Thalamic Relay of Afferent Responses to 1- to 12-Hz Whisker Stimulation in the Rat

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Hartings, Jed A. and Daniel J. Simons. Thalamic relay of afferent responses to 1- to 12-Hz whisker stimulation in the rat. *J. Neurophysiol.* 80: 1016–1019, 1998. Somatosensory cortical neurons in the rat can be entrained to frequencies of pulsatile whisker stimulation up to at least 12 Hz. A recent study proposed that such entrainment depends on oscillatory corticothalamic feedback. According to this model, thalamic relay neurons function as comparators of ascending and descending signals and should vary their response magnitudes and latencies as a function of peripheral stimulation frequency. Here we report, however, that the responses of thalamic relay neurons to 1- to 12-Hz pulsatile whisker deflections are constant in magnitude and latency over these frequencies. In addition, their cycle-by-cycle responses are as invariant as those of primary afferent neurons. These results support the view that thalamic relay neurons are driven primarily by ascending afferent signals and thereby entrain cortical neurons to peripheral stimulation by means of a direct feed-forward mechanism.

INTRODUCTION

The traditional understanding of hierarchical processing in the somatosensory system is that stimulus-evoked signals are relayed sequentially from peripheral receptors to brain stem nuclei and then to thalamic neurons that project to the cortex. Response properties of neurons at each stage are determined mainly by convergence of inputs from the more peripheral neuronal population and local circuit processing of those inputs. In a recent work, Ahissar et al. (1997) refer to this scheme as ''passive'' decoding of peripheral events and reject it in favor of a competing hypothesis of ''active'' decoding, in which corticofugal projections to thalamic and/or brain stem nuclei substantively determine the firing patterns evoked in relay nuclei during periodic peripheral stimulation.

The hypothesis of active decoding is derived from the reported result that thalamic neurons phase-lag both brain stem and cortical neurons during epochs of oscillatory whisker twitches in awake and voluntarily immobile rats (Nicolelis et al. 1995). Employing this result, Ahissar et al. (1997) present a model of the thalamocortical system as a phase-locked loop (PLL) to explain the entrainment of cortical neurons to periodic peripheral stimuli and to propose a mechanism for the coding of object location. According to the PLL model, thalamic relay neurons compare the phases of intrinsic oscillations in the cortex ($\sim$10 Hz) and periodic peripheral input, and signal the phase difference by their firing rates. Thalamocortical input then adjusts the frequency of the cortical oscillations by the amount necessary for the oscillations to match the frequency of the peripheral events. As a consequence, thalamic responses phase-lag the cortical oscillations.

According to the PLL model, the phase difference signaled by thalamic neurons varies with changes in the frequency of peripheral stimulation. Specifically, the model predicts that as the peripheral stimulation frequency increases *1*) thalamic response magnitude will decrease and *2*) thalamic response latencies will increase (Ahissar et al. 1997). A third prediction is that thalamic responses to the first cycle of a periodic stimulus should differ substantially from responses to later cycles of the stimulus, because corticothalamic feedback is proposed to contribute significantly to the attainment of steady-state thalamic responses. Response latencies and magnitudes of primary afferent neurons, however, are presumed to be invariant to the frequency of stimulation. The predictions of the PLL hypothesis are thus inconsistent with the view that thalamocortical neurons are primarily driven by ascending sensory input. As part of an ongoing study, we have recorded the activity of thalamic and primary afferent neurons in response to periodic pulse stimulation of individual whiskers. Contrary to the predictions of the PLL model, we find that, in the range of 1–12 Hz, mean response latencies and magnitudes per stimulus cycle are constant for both populations.

METHODS

Surgical procedures and recordings

Six adult female rats were used in these experiments; four for thalamic recordings and two for trigeminal ganglion (NV) recordings. Animals were prepared for electrophysiological study using methods described previously (Lichtenstein et al. 1990; Simons and Carvell 1989). Briefly, for NV recordings 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 (Frederick Haer, Brunswick, ME), advanced through the brain to the base of the skull by a micromanipulator. Animals were maintained under pentobarbital sodium (Nembutal) anesthesia during surgery and recordings.

For thalamic recordings, halothane anesthesia was used during surgical procedures. A craniectomy was made over the right ventral posterior medial (VPM) nucleus of the thalamus, and the dura mater was resected. After surgery halothane was discontinued, the animal was immobilized by pancuronium bromide, artificially respired through a tracheal cannula, and kept in a lightly narcotized state by continuous infusion of fentanyl (Sublimaze, Janssen Pharmaceuticals; 5–10 $\mu g \cdot kg^{-1} \cdot h^{-1}$). The animal's condition was

assessed by monitoring electroencephalogram, femoral arterial blood pressure, tracheal airway pressure, and pupillary reflexes. Double-barreled glass micropipettes filled with 3 M NaCl (7–15 MΩ) were used for extracellular single-unit recordings. All animals were killed at the end of recording sessions by an overdose of Nembutal. Brains were sectioned in the coronal plane and Nissl stained to confirm the location of electrode tracks through VPM.

Whisker stimulation and data analysis

Hand-held probes were used to identify the whisker that evoked the strongest response (the principal whisker, or PW) from an isolated unit. To determine the preferred direction of whisker movement for the recorded neuron, a piezoelectric mechanical stimulator was attached to the PW 10 mm from the face and used to deflect the whisker in eight directions spanning 360° in 45° increments (Simons 1983). At the preferred direction, pulsatile deflections were applied to the whisker at 1, 2, 4, 8, and 12 Hz for 4, 4, 4, 2, and 2 s, respectively. Ten trials at each frequency were randomly interleaved, with 3 s for recovery between trials. Single pulses consisted of 700-μm deflections of the whisker from its rest position in the unit's preferred direction. Deflections were ~10 ms in duration (rise time = fall time = 5 ms) and were identical for each stimulation frequency. These stimuli were similar to those used by Ahissar et al. (1997).

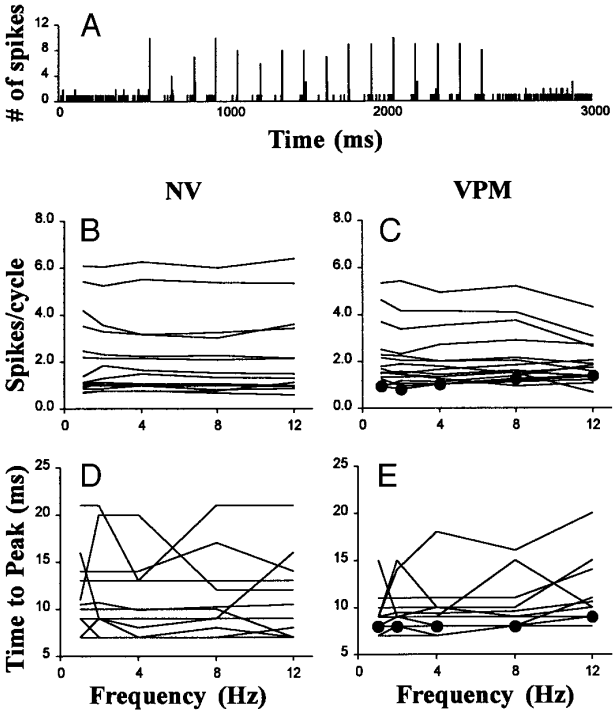

FIG. 1. Single neuron response magnitudes and latencies in trigeminal ganglion neuron (NV) and ventral posterior medial nucleus (VPM). *A*: peristimulus time histogram (PSTH) of a representative thalamic neuron in response to pulsatile whisker stimulation delivered at 8 Hz for 10 trials. Stimulus begins at 500 ms and ends at 2,500 ms. *B* and *C*: mean responses per stimulus cycle of individual NV and VPM neurons, respectively, at each stimulus frequency. *D* and *E*: response latencies as measured by the time of the peak response (see METHODS). The variability in latency among units is partly due to "OFF" responses evoked by the return of the whisker to its rest position, which occur 5–10 ms after the "ON" response (see secondary peaks at 13–14 ms in Fig. 3, *A* and *D*). In some units OFF responses are equal to or greater than ON responses, producing an apparently longer latency response when only the histogram peak is measured. Lines denoted by filled circles in *C* and *E* are data for the neuron whose PSTH is shown in *A*.

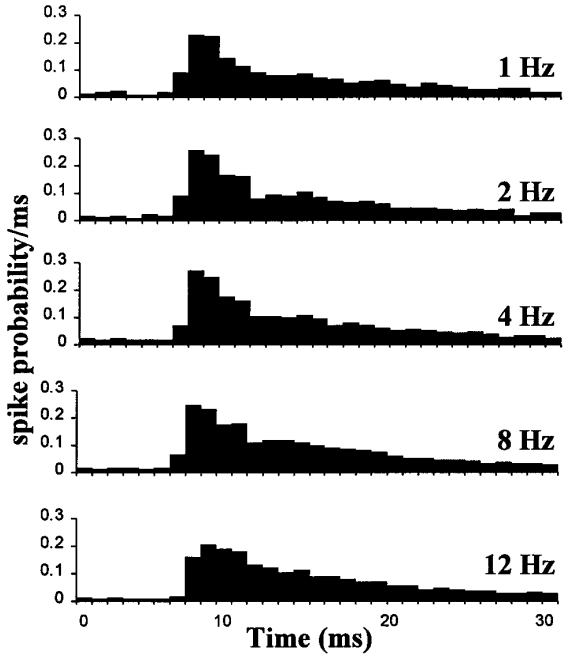

FIG. 2. VPM population cycle time histograms. For each frequency of stimulation, cycle time histograms of all VPM neurons were averaged.

For analyzing neuronal response characteristics per stimulus cycle, cycle time histograms were accumulated for each stimulus frequency by binning spikes over each cycle of all 10 trials into a histogram spanning the period of one cycle. For purposes of comparison, we assessed the latency of the response simply as the time of the peak in the cycle time histogram, as done by Ahissar et al. (1997) for cortical neurons. Response magnitude was determined as the average number of spikes occurring in the time window 5–20 ms after the onset of a cycle. As shown in Figs. 2 and 3, this time window captures the majority of the evoked response.

RESULTS

We recorded from 14 primary afferent neurons in the trigeminal ganglion (NV) and 16 neurons in the thalamic VPM nucleus. In most recorded neurons, spike discharge was well entrained by stimulation at each frequency tested in the 1- to 12-Hz range. Entrainment was obvious by inspection of raster plots (not shown) and peristimulus time histograms (PSTHs; Fig. 1*A*). Autocorrelograms revealed no indication of oscillations in the spontaneous activity of these thalamic neurons.

Figure 1, *B* and *C*, shows that response magnitudes per cycle of both NV and VPM neurons are invariant over the range of stimulus frequencies tested. For both populations, regression lines fit to the mean responses have slopes that do not differ significantly from zero (NV slope = −0.002, $P = 0.80$; VPM slope = −0.012, $P = 0.46$). The mean decrease in thalamic response magnitude over the 1- to 12-Hz range is 0.114 spikes/stimulus (~5%). This is approximately an order of magnitude less than what would be

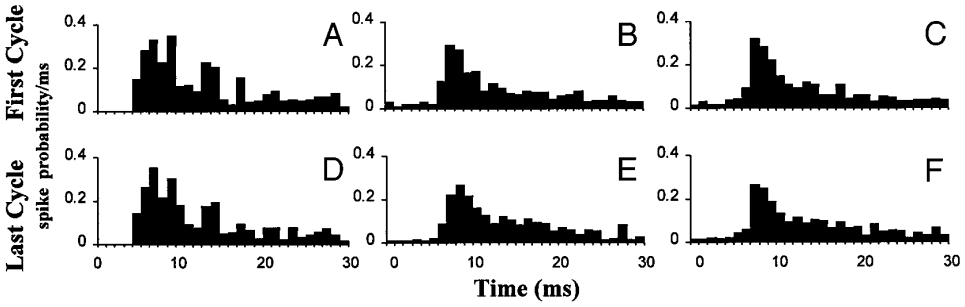

FIG. 3. Invariance of response magnitude and latency within trials. *A* and *B*: PSTHs for the 1st cycle of the 8-Hz stimulus of all NV and VPM neurons. *D* and *E*: same for the last cycles. *C* and *F*: thalamic responses to the 1st and last cycles of the 4-Hz stimulus.

reasonably expected based on the report of Ahissar et al. (1997). We also found that response latencies were constant over all frequencies (Fig. 1, *D* and *E*). The slopes of the regression lines fit to the mean latencies for the NV and VPM populations are 0.002 and 0.113 and do not significantly differ from zero ($P = 0.96$ and $P = 0.12$, respectively). Furthermore, none of the individual neurons had significantly nonzero slopes for either magnitude or latency. The only notable effect of frequency was an increase in the mean thalamic response latency from 9.6 ms at 8 Hz to 10.6 ms at 12 Hz ($P = 0.046$, 1-tail *t*-test). The overall stability of thalamic response timing and magnitude can be appreciated by inspection of the population cycle time histograms in Fig. 2.

Figure 3 shows populations PSTHs constructed from the responses evoked by the first and last cycles of the 4 and 8 Hz stimuli. In VPM, as in NV, response profiles are indistinguishable for the first and last cycles. This is also true for other cycles of the stimuli and for the other frequencies tested (data not shown).

DISCUSSION

We have shown that the response magnitudes and latencies of both primary afferent and thalamic relay neurons to periodic pulsatile whisker movements are constant over the 1- to 12-Hz range. In addition, VPM responses, like those of primary afferent neurons, are similar for the first and last cycles of these stimuli. These results would not be obtained in a circuit governed by feedback occurring on the time scale of the cycle period. These results strongly suggest that under the present experimental conditions VPM responses are determined mainly by their peripheral inputs, not by frequency-dependent corticothalamic feedback. It is thus improbable that VPM neurons act as phase comparators in a feedback-driven PLL as described by Ahissar et al. (1997). Rather, thalamic neurons relay afferent signals and entrain cortical populations to the frequency of peripheral stimulation by direct feed-forward input. Our results are consistent, however, with a facilitatory role for corticothalamic feedback as suggested by Yuan et al. (1986). By suppressing somatosensory cortical activity, these authors demonstrated a decrease in the responses of thalamic neurons to repetitive electrical stimulation of afferent fiber systems in awake rats.

The prediction of Ahissar et al. (1997) that thalamic response latencies and magnitudes would depend on frequency was based on their recordings of layer IV neurons in anesthetized rats, where response latencies increased by 20 ms and magnitudes decreased with increasing stimulus frequency, in the 1- to 12-Hz range. It was argued that the responses of neurons in the thalamocortical recipient zone directly reflect those of their thalamic input neurons, such that the frequency dependence of cortical responses originates in the thalamus. However, thalamocortical response transformations by local cortical circuitry have been demonstrated in a number of systems, including the whisker/barrel pathway (Kyriazi et al. 1994; Simons and Carvell 1989). Thus layer IV circuits are likely to produce the frequency-dependent response properties of their constituent neurons.

The corticothalamic oscillations reported by Nicolelis et al. (1995) immediately preceded and then phase locked to whisker twitches that occurred in rats during voluntary immobility and attentiveness. These whisker twitches are distinct from the high-amplitude whisker movements known as ''whisking,'' which occur during exploratory and discriminative whisker behaviors (Carvell and Simons 1990; Welker 1964); twitching-associated oscillations cease at the onset of whisking. During whisking, neurons in NV, VPM, and barrel cortex do fire rhythmically when the whiskers contact objects (Carvell et al. 1997). Even without object contact, whisking can produce rhythmic cortical firing; such activity depends on actual whisker movement, however, because it is eliminated by anesthetic block of the facial nerve (Fee et al. 1997). Together, these findings suggest that periodic firing in the whisker/barrel pathway during active touch reflects peripheral rather than central influences.

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