

Processing of Periodic Whisker Deflections By Neurons in the Ventroposterior Medial and Thalamic Reticular Nuclei

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Hartings, Jed A., Simona Temereanca, and Daniel J. Simons. Processing of periodic whisker deflections by neurons in the ventroposterior medial and thalamic reticular nuclei. *J Neurophysiol* 90: 3087–3094, 2003; 10.1152/jn.00469.2003. Rats employ rhythmic whisker movements to sample information in their sensory environment. To study frequency tuning and filtering characteristics of thalamic circuitry, we recorded single-unit responses of ventroposterior medial (VPM) and thalamic reticular (Rt) neurons to 1- to 40-Hz sinusoidal and pulsatile whisker deflection in lightly narcotized rats. Neuronal entrainment was assessed by a measure of the relative modulation (RM) of firing at the stimulus frequency given by the first harmonic (F1) of the cycle time histogram divided by the mean firing rate (F0). VPM signaling of both sinusoidal and periodic pulsatile whisker movements improved gradually over 1–16 and was maximal at 20–40 Hz. By contrast, the RM of Rt responses increased over 1–8 Hz, but deteriorated progressively over the 12- to 40-Hz range. In Rt, response adaptation occurred at lower stimulus frequencies and to a greater extent than in VPM. Within a train of high-frequency stimuli, Rt responses progressively decremented, possibly due to the accumulation of inhibition, whereas those of VPM neurons augmented. Mean firing rates in Rt increased 42 spikes/s over 1–40 Hz, providing tonic (low RM) inhibition during high-frequency stimulation that may enhance VPM signal-to-noise ratios. Consistent with this view, VPM mean firing rates increased only 13 spikes/s over 1–40 Hz, and inter-deflection activity was suppressed to a greater extent than stimulus-evoked responses. Rt inhibition is likely to act in concert with actions of neuromodulators in optimizing thalamic temporal signaling of high-frequency whisker movements.

INTRODUCTION

Rats employ their mystacial vibrissae in navigation and exploration by actively “whisking” them through the environment in 6- to 9-Hz cycles of protraction and retraction (Carvell and Simons 1990; Welker 1964). These self-generated whisker movements themselves elicit afferent-evoked periodic firing in central neurons (Fee et al. 1997), where responses to stimuli delivered at these frequencies exhibit response adaptation. The frequencies relevant for sensory processing may be higher, however, because afferent activity is additionally elicited by repetitive object contact. At high frequencies, periodic whisker stimulation causes a reduction in responses of cortical neurons (Ahissar et al. 2001; Garabedian et al. 2003; Simons 1978). These reductions are partly due to processing within the cortex (Castro-Alamancos and Oldford 2002; Chung et al. 2002) but

also reflect response adaptation in relay neurons of the thalamic ventroposterior medial (VPM) nucleus (Diamond et al. 1992; Gottschaldt et al. 1983; Sosnik et al. 2001).

Frequency-dependent characteristics of VPM responses are shaped by several mechanisms and are dependent on an animal's behavioral state (Castro-Alamancos 2002a; Fanselow and Nicolelis 1999). Acetylcholine (ACh) and norepinephrine (NE) released from neurons of the brain stem reticular formation during wakeful states depolarize VPM neurons, enabling their responses to lemniscal-evoked excitatory postsynaptic potentials (EPSPs), which depress for stimuli delivered >2 Hz (Castro-Alamancos 2002b). ACh and NE also depress corticothalamic EPSPs, and this effect is greater during low- than high-frequency stimulation (Castro-Alamancos and Calcajotto 2001). In addition, VPM firing is affected by inhibition from neurons of the thalamic reticular nucleus (Rt), whose GABAergic axons densely innervate thalamic relay nuclei (Cox et al. 1996; Pinault and Deschenes 1998; Scheibel and Scheibel 1966). ACh and NE depress inhibitory postsynaptic potentials (IPSPs) in VPM, facilitating the relay of high-frequency signals (Castro-Alamancos 2002b). However, the response and adaptation properties of Rt neurons themselves have not been characterized for periodic stimuli, and their effects on time- and frequency-dependent VPM relay are unknown.

VPM and Rt neurons differ in the temporal characteristics of their responses to ramp-and-hold whisker deflections (Hartings et al. 2000). VPM neurons respond with synchronous discharges to changes in whisker position but respond only weakly to maintained deflections. Thus they are described as signaling derivatives of whisker position, responding with more synchronous activity to higher velocity movements (Pinto et al. 2000). Rt neurons, on the other hand, have prolonged responses to whisker movements and sustained discharge during maintained deflections, suggesting that Rt inhibition contributes to the transient nature of VPM responses. Indeed, reducing or eliminating Rt inhibition prolongs VPM transient responses and unmasks sustained discharges during maintained deflections (Hartings and Simons 2000; Lee et al. 1994a,b). Hence, low-pass response characteristics of Rt neurons may enhance VPM relay of high-frequency signals.

Previous studies have examined response adaptation and temporal transformations of thalamic relay in the whisker-barrel system by assessing frequency-dependent changes in

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response latency and magnitude. For instance, responses of thalamocortical neurons in the posterior medial (POM) nucleus increase in latency and decrease in magnitude as whisker stimulation frequency increases (Ahissar et al. 2000; Sosnik et al. 2001). These latency shifts may provide a temporal code of deflection frequency. In VPM, on the other hand, frequency may be encoded by changes in response magnitude. Here we take a different, but complementary, approach of examining response transformations in the temporal domain by assessing the relative modulation of neuronal firing, a measure of signal-to-noise ratio, at the stimulus frequency. We recorded from VPM and Rt neurons while applying 1- to 40-Hz stimulation to the recorded unit's principal whisker, employing both sinusoidal and periodic pulsatile stimuli to distinguish among time-, frequency-, and velocity-dependent properties of intrathalamic and thalamocortical signaling. Findings indicate that VPM relay is optimized for signaling of high-frequency sensory stimulation, and that this high-pass transformation is facilitated by the band-pass characteristics and high mean firing rates of Rt neurons. A subset of this data describing invariance of 1- to 12-Hz VPM responses has been previously published (Hartings and Simons 1998).

METHODS

Surgical procedures and recordings

Adult female Sprague-Dawley rats weighing 250–300 g were prepared for thalamic recordings using methods described previously in detail (Hartings et al. 2000; Simons and Carvell 1989). Halothane anesthesia was used during surgical procedures. A steel post was secured to the skull over the left parietal cortex with dental acrylic to hold the animal's head, and a craniectomy was made at stereotaxic coordinates overlying VPM (2.0–4.5 posterior, 1.5–4.0 lateral to bregma) or the somatosensory sector of Rt (1.5–3.5 posterior, 2.5–4.5 lateral to bregma) (Paxinos and Watson 1982; Shosaku et al. 1984). After surgical preparation halothane was discontinued, the animal was immobilized by pancuronium bromide (1.6 mg · kg⁻¹ · h⁻¹ iv), artificially respired through a tracheal cannula, warmed by a servo-controlled heating blanket, and maintained in a lightly narcotized state by a steady infusion of fentanyl (Sublimaze, Jansen Pharmaceuticals; ~10 μg · kg⁻¹ · h⁻¹ iv). The animal's condition was assessed by monitoring electroencephalogram, femoral arterial blood pressure, heart rate, tracheal airway pressure, and pupillary reflexes. Extracellular single-unit recordings were made in VPM or Rt with 10 MΩ stainless steel microelectrodes (Frederick Haer, Brunswick, ME). At the end of recording sessions, animals were deeply anesthetized with pentobarbital sodium (Nembutal, 100 mg/kg iv) and perfused transcardially. Brains were sectioned in the coronal plane and stained with thionin to confirm the location of electrode tracks through the appropriate nucleus.

To assess responses of primary afferent neurons to periodic stimuli, recordings were also made from the trigeminal ganglion (N.V.). For these experiments, the right external jugular vein was cannulated for drug delivery, and a cannula was inserted into the trachea to facilitate respiration. Animals were then maintained under pentobarbital sodium (Nembutal) anesthesia for the remainder of the experiment. A steel post was fixed to the skull with dental acrylic to hold the animal's head, and a craniectomy was made at the stereotaxic coordinates overlying the trigeminal ganglion (6.0 mm anterior to lambda, 2.6 mm lateral to midline). Single-unit recordings were made with tungsten microelectrodes, advanced through the brain to the base of the skull by a micromanipulator. Responses of N.V. cells in anesthetized rats are comparable to those recorded in paralyzed, sedated rats

(Minnery and Simons 2003). Animals were euthanized at the end of recording sessions by an overdose of Nembutal.

Whisker stimulation

Hand-held probes were used to identify the whisker evoking the strongest response from an isolated unit, i.e., the principal whisker. A piezoelectric mechanical stimulator was then attached to this whisker ~10 mm from the face (Simons 1983). Ramp-and-hold whisker deflections were applied first to determine the deflection angle evoking the maximal response to stimulus onset, i.e., the preferred direction. This stimulus consisted of a 1-mm ramp-and-hold deflection of the whisker from its resting position with onset and offset velocities of ~125 mm/s and a plateau duration of 200 ms. Individual trials were separated by 2 s. Ten deflections were applied in each of eight randomly interleaved directions spanning 360° in 45° increments for a total of 80 trials. Peristimulus time histograms (PSTHs) for each of the eight directions were computed and displayed on-line.

Two types of periodic stimulation, sinusoidal and periodic pulsatile deflections, were then applied in each neuron's preferred direction. Sinusoidal deflections were 1 mm in amplitude and began with the resting whisker position as the trough of the sine wave, $\sin(3\pi/2)$, so that the maximum amplitude deflection, $\sin(\pi/2)$, occurs after ½ cycle of the stimulus and the return to rest position completes one full cycle. Sinusoidal stimuli were applied at 1, 2, and 4 Hz for 4 s, 8, 10, and 12 Hz for 2 s, and 16, 20, 30, and 40 Hz for 1 s. Trials at each of the ten frequencies were randomly interleaved, and 2 s was allowed between trials. The battery of 10 frequencies was repeated 10 times, for a total of 100 trials.

Sinusoidal stimulation confounds the effects of whisker movement velocity and frequency on the neural response. Therefore periodic pulse stimulation was used to assess the effects of stimulus frequency alone. Single pulses consisted of 700-μm deflections ~10 ms in total duration (rise time = fall time = 5 ms). Individual pulses applied sequentially and at different frequencies were identical in amplitude and time course. Pulses were applied with the same frequencies and same protocol as sinusoidal stimuli, with the exception that the 10-Hz stimulus was omitted. The smaller amplitude of pulse deflections (700 μm) compared with sinusoidal deflections (1 mm) was necessitated by limitations in the stimulators and a need to keep individual pulses brief in duration. Brief pulses are more likely to evoke only a single neuronal response rather than distinct responses to both stimulus onset and offset. Deflection amplitudes of 700 μm exceed those necessary to evoke a robust thalamic response (Pinto et al. 2000), and mean deflection velocities (140 μm/ms) for pulses were similar to peak velocities of the 40-Hz sinusoidal stimulus (124 μm/ms).

Data analysis

A time/amplitude window discriminator (BAK Electronics) and digital storage oscilloscope were used to isolate single units based on spike amplitude and waveform criteria. Sequential spike event times were recorded with 100-μs resolution on a DEC LSI 11/73. Subsequent data analysis was performed on an IBM PC using Microsoft Excel/Visual Basic and programs written with Interactive Data Language software (Research Systems, Boulder, CO). Statistical tests were performed using Microsoft Excel and the statistics package from SPSS.

Responses to both sinusoidal and pulsatile stimulation were analyzed by constructing cycle time histograms (CTHs). For each unit, CTHs were computed by averaging responses to each stimulus cycle, excluding the first, of each trial. CTHs thus represent firing probability over the period of one "steady-state" stimulus cycle. Entrainment of neuronal firing was measured by applying a fast Fourier transform (FFT) to the CTH. Results of the FFT were normalized to the CTH length and the absolute amplitude of the F1 was taken as a measure of entrainment at the stimulus frequency, $1/(n \times T)$, where n is the

number of time points in the CTH and T is their sampling interval [e.g., 10 Hz = $1/(100 \times 0.001 \text{ s})$]. *Relative modulation (RM)* measures were computed by dividing the F1 by the mean firing rate or DC component of the CTH (F_0). The RM measure reflects the proportion of a unit's activity that is modulated at the stimulus frequency. RM is equivalent to the measure of vector strength used to assess neuronal entrainment (Goldberg and Brown 1969) and presumes a sinusoidal stimulus waveform (Eggermont 1991). However, RM and vector strength have been used to assess entrainment to nonsinusoidal periodic stimulation (Eggermont and Smith 1995; Garabedian et al. 2003).

Peak firing rates evoked by sinusoidal stimulation were measured for each frequency as the maximal firing rate over any 5-ms period in the population CTH. Responses evoked by individual pulse deflections were measured over 15 and 28 ms periods for VPM and Rt, respectively.

RESULTS

Sinusoidal stimulation

Steady state responses of 27 VPM and 42 Rt neurons to 1- to 40-Hz sinusoidal whisker movements were accumulated into population CTHs, which show spike probability as a function of phase within a stimulus cycle (Fig. 1). Responses from 21 primary afferent (N.V.) neurons are also shown for comparison. Activity in both N.V. and VPM populations was entrained by deflections at all frequencies. In VPM, and to a lesser extent in N.V., there are two activity peaks per stimulus cycle, corresponding to the deflection onset, or displacement of the whisker from its rest position, and deflection offset, or return to the rest position. These are analogous to ON and OFF responses to ramp-and-hold deflections (Simons and Carvell 1989).

In Rt, firing rates are also modulated according to the stimulus cycle but differ from VPM responses in several respects.

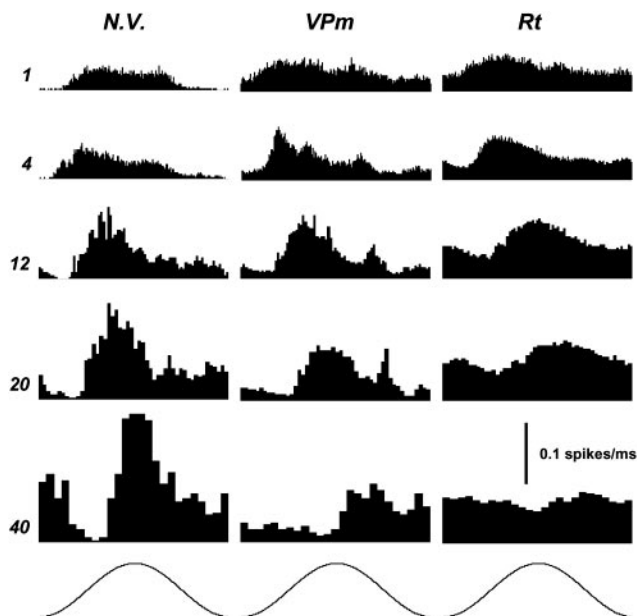


FIG. 1. Population responses to sinusoidal whisker deflections. Cycle time histograms (CTHs) show responses of trigeminal ganglion (N.V., $n = 21$ cells), ventroposterior medial (VPM), and thalamic reticular (Rt) populations as a function of phase within the stimulus cycle. The duration of each CTH is the period of 1 cycle (i.e., 1,000, 250, 82, 50, and 25 ms for 1-, 4-, 12-, 20-, and 40-Hz stimuli, respectively). Bin widths = 1 ms. Each cycle begins at the resting or neutral position of the whisker and reaches a maximum deflection amplitude of 1 mm. The ordinate axes for all CTHs have the same scale.

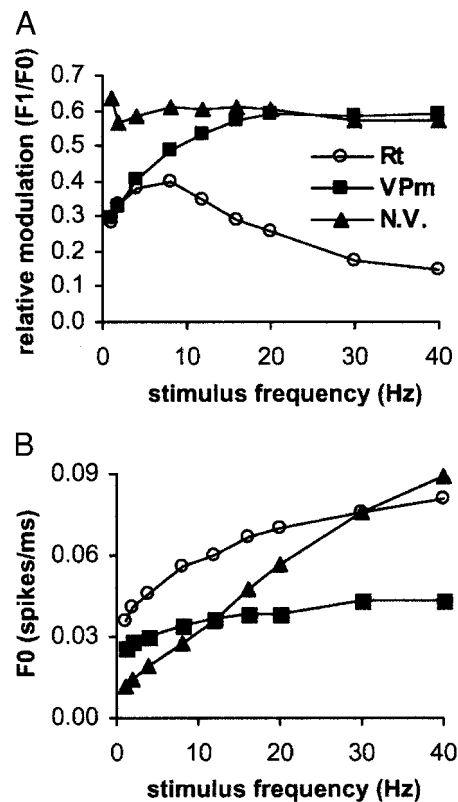


FIG. 2. Fourier analysis of responses to sinusoidal deflections. F_0 and F_1 values were computed for each unit and stimulus frequency based on CTHs. Relative modulation (A) and F_0 (B) values were then averaged over all units in each population. Symbol legend in A applies to both panels.

First, activity peaks have slower rises and decays and are therefore less temporally distinct. Second, Rt responses are monophasic, lacking responses to deflection offset. Third, entrainment declines substantially at higher frequencies (e.g., 40 Hz) as activity remains elevated throughout the stimulus cycle with little phase-specific modulation.

To quantify entrainment to the frequency of stimulation, the amplitude of the first harmonic of a CTH (F_1) was divided by its DC component (F_0) for each unit to yield a measure of relative modulation (RM) of firing rate at the stimulus frequency. Mean RM values were then computed for each population and are shown as a function of stimulus frequency in Fig. 2. In the primary afferent population, RM is high and invariant over the 1- to 40-Hz range. In VPM, by contrast, RM increases with stimulus frequency over the 1- to 20-Hz range and is then maintained at a high level equal to that of N.V. neurons over 20–40 Hz. Rt responses reflect a similar increase in entrainment over 1–8 Hz but, unlike VPM responses, decline in their modulation at higher frequencies. These frequency tuning characteristics, as assessed by RM, depend on the magnitude of peak firing rates as well as overall mean firing rates (F_0). The latter partly account for the decline of RM in Rt at high frequencies (Fig. 2). In Rt, F_0 increased from 35 to 81 spikes/s over the 1- to 40-Hz range (2.31-fold). By contrast, VPM F_0 increased only from 26 to 43 spikes/s (1.65-fold). Peak firing rates (see METHODS) changed similarly for both VPM and Rt, increasing monotonically over 1–8 Hz and remaining relatively constant over 8–40 Hz (not shown).

These population trends in entrainment were also observed

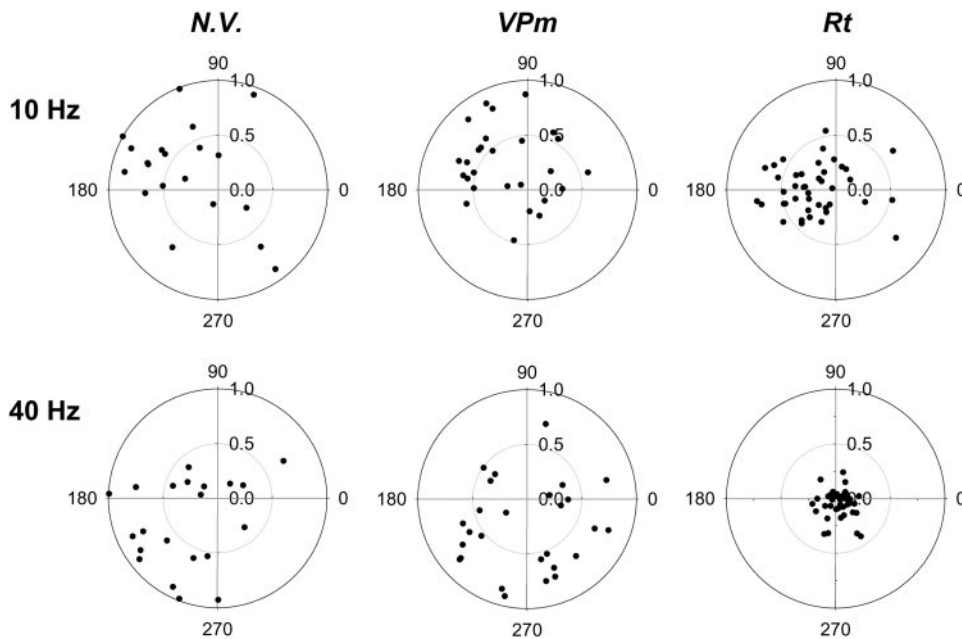


FIG. 3. Entrainment of single units by sinusoidal deflections. Polar plots show the phase (angular coordinate) and strength (distance from origin) of entrainment vectors for neurons at 10 (*top*) and 40 Hz (*bottom*). Each symbol represents an individual unit. 0° is defined as the trough of the sinusoid and the beginning of the cycle (rest whisker position, 0 ms) and 180° is the peak (1-mm deflection, $0.5 \times$ cycle period). See Fig. 1 and METHODS for more details. Frequency-dependent phase shifts reflect constant latencies.

in the firing patterns of individual neurons. Phases and strengths of entrainment for individual neurons are presented for 10- and 40-Hz stimuli in Fig. 3 as vector angles and lengths, respectively. Vector angles reflect the phase of the F1 and lengths reflect the RM value. At 10 Hz, N.V. and VPm neurons were well entrained at similar phases, as indicated by clustering between 90° (25 ms) and 180° (50 ms). Rt units also exhibit entrainment but cluster near 180°, reflecting a phase delay relative to VPm activity. The shorter vector lengths for Rt units reflect poorer relative modulation of activity evident in visual (Fig. 1) and quantitative (Fig. 2) analyses. At 40 Hz, N.V. and VPm units remain well entrained at 180–270° (12.5–18.75 ms) and 270°, respectively, and have similar vector strengths as at 10 Hz. For Rt neurons, however, points lie close to the origin, indicating small or no bias in firing for a particular phase of the stimulus cycle and hence a loss of entrainment.

Periodic pulses

Use of sinusoidal whisker deflections at different frequencies confounds effects of stimulus frequency and velocity on the neural response. Also, the lack of an abrupt stimulus onset time complicates cycle-by-cycle analysis of stimulus-evoked responses. Therefore we employed periodic pulsatile whisker deflections to study the effects of stimulus frequency on response magnitude, adaptation, and frequency tuning. Responses of VPm ($n = 28$) and Rt ($n = 35$) units to 1- to 40-Hz stimuli are shown in PSTHs in Fig. 4. Both populations respond well to each pulse deflection delivered at each frequency. As in the case of sinusoidal whisker deflections, VPm responses to periodic pulses show a high-pass filtering characteristic; RM increases monotonically and approaches asymptotic levels at frequencies ≥ 12 Hz (Fig. 5A). Rt responses, on the other hand, exhibit band-pass responses with peak entrainment occurring at 8 Hz. Overall mean firing rates for both VPm and Rt increased with stimulus frequency (Fig. 5B), reflecting the greater number of whisker deflections. These increases were greater in Rt than VPm, as for sinusoidal stimulation (Fig. 2B).

With periodic pulses, increases in RM over 1–12 Hz are partly attributable to the fixed duration of the neuronal response (see following text, Fig. 6) in relation to the decreasing period of the stimulus; brief, identical responses necessarily contain more power in relation to the cycle duration for shorter cycles (i.e., higher frequencies). Thus RM values increase even for N.V. neurons over 1–12 Hz (Fig. 5A). This effect is minimal in the N.V. population, however, because F0 values increase linearly with stimulus frequency due to lack of adaptation. In the thalamus, the effect is enhanced because adaptation attenuates the frequency-dependent increase of mean firing rates (Fig. 5B).

To understand how adaptation of spike rates contributes to

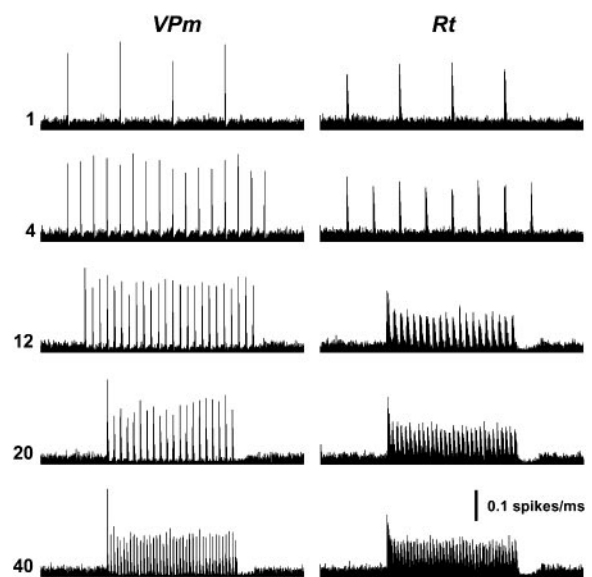


FIG. 4. Thalamic population responses to periodic pulse deflections. Peri-stimulus time histograms (PSTHs) show responses accumulated over all cells in each population. PSTHs are 5 s in duration for 1 and 4 Hz, 3 s for 12 Hz, and 1 s for 20 and 40 Hz. In each case the stimulus period is bracketed by 500 ms of spontaneous activity on both ends. The ordinate axes are scaled the same for all panels.

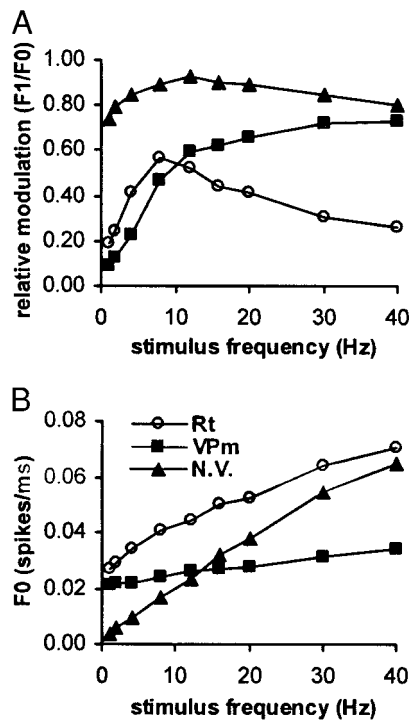


FIG. 5. Fourier analysis of responses to periodic pulse deflections. F0 and F1 values were computed for each unit and stimulus frequency based on CTHs. Relative modulation (A) and F0 (B) values were then averaged over all units in each population. Symbol legend in A applies to both panels.

the different RM frequency tuning curves for VPm and Rt, we examined responses evoked by individual pulse deflections as well as spike rates during periods between whisker deflections. Rt responses to initial pulses averaged 3.7 spikes as compared with 1.1 spikes for VPm cells. PSTHs accumulated over all stimulus cycles excluding the first show that Rt steady-state responses are also greater in magnitude and longer in duration than in VPm (Fig. 6). At high stimulus frequencies, responses of both VPm and Rt neurons depressed relative to their responses to initial pulses (Figs. 4 and 6). Note that response timing, however, is not appreciably affected by stimulus frequency in either nucleus; the peaks and rising phases of responses in steady state PSTHs increase in latency by only 1–3 ms over the 1- to 40-Hz range (Fig. 6) (see also Hartings and Simons 1998; Sosnik et al. 2001).

Evoked response adaptation is examined more closely in Fig. 7. In VPm, responses to later pulses decrement with respect to the first pulse at stimulus frequencies ≥ 16 Hz (inter-stimulus intervals: ≤ 62.5 ms), and the decrement is greater at higher frequencies (Fig. 7, A and C). Note, however, that responses exhibit maximal depression from the first to second cycles and then gradually augment with subsequent stimuli in a train (Fig. 7A). By comparison, Rt responses decrement at frequencies ≥ 8 Hz (≤ 125 ms inter-stimulus interval; Fig. 7, B and C), and these responses progressively decrease, rather than increase, within a train of successive pulses (Fig. 7B). The degree of response suppression also differs between these nuclei (Fig. 7C). In Rt, for instance, steady-state responses to 20-Hz stimuli are suppressed 42% relative to initial responses, whereas VPm responses exhibit only 13% suppression. Thus the greater progressive suppression

of Rt responses partly accounts for their poor RM at high frequencies.

F0, and hence RM, is determined not only by transient stimulus-evoked responses but also by firing rates in the inter-deflection period. This inter-deflection activity was quantified by measuring firing rates during the 5-ms period preceding each pulse deflection. In VPm, inter-deflection activity is enhanced following pulse deflections delivered at 2–10 Hz (Fig. 7D), and stimulus-evoked responses are likewise facilitated at these frequencies (Fig. 7C). At higher frequencies, however, inter-deflection activity is progressively decremented, reaching 67% suppression at 30 Hz (Fig. 7D). In Rt, inter-deflection activity is suppressed progressively from 1 to 12 Hz but then increases with higher frequency stimulation (Fig. 7D). Rt responses to individual punctate whisker deflections are ~ 40 ms in duration (Hartings et al. 2000) and thus can continue throughout the inter-deflection period. At 30 Hz, for instance, activity is enhanced relative to baseline levels. Thus the long duration of Rt responses results in a continuous elevation of firing rates at high stimulus frequencies (Figs. 2B and 5B), even though the number of spikes elicited per whisker deflection are substantially decremented. This results in low RM values. In VPm, by contrast, the greater suppression of inter-deflection activity relative to stimulus-evoked firing enhances signal-to-noise ratios over the high-frequency range.

DISCUSSION

High-pass filtering by thalamic circuits

In this study we have demonstrated that VPm neurons can be optimally entrained to sinusoidal sensory stimulation up to 40 Hz. The strength of this signaling, as assessed by the RM of firing at the stimulus frequency, increases monotonically over 1–16 Hz and is maintained at peak levels over 20–40 Hz. By

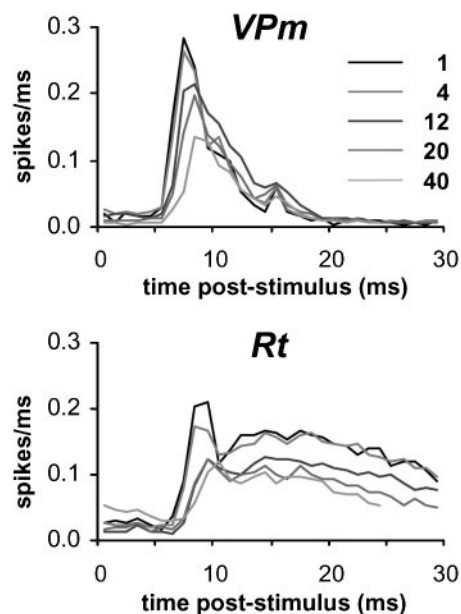


FIG. 6. “Steady-state” evoked responses to periodic pulse stimuli. Population PSTHs for 1, 4, 12, 20, and 40 Hz were constructed by averaging responses to each pulse in a train, excluding the first. PSTHs begin at the time of pulse onset ($t = 0$ ms). The small secondary peaks at 15 ms are responses to stimulus offset, or return of the whisker to rest position.

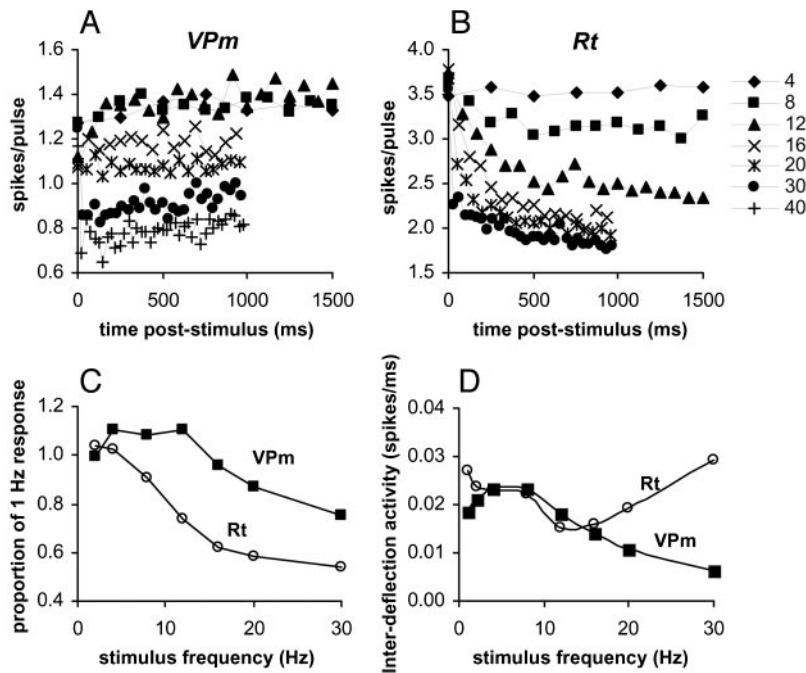


FIG. 7. Time- and frequency-dependent decrement of thalamic responses. *A* and *B*: responses of each cell were computed separately for consecutive pulses within a train and are shown here as a function of time following stimulus onset. Different data series show responses at different stimulus frequencies as indicated in the symbol legend at right. Response magnitudes were computed over 15- and 28-ms periods for VPm and Rt, respectively. Rt responses to 40-Hz stimulation are not shown because the response period extends into subsequent cycles/responses. *C*: mean responses per pulse over 500–1,000 ms after the beginning of stimulus trains were computed for each frequency and are plotted as proportions of responses obtained at 1 Hz. *D*: inter-deflection activity was measured as the mean single-unit firing rate over 5 ms prior to pulse onset for all pulse deflections excluding the first.

contrast, Rt signaling of periodic whisker deflections increases over 1–10 Hz but declines progressively over higher frequencies. Recordings of primary afferent N.V. neurons and VPm-projecting neurons in the brain stem nucleus principalis (PrV) (Minnery 2003) reveal that these response characteristics arise from processing at the thalamic level. RM values for N.V. and PrV populations are constant over 1–40 Hz and do not significantly differ from each other. Recordings of dendritic prepotentials, presumably reflecting inputs to VPm neurons (Gottschaldt et al. 1983), and brain stem recordings in other laboratories (Sosnik et al. 2001) further confirm that response adaptation to successive stimuli occurs within the thalamus itself and not in more peripheral circuits.

High-pass frequency-response characteristics of thalamocortical signaling are attributable to the velocity sensitivity of VPm firing and the effects of Rt inhibition, in addition to neuromodulatory mechanisms. At low sinusoidal frequencies (≤ 2 Hz), low whisker deflection velocity evokes few spikes in VPm relative to background activity, which is maintained at baseline levels. At higher frequencies (e.g., 2–10 Hz), and hence with faster whisker movement velocities, peak evoked firing rates increase, consistent with previous studies demonstrating the velocity sensitivity of VPm responses (Pinto et al. 2000). At 10–40 Hz, peak firing rates are constant, which is likely due to counterbalancing effects of increasing stimulus velocity and greater accumulation of inhibition. However, while inhibition may limit peak firing rates, its net effect is to enhance or preserve high signal-to-noise ratios, as measured by RM, at high stimulus frequencies. While mean firing rates of N.V. and VPm-projecting PrV neurons increase sharply over 1–40 Hz (Fig. 2*B*; see also Minnery 2003), VPm mean firing rates increase in the range of only 26–43 spikes/sec, suggesting that the suppression of mean activity by Rt inhibition contributes to the maintenance of high RM at high frequencies.

Rt firing patterns are consistent with this explanation. Rt firing exhibited poor RM but substantially elevated firing rates

at high sinusoidal stimulation frequencies, thus providing the substrate for phase non-specific (i.e. tonic) inhibition. Such inhibition would enhance the signal-to-noise ratio in VPm by elevating firing thresholds. The decline of Rt entrainment at frequencies >10 Hz reflects the high firing rates and long duration responses of Rt neurons, which limit their ability to modulate temporally their firing according to the timing of rapidly successive whisker deflections. Rt neurons respond to punctate deflections with long-duration responses and discharge continuously during static whisker displacements (Hartings et al. 2000). In response to low-frequency sinusoidal stimulation, Rt responses are temporally dispersed and unimodal. At high frequencies, high rates of Rt discharge from previous stimulus cycles extend to subsequent cycles, resulting in elevated mean firing rates with little modulation. In VPm, phase-specific activity peaks are more temporally focused and are evoked by whisker movements in both directions (i.e., exhibit ON and OFF responses).

An advantage of pulse stimulation is that whisker deflections for each “cycle” of the stimulus have distinct onset times. We therefore analyzed responses to 1- to 40-Hz trains of pulse whisker deflections to examine frequency-dependent changes in stimulus-evoked and inter-deflection activity that contribute to optimization of high-frequency signaling. The use of brief pulses minimized responses to stimulus offset and prevented the confounding effects of period-doubling in the neural response (see Fig. 6). VPm responses to individual pulses adapted in a frequency-dependent manner but reached a peak suppression of only 13% at 30 Hz. At this frequency, inter-deflection activity was suppressed by 67%. The greater suppression of inter-deflection than stimulus-evoked activity suggests that VPm signaling at high frequencies is enhanced by tonic inhibition. In Rt, inter-deflection activity was enhanced at higher frequencies, whereas stimulus-evoked activity was substantially depressed (42%), resulting in low RM values appropriate for phase nonspecific inhibition.

Frequency-dependent adaptation of whisker-evoked responses

In VPM, responses to the second in a pair of sequential deflections decremented at intervals <62 ms (>16 Hz). This is similar to the time course of suppression described for sequential deflections of adjacent and principal whiskers (Simons and Carvell 1989). The extent of this paired-deflection suppression increased with shorter inter-deflection intervals ranging to 25 ms (40 Hz), which was also shown to evoke maximal inhibition in prior studies (Fanselow and Nicolelis 1999; Simons and Carvell 1989). In Rt, on the other hand, the present study demonstrated paired-deflection suppression at intervals as long as 125 ms (>8 Hz). This difference in the time course of suppression in VPM and Rt is consistent with the longer duration of GABA_A receptor-mediated inhibitory postsynaptic currents in Rt than VPM neurons (Hunstman et al. 1999; Zhang et al. 1997). Indeed, suppression of responses in VPM to the second and subsequent stimuli in periodic trains is reversed by application of the GABA_A receptor antagonist bicuculline (Gottschaldt et al. 1983). Depression of lemniscal synapses may also contribute to VPM response suppression, particularly at high stimulation frequencies when tonic inhibition may cause depressed EPSPs to be subthreshold events (Castro-Alamancos 2002a).

After an initial paired-pulse suppression of VPM responses, subsequent stimuli within trains delivered at 30–40 Hz elicited progressively larger numbers of spikes. This augmentation may reflect facilitation at corticothalamic synapses, which is maximal at 20- to 40-Hz frequencies (Castro-Alamancos and Calcagnotto 2001). Augmentation may also be enabled by diminishing levels of inhibition, as Rt responses to pulse deflections progressively decremented for stimulus frequencies ≥ 12 Hz (Fig. 7B). Diminution of Rt responses may be due to long-lasting GABA_A conductances that can shunt excitatory inputs arriving 100–150 ms after a cell responds to a previous stimulus (Sohal et al. 2000). Recurrent inhibition is evidenced in the present data as ~ 200 ms suppressions of Rt activity at the termination of 12- to 40-Hz stimulus trains (Fig. 4). This mechanism of Rt response diminution causes a corresponding decrement of IPSPs in thalamocortical neurons in response to a stimulus train (Huguenard and Prince 1994). VPM IPSPs are reduced progressively in response to periodic whisker deflections, which may also reflect depression of Rt \rightarrow VPM synapses (Castro-Alamancos 2002a).

The frequency tuning characteristics in VPM described here, with the onset of adaptation occurring at frequencies >10 Hz, are similar to those described during attentive wakefulness (Fanselow and Nicolelis 1999) and during stimulation of the brain stem reticular formation (Castro-Alamancos 2002a). By contrast, reduced steady-state responsiveness emerges at lower frequencies (>2 Hz) when animals are anesthetized with pentobarbital (Diamond et al. 1992; see also Chung et al. 2002) or urethan (Castro-Alamancos 2002a; Sosnik et al. 2001) or during awake, quiescent states (>5 Hz) (Fanselow and Nicolelis 1999). In the attentive awake state, sensory relay of high-frequency signals is enhanced by the actions of ACh and NE. These include depolarization of VPM membrane potentials, depression of IPSPs (Castro-Alamancos 2002a,b), and preferential depression of corticothalamic EPSPs at low stimulus frequencies (Castro-Alamancos and Calcagnotto 2001). Our

results show that Rt-mediated inhibition, and its differential effects on VPM versus Rt neurons can act in concert with these mechanisms to enhance transmission of high-frequency inputs.

Implications for processing in cortical layer IV

Under pentobarbital anesthesia, cortical neurons in the thalamic recipient layer IV respond to 1- to 40-Hz periodic stimuli with band-pass characteristics for measures of mean firing rate and relative modulation (vector strength) (Garabedian et al. 2003). In lightly narcotized rats, however, as in the present study, RM is broadly tuned in layer IV regular-spike units (RSUs) with high values maintained at sinusoidal stimulation frequencies up to 40 Hz (unpublished data). This is consistent with previous results demonstrating that the RSU response depends on the temporal contrast, or rate of rise, of the thalamic population input signal, and not its absolute or peak amplitude (Pinto et al. 2000). As demonstrated here, the relative modulation of firing is maintained in thalamocortical neurons up to 40 Hz despite decreases in the number of spikes evoked per whisker deflection.

The sensitivity of layer IV circuitry to thalamic input timing may be enhanced by thalamocortical synaptic depression and local inhibition. With light narcosis, cortical activity is relatively suppressed due to the high spontaneous activity of thalamic neurons and the consequent depression of thalamocortical synapses (Castro-Alamancos and Oldford 2002). Asynchronous spontaneous thalamic activity also activates layer IV fast-spike units (FSUs), which are GABAergic, to a greater extent than glutamatergic RSUs, producing enhanced inhibitory tone (Brumberg et al. 1999). The cortical suppression resulting from these mechanisms may enhance the differential sensitivity of layer IV circuitry to synchronous versus asynchronous thalamic inputs (Pinto et al. 2003) and maintain its *relative* responsiveness to rapid whisker deflections, even when absolute response magnitudes are smaller. These effects would be further enhanced by high-frequency whisker deflections, which cause greater depression of thalamocortical synapses (Chung et al. 2002) by elevating thalamic firing rates (F0) and also engage FSUs more so than RSUs (Simons 1978).

The present findings indicate that thalamic processing preserves and faithfully relays the temporal structure of high-frequency peripheral stimulation. This in turn may enable a similar isomorphic coding in the firing patterns of populations of cortical neurons. High-frequency whisker deflections may be important in texture discrimination and can also improve spatial discrimination. Stimulation of individual whiskers at 10 Hz elicits a spread of cortical activity that is more narrowly focused on the corresponding cortical column than stimulation at lower frequencies (Sheth et al. 1998). Whether higher frequencies can enhance this effect and perhaps further focus activity onto microcircuits within the barrel remains to be determined.

DISCLOSURES

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