Temporal Patterns of Field Potentials in Vibrissa/Barrel Cortex

Reveal Stimulus Orientation and Shape

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ABSTRACT

During environmental exploration, rats rhythmically whisk their vibrissae along the rostro-caudal axis. Each forward extension of the vibrissa array establishes rapid spatiotemporal contact with an object under investigation. This contact presumably produces equally rapid spatiotemporal patterns of population responses in the vibrissa representation of somatosensory cortex (the posterior medial barrel subfield or PMBSF) reflecting features of a stimulus. We used extracellular mapping to identify object features based on spatiotemporal patterns of evoked potentials. Spatiotemporal modeling of evoked potential patterns accurately reconstructed linear versus curved stimuli and detected orientation changes as small is 5 degrees. Whiskers forming arcs in the PMBSF were essential for this reconstruction, and may represent a fundamental processing module. We propose that the PMBSF may function as a spatial frequency analyzer, with intra-row processing integrating a complementary set of spatial frequencies from the arcs in a single whisk.
INTRODUCTION

The somatosensory system of rodents is dominated by afferent input from the vibrissae, which serve as a primary means of close range environmental exploration. The structural arrangement of the vibrissae is highly conserved evolutionarily, suggesting that they may have a unique function in somatosensory information processing (Brecht et al. 1997). The vibrissa system may be separated into two subdivisions, with 25 (5 vertical arcs of 5 vibrissae) large and caudally positioned macro-vibrissae that range in length from one to several cm, and more numerous and rostrally positioned micro-vibrissae with lengths on the order of several mm. Central representation of the macro- and micro-vibrissae occupies a majority of somatosensory cortex, and is characterized by an orderly arrangement of discrete vertically oriented cellular aggregates or cortical columns, often referred to as “barrels” (Woolsey and Van der Loos 1970). The posterior medial barrel subfield (PMBSF) has been most thoroughly studied, and reveals a clear somatotopic organization in approximate register with the macro-vibrissae on the contralateral mystacial pad (Chapin and Lin 1990). A group of micro-barrels, rostral to the PMBSF, receive information from the micro-vibrissae, but their somatotopy has not been explored.

Based on behavioral evidence, it has been suggested that the macro- and micro-vibrissae systems may also be distinguished functionally, with the macro-vibrissae serving as distance detectors, providing head centered spatial information, and the micro-vibrissae reserved as an object recognition sense organ (Brecht et al. 1997). While the function of micro-vibrissae has not been further documented, the hypothesized role of macro-vibrissae in spatial sampling is supported by a number of studies indicating that this system is critical for tasks such as gap detection, distance discrimination and head centered orientation (Harris et al. 1999; Hutson and Masterson 1986; Krupa et al. 2001; Sachdev et al. 2000; Schiffman et al. 1970; Shuler et al. 2002; Vincent 1912).

Yet, other behavioral and physiological studies suggest that the macro-vibrissae may also provide information about object features. During exploration, rats move (“whisk”) the macro-vibrissae in a caudo-rostral direction at approximately 10 Hz, making repeated object contact on each rostral extension (Carvell and Simons 1990). It has been proposed that sensory capacities provided by whisking the macro-vibrissae as a unified sensory array may be analogous to active touch of the primate finger tip (Carvell and Simons 1990; Simons 1995). There is compelling evidence that movement of the macro-vibrissae across an object’s surface provides essential information about fine texture (Andermann et al. 2004; Arabzadeh et al. 2004; Arabzadeh et al. 2005; Carvell and Simons 1990; Guilmage-Robles et al. 1989;
Neimark et al. 2003). However, whisking also establishes a rapid and asynchronous vibrissa contact (Sachdev et al. 2001), presumably producing momentary and complex spatiotemporal patterns of afferent input to the PMBSF that may uniquely reflect spatial features of an object. Single unit studies of single and paired whisker stimulation indicate a temporal consistency between vibrissa contact and cortical response (Ego-Stengel et al. 2005; Sachdev et al. 2001; Simons 1985; Simons and Carvell 1989), introducing the possibility that more complex spatiotemporal afferent patterns evoked by multiple vibrissa contact in the behaving animal could be accurately represented by patterns of electrical activity in larger neural networks of PMBSF. If true, then temporal dynamics of the population response could provide information about not just distance and texture, but about object orientation and shape. However, it is not clear whether the temporal fidelity between vibrissa contact and cortical response demonstrated in unit studies is preserved in the large populations of cells comprising the PMBSF, particularly in the presence of inhibitory and excitatory interactions between barrels when large groups of vibrissae are engaged.

To explore this issue, we used high resolution arrays of epipially placed electrodes to measure population field potentials from the entire PMBSF while stimulating either 1 or 5 arcs of macro-vibrissae using straight and curved edges moved at rostro-caudal velocities mimicking those expected during natural whisking. Our objectives were to: 1) determine what parameters of the somatosensory evoked potential (SEP) complex (timing, amplitude and spatial distribution) are influenced by changing object features, 2) determine if SEP patterns measured from the entire PMBSF are sufficiently stable and unique to accurately classify single presentations of a given stimulus, 3) develop a general method for modeling spatiotemporal SEP patterns that might permit not just classification, but realistic reconstruction, of object features, and 4) determine what the limits this feature estimation may imply about the capacity of the macro-vibrissae/barrel system for object recognition.

MATERIALS AND METHODS

Animals and Surgery

All procedures were performed in accordance with University of Colorado Institutional Animal Care and Use Committee guidelines for the humane use of laboratory animals in biological research. Seven adult male Sprague-Dawley rats (300-400 g) were anesthetized to surgical levels using an intra-peritoneal injection of urethane, placed on a regulated heating pad, and maintained with subsequent injections of a mixture of xylazine (13 mg/kg) and acepromazine (2 mg/kg) throughout the experiment so that the eye blink reflex could be barely elicited. A unilateral craniectomy was performed
over the right hemisphere extending from bregma to lambda and from the mid-sagittal sinus lateral to the temporal bone, exposing a wide region of parietotemporal cortex where the dura was reflected. Animals were sacrificed by anesthesia overdose without regaining consciousness at the conclusion of the experiment.

**Stimulation**

The stimulating apparatus consisted of a smooth 140 mm diameter cylinder (45 mm high) fitted with a straight wire (70 mm length; 0.8 mm diameter) stimulus mounted to surface of the cylinder at its vertical midpoint and attached at the center with a thumb-screw (Fig. 1A). The cylinder was attached to a laboratory built vibration free mount with a play-out <10 µm. The stimulus was manually positioned at various angles in relation to upright (0° in the sagittal plane) using calibrated visual markers. Angles typically included ±45°, 30°, 20°, 10°, and 5° (Fig. 1B). In two animals, a vertically oriented curved wire (same thickness) hemi-circle (25 mm diameter) stimulus was also used (Fig. 1B; dashed red line). Rotation speeds were controlled with a programmable stepping motor (ServoDyne mixer head; 3-180 RPM; controller model 50003-00), and adjusted so that the speed with which the stimulus passed through the vibrissae was either .4 or .2 mm/ms in the rostro-caudal direction. These speeds were chosen to best simulate the 10-20 ms delays between sequential contact of vibrissae in a row observed in behaving animals during rostral whisking motions (Sachdev et al. 2001), assuming an average 4 mm distance between vibrissae in a row. In 3 animals, the 25 macro-vibrissae on the left mystacial pad (see Figure 2A) were trimmed to approximately 2 cm (the remaining whiskers were clipped). Since the angle at which the vibrissa emerges from the face differs from one whisker to the next, the exact length of each whisker was adjusted so that it made light (causing a caudal displacement of approximately 5°) and constant contact with the smooth continuously rotating drum. The stimulus was repeatedly swept through the vibrissae in the rostro-caudal direction and each trial of data sampling was triggered by a tab that interrupted an infrared emitter/detector pair. The beam interrupter was positioned on the inside of the rotating drum at a location that triggered data sampling approximately 50-100 ms before the stimulus made contact with the most rostral vibrissae (Fig. 1A). 85-100 trials of evoked responses were collected in this way before the stimulus was manually repositioned to a new angle and another run performed. In the 4 remaining animals, procedures were the same except that all vibrissae were clipped except for those of the middle arc (consisting of vibrissae B3, C3, D3 and E3). Vibrissa A3 in this arc was also clipped because it typically was difficult to position adequately to make good contact with the stimulus.
Single vibrissa responses were also obtained from all animals and used for consistent alignment of the recording array across animals and for modeling. Single vibrissae were deflected with a solenoid attached to a 4 cm armature by approximately 0.5 mm in the rostro-caudal direction at a speed of approximately 25 µm/ms and the SEP averaged over 100 trials.

**Evoked Potential Recording**

Epipial maps of the vibrissa-evoked SEP complex were recorded using a flat multi-channel electrode array consisting of 64 silver wires arranged in a 8x8 grid (tip diameter: 100 µm; inter-electrode spacing: 500 µm) covering a 3.5 x 3.5 mm area of the cortical surface in a single placement. Light pressure was applied to this flat array so that all of the electrodes made contact with the pial surface. After array placement, surrounding exposed regions of cortex were covered with cotton soaked in artificial cerebrospinal fluid and periodically moistened throughout the experiment to prevent desiccation. Surface field potentials were referenced to a silver ball electrode secured over the contralateral frontal bone, amplified (x1000), analog filtered (band-pass cut-off = -6 dB at 1 to 3000 Hz, roll-off = 5 dB/octave) and digitized at 10 kHz.

**Data Collection and Analysis**

Two hundred ms samples of whisker-evoked responses were recorded, with data from individual trials (N=85-100) stored digitally for subsequent analysis. Averaged responses were plotted on a template of the PMBSF in approximate register with the surface recording sites. The template was derived from previous histology and was used here for illustrative purposes only. Histological verification of precise electrode positions was not performed in the present study since this was not required for interpretation of results. The recording array was aligned to the PMBSF using single vibrissae evoked responses (see Fig. 4A).

Stimulus classification methods used are more easily understood in the context of the results and will therefore only be briefly described here. In animals with 25 intact vibrissae, classification consisted of a template matching procedure. All SEPs were first digitally band-pass filtered (10-1000 Hz) to emphasize their sharp wave components. Relative latencies of averaged SEP sharp waves for each condition (orientation of the stimulus) were compared to the averaged SEPs for an upright stimulus (0° rotation) by recording time lags of the maximum cross-correlation function at each electrode. This resulted in latency templates representing each condition, consisting of 64 time lags (all referenced to 0° response latencies at each of the 64 electrodes). Individual trials for a given condition were then classified according to
their latency pattern, computed in the same way from maximum cross-correlation functions with the averaged SEPs during 0°. Each trial was assigned to the condition whose latency template most closely matched the single trial latency pattern, using a minimum least squared error criterion. Classification accuracy was quantified as percent correct and as magnitude error (in degrees) compared to random classification, and evaluated using unpaired t-tests with significance set to \( p \leq 0.01 \). Results are reported as mean (±SD) unless otherwise noted.

In animals with a single arc of 4 vibrissae intact, classification was based on a more general model that reconstructed the actual orientation and shape of a stimulus based on spatiotemporal patterns of the SEP complex it produced. Averaged SEPs (N=100) from transient (.1 ms; .5 mm deflection) stimulation (using a laboratory built solenoid and 4 cm stick) of each individual vibrissa were first computed. Normalized maps (8x8 electrodes) of root mean squared (RMS) amplitude of the averaged SEP were used to represent the spatial distribution of each vibrissa’s contribution to SEPs evoked when all four vibrissae were subsequently contacted by the stimulus (Fig. 4A). RMS was calculated at each electrode as the average sum of squared values for sample points covering the P1/N1 sharp wave. The multi-vibrissa SEP complex for a given stimulation condition was modeled as a weighted combination of the 4 single vibrissa response patterns for each of 200 sampled time points. This resulted in four time series consisting of 200 regression weights used to model the time course of each vibrissa’s activation during the SEP complex. Regression weights computed in this way for 0° stimulus were compared to similar regression weights modeling SEPs from other conditions, again by recording time lags of the maximum cross-correlation function. Thus, SEPs (either averaged or single trial) evoked by each orientation of the stimulus were represented by four time lags, reflecting the timing of when each vibrissa contacted the stimulus relative to the timing pattern evoked at 0°. Since the velocity of the stimulus was known, and the dorso-ventral positions of each vibrissa where they contacted the stimulus were recorded for each animal, timing differences between each vibrissa response could be converted to relative distances along the drum and thus used to directly reconstruct the orientation (and shape) of the stimulus. Modeling accuracy was quantified as average error (in degrees) and tested for significant differences between means using multiple comparisons with significance set to \( p < 0.01 \).

Single trial SEPs from animals with 4 vibrissa stimulation were also classified with a template matching procedure that used spatial patterns of response amplitude as opposed to time lags as the classification criteria. SEPs were analyzed separately for low, middle and high frequency bandwidths (1-10, 10-200 and 200-1000 Hz, respectively). Within each bandwidth, amplitude template maps were computed from the normalized RMS averaged across trials for each
stimulus orientation. RMS was calculated at each electrode as the average sum of squared values for sample points covering the filtered SEP waveform. Similarly computed RMS amplitude maps of single trials were assigned to a given stimulus condition based on their minimum least squared fit to the templates. Classification accuracy was quantified as per cent correct compared to random classification, and evaluated using unpaired t-tests with significance set to $p \leq 0.01$.

RESULTS

The 25 macro-vibrissae form a 5x5 array with arcs typically labeled 1-5 on the caudo-rostral axis and rows labeled A-E on the dorso-ventral axis (Fig. 2A). The somatotopic representation of these vibrissae in the contralateral PMBSF consists of a similar array of distinct cellular aggregates identifiable in cytochrome oxidase (CO) stained tangential sections of layer IV identifying the PMBSF, with a similar caudo-rostral organization of the vibrissa arcs but an inverted dorso-ventral organization of the rows (Fig. 2B; 1-5 and E-A respectively). An 8x8 epipial electrode array was consistently aligned to the PMBSF using single vibrissa SEPs from vibrissae B3-E3 (see Figure 4A for an example). This alignment procedure has an accuracy of approximately 0.5 mm demonstrated in previous studies (Staba et al. 2005). In this and subsequent illustrations, a standard template of the PMBSF was scaled and appropriately oriented to represent the approximate relationship between the barrel field and recording electrodes in a given recording position (Fig. 2C&D; light traces). However, this template was used only for graphical purposes since histology was not performed in the present study.

Averaged SEPs, evoked by repeatedly brushing an upright stimulus oriented perpendicular to the vibrissa rows (0° orientation) at a velocity of 0.4 mm/ms, formed a distribution of responses centered on and largely constrained to the PMBSF (Fig. 2C). SEP morphology was similar at all recording sites and consisted of a single positive/negative sharp wave whose amplitude peaks are labeled P1 and N1 to reflect their polarity and sequence of occurrence (Fig. 2E). This sweep speed established an approximate 10 ms delay between sequential contact of vibrissae within a row on the rostro-caudal axis. At a slower sweep speed of 0.2 mm/ms, establishing a 20 ms delay between sequential vibrissa contact, the SEP morphology was markedly altered. At rostral electrode sites (Fig. 2Da & Fa), the SEP began with a large P1/N1 sharp wave reflecting the response to initial contact of the stimulus with the rostral vibrissae. This was followed by smaller waves at 20 ms intervals, coincident with contact of progressively more caudal vibrissae. Thus, the rostral barrels appeared to respond both to contact of their principal vibrissae and to more caudal vibrissae. SEPs in the middle of the PMBSF reflected a similar horizontal integration, with similar responses to their principal vibrissae and to those at rostral
and caudal sites (Fig. 2Db-c & Fb-c). SEPs at the most caudal sites (Fig. 2Dd & Fd) consisted of a large P1/N1 sharp wave that was delayed by 50-60 ms to the initial rostral response and was dominated by contact with the principal caudal vibrissae with only little responsiveness to previously contacted rostral vibrissae.

SEPs evoked by 0° stimulus were used as reference for comparison of relative latency shifts in SEPs corresponding to other orientations. For example, when the stimulus was oriented at +10°, it struck ventral vibrissae in the E row slightly earlier and dorsal vibrissae in the A row slightly later than when at 0°. This difference in timing was reflected in the relative latencies of the SEPs. Responses in row A of the PMBSF were later when the stimulus was oriented at +10° (Fig. 3B; left blue trace; single electrode site) as opposed to 0° (Fig. 3B; left black trace; single electrode site). In contrast, SEPs evoked by +10° stimulus were earlier in the E row (Fig. 3B; right blue trace) compared to the 0° response (Fig. 3B; right black trace). An opposite pattern of latency shifts was produced by stimulus at –10° (Fig. 3B, red traces). To quantify relative latency shifts at each electrode site, maximum normalized cross correlation functions were computed (±50 ms lags) between 0° responses and responses to other orientations (shown in Figure 3C for just –10°, 0° and +10° at an electrode site in row A and one in row E). The absolute values of relative latency shifts at each electrode were then averaged across all stimulus orientations (in this example, -45°, -30°, -20°, -10°, -5°, 0°, +5°, +10°, +20°, +30° and +45°) to obtain a composite map reflecting their spatial distribution. At 0.4 mm/ms sweep speeds, the composite map indicated maximum latency shifts across all orientations in the dorsal and ventral regions of the PMBSF (Fig. 3A; left map). Latency shifts were minimal at intervening regions of the PMBSF corresponding to vibrissa rows C and D. These vibrissae were positioned closest to the axis of rotation of the stimulus and thus would be expected to have a minimal change in the timing of vibrissa contact at different orientations. While the waveform morphology of SEPs evoked by slower .2 mm/ms sweep speeds was more complex than that of the faster speed (see Figure 2F), the composite latency map based on maximum cross correlation functions was nearly identical (Fig. 3A; right map).

While composite latency maps reflected regions of the PMBSF most sensitive to timing differences established by all stimulus orientations, the relative SEP latency patterns were quite distinct for each orientation. To determine whether this distinction was sufficient to classify responses at each orientation, latency patterns were computed for averaged responses at each orientation and used as templates for comparison to latency patterns produced by single trials of a given orientation. In this example, 11 templates represented the characteristic latency patterns of the stimulus orientations used. Single trials (85 per orientation in this example) were then classified according to the least squared difference between
their latency patterns and that of each of the templates. Latency patterns were sufficiently stable on a trial to trial basis to yield a 81±6% (p<.001) classification accuracy across animals (78% in the example of Figure 3D). Most notable, when errors in classification did occur, they were not random but instead revealed assignments to orientations immediately adjacent to the target (i.e. –20˚ as opposed to the correct –30˚). This is reflected in minimal off-diagonal classifications shown in the example of Figure 3D. Thus, when the magnitude of errors were taken into account, they were smaller (2.3±4.5 degrees; p<.001) than expected from random classification (27±20 degrees).

Results presented thus far were from 3 animals with 25 macro-vibrissae contacting the stimulus. Yet, they indicated that temporal patterns of the SEP were sufficient for classifying stimulus orientation and that this classification was dominated by latency differences between the rows (thus, within the arcs) of barrels in the PMBSF (i.e. latency differences between rows A-E). To evaluate how well a single arc of vibrissae could perform, all vibrissae except B3-E3 were trimmed for subsequent experiments in 4 remaining animals (A3 was typically oriented too dorsal to make secure contact with the stimulus). In this preparation, a sweep speed of 0.4 mm/ms with the stimulus at 0˚ produced SEPs of largest amplitude over the principal barrels (Fig. 4B; darkened barrels), with some rostral and caudal spread within the PMBSF (Fig. 4B; black traces). It was assumed that this pattern was dominated by activity of barrels B3-E3. Therefore, to simplify the analysis, a model was constructed in which the spatial distribution of SEP amplitude (calculated at each electrode as the RMS for sample points covering the P1/N1 sharp wave) evoked by single vibrissa stimulation of B3-E3, was used to represent the contribution of each vibrissa throughout the recording array during multi-vibrissa stimulation. Thus, each time point of the multi-vibrissa SEP complex was modeled as a weighted combination of only four single vibrissa patterns (Fig. 4A). The model (Fig. 4B; green traces) closely fit the multi-vibrissa response complex and accounted for over 90% of the system variance across all stimulus conditions and animals (92±6.5%). Regression weights associated with each individual vibrissa map were used to represent the time course of activity in their respective barrels during a given stimulation condition (Fig. 4C; left traces). For example, regression weights associated with the 0˚ stimulus (Fig. 4C; left black traces) when multiplied times their respective single vibrissa RMS potential maps (Fig. 4A) and summed, produced a model SEP complex for this condition (Fig. 4B; green traces). Regression weights computed in the same way for +10˚ and –10˚ stimuli (Fig. 4C; left blue and red traces, respectively) had latency shifts of the P1/N1 wave preceding and following the 0˚ weights, with most pronounced shifts for the E3 vibrissa positioned at the greatest distance below the stimulus rotation axis, almost no shifts for the C3 vibrissa nearest the rotation axis, and a reversal of latency
shift for the B3 vibrissa positioned just above the rotation axis. Latency shifts for the four respective regression weights were quantified by computing their normalized cross correlation functions with those from 0° stimulation (Fig. 4C; right traces).

Given the known velocity of the stimulus passing through the vibrissa in the rostro-caudal direction, latency shifts for each vibrissa were converted to relative positions along the rostro-caudal axis where the vibrissa were contacted by stimuli of different orientations. This information, combined with measurements of the locations of the vibrissae on the dorso-ventral axis, permitted a direct estimate of the orientation of the stimulus during a particular stimulation condition. Figure 4D depicts actual (dashed lines) and reconstructed (solid lines) orientations for +10°, 0° and -10° stimuli (blue, black and red lines, respectively) and results for the other stimulus orientations (solid grey lines). Stimulus orientations predicted from temporal SEP responses differed significantly between conditions (multiple comparison test of means; p<.01) during both .4 mm/ms (Fig. 5A) and .2 mm/ms (Fig. 5B) sweep speeds, with an average error of 2.4 ±2.6 and 2.8 ±2.7 degrees, respectively. An advantage of this modeling method was that it allowed us to directly reconstruct any stimulus, without matching templates derived from known stimuli (except that of the 0° stimulus used to calibrate all relative latency shifts). Thus, not only the orientation of linear edges of different orientation could be reconstructed directly from the data, but the curvature of convex and concave stimuli could be estimated as well, as shown in two animals for sweep speeds of .4 mm/ms (Fig. 6A) and .2 mm/ms (Fig. 6B).

In contrast to stimulus pattern classification based purely on SEP latency as demonstrated so far, it was expected that excitatory and/or inhibitory interactions between principal barrels at different latency shifts could also yield unique patterns in the amplitudes of the SEP complex associated with each stimulus condition (i.e. enhanced or attenuated responses depending on latency shift between adjacent barrels). To examine the possibility that amplitude distributions could be used as templates for classification, we mapped the amplitude distributions of the SEP as a function of stimulus orientation. The SEP complex was first segregated into three fundamental frequency bands corresponding to 1-10 Hz (slow waves; Fig. 7A; blue), 10-200 Hz (emphasizing the P1/N1 sharp wave; Fig. 7A; red), and 200-1000 Hz (highlighting small amplitude fast oscillations; Fig. 7A; green). Maps depicted in Figure 7B for the averaged SEP for each stimulus orientation within each frequency band were then used as templates to classify individual trials based only on the similarity of their amplitude distribution with one of the averaged templates. Amplitude maps for the slow waves (Fig. 7B; lower row) appeared quite similar regardless of orientation, and classifications based on these templates did not
perform better than chance (15±8 and 13±8% for .4 and .2 mm/ms speeds, respectively; p>.05). In contrast, maps for the 10-200 Hz band that emphasized the P1/N1 sharp wave appeared to differ subtly between conditions (Fig. 7B; middle row). While classifications based on these templates differed significantly from chance (p<.01), their classification accuracy was modest (36±26 and 37±23% for .4 and .2 mm/ms speeds, respectively). Amplitude maps for the high frequency band, representing small amplitude fast oscillations, revealed the greatest differences between stimulus orientation (Fig. 7B; upper row). However, while classifications based on these templates also performed significantly better than chance (p<.01), their variability on single trials also yielded only modest classification accuracy (25±18 and 24±13% for .4 and .2 mm/ms speeds, respectively).

DISCUSSION

The present results demonstrate that spatiotemporal patterns of epipial SEPs in the PMBSF, evoked by brushing simple stimuli through the macro-vibrissae at speeds similar to those produced by natural whisking, may be used to determine the orientation of linear contours and the shape of large curved objects. The primary feature of the SEP complex supporting stimulus classification is the relative timing of responses between the barrels organized in arcs as opposed to rows within the PMBSF. A general model of SEPs evoked by stimulating vibrissae in a single arc can be used to reconstruct the orientation and shape of each stimulus. Finally, neural integration of temporal activation patterns in the PMBSF produces stimulus specific spatial patterns of SEP amplitude in the 10-100 Hz and 200-1000 Hz bands but not lower frequency bands (<10 Hz). When amplitude patterns alone are used to classify object features, classification performance is poor, but better than chance.

Studies of whisking dynamics in behaving animals indicate that on each forward extension, the vibrissae make sequential contact in the caudo-rostral direction with delays between each vibrissa of a row ranging from 10-20 ms (Sachdev et al. 2001). In this study, we assumed that with an average inter-vibrissa distance along a given row of 4 mm, sweep speeds of .4 - .2 mm/ms would approximate these naturally occurring delays. However, it is conceivable that during exploratory whisking, rats may occasionally group their vibrissae even closer together during forward extension. This possibility, combined with the fact that the rat and/or the object may be moving, suggests even shorter inter-vibrissa delays may occur. While faster sweep speeds were not tested here, stimulus classification should remain accurate even with delays in the millisecond or sub-millisecond range. This conclusion is based on the fact that discriminations were
accurate even for stimulus angles of ± 5° from upright, a circumstance that produced delays between barrels of an arc that were less than 1 ms (see Figure 4D; vibrissae D3-B3).

These data indicate that rapid temporal patterns of SEPs in the PMBSF are the essential parameter in our model for identifying object features. One might expect that synaptic interactions between the barrels of an arc would systematically influence the spatial pattern of SEP amplitude as well. Yet, amplitude maps of the lowest frequency components (1-10 Hz) of the SEP appear unchanged by stimulus orientation (Fig. 7B; bottom row) and perform no better than chance when used as templates for single trial classification. Similar maps of SEP amplitude in the 10-200 Hz frequency range, emphasizing the P1/N1 sharp wave, are clearly influenced by stimulus orientation (Fig. 7B; middle row) but also perform poorly for single trial classification. We recently demonstrated that high frequency (200-1000 Hz) FO are distinctly influenced by phase sensitive interactions between barrels in the sub-millisecond range and may provide a mechanism for high-speed coincidence detection in the PMBSF (Barth 2003). Indeed, spatial maps of FO amplitude measured here are markedly changed by stimulus orientation and typically reveal pairs of amplitude maxima with intervening minima suggestive of phase sensitive interactions between barrels (Fig. 7B; top row). Yet, when these amplitude patterns are used as templates for classification of single trials, their performance was no better than the sharp waves.

The failure of sharp wave, and particularly FO, amplitude patterns to accurately classify single trials may indicate that they do not directly reflect the mechanism for rapid spatiotemporal integration in the PMBSF. Alternatively, our negative results may indicate a sensitivity problem in single trial epipial SEP recordings, particularly for very low amplitude FO, and require further examination with microelectrode unit recording. Laminar recordings have shown that the SEP recorded at the cortical surface is produced by synchronized post-synaptic currents in the aligned apical dendrites of supragranular pyramidal cells (generating the P1 due to proximal depolarization), followed by distal depolarization of apical dendrites of both supra- and infragranular pyramidal cells extending near the cortical surface (generating the N1) (Di et al. 1990; Kulics and Cauller 1986; Kulics and Cauller 1989). The laminar potential pattern conforms to a vertical current dipole for the supra- and infragranular pyramidal cell groups, with polarity reversals in the upper and middle cortical layers, respectively. While field potentials are volume conducted, the strength of the field potential from a current dipole declines with the inverse square of distance from the source (Nuñez 1981). With epipial electrodes, the recording distance from the distal apical dendrites is small (the thickness of the pia), thus potentials from any distance greater than
this are negligible. Evidence for this is the fact that the laminar potential pattern reverses polarity (as expected from a current dipole) at depths as little as 200 µm from the surface for the P1 and at approximately 500 µm depth for the N1 (Di et al. 1990). An advantage of field potential measures is that they show the central tendency of the response of populations of neurons. A disadvantage is that epipial field potentials do not discriminate between supra- and subthreshold post synaptic potentials, and their spatial resolution is not sufficient to discriminate between activation of sub-populations of cells within a barrel. Because they reflect central tendency, it is reasonable to anticipate from field potentials the general response properties of units within a population. For example, the P1 and rising phase of the N1 are closely associated with excitation and cell firing, and the falling phase of the N1 and subsequent slow waves are associated with fast and slow inhibition and with cessation of firing in many units (Purpura 1959; Steriade 1984). Conversely, because unit recording is much more sensitive to distinct responses of individual cells, it is not easy to anticipate what field potentials they will be associated with. Unit studies have mainly examined multi-whisker integration between barrels along the rows of the PMBSF (Carvell and Simons 1988; Shimegi et al. 2000; Shimegi et al. 1999; Simons 1985). However, there have been several reports of supra-linear unit responses when barrels along an arc are sequentially activated with inter-stimulus intervals in the millisecond range similar to timing of barrel activation reported here (Ego-Stengel et al. 2005; Ghazanfar and Nicolelis 1997). Supra-linear responses within the barrel arcs may well reflect integration of object features at the cellular level that cannot be reliably recorded in the epipial field potential.

A single arc of four vibrissae is sufficient to reconstruct not only the orientation of straight edges, but also the curvature of large contours. In this context, an analogy between the macro-vibrissa system and peripheral vision may be appropriate. The macro-vibrissae provide a coarse analysis of an objects’ features (Harvey et al. 2001; Polley et al. 2005), perhaps for more detailed examination by the rostral micro-vibrissae (Brecht et al. 1997), and due to their length, they provide an early warning of object presence for head centered orientation. Yet, it is conceivable that during active “discriminative whisking”, the macro-vibrissae provide even more detailed information than this (Harvey et al. 2001). Sensitivity to orientation changes as small as 5° may actually represent a lower bound on the potential precision of the barrel system given that our model reconstructions were based on epipial field potentials that are no doubt insensitive to more precise temporal patterns recordable at the unit level. We have also ignored the fact that the vibrissae possess a direction sensitivity which could increase their capacity to discriminate form, not based on timing but on directionally sensitive input from each vibrissa (Bruno et al. 2003; Lee and Simons 2004; Simons and Carvell 1989; Wilent and
Contreras 2005). Furthermore, it should be noted that we held constant the distance between the vibrissae on the dorso-ventral axis, a limitation not imposed on the behaving animal. The ability to resolve curves of differing spatial frequency (i.e. number of curves per mm or curvature sharpness) must depend on the spatial sampling frequency of the vibrissae. This is directly analogous to time series analysis, where time varying signals must be sampled at half their shortest period to be resolved. The present experiment held the vibrissae at average dorso-ventral distances of approximately 5 mm, imposing a spatial Nyquist frequency of .1 curves/mm, for want of a better term. This spatial resolution could be improved by decreasing the distance between the vibrissae, an act that may be within the unanaesthetised rat’s behavioral repertoire. Rats have been shown to alter their whisking movement strategies during discriminative task acquisition. Parameters changed include frequency, velocity, amplitude, duration and the amount of whisking (Harvey et al. 2001). The present results suggest that rats may also modify the vertical distance between vibrissae depending on the required spatial resolution of a task.

Our data indicate that rapid temporal interactions between barrels in a single arc are useful for feature extraction in our model and may represent a distinct processing module in the PMBSF. This result is consistent with the expectation that changes in stimulus orientation (and shape) should produce maximum timing differences between vibrissae in an arc versus row during caudo-rostral whisking. Given that a single vibrissa arc can be used for stimulus reconstructions described here, one might wonder why there are five such arcs, with corresponding barrels preferentially interconnected along the rows of the PMBSF (Bernardo et al. 1990; Hoeflinger et al. 1995). Indeed, SEPs evoked by 25 vibrissa stimulation suggest substantial intra-row integration, with a majority of barrels sensitive to sequential activation of the arcs during a rostro-caudal sweep (Fig. 2F). One possibility is that the aggregate barrels in the PMBSF may function as a spatial frequency analyzer. It has been noted that vibrissa length increases exponentially from the rostral to caudal arcs (Brecht et al. 1997). If the entire vibrissa array were adjusted to a common inter-vibrissa angle emerging from the mystacial pad on the vertical axis, systematic differences in length would establish different spatial sampling frequencies for the arcs, with the longest caudal vibrissae sensitive to the lowest frequencies (gradual curvatures), and progressively more rostral and shorter vibrissae sensing higher spatial frequencies (finer detail). In this way, intra-row processing within the PMBSF may integrate a complementary series of spatial frequencies in a single whisk.
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FIGURE LEGENDS

Figure 1. The stimulating apparatus. (A) A smooth plexiglass cylinder (140 mm diameter) was fitted with a straight wire (70 mm length; 0.8 mm diameter) mounted to the outer surface and held at various fixed angles with a thumb screw. The animal was mounted in a nose clamp such that the vibrissae on the left mystacial pad made contact with the cylinder as it repeatedly swept the stimulus in a rostro-caudal direction through the vibrissae. A vertical tab was mounted to the inside of the cylinder that interrupted an infrared photocell emitter/detector to trigger data sampling for each trial. The position of the beam interrupter was adjusted so that data sampling began approximately 50-100 ms before the stimulus made contact with the most rostral vibrissae. The cylinder was kept in constant motion during the collection of trials for a given stimulus orientation. (B) Whiskers of the left face were trimmed to approximately 2 cm such that they made light and continuous contact with the smooth cylinder and were displaced about 5˚ caudally during rotation. The stimulus was manually positioned at various angles in relation to upright (0˚ in the sagittal plane) using calibrated visual markers. Angles typically included ±45˚, 30˚, 20˚, 10˚, and 5˚. In two animals, a curved (25 mm diameter hemicircle) wire was also positioned such that its concave or convex contour faced the direction of rotation.

Figure 2. Somatosensory evoked potentials (SEP) recorded from the posterior medial barrel subfield (PMBSF) during 25 vibrissa stimulation. (A) The 25 macro-vibrissae are arranged in 5 rows labeled A-E and arcs labeled 1-5. According to this labeling scheme, the vibrissae in the third arc consist of A3, B3, C3, D3 and E3. (B) Vibrissae are somatotopically represented as discrete cortical columns in the PMBSF with similar rostro-caudal but inverted dorso-ventral organization. (C) Averaged SEPs recorded from the PMBSF and evoked by repeatedly sweeping a vertically oriented straight edge (0˚) caudally through the contralateral vibrissae at a speed of .4 mm/ms. The SEP (example highlighted with an oval is enlarged in E) consisted of a biphasic sharp wave labeled “P1/N1” to reflect the polarity and sequence of its components. (D) When stimulus sweep speeds were slowed to .2 mm/ms, the SEP morphology changed. To better visualize these traces, 4 responses from electrodes near the D row are enlarged in F (grey). Similar traces near the E (blue), C (red), B (green) and A (purple) rows are superimposed for comparison. Rostral electrodes (a) responded with an initial P1/N1 sharp wave, but continued with smaller responses at 20 ms intervals as vibrissae in subsequent caudal arcs were contacted. SEPs in the middle of the PMBSF (b-c) were multi-phasic, reflecting sequential activation from multiple vibrissa arcs. The caudal SEPs (d) were dominated by a late sharp wave. A similar response pattern was recorded in the other rows.
Figure 3. Single trial stimulus classification based on latency maps of 25 vibrissa SEPs. (A) Composite latency maps, averaged across all stimulus orientations during .4 mm/ms (left) and .2 mm/ms (right) stimulus sweep speeds. These maps reflect the spatial distribution of normalized SEP latency shifts (their absolute values) produced by linear stimuli of different orientations. The dorsal and ventral areas of the PMBSF consistently showed the greatest orientation specific latency shifts, with minimal shifts in the middle barrels responding to vibrissae nearest the axis of stimulus rotation. (B) SEPs from an electrode in the ventral (left) and dorsal (right) PMBSF during −10, 0°, and 10° stimulation (red, black and blue traces). All latency shifts were computed in relation to responses to 0° (vertical) stimuli. The sharp wave preceded and followed 0° responses in ventral and dorsal sites (respectively) during −10° stimulation. The opposite temporal pattern was produced by +10° stimulation. (C) SEP latency shifts at each electrode site were quantified by computing the normalized cross-correlation function between responses of a given stimulus orientation and those evoked by 0° stimulation. Autocorrelations of SEPs from 0° stimulation define 0 ms latency shift (black traces). (D) Latency maps for each trial at a given stimulus orientation were classified by comparing them to templates, computed by separately averaging the latency maps for each orientation. Classification errors typically involved assignments to immediately adjacent orientations, resulting in off diagonal classifications.

Figure 4. Stimulus reconstruction based on latency shifts of 4 vibrissa SEPs. (A) Transient stimulation of each vibrissa (B3-E3) evoked SEPs with maximum root mean squared (RMS) amplitude (summed over the time course of the P1/N1 sharp wave) centered on corresponding barrels in the PMBSF. These responses were used to align the recording array consistently across animals and to align a template of the PMBSF for graphical display. Each map was also used to represent the spatial contribution of a single vibrissa to subsequently evoked multi-vibrissa SEPs. (B) In this example, each time point of SEPs evoked by 0° stimulation at .4 mm/ms (black traces) was modeled (green traces) as a linear combination of the 4 single vibrissa maps shown in A. (C) Modeling reduced the 64 channel data to 4 waveforms that were composed of the regression weights for each vibrissa map over the time course of the SEP. Thus, SEPs from 0° stimulation resulted in 4 regression waveforms (left; black traces) that, when multiplied times their respective single vibrissa maps (A) and summed, produced a model (B; green traces) accounting for over 90% of the variance of the actual SEP complex (B; black traces). Similar regression weights for +10° stimulation (left; blue traces) peaked earlier than those
of 0° in vibrissae E3 and D3 (positioned approximately 8 and 3 mm ventral to the axis of stimulus rotation, respectively), were nearly coincident in vibrissa C3 (nearest the stimulus axis and thus little changed by orientation), and peaked slightly later than 0° in B3 (positioned approximately 2 mm dorsal to the stimulus axis). An opposite pattern of latency shifts in the regression weights was produced by -10° stimuli (left; red traces). Latency shifts relative to responses from 0° stimulation were derived from the lags of maximum cross correlation functions applied to the regression weights (right traces). (D) Estimates of the latency shifts of the 4 vibrissae as they contacted stimuli of a given orientation were combined with information about the vertical location of each vibrissa in relation to the axis of stimulus rotation, and with information about the stimulus sweep speed, to directly estimate horizontal displacements of each vibrissa’s point of contact. These points were connected to reproduce the shape and orientation of the stimulus (shown in red, black and blue for −10°, 0° and +10° orientations, respectively, and grey for the other orientations). Dashed lines indicate actual stimulus orientations.

Figure 5. Reconstructed stimulus orientations from 4 vibrissa SEPs of all animals. (A) Boxes have lines at the lower quartile, median, and upper quartile values of orientations derived from stimuli reconstructed at .4 mm/ms sweep speeds. Bars extending from each end of the box show the extent of the rest of the data. Notches flanking the median lines represent a robust estimate of the uncertainty about the medians for box-to-box comparisons. Boxes whose notches do not overlap indicate that the medians of the two groups differ at the 1% level of significance. Medians across all conditions differed significantly. (B) Same as A, but for .2 mm/ms sweep speeds.

Figure 6. Reconstructed stimulus curvature from 4 vibrissa SEPs of two animals. (A) Reconstructed (black) and actual (grey) stimuli from vertically oriented concave (dashed) and convex (solid) curved edges (diameter = 25 mm) swept at .4 mm/ms. (B) Similar reconstructions from a different animal with .2 mm/ms sweep speeds.

Figure 7. Amplitude patterns of 4 vibrissa SEPs in different frequency bands with .4 mm/ms sweep speeds. (A) The wideband (black; .1-3000 Hz) SEP was separated into low (blue), middle (red) and high (green) frequency bands (1-10, 10-200 and 200-1000 Hz, respectively). Middle frequencies in the 10-200 range emphasized the P1/N1 sharp wave, whereas higher frequencies emphasized fast oscillations (FO) superimposed on the P1 sharp wave. (B) Normalized RMS
amplitude maps of low frequency SEPs (bottom row) showed little difference between stimulus orientations. Middle and high frequency maps (middle and top rows, respectively) showed clear differences in amplitude distributions between conditions. These differences were particularly evident for higher frequencies, where the maps displayed two distinct amplitude maxima separated by low amplitudes, suggesting phase sensitive additive and subtractive interaction patterns between FO produced by timing differences of the SEP in different barrels.
Figure 1
Figure 5

(A) 4 mm/ms

(B) 2 mm/ms
Figure 7

A) Graph showing waveforms with markers PI and N1, and various frequency bands.

B) Heatmaps with orientation in degrees and normalized RMS values.