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Binocular activation elicits differences in neurometabolic coupling in visual cortex

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Abstract

Non-invasive brain imaging requires comprehensive interpretation of hemodynamic signals. In functional magnetic resonance imaging, blood oxygen level dependent (BOLD) signals are used to infer neural processes. This necessitates a clear understanding of how BOLD signals and neural activity are related. One fundamental question concerns the relative importance of synaptic activity and spiking discharge. Although these two components are related, most previous work shows that synaptic activity is better reflected in the BOLD signal. However, the mechanisms of this relationship are not clear. The BOLD signal depends on relative changes in cerebral blood flow and cerebral metabolic rate of oxygen. Oxygen metabolism changes are difficult to measure with current imaging techniques, but it is possible to obtain direct quantitative simultaneous in vivo measurement of tissue oxygen and co-localized underlying neural activity. Here, we use this approach with a specific binocular stimulus protocol in order to activate inhibitory and excitatory neuronal pathways in visual cortex. During excitatory binocular interaction, we find that metabolic, spiking, and local field potential responses are correlated. However, during suppressive binocular interaction, spiking activity and local field potentials are dissociated while only the latter is coupled with metabolic response. These results suggest that inhibitory connections may be a key factor in the dissociation between local field potentials and spiking activity, which may contribute substantially to the close coupling between the BOLD signal and synchronized synaptic activity in the brain.

Keywords

neurometabolic coupling; binocular interaction; visual cortex

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Introduction

Noninvasive brain imaging has become a pervasive tool in both basic and clinical applications. Functional magnetic resonance imaging (fMRI) involves hemodynamic responses which are used to infer neural function. The blood oxygen level dependent (BOLD) signals that are measured depend on cerebral blood flow and volume and the cerebral metabolic rate of oxygen. However, the neurometabolic and neurovascular components of BOLD signals are not understood at a level sufficient for clear interpretation of fMRI signals. One obvious question concerns the nature of neural activity represented by the BOLD signal. The two relevant measures of electrophysiological measurement are action potentials that form the output spiking activity and local field potentials (LFP) that are considered to represent the input of synaptic processes. Although they are related, it is believed that synchronized synaptic activity is better reflected in the BOLD response than spiking output (Logothetis et al., 2001). However, the details of this association are not clear.

In previous studies on relationships between neural and metabolic factors, we made simultaneous co-localized measurements of neuronal activity and tissue oxygen concentration in cerebral cortex (Thompson et al., 2003, 2004, 2005; Li and Freeman, 2007; Viswanathan and Freeman, 2007; Li and Freeman, 2010, 2011). Tissue oxygenation is directly related to the BOLD signal as follows. Activated neurons require oxygen which is provided by cerebral blood flow. The BOLD signal is a measure of deoxyhemoglobin change that occurs as tissue oxygen is increased. In a previous study, we used visual stimuli at high temporal frequencies which activate spiking activity of neurons in the thalamus but not the visual cortex. We found that the LFP signals were correlated with tissue oxygen even when spiking discharge was minimal (Viswanathan and Freeman, 2007). This implies that synaptic activity may contribute more to the BOLD signal than spiking discharge as found in previous work (Logothetis et al., 2001; Bartolo et al., 2011).

To examine underlying mechanisms of the dissociation between the two components of neural activity and their coupling with metabolic response, we use an approach here which clearly dissociates spiking response and synaptic activity in the visual cortex. The visual stream from left and right eyes is first physiologically combined in striate cortex. With dichoptic presentation of sinusoidal gratings at various relative interocular phases, neurons in striate cortex generally exhibit both facilitation and suppression in binocular interaction profiles (Freeman and Robson, 1982; Ohzawa and Freeman, 1986; Freeman and Ohzawa, 1990; Ohzawa et al., 1990; Chino et al., 1994; Truchard et al., 2000). During facilitation, there is an increase in spiking activity, and in suppressive states there is a reduction in action potential events. Because inputs from the lateral geniculate nucleus (LGN) to striate cortex are exclusively excitatory (Ferster et al., 1996), the suppression of spiking activity may derive from intracortical inhibitory drives, which are reported to enhance synchronized gamma oscillation synaptic activity while reducing spiking activity (Traub et al., 1996; Tamas et al., 1998; Whittington and Traub, 2003). Therefore, spiking output and synaptic activity may be dissociated when binocular suppression is activated by visual stimuli at certain interocular phases. We utilize this type of binocular interaction during simultaneous recordings of neural activity and oxygen concentration. We have analyzed spiking multiple

unit activity (MUA) and local field potentials (LFP) along with both components (initial dip and positive peak) of the biphasic tissue oxygen concentration responses. Our results show that LFP and spiking activity are both coupled to oxygen metabolism during excitatory binocular interaction. In marked contrast, during suppressive binocular interaction, there is a dissociation of the two forms of neural activity, such that LFP signals are coupled with oxygen metabolism but spiking activity is not.

Experimental procedures

Surgical procedure and preparation

Mature cats (2.9-4.0 Kg, 5-10 months old) were prepared for physiological experiments as described previously (Thompson et al., 2003; Li and Freeman, 2007). All procedures were conducted in accordance with guidelines by NIH and by the Animal Care and Use Committee at the University of California, Berkeley. For every experiment, the animal was initially anesthetized with isoflurane (2-3%, adjusted individually for each animal). A catheter was inserted into a femoral vein in each leg for intravenous infusion. A tracheal cannula was positioned and the animal was then secured in a stereotaxic apparatus. Isoflurane was stopped and anesthesia was maintained with intravenous infusion of propofol (15-20 mg/(kg·hr)) and fentanyl (10.0 µg/(kg·hr)). 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 above the central representation of the visual field in the striate cortex (Horsley-Clark coordinates P4 L2). The dura was dissected away to allow insertion of a microelectrode. Agar and wax were applied to create a closed chamber. Following surgery, the infusion rate of fentanyl was reduced to 4 µg/(kg·hr) and the rate of propofol was gradually reduced to 6-10 mg/(kg·hr) as determined individually for each animal. After the anesthesia was stabilized, paralysis was then induced to prevent eye movements with pancuronium bromide (0.2 mg/(kg·hr)). Pupils were dilated with 1% atropine sulfate and nictitating membranes were retracted with 5% phenylephrine HCL. Contact lenses with 4 mm artificial pupils were applied to protect the corneas. 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 the experiment, which typically lasted 3-4 days. An overdose of pentobarbital sodium was given to the animal at the end of each experiment.

Visual stimulation

Drifting sinusoidal gratings were presented simultaneously on two CRT monitors. Refresh rate of the monitors was 85 Hz. For each recording site, preliminary subjective estimations were made to determine approximate preferred orientation, spatial and temporal frequency, and size and position of the receptive field (RF) for each eye. We then obtained quantitative tuning functions to be used in experimental protocols. In all cases, contrast level, mean screen luminance, and stimulus duration were 50%, 45 cd/m², and 4 seconds, respectively. Visual stimulus interval values were randomly varied from 30 to 44 seconds to avoid synchrony with spontaneous oscillations in the baseline oxygen signal. We fixed the initial phase of the grating presented to the dominant eye and varied that to the non-dominant eye in 60° steps over a full range of 360°. This results in 6 different relative phases in the

binocular mode. Since gratings for both eyes drift at the same rate, relative phase of the gratings between the two eyes remains constant during stimulus presentation. In addition to dichoptic presentation, a monocular stimulus presented to the dominant eye is included as a control condition in the stimulus protocol. All stimulus conditions were interleaved randomly and sequences were repeated in multiple trials (16 to 80).

Data recording

We used a double barrel APOX microelectrode (Unisense, Aarhus, Denmark) in the experiment. A Clark style (Fatt, 1976) polarographic oxygen sensor and a platinum microelectrode were enclosed in the APOX (30 μm in tip size) for simultaneous measurements of co-localized tissue oxygen and neural responses. The spherical sensitivity region of the oxygen sensor is roughly 60 μm in diameter (Thompson et al., 2003). The APOX microelectrode was advanced into the striate cortex via a micro-manipulator until visually driven spiking activity was identified. 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. A high impedance picoammeter was used for sensing the small currents generated by the oxygen sensor. Sampling rate for tissue oxygen signals was 10 Hz. Impedance of the tungsten electrode for neural signal recording was 0.3-0.8 M Ω 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) and LFP signals (0.7-170 Hz). Sampling rates were 25 kHz and 500 Hz for MUA and LFP signals, respectively.

Data analysis

Data were averaged across multiple trials. For oxygen responses, baseline levels 10 seconds prior to stimulus onset were subtracted from average signals. Oxygen responses were normalized by the mean oxygen levels 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 to obtain binocular phase tuning curves. For the LFP signal, 60 Hz line noise and 85 Hz noise at the monitor refresh rate in the LFP signal were removed by use of a Matlab function (rmlinesc.m, www.chronux.org). A 500 ms window and 50ms step with a multitaper spectral estimation was applied to obtain LFP spectrograms. Average baseline power at each frequency was subtracted from LFP spectrograms. LFP spectrograms were then normalized by average baseline power at each frequency. For each recording site, average LFP power was obtained across all trials. LFP signals occur over a range of frequencies. The major increase in power across our data is between 25 and 115 Hz, which is consistent with findings in previous studies (Logothetis et al., 2001; Henrie and Shapley, 2005; Katzner et al., 2009; Burns et al., 2010). This high-frequency power band in the LFP may carry information that is more local than the low-frequency components (Logothetis et al., 2001; Liu and Newsome, 2006; Katzner et al., 2009). For the data presented here, LFP signals represent average power between 25 and 115 Hz.

Student's t-test was used to determine response significance for tissue oxygen and neural signals. Because of spontaneous low frequency oscillation in tissue oxygen signals, recording sites without significant ($p < 0.05$) initial dips or positive peaks for a given stimulus condition were excluded from data analysis. The significance of interocular interaction at a

specific phase difference is assessed by pairwise comparison of the binocular response and the monocular control (Wilcoxon signed rank test, $p < 0.05$). Error estimates are in the form of standard error of the mean (s.e.m), unless otherwise noted.

Results

Data presented here were obtained from 54 recording sites in the striate cortex of 11 mature cats. Of the total, eight sites were eliminated because of large spontaneous low frequency oscillation in the tissue oxygen signals and another was removed because of an equipment problem. To obtain these data, RFs for each eye were explored subjectively with patches of drifting sinusoidal gratings adjusted for size to be optimal for each recording site. Qualitative and then quantitative tests were conducted to establish preferred parameters of grating stimuli to be used for binocular stimulation. In general, the optimal parameters of a recording site for the two eyes are approximately equivalent. Stimulus parameters, including orientation, spatial frequency, temporal frequency, and size, are determined from the dominant eye.

Binocular spatial phase tuning of tissue oxygen and neural responses

Tissue oxygen and neural responses were measured simultaneously while drifting sinusoidal gratings were presented dichoptically at different relative interocular phases as illustrated in Fig. 1A. Monocular stimulus presentations to the dominant eye, while the other viewed a blank screen, were interleaved with the dichoptic series to serve as a control condition. For the dichoptic stimuli, initial phase of the grating presented to the dominant eye is fixed while that to the nondominant eye is varied randomly in 60° steps (Fig. 1B). Results for a representative recording site are illustrated as MUA histograms in Fig. 2A. Six relative interocular phase conditions are shown along with a separate result for the monocular control. Corresponding LFP spectrograms are presented in Fig. 2B. Here and for other LFP data, we are interested mainly in high gamma frequencies in accordance with previous work (Logothetis et al., 2001; Viswanathan and Freeman, 2007; Li and Freeman, 2010, 2011). Finally, tissue oxygen responses are shown in Fig. 2C. As the data show, all relative interocular phases, plus the control monoptic condition, elicited substantial MUA, LFP, and tissue oxygen response activity. The same variables are shown again in graphical form as a function of relative binocular phase in Fig. 2D-G. In D, a typical phase specific binocular interactive profile is shown in which spiking activity varies considerably as a function of relative interocular phase (Ohzawa and Freeman, 1986; Ohzawa et al., 1990; Freeman and Ohzawa, 1992; Truchard et al., 2000). The monocular control response is indicated by dashed lines. Statistically significant facilitation and suppression are both illustrated in this response. Maximum facilitation and suppression were elicited by binocular stimuli of 240° (Wilcoxon signed rank test, $p = 2.9 \times 10^{-8}$) and 60° ($p = 5.3 \times 10^{-9}$) phase differences, respectively. This type of binocular interaction phase specific profile is typical of previous work (Ohzawa and Freeman, 1986; Ohzawa et al., 1990; Freeman and Ohzawa, 1992; Truchard et al., 2000). Note however that in Fig. 2E a similar profile is not observed for averaged LFP power.

All interocular phase positions, including the condition that causes maximum suppression in MUA measurements, elicit strong LFP responses. Finally, tissue oxygen responses, shown in Fig. 2C, exhibit typical biphasic responses as reported in previous work (Thompson et al., 2003, 2004; Offenhauser et al., 2005; Thompson et al., 2005; Li and Freeman, 2007). Initially, there is a small negative dip which is followed by a relatively large positive peak. The initial dip is tightly coupled with spiking activity and may reflect oxygen consumption of neurons activated by visual stimuli (Thompson et al., 2003, 2004, 2005; Li and Freeman, 2007). Clearly, the coupling between the initial negative oxygen response and spiking activity does not apply to the binocular interaction profiles in the current study (Fig. 2F). In contrast to phase specific modulated MUA, initial dip oxygen responses to all dichoptic stimuli are larger than those to the monocular control stimulus. This also applies to LFP data which are stronger for all binocular stimuli compared to the monocular response (Fig. 2E). We have previously found that the positive oxygen response peak is not well localized to the site of neural activity (Thompson et al., 2004, 2005). In the current measurement, the positive peak shows different binocular phase tuning compared to the initial dip (Fig. 2G). At a phase of 180° , the positive peak response is identical to that of the monocular control stimulus while the initial dip, MUA, and LFP responses are all significantly stronger than that of the monocular control (Wilcoxon signed rank test, $p=0.01$, $p=2.1 \times 10^{-3}$, and $p=0.01$ respectively).

Data similar to those in Fig. 2 are shown in Fig. 3 from another recording site. Unlike the previous example, MUA data indicate nearly uniform overall suppression in the binocular interaction profile. The inter-ocular phase responses shown in Fig. 3D are all below that of the monocular control response. Of the 45 recording sites we have analyzed, 8 show results similar to those of Fig. 3. This kind of suppression profile is well-documented in binocular interaction experiments conducted with single cell recording techniques (Ohzawa and Freeman, 1986). However, in contrast to the MUA result, LFP and the oxygen initial dip have noticeably different profiles. Visual stimuli at all interocular phases elicited strong LFP responses (Fig. 3E) and clear initial dip oxygen responses (Fig. 3F). In both cases, all responses are above those of the monocular control level. Note that LFP and the oxygen initial dip exhibit clear excitatory binocular response profiles while MUA results show suppressive binocular interaction. The oxygen positive peak, again, shows a different binocular phase tuning profile than that for the initial dip. The binocular stimulus at phase 60° elicits a similar positive peak (Wilcoxon signed rank test, $p = 0.11$) to that for the monocular control even though initial dip and LFP responses are strong (Fig. 3G).

This dissociation between MUA, LFP, and tissue oxygen responses is evident for another representative recording site as illustrated in Fig. 4. In this case, interocular phase MUA responses are generally equivalent to those for the monocular control while two binocular phase positions yield slightly positive but significantly stronger MUA values (Wilcoxon signed rank test, $p = 3.9 \times 10^{-3}$ and $p = 0.03$, respectively). No interocular phase positions elicit suppressive MUA responses. For the two interocular phases with stronger MUA responses, both LFP and the initial dip oxygen responses are also stronger compared to control values, which is consistent with the example site in Fig. 2. For the four phases with similar MUA responses to the monocular control, the non-dominant eye did not affect spiking activity while LFP and the initial dip oxygen response are clearly substantially larger

than those of the monocular control. Again, the positive peak exhibits different binocular responses. For the 60° phase position, a significantly weaker positive peak is seen compared to that for the monocular control (Wilcoxon signed rank test, $p = 0.02$) (Fig. 4G). But both the LFP and the initial dip are significantly stronger than that for the monocular control ($p=2.3\times 10^{-3}$ and $p=1.1\times 10^{-3}$, respectively) (Fig. 4E, F). Considered together, when there is a dissociation between MUA and LFP data, initial dip oxygen responses coincide with those of the LFP whereas the positive peak is not associated with either MUA or LFP.

Coupling between changes in MUA, LFP, and tissue oxygen

In the previous figures, we illustrate three representative response patterns for dichoptic neural interaction. These three types of binocular interaction are observed at all cortical depths, and there are no systematic differences. This is consistent with previous results from single-cell recordings (Ohzawa and Freeman, 1986). As illustrated in MUA data, the first type of response exhibits both suppressive and facilitative portions of the dichoptic interaction profile which is typical of single cell results in previous work (Ohzawa and Freeman, 1986). The second type is mainly a suppressive response, and the third shows facilitative dichoptic interaction for part of the profile, while other interocular phase values exhibit very little difference between monocular and binocular tests. In contrast, LFP values to dichoptic stimuli for all three examples are uniformly higher than control monocular data. The initial dip of oxygen response profiles generally mirror those of the LFP while the positive peak is not coupled with changes in either MUA or LFP. For the three examples, MUA is generally comparable to LFP and the initial dip of oxygen measurements when there is facilitative binocular interaction. However, there is a dissociation between LFP and MUA data for interocular phases with suppressive dichoptic interaction. In these cases, initial oxygen dips and LFP profiles are generally associated. To quantify coupling characteristics between MUA, LFP, initial dips and positive peaks of tissue oxygen responses, we use the following normalized change index, which is based on monocular and binocular responses.

$$C_n = \frac{R_{binoc} - R_{monoc}}{R_{monoc}}$$

where C_n indicates normalized change index, and R_{binoc} and R_{monoc} represent binocular and monocular responses, respectively. A positive or negative C_n represents an excitatory or suppressive binocular interaction, respectively.

As we show in the examples here, LFP and the initial dip tend to change with MUA response when there is a facilitative binocular interaction in MUA, but not for suppressive binocular interaction. We therefore compare for all our data, C_n values for MUA, LFP and initial dip oxygen responses during facilitative and suppressive binocular interaction. The analysis of C_n values is shown in Fig. 5A-D which compares MUA, LFP, and oxygen responses for part of the population of data we have studied. Included here are the 37 recording sites which exhibit clear excitatory dichoptic interaction in spiking activity (Wilcoxon signed rank test, $p<0.05$). Of the 37 recording sites, 21 exhibit both facilitative

and suppressive binocular interaction as shown in Fig. 2 and 16 demonstrate only facilitative interaction as shown in Fig. 4. For recording sites with more than one stimulus condition that shows significant excitatory dichoptic interaction, the binocular phase with maximum spike rate is used. The data show close correlations between MUA, LFP, and the initial dip of oxygen response. In the case of MUA and LFP (Fig. 5A), the correlation is 0.4 ($p=0.01$). For MUA and oxygen (Fig. 5B), the correlation is 0.7 ($p=4.2\times10^{-6}$). A similar correlation is shown in Fig. 5C for LFP and the initial dip of oxygen data ($r=0.7$, $p=1.2\times10^{-5}$). No significant difference is found in average C_n values for MUA, LFP and the initial dip of oxygen response as shown in the histograms in Fig. 5D ($p=0.6$, one-way anova test). In summary, MUA, LFP, and the initial dip of oxygen data are well correlated for this group of sites, which exhibit a predominant excitatory component of their dichoptic interaction profiles.

A very different picture of these relationships emerges when there is a predominant suppressive binocular interaction component in the MUA activity as illustrated in Fig. 5E-H. Only sites which exhibit significant suppressive binocular interaction are included here ($n = 29$). Of the 29 recording sites, 21 exhibit both facilitative and suppressive binocular interaction as shown in Fig. 2 and 8 demonstrate only suppressive interaction as presented in Fig. 3. For a recording site with more than one condition which shows significant binocular suppression, the interocular phase with maximum suppression is used for the analysis. C_n values in Fig. 5E, F compare MUA to LFP and MUA to the initial dip of oxygen response, respectively. There is no significant correlation between MUA and LFP ($r = -0.18$, $p = 0.33$, Fig. 5E). A similar lack of association is illustrated for MUA and the initial dip of oxygen data in Fig. 5F. In this case, the correlation is -0.2 ($p=0.3$). In striking contrast to the insignificant correlations in Fig. 5E, F, there is a positive association between LFP and the initial dip of oxygen response as shown in Fig. 5G ($r=0.5$, $p = 5.9\times10^{-3}$). Mean values for the three parameters measured, MUA, LFP and the initial dips of oxygen responses are significantly different as shown in the histograms of Fig. 5H for the population of recording sites we have measured ($p=9.2\times10^{-13}$, one-way anova test). These data show clearly that predominant spiking activity from stimulation of a given stimulated eye is suppressed by activation of the fellow eye. However, neither LFP nor the initial dip of oxygen response follows the suppressive profile of MUA.

Although the initial dip is reliably detected in tissue oxygen measurements with our microsensor, it is generally not considered in fMRI investigations because it is weak or illusive compared to the positive BOLD signal and it is usually not observed (Logothetis, 2000; Buxton, 2001). By contrast, we are generally able to obtain consistent and reliable in vivo initial dip data from tissue oxygen concentration responses and it is well localized to the site of neural activity (Thompson et al., 2004, 2005). Unlike the initial dip, the positive peak is not well localized to the site of neural activity (Thompson et al., 2005). But it is still of clear interest to analyze how the protocol we have used here applies to positive peak data. To see if and how the positive peak changes during facilitative and suppressive binocular interaction, and to examine how it relates to neural activity, we have determined C_n values for it based on monocular and binocular responses as we have considered above for the initial dip.

Fig. 6A-C compares C_n values for MUA, LFP, and the positive peak of oxygen response for 37 recording sites that show significant excitatory binocular interaction in spiking activity (Wilcoxon signed rank test, $p < 0.05$). Fig. 6A, B compare C_n values for MUA and the positive peak, and for LFP and the positive peak, respectively. Although MUA and LFP are clearly associated under excitatory binocular interaction (Fig. 5A), neither MUA nor LFP is correlated with the positive peak. The correlations with the positive peak are -0.3 ($p = 0.1$) for MUA and -0.1 ($p = 0.6$) for LFP, respectively, as shown in Fig. 6A, B. In contrast to the close relationship between the initial dip, MUA and LFP during excitatory binocular interaction (Fig. 5), the positive peak is clearly not associated with the changes in either MUA or LFP. However, on average there is no significant difference between C_n values for MUA (0.56 ± 0.06), LFP (0.65 ± 0.08) and the positive peak (0.58 ± 0.15) as shown in Fig. 6C ($p = 0.83$, one-way anova test) for the overall population.

For suppressive binocular interaction, the C_n values for MUA, LFP and the oxygen positive peak from 29 recording sites are compared in Fig. 6D-F. As shown above in Fig. 5D, MUA and LFP signals are not significantly correlated during suppressive binocular interaction. Similarly, there is no significant correlation between the oxygen positive peak and either MUA (Fig. 6D, $r = 0.1$, $p = 0.7$), or LFP (Fig. 6E, $r = 0$, $p = 0.8$). Average responses for our population of cells for MUA, LFP and the positive peak are -0.44 ± 0.04 , 0.42 ± 0.07 and 0.49 ± 0.16 , respectively, which are highly different (Fig. 6F; $p = 2.4 \times 10^{-9}$, one-way anova test). Although the positive peak is not significantly coupled with changes in either MUA or LFP, its population-average response is similar to LFP ($p = 0.7$) but not MUA ($p = 5.8 \times 10^{-7}$). Taken together, the positive peak does not closely follow the changes in either MUA or LFP. But it has a similar population-average change to MUA and LFP with excitatory binocular interaction, but only to LFP during a suppressive stage.

Discussion

We have conducted experiments intended to elucidate relationships between synchronized synaptic processes, spiking output and metabolic activity in the visual cortex. Our approach is to use a visual stimulus protocol that provides separation of synaptic and spiking activity. This is possible with the dichoptic presentation of drifting sinusoidal gratings at different relative interocular phase positions that we have used. This stimulus protocol permits an assessment of binocular interaction in the visual cortex, which is the basis of stereoscopic depth discrimination (Barlow et al., 1967; Ferster, 1981; Ohzawa and Freeman, 1986; Freeman and Ohzawa, 1990; Ohzawa et al., 1990). We obtained simultaneous measures of neural activity and tissue oxygen response at co-localized regions of the visual cortex. Neural activity is measured as MUA and gamma-band LFP. The major finding is that there are clear differences in the degree to which parameters are related, that depend on whether MUA neural activity is in a suppressed or facilitated condition. Specifically, MUA and LFP are generally correlated and associated with changes in oxygen metabolism during excitatory binocular interaction. In marked contrast, MUA and LFP are not coupled during suppressive binocular interaction. There is generally an increase in LFP during reduced MUA activity and oxygen metabolism is coupled with LFP but not MUA.

Relation between MUA and LFP

The relationship between MUA and LFP has been studied previously in a variety of applications. These two measures of neural activity represent, respectively, high and low frequency components of an electrophysiological signal. LFPs can be measured over a wide range of frequencies. MUA is believed to reflect output spiking activity from a group of neurons. LFP, on the other hand, is thought to represent a combination of synchronized synaptic activity in a localized region (Logothetis et al., 2001; Logothetis, 2002, 2003). A variety of findings have been reported in previous studies. Close correlation has been found between the two measures of neural activity in visual cortex (Logothetis et al., 2001; Nase et al., 2003; Henrie and Shapley, 2005; Liu and Newsome, 2006; Spinks et al., 2008), barrel cortex (Jones et al., 2004; Devor et al., 2005), and auditory cortex (Mukamel et al., 2005). Weak correlation has been observed in other work (Viswanathan and Freeman, 2007; Burns et al., 2010; Lima et al., 2010; Lippert et al., 2010; Bartolo et al., 2011). It is difficult to evaluate the relationship between MUA and LFP signals due to the discrepancies in these results. Our current use of binocular stimulation, which yields both excitatory and suppressive phases in neural discharge, provides some insights. Our recordings are made in primary visual cortex, where information from left and right eyes is first integrated. In this context, excitatory binocular interaction occurs when input from one eye enhances spiking activity to visual stimulation of the other eye. Net facilitation of spiking discharge may be associated with increased synaptic activity that summates linearly in visual cortex (Jagadeesh et al., 1993). This is consistent with close coupling between MUA and LFP during excitatory binocular interaction. On the other hand, during suppressive binocular interaction, spiking activity of one eye is suppressed by visual stimulation of the other. Since projections from LGN to striate cortex are thought to be exclusively excitatory (Ferster et al., 1996), it is not likely that suppressed spiking activity is caused by direct inhibition from relay cells in the LGN. In this case, ocular suppression is likely to arise from cortical inhibitory interneurons. In addition, gamma oscillations are raised during increased inhibitory activity (Traub et al., 1996; Tamas et al., 1998; Whittington and Traub, 2003). This could account for increases in LFP activity during suppression as we have found here and the dissociation between the two measures of neural activity during suppressive binocular interaction.

The nature of MUA and LFP signals

The LFP is thought to represent a weighted average of synchronized dendro-somatic components of input signals of a neural population. In contrast, MUA represents output measured as action potentials (Logothetis et al., 2001; Logothetis, 2002, 2003). To a first approximation, our current results are consistent with an input-output assumption. The visual stimuli used in our experiments are drifting gratings of optimal parameters for spiking activity that are presented dichoptically. Monocular stimulation of each eye is maintained during all interocular phase positions. The total binocular input is obviously stronger than that for each monocular stimulus. As we have shown, LFP signals to binocular stimuli are generally stronger than those of monocular responses, but this is not the case for spiking activity. These results seem to be consistent with the input-output assumption about the nature of LFP and MUA signals. However, MUA and LFP signals are almost certainly more complicated (Burns et al., 2010). In the present study, LFP and MUA signals are recorded

from the same electrode in striate cortex which is a highly recurrent network. Despite different sampling volumes and frequency bands of MUA and LFP signals, these two measures of neural activity may be strongly influenced by recurrent cortical connections. The extent of linearity of a recurrent network may determine the correlation between MUA and LFP signals (Burns et al., 2010). If this is the case, our current results suggest that intracortical inhibition may be a key factor in the linearity of a recurrent network.

The coupling between BOLD signals and neural activity

A current notion is that the BOLD signal in fMRI is more a reflection of LFP than of spiking activity (Logothetis et al., 2001). However, it also seems clear that both LFP and spiking activity are coupled to the BOLD signal (Kim et al., 2004; Mukamel et al., 2005; Nir et al., 2007). A tentative reconciliation of these differing interpretations is available from the findings reported here. We assume that suppressive binocular interaction in spiking activity is generated via intracortical inhibitory interneurons since, as we note above, LGN projections to visual cortex are exclusively excitatory. During excitatory binocular interaction, local inhibitory interneurons are presumably minimally activated. LFP and spiking discharge are well correlated with metabolic activity. On the other hand, during suppressive binocular interaction, inhibitory interneurons are presumably strongly activated. In this case spiking activity is suppressed, but LFP and metabolic activity are increased, since inhibitory input increases LFP (Traub et al., 1996; Tamas et al., 1998; Whittington and Traub, 2003) and metabolic activity (Cauli et al., 2004; Kocharyan et al., 2008; Enager et al., 2009). Considered together, LFP appears to be a more coordinated measure of underlying metabolic activity compared to that of spike discharge. However, it is important to note that the dissociation between these two measures of neural activity, as reported here, is obtained by variable binocular stimulus conditions that selectively enhance a localized excitatory or inhibitory intracortical drive. In experiments with complex stimuli such as checkerboards or natural-scenes, that are commonly used in fMRI studies, excitatory and inhibitory processes are mixed, so that the relationship between LFP and spiking activity may be variable.

Our measure of metabolic function is local tissue oxygen concentration in visual cortex. Oxygenation changes depend on relative alterations in cerebral blood flow and cerebral metabolic rate of oxygen. A clear BOLD signal interpretation depends on detailed knowledge of brain oxygen metabolism and its relation to blood flow (Buxton, 2010). Direct measurement of oxygen response in brain tissue may provide significant relevant information. Here, we find better coupling for synaptic activity between metabolic and LFP data for both excitatory and suppressive binocular interaction. This is consistent with an analogous effect between BOLD signals and synaptic activity in fMRI signals. It may be possible to test this possible analogy because the cerebral metabolic rate of oxygen is reported to be proportional to cerebral blood flow (Sheth et al., 2004). Tissue oxygen consumption measurements estimated by the initial dip may therefore enable predictions of changes in cerebral blood flow. As we have shown in previous work (Thompson et al., 2003, 2004, 2005; Li and Freeman, 2007), tissue oxygen response typically includes the small initial dip followed by a large positive peak. The initial dip is closely coupled with neural activity and may reflect activity-induced oxygen consumption in co-localized regions. On

the other hand, the positive peak is not closely coupled with neural activity and may reflect an activity-induced increase in blood flow. However, the initial dip and the positive peak of oxygen response overlap partially in time (Thompson et al., 2003, 2005) such that the response of each reduces the amplitude of the other. Therefore, measured initial dip amplitude may be lower than actual change in oxygen consumption. Similarly, an increase in blood flow is likely to be underestimated by the measured size of the positive peak. The initial dip has a high resolution of a few hundred micrometers whereas the positive peak has a relatively large spatial scale of ~ 1.4 millimeter (Thompson et al., 2005), which is close to the spatial resolution of the positive BOLD signal in fMRI (Logothetis and Wandell, 2004). Voxel size often used in fMRI is around $3 \times 3 \times 3 \text{ mm}^3$, which is an order of magnitude larger than the spatial scale of the positive oxygen response. As we show here, the positive peak does not closely follow the change in either form of neural activity for an individual recording site, but its population-average response changes similarly with both MUA and LFP for excitatory binocular interaction, but only with LFP under suppressive binocular interaction. Based on population average results, LFP seems to be better reflected in the positive peak than MUA. This is consistent with the close coupling between the BOLD signal and synchronized synaptic activity in fMRI.

Conclusion

In summary, our results demonstrate different coupling characteristics between metabolic, spiking, and LFP levels in visual cortex which are tied to excitatory or inhibitory phases of neural activity. Spiking and LFP activity correlate well with tissue oxygen metabolism when visual input to both eyes generates excitatory binocular interaction. In contrast, when dichoptic input causes suppressive binocular interaction, there is dissociation between spiking activity and LFP. In this case, LFP and oxygen metabolism are closely associated. Collectively, these results suggest that inhibitory drive may be a primary factor that accounts for the different ways that spiking and LFP forms of neural activity are associated with metabolic events.

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Highlights

- Simultaneous O₂ metabolic & neural responses in visual cortex to binocular stimuli
- Synaptic & spiking activity coupled during binocular facilitation
- Synaptic & spiking activity dissociated during binocular suppressive interaction
- O₂ metabolism changes with synaptic but not spiking activity during suppression

Fig. 1.

Visual stimuli used in the experiment. (A) Drifting gratings with sinusoidal luminance profiles are presented at preferred orientation, spatial frequency, temporal frequency and size at different relative interocular phases to both eyes. The vertical lines indicate phase differences between the stimuli. (B) Drifting sinusoidal gratings are depicted at four different interocular phases. The initial spatial phase is fixed at 0° for the dominant eye (right column) while initial phase is varied for the non-dominant eye (left column) over 6 values (0° , 60° , 120° , 180° , 240° , and 300°). For presentation, only 4 phase differences are shown).

Fig. 2.

Neural and tissue oxygen responses are illustrated for a representative recording site in striate cortex. (A-C) MUA histograms, LFP spectrograms, and oxygen responses are shown for 6 binocular stimuli and a monocular control stimulus presented to the dominant eye. "0" on the horizontal axes represents onset of the visual stimulus. Data are averaged across 48 trials. The orientation, spatial frequency and temporal frequency are 115°, 0.7 cpd and 5Hz. The size of the stimuli for both eyes is 4° in diameter. The depth of this recording site is 3,990 μm . MUA histograms prior to the onset of visual stimulus represent spontaneous spiking activity. (D-G) Averaged MUA, LFP, the initial dip and positive peak of oxygen responses during stimulus presentation as a function of relative interocular phase,

respectively. Mean LFP is averaged across a frequency range of 25-115 Hz. A classical phase-specific binocular interaction pattern is shown for the MUA signal in (D). Dashed lines represent responses to the monocular stimulus in the dominant eye. Error bars are ± 1 s.e.m.

Fig. 3.

Results are shown for another recording site in the same format as Fig. 2. Suppressed neural activity (MUA) is exhibited in (D) for all binocular stimuli. Data are averaged across 32 trials. The orientation, spatial frequency and temporal frequency are 140°, 0.5 cpd, and 4Hz. The size of the stimuli for both eyes is 5° in diameter. The depth of this recording site is 2,175 μm . Symbols are identical to those in Fig. 2.

Fig. 4.

Results are shown for a third recording site. In this case, dichoptic presentation yields facilitated MUA levels at two binocular phases and levels similar to the monocular control condition at other relative interocular phases. Data are averaged over 80 trials. The orientation, spatial frequency and temporal frequency 60°, 0.4 cpd and 2Hz. The stimulus size for both eyes is 7° in diameter. The depth of this recording site is 1,220 μm. Symbols are identical to those in Fig. 2.

Fig. 5.

Comparison of normalized changes (C_n , as defined in Results) for MUA, LFP, and the initial dip of oxygen response for recording sites with significant facilitative (A-D) and suppressive (E-H) binocular interaction. Each data point represents a recording site. Comparisons of C_n between MUA and LFP (A, E), MUA and the initial dip of oxygen response (B, F), and LFP and the initial dip of oxygen response (C, G), respectively. (D, H) Average C_n for MUA, LFP, and the initial dip of oxygen response. Error bars represent ± 1 s.e.m. Dashed lines indicate least-squares regression fits.

Fig. 6.

Relationship between C_n values for MUA, LFP, and the positive peak of oxygen response for recording sites with significant facilitative (A-C) and suppressive (D-F) binocular interaction. (A, D) Comparison of C_n values between MUA and the positive peak. (B, E) Comparison of C_n values between LFP and the positive peak. (C, F) Average C_n values for MUA, LFP, and the positive peak of oxygen response.