Adaptation in Thalamic Barreloid and Cortical Barrel Neurons to Periodic Whisker Deflections Varying in Frequency and Velocity

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Layer IV circuitry in the rodent whisker-to-barrel pathway transforms the thalamic input signal spatially and temporally. Excitatory and inhibitory barrel neurons display response properties that differ from each other and from their common thalamic inputs. We further examine thalamocortical response transformations by characterizing the responses of individual thalamic barreloid neurons and presumed excitatory and inhibitory cortical barrel neurons to periodic whisker deflections varying in frequency from 1 to 40 Hz. Both pulsatile and sinusoidal periodic stimulation of fixed deflection amplitude were used to assess stimulus-evoked adaptation of thalamocortical units (TCUs), fast-spikes (FSUs: presumed inhibitory neurons), and regular-spikes (RSUs). Monotonic, frequency-dependent reductions in firing were observed in thalamic and cortical barrels to the second and subsequent stimuli in trains of high (pulsatile) and low (sinusoidal)-velocity deflections. RSUs and FSUs adapted substantially more than their thalamic input neurons, and at all frequencies, FSUs fired at higher rates than the other two cell types. For example at 40 Hz, response magnitudes of TCUs decreased by 34%, FSUs by 72%, and RSUs by 78%. Across frequencies, RSUs and FSUs displayed more cycle-by-cycle entrainment and phase-locked responses for (high velocity) pulsatile than (lower velocity) sinusoidal deflections; for TCUs, phase-locking was equivalent for both stimuli, but entrainment was higher for sinusoidal deflections. Strong feed-forward inhibition, in conjunction with synaptic depression, renders the firing of barrel neurons sparse but temporally faithful to the occurrence of repetitive whisker deflections, especially when they are of high velocity.

INTRODUCTION

Using their whiskers, rats can perform high-resolution tactile discriminations (Brecht et al. 1997; Carvell and Simons 1989; Guic-Robles et al. 1989). Rodents actively whisk their vibrissae back and forth across palpated objects (Welker 1964), creating rapidly changing patterns of neural activity that, by analogy with active touch in other mammalian tactile systems, enhance sensory discrimination (Hellweg et al. 1977). Behavioral arousal and exploration is associated with pronounced adaptation in thalamocortical circuits (Castro-Alamancos 2004), raising the possibility that the vibrissa system is optimized by adaptation to process information at near-whisking frequencies, which occur at ~8 Hz. Consequently, a number of recent barrels in layer IV of the primary somatosensory cortex (Simons et al. 1989; Welker and Woolsey 1974). The whisker/barrel system appears well-suited for conveying and processing sensory information rapidly and reliably. Circuits in the brain stem (Minnery and Simons 2003) and thalamus (Deschenes et al. 2003) faithfully transmit information about temporally precise firing patterns in primary afferent neurons that innervate the whiskers (Jones et al. 2004), and thalamocortical circuitry is preferentially sensitive to the timing of thalamic spikes (Pinto et al. 2003).

Extracellular recordings of barrel neurons have identified two cell types based on spike waveform. Regular-spikes (RSUs) are thought to be excitatory (spiny stellate or pyramidal neurons), whereas fast-spikes (FSUs) correspond to the largest population of inhibitory neurons (see Bruno and Simons 2002). Response properties of RSUs and FSUs differ from each other and from those of thalamocortical units (TCUs) in thalamic barreloids (Simons and Carvell 1989). For example, RSUs have the lowest spontaneous and stimulus-evoked firing rates, FSUs the highest with TCU values intermediate. The highly responsive nature of FSUs is thought to reflect their intrinsic membrane properties (Rudy et al. 1999) and their receipt of highly convergent, strong thalamocortical synaptic input (Bruno and Simons 2002; Swadlow and Gusev 2002). Strong thalamic connections directly onto highly responsive inhibitory barrel neurons renders barrel circuitry highly sensitive to the synchronous arrival times of impulses—on the millisecond scale—from populations of barreloid neurons (Pinto et al. 2003). One consequence is that barrel neurons are preferentially sensitive to deflection velocity but not amplitude; the former affecting thalamic firing synchrony and the latter affecting total thalamic response magnitude but not timing (Pinto et al. 2000).

Responses in thalamocortical circuits are strongly affected by adaptation produced by repetitive sensory stimulation (Fanselow and Nicolelis 1999; Yuan et al. 1986). Periodic stimuli have been employed to examine in vivo effects of central inhibition and synaptic depression, both of which act to reduce responsiveness to repetitive stimuli in a time-dependent fashion (Chung et al. 2002; Hellweg et al. 1977). Behavioral arousal and exploration is associated with pronounced adaptation in thalamocortical circuits (Castro-Alamancos 2004), raising the possibility that the vibrissa system is optimized by adaptation to process information at near-whisking frequencies, which occur at ~8 Hz. Consequently, a number of recent
studies have focused on the encoding of relatively low-frequency periodic whisker deflections (Ahissar et al. 2000; Garabedian et al. 2003; Moore et al. 1999). Yet to be determined is whether the responses of cortical neurons, including their ability to faithfully reflect the temporal signature of the afferent signal, are affected by the velocity of the periodic whisker deflections.

Recently, we used periodic whisker deflections in the range of 1–40 Hz as a probe for studying thalamic circuitry in the whisker-to-barrel pathway (Hartings et al. 2003). Whisker deflections consisted of high-velocity pulses or lower-velocity sinusoids. Results indicate that TCUs faithfully transmit the high-frequency temporal structure originating from afferent sensory input. Here we examine the consequences of this faithful transmission for neural processing in the thalamocortical circuit. We find that adaptation is considerably greater among barrel than barreloid neurons. Results indicate that RSUs and FSUs retain their distinctive response properties during repetitive whisker stimulation and that as a population barrel neurons encode information about repetitive whisker deflections in a temporally faithful fashion. Moreover, differences in thalamic and cortical responses to pulsatile versus sinusoidal stimuli suggest that barrel circuitry retains its sensitivity to thalamic population firing synchrony in the adapted state.

METHODS

Animals and surgical preparation

Surgical preparation and maintenance of the rats during electrophysiological recording were identical to methods described previously (Hartings et al. 2003). Twenty Sprague-Dawley adult female albino rats (200–300 g) were obtained from a commercial supplier. All surgical preparation was performed under halothane anesthesia. A silastic catheter was inserted into the right jugular vein and led out from the nape of the neck for later drug delivery. A short length (~40 mm) of polyethylene tubing was inserted into the trachea for later artificial respiration, and the left femoral artery was cannulated using an angiocath catheter to measure blood pressure. After exposing the skull, small stainless steel screws were placed over the left occipital and frontal cortex for electroencephalographic (EEG) recordings, and a ground screw was placed over the right frontal cortex. Dental acrylic was used to attach a steel post to the skull. The post, which was used to hold the animal’s head without pressure points during the rest of the experiment, permitted unimpeded access to the facial vibrissae. In cortical experiments, the bone overlying the right barrel cortex was thinned, and a small (~1 mm²) craniectomy was made. For thalamic experiments, a craniectomy was made at stereotaxic coordinates overlying VPM (2.0–4.5 mm posterior, 1.5–4.0 mm lateral to bregma). The dura was incised to prevent the brain from dimpling and the left femoral artery was canulated using an angiocath catheter to measure blood pressure. After exposing the skull, small stainless steel screws were placed over the left occipital and frontal cortex for electroencephalographic (EEG) recordings, and a ground screw was placed over the right frontal cortex. Dental acrylic was used to attach a steel post to the skull. The post, which was used to hold the animal’s head without pressure points during the rest of the experiment, permitted unimpeded access to the facial vibrissae. In cortical experiments, the bone overlying the right barrel cortex was thinned, and a small (~1 mm²) craniectomy was made. For thalamic experiments, a craniectomy was made at stereotaxic coordinates overlying VPM (2.0–4.5 mm posterior, 1.5–4.0 mm lateral to bregma). The dura was incised to prevent the brain from dimpling and thus suffering compression damage due to electrode insertion. Last, an acrylic dam was constructed around the skull opening and filled with saline.

Body temperature was maintained at 37°C by a servo-controlled heating blanket (Harvard Apparatus, Holliston, MA). For neural recordings, halothane was discontinued, and the rat was maintained in a lightly narcotized, sedated state by intravenous infusion of fentanyl. To prevent spontaneous movement of facial muscles, which would prevent use of our electromechanical stimulators (in the following text), neuromuscular blockade was induced with pancuronium bromide (1.6 mg · kg⁻¹ · h⁻¹), and the animal inhaled airway pressure waveform. Experiments were terminated if any of the above indicators could not be maintained within normal physiological ranges; this occurred rarely.

Recordings

Data were obtained from cortical barrels and thalamic barreloids in the VPM using high-impedance (5–10 MΩ) stainless steel microelectrodes (Frederick Haer, Brunswick, ME) or beveled glass micropipettes. Glass microelectrodes were made from double-barreled capillary tubes; one barrel, for unit recordings, was filled with 3 M NaCl and the other, for marking penetration sites, with 10% horseradish peroxidase (Land and Simons 1985). Whiskers on the contralateral mystacial pad were stimulated manually during electrode advancement. Extracellularly recorded single units were identified by spike amplitude and waveform criteria using an amplitude discriminator and a digital oscilloscope with a triggered delay. When multiple units were present, only the one having the largest amplitude was discriminated. Spike times were digitized at 10 kHz for subsequent analyses (see following text). In the cortex, we distinguished two types of neurons based on spike waveform, RSUs and FSUs (Bruno and Simons 2002; Kyrzi et al. 1996). These are thought to represent the discharges of excitatory and inhibitory barrel neurons, respectively. In this study, we compared the response properties of RSUs and FSUs with identically studied TCUs from Hartings et al. (2003). Hereafter, the terms barreloid and thalamic neurons are used interchangeably along with TCU.

Histology and recording locations

At the termination of an experiment, the rat was deeply anesthetized with sodium pentobarbital and perfused transcardially for cytochrome oxidase (CO) histochemistry. The cortex was cut tangentially, and the thalamus was sectioned coronally. Alternate tissue sections were reacted for CO or HRP (Land and Simons 1985), and all sections were counterstained with thionine. Using microdrive readings, signs of tissue disruption, HRP spots, and/or electrolytic lesions made with metal microelectrodes, recording sites were localized with respect to individual barrels; data are presented only for units recorded in CO-rich barrel centers. Because of the complex geometry of thalamic barreloids, no attempt was made to identify thalamic recording sites with respect to individual barrels, but all recording sites were confirmed as being located within the ventral posterior medial thalamic nucleus.

Whisker-stimulation protocols

For each unit, we first used hand-held probes to identify the whisker, hereafter denoted the principal whisker (PW), evoking the strongest or most reliable response. The PW was trimmed to 12–15 mm in length, and a multi-angle piezoelectric stimulator was advanced over the terminal 2–5 mm of the cut end of the whisker (Simons 1983). The following stimulation protocols were used.

STANDARD PROTOCOL. The whisker was deflected 1 mm with onset and offset velocities of ~125 mm/s and a plateau duration of 200 ms. Stimuli were delivered randomly in 8 angular directions (spanning 360° in 45° increments), and each randomized battery was repeated 20 times. This protocol enabled quantitative identification of each unit’s maximally effective or “best” deflection angle.

PERIODIC PULSATILE STIMULATION. The PW was deflected repetitively using 10-ms-long deflections of 700 μm; rise and fall times were 5 ms, corresponding to average movement velocities of ~140 mm/s (Fig. 1A). The smaller (<1 mm) amplitude of pulsatile deflections was necessitated by limitations in the stimulators and a need to keep individual pulses brief in duration. These rapid pulses evoke only
FIG. 1. The 2 different types of periodic whisker deflections. A: pulses. B: sines. Pulsatile deflections had a fixed duration of 10 ms with peak amplitude of 0.7 mm and average onset velocity of ~140 mm/s. Sinusoid duration and onset velocity varied with frequency; peak amplitude was 1.0 mm. Waveforms not drawn to scale.

except that for the highest velocity movements (e.g., pulses), the termination of the pulse was associated with two cycles of mechanical ringing at 200 Hz having a maximum peak amplitude of 35 μm (compared with the 700 μm deflection peak). These small deflections occurred immediately on stimulus offset, a period during which thalamic and cortical neurons are refractory to even large-amplitude deflections (Kyriazi et al. 1996).

Data analysis

Sequential spike times were recorded at a resolution of 100 μs using either a DEC LS11 computer or, in later experiments, a PC equipped with a fast A/D converter (National Instruments, Austin, TX). The computers also controlled the whisker stimuli. Spike data from multiple stimulus presentations were first accumulated in 1-ms bins, and responses were quantified by calculating spike counts during specific time windows. For periodic stimuli peristimulus time histograms (PSTHs) were constructed by accumulating responses during the entire length of the trial, including pre- and poststimulus activities. Data were examined also by constructing cycle-time histograms (CTHs) for each frequency’s first cycle and steady-state. The steady-state periods for each cell type and stimulus were determined by examining population cycle-by-cycle spike counts and identifying the first cycle at which changes in firing rate appeared similar to those obtained with the 1 Hz (pulse or sinusoid) stimulus; 1 Hz was chosen as the standard of comparison, because there is virtually no adaptation at this stimulus frequency. The method is illustrated in Fig. 2, which shows cycle-by-cycle spike counts at selected frequencies of pulsatile stimulation for RSUs. Interestingly, it appears that, for all cell types, steady-state occurs at progressively earlier times in the stimulus train as stimulus frequency increases. We did not analyze this in detail, however.

The effects of repetitive pulsatile or sinusoidal whisker stimulation were assessed using an adaptation index (AI). A unit’s response in spikes/deflection was quantified by dividing the mean response to all steady-state stimulus cycles by the mean response to the first stimulus cycle. A value of <1.0 indicates that the unit decreased its firing on repetitive stimulation. We also examined responses on a cycle-by-cycle basis. For pulsatile deflections, which evoked transient responses with distinct onsets and offsets, unit activity was measured during a 25-ms period. The time of response onset for sinusoidal deflections, especially those at low frequencies and thus low velocities, was difficult to determine accurately; therefore spike counts were taken for the entire stimulus cycle. Because of the sparseness of firing of many of the cells with high-frequency stimuli, response durations were computed for TCU, RSU, and FSU populations rather than...
is the total number of evoked spikes, \( n \) is the time between cycle onset and an evoked spike, and \( T \) is the period of the stimulus frequency. Measures of VS were calculated separately for CTHs constructed for first-cycle and steady-state responses. We found that the VS measure is influenced complexly by a number of interacting factors, including period length, level of spontaneous (inter-deflection) activity, response shape, and response duration relative to the stimulus period. As suggested by Eggermont (2002), these complications can be overcome by dividing the VS for the steady-state response by that of the first cycle so as to derive a normalized measure of VS. This procedure also accounts for the overall firing rates of different units. In addition, we normalized the CTHs to a common length corresponding to a full 360° of the stimulus cycle. The normalized VS thus provides a quantification of the extent to which phase-locking during the stimulus cycle changes in the adapted versus nonadapted state. A value of 1 indicates the maintenance of phase-locking in the adapted state, and 0 signifies a complete loss of phase-locking.

We also quantified the degree to which neurons fired periodically at the stimulus frequency or integer multiples thereof (i.e., entrainment). Autocorrelograms were constructed from individual spike trains accumulated over all trials of a given frequency and then analyzed using a discrete Fourier transform (DFT). This analysis was performed on a unit-by-unit basis and on autocorrelograms constructed from all spike trains for a given population. To account for different firing rates among individual units or between populations (e.g., RSUs versus FSUs), the autocorrelogram was normalized to its maximum bin prior to the frequency analysis.

Comparisons among TCUs, RSUs, and FSUs were conducted using ANOVAs followed by post hoc pair-wise comparisons. For the ANOVAs, data were compiled across all frequencies for a given cell type to minimize the number of statistical comparisons. Inspection of frequency-dependent measures (e.g., Fig. 4) revealed that, for virtually all measures, relationships were consistent across frequencies.

RESULTS

Responses to pulsatile periodic stimuli were examined in 29 TCUs, 44 RSUs, and 18 FSUs. Responses to sinusoidal deflections were examined in 27 TCUs, 32 RSUs, and 12 FSUs. Approximately, two-thirds of each population was studied with both stimulus sets. Figure 3 shows population PSTHs illustrating the characteristic responses of the three cell populations to pulsatile stimuli. At low frequencies, each cell type fired relatively uniformly throughout the stimulus train, but with higher-frequency deflections, responses were largest for the first deflection in a series. As quantified in the following text, TCUs and FSUs fire more stimulus-evoked and spontaneous spikes than RSUs. FSUs and RSUs are more similar to each other and different from TCUs, however, in that both show greater response decrements to the second and subsequent stimulus cycles. The extent to which responses decreased after the first cycle was quantified using an AI, wherein the average response evoked by steady-state stimulus cycles is divided by the mean response to the first deflection in the train.

Figure 4B shows frequency-dependent adaptation for the three studied populations. At frequencies where adaptation occurred, adaptation is greater in FSUs and RSUs than in TCUs. For example, at the highest frequency tested (40 Hz), TCU responses decreased by 33% (e.g., AI = 0.67), whereas the responses of RSUs and FSUs were 77 and 72% smaller, respectively. For each cell population, we computed a mean adaptation index across all frequencies tested. An ANOVA indicated that the amount of adaptation differed among the three cell populations (\( P < 0.001 \)). Both RSUs and FSUs
adapted more than TCUs (Student’s unpaired t-test, \( P < 0.001 \)), but FSUs adapted slightly less than RSUs \( (P = 0.02) \). Within each studied population, individual units varied in terms of their adaptation and initial response magnitude. We therefore examined whether the amount of adaptation displayed by an individual cell is related to its response during the first stimulus cycle, i.e., in the nonadapted state. A correlation coefficient was computed by comparing, for the 20-Hz stimulus train, the magnitude (spikes/stimulus) of each unit’s first-cycle response with its calculated adaptation index. For all three cell populations, adaptation was independent of nonadapted response magnitude.

RSUs adapted slightly more than FSUs, and inspection of trial raster-plots suggested that RSU-adapted responses also varied more widely from trial to trial and from cycle to cycle. For each unit, we examined trial-to-trial variability by computing a coefficient of variation \( (CV, \text{mean} \pm \text{SD}) \) for steady-state responses (Fig. 4C). Variability substantially increased in a frequency-dependent fashion for RSUs, but CVs remained relatively constant in FSUs, which in turn were equivalent to those of TCUs. Similar results were observed when CVs were calculated across individual cycles within a train. Again, RSU responses varied most, with FSU and TCU responses being similar to each other. In all three populations, cycle-by-cycle variability was roughly equivalent to trial-by-trial variability.

**Adaptation and response timing**

High-frequency pulsatile stimulation substantially reduced response magnitudes of cortical neurons without greatly degrading the temporal fidelity, or phase-locking, of their responses. This is illustrated qualitatively by the CTHs in Fig. 5. With 4-Hz trains, TCUs and FSUs are similar in the rapid rise of their responses to both the first and subsequent steady-state stimulus cycles; the RSU response develops more slowly. Within each cell type, the time course of the response is similar.
for the first and steady-state cycles. At 20 and 40 Hz, TCU responses are largely similar to those at 4 Hz except that the peak response for steady-state cycles occurs 1–3 ms later than the peak response for the first cycle; this rightward shift causes a reduction in total response magnitude. For RSUs and FSUs at 20 and 40 Hz, the time to peak increases by ~5 ms for the steady-state response, and firing rates are reduced throughout. Responses are briefer as well as smaller (Fig. 6A; also see Fig. 5), however, yielding a CTH that, though scaled down in size, has a distinct, time-locked peak. We quantified the temporal fidelity of responses using a measure of vector strength (Fig. 6B; see METHODS). Vector strengths differed across cells types (ANOVA: P < 0.001). Both TCUs and FSUs were equally phase-locked, and both were better than RSUs (Student’s unpaired t-test: TCUs vs. FSUs, P = 0.61; TCUs vs. RSUs, P < 0.001; TCUs vs. FSUs, P < .001).

Analyses of steady-state CTHs suggest that rapidly repeating stimuli induce cortical neurons to fire periodically, albeit especially sparsely in the case of RSUs. Figure 6A shows an autocorrelogram of spike trains evoked by 40-Hz stimulation in an RSU; this unit was among the best entrained cells, and the data provide a good example of the numerical measures we used. The neuron fired preferentially at intervals of 25 ms or integer multiples thereof. Figure 7B shows a DFT of the autocorrelogram and illustrates the periodicity of spiking at 40 Hz, with a secondary peak at 80 Hz (the 1st harmonic). To quantify this, we divided the power at 40 Hz by the (total) power summed from 5 to 100 Hz; the value for this unit is 0.14. Approximately 20% of RSUs had values >0.14, RSUs displayed lower mean values (0.057 ± 0.010) than FSUs (0.154 ± 0.020) and TCUs (0.160 ± 0.010); variances relative to the means were larger for cortical than thalamic neurons. Thus both quantitative measures, vector strength and autocorrelogram frequency domain, indicate that RSU firing displays the least temporal fidelity for pulsatile whisker deflections. We performed a similar analysis on population autocorrelograms constructed by accumulating spikes across all units and trials. In all three cell types population-level values at 40 Hz were larger than mean values of individual units (compare Fig. 7, C and D), and, interestingly, RSUs (0.21) were virtually identical to FSUs (0.22) and TCUs (0.20).

We also examined whether firing rates at steady-state increased with higher stimulus frequencies (Fig. 4A). For all three cell populations, mean firing rates (MFR) increased only ~50% from 1 to 12 Hz, despite the 12-fold increase in stimulus frequency. MFRs remained relatively constant from 12 to 40 Hz, presumably because the suppressive effects of interdeflection intervals <70 ms counterbalance, and then dominate, the excitatory effects of more frequent whisker deflections. Thus, despite adaptation-induced decreases in responsiveness, populations of thalamic and cortical neurons appear to represent stimulus frequencies ≤40 Hz by the temporal pattern of their firing not by their overall number of spikes.

**Responses to sinusoidal deflections**

In all three cell populations, adaptation to sinusoidal movements increased with higher frequencies (Fig. 8). ANOVA and post hoc analyses revealed that, as in the case of pulsatile deflections, TCUs adapted less than RSUs and FSUs (Fig. 9B), which were equivalent to each other. Effects of deflection velocity are clearly evident in the population CTHs constructed for the first stimulus cycle, i.e., the nonadapted state (Fig. 10). With higher frequency stimuli and thus higher velocity deflections, population responses in the nonadapted state have shorter latency, faster rise times, greater peak magnitudes, and higher mean firing rates. In the adapted state, TCU response latencies are longer, and differences in latency between nonadapted and adapted states are greater for lower frequency sinusoids. Reflecting their thalamic inputs, FSUs and RSUs show similar frequency-dependent latency shifts. In all three populations the number of spikes per cycle in the adapted state increases at higher stimulus frequencies because the velocity of the stimulus increases as well. Effects are modest, however; overall mean firing rates increase only modestly (~25%) with stimulus frequencies from 1 to 12 Hz and then asymptote (Fig. 9A). Response variability as quantified by CVs was virtually identical for FSUs and TCUs with RSU firing being least variable (ANOVA, P < 0.001) perhaps because many cells fired either a maximum of 1 or no spikes per cycle. Mean firing rates were similar for sinusoidal and pulsatile stimuli (compare Figs. 4A and 9A) in spite of the clearly less distinct responses evoked by sinusoidal movements. This reflects the fact that responses to the latter are considerably more temporally dispersed. Autocorrelograms based on unit responses to 40-Hz stimuli were examined using DFTs in a fashion similar to those for pulsatile stimulation. From 5 to 100 Hz, the proportions of power at 40 Hz were 0.035 ± 0.010 for RSUs, 0.094 ± 0.022 for FSUs, and 0.149 ± 0.014 for TCUs. As with pulsatile deflections, population level values were larger in all three cell types and equivalent to each other (0.21) than RSUs (0.22) and FSUs (0.22).

![Fig. 6. Temporal fidelity for pulsatile stimulation. A: response durations of steady-state pulsatile population responses. The TCU response duration remains constant across frequencies, whereas for FSUs and RSUs, responses become briefer with increases of stimulus frequency (see text). B: normalized vector strengths for individual cells calculated by dividing the steady-state VS by that of the 1st cycle.](image-url)
Frequency- and velocity-dependent effects of adaptation

For a given stimulus frequency, the velocity of whisker movement was higher for pulsatile than sinusoidal deflections, even at 40 Hz. This difference enables an assessment of possible interactions between adaptation and deflection velocity. For these analyses, we collapsed data across frequencies to calculate mean values per cell. As illustrated by the bar graph in Fig. 11A, adaptation indices were similar for sinusoidal and pulsatile deflections except that FSUs adapted slightly more for sines (Student’s unpaired t-test, $P < 0.01$). Thus, deflection velocity affects nonadapted (1st cycle) firing rates but not the proportional decrease in steady-state firing. Also, as noted in the preceding text, steady-state mean firing rates reflect stimulus period poorly for both sines and pulses. Tenfold increases in frequency (4–40 Hz) produced maximal increases of $\sim 70\%$ in MFR (Fig. 11B).

In the temporal domain, the timing of spike occurrences remained more faithful to the nonadapted pattern when higher velocity, pulsatile deflections were used. For all cell types, adaptation-induced shifts in peak response times (in milliseconds) were substantially larger for sinusoidal than pulsatile stimuli (Fig. 11C). Phase-locking, as measured by vector strength, was also velocity dependent but only in cortical not thalamic neurons (Fig. 11D). Average vector strengths were greater for pulses than sines in RSUs and FSUs (Student’s unpaired t-test: RSUs, $P < 0.001$; FSUs, $P = 0.003$), whereas TCU phase-locking was equivalent for both types of stimuli (Student’s unpaired t-test: TCU, $P = 0.33$). Similarly, entrainment, as measured by the frequency spectrum of the autocorrelogram, was larger (Student’s unpaired t-test: $P << 0.001$) for pulsatile than sinusoidal deflections for RSUs ($0.057 \pm 0.010$ vs. $0.035 \pm 0.010$) and FSUs ($0.154 \pm 0.020$ vs. $0.094 \pm 0.022$), whereas the converse was the case for TCUs ($0.102 \pm 0.056$ vs. $0.131$ vs. $0.076$), perhaps due to the more slowly adapting-like responses that were tonically modulated by the sinusoidal deflection.
DISCUSSION

The present study investigated thalamocortical response transformations in the whisker-to-barrel pathway using periodic whisker deflections having different frequencies and velocities. Frequency-dependent reductions in firing were observed in thalamic and cortical neurons for both high (pulse) and low (sinusoid) velocity deflections. Consistent with previous reports (Chung et al. 2002; Gottschaldt et al. 1983), we found that cortical neurons adapt more than their thalamic input neurons and that presumed excitatory (RSU) and inhibitory (FSU) barrel neurons adapt equivalently to each other. Greater adaptation in the cortex may reflect more pronounced depression at thalamocortical (Chung et al. 2002) versus trigeminothalamic synapses (Castro-Alamancos 2002), stronger intra-barrel (Goldreich et al. 1999) versus thalamic RT-mediated inhibition (Hartings and Simons 2000), and/or the presence in barrels of recurrent synaptic connections (e.g., excitatory-to-excitatory) that also depress (Egger et al. 1999; Petersen 2002). Despite smaller responses in the adapted state, periodic firing of cortical neurons closely reflects that of thalamic barreloid neurons, especially with higher velocity whisker movement. Thus in an adapted state produced by passive whisker deflection in sedated animals, firing within the barrel is sparse but still temporally faithful to the occurrence of the stimulus and the thalamic input signal. Available evidence suggests that the barrelloid-barrel circuit operates similarly during adaptation that accompanies behavioral arousal. During arousal adaptation to repetitive stimuli is less pronounced because the thalamocortical circuit is already in a suppressed state; nevertheless, periodic stimuli produce a further temporal focusing and magnitude reduction, albeit smaller, of the steady-state response (Castro-Alamancos 2004; Fanselow and Nicolelis 1999).

Responses of thalamic and cortical neurons are determined by the frequency of whisker deflections and by their velocity. In both thalamic and cortical neurons, response onsets and peaks occurred at longer latencies for steady-state compared with first-cycle stimuli, and latency shifts were greater for the lower-velocity (sinusoidal) deflections. With both sinusoidal
and pulsatile deflections, changes identical to those of RSU and FSU response onsets were virtually identical to those of TCUs. Thalamic activity in turn reflects the firing of primary afferent neurons that occurs at longer latency and with less population synchrony for lower velocity deflections (Shoykhet et al. 2000). Our thalamic data are consistent with findings that relatively high-velocity air puffs delivered ≤8 Hz lead to only small increases in VPm response latency (Ahissar et al. 2000).

Adapted TCU vector strengths were equivalent for the sines and pulses despite the more pronounced latency (phase) shifts that accompanied the former. During adaptation, the slopes of TCU population response onsets decreased more with sinusoidal than pulsatile deflections, however. Perhaps as a result, in the cortex the temporal fidelity of the adapted response is velocity dependent; for both RSUs and FSUs sinusoidal whisker movements evoke more temporally dispersed responses (as indicated by smaller vector strength values) than pulsatile stimuli, and even with the highest-velocity (125 mm/s at 40 Hz) sinusoidal deflections the period of the sinusoidal stimulus is represented less well in cortical firing patterns than is the case for pulsatile deflections (140 mm/s). In studies employing single deflections, barrel circuitry has been found to be sensitive to population firing synchrony within thalamic barreloids; whisker stimuli that lead to steeper slopes in thalamic population PSTHs evoke larger responses in barrel neurons (Kyrizzi et al. 1994; Pinto et al. 2000). The present findings therefore suggest that barrel circuitry remains sensitive to thalamic population firing synchrony in an adapted state produced by repetitive whisker deflection.

Previous investigators have described response decrements of somatosensory cortical neurons due to a preceding stimulus at the same location (e.g., Garabedian et al. 2003; Gardner and Costanzo 1980; Kyrizzi et al. 1994; Whitsel et al. 2003). Although details vary, response suppression increases with shorter inter-stimulus intervals and is mostly absent, or weaker, with intervals >100 ms. Suppressive effects have been attributed primarily to local inhibition and more recently to depression at TC synapses (see following text). Recent in vitro evidence indicates that TCU-FSU synapses depress more than TCU-RSU synapses (Beierlein et al. 2004). Our adaptation indices show that in vivo, the combined effects of inhibition and synaptic depression appear to act nearly equivalently on both RSUs and FSUs; if anything, FSUs showed slightly less adaptation with the pulsatile deflections. In cat vibrissa cortex in vivo, cortical excitatory postsynaptic potentials and inhibitory postsynaptic potentials adapt on periodic electrical stimulation of the thalamus (Hellweg et al. 1977). The similarity of adaptation in FSUs and RSUs is consistent with their receiving inputs from similar populations of barreloid neurons (Bruno and Simons 2002) and their extensive interconnections with each other (Petersen and Sakmann 2000). For example, adaptation of RSUs will reduce the amount of recurrent, intra-barrel excitatory input onto FSUs.

Although we found that cortical neurons differed greatly from thalamic neurons in terms of the magnitude of frequency-dependent adaptation, a number of response characteristics are similar at both levels. Across frequencies steady-state mean firing rates remained relatively constant in thalamus and cortex. Up to 8 Hz, mean firing rates increased moderately with increasing frequency of stimulus cycles, but firing rates reached asymptotic levels at ~12 Hz. Indeed, for both pulses and sines, a 10-fold increase in stimulus frequency (4–40 Hz) yielded at most only a 70% increase in MFR (Fig. 11B). This finding appears to be at variance with a recent report (Arabzadeh et al. 2004) in which a robust positive relationship was observed between MFR and the frequency of sinusoidal whisker deflection; for example, MFR doubled with increases in frequency from 19 to 50 Hz. Movement velocities of stimuli used in that study were, however, considerably lower than those of the present study. Interestingly, neurons did not fire periodically even at frequencies comparable to those used here (e.g., ~20–40 Hz) in which substantial phase-locking was observed both at the single cell and population levels. The lack of phase-locking at 20–40 Hz reported by Arabzadeh et al. may be a consequence of the relatively low velocity of the whisker deflections they used; our analyses indicate that higher velocity movements are more likely to evoke temporally focused responses at high stimulus frequencies.

The monotonic, minimal increase in MFR observed in the present study differs from the finding of Garabedian et al. (2003). Although they too observed pronounced frequency-dependent adaptation, MFRs peaked with 8-Hz whisker deflections, whereas firing rates in our cortical cells neared asymptotic values but did not decrease with higher frequencies.
Thalamic neurons were not examined in the former study, and it is therefore difficult to directly compare the two sets of cortical data. Moreover, Garabedian et al. recorded from more deeply anesthetized rats, and anesthesia likely contributed to the perhaps related finding that adaptation was substantially greater in that study. They also employed longer stimulus trains (2 s of adaptation). As suggested by their simulation work, greater response suppression, due perhaps to stronger anesthesia-related inhibition, likely counteracted the effects of more frequent (>8 Hz) excitatory inputs onto thalamic and/or cortical neurons. Garabedian et al. also reported a pronounced band-pass effect on steady-state response vector strengths, such that the timing of individual responses was most faithfully preserved at 6–10 Hz. Our analyses using normalized vector strength measures reveal only subtle changes in entrainment across stimulus frequencies, with no evidence for substantial frequency-dependent filtering ≤40 Hz. Thus at least in lightly narcotized animals, the temporal dynamics of the barreloid-barrel circuit do not appear to appear to be specialized for processing afferent information in the ~8–Hz range.

FSU responses displayed higher firing rates and greater entrainment than RSUs (see also Simons 1978). As in previous studies (e.g., Simons and Carvell 1989), FSU responses were highly similar to those of TCUs, consistent with their receiving strong synaptic inputs from thalamocortical axons (Bruno and Simons 2002; Swadlow and Gusev 2002). Thalamic reticular nucleus (RT) neurons, which provide virtually the only source of inhibition to VPm neurons (Desilets-Roy et al. 2002), also receive monosynaptic inputs from barreloid neurons and are strongly driven by whisker deflection. Interestingly, steady-state responses of these two populations of inhibitory neurons differ substantially. With whisker deflections identical to those used in the present study, the firing of RT neurons becomes increasingly unmodulated and tonic at higher stimulus frequencies (Hartings et al. 2003). Such uniform steady-state RT firing may help to preserve and even enhance VPm response transients (see Minnery et al. 2003), which are initially generated in primary afferent neurons. Unlike RT cells, cortical FS cells fire phasically (see also Mountcastle et al. 1969). The close coupling between phase-locked FSU and RSU responses may ensure that the sensitivity of barrel cortex to thalamic population firing synchrony is maintained on a moment-to-moment basis, especially when stimuli are changing rapidly. Interestingly, mean firing rates of thalamic and cortical neurons were similar across frequencies and for pulsatile and sinusoidal deflections. Thus for the stimuli used here, barrel cortex-based distinctions among high- versus low-velocity periodic deflections likely depend on the sensitivity of thalamocortical circuitry to input timing followed, perhaps, by further transformation to a mean firing rate code elsewhere in the cortical column (e.g., Arabzadeh et al. 2004; Salinas et al. 2000).

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