapped and their summation comprises the full-field stimulus in Fig. 5E. Representative tissue oxygen (left column) and neural responses (right column) are shown for these five stimulus patterns in Fig. 5A–E for a recording site in the striate cortex. The data for each curve are averaged across 64 trials. The diameters of the small circular patches in Fig. 5A, B and the large circular patches in Fig. 5B–D are 2.5° and 5° , respectively. All stimuli elicit clear biphasic tissue oxygen responses with an initial negative phase followed by an extensive large positive peak. Clear neural responses are also observed to stimuli in Fig. 5A–C and E. The full-field stimulus with a blank mask over the CRF elicits weak neural activity, which is probably due to an underestimation of the actual CRF size (Cavanaugh et al., 2002).

To examine the extent of spatial linearity of neural and tissue oxygen responses within the CRF, measured responses to the 5° circular stimulus are compared to the sums of responses to the 2.5° circular (Fig. 5A) and the annular patches (Fig. 5B). As shown in Fig. 5C, the composite oxygen response (left column, gray curve) exhibits a slightly larger initial dip and a stronger positive peak than those from the measured oxygen responses (left column, black curve). Amplitudes of the composite and measured oxygen responses are $3.8 \pm 0.2\%$ and $2.8 \pm 0.2\%$ for the initial dip ($p = 2.0 \times 10^{-5}$, Wilcoxon signed rank test), and $7.8 \pm 0.5\%$ and $2.7 \pm 0.2\%$ for the positive peak ($p = 7.2 \times 10^{-11}$), respectively. Average composite and measured MUA responses are 40.8 ± 1.5 and 34.4 ± 1.2 spikes/s ($p = 1.4 \times 10^{-9}$) (Fig. 5C, right column), respectively. Clearly, these data demonstrate that neural and oxygen responses to a stimulus within this CRF are weaker than what would be predicted by the linear summations of those to each component stimulus. Similarly, to evaluate the spatial linearity of neural and tissue oxygen responses across the CRF and its surround, the measured responses to the full-field stimulus (Fig. 5E) are compared to the composite responses to the 5° circular patch (Fig. 5C) and the full-field with a blank mask covering the CRF (Fig. 5D). The composite oxygen response (gray curve, Fig. 5E, left column) exhibits a similar initial dip but a larger positive peak than the measured (black curve, Fig. 5E, left column) ($4.4 \pm 0.3\%$ vs. $4.3 \pm 0.3\%$ for the initial dip ($p = 0.7$), and $7.3 \pm 0.5\%$ vs. $4.0 \pm 0.3\%$ for the positive peak ($p = 1.6 \times 10^{-7}$), respectively). The average composite MUA (gray curve, Fig. 5E, right column) is also stronger than the measured values (black curve, Fig. 5E, right column) (37.8 ± 1.5 vs. 28.4 ± 1.3 spikes/s ($p = 1.4 \times 10^{-11}$)).

Quantitative analysis of our population data in area 17 confirms the above properties of spatial summation of neural and metabolic responses. For responses within the CRF, the composite oxygen response has significantly larger initial dips (Fig. 6A, $8.0 \pm 0.9\%$ vs. $5.9 \pm 0.7\%$, $p = 4.5 \times 10^{-4}$, Wilcoxon signed rank test ($n=20$)), and positive peaks (Fig. 6B, $18.2 \pm 3.1\%$ vs. $11.6 \pm 2.3\%$, $p = 8.8 \times 10^{-5}$), compared to the measured quantities. Average composite and measured MUA responses are 65.0 ± 9.0 spikes/s and 52.9 ± 7.0 spikes/s respectively (Fig. 6C, $p = 8.9 \times 10^{-5}$). To determine if linearity of neurometabolic coupling holds within the CRF, we obtained impulse response functions by deconvolution of average measured (Fig. 6D) and composite (Fig. 6E) oxygen and MUA responses. Similar to the LGN, the measured and composite impulse response functions are mostly overlapped (Fig. 6F, $R^2 = 0.95$), which suggest that neurometabolic coupling is mostly invariant in space within the CRF in the visual cortex. On the other hand, for responses across the CRF and its surround, the initial negative oxygen response is largely linear in spatial summation, but the positive peak and neural activity tend to be significantly stronger for the composite response. Average amplitudes of the initial dip are $9.0 \pm 2.0\%$ and $8.6 \pm 2.1\%$ (Fig. 7A, $p = 0.1$, Wilcoxon signed rank test ($n=18$)), and average amplitudes of the positive peak are $17.2 \pm 3.3\%$ and $10.0 \pm 1.5\%$ (Fig. 7B, $p = 7.3 \times 10^{-4}$), for the composite and measured oxygen responses, respectively. Average composite and measured MUA responses are 108.3 ± 11.7

spikes/s and 72.1 ± 8.7 spikes/s (Fig. 7C), respectively. To assess the spatial linearity of neurometabolic coupling, we obtain impulse response functions based on the average measured and composite oxygen and neural responses in Fig. 7D–E. As shown in Fig. 7F, the measured impulse response function (black curve) deviates slightly from the linear estimation (gray curve) ($R^2 = 0.85$). These results indicate that neurometabolic coupling is mainly space invariant within the excitatory summation zone (CRF) but becomes variable as the visual stimulus increases in size beyond the CRF.

Discussion

In this investigation, we have studied the relationships between neural and metabolic activity in co-localized regions of the central visual pathway. We showed previously in the LGN that the initial dip, but not the positive peak of tissue oxygen response is approximately linear in temporal integration (Li and Freeman, 2007). We have extended this work in the current study by investigation of spatial summation of neural and oxygen responses in LGN and visual cortex. Our findings can be summarized by two major points. First, when two non-overlapped visual stimuli are presented simultaneously within the CRF, both neural and tissue oxygen responses are generally smaller than the linear summation of those to each stimulus alone. However, neurometabolic coupling between oxygen and neural activity remains mostly linear. Second, for visual stimuli larger than the CRF, nonlinear spatial summation is evident for neural activity while oxygen responses exhibit uncoupled features with neural activity.

The nonlinear spatial summation of neural activity within the CRF in striate cortex in the current study is consistent with previous single-unit measurements in the cat's striate cortex and monkey's middle temporal area (Heggelund et al., 1983; Britten and Heuer, 1999). It is of interest that nonlinear spatial summation of neural activity is found to be closely coupled with tissue oxygen response. Neurometabolic and neurovascular coupling can be modified substantially by activation of inhibitory interneurons (Cauli et al., 2004; Kocharyan et al., 2008; Enager et al., 2009). Linear neurometabolic coupling within the CRF in striate cortex suggests that the contribution to oxygen metabolism by inhibitory interneurons is minimal. Because simple cells in the striate cortex receive thalamic excitatory inputs from the LGN, the decreased oxygen metabolism (compared to a linear prediction) reported here within the cortical CRF may simply reflect reduced activation from LGN to cortical neurons. Note that the extent of spatial linearity of neurometabolic coupling within the CRF may depend on the stimulus patterns. In a previous study, oxygen metabolism was found to be linearly coupled with neural activity when high-contrast stimuli were used and strong neural responses were elicited (Li and Freeman, 2007). However, this linear neurometabolic coupling was not observed for low-contrast stimuli. A nonlinear power-law function describes the relationship between oxygen metabolic and neural responses for low contrast stimuli and weak neural firing rates (Li and Freeman, 2007). This nonlinearity in scaling of neurometabolic coupling may also occur if we had used very small stimulus patches in the current study. Because of the number of variables involved, we chose to use relatively large stimulus patches and high contrast levels for visual stimuli within the CRF.

Although the spatial linearity of neurometabolic coupling within the CRF is clear, it is generally not the case when visual stimuli extend beyond the CRF. Different spatial scales of tissue oxygen and neural responses may account for the uncoupling between these two signals. The positive peak of oxygen response presumably reflects activity-dependent increases in cerebral blood flow over a relatively large distance whereas the initial dip is due to increases in cerebral metabolic rate of oxygen over a range of a few hundred micrometers (Thompson et al., 2005). On the other hand, MUA is believed to be relatively local and spreads in a range of a few hundred micrometers (Legatt *et al.*, 1980). This range is similar

to that of the initial dip but not the positive peak of oxygen responses. This may explain why the initial dip but not the positive peak is coupled with neural activity in the LGN when the visual stimulus is larger than the CRF. Other mechanisms, for example, inhibitory interneurons, may also contribute to the uncoupling between neural and tissue oxygen responses when the stimulus extends into the receptive field surround in the striate cortex (Li and Freeman, 2011).

Despite the different characteristics of neurometabolic coupling within the CRF and the area beyond, we observe nonlinear spatial integration of the positive oxygen response in both regions in the striate cortex. This result suggests a nonlinear spatial summation of fMRI BOLD signals in visual cortex because the positive oxygen response presumably reflects an increase in cerebral blood flow to replenish the oxygen consumed by activated neurons (Malonek and Grinvald, 1996; Mayhew et al., 2001; Thompson et al., 2003). However, this is not consistent with the observation that spatial summation of the BOLD signal is linear in human visual cortex (Hansen et al., 2004). This could reflect different characteristics of these two measurements. The BOLD signal is a complex interaction of cerebral blood flow, cerebral blood volume, and cerebral metabolic rate of oxygen whereas the oxygen responses only reflect changes in tissue oxygen concentration. Moreover, the oxygen sensor used in our measurements is sensitive to localized changes in oxygen concentration, which is presumably substantially finer than the spatial resolution of the BOLD signal (>1 mm). In addition, spatial linearity of the BOLD response may depend on the composition of visual stimuli so that it applies only under some specific conditions (Hansen et al., 2004). Previous reports of fMRI measurements also suggest nonlinear spatial summation of the BOLD signal in visual cortex (Press et al., 2001; Williams et al., 2003; Zenger-Landolt and Heeger, 2003; Nurminen et al., 2009).

Conclusion

By use of selected combinations of visual stimuli, we have explored the extent of spatial linearity of neural and tissue oxygen responses in the LGN and striate visual cortex. We find that the nonlinear spatial summation of oxygen responses within the CRF in both regions is well predicted by analogous spatial integration of neural activity. As visual stimuli extend beyond the CRF, the coupling between oxygen and neural responses deviates from linearity. These results on spatial properties of neural and metabolic activity in the central visual pathway may be relevant to the interpretation of noninvasive neural imaging.

Highlights

- We measure tissue oxygen and neural responses in the central visual pathway.
- Neural and tissue oxygen responses generally sum nonlinearly in space.
- Neurometabolic coupling is invariant within the classical receptive field (CRF).
- Neurometabolic coupling appears to deviate from linearity beyond the CRF.

Acknowledgments

This work was supported by National Eye Institute Research Grant EY01175. We thank N. Yang for help with experiments.

Abbreviations

CRF	classical receptive field
EEG	electroencephalogram
ECG	electrocardiogram
MUA	multiple unit activity
fMRI	functional magnetic resonance imaging
BOLD	blood oxygen level dependent
LGN	lateral geniculate nucleus

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Fig.1.

Tissue oxygen and neural responses to visual stimuli of different spatial patterns for a recording site in the LGN. Oxygen responses (left column) and MUA (right column) to a left semicircular stimulus (A), a right semicircular stimulus (B), a circular stimulus (C), a full-field stimulus with a mask covering the CRF (D), and a full-field stimulus (E). The diameter of the circular patch in (C) is 2°, which is quantitatively determined as optimal by a size tuning function. The diameter of the semicircular patterns in (A), (B) and the blank circle in (C) is also 2°. Data are averaged over 32 trials. “0” in the horizontal axis indicates the start of the visual stimulation. The duration of all stimuli is 4 s. Black and gray curves in (C, E) represent measured and composite oxygen and MUA responses to the circular (C) and

full-field (E) stimuli respectively. The composite responses in (C) are determined as the sums of those to each semicircular stimulus component (A, B). Similarly, the composite responses in (E) are equal to the sums of those to the circular stimulus (C) and the full-field stimulus with a mask covering the CRF (D).

Fig. 2.

Linearity of spatial summation of neurometabolic coupling to visual stimuli within the CRF in the LGN. (A, B) Comparison of measured and composite oxygen responses (A) and MUA signals (B) for a population of recording sites. Each open circle represents a recording site. The measured responses are plotted on the horizontal axis, and the composite responses are plotted on the vertical axis. The unity line represents a perfect correspondence between measured and composite responses. Most points lie above the unity line, indicating that linear predictions of tissue oxygen and neural responses tend to overestimate the measured values. (C, D) Average measured (C) and composite (D) oxygen and neural responses across the population of recording sites. “0” in the horizontal axis indicates the start of the visual

stimulus. The duration of the stimulus is 4 s. Dashed black curves represent ± 1 SEM. Dashed gray curves indicate estimated MUA signals derived by fitting corresponding histograms with an exponent function. (E) Comparison of impulse response functions deconvolved from the average measured and composite oxygen responses and corresponding estimated MUA signals in (C) and (D). A gamma function is used to fit each impulse response function.

Fig. 3.

Spatial summation of neurometabolic coupling to visual stimuli exceeding the CRF in the LGN. (A–C) Comparison of the initial dip (A), positive peak (B), and MUA (C) between measured and composite oxygen and neural responses for the population data. Each unfilled circle represents a recording site. (D–E) Average measured (D) and composite (E) oxygen and neural responses across the population of recording sites. (F) Comparison of measured and composite impulse response functions. Each impulse response function is fit by the difference of two gamma functions. All symbols are identical to Fig. 2.

Fig. 4.

Tissue oxygen and neural responses to visual stimuli beyond the CRF in the LGN. (A) Tissue oxygen (left column) and MUA (right column) to a full-field stimulus with a blank mask over the CRF for the same recording site as shown in Fig. 1. Black and gray curves represent, respectively, measured and composite responses to the stimulus. The composite responses are the sums of those for left half (B) and right half (C) stimuli. Summation of the stimuli in B and C equals the stimulus in A. (D, E) Comparison of measured and composite oxygen responses (D) and neural activity (E) for a population of recording sites. All symbols are identical to those in Fig. 2.

Fig. 5.

Tissue oxygen and neural responses to visual stimuli of different spatial patterns for a recording site in the visual cortex. Tissue oxygen (left column) and MUA (middle column) responses to a circular stimulus of small size (A, diameter = 2.5°), an annular stimulus (B, outer diameter = 5° , inner diameter = 2.5°), a circular stimulus of large size (C, diameter = 5°), a full-field stimulus with a blank mask over the CRF (D, diameter of the mask = 5°), and a full-field stimulus (E). Data are averaged across 64 trials. The optimal size for this recording site is 5° , which is determined quantitatively by a size tuning function. "0" on the horizontal axis indicates the onset of the visual stimulus. The duration of all stimuli is 4s. Black and gray curves in (C, E) represent measured and composite oxygen and MUA

responses to the circular (C) and full-field (E) stimuli, respectively. The composite responses in (C) are determined as the sums of those to the small circular (A) and annular (B) stimuli. Similarly, the composite responses in (E) are equal to the sums of those to the circular stimulus (C) and the full-field stimulus with a blank mask over the CRF (D).

Fig. 6.

Spatial summation of neurometabolic coupling within the CRF in the striate cortex. (A–C) Comparison of the initial dip (A), positive peak (B) and MUA (C) between measured and composite oxygen and neural responses for a population of recording sites. Each unfilled circle represents a recording site. Most points lie above the unity line, indicating deviation from linear spatial summation. (D–E) Comparison of average measured (D) and composite (E) oxygen and neural responses across the population of recording sites. “0” indicates the onset of the visual stimuli. Dashed black lines represent ± 1 SEM. Dashed gray curves represent estimated MUA signals by fitting corresponding histograms with an exponent function. (F) Comparison of measured and composite impulse response functions deconvolved from the average oxygen responses and the estimated MUA signals in (D) and (E). Each impulse response function is fit by the difference of two gamma functions.

Fig. 7. Spatial summation of neurometabolic coupling to visual stimuli extending beyond the CRF in the striate cortex. All symbols are identical to those in Fig. 6.