Representation of Moving Wavefronts of Whisker Deflection in Rat Somatosensory Cortex

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Drew PJ, Feldman DE. Representation of moving wavefronts of whisker deflection in rat somatosensory cortex. J Neurophysiol 98: 1566–1580, 2007. First published June 13, 2007; doi:10.1152/jn.00056.2007. Rats rhythmically sweep their whiskers over object features, generating sequential deflections of whisker arcs. Such moving wavefronts of whisker deflection are likely to be fundamental elements of natural somatosensory input. To determine how moving wavefronts are represented in somatosensory cortex (S1), we measured single- and multiunit neural responses in S1 of anesthetized rats to moving wavefronts applied through a piezoelectric whisker defector array. Wavefronts consisted of sequential deflections of individual whisker arcs, which moved progressively across the whisker array. Starting position (starting arc), direction, and velocity of wavefronts were varied. Neurons responded strongly only when wavefront starting position included their principal whisker (PW). When wavefronts started at neighboring positions and swept through the PW, responses to the PW arc were suppressed by 60% compared with wavefronts that initiated with the PW. Suppression occurred with interarc deflection delays of ≥5 ms, was maximal at 20 ms, and recovered within 100–200 ms. Suppression of PW arc responses during wavefront propagation was largely independent of wavefront direction. However, layer 2/3 neurons showed direction selectivity for responses to the entire wavefront (the entire sequence of SW and PW arc deflection). Wavefront direction selectivity was correlated with receptive field somatotopy and reflected differential responses to the specific SWs that were deflected first in a wavefront. These results indicate that suppressive interwhisker interactions shape responses to wavefronts, resulting in increased salience of wavefront starting position, and, in some neurons, preference for wavefront direction.

INTRODUCTION

Rat whiskers are active tactile detectors that are swept over objects to detect object position, distance, shape, and texture (Vincent 1912; Welker 1964). Using their whiskers, rats’ texture discrimination capability is comparable to human fingertips (Carvell and Simons 1990; Guic-Robles et al. 1989; Moore 2004; Simons and Carvell 1989). During active exploration, rats rhythmically sweep the whiskers at 5–12 Hz over objects (Carvell and Simons 1990; Simons and Carvell 1989; Welker 1964). This rhythmic movement produces two-dimensional “wavefronts” of sequential deflections of arcs as different whiskers brush past specific object features (Carvell and Simons 1990; Sachdev et al. 2001). Moving wavefronts of whisker deflection are therefore likely to be basic components of natural stimuli and may be specially processed in the rat somatosensory system. However, it is not known how the rat somatosensory system processes complex, natural whisker stimuli. Here we studied how moving wavefronts are represented in primary somatosensory (S1) cortex of anesthetized rats.

S1 neurons respond most strongly to deflection of a single, principal whisker (PW) corresponding to the neuron’s location within the topographic S1 whisker map, and more weakly to neighboring surrounding whiskers (SWs) (Armstrong-James et al. 1992; Simons 1978; Welker 1964). Cross-whisker interactions have been studied extensively in the simple case of sequential deflection of single SW and PW. These experiments show cross-whisker interactions in which prior deflection of an SW reduces the response to subsequent deflection of the PW, a phenomenon we term “cross-whisker suppression” (Benison et al. 2006; Brumberg et al. 1996; Civitillo and Contreras 2006; Ego-Stengel et al. 2005; Higley and Contreras 2003, 2005; Kleinfeld and Delaney 1996; Shimegi et al. 1999, 2000; Simons 1985; Simons and Carvell 1989). In contrast, near-simultaneous deflection of several whiskers within a row or an arc generates either supralinear (Ego-Stengel et al. 2005; Ghazanfar and Nicolelis 1997; Shimegi et al. 1999, 2000; Staba et al. 2005) or sublinear (Mirabella et al. 2001; Simons 1985) response summation, depending on laminar location and other, unknown factors. Cross-whisker suppression is maximal 20 ms after SW deflection, and thus is likely to occur during natural whisking onto objects, which produces interwhisker contact intervals of ~24 ms on average (Sachdev et al. 2001). How cross-whisker suppression may shape responses to naturalistic moving wavefronts of ≥2 whiskers is not clear for several reasons. First, moving wavefronts contain both sequential deflections of neighboring whiskers, produced as the wavefront propagates, and simultaneous deflections of multiple whiskers along the leading edge of the wavefront, which may facilitate responses. Whether such facilitation may offset or supersede cross-whisker suppression is unknown. Second, extended temporal sequences of whisker stimuli can generate complex nonlinearities in magnitude and timing of whisker responses (Boloori and Stanley 2006; Webber and Stanley 2004). Third, cross-whisker suppression may be spatially tuned (Brumberg et al. 1996), which could result in selectivity for the direction wavefront propagation.

We studied S1 responses to moving wavefronts in urethane-anesthetized rats using a multi-whisker piezoelectric stimulator that enabled independent deflection of multiple whiskers (in 3 × 3 or 3 × 4 arrays). We measured multiple unit and single-unit responses to moving wavefronts of different start-
ing position, direction, and velocity. Results showed that S1 neurons responded strongly to wavefronts that started with the PW, but responses were powerfully suppressed when wavefronts started at other positions and swept through the PW. Thus cross-whisker suppression powerfully shaped responses to moving wavefronts. This process led to a strongly enhanced representation of wavefront starting position in S1, and, for some neurons, preference for specific directions of moving wavefronts.

METHODS
Surgical preparation

Experiments were done in accordance with National Institutes of Health policies and approved by the UCSD Institutional Animal Care and Use Committee. Twenty-four Long-Evans rats (age, 25–90 days; weight, 70–270 g) of both sexes were used. Rats were anesthetized with urethane (1.5 g/kg, 20% in saline, ip). Atropine methyl nitrate (0.05 mg/kg) and lactated Ringer solution (1–2 ml) were administered intraperitoneally. The scalp was anesthetized with lidocaine and retracted, and a head bolt was attached to the skull with dental cement to secure the head without pressure points. A craniotomy (~2 × 2 mm) was made over S1 (centered 5.5 mm lateral, 2.5 mm caudal of Bregma), and the dura was removed. The brain was kept moist with saline or 1% agar in saline.

Recording procedures

During recording, anesthesia was maintained with supplemental doses of urethane (15% of original dose, ip). Supplemental urethane was given whenever limb withdrawal responses were brisk, whisker movements were observed, or breathing rate exceeded 120 breaths/min. This stage of anesthesia was previously shown by electrocorticogram measurement to correspond to Guedel stage III-3/III-2 anesthesia was previously shown by electrocorticogram measurement to correspond to Guedel stage III-3/III-2 anesthesia (Foeller et al. 2005; Friedberg et al. 1999). Body temperature was maintained at 37°C with a feedback-controlled heating blanket.

Epoxy-insulated tungsten electrodes (4–10 MΩ at 1 kHz, FHC, Bowdoinham, ME) were inserted perpendicularly into S1. Recordings were made 200–1,150 μm below the pia, as determined by microdrive depth readings, which corresponds to layer 2–5 (Celikel et al. 2004). Each recording site was assigned to a layer (2/3, 4, or 5) according to recording depth (L2/3: 200–700 μm; L4: 700–850 μm; L5: 850–1,150 μm). These depths were established in a previous study in our laboratory (Celikel et al. 2004) and verified here by lesion recovery (see Histology). Signals were preamplified (1,000× gain, DAM-50, WPI, Sarasota, FL), band-pass filtered (0.5–10 kHz, Krohn-Hite 3364, Brockton, MA), further amplified (3×, Brownlee 410, San Jose, CA), and digitized at 32 kHz using a 12-bit acquisition board (National Instruments) running custom-written routines in Igor (Waveformics, Lake Oswego, OR). Multunit responses were monitored on-line, and single units were isolated off-line by spike sorting (see Data Analysis).

Whisker stimulation was performed using an array of nine independent, computer-controlled piezoelectric bimorph elements (T215-H4CL-103X, 1.25" × 0.125" × 0.015”; Piezo Systems, Cambridge, MA), attached to individual whiskers in a 3 × 3 array. Whiskers were inserted into lightweight plastic tubes attached to the piezo elements. Individual whiskers were deflected with ramp-hold-return deflections [caudal, rostral, and upward initial deflection were used, piezo located 6.5 mm from the face, 250 μm, equivalent to 2.2°, 4-ms ramp, 1-ms hold, 4-ms return ramp for a maximum velocity of 62.5 mm/s (550°/s)]. Piezo movement was calibrated optically to produce minimal ringing (ringing was <5% of total displacement amplitude) and independence of movement between piezos (attenuation of >20 dB between neighboring elements).

At each recording site, the PW was identified from responses to deflection of nine whiskers using the piezo array (30 repetitions, random order). The PW was identified as the whisker that evoked the greatest number of spikes (5–25 ms after deflection onset) and shortest latency spiking response (Armstrong et al. 1992, Celikel et al. 2004). Only sites with a clear, single PW were used, a criterion that included sites at the centers and edges of barrel columns, but excluded sites in interbarrel septa (as confirmed by recovery of marking lesions). (A few septal recording sites were included for the first experiment in Fig. 10H to assess how wavefront direction was encoded across barrel columns.) PWs included C1, D1-3, E1-2, delta, and gamma whiskers.

Wavefront deflection procedure

Initial experiments measured integration of responses across multiple whiskers deflected synchronously (Figs. 4 and 5). For these experiments, recordings were made in the D2 column, and whiskers were deflected using the nine-piezo array into which D2 and 8 adjacent whiskers were inserted (rows C–E, arcs 1–3). We deflected each whisker individually, each arc individually, and all nine whiskers simultaneously. This entire stimulus set was presented in a randomly interleaved order with an interstimulus interval (ISI) of 1.5 s.

For measurement of responses to moving wavefronts, whiskers were deflected with either the 9-whisker array or a modified 12-whisker array in which four arcs of three whiskers each could be deflected with the addition of a lightweight comb to one of the piezos. The nine-piezo array was used to apply either vertically oriented wavefronts that propagated rostrocaudally across the whisker array, or, in a few cases, horizontally oriented wavefronts that propagated dorsoventrally across the whisker array. The 12-whisker stimulator array was used to apply wavefronts that propagated rostrocaudally, but over a greater range of starting positions than possible with the 9-whisker array. Upward, rostral, and caudal directions of individual whisker deflection were used, and no systematic differences in wavefront responses were observed. Recordings were made from columns representing both central and edge whiskers within the piezo array.

Rostrocaudally moving wavefronts were created by synchronously deflecting all three whiskers within a single arc with the ramp-hold-return deflection, and sequentially deflecting progressively more rostral or caudal arcs at a defined interarc deflection interval (IADI). Dorsoventrally moving wavefronts were created analogously by deflecting three whiskers synchronously within a row and sequentially deflecting superior or inferior rows (Fig. 1). Waves moved with constant velocity (IADI was constant between all arcs of a given rostrocaudally moving wavefront stimulus). Because the E1 whiskers natu-
Each arc deflection is composed of synchronous deflection of 3 whiskers within rows C, D, and E of that arc. Example shows a wavefront initiating at arc 3. Wavefront propagation speed is determined by the interarc deflection interval (IADI). Responses depend on starting position of wavefront. 

Responses at each site were normalized to the highest 1-ms bin and temporally aligned to deflection of the PW arc.

In experiments examining wavefront starting position, responses were suppressed when the wavefront initiated at arc 1 or the Greek arc, and swept through arc 2. Responses to rostrally moving wavefronts at the same site. Responses were suppressed when the wavefront initiated at arc 1 or the Greek arc, and swept through arc 2. 

Data analysis

All data analysis was carried out in Matlab (Mathworks, Natick, MA). Multunit responses were isolated using a threshold crossing algorithm with a 1-ms absolute refractory time after a threshold crossing. The threshold was set ~10 SD on average above background noise, with a minimum of 5 SD above background, typically corresponding to the one to three highest amplitude single units, as revealed by subsequent spike sorting. Most data were collected as multiunit recordings because simultaneous deflection of multiple whiskers tended to elicit highly synchronous firing that made spike sorting unfeasible at many recording sites. However, a subset of sites were determined by visual inspection to contain separable units, and these data were spike sorted to isolate single putative regular spiking units (RSUs; putative excitatory neurons). The spike sorting method was developed by Fee and Kleinfeld (Fee et al. 1996), implemented in Matlab by S. Mehta and S. Jadhav, and used a clustering algorithm to separate units based on shape of the full spike waveform (Celikel et al. 2004; Gabernet et al. 2005). Isolated units were required to have spike amplitude >5 SD above background noise, <1% of spikes with an ISI <1
ms, >200 total spikes, and a clear PW. Spike width was calculated from the start of the spike positivity to the end of the afterhyperpolarizing potential (AHP) negativity (Gabernet et al. 2005). Units with spike width >1.0 ms and that had >400 μs between the peak of the spike and the trough of the AHP were designated putative RSUs. Only putative RSUs were used in single-unit analysis.

Wavefront responses were either analyzed as integrated responses to the entire wavefront (5–655 ms after start of wavefront when the velocity of the wavefront was varied and 5–85 ms after the start of the wavefront for the constant velocity wavefronts with different starting positions) or responses to PW arc deflection only (measured 5–25 ms after PW arc deflection). Spontaneous firing rate was not subtracted. Response latency was defined as the time between stimulus onset and the first of two consecutive 1-ms bins that were 2 SD above the mean background firing rate (assessed over 200 ms at the start of the trial) (Foeller et al. 2005). The somatotopic center of mass of the whisker receptive field (Andermann and Moore 2006) was calculated for each recording site or single unit as the summed response to individual deflection of all three whiskers in the arc rostral to the PW minus the summed response to individual deflection of all three whisks in the arc caudal to the PW, divided by the sum of responses to all nine individual whiskers. Negative values indicate a receptive field weighted toward caudal surround whiskers; positive values indicate weighting toward rostral surround whiskers. Statistical significance was assessed using the two-tailed Kolmogorov-Smirnov (KS) test, using Bonferroni correction for multiple comparisons where appropriate, unless otherwise noted. All numbers are mean ± SE, unless otherwise noted. All plotted peristimulus time histograms (PSTHs) use 1-ms bins unless otherwise indicated.

**Histology**

At the end of the experiment, small electrolytic marking lesions (±5 μA, 10 s, at each lesion site) were made in layer 4 (depth: 700–850 μm). The brain was removed and fixed in 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4) and sunk in 30% sucrose in 4% paraformaldehyde. The cortex was determined by recording depth (480–700 μm). The brain was removed and fixed in 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4) and sunk in 30% sucrose in 4% paraformaldehyde and stained for cytochrome oxidase (Fox 1992). Sixteen of 16 animals where lesions were made at L4 depths (700–850 μm below the pia) were found to lie within L4, as defined by cytochrome oxidase staining, thus verifying our laminar depth criteria.

Lesions were recovered in a subset of animals (11/16), which allowed recording penetration locations to be reconstructed relative to barrel boundaries as defined by cytochrome oxidase staining (Fox 1992). In these animals, 68/82 recording sites were verified to be in the barrel column matched to the PW for the recording site. The exact locations of 11 sites in these animals were not reconstructable, and 3 recording sites whose anatomical location did not match the physiologically measured PW were excluded.

**RESULTS**

We recorded extracellular responses to moving wavefronts of whisker deflection at 132 sites in the S1 cortex. Spontaneous firing rate across multiunit sites (n = 132) was 0.19 ± 0.006 Hz, and PW deflection elicited 2.70 ± 0.11 spikes per stimulus, measured over the interval from 5 to 25 ms after deflection onset. Median PW response latency at multiunit sites in layers 2/3, 4, and 5 was 12.0 ± 0.33 (n = 82), 10.0 ± 0.31 (n = 31), and 9.0 ± 0.81 ms (n = 22), respectively, consistent with previous work (Foeller et al. 2005). Whisker receptive field sharpness, measured as the average response to eight immediate surround whiskers, divided by the response to the principal whisker, was 0.11 ± 0.01 for L2/3 sites and 0.08 ± 0.02 for L4 sites, similar to the sharpness reported for single regular spiking units in a previous study (Gabernet et al. 2005).

**Suppression during wavefronts and effect of wavefront starting position**

We first studied how S1 neurons encoded rostrocaudally moving wavefronts with systematically different starting positions. This experiment was performed at 21 multiunit recording sites. At each site, we first determined the PW using standard single-whisker deflections of nine neighboring whiskers, using an array of nine independent, calibrated, computer-controlled piezoelectric actuators. We then used a 12-whisker piezo array to present rostrocaudally moving wavefronts that either started at the PW arc or started at a neighboring arc and swept through the PW arc. The 12-whisker piezo array allowed independent deflection of four arcs of three whiskers each (either arcs 1–4 or arcs 1–3 plus the Greek arc). Within each wavefront, individual whiskers were deflected in a brief ramp, hold, and return pattern (4-ms ramp upward, 1-ms hold, 4-ms ramp downward, 2.2° amplitude). Whiskers within each arc were deflected simultaneously. Neighboring arcs were deflected sequentially to create moving wavefronts, with the order of deflection determining the direction of movement (rostral or caudal). Wavefront velocity was determined by the IADI (Fig. 1A). For these experiments, velocity was held constant at 20-ms IADI. All wavefronts were presented in random interleaved order.

Wavefront responses at one representative multiunit site are shown in Fig. 1. The site was in L2/3 of the D2 column, as determined by recording depth (480 μm below the pial surface), maximal responsiveness to the D2 whisker (the PW for this site), PW response latency (12 ms), and histological recovery of marking lesions within the penetration (Fig. 1B). This site responded strongly to moving wavefronts that started with arc 2 (the arc that contained the PW; denoted “PW arc”). This can be seen in Fig. 1A, C and D, which show PSTHs of responses to wavefronts starting at different arcs. PSTHs are temporally aligned to the time of PW arc deflection. The site responded strongly to caudally moving wavefronts starting at arc 2 (blue trace), but showed virtually no response to wavefronts that started at arc 3 and swept caudally through arc 2 (green trace; Fig. 1C). A similar effect was observed for rostrally moving wavefronts (Fig. 1D): wavefronts that initiated at arc 2 drove strong responses, whereas those that initiated at arc 1 or the Greek arc and swept through the PW arc evoked much weaker responses. Thus for both movement directions, this site responded strongly to the PW arc whiskers when the wavefront started at the PW arc, but showed strong suppression of PW responses when wavefronts initiated elsewhere and swept subsequently through the PW. This strong...
preference for responding best only when the PW was deflected first held true over the population of sites tested with different wavefront starting positions (Fig. 1, E and F). Wavefront initiation at an adjacent SW arc often evoked a weak direct response to those SW whiskers (Fig. 1E, red and green traces). However, responses to the PW arc and later SW arcs were strongly suppressed during wavefront propagation.

We quantified the response to moving wavefronts over two different time durations. First, we first calculated responses to the PW arc within the overall moving wavefront stimulus as the number of spikes occurring 5–25 ms after PW arc deflection onset (Fig. 2A). Responses at each site were normalized to the maximal PW arc response recorded to any wavefront stimulus. When wavefronts started one arc away from the PW, the PW arc response was reduced to 4.9 ± 12 (for rostrally moving waves) and 6.4 ± 1.3% (for caudally moving wavefronts) of the maximal PW arc response. Similar suppression of PW responses was observed for wavefronts that started two or three arcs away from the PW. Thus this analysis confirms that responses to the PW arc were nearly completely suppressed during wavefronts that began at other arcs for either direction of moving wavefronts.

Second, we calculated the integrated response to the entire wavefront stimulus, as the number of spikes occurring 5–85 ms after onset of first arc deflection (Fig. 2B). This measure includes both responses to early SW arc whiskers during wavefront initiation, as well as PW arc and subsequent SW arc responses as the wavefront propagates. Response suppression was also strongly evident with this measure: compared with wavefronts starting at the PW arc, caudally moving wavefronts that initiated one arc away evoked 59.1 ± 6.8% fewer total spikes, and caudally moving wavefronts that initiated three arcs away evoked 79.3 ± 5.5% fewer total spikes. Thus suppression affects the total number of spikes elicited by wavefront stimuli. Wavefront response suppression was similar across layers (Fig. 2C). Although no preference for direction of wavefront propagation was found, on average, across recording sites (Fig. 2B), some sites did show wavefront direction selectivity caused by differential responses to the earliest SW whiskers in the wavefront (Fig. 1, E and F).

These results show that suppression of whisker responses during wavefront propagation causes wavefront starting position to strongly influence wavefront responses. Recording sites whose PW was in the starting position of a wavefront responded strongly to wavefront onset, whereas sites whose PW was deflected later in the wavefront showed powerful response suppression.

**Summation of individual whisker responses within arcs**

Responses to moving wavefronts may be influenced not only by cross-whisker suppression during wavefront propagation but also by summation of responses to individual whiskers deflected synchronously at the wavefront’s leading edge.

We tested how responses to synchronous deflection of multiple whiskers are integrated to generate responses to simultaneous deflection of multiple whiskers within an arc or within multiple arcs. Responses were measured from 11 single units (putative regular spiking units based on spike width; see METHODS). All units were recorded in the D2 column. Responses of each unit were measured to deflection of nine individual whiskers (rows C–E, arcs 1–3), individual arcs (e.g., C1-D1-E1, deflected simultaneously), and all nine whiskers deflected simultaneously. In layer 2/3 (n = 7), the mean response to isolated deflection of the PW (D2) was 1.54 ± 0.91 spikes/stim (average number of spikes 5–25 ms after whisker deflection), with a background rate of 0.21 Hz. In layer 4 (n = 3), units responded with 2.47 ± 0.05 spikes per PW deflection with a background rate of 0.22 Hz. In layer 5, only one unit was recorded, which fired 0.83 spikes per PW deflection and had a background rate of 0.17 Hz. Mean single unit responses were high because single units were isolated from only the most responsive recording sites, because of the need for a large number of spikes for spike sorting (see METHODS). This biased against less responsive single units.

Figure 3 shows an example unit, recorded in L5 of the D2 column (depth: 949 μm). Responses were measured to all nine whiskers individually (Fig. 3A) and to synchronous deflection of the three whiskers within each arc (Fig. 3B). Evoked responses to each arc were less than (arcs 1 and 2) or equal to (arc 3) the summed response to the component whiskers (Fig. 3, B and C). Thus this unit tended to show sublinear summation of responses within arcs. Similarly, the population of single units (n = 11) showed sublinear response summation in the PW arc (Fig. 4B). Summation within SW arcs (arcs 1 and 3;
Fig. 4, A and C) was closer to linear, particularly for weak responses. The reason for strong sublinear summation within the PW arc appeared to be that deflection of the PW alone (at 2.2°, the standard deflection amplitude) elicited a saturating spiking response. Across the population, D2 whisker responses were 1.04 ± 0.16 times greater than responses to the entire arc 2 and 0.98 ± 0.14 times greater than responses to all nine whiskers. These ratios were not significantly different from 1 (P > 0.40 and P > 0.67, respectively, 2-tailed t-test). Thus the population response magnitude for the D2 whisker alone (the PW) was statistically identical to that for arc 2 deflection (the PW arc) and to that for simultaneous deflection of all nine whiskers (Fig. 4, D and E). These results suggest that because PW responses were already saturating, cross-whisker suppression, rather than facilitation, dominated in determining responses to multi-whisker wavefronts under our conditions.
Effects of wavefront velocity on suppression of PW arc responses

Because the major effect of wavefront stimuli was suppression of responses to noninitial whiskers within the moving wavefront, we next tested how suppression of responses during moving wavefronts was affected by wavefront velocity, by presenting different velocity wavefronts using the $3 \times 3$ piezo array ($n = 111$ multiunit sites). By varying the IADI from 0 (simultaneous deflection of all 9 whiskers) to 200 ms, wavefronts of different speeds were generated (Fig. 5D). Both caudally moving (positive IADI) and rostrally moving (negative IADI) wavefronts were presented. All velocities and directions were interleaved randomly. The direction of individual whisker deflections within the wavefront was held constant (caudal or upward initial deflection), independent of the direction of wavefront propagation. We first present analysis of multiunit spike data, which was available from all recording sites. (Single unit isolation was difficult for this experiment, because rapidly moving wavefronts often drove near-simultaneous spikes from several units at once, making effective spike sorting impossible. We did succeed in isolating single units from a subset of sites, and those data are presented separately below.)

Results from two example multiunit sites are shown in Fig. 5. For the first site (Fig. 5, A–C), the PW was D3, and the piezo array included arc 2 (C2-D2-E2), arc 3 (C3-D3-E3), and arc 4 (C4-D4-E4), so that the PW was in the center of the piezo array. All wavefronts started at the edge of the array (i.e., 1 whisker arc away from the PW) and swept over the PW at varying speeds up to ±20 ms IADI. Responses were quantified...
5–25 ms after onset of PW arc deflection (green lines in Fig. 5C) to generate a wavefront velocity tuning curve (Fig. 5B). Simultaneous deflection of all nine whiskers (IADI = 0 ms) elicited strong responses in this time window, equal to the response elicited by the PW alone (Fig. 5B, asterisk). Near-simultaneous deflections produced by fast moving wavefronts ([IADI] ≤ 2 ms) also produced strong responses. In contrast, slower moving wavefronts ([IADI] = 5–20 ms) elicited greatly attenuated responses to the PW arc. This was true for both wavefront directions. The second example site (Fig. 5, E–G) was tested with a broader range of wavefront velocities (to ±200-ms IADI). This site showed a similar suppressive effect on PW arc responses for [IADI] = 20–50 ms, with recovery for [IADI] = 100 and 200 ms. Thus these sites responded strongly to waves that started with the PW and to fast wavefronts that started elsewhere and swept over the PW in ≤2 ms, but not to wavefronts that swept over the PW 5–50 ms after wavefront initiation.

We quantified velocity tuning for the entire population of multiunit sites with PWs in the center of the piezo array. Like the example sites in Fig. 5, these sites were tested with wavefronts that originated from one arc away from the PW and swept across the PW at varying speeds (Fig. 6a, top). Sites were tested with [IADI] = 0, 2, 5, 10, and 20 ms or with 0, 20, 50, 100, and 200 ms. Data from all sites were merged to create the population wavefront velocity tuning curves in Figs. 6 Figs. 7 Figs. 8. Responses were first calculated for the interval 5–25 ms after PW arc deflection. For each IADI, responses in this interval were normalized to the responses observed during simultaneous deflection of all nine whiskers (IADI = 0 ms). Across the population, multiunit sites responded most strongly to near-simultaneous deflection of all whiskers (IADI ≤ 2 ms) and showed significant suppression below the 0-ms IADI response levels for all [IADI] ≥ 5 ms (Fig. 6A, top, asterisks; P < 0.05). Maximal suppression occurred at [IADI] = 20 ms, with PW arc responses being reduced to 10.3 ± 2.2 and 18.2 ± 2.5% of maximal for rostrally and caudally moving waves, respectively. This suppression is somewhat stronger than the 70% suppression classically observed for single SW and PW whisker deflections (Simons and Carvell 1989). Suppression was less pronounced at [IADI] values of 100–200 ms.

The effect of starting position on wavefront responses was readily apparent from a separate population of recording sites whose PW was on the rostral or caudal edge of the stimulated whisker array and thus was at the starting position of wavefronts moving in one direction. For sites whose PW was on the
caudal edge of the array, rostrally moving wavefronts started at the PW, whereas caudally moving wavefronts started two arcs away and swept through the PW. As a result, these sites responded strongly to rostrally moving wavefronts but were inhibited by caudally moving wavefronts with IADI from 5 to 100 ms (Fig. 6A, bottom). A complementary effect was observed for sites at the rostral edge of the stimulated whisker array, because caudally moving waves initiated at the PW for these sites, whereas rostrally moving waves originated elsewhere and passed through the PW arc. This led to response suppression to rostrally moving wavefronts (Fig. 6A, middle). The magnitude and time-course of wavefront suppression were similar across layers 2/3, 4, and 5 (Fig. 7), although there was a trend toward greater suppression in layer 2/3.

A smaller but similar suppressive effect was observed when integrated responses to the entire wavefront duration were calculated (5–655 ms after 1st whisker deflection), rather than responses to the PW arc alone (Fig. 6B; reanalysis of same data as in Fig. 6A). In this case, for units with PWs in the center of the piezo array, significant suppression below the 0-ms IADI response was observed for all IADIs between 5 and 50 ms and at +100 ms (Fig. 8, top). Similar results were obtained for units with PWs at the rostral and caudal edges of the array (Fig. 8, center and bottom). These data indicate that significant reduction in overall responses to moving wavefronts occurs when wavefronts deflect surround whiskers ~5–50 ms before the PW arc.

In a subset of recording sites, we also presented vertically moving wavefronts by sequentially stimulating three rows of whiskers instead of three arcs. We observed suppressive effects and time-courses for vertically moving wavefronts that were similar to the horizontally oriented wavefronts (data not shown).

Together, these data indicate that sequential deflection of an SW arc followed by the PW arc during wavefronts reduces responses to PW arc deflection for 5- to ~50-ms IADIs. This results in strong selectivity for waves that deflect the PW first, and for near-simultaneous deflection of SW and PW whiskers, such as occur during fast waves with IADI ≤ 2 ms. This process causes neurons to be selective for wavefronts that start with their PW and sweep away, relative to wavefronts that start at other whiskers and sweep over the PW.

**Confirmation of response suppression during wavefronts in isolated single units**

We isolated 39 single units with spike widths ≥1 ms from recording sites in the velocity tuning experiments. Based on spike width, these single units are likely to represent regular spiking (presumed excitatory) neurons (Gabernet et al. 2005). Single units were isolated using the spike sorting algorithm of Fee, Mitra, and Kleinfeld (Celikel et al. 2004; Fee et al. 1996) (see methods). Single units with the PW in the center of the array (n = 25) exhibited 1.67 ± 0.24 spikes per PW deflection (when the PW was deflected in isolation) and 1.66 ± 0.18 spikes per simultaneous deflection of all nine whiskers. Single-unit median response latencies to PW deflection in L2/3, L4, and L5 were 12.5 ± 0.5 (n = 14), 10 ± 0.58 (n = 7), and 9 ± 0.5 ms (n = 4), respectively. Average whisker tuning width (quantified as the mean response to 8 adjacent SWs divided by the PW response) was 0.16 ± 0.02, consistent with excitatory neurons (Bruno and Simons 2002). Single units were biased toward higher firing rates because only the highest responding sites contained sufficient spikes for accurate spike sorting.

We selected the 25 sites whose PW was in the center of the piezo array for further analysis. Like the multiunit sites, moving wavefronts that initiated at a surround arc and swept through the PW arc were found to suppress responses, measured both 5–25 ms after PW arc deflection (Fig. 8A) and over the entire wavefront stimulus (Fig. 8B). The time-course and extent of suppression were highly similar for single-unit and multiunit (gray) sites. Specifically, strong suppression was exhibited when adjacent arcs were deflected 5–50 ms before the PW arc. Thus, although only a small population of single units were sampled, these data suggest that the response suppression observed during wavefronts at multiunit sites is indicative of suppression at the single unit level.

**FIG. 7.** Analysis of speed tuning by layer. Population wavefront velocity tuning curves for responses 5–25 ms after PW arc deflection are plotted. Neurons in layers 2/3 (blue, 200–700 µm below pia), layer 4 (red, 700–850 µm below pia), and layer 5 (black, 850–1,150 µm below pia) were similarly selective to wavefront speed.
Preference for direction of moving wavefronts

We next tested whether neurons showed a systematic preference for the direction of moving wavefronts. For this analysis, we used data from sites in the center of the piezo array, which were presented with wavefronts originating at an SW arc and sweeping either rostrally or caudally through the PW arc.

DIRECTION PREFERENCE FOR SUPPRESSION OF PW ARC RESPONSES. We first asked whether wavefront direction influenced the suppression of responses 5–25 ms after PW arc deflection, which primarily represent PW arc responses. We first calculated for each multiunit site a directionality index (DI_{PWarc,integrated}) defined as the area under the wavefront velocity tuning curve for PW arc responses for wavefronts with 0 ms ≥ IADI ≥ 20 ms (caudally moving waves) minus the area for wavefronts with −20 ms ≤ IADI ≤ 0 ms (rostrally moving waves), divided by the total area. Negative values of DI_{PWarc,integrated} indicate stronger PW arc responses to rostrally moving wavefronts; positive values indicate stronger PW arc responses to caudally moving wavefronts; and 0 indicates no directional selectivity (Fig. 9D). Across all layers, we found a unimodal distribution of DI_{PWarc,integrated} with a mean of 0.04 ± 0.02 (n = 111 multiunit sites), which was slightly but significantly different from zero (Fig. 9E). Similar distributions of DI_{PWarc,integrated} were found for multiunit sites in each layer (layer 2/3: 0.02 ± 0.11, P < 0.27; layer 4: 0.07 ± 0.17, P < 0.02; layer 5: 0.05 ± 0.07, P < 0.17) and for single units (layer 2/3: 0.00 ± 0.14, n = 14, P < 0.90; layer 4: 0.04 ± 0.08, n = 7, P < 0.32). This indicates that, across the population, there was largely similar suppression for rostrally and caudally moving wavefronts, with only a small (4%) tendency for greater PW arc suppression during rostrally moving wavefronts. Consistent with this conclusion, there was no significant difference across the population in magnitude of responses to rostrally versus caudally moving wavefronts of matched speeds (P > 0.4, paired t-test), except for a small directional preference between +20- and −20-ms IADI (P < 0.02), which is visible in the population velocity tuning curve in Fig. 6A.

S1 neurons are tuned for the direction of single whisker deflection and are organized into a map of preferred deflection angle within each S1 column (Andermann and Moore 2006; Simons 1985). In this map, tuning for the direction of single whisker deflection is correlated with the spatial structure of each neuron’s whisker receptive field (somatotopy) and with anatomical location of the neuron in the whisker column (Andermann and Moore 2006). To determine whether a similar map exists for wavefront direction preference, we tested whether rostral versus caudal directional preference of PW arc suppression was correlated with the rostrocaudal somatotopic center of mass of the whisker receptive field. Somatotopy was calculated for each recording site as the summed response to the three individual whiskers within the rostral SW arc minus the summed response to the three whiskers in the caudal SW arc, divided by summed response to all nine whiskers. Negative somatotopy indicates a receptive field bias toward caudal SWs. We found no relationship between DI_{PWarc,integrated} and receptive field somatotopy for multiunit sites in any layer (Fig. 9F). Not only did units with symmetric receptive fields show no substantial direction selectivity for PW arc suppression (e.g., Fig. 5, A–C and E–G), but even units with somatotopic bias typically exhibited PW arc suppression that was symmet-
rostrally moving wavefronts, which deflect the caudal SWs first (Fig. 10, A and B), whereas sites with a somatotopic bias toward rostral SWs responded most strongly to caudally moving wavefronts (Fig. 10, C–F). Correspondingly, DIwavefront, integrated was correlated with receptive field somatotopy for layer 2/3 multiunit sites within barrel column edges and centers (slope: 0.37, y-intercept: −0.03, $r^2 = 0.37$, significantly different from slope 0, $P < 0.05$; Fig. 10H). This correlation was even stronger when a few L2/3 sites overlying septa, which showed very strong somato-
topic bias, were included (slope: 0.44, y-intercept: /H11002 0.01, \(r^2\)/H11005 0.50, \(P\)/H11021 0.05; Fig. 10, E, F, and H). A significant relationship between DIwavefront,integrated and somatotopy was not found in multi-or-single unit sites in layer 4 or 5, or for layer 2/3 single units, perhaps to the lower \(n\) for these measurements (\(P\)/H11022 0.05; Fig. 10 H).

We verified the correlation between somatotopy and wavefront direction preference in L2/3 using a second metric, DIwavefront,20ms, which was defined as the response to the entire wavefront for +20-ms IADI wavefronts minus the response for −20-ms IADI wavefronts, divided by summed response for both wavefront directions. This also yielded a significant correlation (slope 0.80, y intercept −0.04, \(r^2\)/H11005 0.28, \(P\)/H11021 0.05; sites from barrel column edges and centers only). Together, these findings show that L2/3 neurons with somatotopically biased whisker receptive fields exhibit a systematic preference for wavefront propagation direction. This direction selectivity reflected differential responses to initial SW whiskers in the wavefront, followed by strong suppression of PW arc responses. This led neurons to prefer wavefronts that sweep through strong SWs toward the PW and weak SWs rather than the opposite direction.
DISCUSSION

We measured the responses of neurons in the barrel cortex to deflection of multiple whiskers in moving wavefront patterns to determine how these naturalistic patterns may be represented in S1. During moving wavefronts, single columns either experience PW deflection first, if the PW is in the initial position of the wavefront, or experience SW deflection before PW deflection, if wavefronts initiate elsewhere and propagate through the PW. We found that SW arc deflection during horizontally moving wavefronts elicits strong cross-whisker suppression of subsequent PW arc responses and that this suppression is a dominant process shaping the neural response to moving two-dimensional wavefronts. This suppression leads to a strong representation of initial wavefront position in S1 and greatly reduced representation of subsequent whisker deflections as the wavefront propagates.

Cross-whisker suppression has been previously characterized using pairs of whiskers, in which deflection of a single SW reduces subsequent responses to the PW (Brumberg et al. 1996;Civillico and Contreras 2006;Ego-Stengel et al. 2005;Higley and Contreras 2003, 2005;Shimegi et al. 1999, 2000;Simons 1985;Simons and Carvell 1989). Suppression of PW arc responses during wavefronts was maximal at 20-ms interwhisker delay and recovered at ~100-ms interwhisker delay, similar to suppression evoked by single SWs (Simons 1985). Suppression during moving wavefronts reduced PW arc responses by ≤90% (at 20-ms interarc deflection interval; Fig. 6A, top), which is greater than the ~70% suppression typically elicited by single SW deflections (Higley and Contreras 2005;Simons and Carvell 1989). This is likely because of the larger number of surround whiskers deflected during wavefronts, which is known to nonlinearly recruit suppressive mechanisms (Brumberg et al. 1996).

At the outset of this study, it was unknown whether suppression would be a dominant process in shaping responses to moving wavefronts, because response facilitation between whiskers deflected synchronously during wavefronts could have reduced or outweighed cross-whisker suppression. Such facilitation has been observed previously (Ego-Stengel et al. 2005;Shimegi et al. 1999, 2000) and may be expected given the ability of remote single whiskers to evoke subthreshold, depolarizing responses in S1 neurons (Brecht and Sakmann 2002;Brecht et al. 2003;Manns et al. 2004;Moore and Nelson 1998;Zhu and Connors 1999). However, facilitation was not observed under our conditions, probably because single-whisker PW deflections already saturated neuronal responses (Fig. 5). As a result, synchronous whisker responses added sublinearly, consistent with another recent study of whisker-evoked activity (Rodgers et al. 2006). Thus our results showed that suppression was the dominant mechanism governing responses to moving wavefronts, at least when large whisker deflections are used.

The cellular basis for suppression is not known and may involve recruitment of inhibition or excitatory synaptic depression at cortical and/or thalamic synapses by prior whisker deflection (Brumberg et al. 1996;Wilent and Contreras 2005). Because the extent of suppression during wavefronts was found to be similar across cortical layers 2/3, 4, and 5 (although there was a tendency for stronger suppression in layer 2/3), our results suggest that cross-whisker suppression is largely computed at or before thalamocortical input to L4.

Preference for direction of moving wavefronts

Suppression of PW arc responses during moving wavefronts was largely independent of the direction of wavefront propagation when wavefronts initiated at an immediately adjacent SW arc and propagated through the PW (Fig. 9). A small directional bias was observed in which rostrally moving wavefronts suppressed PW arc responses slightly (4%) more than caudally moving wavefronts (Fig. 9), consistent with the prior finding that cross-whisker suppression is maximal when single caudal SWs are deflected before more rostral single PWs (Brumberg et al. 1996). However, this 4% difference is slight compared with the ~90% general suppression of PW arc responses, and we therefore conclude that PW arc response suppression is largely independent of wavefront direction.

In contrast to the direction-independent suppression of PW arc responses, many neurons showed direction-dependent responses to the entire wavefront (the complete spatiotemporal sequence of SW and PW deflections). This wavefront direction preference was correlated with the somatotopic structure of the whisker receptive field (Fig. 10); neurons with somatotopically biased receptive fields responded most strongly to wavefronts that initiated at the most responsive SWs and traveled through the PW toward the least responsive SWs. This effect occurred because SW deflection greatly reduced responses to subsequent PW deflection during moving wavefronts, and as a result, responses to initial SWs in the wavefront were a dominant part of the entire wavefront response. Thus suppression of later responses during wavefronts leads to both a heightened representation of initial wavefront position (Figs. 1 and 2) and to wavefront direction preference in some neurons (Fig. 10).

Receptive field somatotopy is mapped across each S1 barrel column, with neurons located at column edges having strongly asymmetric receptive fields dominated by the nearest neigh-
Cross-whisker suppression suppression has been hypothesized to serve as a classical contrast enhancement mechanism (Brumberg et al. 1992). Our data show that, during moving wavefronts, this suppression functions particularly strongly to enhance representations of wavefront starting position. S1 neurons responded most strongly to moving wavefronts only when their PW was deflected first during the wavefronts or within a few milliseconds of the first whiskers in the wavefront. Later whisker deflections in the moving wavefront were powerfully suppressed. As a result, neurons in S1 columns representing the whiskers that were deflected first fired strongly during wavefronts, whereas neurons in S1 columns representing whiskers deflected later fired very little, both to PW deflection and over the entire duration of the moving wavefront. Thus suppression during wavefronts transforms a temporal code for the origin of moving wavefronts (which columns fire first?) into a firing rate code (which columns fire most?) (Fig. 11). This transformation is expected to increase the salience in S1 of the whisker(s) that are deflected first during wavefronts. Recent evidence in monkey S1 indicates that a rate code integrated over a scale of hundreds of milliseconds is in fact used for discrimination of vibrotactile stimuli (Luna et al. 2005), suggesting that transformation of temporal codes into rate codes may be useful in the somatosensory system.

Whether cross-whisker suppression, observed here in anesthetized rats, occurs during natural palpation in awake, behaving rats is debated (Castro-Alamancos 2004; Fanselow and Nicolelis 1999). However, interwhisker contact times during natural exploration are in the range of a few to a few tens of milliseconds, appropriate to drive cross-whisker suppression (Sachdev et al. 2001). If cross-whisker suppression does occur, it would be expected to enhance coding of any object feature read out by either the temporal order of whisker contact or by which of a set of whiskers contacts an object first. This could include edge orientation, which may be encoded by the relative timing of contact by different whiskers onto different regions of the oriented object. Cross-whisker suppression during wavefronts would attenuate responses to later contacts, resulting in higher firing rates in cortical columns that represent the whiskers that contacted the edge first. Similarly, suppression could improve coding of distance (range) to an object, which has been proposed to be encoded by which whisker contacts the object first, because caudal whiskers are progressively longer than more rostral whiskers (Brecht et al. 1997).

The magnitude of cross-whisker suppression is thought to depend on the behavioral state of the animal, with sensory-evoked suppression being reduced in active brains because of partial saturation of suppression mechanisms by background activity (Castro-Alamancos 2004; Ego-Stengel et al. 2005). Consistent with this idea, less whisker-evoked suppression is observed in awake, active rats than in awake, resting rats (Fanselow and Nicolelis 1999). We hypothesize that, if the effectiveness of cross-whisker suppression is dynamically regulated by activity, it may be regulated by repetitive whisker activation, as occurs for other inhibitory processes in S1 (Gabernet et al. 2005). Such dynamic regulation of suppression could have important effects for sensory coding during whisking, which is organized into bouts of multiple whiskers (single protraction-retraction movements) against objects. During the first whisk onto an object, cross-whisker suppression may not yet be fully saturated, resulting in relatively strong responses to first whisker contact but suppression of subsequent contacts during the whisk. During later whisks in the bout, cross-whisker suppression may be saturated, causing weaker responses, but more temporally faithful transmission of high spatial-frequency information across whiskers. Thus the first whisk in a bout may preferentially encode the position of the object (or its closest edge), whereas later whisks could encode higher spatial-frequency information including texture (Anderman et al. 2004) and detailed shape.

Possible functional relevance of cross-whisker suppression

Cross-whisker suppression could have important effects for sensory coding during whisking, which is organized into bouts of multiple whiskers (single protraction-retraction movements) against objects. During the first whisk onto an object, cross-whisker suppression may not yet be fully saturated, resulting in relatively strong responses to first whisker contact but suppression of subsequent contacts during the whisk. During later whisks in the bout, cross-whisker suppression may be saturated, causing weaker responses, but more temporally faithful transmission of high spatial-frequency information across whiskers. Thus the first whisk in a bout may preferentially encode the position of the object (or its closest edge), whereas later whisks could encode higher spatial-frequency information including texture (Anderman et al. 2004) and detailed shape.

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