Effect of Subthreshold Up and Down States on the Whisker-Evoked Response in Somatosensory Cortex

Robert N. S. Sachdev,1 Ford F. Ebner,2 and Charles J. Wilson1

1Department of Biology, University of Texas, San Antonio, Texas 78249-0662; and 2Department of Psychology, Vanderbilt University, Nashville, Tennessee 37240

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Sachdev, Robert N. S., Ford F. Ebner, and Charles J. Wilson. Effect of subthreshold up and down states on the whisker-evoked response in somatosensory cortex. J Neurophysiol 92: 3511–3521, 2004. First published July 14, 2004; doi:10.1152/jn.00347.2004. Changes in spontaneous activity within the cortex recognized by subthreshold fluctuations of the membrane potential of cortical neurons modified the response of cortical neurons to sensory stimuli. Sensory stimuli occurring in the hyperpolarized “down” state evoked a larger depolarization and were more effective in evoking action potentials than stimuli occurring in the depolarized “up” state. Direct electrical stimulation of the thalamus showed the same dependence on the cell’s state at the time of the stimulus, ruling out a strictly thalamic mechanism. Stimuli were more effective at triggering action potentials in the down state even during moderate de- or hyperpolarization of the somatic membrane potential. The postsynaptic potential (PSP) evoked from the down state was larger than the up state PSP but achieved about the same peak membrane potential, which was also near the reversal potential of the PSP (about −51 mV). Chloride loading shifted the reversal potentials of both the up state and the whisker-evoked PSP toward a more depolarized membrane potential. In addition, the threshold for action potentials evoked from the down state was lower than for spikes evoked in the up state. Thus the larger PSP from the down state may be caused by its larger driving force, and the state dependence of action potential generation in response to whisker stimulation may in part be related to a shift in threshold. Different mechanisms are therefore responsible for the state-dependence of PSP amplitude and the spike frequency response to the whisker stimulus.

INTRODUCTION

The cortical response to a sensory stimulus is variable; an identical stimulus can evoke a different response in cortex from trial to trial. In this study, we examine the role of autonomous activity within cortical circuits—i.e., activity that occurs even in the absence of subcortical inputs—in producing the trial-to-trial variability. Earlier work has shown that intracortical recurrent excitation can sustain autonomous activity, and this activity can govern the cortical response to thalamic inputs (Arieli et al. 1996; Bernander et al. 1991; Burns et al. 1979; Connors 1984; Cossart et al. 2003; Mao et al. 2001; Sanchez-Vives and McCormick 2000; Shu et al. 2003; Steriade et al. 1998). One consequence of activity within cortical circuits is that in the absence of desynchronizing inputs the membrane potential of cortical neurons spontaneously fluctuates between two subthreshold values, a hyperpolarized down state and a depolarized up state (Cowan and Wilson 1994; Lampl et al. 1999; Steriade et al. 1993a, b). During the up state, cortical neurons are spontaneously active and can fire action potentials (Cowan and Wilson 1994; Destexhe and Pare 1999; Landry et al. 1987; Pare et al. 1998; Steriade et al. 1993a, b; Stern et al. 1997). During the hyperpolarized down state, both the excitatory and inhibitory cortical neurons are mostly inactive (Contreras and Steriade 1995; Contreras et al. 1996; Timofeev et al. 2001) as indicated by an increase in the input resistance in the down state input compared with the up state (Cowan and Wilson 1994; Pare et al. 1998). The intracortical generation of these states has been established by experiments showing that thalamic ablations do not abolish cortical up and down states (Steriade et al. 1993b) and cortical ablations prevent up states from occurring in the striatum and the thalamus (Steriade et al. 1993b; Timofeev and Steriade 1996; Wilson et al. 1983).

These naturally occurring state changes observed during slow wave sleep, in the anesthetized animal and in the awake rat (Petersen et al. 2003; but also see Timofeev et al. 2001), present an opportunity for examining the ability of ongoing activity within cortical circuits to modify the cortical response to sensory stimulation. The response to sensory stimulation during the up state when cortical circuits are active can be compared with the response to the same stimulus during the down state when these circuits are relatively inactive. If the up state is more excitable because regenerative mechanisms in cortical neurons or circuits render them more excitable or because cortical neurons are closer to action potential threshold, then sensory stimulation should be more effective in evoking responses in the up state. If the up state is less excitable and the response to sensory stimulation smaller, inhibitory circuits in the trigeminal or thalamic pathways could be gating the response to sensory stimulation. Alternatively synaptic inhibition from intracortical circuits may shut the excitatory postsynaptic potentials (EPSPs) in the up state (Anderson et al. 2000; Berman et al. 1991; Borg-Graham et al. 1998; Contreras et al. 1996; Cowan and Wilson 1994; Mancilla and Ulinski 2001; Pare et al. 1998).

The up and down states reflect the state of the circuit as they occur only when the circuits are relatively intact (e.g., Sanchez-Vives and McCormick 2000; Timofeev et al. 2000). One goal of this study is to determine whether the changes in sensory responses between the up and down state are due to single neuron or circuit mechanisms. The response to sensory stimulation depends on several factors, including both synaptic
excitability of the individual cell under study and the state of the circuit (excitation, inhibition and disfacilitation) in which it is embedded. We used in vivo intracellular recordings from neurons in the primary somatosensory cortex during the presentation of a whisker deflection to test these possibilities.

The vibrissae are ideal for these studies because whiskers are sensitive tactile organs of great importance to the animal (Carvell and Simons 1990; Vincent 1912). Stimulation of the vibrissae produce graded responses in identified clusters of neurons in cortex (Armstrong-James and Fox 1987; Simons 1978; Welker 1971; Woolsey and Van der Loos 1970). Extracellular recordings show that stimulation of both principal and surround whiskers can evoke action potentials (Armstrong-James and Fox 1987; Nicolelis et al. 1995; Simons 1978; Welker 1971) and suppress action potentials (Krupa et al. 2004; Sachdev et al. 2000; Simons 1985; Simons and Carvell 1989; Swadlow 1989). Intracellular recordings from neurons in S1 barrel cortex neurons show that on average depolarizing PSPs evoked by principal whiskers begin \( \sim 8-10 \) ms after whisker deflection (Brecht and Sakmann 2002; Chung et al. 2002; Higley and Contreras 2003; Moore and Nelson 1998; Zhu and Connors 1999) followed several milliseconds later by inhibitory PSPs (IPSPs) (Higley and Contreras 2003; Moore and Nelson 1999). The spectrum of excitatory and inhibitory synaptic activity can be repeatedly activated by stimulation of a single whisker.

METHODS

All procedures were carried out in accordance with animal care guidelines of the National Institutes of Health, and the University of Texas at San Antonio.

Surgery

Thirty adult male long-Evans rats weighing 270–495 g were used in these experiments. Animals were anesthetized with urethane (20% wt:vol; 1.0 g/kg body wt ip) and supplemented with ketamine/xylazine (35 mg/kg ketamine, 7 mg/kg xylazine im, hourly) respectively. A craniotomy was made over the somatosensory barrel cortex, and the cisterna magna was drained to relieve intracranial pressure.

Electrophysiology

The recording electrodes were either thin walled glass micropettes or standard walled (2 mm diam) glass micropettes filled with 1 M potassium acetate or 1 M potassium chloride and 4% biocytin. In experiments where action potentials were blocked, 5–25 mM lidocaine N-ethyl bromide (QX-314) was also present in the microelectrode. Electrodes were advanced into the brain using a Narashige micromanipulator. The electrode impedance was determined when the electrode entered the brain. Electrode impedances ranged between 18 M\(\Omega\)H9024 and 60 M\(\Omega\)H9110 measured in the brain. A Neurodata IR 283 (Cygnus) active bridge amplifier was utilized for intracellular recording. Capacitance compensation, DC offset, and bridge balance were monitored and carefully adjusted as the electrode was advanced into the brain. The signal from the electrode was digitized at a rate of 10 or 20 kHz. After recording from the neuron, the electrode was retracted, and a record of the DC offset was saved. This DC offset was removed from the final membrane potentials reported in this manuscript.

A probe attached to a piezo-electric stimulator was positioned next to a single whisker that evoked the best extracellular response for that penetration. Whiskers were stimulated at low frequencies (0.7 Hz), moved by \( \sim 150 \) \(\mu\)M caudally and upward at a 15\(^\circ\) angle from vertical. Forty to 70 trials were collected to construct post stimulus time histograms. The stimulus duration used in this study ranged between 5 and 500 ms with the 500-ms stimulus used for all neurons. Long-duration stimuli were used because these stimuli were more likely to produce OFF responses that could also be investigated for their state dependence. For each neuron and each stimulus condition (current injection, stimulus duration), an equivalent number of traces of spontaneous activity were also collected. Only neurons with a membrane potential more negative than \(-55\) mV and action potentials that crossed zero were included in the sample. Some cells were stained for biocytin by passage of depolarizing current pulses (1–1.5 nA, 200-ms duration). The purpose of staining was to determine the cell type and lamina of the recovered neurons. All 16 neurons recovered were pyramidal cells, located either in layer II–III or V. Data were collected 0.2–1.6 mm from the pial surface; recordings in layer VI were therefore unlikely.

Electrical stimulation of the thalamus

Pairs of stainless steel 000 insect pins (diameter at tip 10 \(\mu\)m, diameter at shaft 200 \(\mu\)m, insulated with epoxylite except for 0.2–0.5 \(\mu\)m at the tips, separated by 0.5–1 mm) were stereotaxically implanted in VB thalamus (AP 3.3, L 2.1, Z 6.7). Thalamic stimuli were square wave pulses, brief in duration (0.1 or 0.5 ms) ranging from 0.1 to 2 mA in amplitude. In neurons that fired spikes, the stimulus strength was adjusted to produce at least one spike on half the trials.

Data analysis

All points histograms of the membrane potential were prepared by plotting the membrane potential at all digitized points during 10 s of spontaneous membrane potential fluctuations. Post stimulus time histograms and raster plots were prepared using standard methods and were implemented in Mathematica. For each trial, 1 s of data were collected with the stimulus applied 50–200 ms after the start of a trial and the rest in post stimulus activity. The prestimulus membrane potential trajectory was used to determine whether the neuron was in the up or the down state at the time of stimulus onset. Traces were sorted according to whether the stimulus was delivered in the up or the down state (meaning that they had been in that state for \( \geq 50\) ms at stimulus onset). Trials in which the membrane potential was in transition between the two states at or just before stimulation were discarded.

Trials in which only spontaneous activity was collected were similarly sorted, based on their membrane potential trajectory in the first 50 ms. Because there is some periodicity of up and down state transitions, cells in the up state at the beginning of the trial are very likely to show a down state transition in the subsequent 500 ms regardless of stimulation. We used spontaneous firing to estimate the expected poststimulus histograms for each set of sorted trials. Up and down state histograms of spontaneous activity were subtracted from the post stimulus time histograms before doing the paired t-test in Mathematica. For the calculation of the rate of firing during the response and for the rate of spontaneous activity, the data were binned into 10-ms bins.

Reversal potentials for the PSPs evoked by whisker stimulation were estimated in the presence of QX-314. For each cell, representative up and down state trials were selected in the presence and in the absence of constant current. A representative trial for each state and for each level of current injection (0 current and reversed) was smoothed using a nine-point moving average. Response amplitude at each time point was defined as the difference between the membrane potential achieved and the average baseline membrane potential for the 100 ms before the stimulus onset. The reversal potential was estimated from the intercept of the line between the zero current and the reversed traces. The change in reversal potential over the course of the response could be measured in this way for responses evoked in...
the down state. Because of its small amplitude compared with the spontaneous membrane potential fluctuations of responses, the reversal potential of the up state response could only be accurately estimated at the peak of the response.

The action potential threshold was defined as the voltage at which the rate of change of voltage exceeded 10 mV/ms. The rising phase of action potentials was identified using a simple voltage threshold. The threshold was the voltage at the last point with a ±10 mV/ms voltage derivative on the rising phase of the action potential.

**RESULTS**

All neurons in the sample responded to stimulation of at least one whisker. The peak amplitude of the PSP and the number of spikes evoked in the first 50 ms after whisker-stimulus onset was measured in 23 neurons recorded with 1 M potassium-acetate-filled electrodes and in 20 neurons recorded with 1 M potassium-chloride-filled electrodes. The PSP amplitude was also measured in 12 neurons with electrodes filled with QX-314 and 1 M KAc solution and in 17 neurons with electrodes filled with QX-314 electrodes and 1M KCl. In an additional seven neurons, recorded with QX-314 (KCl or KAc) whisker stimulation was presented during application of a series of constant current steps to determine the PSP reversal potential.

**Effect of states on whisker-evoked responses**

The membrane potential of all neurons spontaneously switched between a hyperpolarized down state and a mostly subthreshold depolarized up state (Fig. 1 A and B, top). To examine the state dependence of spikes evoked per trial, trials were sorted on the basis of the state of the neuron at stimulus onset. If a neuron was in the down state for ≥50 ms prior to the stimulus onset, the trial was included for the down state histogram (Fig. 1C). Similarly, the up state trials were included if the neuron had been in the up state for ≥50 ms before stimulus onset. Trials with state transitions within 50 ms before stimulus onset were discarded.

In all neurons tested, (n = 23) the synaptic potentials evoked during the down state were larger than those evoked in the up state. In six neurons whisker-stimulation-evoked PSPs were subthreshold for action potential generation, both in the up and down states. For these six neurons, the mean PSP amplitude evoked from the down state was greater than that from the up state. The average synaptic potential in response to whisker stimulation was 3.6-fold larger from the down state (14.8 ± 1.4 mV) than from the up state (4.1 ± 0.74 mV).

Seventeen of the 23 neurons exhibited suprathreshold responses to whisker stimuli; of these cells, all but three cells fired more action potentials in response to the stimulus presented in the down state than in the up state. When the neurons were in the up state (and firing spontaneously), the whisker stimulus evoked fewer action potentials and a much smaller PSP in response to the stimulus (Fig. 1C) than the same stimulus presented in the down state. Figure 1D shows the same result for all trials in rasters and poststimulus time histograms (PSTHs). Note the lower spontaneous activity in the 50-ms period before the stimulus in the down state raster and histogram (Fig. 1D). This results from sorting trials. Consequently, histograms of similarly sorted trials in the absence of whisker stimulation also show very different histograms for the up and down states. Because of spontaneous activity, the probability of spikes early in the trial is higher in the up state histogram; the down state histogram on the other hand has no spikes early in the trial. Later in each trial, a state transition and accompanying change in firing rate is likely, producing abrupt changes in spike rate unrelated to the stimulus. To take this sorting artifact into account, spontaneous activity was collected (n = 13 neurons) in 1-s periods and sorted into up and down state trials as if stimuli had been delivered. The difference between the histograms for stimulus and spontaneous trials had a zero firing baseline and showed only firing related to the stimulus. These residual histograms (Fig. 1E) were prepared for every neuron for each state before subtracting the up and down states. Sensory stimuli delivered in the down state were significantly (P < 0.001, paired t-test, t = 7.24, df = 7) more likely to evoke action potentials within 50 ms after stimulus onset than the same stimuli delivered in the up state. After subtraction of the spontaneous firing rate, the sample average peak firing rate for the response (averaged in 50-ms post stimulus) from the down state was 27.0 ± 3.1 versus 4.2 ± 2.2 spikes/s from the up state. The absolute stimulus-evoked firing rate (without subtraction of spontaneous firing) was also greater in the down state (28.3 ± 3.3 spikes/s) than in the up state (21.2 ± 3.2 spikes/s, t = 4.44 df = 12, P < 0.001). Thus when assayed by the number of spikes generated by the whisker stimulus, neurons were more responsive to sensory stimulation in the down state than the up state. Responses that occur at stimulus offset (off responses) had similar state dependence as the on responses (data not shown).

Chloride loading is expected to make IPSPs depolarizing and reverse direct inhibition to the cell under study. In the absence of stimulation, the mean spontaneous firing rate for neurons recorded with 1 M KAc and 1 M KCl was 4.27 ± 1.43 and 7.01 ± 1.03 Hz, respectively. Chloride loading did not abolish spontaneous up and down states and did not abolish the down state dependence of the whisker-evoked spikes (Fig. 2). The average spike rate evoked in the 50-ms post whisker stimulation was significantly greater from the down state (n = 20, P < 0.001, t = 4.91, df = 19), evoking 27.7 ± 5.5 compared with 3.6 ± 3.12 spikes/s from the up state (after subtraction of the sorted spontaneous histograms). The state dependence of spikes evoked by the stimulus was apparent in 18/20 neurons loaded with chloride. Of the remaining neurons, one had a twofold higher firing rate from the up state, 10.6 versus 4.6 spikes/s, and the second one had an almost equal response from the up and down state (5 versus 4.4 spikes/s) in the 50-ms post stimulus after spontaneous histogram subtraction.

**Effect of up and down states on thalamic-evoked response**

Earlier work established that cortical up and down states generate changes in activity in nuclei that receive input from cortex, including the striatum and thalamus (Steriade et al. 1993b; Stern et al. 1997; Wilson and Kawaguchi 1996). We therefore considered the possibility that during the cortical up state the thalamus, not the cortex, might be less responsive to the whisker stimulus. Changes in thalamic excitability were bypassed by electrical stimulation with a bipolar stimulating electrode stereotaxically implanted in the ventrobasal thalamic
The average latency to PSP onset (in the presence of QX-314, from the down state) was 3.89 ± 0.41 and 9.72 ± 0.54 ms to VB and whisker stimulation, respectively. The latency to PSP values corresponds well to values for principal whisker stimulation (Brecht and Sakmann 2002; Moore and Nelson 1999; Zhu and Connors 1999) and for thalamic stimulation (Chung et al. 2002). The average latency to spike onset was 10.4 ± 1.06 ms for the thalamic stimulus and 21.96 ± 2.73 ms for whisker stimulation. These numbers are somewhat longer than numbers reported in extracellular recordings, and the latency to action potentials is more variable. The latency numbers are mixed across layers reflecting the fact that the results of this study do not depend on the layer or on the particular (principal or surround) whisker stimulated (not shown). Also note that for thalamic stimulation, the stimulus intensity was adjusted so that spikes were evoked on approximately half the trials.

With KAc electrodes, the PSP generated by thalamic stimulation in the down state was larger than the PSP evoked from the up state, (Fig. 3A). More spikes were generated from the down state (compare the middle PSTH to the PSTH on the right) than from the up state (without spontaneous histogram subtraction). The average firing rate evoked by thalamic stimulation was 3.34 ± 3.0 spikes/s in the up and 10 ± 3.1 spikes/s in the down state (after spontaneous histogram subtraction). After current injection to hyperpolarize the neuron (Fig. 3B, right) the up state PSP amplitude increased, but the down state PSP evoked without current injection still had a larger relative amplitude (Fig. 3B, left). Nevertheless, the absolute membrane potential achieved by the PSP evoked from the down state was

**FIG. 1.** Sensory stimuli delivered in the down state evoke a postsynaptic potential (PSP) and spike. A: spontaneous activity during no current injection is shown at top, during depolarizing current is shown in the middle, and hyperpolarizing current is shown at the bottom. The 2nd long record of the spontaneous activity shows the 2 states, a hyperpolarized down state and a depolarized up state at the 3 levels of current injection. Depolarization by current injection (0.25 nA) increases the firing rate (A, middle) and hyperpolarization by current injection (0.25 nA) decreases the rate of firing (A, bottom) but current injection has no effect on the occurrence of up and down states. Scale bars are shown at the bottom. B: all points histograms of the membrane potential. The all points histograms are bimodal showing that the neuron has 2 preferred membrane potentials. Current injection has no effect on the occurrence of up and down states but shifts the average membrane potential to the right (middle) or left (bottom). All points histograms are made from 10 s of activity sampled at 20 kHz. C: stimulus evoked response in 3 trials. Trials in which the whisker stimulus occurred when the neuron was in the down state (left) are separated from trials when the stimulus occurred in the up state (right). These records indicate that independent of whether the neuron is depolarized or hyperpolarized this neuron is more responsive in the down state. The different colored traces are different trials. Action potentials are truncated. The arrows point to stimulus onset; the stimulus stayed on throughout these records. The stimulus artifact at stimulus onset looks like an action potential and has not been removed or truncated. Scale bars are shown in the middle. The dashed lines mark the membrane potentials –90 and –65 mV. D: rasters and poststimulus time histograms of the whisker evoked response. These show the down state dependence of the whisker stimulus evoked response in the all trials used in the down and the up state histograms. In the up state, there is just spontaneous activity. Poststimulus time histogram (PSTH) bins are 10 ms. Lines in the rasters indicate stimulus onset. E: residual PSTHs. Spontaneous activity records for this neuron at each level of current injection have been sorted into up and down state records and resulting histograms have been subtracted from the post stimulus time histograms shown in D. Stimulus duration is 500 ms. Neuron recorded with 1 M KAc in the pipette.
more polarized than the membrane potential achieved by the PSP evoked in the up state. In neurons loaded with chloride (in the absence of QX-314, n = 23, Fig. 3C), the average number of spikes evoked per second, in the down and up states (after spontaneous histogram subtraction) was 24.4 ± 2.62 and 11.0 ± 3.0 spikes/s, respectively. The down state response was significantly larger than the up state response (P = 0.001, paired t-test, t = 3.69, df = 22). Of these neurons, three had more spikes (double the response level after subtraction of the spontaneous activity) in the up state. These experiments confirm that the cortical circuit is less responsive to both natural and artificial thalamic inputs in the up state but do not rule out a thalamic role in the decreased excitability in the cortical up state response to whisker stimuli.

Driving force in the up and down states

The difference in PSP amplitude of responses evoked in the up and down states may occur due to a difference in the synaptic driving force caused by depolarization in the up state. If the synapses responsible for the PSP were somatic, the moderate membrane potential changes in Figs. 1–3 might be sufficient to test this. But the effects of constant current injection into the soma may be attenuated at the distal dendritic synapses, and larger current injections would be required to ensure that the distal dendritic synapses were affected by current injection at the soma. To ensure that the membrane potential at the relevant dendritic synapses was altered by current, neurons were depolarized to the reversal potential for whisker responses and state transitions in the presence of the sodium channel blocker QX-314. When applied internally, QX-314 blocks sodium currents in a use-dependent manner that develops over several minutes (Cahalan 1978; Cahalan and Almers 1979; Connors and Prince 1982; Wilson and Kawaguchi 1996). The blockade of action potentials by QX-314 made it possible to measure the amplitude of whisker-evoked PSPs in all cells.

The use of QX-314 did not change the basic observation that the PSP evoked by whisker stimulation is larger for stimuli that begin in the down state than for those that begin in the up state. In 10 neurons recorded with electrodes filled with KAc/QX-314, the depolarizing PSP evoked by whisker stimulation was significantly larger (P < 0.001, paired t-test, t = 7.28, df = 9) in the down state (14.2 ± 1.43 mV, Fig. 4A, left and bar graphs) than in the up state (2.4 ± 0.9 mV; Fig. 4A). When the up and down states were reversed by current injection (Fig. 4A), the whisker-evoked PSPs were similarly reversed, but those from the down state were still significantly larger (P < 0.001, paired t-test, t = 4.91, df = 9) than the PSPs evoked from the up state. The down state PSP amplitude to whisker stimulation was −35.7 ± 4.2 mV; the up state PSP was −14.4 ± 1.5 mV (Fig. 4A, bar graphs). Likewise, thalamic stimulation...
elicited a PSP of $2.4 \pm 0.9$ and $16 \pm 3.1$ mV from the up and down state, respectively, in recordings made with QX-314/KAc-filled pipettes (Fig. 4A, right, $n = 5$). With QX-314/KCl electrodes, thalamic stimulation ($n = 10$) in the Down state evoked a significantly ($P < 0.001$, paired t-test, $t = 7.9, df = 9$) larger PSP ($17.1 \pm 1.4$ mV) from the down state versus ($7.5 \pm 2.1$ mV) the up state. In the presence of QX 314 ($n = 12$) dissolved in 1 M KCl, whisker stimulation evoked a $20.01 \pm 1.7$ mV depolarization from the down state and a $3.1 \pm 1.1$ mV depolarization from the up state (Fig. 4A, middle). Despite artificial depolarization of the membrane to the reversal of the PSPs in 9 neurons, the PSP evoked from the up state continued to be smaller than from the down state ($P < 0.001$, paired t-test, $t = 5.84, df = 8$).

The reversal potentials for the PSPs were determined in 19 neurons treated with QX-314 (Fig. 4). The effects of various levels of constant current on the up and down state membrane potential in the up and down states was assessed (Fig. 4, B and

![Figure 4](https://example.com/fig4.png)

**FIG. 4.** Current induced changes in PSP amplitude and reversal of the PSP. A: using QX-314 dissolved in KAc (left) or KCl (right) and depolarizing the neurons by current injection. Whisker stimulation (500-ms stimulus, arrows indicate the onset) evokes a larger depolarizing PSP from the down state (black) compared with the PSP evoked from the up state (gray) with both KAc and KCl. Reversing the up and down states by current injection has no effect on the relative PSPs. The average down state PSP is larger even after the neuron has been depolarized and the responses reversed (bar graphs). The PSPs evoked by thalamic stimulation (right) in the presence of QX-314 show a similar state dependence as those evoked by whisker stimulation. The error bars are standard errors of the mean PSP. B: effect of current injection on membrane potential and PSP amplitude with KAc and QX-314. Negative current injection hyperpolarized both the up and down states (left). The up and down state PSP amplitude increases with hyperpolarization (middle) and rectifies with large hyperpolarizing current injection. Depolarizing current reverses the up state PSP; the PSP remains reversed throughout the entire depolarizing current regime. The down state PSP reversed later. The reversal potential for a group of neurons was estimated by plotting the PSP amplitude from the up (open squares, $n = 10$) and down (closed squares, $n = 10$) state with and without current injection (right). The intersection with the 0 PSP amplitude was considered the reversal potential. The reversal potential for the PSP in the up and down state was similar. The reversal potential was $–51$ mV. C: effect of current injection on membrane potential and PSP amplitude with KCl and QX-314. Negative current injection hyperpolarized the down state but had little effect on the up state membrane potential (left). The down state PSP amplitude increased with hyperpolarization. Depolarizing current reversed the up state PSP, but it took more current to reverse the down state PSP. The PSP evoked in the up and down state reversed at $–34$ mV (more depolarized than the reversal potential in B), suggesting that IPSPs normally contribute to the PSP in both the up and down state. This reversal potential was similar for the up and down state. Error bars are SE.
C, left). To measure the reversal of the peak response, the amplitude of the PSP at 20-ms post stimulus onset was measured relative to average membrane potential in the 100 ms before stimulus onset (Fig. 4, B and C, middle). The choice of 20-ms post stimulus was based on two factors: one, the peak of the PSP evoked by whisker stimulation occurs at 20 ms and second, (in the absence of QX-314) action potentials evoked by whisker stimulation were triggered at this latency. The reversal potential was estimated by interpolation (Fig. 4, B and C, right). At zero injected current with KAc electrodes (Fig. 4B), the relative amplitude of the down state PSP was larger than the up state PSP but the absolute potential achieved at the peak of the PSP in the two states was similar. Hyperpolarization increased the amplitude of the PSP in both the up and the down states but had a larger effect on the down state. Small depolarizing currents (0.25 –0.85 nA) reversed the PSP in the up state, indicating that in the up state the neuron was close to the reversal potential for the whisker-evoked PSP. The reversal potential for responses evoked from the up and down states were approximately the same; these were the same as the reversal point for the up and down states themselves (compare Fig. 4B, left and middle). The reversal potential from the up and down state is illustrated in Fig. 4B (right) for a sample of 10 neurons measured at two levels of injected current one below and one above the reversal point for down state PSPs. The mean interpolated reversal point for the whisker evoked PSP was –51 mV and was similar for both up and down states. Chloride loading shifted the reversal potential in the positive direction to –34 mV; in this case both the up and down state PSPs reversed at the same point (Fig. 4C). The amplitude of the PSP simply depends on the membrane potential at stimulus onset and the reversal potential of the PSP; consequently, even after reversal of the up and down states the PSP evoked from the down state is larger.

The time course of the changes in reversal potential were examined for whisker stimulation in the down state for 19 cells recorded with either KAc/QX-314- or KCl/QX-314-filled electrodes (Fig. 5); the up state spontaneous fluctuations were too large and the up state PSP too small for this analysis. Single-trial data at two membrane potentials— with and without depolarizing current injection—were used for this analysis. The whisker-evoked PSP shown in the example in Fig. 5 began 7 ms after the stimulus onset. For the same neuron, the reversal potential immediately after the response began was depolarized (>0 mV (Fig. 5), but by 20 ms the reversal potential of the response was relatively hyperpolarized (Fig. 5; also note Fig. 4, B and C, right). The average reversal potential for the 19 neurons, 1 ms after the responses began was –5 ± 27 mV. These results are consistent with earlier observations by Moore and Nelson (1999) and Higley and Contreras (2003) showing that the reversal potential for the early portion of the PSP evoked by principal whisker stimulation is positive to 0 mV. The results are also consistent with the work done in cat visual cortex showing that at the maximal conductance the average values for the reversal potential were hyperpolarized (~63 mV) (Borg-Graham et al. 1998; Monier et al. 2003). The early depolarized reversal potential indicates that excitatory conductances dominate inhibitory conductances immediately after the response begins.

State-dependent variability in action potential threshold

Because the membrane potential of the neuron fluctuates slowly and because the neuron dwells in each state for ~500 ms (Stern et al. 1997), we considered the possibility of a change in firing threshold in the up and down states. Note that in the up state action potentials were rarely triggered by whisker stimulation. To examine the shift in threshold, we therefore used cells and trials (data from recordings made with KAc- and KCl-filled electrodes are both included for this analysis) in which the whisker stimulus was presented in the down state and evoked more than one spike in the 100-ms poststimulus onset. Spike thresholds were measured for action potentials evoked at the beginning of the PSP and for subsequent spikes that occurred in the same PSP when the cell was at peak depolarization comparable to the up state membrane potential (Fig. 6, black). The thresholds for spikes that occurred in the 100 ms before stimulus onset (i.e., in the up state) were also measured (Fig. 6, red, orange). In 21 cells (225 trials), whisker stimulation evoked more than one spike in the 100 ms poststimulus (Fig. 6, black and blue). The mean latency for the first spike was 19 ± 3 ms and the latency to the second spike was 79 ± 8 ms (median latency for 2nd spike was 31 ms). The threshold for the first spike was ~49.8 ± 1.2 mV; the threshold for the second spike was significantly more depolarized (paired t-test, t = –2.08, P < 0.05) by 3.2 to
evoked response to become subthreshold for spiking. If for some reason in these other studies, the balance between excitation and inhibition in the PSP were altered relative to that responsible for the up state, the excitatory component of the response might become dominant, the stimulus may be even more effective when combined with the depolarization of the up state.

State dependence of PSP amplitude is caused by the change in driving force

It is an apparent paradox of this study that the depolarization that occurs as part of the up state brings the neuron close to action potential threshold, an action expected to make it easier to evoke action potentials, but instead action potentials become less likely. This could be caused by a decrease in the amplitude of PSPs, as observed in the present study, or a positive shift in action potential threshold, as also observed here, or both.

On the basis of our manipulations of membrane potential, we conclude that the larger PSP evoked by whisker stimulation from the down state as compared with the up state is produced by the difference in driving force and can be entirely explained by the difference in membrane potential. We found that the reversal potential for the PSP evoked by whisker stimulation is the same when stimuli are evoked from both up and down states. Because the input resistance of the neuron is different in the up and down states and synaptic input is distributed over the surface of cortical pyramidal cells, it was necessary to block action potentials and apply large somatic currents to reverse the evoked PSPs for this measurement. The somatic location of the recording electrode and the dendritic location of most synapses introduce some known errors in the estimate of synaptic reversal potentials. To ensure that synaptic membrane potential was altered by somatic current injection, we poisoned sodium action potentials with QX-314 and injected larger amounts of current to depolarize (by 50–70 mV) and hyperpolarize (by 20–40 mV) the neuron while measuring the PSP amplitude evoked by whisker stimulation. If the membrane properties (i.e., the input resistance) of the cell were similar in the up and down states, the errors in the estimate of the reversal potentials would be similar in the up and down state. However, earlier work (Cowan and Wilson 1994; Pare et al. 1998) showed that pyramidal cells have a lower input resistance in the up state than in the down state. This difference in input resistance is attributed to the difference in cortical circuit (synaptic) activity in the two states. Thus there is likely to be a difference in the estimated errors of the reversal potentials in the up and down states. These errors cannot account for the polarized reversal potential we observed (about −51 mV, at 20 ms) for whisker-evoked PSPs in both the up and down states. The relatively negative reversal potential at the peak of the depolarizing PSP indicates that the whisker-evoked PSP contains a large inhibitory component. After chloride loading to shift the IPSP reversal potential positive, firing was still best evoked by stimuli delivered from the down state, and the whisker-evoked PSPs in the up and the down states were shifted similarly. The effect of chloride loading supports the view that the difference in PSP amplitude in the up and down states is primarily due to the difference in driving force in the two states and that in the up state the membrane potential is near the reversal potential for the PSP.
The time course of the reversal potential indicates that for a brief period after the onset of stimulus excitatory conductances dominate inhibitory conductances. A brief dominance of excitation could occur if inhibitory circuits were delayed relative to the excitatory PSPs and the depolarization in the up state allowed inhibitory circuits to respond more rapidly (perhaps because they were already active). In our results, action potentials evoked by the whisker stimulation occurred at ~20 ms, usually at the peak of the PSP, at which time our measurement of reversal potential was most accurate.

It is possible that the measurement of reversal potentials using QX-314 fundamentally alters the PSP. QX-314 has a wide range of effects, including blockade of currents activated by the GABAB receptor (Andrade 1991; Perkins and Wong 1995). We varied the concentration of QX-314 in the electrode from 5 to 25 mM to find the concentration just sufficient for blockade of action potentials, but it is conceivable that it still might alter synaptic reversal potentials. This is an important issue because our best measurement of similar reversal potentials of whisker-evoked responses was obtained in the presence of QX-314, but the observations on firing in response to synaptic input were necessarily obtained in its absence. However, even in the absence of QX-314, it was evident that the absolute amplitudes of PSPs evoked from the up and down states were similar.

One key feature of the present results is that up and down states and the PSPs evoked by whisker stimulation reverse at similar membrane potentials (Fig. 4); that is, the mixture of EPSPs and IPSPs occurring spontaneously is similar to that evoked by whisker stimulation. The moment-to-moment balance between excitation and inhibition in the up state largely determine the up state membrane potential. The momentary decrease in inhibition or the increase in excitation allows spontaneous spikes to occur. Excitatory and inhibitory cortical neurons are spontaneously active in the up state (Steriade et al. 1993a,b) and during the up state fast IPSPs occur at a frequency of 30–40 Hz in cortical pyramidal neurons (Cowan and Wilson 1994; Timofeev et al. 2001). During the cortical up state, there is more activity in both the excitatory and the inhibitory interneurons.

Whisker stimulation activates both excitatory and inhibitory neurons in layers I–V of cortex (Armstrong-James and Fox 1987; Armstrong-James et al. 1992; Simons 1978). Several earlier studies have examined the time course of excitation and inhibition after whisker stimulation (Higley and Contreras 2003; Moore and Nelson 1998). These studies show that at response onset EPSPs are followed by IPSPs and that except for the initial few milliseconds, IPSPs and a reduction in circuit excitation dominate the response to both the principal and the surround whiskers (Higley and Contreras 2003). Stimulation of the principal whisker evokes a short lasting (1–2 ms) EPSP that reverses at 0 mV; the surround whisker does not evoke a similar EPSP. In the present study, the reversal potential of the PSP evoked from the down state was close to 0 mV in the first few milliseconds; at later time points, the reversal potential shifted to hyperpolarized membrane potentials. This shift in the reversal potential of the PSP is consistent with the results of previous studies (Borg-Graham et al. 1998; Higley and Contreras 2003; Moore and Nelson 1998). The whisker-evoked latency to PSP reported in the present study is also comparable to the latencies reported for PSPs evoked by principal whiskers (Brecht and Sakmann 2002; Higley and Contreras 2003; Moore and Nelson 1998; Zhu and Connors 1999).

Excitatory and inhibitory neurons are activated by thalamic inputs directly. In vitro, thalamic inputs preferentially excite and evoke action potentials in a group of inhibitory cortical neurons not in pyramidal neurons (Porter et al. 2001). In vivo, a single thalamic action potential can be related to widespread inhibition in barrel cortex (Swadlow and Gusev 2000). Inhibition spreads because of powerful interconnections and coupling between inhibitory interneurons (Galarreta et al. 1999; Gibson et al. 1999).

Increase in inhibition or decrease in excitation could be related to short-term synaptic plasticity. Cortical synapses depress the excitatory synapses more than the inhibitory (Galarreta and Hestrin 1998; Thomson et al. 1993; Tsodyks and Markram 1997; Varela et al. 1999). Thalamocortical synapses can be depressed or facilitated (Castro-Alamancos and Connor 1996, 1997; Chung et al. 2002; Gil et al. 1997). With increasing stimulation frequency, response levels in cortex and thalamus decrease (Ahissar et al. 2000; Hartings and Simons 1998; Kleinfeld et al. 2002; Simons 1978). Here we use a low stimulus frequency, so stimulation is not likely to induce depression in any of these synapses; however, the higher levels of spontaneous activity in the up state may depress the cortical and thalamic synapses. Synaptic depression could therefore in theory provide an explanation for the decreased number of action potentials in the up state (Petersen et al. 2003). Taken together the inhibitory interneuron activity in the up state, preferential activation of inhibitory interneurons by thalamic inputs, and the hyperpolarized reversal potential of the PSP evoked by whisker stimulation all indicate that in up state inhibitory cortical neurons will fire action potentials at stimulus onset.

**Mechanisms suppressing firing in the up state**

Action potential threshold is not constant. The threshold varies with repetitive firing and depolarization induced by synaptic inputs (Kolmodin and Skoglund 1958) or by intracellular current injection (Granit et al. 1963; Schwindt and Crill 1982). The voltage trajectory just before an action potential influences threshold (Azouz and Gray 1999; Henze and Buzsaki 2001; Wickens and Wilson 1998). Gradual depolarization reduces the availability of sodium channels (Bezania 2000; Hodgkin and Huxley 1952; Noble 1966). Alternatively, the threshold could be lowered in a state-dependent manner as reported for cat motor neurons (Krawitz et al. 2001) where the threshold is lowered during fictive locomotion whether the neuron is in the depolarized or the hyperpolarized phase of the cycle (Dai et al. 2002; Krawitz et al. 2001). Altering the maximum sodium or delayed rectifier potassium conductance or shifting the voltage dependency of sodium and potassium channel opening is effective in shifting action potential threshold. In the present study, spike threshold is lowered, but only for the first spike evoked from the down state. Spikes evoked by whisker stimulation in the 50-ms post stimulus in the up state did not occur very often, but the spikes that occurred in the up state had a higher threshold than the first spike evoked from the down state. Spike threshold accommodation accounts for the state-dependent decrease in firing in the up state.
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