Transient Cortical Excitation at the Onset of Visual Fixation

Primates actively examine the visual world by rapidly shifting gaze (fixation) over the elements in a scene. Despite this fact, we typically study vision by presenting stimuli with gaze held constant. To better understand the dynamics of natural vision, we examined how the onset of visual fixation affects ongoing neuronal activity in the absence of visual stimulation. We used multiunit activity and current source density measurements to index neuronal firing patterns and underlying synaptic processes in macaque V1. Initial averaging of neural activity synchronized to the onset of fixation suggested that a brief period of cortical excitation follows each fixation. Subsequent single-trial analyses revealed that 1) neuronal oscillation phase transits from random to a highly organized state just after the fixation onset, 2) this phase concentration is accompanied by increased spectral power in several frequency bands, and 3) visual response amplitude is enhanced at the specific oscillatory phase associated with fixation. We hypothesize that nonvisual inputs are used by the brain to increase cortical excitability at fixation onset, thus "priming" the system for new nonvisual inputs. We used multiunit activity and multiunit activity (MUA) profiles in area V1 in awake behaving macaque monkeys. CSD analysis indexes the first-order neuronal firing. We evaluated the hypothesis that nonvisually mediated increase in firing, coupled with the onset of fixation, may reflect an underlying active process that amplifies neuronal responses to retinal inputs generated at fixation. Evaluation of this "Fixation-Amplifier" hypothesis is of fundamental importance because, aside from a few other forms, such as pursuit eye movements, we found that brief, snapshot-like fixation provides the major means of sampling the visual environment.

Data were collected with the monkeys in complete darkness to eliminate the possibility that effects stemmed from visual stimulation consequent to eye movements. We analyzed fixation-related changes in laminar current source density (CSD) and multiunit activity (MUA) profiles in area V1 in awake behaving macaque monkeys. CSD analysis indexes the first-order synaptic response in a neuronal population. Multielectrode sampling of CSD and its action potential (MUA) correlates provide an efficient method that is sensitive to subtle processes, such as subthreshold inputs and balanced excitation/inhibition inputs. Our results confirm that nonvisually mediated neuronal excitation occurs in V1 at fixation onset. We hypothesize that this effect may reflect modulation of the local neuronal ensemble, preparatory to the arrival of visual inputs generated at fixation.

Introduction

In natural vision, information is actively acquired by directing gaze toward or "fixating" on points of interest (Yarbus 1967). Humans and other primates typically scan a visual scene with a large number of brief fixations, at a rate of 2–3/s, separated by rapid "saccadic" eye movements. At each fixation, a volley of retinal outputs courses into the system and produces a spatiotemporal pattern of brain activation determined by the interaction of stimulus qualities with properties of neurons within each of the visual pathways. We were interested in the influence of eye movement dynamics on visual processing and perception (Gallant et al. 1998; Vinje and Gallant 2000, 2002). "Perisaccadic" modulation of neuronal firing, usually suppression during the saccade, followed by enhancement starting at the onset of fixation, has been observed throughout the visual pathways from the lateral geniculate nucleus (LGN) to prefrontal cortex (reviewed by Purpura et al. 2003). Interestingly, perisaccadic modulation is also observed in total darkness and, thus, is at least partially nonvisually mediated (Ringo et al. 1994; Sobotka and Ringo 1997; Lee and Malpeli 1998; Nakamura and Colby 2000; Sylvester and Rees 2005; Sylvester et al. 2005).

Although much of the recent interest in perisaccadic modulation has focused on the neural basis of perceptual saccadic suppression (Ross et al. 2001; Reppas et al. 2002; Thiele et al. 2002), we focused on the following fixation-related increase in neuronal firing. We evaluated the hypothesis that nonvisually mediated increase in firing, coupled with the onset of fixation, may reflect an underlying active process that amplifies neuronal responses to retinal inputs generated at fixation. Evaluation of this "Fixation-Amplifier" hypothesis is of fundamental importance because, aside from a few other forms, such as pursuit eye movements (Lisberger and Nusbaum 2000; Gardner and Lisberger 2001), the brief, snapshot-like fixation provides the major means of sampling the visual environment.

Materials and Methods

Data for this study were collected during the course of experiments examining mechanisms by which nonretinal influences modulate neuronal ensemble activity in the visual and auditory systems. The effects related to stimulus processing are reported elsewhere (Mehta et al. 2000b; Shah et al. 2004). This report concerns the eye movement-related activity measured in V1, while the animal waited in total darkness, during time periods between stimulus trials.

Subjects and Preparation

Complete details of the surgical procedures can be found in earlier reports (Schroeder et al. 1998; Mehta et al. 2000a). Briefly, 2 male macaques (Macaca fascicularis), weighing 6–9 kg, were surgically prepared for chronic, awake intracranial recordings. All animal care and procedures were approved by the Institutional Animal Care and Use Committee of the Nathan Kline Institute and were in accordance with the Principles of Laboratory Animal Care (the National Institutes of Health Publication no. 86-23, revised 1985). Preparation of subjects for chronic awake recording was performed using aseptic techniques, under general anesthesia. To provide access to the brain and to promote an orderly pattern of sampling across the surface of visual areas, matrices

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of 18-gauge stainless steel guide tubes were positioned normal to the brain surface for orthogonal penetration of the lateral striate operculum, targeting the foveal representation of area V1. Individual epidural guide tubes were positioned over central and frontal sites to serve as ground and reference electrodes. The matrices were placed within small, appropriately shaped craniotomies to rest against the intact dura. Together with socketed Plexiglas bars (to permit painless head fixation), they were secured to the skull with orthopedic screws and embedded in dental acrylic. Recovery time of 2 weeks was allowed before the beginning of data collection.

Electrophysiological Recordings
Animals sat in a primate chair in a dark, isolated, electrically shielded, sound-attenuated chamber with heads fixed in position. Laminar profiles of field potentials (i.e., local electroencephalography [EEG] signals) and concomitant MUA were obtained by recording with a linear array multicontact electrode (14 equally spaced contacts; Fig. 1) constructed with an intercontact spacing of 150 μm and positioned to sample all the layers simultaneously (Barna et al. 1981). The impedance at each contact was 0.1-0.3 MΩ. Each intracortical electrode referenced to an epidural electrode at the frontal midline. Signals were amplified with a bandpass of 1-3000 Hz. MUA were obtained by band-pass filtering the signals (0.5-2 kHz), full-wave rectifying and digitizing at 4 kHz. For field potential recordings, the amplifier outputs were sampled at 2 kHz. Although this procedure can cause aliasing of high-frequency signals, analysis of power spectra showed that most of the power in the signal is concentrated below 50 Hz and there is negligible power above 1 kHz (Schroeder et al. 1998). Signals were processed using PC-based data acquisition system (Neuroscan, El Paso, TX) and analyzed using custom-made code in MATLAB (The Mathworks Inc., Natick, MA). One-dimensional CSD profiles were calculated using a 3-point formula for estimation of the second spatial derivative of voltage (Nicholson and Freeman 1975). CSD analysis provides an index of the location, direction, and density of transmembrane current flow; this is the first-order synaptic process that in turn generates postsynaptic potentials and the extracellular distribution of local field potentials (Schroeder et al. 1998).

Determination of Visual Fixation Effects
Eye position was monitored using a Stoelting, Model 4100/4500 infrared system, which tracked one eye with a resolution of 1.0° of visual angle and a 60-Hz sampling rate. The monkeys in this study were trained on a fixation task for the purposes of studying selective attention effects (Mehta et al. 2000a) and mechanisms of event-related potential (ERP) generation (Shah et al. 2004). During the training session of the experimental paradigm, monkeys were first trained to fixate within a 4.5° window around a light-emitting diode (LED) and then trained to hold the gaze within the fixation window when the LED was off. The LED was used only during the training session but not during the experiments reported in the present study. Visual stimuli (10 μs light flashes at 2/s) generated by a Grass PS22 Photo Stimulator projected onto a diffuser in front of the monkey at the viewing distance of 43 cm, subtending 11.8° of the visual field. Neither the stimulator nor the diffuser had background luminance, and there were no other light sources (e.g., equipment LED indicators, etc.) in the chamber during the experiments. Further paradigmatic details are available in Mehta et al. (2000a). However, critical to the present report is the fact that stimulation paused whenever the monkey released the switch or broke fixation. As the task was self-paced, the monkeys would often “take a break” by releasing the switch and/or gazing around outside of the fixation window. Data used in the present study were acquired during these periods. The animals were spontaneously looking all around the chamber. There was no apparent preferred plane or directional bias of fixation. While stimulus trains were being presented, monkeys were holding fixation and there were no saccades prior to or after visual stimulation for at least 500 ms.

Data Analysis
Data used in the present study were obtained from 13 experimental sessions (8 from one monkey and 5 from the other), each entailing acute positioning of an electrode array in V1, followed by extensive sampling

Figure 1. Laminar profile of stimulus and visual fixation-related neuronal activity in area V1 from one experimental session. On the left is a schematic of the linear array multielectrode used to record field potential and MUA from V1. (A) Stimulus-related (foveal light flash) averaged CSD profile, with overlay of concomitant MUA traces from a subset of the electrodes. CSD profile is color coded; current sinks (red) reflect net inward flowing transmembrane currents in the local neuronal population; current sources (blue) reflect net outward current flow. Bottom trace shows the time of stimulation. Arrow indicates the major excitatory response to thalamic input in lamina 4C; the earlier oscillation in MUA, unaccompanied by prominent CSD features, reflects thalamic input (see Schroeder et al. 1998). CSD profiles were calculated from the field potential profiles, and laminar boundaries (indicated by horizontal dotted lines) were determined using functional criteria derived from prior studies (see Materials and Methods). (B) CSD and concomitant MUA profiles associated with the onset of fixation. Bottom traces represent a subset of horizontal eye position traces. Average (n = 100 trials) was made from single trials aligned at the onset of fixation. Note that the CSD and MUA profiles in (B) are displayed at a larger gain than in (A). Both stimulus- and fixation-related activities were recorded during the same experimental session with the same electrode position.
of both ambient- and stimulus-related activity. Each experiment corresponded to one multielectrode penetration. Saccade onset and end points were defined based on the velocity of eye movement. The maximum eye velocity was determined for each saccade that exceeded 40°/s threshold. Saccade onset was defined as the time when eye speed reached 10% of the maximum velocity. Fixation onset was defined as the time when eye velocity had decreased to 10% of the maximum. Saccade duration was considered as the interval between these 2 points. Only epochs with fixations maintained for at least 500 ms after the saccade were involved in the analyses. Trials containing blink or muscle artifact were discarded.

In the first part of the data analysis, averages were made from data epochs from -500 to +500 ms, with time zero set as 1) the onset and 2) end of saccade (i.e., onset of fixation) and 3) to the onset of visual stimulation, respectively. These zero points allowed us to look for saccade-related, fixation-related, and stimulus-evoked effects, respectively. CSD profiles were calculated from averaged field potentials profiles, and the laminar assignment of the CSD channels was defined as supragranular, granular, and infragranular according to functional criteria established by earlier studies (Schroeder et al. 1991, 1998; Givre et al. 1999a, b) to make the extensive single-trial analysis manageable. Data reduction was necessary (see also Shah et al. 2004). We full-wave rectified the signals from all channels in the CSD profile for each of the 13 experiments and selected one channel from each laminar division (supragranular, granular, and infragranular), that is, the channel with the largest integral area of the full-wave-rectified CSD. Summary Averaged REtified Current (sAVREC) and summary Averaged MultiUnit Activity (sAVMUAA) values were calculated by averaging together the rectified CSD and MUA signals for each single trial (note that this signal contains both phase-locked and nonphase-locked activity).

In the second part of the analysis, phase distribution across trials was determined by assigning a phase to each time point of the data by using Hilbert Transform for each single trial. Phase was determined with Hilbert Transform in broadband without narrow band filtering of the signal. This capitalizes on high time resolution that is not ensured with narrow band filtering the data or with wavelet transforms, which also can generate artifacts during phase assignment (Netoff and Schiff 2002; Kruglikov and Schiff 2003). Hilbert Transform is a powerful tool to assign phase to raw signals like EEG/CSD. This method has been used extensively in EEG studies to analyze such signals (Netoff and Schiff 2002; Kruglikov and Schiff 2003). Its advantage is that phase for a broadband signal can be found. The Hilbert Transform extracts the phase of the dominant cortical cycle regardless what dominant cycle is present (e.g., alpha, theta, etc.). Even if the signal is broadband, the phase denotes the position of a peak or trough in the signal "and can be used to characterize the oscillation’s dynamics." Phase is also physiologically relevant because neuronal excitability is regulated according to the phase of ongoing local oscillations (Bishop 1935; Kruglikov and Schiff 2003; Lakatos et al. 2005, 2007). To avoid being biased by large transient fluctuations in the raw signal, we used the Rayleigh test for statistical evaluation of the results of the Hilbert Transform. This determined if the distribution of the phase of CSD was significantly different from a uniform (random) distribution for each data point from -500 ms pre-fixation onset to +500 ms postfixation onset, using an analysis window 1 data point (0.5 ms) wide. Also, in order to more directly relate the results of the Hilbert Transform analysis to neuronal oscillatory activity, we took the additional step of analyzing oscillation amplitude and phase concentration of prefixation and postfixation (-300 and 77.5 ms, respectively) in 3 EEG bands (i.e., 3-8 Hz for delta/theta band, 8-20 Hz for alpha/beta band, and 20-80 Hz for beta/gamma band) in supragranular, granular, and infragranular layer. In each layer, we first filtered the signals into 3 bands and ran the Hilbert Transform in each band. After the transform, we got phase and amplitude information of each band. Second, we used Rayleigh tests for determining phase concentration (i.e., calculating Rayleigh statistics parameter R intertrial coherence, and their P values) in each band. Third, paired t-tests were used to determine significant prefixation to postfixation amplitude increase.

In the final part of the analysis, we calculated visual-evoked response amplitudes as a function of prestimulus phase by first sorting the phase values gained by Hilbert Transform from -π to π radians. Then the permutation vector obtained during the phase sorting procedure was applied to the event-related response amplitude values (Lakatos et al. 2005). A whole cycle was divided into 6 even parts, and response amplitudes falling into the same phase division were averaged together (Fig. 5). By sorting the visual-evoked response amplitudes and then applying the permutation vector to the phase values, the phase associated with the largest response amplitude (called "ideal" phase) can be determined. Similarly, the phase associated with the smallest response amplitude (called "worst" phase) was assessed. CSD amplitude difference between ideal and worst phase was determined by subtracting the amplitude values obtained in ideal and worst phase. This CSD amplitude difference was determined for the prestimulus period (from -170 to 0 ms relative to stimulus onset) and also for the poststimulus period (from 30 to 200 ms relative to stimulus onset). Amplitude and frequency values were statistically analyzed by Student’s t-test. Phase was analyzed by circular statistics. To test the difference between 2 phase distributions, Watson U² test was used. The Rayleigh statistic was used as a test of uniformity of phase distribution (Fig. 3).

**Results**

**Effect of Fixation on Neural Activity**

Figure 1A displays the averaged laminar CSD profile, time locked to the onset of a foveal light flash, obtained from a site in the foveal representation of V1. On the extreme left is a schematic of the electrode positioned with respect the laminar expanse of V1 (depicted with the cytochrome oxidase section). In the CSD profile, extracellular current sinks (representing net inward flowing current) are colored red-yellow and sources (representing net outward flowing current) are colored blue. Superimposed on the CSD plot are selected MUA recordings from the supragranular, granular, and infragranular laminae, acquired simultaneously with the CSD profile. This profile is typical of the V1 response to this stimulus, as established by earlier studies (Schroeder et al. 1991, 1998; Givre et al. 1995; Melha et al. 2000a, 2000b). The initial major postsynaptic response, a current sink with a concomitant increase in action potentials (arrow), occurs in lamina 4C and is followed by a smaller activation in the supragranular laminae; this granular to supragranular activation sequence signifies feedforward activation by afferents from LGN (Schroeder et al. 1991, 1998; Givre et al. 1995).

Figure 1B depicts the averaged laminar CSD and MUA profiles phase locked to the onset of fixation, at the same recording site; the conventions are identical to Figure 1A, though the amplitude calibrations are different. Like the local stimulus-evoked response, this activity appears excitatory, but the fixation-related excitation appears smaller (see MUA and CSD amplitude calibrations). Comparison of the peak amplitudes of fixation-related and stimulus-evoked excitatory responses across all 13 experiments (see Materials and Methods) revealed that this difference is significant (paired t-test, P < 0.05 for CSD; P < 0.01 for MUA). Also in contrast to the stimulus-evoked response (described above), fixation-related response does not fit the simple feedforward (granular followed by extragranular excitation; Schroeder et al. 1998) pattern, based on either the timing or laminar distribution of activity. We did not attempt to quantitatively analyze the laminar timing pattern of the fixation-evoked response; given the relatively coarse (约17 ms) temporal resolution of the eye tracker. Finally, prior to fixation onset (during the saccade), there is mild suppression of local neuronal firing, whereas no such effect is visible in the stimulus-evoked profile.

To describe fixation effects more quantitatively, we computed (Fig. 2) the grand mean of the normalized single-trial-rectified CSD (sAVREC) and grand mean of the corresponding MUA.
measure (sAVMUA). This analysis indicates that prior to fixation, there is actually CSD suppression accompanying MUA suppression. This is a subtle effect compared with the subsequent fixation-related enhancement, but whenever saccade-related MUA suppression was detected, it was accompanied by CSD suppression. No such “prior” suppression has ever been observed in similar analyses of the stimulus-evoked response in V1 (Givre et al. 1994, 1995; Schroeder et al. 1998; Mehta et al. 2000a, 2000b; Shah et al. 2004). Significantly, the quantification also shows that neuronal suppression peaking during the saccade and followed by enhancement at fixation onset is typical of the entire data set. The prefixation suppression peaked in both AVREC and AVMUA around the time of saccade onset (saccade duration: 84.0 ms [mean] ± 33.6 [standard deviation {SD}]). The postfixation enhancement peaked at 77.5 and 51.0 ms (relative to the fixation onset) in AVREC and AVMUA, respectively. Neuronal activity increase after fixation was significant at P < 0.01 for AVREC and P < 0.05 for AVMUA (paired t-tests). These effects were similar when the averaging of single trials was synchronized to saccade onset (not shown). However, comparison of fixation-related and saccade-related excitatory response peak amplitudes across all 13 experiments showed that the fixation-related response is significantly larger (paired t-test, P < 0.05 for CSD; P < 0.05 for MUA).

**Physiological Mechanisms of Visual Fixation Effects**

The foregoing analysis indicates that in the absence of visual stimulation of any sort, there is neuronal excitation immediately following the onset of fixation. The second goal of this study was to evaluate the underlying mechanisms. An obvious possibility is that the effect is a local excitation, akin to an “evoked” response, triggered by some form of input, for example, an efferent copy of the eye movement command. Another possibility is that the effect is generated by reorganization of ongoing activity without addition of energy to the system. This type of effect is referred to as “phase resetting” or “phase modulation” (Makeig et al. 2004; Shah et al. 2004; Lakatos et al. 2005, 2007). Both types of mechanism predict a shift from a random distribution of oscillatory phase prior to the onset of fixation, to an organized or “phase concentrated” state with fixation. However, in an evoked response, phase concentration would accompany an increase in spectral power. To assess phase concentration, we examined the uniformity of phase distribution by using the Rayleigh test for each single time point over the interval from –500 ms prefixation onset to +500 ms postfixation onset (Fig. 3A). Initial analyses revealed that the phase distribution pattern was similar in the supragranular, granular, and infragranular laminar divisions; therefore, the data are collapsed across the layers. In the prefixation period (including the time frame of the saccade), the phase distribution did not differ significantly from a random (uniform) distribution (Rayleigh tests, P > 0.05). However, beginning at fixation onset and continuing for about 200 ms postfixation, phase distributions were significantly different from uniform (P < 0.001). This time frame brackets the arrival time of retinal inputs to V1 (Maunsell and Gibson 1992; Schrolesky et al. 1998; Schroeder et al. 1998; Chen et al. 2006), and thus, these findings are in line with our hypothesis that oscillatory phase concentration reflects modulation of the local neuronal ensemble, preparatory to the arrival of visual inputs generated at fixation.

To further characterize this effect, we chose 2 time points, fixation onset (the beginning of phase concentration) and 300 ms prefixation onset (just before the beginning of saccade-related influences—see Fig. 2). We then calculated the phase distribution of single trials at –300 and 0 ms, for the entire data set, collapsing across experiments and cortical layers (Fig. 3B). At the time of fixation the grand mean (n = 13) phase (θ) was 1.85 rad and the pooled angular deviation (s) was 0.91. Pooled (grand mean) phase distribution for a particular time point tells us how uniform the distribution is for the whole data set, as well as the most common phase at that time point. Pooled angular deviation (s) gives information about the variability from session to session at the time of fixation.

These findings raise 2 additional questions. First, is this phase concentration indicative of “pure” phase resetting? According to earlier formulations (Shah et al. 2004), pure phase resetting produces event-related responses without attendant increase in power at relevant frequencies. Second, given that the Hilbert Transform extracts phase without reference to oscillation frequency, how exactly are we to interpret these findings with respect to the well-known EEG bands? In order to address these issues, we examined the degree to which fixation-related phase concentration (indexed by Rayleigh test) is accompanied
by change in amplitude (indexed by Hilbert Transform) in 3 bands (3–8 Hz for delta/theta band, 8–20 Hz for alpha/beta band, and 20–58 for beta/gamma band). We keyed the analysis to the prefixation (−300 ms) and postfixation (77.5 ms, the postfixation enhancement peak in AVREC) values. This analysis (Fig. 4) reveals significant prefixation to postfixation phase concentration in the 3- to 8-Hz “delta/theta” band, with no significant effects in the other bands (Fig. 4A). Accompanying this pattern of effects, significant prefixation to postfixation amplitude increase is largely confined to the delta/theta band (Fig. 4B).

Effects of Fixation-Biased Phase on Stimulus Processing
Data presented in Figures 1 and 2 reveal increases in ambient CSD and MUA amplitudes following fixation onset, and these increases are associated with oscillatory phase concentration (Figs 3 and 4) and a general increase in spectral power (Fig. 4). Do these effects reflect an increase in local cortical excitability? Excitability could not be directly addressed here, as the foregoing analyses are based on analyses of time periods between stimulus presentation trials. However, we could address this question indirectly using the accompanying trials in which stimuli were presented, by examining the relationship between the phase of the ongoing EEG at the time a stimulus is delivered and stimulus-evoked response amplitude in that trial. Therefore, we asked whether the observed phase distribution at the time of fixation is associated with enhanced excitability.

To isolate the effects of oscillation phase from those of fixation onset, we analyzed only the responses to visual stimuli presented during periods of stable fixation (see Fig. 5). Mean rectified CSD and MUA amplitudes were calculated for the 30- to 200-ms poststimulus interval of each single trial; 30 ms is...
the approximate onset latency in V1 under these stimulation conditions (Givre et al. 1995). To determine if there is a systematic relationship between these single-trial response amplitude values and the phase of the ongoing activity, we sorted the amplitudes (CSD and MUA values, respectively) as a function of prestimulus phase. The systematic relationship between prestimulus oscillatory phase and visual response amplitude is depicted in a polar plot in Figure 5A. The results are quantified as a pooled amplitude distribution as a function of prestimulus phase across sessions in Figure 5B. These data show that the phase of the ongoing local neuronal oscillation is consistently related to the amplitude of the stimulus-evoked response.

Following Lakatos et al. (2005), we designated the phase associated with the largest response amplitudes the ideal (\(\phi\) mean: 1.92; \(s\): 1.13) and that related to the smallest response amplitudes the worst phase (\(\phi\) mean: -1.73; \(s\): 0.98). As described above in relation to Figure 3, at the time of fixation, the grand mean (\(n = 13\)) phase (\(\phi\)) was 1.85 rad and the pooled angular deviation (\(s\)) was 0.91. The mean phase at visual fixation onset did not differ significantly from the ideal excitability phase identified in this way (Watson \(U^2\) test, \(P > 0.05\)).

To determine whether the enhanced evoked response amplitude reflects a simple linear summation of the ongoing oscillation with the evoked response as opposed to an interaction between input and local oscillation phase (i.e., interaction between stimulation and excitability state), we compared the amplitude differences between ideal and worst phase of the ongoing oscillation in the prestimulus period with that of the poststimulus interval. A significant change in the maximal (ideal–worst phase) amplitude difference from the prestimulus to poststimulus interval would indicate the presence of an interaction. The prestimulus (from -170 to 0 ms relative to stimulus onset) CSD amplitude difference between ideal and worst phase was 0.082 mV/mm² (mean \(\pm\) 0.063 (SD) and the poststimulus (from 30 to 200 ms relative to stimulus onset) CSD amplitude difference was 0.114 mV/mm² (mean \(\pm\) 0.071 (SD). We found that the maximal amplitude difference between the 2 phases (i.e., ideal and worst) is significantly greater poststimulus than prestimulus (paired \(t\) test, \(P = 0.0015\)).

In sum, the oscillatory phase associated with the onset of fixation (in the absence of stimulation) is not discriminably different from the ideal phase, that at which maximal visually evoked responses occur. The ideal phase does appear to reflect a high excitability state in local cortical neurons. Overall, these findings are consistent with the hypothesis that the onset of fixation is associated with an increase in cortical excitability.

**Discussion**

The results of this study clearly demonstrate that neuronal modulation at the onset of fixation in the dark reflects an underlying nonvisual process that produces local neuronal excitation in V1. Additional analyses suggest that this effect stems from a transient increase in local neuronal excitability. We propose that in a lighted environment, this increased excitability will amplify responses to visual inputs generated at fixation. Consistent with this proposition, the effect persists until about 150 ms postfixation, which allows ample time for even the slowest retinal outputs to transit through V1 into the higher order visual areas including inferotemporal cortex (see e.g., Schmolesky et al. 1998; Schroeder et al. 1998; Chen et al. 2006).

Our single-trial analyses reveal that fixation onset is associated with significant oscillatory phase concentration, which is consistent with the possibility that the fixation effect operates through phase modulation of ongoing neuronal oscillations in visual cortex. The idea is that fixation-related phase resetting of an ongoing neuronal oscillation can place new retinal input in an ideal (optimal excitability) phase, so that it is amplified relative to inputs that are not synchronized to fixation. This is an appealing mechanism as it makes elegant use of the large amounts of energy present in neuronal oscillations. Moreover, recent evidence suggests that oscillatory phase modulation may be a general mechanism of predictive, as well as adaptive, cortical operation (Lakatos et al. 2007). However, the accompanying power increase rules out a pure phase resetting interpretation according to current formulations of how such a mechanism operates (Makeig et al. 2004; Shah et al. 2004). We will continue to investigate this hypothesis.
“Are these effects perceptually relevant?” Because fixations provide the major means of visual data collection in humans and other primates (Yarbus 1967), it is logical to suppose that the system might operate to enhance the perceptual impact of visual inputs occurring just after fixation, and there is evidence for this idea. The perceptual impulse response accelerates immediately following eye movements (i.e., at fixation; Ikeda 1986; Burr and Morrone 1996). Also, perceptual sensitivity, particularly for colored patterns, is enhanced at fixation (Burr et al. 1994).

**Mechanisms of Phase Modulation**

One important mechanistic issue concerns the underlying brain circuitry. There are numerous potential anatomical sources of both direct and indirect saccade/fixation-related inputs to V1. These include cortical feedback projections from parietal cortex (Barash et al. 1991a, 1991b; Goldberg et al. 2002) and frontal/prefrontal sites (Funahashi et al. 1991; Schall 1991; Stanton et al. 1995), as well as subcortical feedforward inputs from lateral pulvinar (Benevento and Rezak 1976), intralaminar thalamic nuclei (Schlag-Rey and Schlag 1984), and brainstem (Doty et al. 1973; McCormick and Pape 1988; Lu et al. 1993). Distinguishing these alternatives will require additional experimentation.

Whatever the input route is, the local physiology of fixation effects is an important question. Excitatory and inhibitory mechanisms are both possible. Although long distance afferents, such as cortical feedback projections, have generally excitatory (glutamatergic) effects, they can also operate locally by activating γ-aminobutyric acidergic (GABAergic) inhibition (Gonchar and Burkhalter 1999). Direct enhancement of local cortical excitability could account for both increase in spectral power and MUA amplitude that occur at fixation fit (Sherman and Guillery 2002). The nontrivial increases in tend to operate as “modulatory,” as opposed to “driving inputs” feedforward input sources that appear likely (above) would and any accompanying phase modulation. Detracting slightly and Burkhalter 1999). Direct enhancement of local cortical activation of GABA_α receptors should not result in large trans- chloride ions is near typical membrane resting potential values, account for our observations. Because the reversal potential for (e.g., Nicoll et al. 1990) during the prior saccade could also account for our observations. Because the reversal potential for chloride ions is near typical membrane resting potential values, activation of GABA_α receptors should not result in large transmembrane currents. Thus, the reduction in ongoing MUA during the saccade would have little or no correlate in the concomitant CSD profile, which is what we observe. However, the underlying phasic inhibition could itself contribute to the effects we note at fixation onset. That is, with release of local inhibition, ongoing activity in inputs from outside of the immediate region, trigger a resumption of ambient activity, with a phase tied to the offset of inhibition. A similar logic appears implicit in the use of the term “pause rebound” to describe neuronal activity related to generation of eye movement potentials (e.g., Purpura et al. 2003).

**Consolidation and Extension of Prior Findings**

Our results are consistent with the hypothesis that the increased neuronal activation following fixation (Lee and Malpeli 1998; Park and Lee 2000; Reppas et al. 2002) reflects an actual enhancement of neuronal excitability (i.e., increased neuronal sensitivity to stimulation). Also, the CSD profiles associated with this effect index the neuronal generators of local field potentials (Schroeder et al. 1995), thus providing physiological underpinnings for eye movement–related potentials previously observed within V1 and extrastriate cortex (Purpura et al. 2003), as well as effects found with scalp ERPs (Evans 1953; Marton et al. 1983; Skrandies and Laschke 1997) and functional magnetic resonance imaging (Sylvester and Rees 2005; Sylvester et al. 2005). The fixation effects we report would likely contribute to previously hypothesized functions such as synchronizing retinal inflow with ongoing functions in higher order regions (Sobotka et al. 1997) and synchronizing activation onset across areas (Purpura et al. 2003). For optimal results, fixation-induced phase resetting (and amplification) should be coordinated throughout the visual pathways. This prediction is consistent with the distribution of perisaccadic modulation effects across the visual pathways from lateral geniculate nucleus (Lee and Malpeli 1998; Reppas et al. 2002), through V1 (Purpura et al. 2003), V3a (Nakamura and Colby 2000), middle temporal (MT; Bair and O’Keefe 1998), inferotemporal cortex (Ringo et al. 1994; Sobotka et al. 1997; Purpura et al. 2003), medial temporal cortex/hippocampus (Ringo et al. 1994; Sobotka and Ringo 1997; Sobotka et al. 1997), and frontal eye fields (Dejardin et al. 1998). Although it is not certain that the same process is operating across all of these stages, preliminary findings in our laboratory (Rajkai et al. 2005) suggest that this is the case.

**Relationship to Attention**

Strong links have been established between the brain circuits controlling gaze and those controlling spatial selective attention (e.g., Goldberg et al. 2002; Bisley and Goldberg 2003). It is possible, particularly with saccades during voluntary visual search, that the effects we report reflect some modulation due to attention. On the other hand, it is clear that attentional allocation can be divorced from eye position (Harter et al. 1982; Hillyard and Munte 1984; Moran and Desimone 1985; Treue and Maunsell 1996). The present study was not designed to examine attentional involvement per se. Because we analyzed data gathered during periods of unconstrained eye movements in the dark, the involvement of attention in the effects we describe is an open question. It will be very interesting to learn if fixation-related amplification reflects the influence of attention or if it reflects a more automatic sensory-motor effect (e.g., a corollary discharge) that could be utilized by attention. In any case, it merits emphasis that although precise determination of the moment-to-moment locus of attention in unconstrained natural viewing may remain problematic, eye position is a robust dependent measure whose spatiotemporal dynamics can be precisely described and related to the internal cortical state.

**Implications**

Our findings bridge the gap between 2 emerging themes in systems neuroscience. On one hand, studies in a variety of disciplines including optical imaging, single-unit recording, and field potential/EEG recording have dramatically underscored the effects of ambient activity on sensory processing (Pfurtscheller 1976; Rahn and Basar 1993a, 1993b; Steriade et al. 1993; Arieli et al. 1996; Contreras et al. 1996; Polich 1997; Kisley and Gerstein 1999; Sanches-Vives and McCormick 2000; Truccolo et al. 2002; Kruglikov and Schiff 2003; Fiser et al. 2004; Lakatos et al. 2005).
For natural vision in particular, it appears that any incoming visual stimulus will have a very small impact on the system unless it falls into an ideal excitability phase of the ongoing activity (Fiser et al. 2004). Findings like these led (Arieli et al. 1995) to conclude that ongoing oscillatory activity forms the "context" for the processing of new sensory "content." On the other hand, there is rapidly growing interest in the use complex natural stimuli to study brain mechanisms of vision (Kayser et al. 2003; Long and Purves 2003; Kayser and Konig 2004; Lesica and Stanley 2004; Salazar et al. 2004; Yang and Purves 2004; Howe and Purves 2005), coupled with a recognition that adequate understanding of natural vision requires incorporation of eye movement dynamics (Gallant et al. 1998; Reppas et al. 2002; Vinje and Gallant 2002). The integration of these themes is both technical and conceptual.

On a purely technical level, it will be of interest to explore the use of fixation triggered averaging of EEG as a novel paradigm for recording visual event-related responses. Fixation-related responses to visual stimuli offer the advantage of observing visual processing under conditions that closely approximate natural vision. Moreover, our findings suggest that because of the amplifying effects of fixation, these responses will have a higher signal-to-noise ratio than traditional measures.

On a more fundamental scientific level, our findings reinforce the view that the sensory and motor systems of the brain work in close coordination. Fixation effects reflect the ability of the brain’s gaze control systems to "prime" or otherwise predictively prepare the visual system for a temporal pattern of visual input that is a straightforward consequence of the way in which the eyes are used to actively sample the visual environment. This ability is exploited to amplify the processing of visual stimuli that become the targets of visual fixation.

**Notes**

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