Enhancement of Visual Responsiveness by Spontaneous Local Network Activity In Vivo

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Enhancement of visual responsiveness by spontaneous local network activity in vivo. J Neurophysiol 97: 4186–4202, 2007. First published April 4, 2007; doi:10.1152/jn.01114.2006. Spontaneous activity within local circuits affects the integrative properties of neurons and networks. We have previously shown that neocortical network activity exhibits a balance between excitatory and inhibitory synaptic potentials, and such activity has significant effects on synaptic transmission, action potential generation, and spike timing. However, whether such activity facilitates or reduces sensory responses has yet to be clearly determined. We examined this hypothesis in the primary visual cortex in vivo during slow oscillations in ketamine-xylazine anesthetized cats. We measured network activity (Up states) with extracellular recording, while simultaneously recording postsynaptic potentials (PSPs) and action potentials in nearby cells. Stimulating the receptive field revealed that spiking responses of both simple and complex cells were significantly enhanced (>2-fold) during network activity, as were spiking responses to intracellular injection of varying amplitude artificial conductance stimuli. Visually evoked PSPs were not significantly different in amplitude during network activity or quiescence; instead, spontaneous depolarization caused by network activity brought these evoked PSPs closer to firing threshold. Further examination revealed that visual responsiveness was gradually enhanced by progressive membrane potential depolarization. These spontaneous depolarizations enhanced responsiveness to stimuli of varying contrasts, resulting in an upward (multiplicative) scaling of the contrast response function. Our results suggest that small increases in ongoing balanced network activity that result in depolarization may provide a rapid and generalized mechanism to control the responsiveness (gain) of cortical neurons, such as occurs during shifts in spatial attention.

INTRODUCTION

Neocortical neurons are bombarded by ever-changing patterns of synaptic input, generating spontaneous activity in the absence of sensory stimulation. Such activity patterns may take the form of synchronized oscillations, as seen during anesthetic and natural sleep (Lampl et al. 1999; Steriade et al. 1993, 2001), or may appear as persistent background activity in the awake cortex that maintains the membrane potential of cortical neurons in a depolarized yet highly variable state (Chen and Fetz 2005; Crochet and Petersen 2006; Steriade et al. 2001). Studies of rhythmic cortical activity during visual processing have mainly focused on fast oscillations (Singer and Gray 1995); however, slower fluctuations in background activity across widespread regions of the cortex have been implicated in changes of functional connectivity and neural response variability to sensory stimuli (Anderson et al. 2000a; Arieli et al. 1996; Azouz and Gray 1999; J. Massimini et al. 2005).

Particularly interesting is the finding that increases in background activity and responsiveness occur in primate visual areas during selective attention (Lluck et al. 1997; Reynolds et al. 2000; Williford and Maunsell 2006) a finding that can be explained by an increase in synaptic bombardment of these cells (Murphy and Miller 2003). Thus study of the cellular mechanisms associated with modulation of background activity levels may be relevant not only for sensory processing, but also for complex behaviors (Raichle 2006).

The cortical slow (<1 Hz) oscillation is a readily studied form of background activity, characterized by rhythmic cycles of synchronically mediated depolarization and firing (Up states), followed by diminution of synaptic barrages, hyperpolarization, and near cessation of firing (Down states). Examination of such states has yielded valuable information about basic mechanisms of cortical function during ongoing activity (Contreras and Steriade 1995; Cowan and Wilson 1994; Haider et al. 2006; Hasenstaub et al. 2005; Pare et al. 1998; Shu et al. 2003b, 2006; Waters and Helmchen 2004). Importantly, characteristics of the membrane potential (depolarization, variability, and increased conductance) observed during Up states are similar to those in awake animals (Crochet and Petersen 2006; Steriade et al. 2001). What is the effect of spontaneous network activity on cortical sensory responses? Up state network activity in vitro strongly facilitates responsiveness of cortical neurons to local inputs (Destexhe et al. 2003; Hasenstaub et al. 2005; Ho and Destexhe 2000; McCormick et al. 2003; Shu et al. 2003a) and also to thalamic activation (MacLean et al. 2005). Similarly, studies in cat sensori-motor systems in vivo showed increased responsiveness to electrical stimulation of prethalamic pathways (Timofeev et al. 1996) and to peripheral nerve stimulation (Rosanova and Timofeev 2005) during Up states, whereas studies in the rodent somatosensory system have shown strongly diminished responsiveness of cortical neurons during Up states (Petersen et al. 2003; Sachdev et al. 2004). Thus the effects that activated network states have on sensory responsiveness remains disputed. Here, using combined extra- and intracellular recordings, we first systematically examined whether the presence (Up states) or absence (Down states) of spontaneous local network activity enhanced or diminished the neuronal response to sparse visual stimulation in cat primary visual cortex. We found that, indeed, spiking responses to visual receptive field stimulation, and spiking responses to intracellular injection of excitatory...
postsynaptic potential (EPSP)-like conductances are significantly enhanced when stimuli are presented during Up versus Down states, because of the depolarization associated with the Up state. Closer examination of membrane potential dynamics during network activity revealed that this response enhancement varies smoothly with gradual increases in extracellular network activity and in parallel with progressive membrane potential depolarization. Importantly, we found that these spontaneous increases in network excitability also enhanced the responses to stimuli of varying contrasts, resulting in an upward scaling of the contrast response function, similar to the contrast response enhancement observed with attention in behaving primates. These results indicate that coherent increases in the level of background network activity are transformed into depolarizations that may enhance neuronal responsiveness to a wide variety of stimuli. Such a mechanism for gain modulation may be a basic feature of flexible cortical network operations.

METHODOLOGY

Anesthesia and surgical preparation

Acute experiments were conducted on young adult female cats (Felis catus), weighing between 2.5 and 3.5 kg. Animals were initially anesthetized intramuscularly with a ketamine (15–20 mg/kg) and xylazine (1 mg/kg) mixture, and atropine (0.5 ml, sc) was administered to reduce secretions. A forelimb vein was cannulated for continuous intravenous infusion of ketamine-xylazine dissolved in lactated dextrose Ringer solution (1.8 mg ketamine and 0.04 mg xylazine/ml solution, respectively, infusion rate 16.0 ml/h). Auffed endotracheal tube was inserted for active ventilation, and the EKG was monitored continuously. Additionally, we performed experiments in which the animal was anesthetized only with intramuscular supplements (instead of intravenous infusion) of the above described ketamine-xylazine mixture to replicate the anesthetic regimen that we used in previous studies (Haider et al. 2006; Hasenstaub et al. 2005). We found no major difference in expression of the slow oscillation or in visual response properties between the two differing anesthetic delivery methods. We therefore performed the majority of the experiments using the continuous intravenous regimen as described above. The animal was artificially respirated (~20 cycles/min) with oxygen, and end-tidal CO2 was maintained between 3.5 and 4.5%. A bilateral pneumothorax was performed to minimize brain pulsations arising from respiration. Depth of anesthesia was assured by continuously monitoring EKG wavesforms along with heart rate (maintained at 120–180 bpm), rectal body temperature (maintained at 37–39°C), and by observing reactions to noxious stimuli (toe pinch) and reflexes. The animal was placed in a stereotaxic apparatus, the ribcage was elevated, a midline scalp incision was made, and the skin, underlying fascia, and muscles were retracted. A small craniotomy (~2 mm diam) was performed to expose the cortex 200–500 μm away from the electrode. Intracellular electrodes were filled with 2 M potassium acetate and beveled to have a final impedance of 55–110 MΩ. Current-clamp recordings were performed with an AxoClamp 2B amplifier (Molecular Devices, Sunnyvale, CA). Extracellular broadband, LFP, MU, membrane potential (Vmem), and current (I) were sampled at 20, 1, 10, 20, and 20 kHz, respectively, and recorded using the Spike2 System (Cambridge Electronics Design, Cambridge, UK). Intracellularly recorded cells were classified as RS, FS, intrinsically bursting (IB), or chattering (CH), according to standard criteria (McCormick et al. 1985; Nowak et al. 2003). Recorded cells had to display robust Up states (>10 mV in amplitude) and had to exhibit stable (Up state negative to ~50 mV) membrane potentials with minimal (0 to ~1 nA) current injection. If a neuron failed these criteria, it was excluded from subsequent analysis. Every intracellularly recorded cell exhibited Up and Down states. When these oscillations were less robust or asynchronous in the intracellular recording, it was invariably also the case in the local MU and LFP network activity. This indicates that changes in the oscillations were likely related to the anesthetic state of the animal and not due solely to recording from a particular cell type, as has been previously suggested (Anderson et al. 2000a). Spiking responses to visual stimulation were recorded with zero current injection, whereas synaptic responses were recorded by hyperpolarizing the Up state membrane potential to near ~75 mV (reversal for GABAergic Cl−-mediated inhibition) with negative DC injection. At this membrane potential, synaptic potentials should be dominated by glutamatergic EPSPs.

Dynamic clamp methods

Our dynamic clamp methods have been published previously (McCormick et al. 2003; Shu et al. 2003a). Briefly, using a real-time Linux system (Dorval et al. 2001) and a DAP-5216a board (Microstar Laboratory, Bellevue, WA), we injected artificial EPSPs at 10 Hz, in discontinuous current-clamp mode (to minimize any errors associated with series resistance compensation) with a minimum switching frequency of 2 kHz. During the protocols, the headstage output was continuously monitored with an oscilloscope to ensure adequate
settling of the electrode voltage between samples. Injected conductances varied in size, from 2 to 80 nS, in steps of 2 nS. The amplitude time-courses of the artificial EPSPs were defined by a kinetic model of synaptic transmission, using only AMPA receptor kinetics (Destexhe et al. 2001).

**Visual stimulation and analysis**

Detailed descriptions of the visual stimulation protocol have been reported previously (Nowak et al. 2005; Sanchez-Vives et al. 2000). Briefly, size, orientation, and direction preference of spiking responses for either intracellular or extracellular units were first determined by hand-mapping, after which a series of automated routines (VSG Series 3, Cambridge Research Systems) displayed on a 19-in color monitor (80 Hz noninterlaced refresh rate, 1.024 × 768 resolution; Sony, Tokyo, Japan) were used to quantitatively determine the neuron’s optimal stimulus. We used a sparse noise stimulation technique similar to that developed by others (DeAngelis et al. 1993, 1994; Jones and Palmer 1987), and used previously in our laboratory (Nowak et al. 2005) to explore the role that ongoing network activity has on responses to stimulation within the receptive field (RF). A single bright (or dark) bar of 80% contrast and optimal direction, length, and spatial frequency was flashed once every 50 or 62.5 ms (stimulus duration, 50 ms) in 1 of 16 different randomly selected locations within the RF along a single axis that was perpendicular to the preferred orientation of the neuron. These single bright or dark bars, varying randomly in position, were presented in 2-s blocks that were followed by 2 s of uniform gray screen to record spontaneous activity in the absence of stimulation (Fig. 1).

To facilitate comparison of the suprathreshold spiking responses to the subthreshold synaptic responses, spike events were converted to a continuous spike density function by replacing each spike with a raised cosine bell (10 ms width). During recording, the responses to bright and dark bars at all locations were totaled cumulatively, and the average response to each bar at each location (forward correlation) was displayed as a peristimulus time histogram (PSTH) on-line (Fig. 1G). Note that visual stimulation was not triggered by any particular network state; stimuli were presented randomly during ongoing activity. Records were segregated off-line into Up/Down states in conjunction with the locally recorded MU network activity, as detailed previously (Haider et al. 2006). We were assured of the proximity of our extracellular and intracellular recordings by the close relationship between the level of network activity and the intracellular membrane potential (see Fig. 6) and also by the observation that both recordings exhibited similar orientation and direction preference (data not shown). Bar responses, sorted by state, were used to construct separate three-dimensional space-time maps (Figs. 1, B–E, S1, and S2) in a manner similar to that described above for the on-line analysis. Each space-time plot shows J time on the z-axis, 2) spatial location on the y-axis, and 3) response amplitude (either spikes or membrane potentials) as color-coded values in the z-axis. For display purposes, space-time plots were smoothed with a normalized Gaussian filter (width, 3.6 ms; e.g., Fig. 1, B–E). RF responses elicited by bright or dark bars presented during Down states were compared with the responses generated by these same stimuli that were presented during Up states. Cells were classified as simple and complex based primarily on the space-time plots. Cells exhibiting either a single dark or single bright excitatory subfield (S1 cells) or multiple (usually 2–4) bright and dark excitatory subfields that are separated in space (DeAngelis et al. 1993; Field and Tolhurst 1986; Heggelund 1986; Hubel and Wiesel 1962; Jones and Palmer 1987; Movshon et al. 1978a,b) were classified as simple, whereas cells exhibiting bright and dark excitatory subfields overlapping in both space and time were classified as complex. In a subset of recordings, we also presented cells with optimally oriented sine-wave drifting gratings (data not shown) and found that categorization of both simple and complex cells based on the space-time plots was consistent with classification based on the ratio of the modulated to maintained (F1/F0) portions of the spike rate response to drifting gratings (Nowak et al. 2005; Skottun et al. 1991). For a subset of experiments, we also presented small stationary gratings of the optimum phase, orientation, and spatial frequency, restricted in space to the center of the receptive field, as determined by the above one-dimensional mapping protocol. These grating patch stimuli were on screen for 85 ms with a 200-ms interstimulus interval, whereas the contrast for each stimulus was randomly assigned to be either 0, 5, 10, 20, 40, or 80%.

For our present purposes, we were primarily interested in the effect of network activity on responses to the optimal stimulus within the RF. This was quantitatively defined as the single flashed bar (either bright or dark) that elicited the shortest latency and greatest amplitude response (optimal bar) during the Up state. This bar was nearly always located at the center of the main excitatory subfield in the space-time plot (deemed “best location”) and was also always the same bar polarity and location that elicited the best response from the Down state. This best location response was compared with responses in “null locations,” which were defined as locations in which bar presentations did not elicit activity that was more than ±1.5 SD from the average baseline activity (measured during the period spanning 25 ms before to 25 ms after stimulus onset). Only a fraction of the spatial locations in which stimuli were presented qualified as “null” locations, because of the presence of sometimes multiple inhibitory and excitatory subfields (Fig. 1). We combined and took the average activity of all of the null locations and compared them to the activity at the best location (Figs. 3, 5, and 7). The results were not quantitatively different if we randomly selected only one of the null locations for comparison to the best location. On-line and off-line analysis was initially performed in Spike2, and space-time plots were constructed using Origin (Microcal, Northampton, MA). All further analyses were performed using MATLAB (The Mathworks, Natick, MA). Population means reported in text are ±SE, whereas population plots are shown as ±SE (dashed lines), unless otherwise noted.

**RESULTS**

**Up states enhance network responsiveness to visual stimulation**

To study the dynamics of sensory responses in active cortical networks, we employed simultaneous extracellular and intracellular recordings in the primary visual cortex of ketamine-xylazine anesthetized cats while flashing optimal bright and dark bars. Ketamine-xylazine anesthesia produces robust, rhythmic slow oscillations, as shown in Fig. 1A. The recording configuration shows the LFP and the MU activity recorded from the same electrode. The Up state (solid horizontal lines) is characterized by local network MU firing and low-voltage, high-frequency fluctuations in the LFP. This period of activity is followed by a rapid transition into the Down state (dashed lines) where network firing ceases and is associated with a large, positive-going deflection of the LFP. The top vertical lines in Fig. 1A indicate the times of flashed bright and dark bars. Notice that the structure, frequency, and occurrence of Up and Down states are not obviously altered by presentation of visual stimuli.

We quantified the responsiveness of the network to visual stimulation by constructing two-dimensional space-time maps from the MU responses to flashed bars that were presented during Down and Up states (n = 5 MU recordings). Both the structure (mean Up duration, 721.6 ± 372.0 ms; mean Down
duration, 335.9 ± 157.4 ms) and the frequency (mean frequency, 0.39 ± 0.12 Hz) of network slow oscillations we recorded in cat area 17 are in agreement with previous reports in cat association cortex (Steriade et al. 1993), and broadly agree with a variety of other preparations (Anderson et al. 2000a; Cossart et al. 2003; Petersen et al. 2003; Sachdev et al. 2004; Timofeev et al. 2000; Waters and Helmchen 2004), including what we have observed both in vitro and in vivo in ferret prefrontal and visual cortical areas (Haider et al. 2006; Hasenstaub et al. 2005; Sanchez-Vives and McCormick 2000; Shu et al. 2003b). Throughout our study, visual cortical cells typically responded to the onset, and sometimes the offset, of the bright and dark bars. This was especially evident in the MU records (Fig. 1, C and E), which reflect the ensemble activity of cells with differing RF properties. We focused our analysis on the shortest-latency response to the onset of the bright or dark bar, particularly because the offset response to bars presented during the Down state often occurred during the spontaneous transition to the Up state and therefore should not be considered as a response that occurs during the Down state.

We observed that when bright and dark bars were presented during the Up state, the MU response to the onset of the bright or dark bar, particularly because the offset response to bars presented during the Down state often occurred during the spontaneous transition to the Up state and therefore should not be considered as a response that occurs during the Down state.
enhancement during the Up state. Similar results were obtained in all MU recordings \( (n = 5) \).

Because of the relatively short duration of the Down state (e.g., average of 336 ms for MU recording in Fig. 1), space-time plots constructed from visual stimuli presented during this state exhibit a general increase in activity over time, especially during the second half of the period analyzed. This nonspatially localized increase in activity is caused by an increase in the probability of spontaneously entering into the Up state (dashed lines above space-time plots in Fig. 1, B and D). We found that in MU recordings, the network is capable of responding to the visual stimulus in both the Up and Down states, although the peak response rate to the optimal stimulus (best bar) is approximately doubled when stimuli are presented during Up states. Figure 1, F and G, shows the response amplitude as a function of time since stimulus presentation, where each of the curves represents the MU response to bright or dark bars in a particular spatial location (corresponding to the horizontal dashed lines on the space-time plots in Fig. 1, B and C). Because the response profile is constructed from multiple neurons with differing receptive field properties (i.e., increases or decreases in firing in response to the same bright or dark stimulus), the overall response profile exhibits phasic increases and decreases at the frequency of stimulus presentation. This in no way affects the main observation that the peak response to the best bar is significantly elevated during Up states. Furthermore, the response enhancement to bars presented in the Up state is not limited to the best bar in the optimal location but also applies to the suboptimal bars presented in adjacent spatial locations within the excitatory subfield (Fig. 1, F and G).

**Up states enhance visual responses in extracellularly recorded RS and FS neurons**

We next examined whether well-isolated SU recordings also exhibit response enhancement when visual stimuli are presented during Up states. Figure 2 shows representative examples of electrophysiologically identified RS (presumably pyramidal) and FS (presumably inhibitory interneuron) cell responses to bars presented during both Up and Down states. The RS cell responses to the optimal bar are clearly enhanced by network activity (peak in Up state, 15.6 Hz; Fig. 2B) compared with when stimulated during the Down state (peak in Down state, 1.2 Hz; Fig. 2A). As observed with MU activity, this response enhancement during the Up state is not limited to the best bar in the optimal location (Fig. 2B, arrow), but extends to multiple locations across the RF. Similar response enhancement was seen in the population of RS cells \( (n = 10) \). FS cells also show markedly enhanced responses to the optimal bar presented during the Up state (peak in Up state, 84.9 Hz; Fig. 2D) compared with responses to optimal bar presentation during Down states (peak in Down state, 23.6 Hz; Fig. 2C). As we have observed in frontal cortex (Hasenstaub et al. 2005), FS neurons typically exhibit a high spontaneous discharge rate during the Up state, which we also observe here in visual
cortex (Fig. 2D). Similar response enhancement was seen in all identified FS cells (n = 3).

**Up states more than double the population spiking response to visual stimulation**

We next compared the average response elicited by presentation of the optimal bar (either bright or dark) in the best location, to the average response elicited to bars in “null” locations (locations that did not exhibit a significant change in activity; see METHODS) for the population of extracellular SU recordings (n = 14 neurons; 3 FS, 10 RS, 1 unclassified; 12 simple (6 S1), 2 complex). On the population level, the neurons were responsive in both the Up and Down states, and as expected, the peak responses to the optimal location (Fig. 3, A and B, solid red lines) were significantly greater than the responses in null locations (Fig. 3, A and B, solid blue lines; P < 0.01, paired Wilcoxon sign rank test). However, as with MU responses, the SU responses were on average significantly greater during the Up state (13.1 ± 10.3 Hz; Fig. 3A, solid red line), than during the Down state (5.0 ± 6.2 Hz; Fig. 3B, solid red line; P < 0.01, paired Wilcoxon sign rank test).

One difficulty with our analysis is that the Down states are relatively short, and for at least some visual stimuli presenting during the Down state, the visual cortical response occurred during the transition into the subsequent Up state, which may have artificially inflated our Down state responses. Likewise, Up state responses that occur during the transition to the Down state may be altered by this change in network activity (Leger et al. 2005). To examine these possibilities, we pooled those SU and MU recordings that exhibited any action potential responsiveness during the Down state and performed a similar analysis to that shown in Fig. 3, A and B, with the restriction that the action potential response for each neuron had to occur entirely during the Up or Down state (Fig. S3B). This analysis revealed that the magnitude of the Up state response was nearly identical, whether the criterion for analysis was the stimulus or the response occurring in the Up state. In contrast, for data collected during the Down state, responses that were entirely confined to the Down state were significantly smaller than those that were allowed to contain periods of transition to the Up state (Fig. S3). These results indicate that, in our experiments, the magnitude of Down state responses is likely an upper bound and add further support to our hypothesis that the presence of network activity significantly enhances visual responsiveness.

**Flashed bar stimulation does not evoke transitions into or out of Up states**

It is conceivable that visual stimulation with flashed bright and dark bars affected the patterns of ongoing activity by changing the probability of Up/Down state transitions compared with spontaneous transitions occurring in the absence of stimulation. For example, if presentation of the best bar increased the probability of starting an Up state, one would expect the best bar triggered histogram to display a sustained elevation of firing. That is, the response profile after best bar stimulation in the Down state (e.g., trace with arrow in Fig. 1F) would be followed by a sustained increase in firing that was greater than that occurring spontaneously at null bar locations. Note that in both the MU and SU space-time plots constructed from Down state stimulation, we do not observe a sustained increase in firing after best bar stimulation (i.e., Figs. 1F and 1H).

![FIG. 3. Extracellular population visual responses are enhanced during Up states, and flashed bar stimulation does not induce transitions in local network. A: population histogram (n = 14) of response amplitude as a function of time during Up states, from presentation of best bar (bright or dark) in best location during Up states (best location, solid red line), compared with bars in locations that do not elicit significant visual responses (null location, solid blue line). B: population histogram of response amplitude as a function of time since best (solid red line) or null (solid blue line) bar presentation during Down states. C: histogram of probability of Up state ends as a function of either presentation of best bar in best location (red line) or presentation of bars in null locations (blue line), for population of SU recordings as above, along with 2 MU recordings (total n = 16). Bar presentation at 0 ms. There is no significant difference in Up to Down transition probability for best vs. null location bar presentations. D: histogram of probability of Up state starts as a function of either presentation of best bar in best location (red line) or presentation of bars in null locations (blue line) for same recordings as above. There is no significant difference in Down to Up transition probability for best vs. null location bar presentations.](http://jn.physiology.org/)
However, it is possible that transitions were occurring without being visible with this analysis. Therefore, to further examine the possibility that bar presentations caused transitions between Up and Down states, we first calculated the rate of state transitions before and after visual stimulation and compared the transition rate after best bar stimulation to the transition rate after bar presentation in null locations. Bar presentation in null locations not only provides an estimate of the average spontaneous spiking activity (Fig. 3, A and B, solid blue lines) but also provides an estimate of the spontaneous transitions between states (either Down to Up or Up to Down) occurring in the local network (Fig. 3, C and D, solid blue lines). We thus examined whether presentation of the optimal stimulus (Fig. 3, C and D, solid red lines) could affect the probability of starting or ending an Up state in the local network. We found no significant difference in the probability of generating an Up state in the local network after presentation of the optimal bar in the best location compared with the spontaneous transition probability after bar presentation at the null spatial locations (Fig. 3Dc; >4,000 best bar presentations in Down state, $P > 0.05$, paired Wilcoxon sign rank test; $n = 12$ SU and $n = 2$ MU). Similarly, Fig. 3C shows that the probability of ending the Up state after presentation of the best bar is not significantly different from the average probability of Up state ends after presentation of bars at the null locations ($>4,000$ bar presentations during Up state, $P > 0.05$, paired Wilcoxon sign rank test).

Given the rapid delivery of stimuli in our mapping protocol (see Methods), it is conceivable that the probability of stimulus evoked transitions was affected by multiple sequential stimuli. To control for this possibility, we also presented the single best bright or dark bar in the best location at long interstimulus intervals (1.5 s), while keeping the stimulus duration (50 ms) the same as in the mapping protocols. Again, we found no significant effect of single flashed bars on the probability of evoking or terminating Up states in the local network ($n = 3$ neurons, 500 best bar presentations, $P > 0.05$, paired Wilcoxon sign rank test for both probability of ending the Up state after presentation of the best bar and for probability of starting the Up state after presentation of the best bar compared with spontaneous transition rates; data not shown). Importantly, because we recorded and segregated the local network activity into Up or Down states using the MU/LFP from the same electrode as that used to record visual responses of single cells (see Methods), these findings strongly suggest that presentation of flashed bright and dark bars within the RF does not reliably evoke Up or Down state transitions in local cortical networks.

Up states enhance visually evoked intracellular spikes in simple and complex cells

Having established that extracellularly recorded responses to visual stimuli are enhanced by ongoing network activity, we next examined the effect that network activity has on intracellular spiking responses and synaptic potentials resulting from visual stimulation. We recorded local network activity with LFP and MU recordings (Fig. 4A, top and bottom traces) simultaneously with intracellular recordings from nearby cells ($V_m$; Fig. 4A). As previously described (Contreras and Steriade 1995; Cowan and Wilson 1994; Lampl et al. 1999; Metherate and Ashe 1993; Steriade et al. 1993), when the local network is silent (Down state, dashed lines below LFP trace), the intracellular membrane potential is markedly hyperpolarized, whereas during increased levels of network firing during Up states (solid line underneath LFP trace), the membrane potential of nearby neurons becomes depolarized to levels near firing threshold and is highly variable. The values that we measured here in cat primary visual cortex (mean depolarization of Up state, $14.8 \pm 7.2$ mV; SD of membrane potential during Up state, $2.4 \pm 0.7$ mV; SD of membrane potential during Down state, $0.6 \pm 0.3$ mV; average maximal range from Down$_{min}$ to Up$_{max}$, $28.4 \pm 7.2$ mV; $n = 7$ neurons recorded in absence of visual stimulation and hyperpolarized to prevent APs) are similar to those measured in prefrontal cortical neurons (Haider et al. 2006; Shu et al. 2003b). Figure 4A is a representative example of an electrophysiologically identified RS simple cell (see Fig. S1 for space-time plot). The presentation of the best bright bar (Fig. 4A, arrow above tick mark at top) during the Down state (Fig. 4A, dashed line) results in subthreshold PSPs (Fig. 4A, arrow). These Down-state evoked PSPs do not reach spike threshold, and the MU and LFP traces show very little activity. Presentation of this same optimal bright bar during the Up state (Fig. 4A, 2nd arrow at top) results in a PSP that reaches threshold and produces an AP (Fig. 4A, $V_m$ trace, 2nd arrow).

Figure 4B shows a representative example of another electrophysiologically identified RS cell, but with a complex RF (Fig. S2). As with simple cells, the membrane potential of complex cells ($n = 4$) depolarized and hyperpolarized with local Up and Down states (Fig. 4B, LFP and MU traces). Similar to simple cells, the presentation of the optimal stimulus (dark bar in this neuron, 1st arrow at top, Fig. 4B) leads to subthreshold PSPs during the Down state (1st arrow, $V_m$ trace at bottom), whereas presentation of this same dark bar during the Up state leads to two APs (2nd arrow, $V_m$ trace at bottom).

Intracellularly recorded population spiking responses to visual stimulation are significantly enhanced by Up states

On the population level, the average intracellular spiking response to the onset of a bar in the optimal location was significantly enhanced during the Up state (36.3 \pm 9.1 Hz; Fig. S3A, solid red line) in comparison with the Down state (Fig. S3A, solid blue line; 14.6 \pm 3.7 Hz, $P < 0.01$, paired Wilcoxon sign rank test; $n = 16$ neurons; 2 FS, 1 CH, 12 RS, 1 unclassified; 12 simple (4 S1), 4 complex). We next compared the visually evoked change in firing rate, relative to the ongoing background activity, for the entire population (both extracellular and intracellular) of recorded neurons. As previously shown (Fig. 3, C and D), we were confident that the presentation of the optimal bar was not inducing transitions in the local network, and therefore used the activity levels present during the presentation of bars in null locations as estimates of the background activity present during the Up state (Fig. S3A, solid orange line) and also as an estimate of the spontaneous increase in firing caused by transition from the Down state into the Up state (Fig. S3A, solid light blue line). Thus for every neuron that we recorded, we subtracted the activity present during presentation of bars at null locations from the activity evoked by the best bar. The resulting plots of spiking responses take into account the background activity and state transitions occurring spontaneously over time, both during Up and Down states (Fig. 5A, baseline activity near 0 Hz for both Up and Down states).
Down states, solid red and blue traces, respectively). For the entire population (combined extracellular and intracellular, n = 30), the relative magnitude of the onset (1st peak) spike response evoked by the best bar presented during the Up state (17.5 ± 4.3 Hz, Fig. 5A, solid red line) was more than double the relative spike response evoked by best bar presentations during the Down state (7.1 ± 2.1 Hz; Fig. 5A, solid blue line; P < 0.01, Wilcoxon sign rank test). Note that the peak population firing rate change evoked from the Down state occurs slightly later than the peak population response evoked by the same best bar presentations during the Up state. It is possible that elevated spiking responses occurred during Down state presentations, but with more temporal variability than the analogous responses evoked during Up states (resulting in a lower peak in the average Down state histogram response). To control for this, we examined the individual raw peak firing rates evoked by best bar stimulation in both Up and Down states for each neuron for the entire time period from the first significant deviation from baseline to return to baseline (i.e., from ~30 ms to 125 ms in Fig. 5A). The average peak rate evoked by best bar stimulation during the Up state is significantly greater than the average peak rate evoked by stimulation during the Down state at any time-point during the initial visual response (Fig. 5B, mean of individual differences, solid dot, bars indicating 95% CIs; Kruskal-Wallis nonparametric ANOVA, P < 0.01). Examination of the individual times of this firing rate peak for the population revealed that the peak response to Up state best bar stimulation occurred significantly earlier (mean difference, 11.8 ms) than the corresponding peak response to Down state best bar stimulation (Fig. 5C, same convention as Fig. 5B; Kruskal-Wallis nonparametric ANOVA, P < 0.01).

Previous studies have shown that increases in the rate of change of the membrane potential immediately preceding an AP is associated with decreases in spike threshold (Azouz and Gray 1999, 2003). We made a similar finding here. Examination of the spike-triggered average of the membrane potential for the best stimulus evoked spikes showed that spike threshold exhibits significant variability and is well correlated with the rate of change of the membrane potential (dv/dt) before spike onset (Shu et al. 2006). For a window of 10 ms before spike onset, threshold was lowest (about ~55 mV) for those spikes that were preceded by high values of dv/dt (~1 mV/ms; data not shown). Taken together, these population results strongly support the hypothesis that synaptic bombardment during Up states is associated with more robust and more rapid spiking responses of cortical neurons to visual stimulation of the receptive field.

Visually evoked PSPs do not differ in amplitude in Up versus Down states, but Up state PSPs bring V_m closer to spike threshold

Having established that the spiking output of neurons is enhanced by network activity during Up states, we next exam-
We also examined the synaptic potentials arriving because of optimal bar presentation in complex cells (Fig. 6, C and D; same cell as shown in Fig. 4B). Again, note that robust PSPs are evoked from visual stimulation occurring in either the Up or Down state. Similar to the previous example simple cell, the peak depolarization achieved by visual stimulation during the Up state in the complex cell (Fig. 6D, arrow, peak dark bar response in Up state, $-64.4$ mV) is significantly greater than the peak depolarization to the same visual stimulus presented during the Down state (Fig. 6C, arrow, peak dark bar response in Down state, $-76.2$ mV), even though for this particular cell, the amplitude of the evoked PSP is slightly larger in the Down versus the Up state.

We examined the effects of Up and Down states on evoked PSP responses more thoroughly on the population level (Fig. 7). We found that, in our sample of cells ($n = 10$; 1 FS, 9 RS; 6 simple (1 S1), 4 complex), optimal bar presentation evoked a response in the Down state that was $7.4 \pm 9.5$ mV with respect to the membrane potential at the onset of the PSP (Fig. 7B), which seems to be larger than that evoked by the visual stimulus during the Up state ($3.8 \pm 5.0$ mV; Fig. 7A). However, a significant component of the Down state--evoked response is intermixed with the depolarization of the membrane potential associated with spontaneous transition to the Up state. To control for this Down-to-Up transition, we performed two manipulations. First, we subtracted null location membrane potential responses from best bar locations and found no significant difference in magnitude or latency of the evoked synaptic potentials between Up and Down states (Fig. 7C; $P > 0.05$, paired Wilcoxon sign rank test). However, on average, bar presentations in null locations (see METHODS) during the Up state resulted in a small ($1.8 \pm 0.5$ mV) hyperpolarization, presumably because of surround inhibition that was difficult to detect at any particular bar location. Subtracting this hyperpolarization increases the apparent magnitude of the absolute evoked synaptic potential in Fig. 7C. Additionally, the population plots of membrane potential versus time since stimulus onset (Fig. 7, A ad B) combine the responses of neurons with varying latencies to peak membrane potential value.

To exclude the effect of the hyperpolarization in the null locations, we linearly interpolated the membrane potential of null location trials for the times spanning the initial best location visual response (25–150 ms). This procedure preserved the overall decrease in membrane potential over time during the Up state (Fig. 7A, dashed line with endpoints) and also preserved the increase in membrane potential depolarization for these same time-points during the spontaneous transition to the Up state (Fig. 7B, dashed line with endpoints). We compared the peak magnitude of the optimal bar evoked synaptic potentials at any time during the initial visual response (25–125 ms) for each neuron relative to the interpolated membrane potential value in the null location at the time of the stimulus evoked peak (Fig. 7D). Again, we found no significant difference in the population average for the peak amplitude of the evoked PSP in the Up state ($5.7 \pm 4.8$ mV) compared with the Down state ($6.1 \pm 6.2$ mV; Fig. 7D, bottom; $P > 0.05$, paired Wilcoxon sign rank test), and there was also no difference in the latency of onset or time to peak for the population PSPs ($P > 0.05$, paired Wilcoxon sign rank test).
Finally, by examining the level of depolarization evoked by visual stimulation in our population, we found that the peak membrane potential value of synaptic potentials evoked by the optimal bar during the Up state was significantly more depolarized than the same peak depolarization achieved from the Down state (Fig. 7E, bottom; Up state mean membrane potential value, $-73.7 \pm 9.1$ mV; Down state mean membrane potential value, $-81.6 \pm 8.5$ mV; $P < 0.01$, paired Wilcoxon sign rank test; hyperpolarizing DC was injected to prevent AP generation). These results indicate that, although the magnitude of the PSPs evoked by optimal visual stimulation during the Up and Down states was not significantly different, the peak depolarization achieved by these visually evoked synaptic potentials is greater during the Up state and significantly closer to the threshold for action potential generation.

Responses to artificial conductance stimuli are significantly enhanced by Up states

Our results suggest that the enhancement of AP visual responses may occur through increases in neuronal excitability, as opposed to increases in the amplitude of synaptic responses. To test this hypothesis, we explored the input-output relationship of neurons by using the dynamic clamp technique to precisely control the conductance input into neurons, while the activity in the local network continued to oscillate between Up and Down states ($n = 8$ RS neurons). For these experiments, we recorded local network activity (Fig. 8A, LFP and MU, top traces), while simultaneously intracellularly injecting artificial conductance stimuli of various amplitudes (Fig. 8A, $G_{syn}$) that mimicked single (AMPA receptor mediated) EPSPs into nearby neurons (Fig. 8A, $I_{inj}$), and recorded the ensuing APs (Fig. 8A, $V_{m}$, bottom trace). Note that when the local network is silent and the membrane potential is hyperpolarized, a small amplitude artificial conductance stimulus results in an injected current that produces an $\sim 10$ mV depolarization that does not result in APs (Fig. 8A; 1st arrow), whereas the same conductance input presented during the Up state results in a slightly smaller injected current (caused by decreased driving force) but leads to a depolarization that does result in APs (2nd arrow). We quantified the probability of generating a spike in response to these varying amplitude conductance stimuli and found that the probability of an EPSP-like input initiating a spike was greatly enhanced in this neuron when the stimuli were injected during Up versus Down states (Fig. 8B; cf. red and blue traces). In addition, the Up state also caused a decrease in slope of the input-output curve (Fig. 8B). For this neuron, both the latency (Fig. 8C, top) and the jitter (SD of latency; Fig. 8C, bottom) of spikes were reduced in response to conductance stimuli injected during Up states (red trace) compared with Down states (blue trace; paired Wilcoxon sign rank test, $P < 0.01$).

We quantified these effects for each neuron ($n = 7$) by normalizing injected conductance magnitudes to the stimulus.
value that evoked spikes on 50% of the trials during Down states (Fig. 8D; normalized value of 1). The normalized response profiles of the population were combined for both Up and Down states. It is evident that the probability of responding to artificial conductance stimuli was significantly enhanced by network activity during the Up state, indicated by a leftward shift of the input-output curve (Fig. 8D; 50% spike probability decreased by 0.63 ± 0.25 normalized conductance during the Up state, solid red line; P < 0.01, paired Wilcoxon sign rank test), with an accompanying 41% decrease in the slope of the linear portions (spike probabilities from 0.3–0.7) of the input-output curve (P < 0.01, paired Wilcoxon sign rank test). Similarly, for all artificial conductance magnitudes, the latency for AP generation during the Up state was significantly shorter than in the Down state (Up state latency, 13.8 ± 3.4 ms; Down state latency, 15.6 ± 2.9 ms; P < 0.05, paired Wilcoxon sign rank test; data not shown), but without a significant decrease in jitter (Up state jitter, 5.12 ± 3.8 ms; Down state jitter, 5.5 ± 4.7 ms; P > 0.05, paired Wilcoxon sign rank test; data not shown). Overall, these results clearly showed that, although neurons are capable of responding to inputs during the Down state, the presence of network activity during Up states greatly facilitates responsiveness to stimuli of varying amplitudes, particularly to those stimuli which produce small- to medium-sized depolarizations. This response enhancement by local network activity is in agreement with our previous (and more detailed) studies in vitro (McCormick et al. 2003; Shu et al. 2003a).

Depolarization induced by network activity enhances responses to both artificial conductances and to visual stimulation in a smoothly graded manner.

We next explored the enhancement of cellular responsiveness caused by network activity by examining the probability of evoking spikes to a medium-sized artificial PSP as function of membrane potential. Rather than segregating each occurrence of this single PSP into Up or Down state trials, instead, the number of spikes per trial was examined as a function of membrane potential and probability of the EPSP evoking an AP. A similar analysis examining the number of spikes per trial as a function of membrane potential level immediately preceding the injection of an artificial EPSP (n = 4; Fig. 9A). As can be seen in Fig. 9A, the probability of a given sized PSP evoking an AP is a smooth function of the membrane potential just before injection of the artificial EPSP. These data were well fit by linear regression (dashed lines), and across the population of neurons (n = 7), there was a highly significant correlation (r = 0.94 ± 0.05, \( \chi^2, P < 0.05 \) for each neuron) between membrane potential and probability of the EPSP evoking an AP. A similar analysis examining the number of spikes per trial as a function of membrane potential was performed for flashed, optimal bars. Again, increased depolarization gradually increased the number of spikes evoked by the flashed, optimal bar (Fig. 9B). This was especially true for the range of membrane potential values that occurred in each neuron during the Up state (membrane potential histograms for each neuron in Fig. 9C). The gradual increase in spike output to the best stimulus as a function of membrane potential was evident for a representa-
tive sample of neurons \((n = 5)\), and the average correlation coefficient to a linear regression best fit line was highly significant \((r = 0.90 \pm 0.07; \chi^2, P < 0.05\) for each neuron). Taken together, these results showed that increases in depolarization of the membrane potential are highly correlated with an increased probability of generating spikes to the same stimulus.

**Depolarization induced by network activity enhances responses to visual stimuli at multiple contrast levels**

Finally, we determined whether the ongoing fluctuations seen in the network during the Up state correlate with increased neural responsiveness to optimal stimuli of different contrasts. For these experiments, a stationary grating patch of the best orientation (optimized for spatial frequency and phase) was randomly varied in contrast, and the responses to each contrast were plotted as a function of both the membrane potential of the recorded neuron and the level of network activity at the time of visual stimulation. Data obtained from a representative cell show the basic findings. Figure 10A shows a chattering (CH) neuron in which these varying contrast stimuli were presented during ongoing network activity of the activated (Up) state. The probability of generating spikes to a 40% contrast stimulus is markedly affected by membrane potential level at the time of visual stimulation, with a burst of spikes generated when the membrane potential was at \(-63\) mV (1st arrow), whereas this same stimulus results in a subthreshold PSP and no spikes when the neuron is spontaneously less depolarized about 200 ms later (\(-68\) mV, 2nd arrow). Shortly afterward, the neuron again becomes spontaneously depolarized to \(-63\) mV, and a high-contrast stimulus (80%, 3rd arrow) results in more spikes than the 40% contrast stimulus delivered previously at the same membrane potential. Note that we included only responses that occurred during the body of the Up state, and therefore our visual responses do not represent the initiation or termination of this state of recurrent network activity. During the activated (Up) state, fluctuations in membrane potential are significantly correlated with fluctuations in the activity of the local network, as measured simultaneously with nearby LFP recording (Fig. 10A, red trace at top). During the body of the Up states, the LFP and membrane potential fluctuations were significantly correlated, and the relationship between these two variables was well described by a linear regression (Fig. 10B; \(r = -0.61; \chi^2, P < 0.01; 30\) sequential Up states).

We found that spontaneous changes in membrane potential and network activity are associated with enhanced neuronal responsiveness to visual stimuli at multiple contrast levels (Fig. 10C). In extracellular SU recordings, we found that stimuli of all contrasts yielded larger AP responses during periods of greater LFP negativity \((n = 6, \text{data not shown})\), which is correlated with increased network activity and depolarization of single neurons. In addition, we also observed enhanced visual spike responses of intracellulary recorded neurons during periods of network-mediated membrane depolarization (Fig. 10, C and D). Changes of a few millivolts in a single cell can markedly enhance the number of spikes generated (above background firing rates) to low- and medium-contrast stimuli (Fig. 10C) and also enhance responses to high-contrast stimuli, although often by a lesser degree (e.g., compare enhancement of 80% stimulus to 10% stimulus). For the population of intracellular recordings \((n = 5)\), the change in firing rate above baseline was normalized to the maximum elicited response for...
each neuron, and the relationship between contrast and firing rate was fit by sigmoidal curves (Fig. 10D; \( \chi^2, P < 0.01 \) for both fits using a modified Hill equation). These results showed that a greater level of depolarization during network-activated states significantly scales upward the entire contrast response function at all contrast levels (from \( R_{max} = 0.60 \pm 0.2 \) to \( R_{max} = 0.91 \pm 0.15; P < 0.01, t\)-test) without a statistically significant change in the contrast needed to produce a half-maximal response (hyperpolarized: \( C_{50} = 19.28 \pm 2.24 \); depolarized \( C_{50} = 16.86 \pm 10.96; P > 0.05, t\)-test).

**DISCUSSION**

We have shown here that Up state network activity is associated with significant increases in cortical AP responses to visual RF stimulation compared with responses elicited by stimulation of quiescent networks during Down states. Visual response enhancement is paralleled by an increased ability of EPSP-like conductance injections during Up states to elicit APs. PSPs evoked by visual stimulation during Up/Down states are not, on average, significantly different in amplitude, but because Up state PSPs reach more depolarized levels because of ongoing network activity, they bring the membrane potential closer to threshold, and thus are more likely to initiate spikes. Furthermore, graded visual response enhancement mediated by spontaneous depolarizations is observed for both spatially sparse stimuli and for optimal stimuli of varying contrasts. The amplitude of this enhancement is strongly correlated with ongoing network fluctuations during the Up state. We conclude that such modulation of intracortical network activity levels provides a rapid and powerful mechanism to impact visual responsiveness.

**Agreement with previous findings**

These results are, to our knowledge, the first to show that relatively natural network activity enhances the spiking response elicited by brief stimulation within visual RFs and also increases the gain of the contrast response function. Furthermore, we showed that visual response enhancement is observed in both excitatory and inhibitory neurons exhibiting either simple or complex RFs. These results are in agreement with our previous studies showing that network activity enhances spiking responses to artificial EPSP-like conductance stimuli in vitro (McCormick et al. 2003; Shu et al. 2003a). Here, the change in spike rate on visual stimulation was more than two-fold greater during network activated (Up) versus network quiescent (Down) states, even though these visual stimuli were capable of initiating PSPs (Fig. 7) and spikes (Fig. 5A) in both states. Moreover, our results showed that the ongoing fluctuations in network activity level during the Up state are mirrored by fluctuations in neuronal responsiveness. These “analog” fluctuations in network activity translate to continual changes in the membrane potential, whereby increased depolarization progressively enhances spike probability to both artificial PSPs and to natural PSPs elicited by visual stimulation (Fig. 9). Our results are consistent with two previous findings in cat visual cortex: both spontaneous depolarization and increased network activity correlate with larger evoked responses (Arieli et al. 1996; Azouz and Gray 1999). However, these previous studies used high-contrast whole field

**FIG. 9.** Spontaneous depolarization during Up states linearly increases spike probability to both artificial PSPs and natural visual stimulation. A: spikes produced per injection of the same-sized dynamic clamp PSP as a function of prestimulus membrane potential. Plots for 4 representative neurons. Dashed line indicates linear regression best fit line, with correlation coefficient \( r \) and accompanying \( \chi^2 \) value for each neuron. B: spikes produced per presentation of the optimal visual stimulus as a function of prestimulus membrane potential for 4 example neurons. Same conventions as in A. Cell 7 presented both dynamic clamp and visual stimuli. C: distribution of membrane potentials for each neuron in B. For each neuron, increasing depolarization within range of membrane potentials exhibited during Up state gradually increases number of spikes elicited by same visual stimulus.
drifting gratings, where visual stimulation lasted for many seconds, and did not examine responses at different contrasts. Our present results showed that responses elicited from receptive field stimulation by either sparsely flashed bars or varying contrast stationary grating patches are potently enhanced by spontaneous depolarizations composed of patterns of balanced synaptic excitation and inhibition (Haider et al. 2006). Interestingly, our results show a depolarization-induced enhancement of weak (e.g., nonoptimal spatial location or contrast) and strong (best location or highest contrast) visual stimuli. Our results agree with studies of active and silent periods in cat sensori-motor cortex that showed strong Up state facilitation of cortical responses to peripheral nerve electrical stimulation (Rosanova and Timofeev 2005) and to electrical stimulation of the thalamus (Sachdev et al. 2004) versus decreased Down state responsiveness in cat sensori-motor cortex on electrical stimulation of thalamic pathways (Timofeev et al. 1996).

Comparison with rodent somatosensory system

In contrast to the results presented here and in prior studies of the cat visual cortex, studies in the rodent somatosensory system showed that AP and PSP responses to brief whisker stimulation are largest and most reliable when evoked from specialized cytoarchitecture (i.e., barrels). Barrel cortical PSPs are also influenced by the strong convergence and synchrony of subcortical structures (Bruno and Sakmann 2006). We hypothesize that, in the rodent vibrissal system, rapid and temporally succinct patterns of APs in subcortical pathways facilitate initiation of large PSP responses in barrel cortical networks during Down states, whereas comparatively more sluggish responses to flashed bars in the cat visual system do not activate such facilitated PSP responses. However, this hypothesis does not explain increased Down state responsiveness of barrel cortical neurons to electrical stimulation of the thalamus (Sachdev et al. 2004) versus decreased Down state neuronal responsiveness in cat sensori-motor cortex on electrical stimulation of thalamic pathways (Timofeev et al. 1996). One further possibility is that rodent somatosensory cortical responses are strongly influenced by this area’s specialized cytoarchitecture (i.e., barrels). The lack of augmentation of visually evoked Up state PSP amplitudes in this study is somewhat unexpected, because cortical spiking responses are larger during Up states and because intracortical synapses should make a significant contribution to visually evoked PSPs in these neurons (Douglas et al. 1995; Ferster et al. 1996). However, the Down state is associated with decreased membrane conductance and hyperpolarization, both of which will enhance the amplitude of evoked PSP barrages. Also, because neurons are less active during Down versus Up states, the synaptic potentials they
evoke may be larger because of removal of synaptic depression. An additional factor to consider is a possible increase in bursting in thalamic relay neurons during Down states (Contreras and Steriade 1995; Rosanova and Timofeev 2005; Timofeev et al. 1996), perhaps enhancing thalamocortical communication through temporal summation and facilitation of synaptic transmission (Gil et al. 1999; Swadlow et al. 2005).

One further intriguing difference exists between cat visual and rodent vibrissal system responsiveness. Similar to previous studies in anesthetized cats (Arieli et al. 1996; Tsodyks et al. 1999), our experiments using flashed bars did not initiate state transitions (but see Anderson et al. 2000a), whereas whisker stimulation often initiates transitions between Up and Down states (A. R. Hasenstaub, R. N. Sachdev, and D. A. McCormick, unpublished observations) and can elicit propagating waves of excitation within and across the barrel field during wakefulness (Ferezou et al. 2006; Petersen et al. 2003).

Effects of increased background activity in vivo

Synaptic barrages can have several effects on neuronal responses, through changes in membrane potential, conductance, and variance, in a spatially complex manner for individual neurons (Chance et al. 2002; Ho and Destexhe 2000; Rudolph and Destexhe 2003; Shu et al. 2003a). Transitions from Down to Up states in vivo are associated with ~15 mV of depolarization, increased membrane conductance, and increased membrane potential variability. Although our previous studies of visual cortical responsiveness have shown that intracellular current injection, resulting in depolarization of only a few millivolts, can significantly enhance the probability of spiking on visual stimulation (see Fig. 11 in Sanchez-Vives et al. 2000), we show here that enhancement of visual responsiveness is also mediated by depolarizing barrages of synaptic activity generated within the local network itself. Furthermore, during Up states in vivo, increased membrane potential variability facilitates responses to small- and medium-sized artificial PSP inputs (Fig. 5D; see also McCormick et al. 2003; Shu et al. 2003a) and may facilitate responses to low-contrast stimuli (Fig. 10). More detailed study of the effects of membrane conductance and variability on responses to low-contrast stimuli remains an important issue (Anderson et al. 2000b; Murphy and Miller 2003). Nonetheless, our main finding—that Up states facilitate neuronal AP responses to visually evoked PSPs and to artificially injected EPSP-like conductances—strongly suggests that the depolarization and increased membrane potential variability of Up states overcomes increased membrane conductance (Borg-Graham et al. 1998), resulting in enhanced spike probability. Furthermore, the amount of enhancement scales with the level of membrane depolarization, and both are highly correlated with the level of ongoing LFP/MU activity in the network. Importantly, our preparation preserves activity that is naturally expressed during slow wave sleep (Steriade et al. 2001) and also exhibits synchronized high-frequency content as seen in behaving animals (Hasenstaub et al. 2005; Womelsdorf et al. 2006), suggesting that the tight link between ongoing network activity fluctuations and membrane potential dynamics that we observe may extend beyond sensory processing.

Background activity and gain modulation with attention

The rapid modulation of neuronal responsiveness (e.g., gain) is a defining feature of cortical computations and may underlie diverse cognitive phenomena (Salinas and Sejnowski 2001). One prominent example is the enhancement of visual responses in primates during attention (Maunsell and Treue 2006; Reynolds and Chelazzi 2004; Womelsdorf et al. 2006). Importantly, multiple studies have shown that attention enhances the response to low-contrast visual stimuli (Martinez-Trujillo and Treue 2002; Reynolds et al. 2000; Williford and Maunsell 2006), although no single mechanism consistently accounts for all of these data. One model of visual attention posits a leftward shift in the contrast response function (the “contrast model”; Reynolds et al. 2000), whereas an alternative model explains attentional response enhancement as arising from a multiplicative gain change at all contrasts, including an increase in spontaneous action potential activity (the “activity gain” model; Williford and Maunsell 2006). Interestingly, the activity gain model accounts for the observed 30–40% baseline increase in spontaneous activity when spatial attention is directed within the RF, even in the absence of any visual stimulus (Luck et al. 1997; Reynolds et al. 2000; Williford and Maunsell 2006). We have shown here that, consistent with the activity gain model, the gain of the contrast response function is scaled up at all contrast strengths along with an increase in baseline activity during small depolarizations associated with spontaneous increases in network activity (Fig. 10). The range of fluctuations in membrane potential that we studied here in our anesthetized animals is similar to that found in naturally waking animals (Chen and Fetz 2005; Crochet and Petersen 2006; Lee et al. 2006; Steriade et al. 2001). In addition, our findings are in agreement with our previous results, which showed a similar multiplicative enhancement of the contrast response function with depolarization induced through the intracellular injection of DC (Fig. 12 of Sanchez-Vives et al. 2000). We propose that membrane potential depolarization, through either increases in synaptic bombardment or through alterations in the balance of excitatory and inhibitory potentials (Gabernet et al. 2005; Hasenstaub et al. 2005; Murphy and Miller 2003; Wilent and Contreras 2005), is the main mechanism underlying rapid changes in responsiveness among functionally interconnected cortical neurons.

An alternative hypothesis is that decreases in membrane conductance, through precise coordination of excitatory and inhibitory synaptic barrages, so as not to alter the average membrane potential, could underlie changes in neuronal gain (Chance et al. 2002). An important observation is that our measure of SD of $V_m$ during Up states (2.4 ± 0.7 mV) is considerably less than that previously reported (4 ± 2 mV; Pare et al. 1998; Rudolph et al. 2005), and is one half of that used for gain modulation in computational models (Chance et al. 2002). Although the sources of these discrepancies in neuronal variance are not precisely known, we were careful to measure SD of $V_m$ on neurons hyperpolarized with DC that prevented APs and their associated conductances, because these may have significant effects on membrane potential variability, even after filtering or manual AP removal. Such limitations on membrane variance naturally constrain the degree to which gain modulation may operate in cortical neurons through combined changes in membrane potential variability.
Relevance to awake behaving animals

The natural patterns of spontaneous activity that we used here are a widely observed phenomenon, present during slow wave sleep, anesthesia in vivo, and in slices in vitro (Cowan and Wilson 1994; Massimini et al. 2004; Sanchez-Vives and McCormick 2000; Steriade et al. 2001). Simultaneous EEG and intracellular recordings from unanesthetized cats (Steriade et al. 2001) indicate that the membrane potential in awake animals is tonically depolarized (near −57 mV), with a SD of −2 mV (their Fig. 7), and does not exhibit overt, rhythmic slow oscillations. Similarly, intracellular recordings from monkey motor cortex also display tonically depolarized, but variable, membrane potential trajectories lacking spontaneous slow oscillations during behavior (Chen and Fetz 2005). Recent recordings, however, from mouse barrel cortex have shown large (∼20 mV) V_m excursions during quiet waking that are reduced, but not eliminated, during active behaviors (Crochet and Petersen 2006). These fluctuations may be similar to activity we have measured here, although it is important to determine if membrane potential dynamics are unique for different cortical areas, modalities, species, and behavioral states. Nonetheless, all studies thus far indicate that the membrane potential of awake cortical neurons is considerably depolarized and moderately to highly variable. Because the overwhelming majority of inputs to cortical neurons arise from other nearby cortical neurons, these observed changes in depolarization and variability are likely consequences of changing activity patterns in the local cortical network itself. Therefore our studies here of visual response enhancement during spontaneously varying levels of network activity may be similar in many regards to the local network events surrounding visual processing in awake animals. The modulation of neuronal excitability—by active reconfiguration of the functional state of neural networks—may underlie diverse cognitive phenomena, such as attention, sensory-motor coupling, and contextual effects. Concerted changes in background activity not only provide a rapid and specific mechanism to control the gain and conductance (Chance et al. 2002; Murphy and Miller 2003).

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