Response Properties of Whisker-Associated Trigeminothalamic Neurons in Rat Nucleus Principalis

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Minnery, Brandon S. and Daniel J. Simons. Response properties of whisker-associated trigeminothalamic neurons in rat nucleus principalis. J Neurophysiol 89: 40–56, 2003; 10.1152/jn.00272.2002. Nucleus principalis (PrV) of the brain stem trigeminal complex mediates the processing and transfer of low-threshold mechanoreceptor input en route to the ventroposterior medial nucleus of the thalamus (VPM). In rats, this includes tactile information relayed from the large facial whiskers via primary afferent fibers originating in the trigeminal ganglion (NV). Here we describe the responses of antidromically identified VPM-projecting PrV neurons (n = 72) to controlled ramp-and-hold deflections of whiskers. For comparison, we also recorded the responses of 64 NV neurons under identical experimental and stimulus conditions. Both PrV and NV neurons responded transiently to stimulus onset (ON) and offset (OFF), and the majority of both populations also displayed sustained, or tonic, responses throughout the plateau phase of the stimulus (75% of NV cells and 93% of PrV cells). Average ON and OFF response magnitudes were similar between the two populations. In both NV and PrV, cells were highly sensitive to stimulus onset (ON) and offset (OFF), and the majority of both populations also displayed sustained, or tonic, responses throughout the plateau phase of the stimulus (75% of NV cells and 93% of PrV cells). Average ON and OFF response magnitudes were similar between the two populations. In both NV and PrV, cells were highly sensitive to the direction of whisker deflection. Directional tuning was slightly but significantly greater in NV, suggesting that PrV neurons integrate inputs from NV cells differing in their preferred directions. Receptive fields of PrV neurons were typically dominated by a “principal” whisker (PW), whose evoked responses were on average threefold larger than those elicited by any given adjacent whisker (AW; n = 197). However, of the 65 PrV cells for which data from at least two AWs were obtained, most (89%) displayed statistically significant ON responses to deflections of one or more AWs. AW response latencies were 2.7 ± 3.8 (SD) ms longer than those of their corresponding PWs, with an inner quartile latency difference of 1–4 ms (±25% of median). The range in latency differences suggests that some adjacent whisker responses arise within PrV itself, whereas others have a longer, multi-synaptic origin, possibly via the spinal trigeminal nucleus. Overall, our findings reveal that the stimulus features encoded by primary afferent neurons are reflected in the responses of VPM-projecting PrV neurons, and that significant convergence of information from multiple whiskers occurs at the first synaptic station in the whisker-to-barrel pathway.

INTRODUCTION

The whisker-barrel pathway of the rodent trigeminal system is characterized at each level of the neuraxis by the presence of distinct cellular aggregates that correspond in a one-to-one fashion to the large vibrissae of the mystacial pad. Over the past several decades, anatomical and physiological studies of cortical barrels have yielded abundant insights into how the CNS processes sensory information (reviewed in Simons 1995). This scrutiny has also extended to subcortical stages of the whisker afferent pathway, where investigators have studied the responses of third-order neurons within thalamic “barreloids” (Armstrong-James and Callahan 1991; Simons and Carvell 1989), as well as the responses of primary afferent (first-order) neurons of the trigeminal ganglion (NV) (Gibson and Welker 1983a,b; Lichtenstein et al. 1990; Shoykhet et al. 2000; Zucker and Welker 1969). Far less is known, however, regarding the physiology of second-order neurons within the brain stem trigeminal complex.

In the rodent brain stem, whiskers are represented in cytochrome oxidase-rich patches termed “barrelettes,” which are present in both nucleus principalis (PrV) and in two of the three subdivisions of the spinal trigeminal nucleus (SpV) (Belford and Killackey 1979; Durham and Woolsey 1984; Ma 1991; Ma and Woolsey 1984; Sikich et al. 1986). The barrelettes of PrV receive a dense innervation of low-threshold mechanoreceptor input from NV (Clarke and Bowsher 1962; Hayashi 1980; Jacquin et al. 1993), and in turn, provide the bulk of ascending afferents to the barreloids of the ventroposterior medial (VPM) thalamic nucleus (Bruce and McHaffie 1987; Chiaia et al. 1991; Erzurumlu et al. 1980; Peschanski 1984; Smith 1973; Veinante and Deschénes 1999; Williams et al. 1994). Previous studies have demonstrated a critical role for PrV in the relay of afferent information (Rhoades et al. 1987) and in the establishment of whisker-patterned somatotopy within VPM (Killackey and Fleming 1985). However, despite the importance of PrV inlemniscal transmission, few studies have examined in quantitative detail the response properties of trigeminothalamic PrV neurons.

Until relatively recently, a major handicap in the investigation of second-order trigeminal circuitry has been a poor understanding of the anatomical structure of PrV and of the synaptic wiring of its component cells. Nevertheless, a consensus has emerged that PrV mediates the processing of the fine spatial details of tactile stimuli within the ascending lemniscal pathway. In accordance with this notion, the majority of PrV cells have been shown to project to contralateral VPM, where their axon terminals ramify within dimensions consistent with the size of a single barreloid (Chiaia et al. 1991; Peschanski 1984; Veinante and Deschénes 1999; Williams et al. 1994). Recent studies have also shown that PrV neurons
respond with high temporal fidelity to periodic whisker deflections (Ahissar et al. 2000; Sosnik et al. 2001). Understanding the sensory signal transmitted by PrV to VPM is a critical first step toward developing accurate models of thalamic function and will also be key to determining how second-order circuits themselves transform afferent information.

Of the few studies that have examined response properties of PrV neurons, most report that the majority of PrV cells respond phasically to deflections of only one whisker (Doherty et al. 1993; Jacquin et al. 1988; Shipley 1974; Veinante and Deschenes 1999; but see also Chiaia et al. 2000). The use of different types and levels of anesthesia, as well as differences in the nature of whisker stimuli, make it difficult to compare these reports with one another and with neuronal responses recorded in NV and VPM. The availability of directly comparable data sets for pre- and postsynaptic populations will greatly enhance our understanding of the role of PrV in processing afferent information and should enable the implementation of physiologically realistic models of thalamic circuitry (see for example Kyriazi and Simons 1993). Recently, Ahissar and colleagues have adopted such an approach for defining functional differences between whisker-associated neurons in the lemniscal and paralemniscal pathways (Ahissar et al. 2000; Sosnik et al. 2001).

The present study was motivated specifically by our ongoing interest in thalamic processing and more generally by our longer-term goal of developing realistic circuit models to account for response transformations at different levels of the whisker-barrel pathway. Here we describe quantitative receptive field properties of PrV and NV cells recorded under conditions identical to those of our previous investigations of thalamic and cortical neurons. We provide evidence that significant processing of afferent information occurs at the first synaptic station of the whisker-barrel pathway, and that the nature of the signal output from PrV to VPM is consistent with the known response properties of VPM neurons.

METH ODS

Preparation for recording

PrV data were collected from adult female Sprague-Dawley rats weighing approximately 300 g. Animals were prepared for electrophysiological recordings using methods described previously in detail (Simons and Carvell 1989). In brief, a surgery was conducted under halothane anesthesia during which a tracheotomy was performed, the right jugular vein was catheterized, and a cannula was inserted into the left femoral artery. A steel post was then affixed to the skull for the purpose of stabilizing the head. This method was preferred to the use of a stereotaxic device in that it allowed unimpeded access to the whiskers and did not require the application of acute pressure points. Two small craniotomies were made in regions overlying the right ventropascal thalamus and the anterior portion of the left brain stem trigeminal complex. Following surgery, animals were transferred to a vibration isolation table and were placed on a servo-controlled heating blanket. Halothane was discontinued and rats were maintained in a lightly narcotized state via a steady infusion of fentanyl delivered through the jugular catheter (Sublimaze, Jansen Pharmaceuticals; approximately 10 μg · kg⁻¹ · h⁻¹). The responses of cortical barrel neurons (Simons and Land 1985; Simons et al. 1992) and thalamic barreloid neurons (Simons and Carvell 1989) recorded under light narcosis have been shown to be similar to those of awake or lightly anesthetized animals (Armstrong-James and Callahan 1991; Friedberg et al. 1999). To prevent spontaneous movements that occur with light narcosis, animals were immobilized by intravenous infusion of pancuronium bromide and were artificially respired for the remainder of the experiment. A computer continuously monitored femoral arterial blood pressure, heart rate, and tracheal airway pressure waveform. Core body temperature was maintained at 37°C. NV data were collected from a separate group of rats (approximately 300 g). Surgical preparation and experimental procedures were the same as described above, with the exception that a craniotomy was made over the left NV. Previous studies of NV neurons in our laboratory have been carried out using sodium pentobarbital anesthesia and without neuromuscular blockade (Kyriazi et al. 1994; Lichtenstein et al. 1990; Shoykhet et al. 2000). However, to facilitate the explicit comparison of response parameters of populations of neurons separated by a single synapse (NV→PrV), we decided to acquire a new body of NV data under conditions identical to those from which PrV data were obtained.

Electrophysiological recordings

PrV/VPM. Coordinates for PrV and VPM were derived from the atlas of Pellegrino et al. (1979). Positioning the animal’s head in the approximate plane of this atlas allowed us to advance the PrV recording electrode at an oblique angle through occipital cortex and thus enabled us to avoid damaging the transverse sinus located directly dorsal to PrV. Recording of single PrV neurons was accomplished using either stainless steel microelectrodes (500 kΩ–3 MΩ; Frederick Haer, Brunswick, ME) or low-impedance (approximately 500 kΩ) platinum-tungsten wire electrodes encased in quartz glass (Thomas Recording, Giessen, Germany). In VPM, receptive field (RF) mapping and antidromic stimulation were conducted through the same electrode, which in most experiments was a single, low-impedance stainless steel microelectrode (100–400 kΩ). In several earlier experiments, we employed a bipolar electrode comprised of two stainless steel microelectrodes cemented together with dental acrylic (tip separation approximately 300 μm).

During an experiment, the location of the recording electrode was inferred by the stereotyped somatotopy of the brain stem trigeminal nuclei (Jacquin et al. 1993). However, since the somatotopic map within PrV is continuous with that of the caudally adjacent subnucleus oralis (SpVo), it was often difficult to determine recording location based on this criterion alone. To address this problem we developed the following strategy. The first mapping penetration was made deliberately caudal to the suspected location of PrV so as to increase the likelihood that some portion of the trigeminal complex would be encountered. In subsequent penetrations, the electrode was advanced rostrally in approximately 300-μm increments until the anterior extent of the complex was surpassed, as was evidenced by a lack of responses to orofacial stimuli. At this point the electrode was repositioned several hundred micrometers caudal to the previous penetration. This last step usually ensured that we were recording in PrV. The successful evocation of antidromic spikes through electrical stimulation of contralateral VPM provided additional confirmation that the electrode was positioned within PrV, since SpVo projects only sparsely to thalamus and innervates primarily the posterior group (PO) (Jacquin and Rhoades 1990; Veinante et al. 2000). As was the case for the recording electrode, the location of the thalamic stimulating electrode was inferred through somatotopy (Land et al. 1995).

NV. The procedure for recording from NV cells has been described previously (Lichtenstein et al. 1990; Shoykhet et al. 2000). Although the details of our experimental preparation differ from those of earlier studies, the recording methods were the same and will not be fully recounted here. To summarize, the animal’s head was positioned in the plane of the atlas of Paxinos and Watson (1998). High-impedance stainless steel microelectrodes (1–10 MΩ; Frederick Haer) were then used to isolate and record the responses of whisker-associated primary afferent neurons.

J Neurophysiol • VOL 89 • JANUARY 2003 • WWW.JN.ORG
Antidromic identification of VPM-projecting neurons

We used antidromic stimulation of VPM in conjunction with a collision test protocol to identify VPM-projecting PrV neurons. Once the whisker representation in contralateral VPM was located (preferably a C, D, or E row barreloid), a search stimulus consisting of brief current pulses (0.05-ms duration, approximately 50 μA) was applied through the thalamic electrode while the recording electrode was simultaneously advanced through PrV. A spike occurring in response to thalamic stimulation was identified as potentially antidromic if it met the following criteria: 1) constant latency response (<0.1 ms jitter; see Fig. 2B) at suprathreshold stimulation and 2) the ability to follow a high-frequency stimulus. For the first criterion, latency was measured as the time between onset of the stimulus artifact and onset of the antidromic spike (Fig. 2A, inset). The second criterion was deemed satisfied if the recorded spike was able to follow twin stimulus pulses administered in brief (approximately 1 ms) succession. Putative antidromic spikes were then further isolated using a time/amplitude dual-window discriminator (BAK Electronics). After a spike was isolated, a collision test was performed in which the antidromic stimulus was triggered by the isolated PrV spike (Fig. 2C). A consistently successful collision between the spontaneously occurring PrV spike and the antidromic spike was taken as evidence that the isolated PrV cell was indeed a VPM-projecting neuron. All PrV cells described here met the criteria for a successful collision test. Although we often encountered cells that we were unable to activate antidromically, we did not conclude that these were non–VPM-projecting neurons, since the antidromic stimulus might simply have failed to depolarize all PrV axons terminating within the stimulated barrel. For this reason we did not attempt to infer from negative findings the proportion of PrV cells that projected to VPM.

In one recent study of PrV projection neurons (Veinante and Deschênes 1999), it was suggested that antidromic stimulation of VPM may not be sufficient to distinguish VPM-projecting PrV cells from the minority of PrV projection neurons whose axons innervate the adjacent posterior group (PO) of the thalamus. Indeed, the broad-tipped bipolar stimulating electrodes (tip diameter approximately 50 μm; tip separation approximately 1 mm) employed in the aforementioned study may have been too large to ensure selective stimulation of VPM. We therefore chose to use single microelectrodes (tip diameter <5 μm) so that areas of high current density would be localized near the electrode tip during stimulation. In the several experiments in which we used a bipolar stimulating electrode, tip diameters were also small (<5 μm), and the tip separation (approximately 300 μm) was well within the dimensions of VPM. Although we did not systematically attempt to measure the spread of our antidromic stimulus currents, the small radius of the stimulus was evidenced by the fact that successful antidromic activation of PrV cells required close somatotopic alignment between stimulating and recording electrodes (i.e., no more than a 2-whisker disparity in alignment was usually allowable). Moreover, once an antidromic spike was isolated, subsequent movements of the stimulating electrode more than 200 μm in either direction along the dorsal-ventral axis typically resulted in the loss of the antidromic response even in cases where stimulating currents were set to well above threshold.

Histology

In the case of NV recordings, rats were administered a lethal dose of sodium pentobarbital at the conclusion of an experiment. Histological analyses were not conducted since the relative anatomical isolation of the ganglion precluded accidental recording of whisker-associated responses from nonprimary sources. Following PrV recording sessions, electrolytic marker lesions were made through both the PrV and VPM electrodes (7 μA for 7 s, anodal current). Animals were then deeply anesthetized with sodium pentobarbital and perfused transcardially. Brains were frozen, serially sectioned (60 μm) in an oblique coronal plane, reacted for cytochrome oxidase (CO), and counter-stained with thionin. Standard anatomical landmarks were used to determine the borders of VPM and PrV (Paxinos and Watson 1998), and tissue was visually inspected to confirm the presence of electrode tracks within the two nuclei (Fig. 1). Histological confirmation of PrV recording sites was successfully completed in all but 1 of 16 experimental animals (n = 3 cells). In a separate animal, procedural complications prevented reconstruction of electrode tracks in VPM (n = 3 cells). For these data, physiological criteria alone were used to determine recording/stimulation sites.

No attempt was made to correlate a PrV cell’s response properties with its rostrocaudal location within the nucleus. Several cells located within the PrV/SpVo transition zone in caudal PrV were included in the present analyses and were presumed to be PrV neurons based on their projection target (VPM) and their physiological similarity to confirmed PrV cells. Occasionally electrode tracks were observed in rostral SpVo, but these typically corresponded to initial mapping penetrations which did not yield any data. Electrode tracks were never observed in the more caudal SpV subnuclei.
Whisker stimulation

A hand-held probe was used to identify the whisker most effective at evoking activity in an isolated cell, i.e., the principal whisker (PW). A piezoelectric mechanical stimulator (Simons 1983) was then attached to the whisker approximately 10 mm from the skin surface. Ramp-and-hold stimulus waveforms were similar to those employed in previous studies in our laboratory and were generated via D/A converters using custom-written software in LabView (National Instruments, Austin, TX). Whisker deflections were applied 10 times in each of eight directions differing in 45° increments, for a total of 80 stimuli. Deflections were 1 mm in amplitude, with onset/offset velocities of 125 mm/s and a plateau duration of 200 ms. The angle of deflection was varied in a pseudorandom order throughout the stimulus presentation. This procedure was then repeated for several whiskers adjacent to the PW.

When possible, we recorded the responses of the rostral, caudal, ventral, and dorsal adjacent whiskers (AWs). Although in a few instances the surround component of a neuron’s receptive field (RF) clearly extended beyond the four immediate AWs, we did not systematically investigate the responses of nonadjacent whiskers. For two PrV cells whose PW consisted of the Δ “straddler” whisker situated between rows D and E, only the dorsal AW (γ) response is described here. No straddler whiskers were included as AWs for any cell whose PW was not itself a straddler whisker. In the case where a cell responded robustly to multiple whiskers, the PW was taken to be the whisker that evoked the strongest response to stimulus onset averaged over all deflection angles.

The data collection protocol for a given cell typically lasted 25–40 min for PrV neurons and <10 min for NV neurons. Total recording sessions lasted 5–9 h for both NV and PrV recordings. This range does not include surgical preparation of the animal or time spent mapping the somatotopy of the respective nuclei, procedures that together required 5–7 h for PrV experiments, but no more than 5 h for NV experiments.

Data collection and analysis

All data in this study were derived from single-unit recordings. Spikes were isolated on-line by means of a time/amplitude dual-window discriminator. Many of the data analysis procedures used in this study have been described previously (Simons and Carvell 1989; Lichtenstein et al. 1990). Spike events were captured with 100-μs resolution and were compiled on-line into peristimulus time histograms (PSTHs) with 1-ms bin width. For each deflection angle, ON and OFF responses were calculated over a 20-ms window and were defined as the average number of spikes occurring in response to, respectively, stimulus onset and offset. Plateau responses were defined as the average number of spikes occurring within the middle 100 ms of the plateau epoch. Latencies to response onset were calculated from the total PSTH (all angles) and were defined as the earlier of two consecutive 1-ms bins in which the number of spikes significantly exceeded prestimulus firing rates (P < 0.02, assuming a Poisson distribution for prestimulus activity). All deflection angles were included in the PSTH since most neurons exhibited significant responses at multiple angles. In the case of many AW data files, no two consecutive bins met the criterion for significance for the ON and/or OFF window. These AWs were deemed unresponsive, and the corresponding files were excluded from subsequent analyses involving response latencies. All other AWs were classified as responsive. To test whether the criterion for two consecutive bins might have failed to detect a number of substantial yet very transient (i.e., <2-ms duration) responses, we examined the population PSTH for those AWs classified as unresponsive (ON and OFF PSTHs computed separately). Inspection of the PSTHs revealed that, as expected, responses were virtually absent, indicating that relatively few if any cells were misclassified as unresponsive.

For comparison with previous studies (Simons and Carvell 1989), we also tested for the presence of responses by comparing firing rates within the entire 20-ms response window to prestimulus firing rates estimated over a 100-ms period (α = 0.05; 1-tailed t-test). This criterion proved slightly more liberal as it enabled the detection of broader, more temporally dispersed ON responses. The t-test method for detecting responses was used in this study to determine the number of “null angles” for a given cell, i.e., the number of deflection angles for which no responses were elicited.

Spontaneous activity levels for a given PrV cell were estimated using a 100-ms window of prestimulus activity collected from one of several AW data files for that cell. Approximately 50 ms separated the end of the prestimulus window from the beginning of stimulus onset. For reasons of consistency, the caudal AW was selected; but in cases where caudal AW data were absent, one of the other three AWs was substituted instead. We chose to use AW rather than PW data to calculate spontaneous activity since, due to the exquisite sensitivity of PrV cells, mere attachment of the stimulator to the PW often resulted in an elevation in spike discharges during the prestimulus period. For a number of PrV cells (38 of 72), we also recorded spontaneous firing rates by running the data collection protocol with the stimulator unattached to any whisker. Average spontaneous activity levels calculated using the latter method were nearly identical to those derived from AW prestimulus data. For NV, average spontaneous activity of the population was computed using data collected (stimulator unattached) from 50 of the 64 recorded cells.

A cell’s adaptive properties were assessed through comparison of the plateau response with prestimulus activity measured over an equivalent period (Lichtenstein et al. 1990). If the plateau response at the maximally responsive angle exceeded prestimulus activity, the cell was classified as tonic (1-tailed t-test; P < 0.025). This criterion tended to be conservative because, as stated above, prestimulus activity occasionally yielded an inflated estimation of actual spontaneous firing rates. Those cells not classified as tonic were termed phasic by default.

Several analyses were used to characterize the degree to which PrV cells responded selectively to different deflection angles (i.e., directional tuning). The simplest of these, the tuning ratio, was calculated for each cell by dividing the mean ON response (averaged over all angles) by the ON response at the maximally responsive angle. In addition, we computed a tuning index for each neuron. Cells were categorized according to the number of deflection angles (0–7) that elicited a response that was statistically smaller than the maximum angle response (P < 0.05; 1-tailed t-test). To facilitate graphical comparison of directional tuning properties between populations, we also constructed normalized, rotated population polar plots. For a given population, each neuron’s polar plot was first rotated so that maximally responsive angles were aligned for all cells. For a given cell, the response at each angle was normalized with respect to the maximum angle response for that cell, and average responses were then computed for each angle based on the whole population. All data analyses described above were implemented on an IBM PC using Microsoft Excel/Visual Basic software.

Results

We recorded and analyzed the response properties of 72 whisker-associated, VPM-projecting PrV neurons in 16 fentanyl-sedated rats. Of our sample, the majority responded most robustly to stimulation of whiskers located within the ventral-most rows (rows D and E: 64 cells) and caudal-most arcs (arcs 1–3; 48 cells). Two cells had receptive field (RF) centers consisting of the Δ “straddler” whisker situated between rows D and E, caudal to arc 1. For the 72 PrV neurons described here, a total of 197 adjacent whisker (AW) responses were also recorded. In addition, we recorded the responses of 64 whisker-
associated trigeminal ganglion (NV) neurons in a separate
group of four fentanyl-sedated rats. As was the case for the PrV
recordings, the majority of NV data were collected from cells
with RFs consisting of the large, caudal whiskers.
VPM-projecting PrV neurons were identified through anti-
dromic stimulation of contralateral VPM (Fig. 1) and projec-
tion status was verified by means of a collision test (Fig. 2C;
see also METHODS). All PrV data discussed hereafter were
derived from confirmed VPM-projecting cells. Consistent with
previous reports (Chiaia et al. 2000; Jacquin et al. 1988), the
axons of PrV cells in our sample conducted rapidly, with an
average antidromic latency of 0.89 ± 0.29 (SD) ms (see Fig.
2A). This value may in fact represent a slight underestimate
given that our measurements did not take into account the
delay between application of the antidromic stimulus and the
evocation of an action potential (Takahashi 1965). Assuming
an approximate point-to-point distance of 9 mm between ven-
tral PrV and contralateral VPM (Paxinos and Watson 1998),
average axonal conduction velocity for VPM-projecting PrV
cells was approximately 10 m/s. No significant correlations
were observed between conduction velocity and several of the
standard response parameters we examined, including response
magnitude and RF size.

Stimulus-evoked activity of PrV neurons

For each PrV cell, we identified the whisker that evoked the
maximum excitatory response, termed that cell’s “principal
whisker” (PW). Figure 3A shows the response of a representa-
tive PrV neuron to ramp-and-hold deflections of its PW in
eight directions differing in 45° increments. The firing rate for
each deflection angle is represented in peristimulus time his-
tograms (PSTHs). As was the case for the majority of our
sample, the cell shown here responded transiently to both
stimulus onset and offset (on and off responses, respectively)
and exhibited a sustained response during the plateau phase of
the stimulus for deflections in at least one direction (compare
Fig. 3A with the population response profile in Fig. 3C). On and
off responses were each determined using a 20-ms window,
while plateau responses were calculated over the middle
100-ms period of the stimulus plateau (Fig. 3C). Note that, for
the cell in Fig. 3A, on response magnitudes on average ex-
ceeded those of off responses. This disparity between on and
off responses was observed often both in PrV and NV and is
clearly seen in the PrV population PSTH (Fig. 3C). Response
magnitudes for the cell in Fig. 3A also varied with deflection
angle (Fig. 3B), a characteristic that is discussed in the follow-
ing section. Distributions of response amplitudes at the maxi-
mally responsive angle for PW deflections (n = 72) are pre-
sented in Fig. 4 (A–C, dark bars), with on, off, and plateau
response epochs plotted separately. All recorded responses
were included in the histograms irrespective of whether or not
the criteria for statistical significance were met. Table 1 lists
separately the means and SDs of statistically significant re-
ponses.

Comparison of activity: PrV versus NV

Spontaneous firing rates of PrV cells varied widely, but in
most instances were great enough that the antidromic stimulus
was not necessary to disclose the presence of a neuron. In

\[ \text{stimulus-evoked activity of PrV neurons} \]

\[ \text{Comparison of activity: PrV versus NV} \]

\[ \text{FIG. 2. Antidromic identification of VPM-projecting cells. A: distribution} \]

\[ \text{of antidromic latencies. Latencies were measured from the onset of the} \]

\[ \text{stimulus artifact to the onset of the antidromic spike (inset). Arrow above} \]

\[ \text{histogram indicates bin containing the mean antidromic latency for all cells} \]

\[ \text{(n = 72). B: 4 superimposed traces of an antidromic spike demonstrating small} \]

\[ \text{variability in latency. *Stimulus artifact. C: example of a collision test for the} \]

\[ \text{cell shown in B. Top: when the delay (Δt) between the spontaneously occurring} \]

\[ \text{spike and the antidromic stimulus was greater than the critical "collision} \]

\[ \text{interval" (CI), the spontaneous and antidromic spikes failed to collide. Bottom:} \]

\[ \text{when Δt was less than CI, the antidromic spike collided with the spontaneous} \]

\[ \text{spike and therefore did not appear following the stimulus artifact. CIs mea-} \]

\[ \text{sured in this study were typically close to theoretical values as given by the} \]

\[ \text{formula: CI = antidromic latency + axonal refractory period. Axonal refrac-} \]

\[ \text{tory periods were assessed using the twin pulse paradigm described in metho-} \]

\[ \text{ds. (Note: 3 superimposed traces are shown for each scenario in C. Raw} \]

\[ \text{signals were high-pass filtered at 10 kHz to minimize the stimulus artifact.)} \]
contrast with earlier studies in deeply anesthetized rats, where PrV cells were described as relatively silent in the absence of peripheral stimuli (Jacquin et al. 1988; Kwan et al. 1999; Veinante and Deschénes 1999), mean spontaneous activity for our sample was $11.9 \pm 12.6$ Hz (Fig. 4D). To determine whether the high spontaneous firing rates of PrV cells might in part reflect input from NV, we recorded the spontaneous activity of 50 NV neurons (Fig. 4D). The mean spontaneous firing rate of the NV population, $6.9 \pm 13.7$ Hz, was less than that of PrV ($P < 0.001$; Mann-Whitney test), but greater than has been observed in previous NV studies conducted using sodium pentobarbital anesthesia without neuromuscular blockade (see Discussion). It is therefore likely that afferent input from NV contributes to the high spontaneous firing rates of PrV cells observed under our experimental conditions.

Mean on and off responses averaged over all deflection angles, although slightly greater in NV than in PrV, did not differ significantly ($\alpha = 0.05$) between the two populations (on: $P = 0.15$; off: $P = 0.07$; Mann-Whitney test). However, maximum angle on and off responses were on average larger in NV, whereas minimum angle on and off responses were greater in PrV (see Table 1 for summary; all $P < 0.05$; Mann-Whitney test). In other words, the range of response magnitudes over all eight deflection angles was larger for NV neurons than for PrV neurons. To test the extent to which this finding was also reflected at the single-cell level, we computed for each neuron and each response window a coefficient of variation (CV) for responses over all eight angles. For both on and off windows, CVs were greater for NV neurons ($P < 0.0001$ for both on and off windows; Mann-Whitney test). As discussed in the following section, differences in response ranges between PrV and NV are reflected as differences in directional tuning between the two populations.

Inspection of PrV and NV population responses yielded readily observable differences in the shape of the PSTH for the on and off responses. Figure 5 shows magnified population PSTHs for the maximally responsive on (left) and off (right) angles. Also depicted is the temporal distribution of the NV-PrV response differential within the on and off windows. The difference in maximum angle on responses is notable in that, while the rise time and peak amplitudes were nearly identical for both populations, the NV on response decayed gradually into the plateau phase of the response, whereas the PrV on response declined rapidly following the initial several milliseconds. The source of the precipitous drop off in the PrV on response is unclear, though it may involve rapid pre- and/or postsynaptic inhibition mediated by local GABAergic neurons within PrV (see Discussion).

**Directional tuning**

In previous studies, whisker-associated primary afferent neurons in the rat were shown to possess a high degree of sensitivity to the direction of whisker deflection (Gibson and Welker 1983a; Lichtenstein et al. 1990; Zucker and Welker 1969). We explored the extent to which PrV responses reflect the directional selectivity of primary afferent inputs. Figure 6 shows that, like NV neurons, most PrV cells responded differentially based on deflection angle. To characterize directional tuning within the PrV population, we calculated a tuning ratio for each cell, defined as the ratio of the mean (all angles) on response to the maximum angle on response. Lower tuning ratios correspond to greater directional tuning. The distribution of tuning ratios for PrV is illustrated in Fig. 6A ($0.61 \pm 0.15$). Of 72 PrV neurons, 20 (28%) had tuning ratios of 0.5 or less, indicating a maximum angle on response that was at least double that of the average on response.
We also computed a directional tuning index for each neuron (Fig. 6B). Cells were placed into one of eight categories (0–7) based on the number of angles having ON responses that were significantly less than the ON response at the maximally responsive angle \( P < 0.05 \); 1-tailed t-test). An index of 0 therefore represents a poorly tuned cell, whereas 7 indicates a highly tuned cell. The cell shown in Fig. 3A, for example, had a tuning index of 6. Nearly one-half of our sample (46%; 33 of 72 cells) was assigned an index of 6 or 7. Conversely, only 9 of 72 neurons (13%) were placed in the two lowest categories, 0 and 1. Despite the high variation in ON response magnitude across deflection angles for a given PrV cell, most angles typically exhibited statistically significant responses \( (P < 0.05; \text{1-tailed } t\text{-test}) \), and the mean number of angles that exhibited no response, termed “null angles,” was small \((1.33 \pm 1.72)\). Also, although most PrV cells were directionally selective, mean responses at each angle averaged across all cells revealed no preference for a particular angle among the PrV population \((P > 0.7; \text{ANOVA})\).
TABLE 1. Stimulus-evoked and spontaneous activities of PrV and NV neurons

<table>
<thead>
<tr>
<th></th>
<th>PrV (n = 72)</th>
<th>NV (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PW (n = 72)</td>
<td>AW (n = 197)</td>
</tr>
<tr>
<td>on†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max</td>
<td>4.33 ± 2.21</td>
<td>1.30 ± 1.14</td>
</tr>
<tr>
<td>Min</td>
<td>1.31 ± 1.35</td>
<td>0.33 ± 0.59</td>
</tr>
<tr>
<td>All</td>
<td>2.65 ± 1.57</td>
<td>0.75 ± 0.83</td>
</tr>
<tr>
<td>OFF‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max</td>
<td>2.68 ± 1.32</td>
<td>1.13 ± 0.85</td>
</tr>
<tr>
<td>Min</td>
<td>0.79 ± 0.83</td>
<td>0.21 ± 0.37</td>
</tr>
<tr>
<td>All</td>
<td>1.70 ± 1.00</td>
<td>0.59 ± 0.55</td>
</tr>
<tr>
<td>Plateau†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max</td>
<td>8.92 ± 4.42</td>
<td>3.70 ± 2.39</td>
</tr>
<tr>
<td>Min</td>
<td>1.99 ± 2.43</td>
<td>1.42 ± 1.34</td>
</tr>
<tr>
<td>All</td>
<td>5.00 ± 2.68</td>
<td>2.51 ± 1.89</td>
</tr>
<tr>
<td>Spontaneous§</td>
<td>11.90 ± 12.70</td>
<td>6.90 ± 13.68</td>
</tr>
</tbody>
</table>

All values are means ± SD. PrV, nucleus principalis; NV, trigeminal ganglion; PW, principal whisker; AW, adjacent whisker; ON, onset; OFF, offset.

* Includes only AWs that elicited statistically significant responses within the given window (ON: n = 129; OFF: n = 122). † Responses are defined as the average number of spikes occurring within the 20-ms response window (n = 10 deflections). "Max" and "min" refer to, respectively, maximally and minimally responsive deflection angles. "All" is the average of the mean response magnitudes for all angles (n = 8). ‡ Responses are the number of spikes occurring within the middle 100-ms period of the stimulus plateau. Only statistically significant (i.e., "tonic") responses are included (PW: n = 67; AW: n = 71; NV: n = 48). Responses were not adjusted for background activity. § Units are spikes/s.

Directional tuning of primary afferent responses was even more pronounced than in PrV. A larger percentage of NV neurons (55%; 35 of 64 cells) had directional tuning indices of 6 or 7 (Fig. 6B). Likewise, the average tuning ratio in NV was 0.51 ± 0.14 (see Fig. 6A for distributions), a value that was slightly but significantly less than that of the PrV population (P < 0.001; Mann-Whitney test). Compared with PrV, NV neurons also exhibited significantly more null angles (2.07 ± 1.89; P < 0.01; Mann-Whitney test). Figure 6C shows normalized, rotated population polar plots for NV and PrV (see METHODS). The sharper tuning of the NV population with respect to PrV is evident from the superposition of the two plots on the same axes. This difference in ON response tuning between the two populations was expected and reflects our earlier finding that the difference between maximum and minimal angle ON responses was on average less for PrV than for NV. Although OFF responses in PrV and NV also displayed clear directional selectivity, we did not conduct quantitative analyses of OFF response tuning (however, see Lichtenstein et al. 1990 for a discussion of OFF response tuning in NV).

For many well-tuned units, the preferred angle of deflection was independent of the position of the whisker at the onset of deflection. For example, a neuron that discharged vigorously to the onset of deflections in the caudal direction (0°) might also respond robustly to deflection offsets in the same direction, even though in the latter instance the whisker begins the movement from a rostrally deflected (180°) position. Such neurons are termed directionally consistent (Lichtenstein et al. 1990). As a measure of directional consistency, we computed for each cell a correlation coefficient (r) between the ON and OFF responses for all eight angles (n = 8; Fig. 7A). For a directionally consistent cell, the ON and OFF responses are inversely related, and r is therefore negative. Figure 7B shows the distribution of correlation coefficients for the PrV and NV populations (PrV: −0.26 ± 0.45; NV: −0.39 ± 0.52). Of the 72 PrV cells, 53 (74%) had negative correlation coefficients. However, like directional tuning, directional consistency for the PrV population was less pronounced than in NV (P < 0.05; Mann-Whitney test).

Population PSTHs further illustrate differences in directional consistency between NV and PrV. Figure 7C shows PSTHs for the angle evoking the maximum OFF response and for the angle opposite to it. Note that selecting for the angle opposite to the maximum OFF angle yields a comparatively larger ON response. This is to be expected if responses overall are directionally consistent, since the deflection offset at the angle evoking the maximum OFF response occurs in the same direction as the deflection onset at the opposite angle. Within NV this relationship was even more prominent than in PrV (compare left vs. right panel). Although little is known about the convergence ratio of NV inputs onto PrV neurons, our finding of broader directional tuning and less directional consistency within the PrV population is consistent with the notion that PrV cells integrate multiple inputs differing in their preferred directions.

Adaptive properties

Many studies of neurons along the whisker-barrel neauraxis have employed a binary, “tonic-versus-phasic” classification scheme for describing the adaptive properties of neuronal responses. Of our current sample of NV neurons, 75% (48 of 64 cells) were found to respond in slowly adapting, or tonic, fashion to whisker deflections. This percentage is identical to that recorded by Lichtenstein et al. (1990), using similar stimuli under different anesthetic conditions. The remaining pro-
NV neurons as indicated by their smaller tuning ratios. B: directional tuning indices (see METHODS) for ON responses. Index of 7 indicates projecting PrV cells with respect to NV cells (see METHODS for description of rotated population polar plots illustrating broader directional tuning of VPM-highly tuned response; 0 indicates lack of directional tuning. C: less directionally tuned ON responses and exhibit less directional consistency (Gottschaldt et al. 1973; Lichtenstein et al. 1990). During our NV recording sessions, these differences were easy to recognize even without careful quantification. In the course of PrV recording sessions, however, it became apparent that such a clear dichotomy did not exist among PrV cells, as strictly phasic responses were virtually absent. When the tonic-phasic classification scheme (see METHODS) was applied to our sample of PrV neurons, a significantly larger percentage than in NV (93%; 67 of 72 cells) were found to respond tonically (P < 0.005; χ² test; see Fig. 8A).

The paucity of phasic neurons in PrV precluded meaningful tonic-versus-phasic comparisons for PrV responses. We therefore focused on comparing plateau responses between tonic NV and PrV populations. Despite a larger proportion of tonic PrV cells, we found that the maximum angle plateau responses of tonic neurons were on average weaker in PrV than in NV (P < 0.05; Mann-Whitney test; see also Table 1). This is evident from the population PSTHs in Fig. 8B, which show the background-subtracted, maximum angle plateau responses for the subpopulations of tonic NV and PrV cells. In contrast, minimum angle plateau responses were greater in PrV than in NV for tonic cells (P < 0.001; Mann-Whitney test; PSTHs not shown). Note that the pattern of plateau response differentials between NV and PrV parallels that described earlier for ON and OFF responses. As was the case for ON response tuning, the greater differential between maximum and minimum angle plateau responses in NV versus PrV suggests that the plateau responses of tonic PrV cells were less directionally tuned than their counterparts in NV. This disparity in tuning, which is evident in the population polar plots (Fig. 8C), is more pronounced than the difference in ON response tuning between NV and PrV (see Fig. 6C). The most parsimonious explanation for the broader plateau response tuning of tonic PrV cells is that these cells integrate multiple tonic inputs having slightly different preferred directions.

For each tonic cell in PrV and VPM, we also computed an adaptation index to assess the relative strength of the plateau response with respect to the ON response. The adaptation index was defined as a cell’s maximum angle plateau response divided by its maximum angle ON response. Figure 8D shows the distributions of adaptation indices for NV and PrV. Despite the fact that both maximum angle ON and plateau responses differed between the two populations, adaptation indices were similar. Although the tendency was for PrV cells to have slightly greater indices than NV cells, this difference was not significant (P = 0.11; Mann-Whitney test).

**Adjacent whisker responses**

An important goal of the present study was to provide a quantitative description of the surround component of PrV RFs. For each PrV cell that we recorded, we applied our stimulus protocol successively to as many as four AWs. Of the 197 AWs tested, the majority resulted in statistically significant ON (65%; 129 AWs) and OFF (62%; 122 AWs) responses according to the criterion described in METHODS. Most PrV cells responded to deflections of one or more AWs. Of the 65 neurons for which data from at least two AWs were obtained, 58 (89%) exhibited statistically significant responses to deflections of at least one AW. However, average AW responses for all response windows were uniformly weaker than those of...
PWs (Fig. 4, A–C, light bars; see also Table 1 for means and SDs). Differences in trial-by-trial variability between PW and AW responses were assessed by comparison of CVs calculated from maximum angleON responses. AW responses were found to exhibit considerably more variability than PW responses (P < 0.0001; Mann-Whitney test; data not shown). This finding was not unexpected, however, given that AW responses were overall much weaker than those of PWs and therefore were more likely to be influenced by fluctuations in background activity. Indeed, linear regression analysis showed a significant negative correlation between CV and ON response magnitude (r² = 0.42; P < 0.0001). AW ON responses, when present, occurred on average at longer latencies than those of PWs (Fig. 4E). Mean latencies with respect to stimulus onset for PWs and AWs were, respectively, 4.1 ± 1.9 and 6.6 ± 4.0 ms (P < 0.001; Mann-Whitney test; data not shown). This finding was not unexpected, however, given that AW responses were overall much weaker than those of PWs and therefore were more likely to be influenced by fluctuations in background activity. Indeed, linear regression analysis showed a significant negative correlation between CV and ON response magnitude (r² = 0.42; P < 0.0001).

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During recording sessions, it was apparent that a number of AW responses were not only statistically significant but were also quite robust relative to their corresponding PW responses. Figure 9A presents PSTHs for one cell in which multiple AWs elicited vigorous activity. To assess the relative strength of AW with respect to PW responses within the PrV population, we compared every AW response to that of its corresponding PW by calculating the ratio of their ON responses. The distribution of AW:PW ON response ratios is shown in Fig. 9B (0.29 ± 0.25). Of the 197 AWs stimulated, 41 (21%) evoked ON responses that were ≥50% that of their matched PW. However, approximately one-half (47%; 93/197) elicited responses that were ≤20% that of the corresponding PW. No relationship was found between the magnitude of a cell’s PW ON response and its associated AW:PW ON response ratios. The distributions of AW:PW ON ratios for the four AWs are shown as cumulative probability plots in Fig. 9C. Mean values ± SD for the four whiskers were as follows: caudal, 0.32 ± 0.24; rostral, 0.31 ± 0.27; ventral, 0.30 ± 0.26; and dorsal, 0.23 ± 0.25. Although the dorsal AW evoked on average weaker responses relative to the PW than did the other whiskers, ANOVA revealed no significant differences among the four whiskers in terms of their AW:PW ON ratios (P > 0.1).

We next asked whether robust AW responses were distributed evenly across the population of PrV cells or whether they
were concentrated in a small subpopulation of our sample. We specifically examined those 65 PrV neurons for which data from at least two AWs had been collected. Of these, nearly one-half (49%; 32 cells) had at least one AW whose AW:PW ON ratio was $H_{0.5}$. However, only nine cells (13%) had more than one AW with an AW:PW ON ratio $H_{0.5}$. These findings indicate that the robust AW responses observed in our study were not confined to a small number of strongly multi-whisker cells, but instead were relatively common throughout PrV.

**Comparison of responses: AW versus PW**

We also investigated whether AW and PW responses differed with respect to parameters other than response magnitude and latency. A comparison of directional tuning properties between the two populations revealed that most AW ON responses, although somewhat less tuned than those of PWs, were nonetheless sensitive to stimulus direction (Fig. 10, A and B; cf. Figs. 10A and 6B). Likewise, when directional consistency was examined, the mean correlation coefficient ($H_{0.33} - H_{0.38}$) for AWs having both ON and OFF responses ($n = 107$) was found not to differ significantly from that of PWs ($P > 0.2$; Mann-Whitney test; distribution not shown).

Another similarity between AW and PW ON response tuning was the lack of preference among the AW population for a particular angle ($P > 0.7$; ANOVA). This finding also held true when each of the four AW subpopulations (i.e., caudal, rostral, etc.) was examined separately (all $P > 0.15$; ANOVA). When individual AW ON response polar plots were compared with those of their corresponding PWs, no correlations in directional tuning were observed (average correlation coefficient, $0.11 - 0.48$), indicating that AW and PW inputs onto individual PrV cells are not matched for preferred direction.
Despite similarities in directional tuning, AW and PW responses differed markedly in terms of adaptive properties. Of those AWs that exhibited statistically significant ON responses, approximately 55% (71 of 129 AWs) also displayed significant plateau responses, i.e., were classified as tonic. Compared with the 93% of PW responses found to be tonic, the proportion of tonic responses was significantly lower for AWs ($P < 0.001$; $\chi^2$ test). It is unclear from these findings, however, whether AW responses arise from disproportionately phasic input or whether tonic inputs are present but are differentially filtered with respect to more synchronous, transient (ON and OFF) responses.

**DISCUSSION**

We employed controlled whisker deflections to assess the response properties and receptive field characteristics of VPM-projecting PrV neurons. To understand how second-order cir-
cuits involving PrV transform afferent inputs, we also recorded the responses of whisker-associated NV neurons using the same stimuli applied under identical conditions. Our essential finding is that the encoded features of a tactile stimulus, as represented in NV responses, are for the most part preserved in the responses of VPM-projecting PrV cells. Overall response magnitudes were comparable in both populations, and both populations exhibited a high degree of directional tuning and a majority of tonic responses. However, response magnitudes occurred over a narrower range in PrV, a finding that was reflected in the broader directional tuning of PrV with respect to NV neurons. Both in NV and PrV, receptive fields were dominated by a single whisker. Unlike in NV, however, where neurons invariably possess single-whisker receptive fields (Gibson and Welker 1983a; Gottschaldt et al. 1973; Lichtenstein et al. 1990; Zucker and Welker 1969), numerous PrV cells responded to stimulation of adjacent whiskers, albeit with much lower firing rates than were elicited by PW stimulation.

Response magnitude and tuning

Despite the finding that mean ON and OFF responses averaged over all deflection angles were slightly larger in NV than in PrV, these differences were not significant, indicating that afferent signal gain is not a major function of the second-order synapse. Interestingly, response magnitudes of individual PrV cells occurred within a narrower range, on average, than those of NV neurons. Several explanations are possible as to how PrV circuitry accomplishes this reduction in response range. The boosting of minimum angle responses, for instance, might be accounted for by the convergence onto individual PrV cells of inputs having different preferred directions. Our finding that fewer PrV responses exhibited “null” angles supports this contention. For example, a PrV neuron receiving input from two NV cells, each with a single, distinct null angle, would itself likely display no null responses, since afferent drive is provided at all deflection angles.

Inspection of maximum angle ON response PSTHs revealed that, following an initial rapid rise, the PrV response is rapidly truncated with respect to the NV response. GABAergic inhibition offers one possible mechanism by which second-order circuits might attenuate high-amplitude responses. Inhibition has been shown to influence responses of neurons in the rat dorsal column nuclei (Nunez and Buno 1999), and anatomical substrates for inhibitory interactions are known to exist also within PrV (Ginestal and Matute 1993; Haring et al. 1990; Lo et al. 1999). In the rat, Bae et al. (2000) showed that most NV terminals presynaptic to PrV cells are themselves recipients of axo-axonal GABAergic synapses. In addition, GABAergic synapses onto PrV dendrites postsynaptic to primary afferent terminals have been shown to occur frequently in the mouse (Xiang et al. 2000). A recent in vitro study by Lo et al. (1999) demonstrated that for a given barrelette cell, electrical stimulation of the trigeminal tract resulted in an excitatory postsynaptic potential followed immediately (<1 ms) by an inhibitory postsynaptic potential. This fast-onset inhibition is consistent with the rapid dip in ON response magnitude observed in the present study.

The onset time and duration of the PrV ON response suppression observed in our study are, however, notably shorter than the time course of GABA-mediated suppression of VPM responses, which occurs exclusively via feedback inhibition from the thalamic reticular nucleus (RT) (reviewed in Guillery et al. 1998). In the latter case, suppression of VPM responses persists throughout the plateau phase, and sometimes results in the “phasic” classification of cells that, absent inhibition, would otherwise express tonic properties (Hartings and Simons 2000). Although PrV plateau responses are on average weaker than those of NV cells, the magnitude and duration of inhibitory interactions in PrV are apparently insufficient to mask the expression of statistically significant plateau responses.

One consequence of the decrease in response range from NV to PrV is that PrV cells exhibit broader directional tuning profiles than do NV cells. This fact notwithstanding, most PrV cells in our study remained highly sensitive to the direction of deflection. Similarly, both Chaia et al. (2000) and Veinante and Deschénes (1999) reported that the majority of VPM-projecting PrV cells in their respective samples responded selectively according to deflection angle. Although Jacquin et al. (1988) observed no directional tuning in labeled VPM-
projecting neurons, their sample size was small (n = 10), and
tuning was not assessed through controlled stimuli or quanti-
tative criteria. Other earlier studies are more difficult to com-
pare with our own, since neuronal projection status was not
identified. Nevertheless, most describe directionally tuned re-
sponses in PrV (Doherty et al. 1993; Kwan et al. 1999; Shipley
1974).

Adaptive properties

PrV receives both slowly (tonic) and rapidly (phasic) adapt-
ing inputs from NV, where the adaptive properties of primary
afferent neurons are thought to reflect their specialized receptor
endings at the periphery (Gottschaldt and Vahle-Hinz 1981;
Gottschaldt et al. 1973; Rice et al. 1986). Previous studies have
differed in their classifications of the adaptive properties of
PrV cells, but most describe predominantly phasic responses.
Shipley (1974) reported that in the anterior half of the trigem-
inal complex, 63% of cells responded in phasic fashion. Simi-
larly, Veinante and Deschénes (1999), Jacquin et al. (1988),
and Doherty et al. (1993) all reported a majority of phasic
responses. In striking contrast, we found that more than 90% of
VPM-projecting PrV neurons displayed tonic responses. Our
results support the prevailing view that a major function of
thalamic circuitry is the transformation of tonic input into
phasic output via feedback inhibition from Rt (Gottschaldt et
al. 1983; Hartings and Simons 2000; Lee et al. 1994a,b).

It is probable that the higher percentage of tonic PrV cells
observed in our study is related in part to our stimulus regimen
and to our statistical classification criteria. By deflecting whis-
kers in eight different directions, we are unlikely to have
overlooked plateau responses that were directionally tuned to
a small number of deflection angles. Also, the use of a quanti-
tative classification scheme (see METHODS) led us to identify as
tonic a number of cells whose plateau responses were other-
wise difficult to discern via the audio monitor alone. Chiaia et
al. (2000), who also used controlled, multi-angle deflections
and quantitative criteria to assess adaptive properties, found
that a majority (57%) of VPM-projecting PrV cells responded in
tonic fashion. Furthermore, during their recordings the in-
vestigators carefully monitored vital signs to ensure that anes-
thetic doses were no greater than were required to maintain
analgies. The similarity between their findings and those
recorded in fentanyl-sedated animals suggests that deeper lev-
eels of anesthesia, such as those employed in earlier studies,
may mitigate the expression of sustained responses in PrV and
therefore result in the classification of a larger percentage of
neurons as phasic.

Unlike in some species, where tonic and phasic channels
remain segregated along the afferent pathway (Dykes et al.
1981; Poggio and Mountcastle 1963; Powell and Mountcastle
1959), it is not known whether similar separation is maintained
in the rodent trigeminal system. Detailed studies of the central
terminals of whisker-associated primary afferent collaterals
differing in adaptive properties have shown that this quality
does not predict the distribution of synapses within rat PrV
(Jacquin et al. 1993; Shortland et al. 1995, 1996). It is therefore
unclear whether or to what extent phasic and tonic primary
afferents converge onto individual PrV cells. The large per-
centage of tonic responses within PrV makes plausible the
argument that at least some of those PrV cells that receive tonic

NV input also receive input from phasic NV cells. Indeed, the
broader directional tuning and less pronounced directional
consistency of PrV responses with respect to those of NV could
be explained by a widespread contribution of phasic NV in-
puts, which, compared with tonic NV responses, are less di-
rectionally tuned and less directionally consistent (see RESULTS
for references). An alternative explanation is that these differ-
ences between PrV and NV responses result from the conver-
gence within PrV of multiple, directionally consistent, tonic
NV inputs differing in their preferred directions. This latter
hypothesis does not require convergence of tonic and phasic
inputs onto individual second-order neurons. Ultimately, si-
multaneous recordings from synthetically coupled pairs of NV
and PrV neurons will be required to settle this question.

Multi-whisker receptive fields

Several groups reporting multi-whisker responses in VPM
neurons have suggested that VPM receptive fields reflect input
from multi-whisker trigeminothalamic neurons in SpV (Arm-
strong-James and Callahan 1991; Friedberg et al. 1999; Lee et
al. 1994a; Rhoades et al. 1987). The most surprising result of
the present study was that individual PrV neurons themselves
provide multi-whisker inputs to thalamic barreloids. Nearly
one-half of all VPM-projecting PrV neurons in our sample
exhibited strong responses (defined as ≥50% of PW response)
that a majority (57%) of VPM-projecting PrV cells responded
to stimulation of at least one whisker adjacent to the receptive
field center. These findings contrast with those of earlier studies
reporting a majority of single-whisker receptive fields in
PrV (Doherty et al. 1993; Jacquin et al. 1988; Shipley 1974;
Veinante and Deschénes 1999; but see also Chiaia et al. 2000).

It is conceivable that some of the antidiromic responses in
the present study may have resulted from accidental activation
of PO-projecting PrV cells, whose axons frequently terminate
near the PO/VPM border and whose receptive fields have been
reported to include multiple whiskers (Veinante and Deschénes
1999). It is unlikely, however, that our sample was signifi-
cantly contaminated by PO-projecting cells, as we employed
eine-tipped stimulating electrodes to minimize current spread.
As described in METHODS, the effective radius of our antidiromic
stimulus was likely small, since antidiromic responses could be
obtained only when the stimulating electrode in VPM and the
recording electrode in PrV were in close somatotopic align-
ment. Despite these precautions, we cannot entirely rule out the
occasional activation of PO-projecting axons traveling en pas-
sant through VPM (M. Deschénes, personal communication).
Nevertheless, most previous reports agree that the large ma-
jority of cells in PrV project to VPM, with estimates ranging
from approximately 70% (Veinante and Deschénes 1999) to
approximately 90% (Chiaia et al. 1991). Thus even without the
aid of antidiromic stimulation, a random and unbiased sam-
pling of neurons in PrV would result in the classi-

J Neurophysiol • VOL 89 • JANUARY 2003 • www.jn.org
different whiskers converge onto the same PrV neuron. Although this explanation cannot be ruled out, Jacquin et al. (1993) showed that labeled NV axons tend to terminate within the histochemically defined boundaries of single barrelettes. Furthermore, dendritic fields of most barrelette neurons are small in the transverse plane, rendering them unlikely to sample adjacent whisker inputs from neighboring barrelettes (Jacquin et al. 1988; Lo et al. 1999; Ma 1991; Veinante and Deschénes 1999). Recurrent excitatory collaterals of PrV neurons provide an equally implausible explanation for multi-whisker responses, as they do not appear to exist in significant numbers in the rat (Jacquin et al. 1988; Veinante and Deschénes 1999). Perhaps the most likely source of adjacent whisker input derives from the dense network of intersubnuclear projections within the brain stem trigeminal complex itself. The majority of intersubnuclear fibers originate in the spinal trigeminal complex (SpV) and project both to PrV and to other SpV subnuclei (Jacquin et al. 1990a). Haring et al. (1990) demonstrated that none of the SpV neurons retrogradely labeled by tracer injections into PrV were immunoreactive for GABA or GAD, thus indicating that, at least in the case of PrV, intersubnuclear input serves an excitatory role. At present, no comprehensive study has investigated the contribution of SpV inputs to the receptive fields of trigeminothalamic projection neurons in PrV (but see Doherty et al. 1992).

The mean paired latency difference of 2.7 ms between adjacent and principal whisker responses is consistent with the notion that, at least in some cases, adjacent whisker input arrives via a long distance, multi-synaptic pathway. PrV receives input from each of the SpV subnuclei. The most caudal of these, subnucleus caudalis (SpVc), is located ≥3.5 mm caudal to PrV. The nearest SpV subnucleus, oralis (SpVo), lies in caudal continuity with PrV. It is therefore difficult to estimate a single intersubnuclear conduction time with which to compare our measured latency differences. The origin of adjacent whisker responses becomes even less clear when one considers that the modes of the adjacent and principal whisker latency distributions differed only by a millisecond (see Fig. 4E). Such a small difference could be accounted for by fast multi-synaptic transmission, but might also be explained by relatively longer integrative delays within PrV neurons of monosynaptic NV inputs from adjacent whiskers. How do we reconcile our finding of multi-whisker responses in PrV-projecting PrV neurons with earlier reports of single-whisker receptive fields? The answer may lie in the anesthetic agent used in the present study. Under fentanyl-induced narcosis, animals were maintained on a lighter anesthetic plane than has been the case for previous PrV recordings conducted under sodium pentobarbital (Doherty et al. 1993; Jacquin et al. 1988; Shipley 1974), ketamine-xylazine (Chiaia et al. 2000; Veinante and Deschénes 1999), and urethane (Ahissar et al. 2000; Kwan et al. 1999). Investigations of the effects of anesthesia on the receptive field sizes of VPNV neurons have shown that multi-whisker receptive fields are manifested at light anesthetic depths (Armstrong-James and Callahan 1991; Friedberg et al. 1999; Simons and Carvell 1989). However, it is presently unknown whether receptive fields of brain stem trigeminal neurons, like those of VPNV, are affected by general anesthesia. Discrepancies in reported PrV receptive field sizes might also be due to the fact that most earlier studies failed to apply controlled stimuli and quantitative criteria in assessing adjacent whisker responses. To date, Chiaia et al. (2000) are the only other group to have measured the receptive fields of confirmed VPNV-projecting PrV neurons by applying controlled deflections to individual whiskers outside the receptive field center. Overall receptive field sizes in the former study were found to be small, but were on average greater than one whisker (mean = 1.3 whiskers).

It is important to point out that our finding of multi-whisker receptive fields in PrV neurons should not be interpreted as a refutation of the prevailing hypothesis that PrV mediates the transmission of spatially precise information to the thalamus. Nor are our findings incompatible with those of other investigators reporting contrasting roles oflemniscal (PrV→VPM) and paralemniscal (SpV→PO) pathways in the processing of, respectively, the spatial and temporal features of a tactile stimulus (Ahissar et al. 2000). On average, principal whisker responses in our study were more than three times greater than those of their associated adjacent whiskers. Thus the spatial resolution of a PrV cell’s receptive field was usually high despite the inclusion of significant surround components. In contrast, second-order thalamic projection neurons of the paralemniscal pathway, such as those of SpV subnucleus interpolaris, typically exhibit robustly multi-whisker receptive fields even at relatively deep levels of anesthesia (Jacquin et al. 1986, 1989; Veinante et al. 2000). Although single-whisker receptive fields have been observed in each of the three SpV subnuclei, these cells appear not to project substantially to the diencephalon (Veinante et al. 2000). What then is the role for multi-whisker receptive fields in lemniscal transmission? It is possible that the inclusion of adjacent whiskers in PrV receptive fields enables the dynamic regulation of spatial resolution in a behaviorally context-specific manner. This hypothesis is supported by the presence of extensive anatomical channels for higher-order control of trigeminothalamic transmission, both through corticobulbar inputs (Jacquin et al. 1990b) and through intersubnuclear pathways.

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