Balanced Excitation and Inhibition Determine Spike Timing during Frequency Adaptation

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In layer 4 (L4) of the rat barrel cortex, a single whisker deflection evokes a stereotyped sequence of excitation followed by inhibition, hypothesized to result in a narrow temporal window for spike output. However, awake rats sweep their whiskers across objects, activating the cortex at frequencies known to induce short-term depression at both excitatory and inhibitory synapses within L4. Although periodic whisker deflection causes a frequency-dependent reduction of the cortical response magnitude, whether this adaptation involves changes in the relative balance of excitation and inhibition and how these changes might impact the proposed narrow window of spike timing in L4 is unknown. Here, we demonstrate for the first time that spike output in L4 is determined precisely by the dynamic interaction of excitatory and inhibitory conductances. Furthermore, we show that periodic whisker deflection results in balanced adaptation of the magnitude and timing of excitatory and inhibitory input to L4 neurons. This balanced adaptation mediates a reduction in spike output while preserving the narrow time window of spike generation, suggesting that L4 circuits are calibrated to maintain relative levels of excitation and inhibition across varying magnitudes of input.

Key words: barrels; whisker; intracellular; cortex; synaptic; conductance

Introduction

Cortical representation of sensory information across modalities is shaped by the local balance of excitation and inhibition (Kyriazi et al., 1996; Fox et al., 2003; Wehr and Zador, 2003; Zhang et al., 2003; Wilent and Contreras, 2004; Marino et al., 2005; Priebel and Ferster, 2005). In the rodent whisker system, ascending thalamic input engages neuronal circuits in cortical layer 4 (L4), consisting of excitatory spiny stellate and pyramidal cells as well as aspiny interneurons (White and Rock, 1981; Beierlein et al., 2002, 2003; Bruno and Simons, 2002) that provide feedforward and feedback inhibition to the local network (Agmon and Connors, 1991; Swadlow and Gusev, 2000; Porter et al., 2001; Swadlow, 2003; Staiger et al., 2004). This functional architecture leads to a precise sequence of excitation followed by inhibition in response to whisker deflection that may serve to sharpen the spike timing of suprathreshold responses and limit the time for integration of excitatory inputs (Pinto et al., 2000, 2003; Pouille and Scanziani, 2001; Wehr and Zador, 2003; Wilent and Contreras, 2004; Blitz and Regehr, 2005; Mittmann et al., 2005).

Most studies have examined the relationship between synaptic input and spike timing using single sensory stimuli. However, sensation is an active process that often involves repeated sampling over time (Ahissar and Arieli, 2001). Awake rats repeatedly sweep their whiskers across objects in the environment at frequencies ranging from 5 to 20 Hz (Welker, 1964; Carvell and Simons, 1990), resulting in periodic firing of barrel cortex neurons in phase with the movement (Fee et al., 1997). Whisker-evoked suprathreshold responses exhibit frequency-dependent adaptation (Fanselow and Nicolelis, 1999; Ahissar et al., 2000, 2001; Garabedian et al., 2003; Castro-Alamancos, 2004; Khatri et al., 2004) that is at least partially dependent on thalamocortical synaptic depression (Chung et al., 2002). However, both excitatory and inhibitory corticocortical synapses within L4 also undergo frequency-dependent short-term depression (Thomson and West, 1993; Petersen, 2002; Beierlein et al., 2003; Cowan and Stricker, 2004; Staiger et al., 2004), making it difficult to predict the net changes in excitation and inhibition after periodic whisker deflection. Using extracellular recordings, Khatri et al. (2004) found that putative excitatory and inhibitory units in L4 exhibited a similar magnitude of frequency adaptation. However, intracellular recordings are necessary to determine the relative postsynaptic changes in excitatory and inhibitory input.

In the present study, we show that, for L4 cells, whisker deflection evokes overlapping excitatory and inhibitory synaptic conductances that long outlast the duration of the suprathreshold response. The relative magnitude and timing of excitation and inhibition define a narrow window over which input integration and spike output can occur. Moreover, repetitive whisker deflection results in a balanced decrease in both excitatory and inhibitory inputs, reducing spike output while maintaining a narrow spike timing window. By exploring responses in a model L4 neuron, we also show that unbalanced adaptation of excitation and inhibition results in disruption of both the reliability and timing of L4 spike output.
more, our values for VS (see Fig. 2D) did not differ appreciably from those of Kathri et al. (2004) using the longer interval.

For synaptic response analysis, spikes were removed by detecting spike threshold at the base of the action potential and extrapolating the \( V_m \) values from the start to the end of the spike, followed by smoothing with a three-point running average. Baseline membrane potential (\( V_m \)) was calculated as the mean \( V_m \) for the 10 ms preceding each stimulus in a train. Evoked postsynaptic potential (PSP) onset latency was defined as the first time point at which the \( V_m \) clearly deviated from baseline at the start of the response, and amplitude of the PSP was measured from baseline to the peak depolarization. The rate of rise for each PSP was measured by calculating the slope of the line connecting the points of 10 and 90% peak amplitude for each response. For all measures, values reported are the mean ± SEM.

**Calculation of synaptic conductances.** For the cells in which fast sodium spikes were blocked using internal QX-314, the total membrane conductance of the cell was calculated at each time point immediately before and during a whisker-evoked PSP. We used the membrane equation: \( C_m \times \frac{dV_m}{dt} = -g_f(V_m - V_{rev}) + I_m \), where \( C_m \) is the membrane capacitance of the cell, calculated by measuring the time constant from short hyperpolarizing current pulses, \( g_f \) is the total membrane conductance, \( V_m \) is the weighted combined reversal potential of all membrane conductances, and \( I_m \) is the injected current. This equation can be rewritten as: \( V_m = V_m^0 + I_m g_f + V_{rev} \), where \( V_m^0 \) is a linear function of the injected current corrected for the capacitative current (\( I_m^c = C_m \times \frac{dV_m}{dt} \)). By evoking a synaptic response while holding the cell at multiple \( V_m^0 \) levels during DC current injection, we could construct a \( V-I \) plot (using the corrected \( I_m \) value), where the inverse slope of the best-fit line was \( g_f \). By subtracting the value of \( g_f \) measured before the onset of the evoked response (effectively, the resting leak conductance) from the value of \( g_f \) at each point during the response, we derived a measurement of the total evoked synaptic conductance (\( g_{syn} \)) over time.

To decompose the total synaptic conductance, \( g_{syn} \), into excitatory and inhibitory components, we used the following simplification: \( I_m = g_f(V_m - V_E) + g_i(V_m - V_I) \), where \( I_m = \) the total synaptic current, \( g_f \) and \( g_i \) are the total excitatory and inhibitory conductances, respectively, and \( V_E \) and \( V_I \) are the reversal potentials for excitation and inhibition, respectively. At the synaptic reversal potential, \( V_{rev} \), \( I_m = 0 \), resulting in: \( 0 = g_f(V_m - V_E) + g_i(V_m - V_I) \). Using the simplification that \( g_{syn} = g_f + g_i \), we obtain: \( g_f = \frac{g_{syn}(V_m - V_{rev})}{(V_m - V_E)} \) and \( g_i = \frac{g_{syn}(V_m - V_{rev})}{(V_m - V_I)} \). \( V_{rev} \) can be calculated as the \( y \)-value of the intersection of the \( V-I \) plot made at baseline with the \( V-I \) plot made at each point in the synaptic response. Using the previous calculation of \( g_{syn} \) over time and assuming values of 0 mV and –80 mV for \( V_E \) and \( V_I \), respectively, we can use these equations to obtain measures of the excitatory and inhibitory currents as a function of time during the evoked response.

A potential source of error in the present analysis is the uncertainty in the assumption of values for synaptic reversal potentials, particularly in sharp electrode recordings where the internal solution is not concentration clamped. However, this uncertainty has only a minor quantitative and not qualitative effect on the results. In additional analyses (data not shown), we found that a 10 mV change in \( V_I \) resulted in only a 15% change in the corresponding calculated conductance magnitude and no alteration of the conductance timing.

**An additional source of error is the assumption of a linear, isopotential neuron inherent in the method of conductance calculation.** The deviation of the actual membrane from these assumptions results in an underestimate of conductance magnitudes that is greater for inhibitory than excitatory inputs (Wehr and Zador, 2003). We have attempted to minimize the impact of this underestimate by only considering relative changes in conductance magnitude across trains of stimuli. In addition, theoretical work in the auditory system demonstrates that the relative timing of excitatory and inhibitory conductances is much less affected by the cable properties of the cell (Wehr and Zador, 2003), suggesting our calculations for the temporal aspects of whisker-evoked conductances are also valid.

**Simulation.** We used a single-compartment model developed by Destexhe et al. (2001) run in the NEURON simulation environment (Hines and Carnevale, 1997). Briefly, the model included Hodgkin and Huxley-
type voltage-dependent sodium and potassium conductances to generate spikes. Realistic background activity was generated using two independent conductances for excitation and inhibition, simulated as one-variable stochastic processes (Destexhe et al., 2001). The average resting conductance of the model cell was 70 nS, and the membrane capacitance was 0.35 nF. The complete model can be obtained from http://cns.iaf.cnrs-gif.fr/alain_demos.html.

The sensory-evoked excitatory and inhibitory input conductances, \( g_E(t) \) and \( g_I(t) \), were modeled using an \( \alpha \) function, \( g(t) = g_{\text{max}} \exp(-t/\tau) \times \exp(-t/\tau_I) \), for the rising phase and a double exponential, \( g(t) = g_{\text{max}}/2 \times \exp(-t/\tau) + g_{\text{max}}/2 \times \exp(-t/\tau_I) \), for the decay. We found this combination of curves most closely fit the actual measured conductances. The peak magnitudes and time constants for the simulated input conductances (Table 1) were set using the average values from the 10 cells recorded in the present study. Because the model cell exhibited a higher resting conductance than that seen in our recordings, we increased the peak magnitudes for \( g_E \) and \( g_I \), maintaining their relative values, until simulated responses to a single stimulus matched those seen in the data. The temporal features of the simulated conductances were set by normalizing each individual in vivo trace to the average peak magnitude and then visually adjusting the time constants to obtain curves that closely approximated the actual data (see Fig. 5A). To simulate unbalanced adaptation of inhibition, the \( g_{\text{max}} \) values for \( g_I \) were adjusted as indicated here. For the simulation, \( V_A \) and \( V_I \) were set to 0 mV and \(-80\) mV, respectively.

Histology. Neurobiotin-filled cells were processed using cyanine 3 as described previously (Higley and Contreras, 2005) and imaged using confocal microscopy (40×, 1.25 numerical aperture objective; TCS 4D system; Leica, Nussloch, Germany). Single-plane projections were assembled from stacks of images. Brain slices from the contralateral hemisphere were processed for cytochrome oxidase reactivity as described previously (Wilent and Contreras, 2004) and photographed using an Olympus BX51 microscope (Olympus America, Melville, NY).

**Results**

We recorded intracellularly from 31 regular spiking neurons in L4 of the barrel cortex of 19 isoflurane-anesthetized rats. Mean resting \( V_m \) was \(-69.7 \pm 5.4\) mV and mean resting input resistance was \(34.1 \pm 5.6 \) MΩ. Cells were located at a cortical depth between 500 and 850 μm and had PSP onset latencies of \(<5.5\) ms (range, \(4.0–5.5\) ms), corresponding to L4 neurons receiving monosynaptic thalamic input. The short onset latencies suggest that all cells in the present study were located within L4 barrels, as L4 septal cells exhibit PSP onset latencies >10 ms (Brecht and Sakmann, 2002). During recording, neurons were filled with neurobiotin for histological verification of depth. We recovered 14 of 31 neurons, including both spiny stellate and pyramidal cells. No differences were observed in the evoked responses between groups, and all data were pooled for additional analysis. Figure 1E shows examples of a L4 spiny stellate cell and an L4 pyramidal cell recorded in a single micropipette track. The figure also shows a supragranular pyramidal cell.

<table>
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<th>Table 1. Summary of model parameters</th>
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<tr>
<td>Control</td>
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<tr>
<td>( g_{\text{max}} )</td>
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<tr>
<td>40 nS</td>
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No differences were found, and therefore, suprathreshold data from all recordings were combined.

Frequency adaptation of spike output and synaptic responses in L4

We studied the frequency adaptation of L4 whisker-evoked responses by deflecting the PW at 10 Hz with 1 s trains. Example traces of a single extracellular unit and an intracellular recording are shown in Figure 1, A and B, respectively. As illustrated by the corresponding PSTHs, both cells reliably fired action potentials in response to the first whisker deflection in the train (1.2 and 1.6 spikes/stimulus, respectively). The magnitude of the suprathreshold response to the 10th deflection decreased to 0.9 spikes/stimulus (extracellular) and 0.7 spikes/stimulus (intracellular). Figure 1D shows the average spike output across the stimulus train, normalized to the magnitude of the first response, for all cells with suprathreshold responses (n = 32) (open squares). The reduction in spike counts was significant, with the mean spike output to the 10th deflection decreased to 67.3 ± 7.4% of the first response (Student’s paired t test; p < 0.001).

Intracellular recordings enabled us to characterize the synaptic changes occurring during frequency adaptation. For the cell in Figure 1B, responses to the first deflection in the train showed little variation in amplitude and rate of rise across trials (Fig. 1B, bottom left detail). In contrast, responses to the last deflection were more variable (Fig. 1B, bottom right detail). The average synaptic responses (n = 30 trials) showed a 29.6% reduction of the PSP amplitude, from 11.5 to 8.1 mV (Fig. 1C, first and 10th responses highlighted in bold). This change was accompanied by a broadening of the PSP, a reduction in the rate of rise, and a decrease in the amplitude of the delayed hyperpolarization. The reduction in PSP amplitude was significant for the population of recorded cells (n = 31), decreasing to 78 ± 7% (p < 0.05) of the first response by the 10th deflection (Fig. 1D, filled circles).

Repetitive whisker deflection also resulted in changes in the temporal features of the evoked response. Figure 2A illustrates the synaptic responses (overlaid traces) and spike output (rasters) to the first and 10th deflection over 10 sequential presentations of a 10 Hz train for a different cell. We quantified spike timing using three measures: the mean spike latency as well as the SD and vector strength of individual spike times. The SD gives the exact temporal scatter of evoked spikes, whereas the vector strength provides a normalized value ranging from 0 to 1 of the phase-locking of spikes to a particular latency (see Materials and Methods). Spikes to the first stimulus were highly reliable (1.1 spikes/stimulus) and occurred within a brief poststimulus window as measured by the SD (σ = 1.0 ms) and the vector strength (VS, 0.77). Spikes to the 10th stimulus were less reliable (0.6

in the same track that was not included in the present analysis. To confirm that L4 corresponds to depths of 500–850 μm, the inset illustrates a lower magnification image of the contralateral barrel cortex stained for cytochrome oxidase. L4 barrels are indicated by the darkened staining and are highlighted by the dashed lines.

For all cells, PW deflection evoked a PSP from resting V_m consisting of an initial fast-rising depolarization that often evoked a single spike or less often spike doublets and that was quickly quenched by a longer-lasting (50–100 ms) hyperpolarization. Fifty-five percent of the cells recorded with pipettes containing the control solution of potassium acetate exhibited suprathreshold responses. We also made extracellular recordings from 20 single units, located at similar depths as the intracellular recordings. All extracellular spike waveforms were >0.7 ms in duration and had first-spike latencies <6 ms, corresponding to thalamocortical-receiving L4 regular-spiking units (Simons, 1978; Armstrong-James et al., 1993; Bruno and Simons, 2002). For all measures of spike output, we analyzed separately data from intracellular and extracellular recordings (data not shown).
spikes/stimulus) and occurred within a slightly broader window ($\sigma = 2.0$ ms; $\mathrm{VS}, 0.70$). Additionally, spike responses shifted from a mean latency of 7.1 ms for the first deflection to 11.8 ms for the 10th. Adaptation encompassed corresponding changes in the timing of the underlying PSP (Fig. 2A, bottom traces). Across the stimulus train, the average synaptic response for this cell exhibited an increased onset latency from 4.2 to 4.9 ms and a larger increase in latency to peak from 9.1 to 16.1 ms. The increased latency to peak coupled with a reduction in peak amplitude gave rise to a decrease in the average rising slope of the PSP of $\sim 50\%$ from 2.9 to 1.5 mV/ms ($dV/dt$) (Fig. 2A, indicated by the dashed lines).

We quantified the changes in response timing for the population. Figure 2B illustrates the overlaid PSTHs for the first and 10th stimuli of all cells with suprathreshold responses. In addition to a decrease in total spike output (Fig. 1D), mean spike latency for the population increased significantly from 7.5 ± 0.2 to 10.5 ± 0.3 ms (Fig. 2D, top graph) ($p < 0.001$). The mean SD of the spike times also increased from 1.3 ± 0.1 to 2.2 ± 0.2 ms (Fig. 2D, middle graph) ($p < 0.001$), and the mean vector strength decreased from 0.72 ± 0.04 to 0.62 ± 0.04 (Fig. 2D, bottom graph) ($p < 0.05$). Although we used a shorter poststimulus window than did previous authors for the calculation of vector strength (see Materials and Methods), our values were similar to the proportional change found previously (Khatri et al., 2004). For the synaptic responses, mean onset latency increased from 4.8 ± 0.1 to 5.5 ± 0.1 ms (Fig. 2C, top graph) ($p < 0.001$), latency to peak increased from 9.6 ± 0.5 to 13.9 ± 0.7 ms (Fig. 2C, middle graph) ($p < 0.001$), and $dV/dt$ decreased from 3.0 ± 0.3 to 1.3 ± 0.2 mV/ms (Fig. 2C, bottom graph) ($p < 0.001$). In summary, the data indicate that frequency adaptation in layer 4 results in a slower and smaller synaptic response, which produces weakened and delayed output. Despite a small decrease in spike precision, spike output remained confined to a narrow poststimulus time window.

Relative adaptation of excitatory and inhibitory synaptic conductances

Although the observed decrease in PSP amplitude and spike output might be explained by a simple withdrawal of excitatory input, such as thalamocortical synaptic depression, the reduction in the delayed hyperpolarization (Fig. 1C) suggests a simultaneous weakening of inhibition. To determine whether repetitive whisker stimulation differentially alters the excitatory and inhibitory inputs to L4 neurons, we recorded 10 cells with the sodium channel blocker QX-314 (25 mM) in the pipette. QX-314 also partially blocks calcium and potassium channels (Nathan et al., 1990; Perkins and Wong, 1995; Talbot and Sayer, 1996). However, in previous studies without QX-314, we found that the $V_m$ behaved linearly over the ranges studied here (Higley and Contreras, 2003, 2005), suggesting these voltage-dependent currents do not play a large role in mediating whisker-evoked responses. Spikes were typically eliminated within 10 min of cell penetration, after which $V_m$ and input resistance remained stable for the duration of the recording. This allowed us to measure synaptic responses while altering the $V_m$ of the cell over a wide range via DC current injection through the recording pipette.

An example of this method is shown in Figure 3A, where the average response to PW deflection in a L4 neuron is shown at five different $V_m$ levels. By plotting the $V_m$ value against the injected current corrected by the capacitative current ($I_{\text{inj}} - I_{\text{cap}}$; see Materials and Methods), we calculated the total membrane conductance at each time point during the response. For all cells in the present study, no rectification of the current–voltage relationship was observed over the ranges of $V_m$ studied (Fig. 3B), suggesting our derivation of membrane conductance was not contaminated by voltage-dependent processes. This calculation is illustrated for two points in Figure 3B: baseline (black squares) and near the peak value for evoked synaptic conductance (gray squares). The inverse slope of each line gives the value of the total membrane conductance at that time. Subtraction of the baseline conductance from the total membrane conductance yields the synaptic conductance during the response. Furthermore, the intersection of each line calculated during the response with the line calculated at the baseline gives the apparent synaptic reversal potential at that time. Plots of total synaptic conductance (black trace) and apparent reversal potential (blue trace) for all points in the response are shown in Figure 3C. Because the calculation for reversal potential is not reliable for the initial portion of the response where there is little deviation from baseline $V_{m}$, this interval is shown as a dashed line. The synaptic response exhibited an early peak in the apparent reversal potential reaching 0 mV that coincided with a small increase in synaptic conductance, corresponding to an initial depolarization from all $V_m$ levels and consistent with an excitatory ionotropic glutamatergic (AMPA) synaptic input. This early excitation was followed by a larger increase in synaptic conductance to 53.2 nS (130% of the resting conductance, 40.9 nS) that coincided with an abrupt decrease in the apparent reversal potential to $-60$ mV, consistent with GABA$\alpha$-mediated inhibition. The time point corresponding to the gray squares in Figure 3B is indicated (arrow).

Measuring the apparent reversal potential and total synaptic conductance allowed us to dissect the contributions of excitatory and inhibitory conductances to the response (see Materials and Methods), also shown in Figure 3C (green and red traces, respectively). After whisker deflection, excitation preceded inhibition, consistent with monosynaptic thalamocortical input rapidly followed by feedforward disynaptic inhibition. The much stronger inhibition (peak inhibitory conductance, 40.5 nS; peak excitatory conductance, 14.2 nS) accounted for $\sim 75\%$ of the total peak synaptic conductance and quickly overtook the excitation. The calculated reversal potential reached 0 mV immediately after the synaptic and excitatory conductances began to rise (Fig. 3C, inset, left vertical line). The reversal potential then hyperpolarized as the inhibitory conductance began (Fig. 3C, inset, right vertical line). Similar results were found for all cells recorded with QX-314, where the mean peak conductances were 16.2 ± 3.9 and 43.7 ± 4.4 nS for excitation and inhibition, respectively. The data revealed that the duration of whisker-evoked excitation typically lasts much longer than the duration of the observed suprathreshold responses (Fig. 2B), confirming that local inhibition is critical in limiting neuronal output from L4.

To study the relative frequency adaptation of excitation and inhibition, we deflected the PW in trains of 10 Hz while holding the cell at multiple $V_m$ levels via current injection (Fig. 4A). The average responses to the first and 10th deflection are shown in detail below (Fig. 4B). The color-coded $V_m$ levels are offset to highlight the $V_m$-dependence of the PSP shape because of different reversal potentials for the synaptic components comprising the response. Although the cell was filled with QX-314, early in the recording session, a small number of spikes were evoked, and the rasters and corresponding PSTHs of these suprathreshold responses are shown. As with the example in Figure 2A, frequency adaptation from the first to the 10th stimulus resulted in fewer spikes (0.6–0.5 spikes/stimulus) that occurred at longer latency (5.7–7.5 ms) but with only a submillisecond change in the spike window ($\sigma = 0.2–0.6$ ms; $\mathrm{VS}, 0.98–0.90$). Below the
Contribution of excitatory and inhibitory conductances to the PW-evoked synaptic response. For this cell, frequency adaptation resulted in a decrease in both excitatory (16.8–9.7 nS) and inhibitory (50.2–30.6 nS) conductance over the course of the stimulus train (resting conductance was 32.9 nS). Furthermore, for both excitation and inhibition, there was an increase in the onset latency (4.0–5.0 ms and 4.6–6.3 ms, respectively) and the latency to peak (5.5–7.2 ms and 6.3–8.6 ms, respectively).

We quantified the relative adaptation of excitatory and inhibitory conductances for the population of cells studied with QX-314 (n = 10). Reduction of the magnitude of excitatory and inhibitory inputs was balanced over repetitive whisker deflection (Fig. 4C, left, filled triangles), with peak excitation decreasing to 52 ± 6% (p < 0.001) and peak inhibition decreasing to 48 ± 6% (p < 0.001) of their respective first-deflection magnitudes. The proportional change in excitation and inhibition was not significantly different across cells (paired t test). To assess the relative balance of excitation and inhibition across individual cells, we also plotted the normalized peak magnitude of excitation versus inhibition for the responses to the 10th stimulus (Fig. 4D, left). The dashed lines indicate unity ± 20%, demonstrating that adaptation of conductances for each cell remained balanced within this margin. In addition to a reduction in magnitude, both excitation and inhibition exhibited increases in onset latency (from 4.6 ± 0.2 to 5.2 ± 0.1 ms, p < 0.005 and from 5.1 ± 0.2 to 6.5 ± 0.2 ms, p < 0.001, respectively) (Fig. 4C, right, open squares) and latency to peak (from 6.5 ± 0.3 to 8.4 ± 0.3 ms, p < 0.001 and from 7.3 ± 0.3 to 9.9 ± 0.6 ms, p < 0.001, respectively) (Fig. 4C, right, filled circles). The onset latency increase for inhibition was significantly greater than for excitation (p < 0.001).

A comparison of the PSTHs and synaptic responses in Figure 4B revealed that the window for suprathreshold responses (gray boxes) corresponded to the PSP width measured at −50 mV. At this V_m, the depolarization is expected to consist exclusively of excitation (rather than reversed inhibition). Thus, we took the PSP width at −50 mV as an approximate measure for the window of potential spike output in cells recorded with QX-314. In Figure 4D (right), we plotted this value against the “excitation dominance window,” taken as the interval between excitation onset and peak inhibition, for responses to the first (open circles) and 10th (open squares) stimuli in all cells. The parameters were well correlated (Fig. 4D, solid line) (Pearson’s correlation; r^2 = 0.62). The average values of PSP width (2.2 and 4.0 ms) and excitation dominance window (2.6 and 4.9 ms) for the first and 10th stimuli, respectively, are shown as filled symbols. Notably, these average values indicate temporal windows, the durations of which are approximately twice the SDs of spike times shown in Figure 2D (2.6 ms and 4.4 ms, first and 10th stimuli, respectively), strongly suggesting that the timing of whisker-evoked spike output in L4 can be explained

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**Figure 3.** Contribution of excitatory and inhibitory conductances to the PW-evoked synaptic response. A, Response to PW deflection of an L4 neuron (680 μm) filled with QX-314 and recorded at multiple V_m levels via current injection through the recording pipette. Stimulus time is indicated by an arrowhead. B, Plot of V_m versus injected current corrected for capacitative current (V_m−I_cap). C, Calculated apparent reversal potential (blue trace) and total synaptic (black trace), excitatory (green trace), and inhibitory (red trace) conductances for the PW-evoked response from A. The inset highlights the changes in reversal potential and conductances early in the response.
precisely by the relative magnitude and timing of the excitatory and inhibitory synaptic conductances.

Simulation of evoked responses with balanced and unbalanced adaptation of excitation and inhibition

The balanced adaptation of the magnitude and timing of excitation and inhibition was surprising given the multiple independent sources of inputs to L4 neurons. To further explore the importance of this balance in shaping L4 output, we simulated a L4 cortical neuron using a single-compartment model that included in vivo-like background activity and Huxley-type fast conductances (see Materials and Methods). Our goal was not to distinguish the specific mechanisms underlying balanced adaptation but to characterize phenomenologically the sensitivity of spike output magnitude and timing to changes in the relative amounts of synaptic excitation and inhibition. Therefore, we modeled sensory-evoked responses using one excitatory and one inhibitory conductance representing the combined evoked synaptic input to the cell. The simulated input conductances were matched to our in vivo data. Figure 5A illustrates the excitatory and inhibitory conductances (bold traces) used to simulate synaptic inputs for the first and 10th stimuli overlaying the individual conductance traces from the 10 cells in the present study, normalized to the peak magnitudes for the simulated traces.

The simulated whisker-evoked responses are depicted in Figure 5B. The top histograms show the total spike output of the model for 100 sequential stimuli, whereas the middle traces illustrate 20 representative Vm responses. The bottom traces depict the excitatory (green) and inhibitory (red) conductances used to generate the data. The left panel shows the response to the first stimulus in a train, which evoked 0.89 spikes/stimulus with a mean spike latency of 7.1 ms. The SD of spike latencies was 1.9 ms, and the vector strength was 0.86. We then simulated the evoked response under conditions of balanced adaptation for excitation and inhibition. The second panel shows the control response to the 10th stimulus in a train where both excitation and inhibition have been reduced to 50% of the first stimulus magnitude. In this case, the spike response decreased to 0.70 spikes/stimulus with an increase in latency to 9.2 ms. There was also a small increase in the spike SD to 2.4 ms, and the vector strength decreased to 0.69. These values are similar to those obtained from the in vivo data and suggest that the model accurately captures the basic elements of evoked responses of L4 neurons to both single and repetitive stimuli. We then explored the consequences of unbalanced adaptation in the model. The third panel shows the case in which adaptation of excitation is maintained at control levels (50%), but inhibition is reduced to 25% of the first stimulus magnitude. Under these conditions, spike output increased to 1.1 spikes/stimulus. Furthermore, the window for spike generation increased sharply (V = 3.6 ms; VS, 0.43). This outcome was a result of the inability of inhibition to suppress longer-latency spikes generated by the unbalanced excitatory drive. The fourth panel illustrates the case in which inhibition is reduced to 75% of the first stimulus magnitude. Under these conditions, inhibition rapidly quenched the excitatory response, and spike output was reduced to 0.57 spikes/stimulus, although spikes occurred in a narrower window (V = 1.2 ms; VS, 0.93). Figure 5C shows the data for the spikes/stimulus (filled circles), SD (filled triangles), and vector strength (open squares) versus the percentage of inhibitory conductance relative to the first stimulus magnitude. The graph indicates that the relative amount of inhibition is directly related to spike precision and inversely related to the magnitude of spike output. This finding suggests that, during frequency adaptation, the balanced change in excitation and inhibition allows cortical cells to preserve a narrow window for spike output with a moderate reduction in response magnitude.

Discussion

Our central finding was that spike output in response to both single and periodic whisker deflection is critically shaped by the dynamic interaction of excitation and inhibition. We have shown that frequency adaptation involves a balanced reduction in excitatory and inhibitory synaptic conductances, resulting in a reduction in total spike output without substantial degrading of the narrow window for spike timing.

Cortical response adaptation has been observed in a variety of sensory systems after visual (Ohzawa et al., 1982), auditory (Shu
As with previous findings (Garabedian et al., 2003; Khatri et al., 2004), repetitive PW deflection produced a significant increase in the mean spike latency. We also observed a significant but submillisecond reduction in spike precision, consistent with reports that repetitive whisker deflection reduces response magnitude without greatly degrading the phase-locking of spike output (Khatri et al., 2004). Moreover, the spike output in response to both single and periodic stimuli occurred within a window that is shorter than the membrane time constant of barrel cortex neurons in vivo, which ranged from 5 to 12 ms in the present study (data not shown). Thus, the increased spike jitter may not constitute a functional loss of precision.

Accompanying the adaptation of spike output were corresponding changes in the underlying synaptic response, including a 22% decrease in PSP amplitude. As with the spike data, this value is less than that reported previously (Chung et al., 2002). However, our study differs from previous studies in limiting our recordings to neurons in L4. This distinction is important, because other studies have found differences in adaptation across layers (Ahissar et al., 2000, 2001). We also observed a 57% reduction in the PSP slope that may explain the increased SD of spike times, because spike precision has been shown to correlate inversely with transient $dV/dt$ (Mainen and Sejnowski, 1995; Amsterlaher and Miles, 2004). The decrease in slope is similar to that observed in previous studies of cross-whisker suppression, in which preceding whisker deflection caused a reduced magnitude and $dV/dt$ of a subsequent synaptic response because of withdrawal of input to the cell (Higley and Contreras, 2003, 2005).

The similar reduction in spike output and PSP amplitude was somewhat surprising given the nonlinearity of $V_m$ behavior imposed by spike threshold. However, this finding is partially explained by the increased variability in PSP amplitude for the 10th versus first stimulus in a train (Fig. 1B). Thus, although the average PSP amplitude was reduced, a number of individual trials remained near control amplitude, resulting in less reduction in spike output than might have been observed if all individual PSPs were reduced to the average value. In addition, spike output is generally considered to be a monotonic saturating function of $V_m$ (Koch, 1999). With the high-velocity stimuli used in the present study, it is likely that we are far to the right on such a spike frequency–$V_m$ curve (Wilent and Contreras, 2004), resulting in small reductions in spike output for a given reduction in PSP amplitude.

Using methods similar to those described in the visual (Marino et al., 2005; Priebe and Ferster, 2005) and auditory (Wehr and Zador, 2003, 2005; Zhang et al., 2003) systems, we showed that PW deflection evoked a short-latency excitatory conductance that was rapidly overtaken by a much larger inhibitory conductance. This finding is consistent with the canonical view of L4 activity where thalamocortical excitation triggers strong disynaptic feedforward inhibition (Agnon and Connors, 1991; Swadlow and Gusev, 2000; Porter et al., 2001; Swadlow, 2003; Stagier et al., 2004). The temporal window established between the onset of excitation and the peak of inhibition closely agreed with the excitatory PSP width and the duration of suprathreshold responses observed in spiking neurons, suggesting that the timing of spike output in L4 is a direct consequence of the dynamic interaction between excitatory and inhibitory inputs. A similar sequence of excitation followed by inhibition also plays a role in shaping spike timing in the auditory cortex (Wehr and Zador, 2003), hippocampus (Pouille and Scanziani, 2001), cerebellum (Mittmann et al., 2005), and thalamus (Blitz and Regehr, 2005). Our present findings, combined with this accumulated
data, strongly indicate that feedforward inhibition is a general mechanism used across brain regions to regulate the precision of spike timing.

Synaptic inputs to L4 originate from corticocortical and thalamocortical sources (Porter et al., 2001; Beierlein et al., 2002, 2003; Bruno and Simons, 2002; Schubert et al., 2003). Numerous studies in vivo have shown that local excitatory and inhibitory synapses between L4 neurons depress at the frequency studied here (Thomson and West, 1993; Petersen, 2002; Beierlein et al., 2003; Cowan and Stricker, 2004; Staiger et al., 2004). Furthermore, thalamocortical synaptic inputs to both excitatory and inhibitory neurons also exhibit short-term depression (Gil et al., 1997; Gibson et al., 1999; Beierlein et al., 2002; Castro-Alamancos and Oldford, 2002). Chung et al. (2002) showed that frequency adaptation in the barrel cortex in vivo is partially dependent on thalamocortical synaptic depression. However, this work did not address whether a reduction in thalamic input might differentially impact the activity of local excitatory and inhibitory circuits. Our experiments demonstrated that repetitive PW deflection resulted in a balanced reduction in the magnitude of excitatory and inhibitory postsynaptic conductances. This result is consistent with a recent extracellular study showing L4 putative excitatory and inhibitory units exhibited similar amounts of frequency adaptation (Khatri et al., 2004). Together, these data indicate that adaptation results from a reduction in total synaptic drive to L4 cortical neurons rather than shift in the relative contribution of excitation versus inhibition. Our findings further suggest that cortical circuits are calibrated to maintain an appropriate balance of excitation and inhibition despite changes in input magnitude, such as occurs with thalamocortical synaptic depression. This conclusion is supported by recent findings that spontaneous cortical activity maintains a strong proportionality of excitatory and inhibitory conductances, even during large fluctuations in total input (Shu et al., 2003).

In addition to a reduction in magnitude, we observed a small increase in onset latency for excitation (0.6 ms), consistent with previous reports of minimal change in the mean latency of thalamocortical spike output after repetitive PW deflection (Hartings and Simons, 1998; Ahissar et al., 2000; Sosnik et al., 2001; Khatri et al., 2004). We also observed a larger increase in onset latency for inhibition (1.4 ms), suggesting that interneurons in L4 also experience increases in spike latency. This conclusion is supported by previous extracellular studies that found slightly larger increases in the earliest spike latency of putative cortical interneurons versus thalamic units [Khatri et al. (2004), their Fig. 5]. The latency to peak increased for both excitatory and inhibitory conductances, resulting in a slight broadening of the “excitation dominance window” that was correlated with an increased PSP width and agreed with the increased duration of spike output. Thus, frequency adaptation allows a direct test and confirmation of the hypothesis that the interplay of excitatory and inhibitory inputs to L4 neurons directly and precisely determines the window for spike timing. Simulated whisker-evoked responses further suggested that L4 neurons are optimized to exhibit moderate response adaptation without substantial loss of spike precision. Nevertheless, cortical circuits may have the ability to promote either response magnitude or precision at the expense of the other depending on local network conditions. Whether this trade-off occurs in vivo remains to be determined.

One remaining unknown is the function of the narrow spike window established by the dynamic interaction of excitatory and inhibitory conductances. Others have proposed that L4 neurons act as temporal contrast detectors, responding preferentially to highly synchronized thalamic input (Kryziari and Simons, 1993; Pinto et al., 2000, 2003). In this view, L4 functions as a gate to other cortical layers, filtering out nonoptimal inputs such as whisker deflections with low velocity (Wilen and Contreras, 2004), nonpreferred direction (Kryziari et al., 1996), or of non-principal whiskers (Kryziari et al., 1996; Fox et al., 2003). Our findings strongly support the proposed view of L4 function and argue that this role is maintained both for single sensory stimuli and for the behaviorally relevant context of repetitive whisking.

References