Spatio-Temporal Subthreshold Receptive Fields in the Vibrissa Representation of Rat Primary Somatosensory Cortex

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Moore, Christopher I. and Sacha B. Nelson. Spatio-temporal subthreshold receptive fields in the vibrissa representation of rat primary somatosensory cortex. J. Neurophysiol. 80: 2882–2892, 1998. Whole cell recordings of synaptic responses evoked by deflection of individual vibrissae were obtained from neurons within adult rat primary somatosensory cortex. To define the spatial and temporal properties of subthreshold receptive fields, the spread, amplitude, latency to onset, rise time to half peak amplitude, and the balance of excitation and inhibition of subthreshold input were quantified. The convergence of information onto single neurons was found to be extensive: inputs were consistently evoked by vibrissa one- and two-away from the vibrissa that evoked the largest response (the “primary vibrissa”). Latency to onset, rise time, and the incidence and strength of inhibitory postsynaptic potentials (IPSPs) varied as a function of position within the receptive field and the strength of evoked excitatory input. Nonprimary vibrissae evoked smaller amplitude subthreshold responses [primary vibrissa, 9.1 ± 0.84 (SE) mV, n = 14; 1-away, 5.1 ± 0.5 mV, n = 38; 2-away, 3.7 ± 0.59 mV, n = 22; 3-away, 1.3 ± 0.70 mV, n = 8] with longer latencies (primary vibrissa, 10.8 ± 0.80 ms; 1-away, 15.0 ± 1.2 ms; 2-away, 15.7 ± 2.0 ms). Rise times were significantly faster for inputs that could evoke action potential responses (suprathreshold, 4.1 ± 1.3 ms, n = 8; subthreshold, 12.4 ± 1.5 ms, n = 61). In a subset of cells, sensory evoked IPSPs were examined by deflecting vibrissa during injection of hyperpolarizing and depolarizing current. The strongest IPSPs were evoked by the primary vibrissa (n = 5/5), but smaller IPSPs also were evoked by nonprimary vibrissae (n = 8/13). Inhibition peaked by 10–20 ms after the onset of the fastest excitatory input to the cortex. This pattern of inhibitory activity led to a functional reversal of the center of the receptive field and to suppression of later-arriving and slower-rising nonprimary inputs. Together, these data demonstrate that subthreshold receptive fields are on average large, and the spatio-temporal dynamics of these receptive fields vary as a function of position within the receptive field and strength of excitatory input. These findings constrain models of suprathreshold receptive field generation, multivibrissa interactions, and cortical plasticity.

INTRODUCTION

The rodent primary somatosensory cortex (SI) contains a detailed representation of the vibrissae on the face. For each vibrissa, there is a corresponding cluster of layer IV neurons in SI (a “barrel”) (Woolsey and van der Loos 1970). This unique anatomic homology in layer IV has inspired a number of physiologic studies of action potential

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METHODS

Surgical methods

Adult Sprague-Dawley rats (190–330 g) were anesthetized with urethane (20% in saline solution, 1.25 g/kg ip initial dose). Dexamethasone 8 mg/kg ip was given to reduce pial and cortical swelling (Istvan and Zarzecki 1994; Iwata and Zarzecki 1994), and atropine 8 µg/kg ip was given to reduce respiratory secretions. During the experiment, a 0.125 g/kg supplement of urethane was administered if needed to maintain a level of anesthesia at which animals were unresponsive to hind-paw pinch and had a breathing rate of 80–120 breaths/min (Armstrong-James and Callahan 1991). Core body temperature of 36°C was maintained with a servo-controlled heating blanket and rectal thermometer (Harvard apparatus).

After induction of anesthesia, the animal was placed in a stereotaxic device on an air table, and the right scalp and temporal muscle were resected. Using a dremel tool bit, the skull over the vibrissae representation was thinned until translucent, allowing an accurate reconstruction of the cortical vasculature. Using the vascular pattern as a guide, a small (1–3 mm) craniotomy and durotomy were made over the right SI vibrissa representation at ~2 mm posterior and 5.5 mm lateral to bregma (Armstrong-James et al. 1992; Simons and Carvell 1989). To improve recording stability, cerebrospinal fluid was removed through the foramen magnum. The craniotomy and durotomy were enlarged later in the experiment to allow for further electrode penetrations.

Whole cell recording techniques

All recordings reported here were obtained using “blind” in vivo whole cell recording techniques (Ferster and Jaggadeesh 1992; Nelson et al. 1994). Recordings were made in bridge mode (Axoclamp 2A amplifier), and the bridge balance was adjusted to compensate series resistance. Whole cell recording pipettes were lowered through the cortex with a hand-operated Leitz micromanipulator [electrode resistance, 5–7 MΩ; tip width, 2 µm; recording solution contained (in mM) 120 potassium gluconate, 20 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 1 ethylene glycol-bis(β-aminoethoxy ether)-N,N,N’,N’-tetraacetic acid, 10 KCl, 5 MgSO4, 3 ATP, and 1 GTP]. A current step (−440 nA, 500 ms, 0.5 Hz) was passed through the electrode tip while descending through the cortex. Penetrations continued until an increased resistance was observed, at which time back pressure on the electrode tip was removed, and a high resistance seal (1–10 g) was allowed to form spontaneously or with application of gentle suction. Additional suction then was used to achieve whole cell recording (Fig. 1). Neurons with a resting membrane potential of less than −50 mV and a spike height of >20 mV were accepted for analysis [Rc = 87.2 ± 68.5 (SD) MΩ]. Recordings lasted 3–8 h.

Electrode depth was measured as the electrode tip progressed through the cortex. Based on cytochrome oxidase staining in coronal section (60 µm; n = 5 animals) (A. Basu and C. Moore, unpublished observations) and correspondence with the Paxinos and Watson (1986) rat atlas, we made an adjustment for the angle of the penetrating electrode (electrode depth was multiplied by 0.86). This calculation was confirmed by biocytin staining in two neurons from which intracellular recordings were obtained and in five small extracellular deposits of biocytin made using whole cell electrodes (data not shown). We could not estimate accurately the depth in 16/45 neurons because of difficulty in precisely determining the point of contact with the surface of the cortex. The segmentation of cortical depths into supragranular (0–500 µm), granular (500–850 µm), and infragranular divisions (>850 µm) was made from cytochrome oxidase-stained sections.

Classification of the intrinsic firing properties of neurons were conducted as previously described (Agmon and Connors 1992; Istvan and Zarzecki 1994; McCormick et al. 1985; Pockberger 1991). Briefly, on depolarization with current injection, intrinsically bursting (IB) neurons demonstrated a burst of two to four action potentials riding on a broad triangular depolarization; fastspiking (FS) neurons demonstrated a high-frequency (>150 Hz) train of nonadapting action potentials with pronounced afterhyperpolarizations; and regular-spike neurons (RS) demonstrated a train of slowly adapting action potentials.

Receptive-field mapping

Sensory stimuli were provided by a Chubbuck stimulator (Chubbuck 1966) (n = 8 recordings) and a piezoelastic bimorph wafer attached to a thin-wall glass capillary (Simons 1983; M. Armstrong-James, personal communication) (n = 16). Both stimulators employed a 100- to 300-ms stimulus of 1 mm (initial rate of rise: Chubbuck = 120 mm/s; piezoelastic = 100 mm/s). To prevent ringing in the piezoelectric stimulus, the voltage step driving the piezoelastic stimulator was filtered through an RC filter (τ = 10 ms), and a section of sponge that expanded in 10 ms was applied over the right SI vibrissa representation at ~1 cm and the deformation point of the vibrissa was established at 5–7 mm from the base of the vibrissa. After entering the cell and establishing its intrinsic firing properties, the center of the receptive field was determined using a hand-held probe and prepositioned stimulators. In nine experiments, sensory-evoked field potentials were recorded before whole cell recording to preposition stimulus(s) in the center of the receptive field accurately. For quantitative receptive field mapping, each vibrissa was stimulated a minimum of 10 times at 2-s intervals (Armstrong-James et al. 1993). Up to 18 vibrissae were mapped quantitatively during a single neuronal recording.

The latency to the initial postsynaptic potential (PSP) was measured from the onset of vibrissa deflection to the first positive deviation from resting membrane potential. The amplitude of evoked subthreshold responses was measured as the peak depolarization of the average response subtracted from baseline (the period 1–5 ms before vibrissa deflection). The primary vibrissa was defined as the vibrissa that evoked the largest depolarization from resting membrane potential. If action potentials were evoked by vibrissa deflection, subthreshold amplitude was assessed as the peak of the postsynaptic potential not including the amplitude of the action potential. The onset of the action potential was defined by its fast depolarizing rise time relative to the rise of the postsynaptic potential. Suprathreshold responses were defined as action potentials evoked within 100 ms of deflection of onset (Armstrong-James et al. 1991) on a minimum of 20% of trials after subtracting the probability of spontaneous action potential firing during the period 100 ms before deflection (spontaneous action potential firing was relatively rare in our sample and was not present in the baseline period of neurons possessing action potential receptive fields). Rise time to half-maximal depolarization was measured as the time from onset of the evoked response to the point one-half the amplitude of the peak depolarization. For analyses requiring identification of a primary vibrissa, only neurons in which four or more vibrissae were quantitatively mapped were analyzed. This criterion was used to further ensure that the center of the receptive field had been identified. Probability statistics are reported for two-tailed t-test comparisons except where otherwise noted.

RESULTS

We obtained stable whole cell recordings from 45 neurons in 20 animals (mean resting membrane potential, −62.1 ± 9.2 mV; mean spike height, 52.5 ± 16.6 mV). Neurons in
FIG. 1. Seal formation and intrinsic firing properties. A: current-clamp recording before and immediately after (+) break through to achieve whole cell recording configuration. Initial seal resistance was > 9 GΩ. Note resting membrane potential (−55 mV) and spontaneous synaptic potentials after break through. Scale bar 100 ms, horizontal and 10 pA and 10 mV, vertical. Right traces: examples of an intrinsically bursting (B) and a regular-spiking neuron (C). Scale bar 100 ms, horizontal, and 500 pA and 10 mV, vertical.

this population exhibited RS (n = 35), IB (n = 9), and FS (n = 1) firing characteristics (Fig. 1) (Agmon and Connors 1992; Istvan and Zarzecki 1994; McCormick et al. 1985; Pockberger 1991). Approximately one-third of the neurons classified as RS (n = 13) also fired a burst-like cluster of action potentials riding on a broad depolarization (Amitai and Connors 1995; Friedman and Gutnick 1987; Istvan and Zarzecki 1994; Montoro et al. 1988). This bursting mode usually was observed during periods of membrane oscillations that are common during urethane anesthesia (0.2–5 Hz) (Wilson 1993). Two neurons demonstrated prolonged, complex action potentials suggestive of dendritic recordings (data not shown) (Amitai and Connors 1995; Kim et al. 1995). These neurons were not included in receptive field analyses.

Receptive field analyses

We quantitatively assessed the vibrissa receptive field in 24 neurons and 126 vibrissae. In four neurons, including eight vibrissae, we did not observe subthreshold or supratreshold responses within the vibrissa field. For the 20 neurons in which we observed an evoked response, 96 of 118 (81.4%) vibrissae demonstrated subthreshold receptive field inputs (Fig. 2, gray bars within histogram). Supratreshold responses were observed in 7 of 20 neurons (35%) quantitatively mapped and in 13 of 118 vibrissae (10.2%; Fig. 2, black bars within histogram). There was no significant difference in the mean resting membrane potential of neurons with supratreshold and purely subthreshold receptive fields [supratreshold, −60.2 ± 2.0 (SE) mV; subthreshold, −61.6 ± 3.1 mV; P > 0.10], and both populations readily fired action potentials in response to injection of depolarizing current.

Extensive subthreshold receptive fields were observed in
neurons throughout the depth of rat SI. An example of a subthreshold receptive field recorded at a depth of 645 μm, corresponding to layer 4, is shown in Fig. 3. In this neuron, responses were evoked from 10 of 10 vibrissae stimulated, and deflection of vibrissae more than two-away from the primary vibrissa (e.g., the D4) evoked consistent PSPs. To assess the relative strength of input throughout the receptive field, we normalized the amplitude of surround vibrissa responses to the response of the primary vibrissa (for a similar approach applied to suprathreshold receptive fields, see Armstrong-James et al. 1991). The majority of neurons had receptive fields with a single peak and response amplitudes that diminished with increasing distance from the peak (n = 14/15 receptive fields in which ≥4 vibrissae were assessed, including n = 6 suprathreshold and n = 8 exclusively subthreshold receptive fields). The single exception to the single-peaked receptive field pattern was an infragranular bursting neuron (depth = 860 μm) that showed two distinct peaks (the D4 and the B3) with a trough of nonresponsive vibrissae between them. Similar multifoci receptive fields have been reported for extracellular recordings from infragranular neurons (Armstrong-James and Fox 1987; Armstrong-James et al. 1992; Chapin 1986; Chapin and Lin 1984; Innocenti and Manzoni 1972). Because we were unable to define a primary vibrissa for this neuron, it was excluded from group analyses described later.

Stimulation of vibrissae one- and two-away (in the same row, arc, or diagonal) from the primary vibrissa consistently evoked sensory responses [Fig. 4A; primary vibrissa, 9.1 ± 0.84 (SE) mV, n = 14; 1-away, 5.1 ± 0.5 mV, n = 39; 2-away, 3.7 ± 0.59 mV, n = 20; P < 0.001 for 2-away]. The response to stimulation three vibrissa away was not, over the population tested, significantly different from baseline [1.3 ± 0.70 (SE) mV; P > 0.10], although our limited sample (n = 8) included clear subthreshold responses from vibrissa three-away in neurons from the supragranular, granular, and infragranular depths (e.g., the D4 vibrissa input in Fig. 3). To assess the spatial organization of subthreshold receptive field input, we normalized as described above and averaged across vibrissae (n = 14 neurons and 61 vibrissae). Previous mapping studies of cortical and thalamic suprathreshold receptive fields (Armstrong-James et al. 1992; Chapin 1986; Lee et al. 1994) have reported a bias toward elongation of the receptive field within a vibrissa row. We compared the spread of subthreshold signal from the PV to the neighboring vibrissae along the row, arc, and diagonal directions using a one-way analysis of variance. This analysis revealed a significant difference between within row and within diagonal spread of signal [1-away within row, 67.8 ± 6.8% (mean ± SE); within arc, 51.9 ± 9.3%; within diagonal, 41 ± 6.1%; P < 0.05, Tukeys HSD], but no significant difference between within row and within arc (P > 0.10, Tukeys HSD).

Latency and rise time of subthreshold inputs

Subthreshold latencies after peripheral stimulation varied with cortical depth as previously reported (Armstrong-James et al. 1992; Carvell and Simons 1988). The shortest latencies (≤8 ms) arose from inputs to the granular and the middle of the infragranular depths (Fig. 5), the primary termination zones of ventral posterior medial nucleus thalamocortical inputs (Keller 1995; Koralek et al. 1988). Latency to onset was more rapid for primary than for nonprimary vibrissa inputs [primary vibrissa, 10.8 ± 0.80 (SE) ms, n = 14; 1-away, 15.0 ± 1.2 ms, n = 35; 2-away, 15.7 ± 2.0 ms, n = 16; P < 0.05; Fig. 5]. The most rapid latency input was not, however, always or exclusively from the primary vibrissa: In 10 neurons, the onset latency to the primary vibrissa occurred within 1 ms of onset of input from a neighboring vibrissa or after the onset of another input. The rise time needed to reach one-half amplitude and the initial slope of the sensory response were measured across vibrissae. A significant correlation (r² = 0.44; P < 0.05;
PSP onset and the rise time, and no correlation ($R^2$ = 0.46) between the expected reversal potentials for excitatory (-45 mV) and inhibitory (about -60 mV) currents. At -30 mV, the driving force on excitatory and inhibitory conductances was assumed to be approximately equal. Our experiments did not permit isolation of pure EPSPs and IPSPs, and the presumed reversal potentials of 0 and -60 mV are necessarily approximate. Nevertheless, this method provided an estimate of the relative magnitude of inhibitory and excitatory conductances evoked at various times after stimulation of vibrissa throughout the receptive field. Figure 8 depicts these

Inhibitory and excitatory subthreshold receptive fields

To assess excitation and inhibition within the subthreshold receptive field, vibrissae were deflected while depolarizing and hyperpolarizing current was injected into the neuron ($n = 5$ neurons). In all five neurons, inhibitory postsynaptic potentials (IPSPs; defined as evoked responses that reversed at a membrane potential of about -60 mV or greater) were evoked in both primary ($n = 5$/5 vibrissae) and nonprimary vibrissae ($n = 8$/13). Figure 7 (top 2 sets of evoked responses) displays responses in a supragranular neuron during stimulation of the D2 and D3 vibrissae. After deflection of either vibrissa, an initial excitatory postsynaptic potential (EPSP) followed by an IPSP is revealed in the depolarized traces. In the case of the D3 input, the initial EPSP was sufficient to elicit an action potential (see Fig. 7A, top, D3 deflection). These recordings were used to reconstruct the membrane reversal potential at different latencies after vibrissa deflection. For D3, the projected reversal potential 1–3 ms after the onset of the PSP was -10 mV, close to that expected for a glutamatergic EPSP (black circles and black line, Fig. 7B, right). At 10–11 ms (dark gray circles) and 20–21 ms (white circles) after onset of the PSP, the reversal potential was -60 and -62 mV, respectively, indicating the dominance of an inhibitory, $\gamma$-aminobutyric acid-A (GABA$_A$)-mediated IPSP. This transition from excitation to inhibition was detailed in Fig. 7C, where the reversal potential was plotted for both D2 and D3 responses as a function of time. While both vibrissae evoked strong inhibition that began within 3 ms of the onset of excitation, the stronger initial response of the D3 vibrissa permitted a window within which action potential firing could occur before IPSPs reached peak amplitude, whereas the D2 input was unable to fire an action potential before the onset of inhibition.

To assess the evolution of excitation and inhibition within the receptive field, we measured the amplitude of evoked responses at or projected to an initial membrane potential of -30 mV, a membrane potential approximately half-way between the expected reversal potentials for excitatory (-0 mV) and inhibitory (about -60 mV) currents. At -30 mV, the driving force on excitatory and inhibitory conductances was assumed to be approximately equal. Our experiments did not permit isolation of pure EPSPs and IPSPs, and the presumed reversal potentials of 0 and -60 mV are necessarily approximate. Nevertheless, this method provided an estimate of the relative magnitude of inhibitory and excitatory conductances evoked at various times after stimulation of vibrissa throughout the receptive field. Figure 8 depicts these
FIG. 6. Relationship between rise time and latency in suprathreshold and subthreshold inputs. A: latency to onset and amplitude are plotted as a function of rise time to half-maximal response. ○, inputs that evoked suprathreshold responses. There was a significant correlation between latency to onset and rise time ($r^2 = 0.44$) but no correlation between amplitude and rise time ($r^2 = -0.02$). B, left: rise time to half-maximal and slope are shown for suprathreshold and subthreshold evoked responses. Subthreshold inputs had significantly longer rise times and lower slopes (rise time to half-maximal; suprathreshold, 4.1 ± 1.3 (SE) ms; subthreshold, 12.4 ± 1.5 ms; slope, suprathreshold, 2.57 ± 0.58 mV/ms; subthreshold, 0.46 ± 0.14 mV/ms; *$P < 0.01$). Right: evoked responses from 2 vibrissae (C3 and C4) with identical latency to onset (11 ms). Faster rising C3 response (gray traces, 4 ms to half amplitude; 1.6 mV/ms slope) evokes a suprathreshold response, while the more slowly rising C4 response (black traces, 20-ms rise time to half amplitude; 0.24 mV/ms slope) remains subthreshold.

maps for a supragranular (top; same neuron as in Fig. 7) and an infragranular neuron (bottom). As both examples illustrate, the strongest inhibitory input was centered on the vibrissa that evoked the strongest excitatory input (i.e., those vibrissa capable of firing an action potential in the nondepolarized state). The inhibition had a delayed onset, but quickly overpowered any concurrent excitation, leading to a functional inversion of the center of the receptive field, with inhibition reaching full amplitude at 10–20 ms after the onset of the fastest excitatory response. Stimulation of surrounding vibrissa that previously evoked only subthreshold PSPs could, on depolarization, evoke action potentials (e.g., the 10 mV upward light gray columns without a dot in the infragranular neuron map). Unlike responses to primary vibrissae, responses to surrounding vibrissae did not always contain prominent inhibitory components. In addition to rapid EPSPs and IPSPs, some responses contained late excitatory components that were more variable in duration and amplitude and that were more prominent at depolarized membrane potentials (Fig. 7, D2 and D3 depolarized traces). The appearance of IPSPs at only a subset of surround vibrissae and the occurrence of later or longer-duration EPSPs at other vibrissae led to a spatial asymmetry in the temporal progression of excitation and inhibition throughout the receptive field.

**DISCUSSION**

**Organization of subthreshold receptive fields**

Subthreshold inputs to rat SI neurons could be evoked from a large number and a wide spatial extent of vibrissae. Stimulation one- and two-vibrissa away from the primary vibrissae consistently evoked prominent synaptic responses. Further, subthreshold receptive fields spanning three vibrissae from the primary vibrissae were observed at depths corresponding to supragranular, granular, and infragranular layers. In the context of the vibrissa map, our data predict that the population of neurons within the cortical column corresponding to the C3 vibrissa integrate information from the A5 to the E1 vibrissae, and from ≥25 vibrissae within the vibrissa pad. From the perspective of divergence of information entering the cortex, subthreshold input from a single vibrissa spreads over at least five rows and arcs of cortical vibrissa columns.

The extensive subthreshold receptive fields we observed are in agreement with recent intracellular recordings in the rat SI forepaw representation (Li and Waters 1996), which demonstrate multidigit subthreshold receptive fields, and in the rat vibrissa representation (Zhu and Connors 1994; personal communication; see also Istvan and Zarzecki 1994 for similar receptive fields recorded in raccoon SI). In both
FIG. 7. Mapping inhibitory and excitatory subthreshold receptive fields. A: D2 (left) or the D3 vibrissa (right) was deflected during depolarizing and hyperpolarizing current injection in a supragranular neuron. In response to deflection of either vibrissa, an excitatory postsynaptic potential (EPSP) followed by an inhibitory postsynaptic potential (IPSP) is revealed in the more depolarized current traces. An additional long-latency depolarizing potential is apparent in the depolarized traces from both sets of responses. This response occurred at a latency of ~100 ms after the onset of stimulation and may reflect voltage-dependent activation of N-methyl-D-aspartate (NMDA) receptors or active inward conductances. B: hyperpolarizing or depolarizing amplitude of evoked responses is plotted as a function of the initial membrane potential. Responses were measured 1–3 ms (black line, black circles), 10–11 ms (dark gray line, dark gray circles), and 20–21 ms (light gray line, white circles) after response onset. C: PSP reversal potential for the D2 (gray circles) and D3 vibrissa (white circles) plotted at 1- and 2-ms intervals postonset of response.
studies, animals were anesthetized with pentobarbital sodium, suggesting that large subthreshold receptive fields are not unique to urethan anesthesia. Further, the stimulus parameters we employed were identical in amplitude to those previously employed in suprathreshold studies of the vibrissa representation (Simons 1985), suggesting that the divergence of information we observed was not the result of artificial stimulation of neighboring vibrissae.

The predominance of purely subthreshold receptive fields within our sample may result from several factors. Although all neurons readily fired action potentials after current injection, our intracellular recordings may have damaged the spike initiation machinery of the neuron, thereby limiting output to only the most robust responses. Our findings also may result from the depth of anesthesia we employed: our animals were maintained at a level just subresponsive to reflexive withdrawal, whereas previous suprathreshold studies under urethan anesthesia were performed at levels of slow withdrawal (Armstrong-James and Callahan 1991; Armstrong-James et al. 1992; Simons et al. 1992; see also Armstrong-James and George 1988; Chapin and Lin 1984). Third, the stimulus we employed, while able to evoke suprathreshold responses in rate of rise and amplitude of deflection (Armstrong-James and Fox 1987; Simons 1985), was administered in a single direction, potentially leading to an undersampling of the optimal direction for suprathreshold activity (Simons 1985). A fourth possibility is that we have recorded from neurons usually undetected in extracellular recording studies. The recording bias introduced by our whole cell recordings was toward neurons in which a satisfactory seal and intracellular recording could be achieved, not neurons that demonstrated spontaneous action potential firing or suprathreshold receptive fields. The subthreshold receptive fields we observed may exist without recognition in studies of suprathreshold receptive fields. Using extracellular injection of glutamate and bicuculline to reveal nonslowly active neurons, Dykes and Lamour (1988) reported that the majority of neurons recorded in SI of the urethan-anesthetized rat did not demonstrate suprathreshold receptive fields, while in the presence of bicuculline methiodide, more than half of the nonresponsive neurons revealed suprathreshold output. Our findings are in good agreement with this report and with other reports of purely subthreshold receptive fields in somatosensory neurons (Innocenti and Manzoni 1972; Swadlow 1992).

The amplitude and temporal characteristics of subthreshold responses varied as a function of position within the receptive field. By definition, nonprimary vibrissae evoked smaller amplitude depolarizations from resting membrane potential than the central, primary vibrissa, and the amplitude of evoked inputs decreased with distance from the primary vibrissa. Nonprimary inputs also evoked, on average, subthreshold responses with increasing latency to onset with increased distance from the primary vibrissa. The rise time of evoked subthreshold responses was not closely correlated with the primary versus nonprimary vibrissa distinction. However, rapid rise times (≤4 ms) were found to be well correlated with the presence of suprathreshold-activated activity, which occurred in the primary vibrissa and in the vibrissa adjacent to it within the receptive field.

Sensory-evoked inhibition also varied as a function of the spatial position of sensory input and the time poststimulus. In our limited sample (n = 5), and in previous investigations of rat SI vibrissa-evoked responses (intracellular: Carvell and Simons 1988; extracellular: Simons 1985; see also Istvan and Zarzecki 1994 for similar findings in raccoon SI and Hellweg et al. 1977; Innocenti and Manzoni 1972 in cat SI), inhibition developed during the first 10 ms after the arrival of excitation within the cortex. Rapid cortical inhibition arises from the disynaptic (or polysynaptic) activation of intracortical interneurons after the arrival of excitatory input to the cortex, and our latencies to the onset of inhibition are in agreement with this pattern of connectivity (Agmon and Connors 1992; Istvan and Zarzecki 1994; Simons 1995). As in previous reports (Carvell and Simons 1988), we observed that the strongest inhibition was centered on the input that
also evoked the strongest excitation and that less robust inhibition also was evoked by a subset of nonprimary vibrissae. In our sample, nonprimary vibrissa inhibition that was organized asymmetrically in neurons from all three cortical depths, with a bias toward spread within a row (e.g., Fig. 8). Prior investigations of barrel cortex circuitry (Agmon and Connors 1992) and cat somatosensory cortex (Hellweg et al. 1977; Innocenti and Manzoni 1972) have reported ‘pure’ IPSPs in some neurons. We have not observed an IPSP evoked without an EPSP but did observe one example of an IPSP arriving before an EPSP (vibrissa B4 in the infragranular neuron) (Hellweg et al. 1977).

Implications for the spatio-temporal organization of suprathreshold receptive fields

The spatial organization of the amplitude, onset latency, rise time and slope, and the balance between excitatory and inhibitory inputs over time, suggest the following model for the spatio-temporal evolution of suprathreshold evoked responses. Deflection of the primary vibrissa (or 1 of the adjacent vibrissa providing strong input) causes a large-amplitude, short-latency response, while, conversely, deflection of a more peripheral vibrissa evokes a smaller amplitude response that arrives at either the same latency as the primary input or later and that has a slower rise time to peak amplitude. The fastest-rising, large-amplitude inputs can fire an action potential before the peak of inhibition, while the slower rise time and longer latency of the smaller inputs make them more susceptible to cortical inhibition. There was, then, a dynamic reorganization of the receptive field over time. An initial strong, focal excitatory input was followed by weaker more diffuse excitation and by inhibition that was strongest at the primary vibrissa and spread asymmetrically through the receptive field. The fact that peripheral inputs are more susceptible to inhibition may explain the finding that administration of bicuculline methiodide, a GABA A blocker, causes expansion of the receptive field and differential amplification of suprathreshold inputs that are initially less robust (Kyrazi et al. 1996; see also Dykes et al. 1984).

Extracellular mapping studies have found repeatedly that weaker, more peripheral responses occur later but have disagreed as to whether this pattern reflects underlying inputs that have a longer latency to onset or inputs that have similar latencies but rise more slowly (Armstrong-James et al. 1992; Armstrong-James and Fox 1987; Armstrong-James et al. 1992; Simons et al. 1992). Our results suggest that both factors are likely to play a role. Peripheral inputs do have longer average latencies (by ~5 ms; see for example D4 vs. B1 in Fig. 3), but even inputs that have the same latency can have dramatically different initial rates of rise (e.g., B1 vs. D1 in Fig. 3 and C3 vs. C4 in Fig. 6). Differences in this initial slope are likely to be due to differences in both amplitude and kinetics (i.e., rise time) of the underlying EPSP. In our recordings, more peripheral inputs that did not rise rapidly enough to escape concurrent inhibition remained subthreshold unless the neuron was artificially depolarized. Presumably, under other recording conditions (see preceding text) these inputs may be expressed as long-latency suprathreshold responses.

Implications for multivibrissa interactions

Rat vibrissae are seldom deflected in isolation in the natural environment: an object usually is contacted by several whisks of multiple vibrissae (Carvell and Simons 1990; Nicolelis et al. 1995; Simons 1995). Our data demonstrate that rat SI cortical neurons possess the large subthreshold receptive fields necessary to integrate input from a spatially extensive peripheral area rapidly. Further, the dynamic shift in the organization of the subthreshold receptive field, with the later arrival and peak of more peripheral inputs and of inhibition, positions these neurons to amplify stronger excitatory inputs differentially. Simons and colleagues (Brumberg et al. 1996; Simons 1985) have shown this type of context-dependent modulation by demonstrating that preceding stimulation of the same or a neighboring vibrissa typically inhibits the output of suprathreshold receptive fields, with peak inhibition occurring 10–20 ms after vibrissa input. In concordance with these findings, and with theoretical modeling of thalamocortical circuitry (Kyrazi et al. 1993; Pinto et al. 1996), the observations presented here suggest that during simultaneous or nearly simultaneous contact of multiple vibrissae, the spatio-temporal organization of the subthreshold receptive fields serves to dampen weaker responses selectively.

The asymmetry we observed in the organization of excitation and inhibition within the receptive field is in agreement with previous extracellular (Armstrong-James et al. 1992; Chapin 1986; Lee et al. 1994; Simons 1985) and intracellular reports (Carvell and Simons 1988). The asymmetry in the spread of excitatory subthreshold input within a row may provide the substrate for the construction of asymmetric suprathreshold receptive fields, because the firing threshold is likely to sharpen subtle differences between surround inputs significantly. The asymmetric spread of inhibition (e.g., Fig. 8) may provide the substrate for asymmetric inhibitory multivibrissa interactions (Simons 1985). Receptive fields in which the latency and duration of excitatory and/or inhibitory influences vary systematically with spatial location have been shown in the visual system to be an important mechanism for generating selectivity for the direction and speed of a moving stimulus (Jagadeesh et al. 1993; Reid et al. 1991). Similarly, the spatio-temporal asymmetry observed here may play a role in generating suprathreshold receptive fields tuned for the direction of motion across several vibrissae (Simons 1995) and, hence, may represent a general feature of sensory cortical organization.

Implications for receptive field plasticity

The subthreshold receptive fields we observed are consistent with many of the mechanisms that have been hypothesized to underlie the short- and longer-term reorganization of cortical receptive fields (Moore and Sur 1997). First, the broad subthreshold spread of input to SI cortical neurons may define the region over which expansion via Hebbian mechanisms can occur. Temporal correlation of weak subthreshold inputs with stronger inputs may lead to the emergence a new suprathreshold response (Allard et al. 1991; Clark et al. 1988; Delacour et al. 1987; Diamond et al. 1992; Wang et al. 1995). The broader the subthreshold input to a
neuron, the larger the region over which such correlations can be effective. Second, the selective alignment of the peak of inhibition with that of smaller, more slowly rising excitatory inputs suggests that relatively subtle changes in the strength of inhibition should lead to the emergence of longer latency suprathreshold responses to stimulation of nonprimary inputs. Longer latency suprathreshold responses to stimulation of vibrissae that previously evoked little or no suprathreshold response have been reported after prolonged alterations in the pattern of input from the vibrissae (Armstrong-James et al. 1994; Diamond et al. 1992, 1994). Third, increased temporal synchrony of presynaptic inputs to a cortical neuron (leading to a sharper rate of rise) would allow emergence of new suprathreshold responses without requiring changes in the relative strength of inhibition (Pinto et al. 1996; see also Zarzeczk et al. 1993, who report changes in rise time of evoked responses in a deafferented regions of the cortical map).

Taken together, our results and those of prior studies suggest that even minor alterations in the intrinsic excitability of cortical neurons, or in the balance of excitation and inhibition, are likely to alter the size of the suprathreshold receptive field substantially. Presumably, changes in receptive field size with anesthesia (Armstrong-James and George 1988; Chapin and Lin 1984) or after sensory manipulation (Armstrong-James et al. 1994; Delacour et al. 1987; Diamond et al. 1992, 1994; Faggin et al. 1997; Welker et al. 1989) may occur as the result of one of the above-mentioned mechanisms. The precise mechanisms by which receptive fields reorganize on short- and long-time scales are difficult to disambiguate with suprathreshold recording techniques, suggesting that a full mechanistic understanding of cortical reorganization will include information gathered using intracellular in vivo recording techniques.

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