Spatial Gradients and Inhibitory Summation in the Rat Whisker Barrel System

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SUMMARY AND CONCLUSIONS

1. Extracellular single-unit recordings and controlled whisker stimuli were used to compare response properties of cells in the barrels of the ventral posterior medial nucleus of the thalamus and the barrels in the rat primary somatosensory cortex. Whiskers were deflected alone or in combinations involving up to four immediately adjacent whiskers to assess their relative inhibitory and excitatory contributions to individual receptive fields. Quantitative data were obtained from 51 thalamocortical units (TCUs), 79 “regular-spiking” barrel neurons (RSUs), and 5 “fast-spiking” barrel neurons (FSUs) in 28 normal female adult rats.

2. A random-noise generator was used to produce small, continuously varying whisker movements that were applied to one to four adjacent whiskers while the principal (columnar) whisker was displaced with the use of a ramp-and-hold deflection. RSUs displayed adjacent whisker-evoked inhibition that increased as the number of adjacent whiskers stimulated was incremented. Asymptotic levels of inhibition were reached with the application of the noise stimulus to two or three adjacent whiskers depending on which particular combinations were deflected. By contrast, TCUs and FSUs showed weak, or no, surround inhibition.

3. As the number of adjacent whiskers stimulated increased, the background (prestimulus) activity in TCUs and FSUs increased, whereas displayed background activity in RSTs was relatively unaffected. The increase in background activity observed in the FSUs is hypothesized to mediate adjacent whisker-evoked inhibition in the RSUs.

4. A spatial gradient of adjacent whisker inhibition was observed in RSUs. The caudally adjacent whisker evoked more inhibition than the rostrally adjacent whisker, and the ventral more than the dorsal. A cortical origin for the gradient is suggested by the finding that TCUs did not show a spatial inhibitory gradient.

5. As the noise stimulus was applied to an increasing number of adjacent whiskers, RSUs became more sharply tuned for deflection angles. Neither TCUs nor FSUs showed increases in angular tuning.

6. Inhibition worked disproportionately in RSUs to inhibit those responses that were initially the least robust. For example, inhibition was most effective at reducing responses to nonpreferred versus preferred whisker deflection angles.

7. To assess the principal whisker’s effect on adjacent whisker excitatory responses, the noise stimulus was applied to the principal whisker. In RSUs, principal whisker evoked inhibition was more potent than adjacent whisker-evoked inhibition. FSUs were excited to a greater extent by the application of the noise stimulus to the principal whisker than to adjacent whiskers. TCUs did not display principal whisker-evoked inhibition.

8. Inhibition within the barrel serves as a contrast enhancement mechanism to differentiate small versus large magnitude responses. Less vigorous responses, such as those associated with perturbations of noncolumnar whiskers and inputs from nonoptimal deflection angles, are more strongly suppressed. During active touch, when many whiskers simultaneously palpate an object, these inhibitory interactions could effectively increase the “principal whiskerness” of the cortical column.

INTRODUCTION

Neurons within the dorsal column-medial lemniscus system characteristically display both excitatory and inhibitory receptive-field properties. Stimulation of the skin surface near the receptive-field center evokes both excitation and inhibition, whereas stimulation of more peripheral regions of the receptive field evoke inhibition only (Gardner and Costanzo 1980; Mountcastle and Powell 1959). A similar organization has been described in the rodent somatosensory cortex, where the face representation contains identifiable groups of neurons in layer IV, called barrels, that correspond one to one to individual whiskers on the mystacial pad (Welker 1976; Woolsey and Van der Loos 1970). Barrel circuitry transforms multiwhisker thalamic receptive fields having weak inhibitory surrounds into predominately single-whisker cortical fields having strong inhibitory surrounds (Simons and Carvell 1989). This transformation is thought to be mediated by circuitry intrinsic to the barrel whereby spiny (excitatory) cells and smooth (inhibitory) cells, which both receive similar monosynaptic inputs from thalamic “barreloid” neurons, synapse on each other in an interconnected neuronal network (for reviews see Keller 1995; White 1989). These two classes of barrel neurons can be distinguished electrophysiologically by the time course of their action potentials (Simons 1978); spiny barrel neurons are thought to correspond to regular-spike units (RSUs), and smooth neurons have been shown to correspond to fast-spike units (FSUs) (McCormick et al. 1985). RSUs have focused receptive fields, being strongly excited by a particular vibrissa, the principal whisker or PW, that corresponds anatomically to the barrel in which the unit is located. In unanesthetized or lightly narcotized animals, effects of adjacent whiskers within the barrel are predominantly inhibitory. For example, deflection of an adjacent whisker before movement of the PW diminishes the strength of the PW evoked response. By contrast, FSUs typically respond to deflections of both principal and adjacent whiskers, and their PW-evoked responses are not strongly inhibited by adjacent whisker deflections (Simons 1985; Simons and Carvell 1989).

Studies of the whisker-to-barrel system benefit from a clear demarcation between the receptive-field center, the PW, and its surround, the adjacent whiskers. Each whisker represents an identifiable subregion that can be stimulated independently of the others. Experiments in which individual
adjacent whiskers were deflected in paired combination with the PW revealed that the PW's four immediately adjacent neighbors are not equally inhibitory. The caudally adjacent whisker evokes more inhibition in barrel neurons than the rostrally adjacent one, and the ventral more than the dorsal (Simons and Carvell 1989). Correspondingly, FSUs respond more vigorously to deflection of the caudally adjacent than rostrally adjacent whiskers, and whiskers ventral to the PW evoke stronger responses than dorsal ones (Simons 1995). Comparable results are obtained in behaving mice with the use of 2-deoxyglucose (2-DG) (Durham and Woolsey 1985; McCasland and Woolsey 1988); caudal barrels are more active than rostral barrels and ventral more than dorsal. In mice that had all but one of their whiskers acutely trimmed, the gradient of 2-DG labeling reversed. These metabolic findings in behaving animals can be correlated with the aforementioned electrophysiological results (McCasland et al. 1991). Importantly, findings from the same electrophysiological studies suggested that such inhibitory gradients were weak or absent altogether in thalamocortical neurons, suggesting that the gradient is a cortical phenomenon (Simons and Carvell 1989).

The results of these studies raise questions about how inhibitory influences from different subregions within a receptive field summate, a general issue that is elsewhere receiving experimental attention in the study of visual cortical neurons (Jagadeesh et al. 1993). Previous work in the whisker/barrel system has focused on the integration of inhibitory influences from different subregions within a receptive field (a single adjacent whisker). In the present study we examined how thalamic and cortical neurons integrate simultaneous stimulation of up to four adjacent whiskers. To study the effects of multiple subregions without reaching asymptotic levels of inhibition, small randomly varying vibrations were employed as conditioning stimuli. Inhibitory influences of multiple adjacent whiskers were found to summate in RSUs but not FSUs or thalamic neurons, and the effects of each whisker were consistent with the previously described spatial inhibitory gradients.

METHODS

Preparation

Twenty-eight adult female rats weighing between 294 and 468 g (Sprague-Dawley strain, Zivic Miller, Zelienople, PA) were used for these experiments. The rats were initially sedated with the use of mefetane (mefentanylurethane, Pitman-Moore, Mundelein, IL). Under subsequent halothane anesthesia, 0.020-in. silastic catheters were inserted into both external jugular veins (Harms and Ojeda 1974) and tunneled under the skin and through the nape of the neck. A short length (40 mm) of polyethylene tubing (1.67 mm ID) was inserted into the trachea as a catheter. The femoral artery was cannulated with the use of a 22-gauge angiocath catheter and attached to a pressure transducer (World Precision Instruments, Sarasota, FL) to monitor mean arterial pressure. Stainless steel skull screws were placed over the left frontal and occipital cortex for electrencephalographic recordings, and a ground screw was placed over the right frontal cortex. A steel post was affixed to the left side of the skull with dental acrylic to hold the rat's head without pressure points and in order to allow unimpeded access to the face.

For thalamic recordings a small craniectomy was made in the skull overlying the ventral posterior medial nucleus (VPM) with the use of skull sutures as landmarks according to the atlas of Paxinos and Watson (1982). The underlying dura was resected to allow for later insertion of glass micropipettes. For cortical experiments the skull overlying the barrel cortex was carefully thinned by drilling, and a drawing was made of the surface vasculature. With the use of a previously described triangulation procedure (Simons and Carvell 1989), a barrel of interest was selected for study, and the overlying dura was incised. For both thalamic and cortical experiments, an acrylic dam was constructed around the craniectomy and was filled with saline. All wound margins were sutured closed, and ophthalmic ointment was applied to prevent drying of the corneas.

The rat was placed on a vibration isolation table, and its core body temperature was maintained at 37°C by a servo-controlled heating blanket (Harvard Apparatus, Cambridge, MA). After all surgical procedures were complete, halothane was discontinued, and the rat was maintained throughout the recording session in a lightly narcotized and sedated state by means of continuous intravenous infusion of fentanyl (Sublimaz, Jansen Pharmaceuticals; 5–10 mg·kg⁻¹·h⁻¹). To prevent spontaneous movements of the whiskers, the rat was immobilized with pancuronium bromide (1.6 mg·kg⁻¹·h⁻¹) and artificially respirated with the use of a positive pressure respirator. The condition of the rat was monitored throughout the experiment by assessing its electroencephalogram, mean arterial pressure, arterial pulse rate, pupillary reflexes, perfusion of glabrous skin, and tracheal airway pressure. Experiments were terminated with an intravenously injected overdose of barbiturate if any of the above indicators could not be maintained within normal physiological ranges.

Electrophysiological recordings

Extracellular single unit recordings were obtained with the use of double-barrel glass micropipettes (Simons and Land 1987). One barrel was filled with 3 M NaCl (~1 μm tip diameter; 5–10 MΩ impedance at 135 Hz) and was used for unit recordings; the other barrel was filled with 10% horseradish peroxidase (HRP) in 0.5 M Tris-HCl. The HRP was ionotophoretically ejected to mark recording sites and/or the termination of electrode tracks. Some early cortical experiments employed tungsten microelectrodes (9 MΩ, Frederick Hare, Brunswick, ME). For thalamic recordings the electrode was passed through VPM in the approximate dorsal/ventral plane used in the Paxinos and Watson atlas (1982). For cortical recordings, the electrode was positioned perpendicular to the pial surface overlying the barrel field. In both cases, the electrodes were advanced in 4-μm steps by means of a hydraulic microdrive equipped with a digital counter. To isolate units that were not spontaneously active, whiskers on the contralateral mystacial pad were stimulated during electrode advancement manually or using electromechanical whisker stimulators (see below).

Extracellular single units were determined by spike amplitude and waveform criteria. A digital oscilloscope with time delay was used to visualize the entire spike waveform, and an amplitude discriminator digitized the impulse events. All of the data were obtained from spikes whose waveforms were initially negative. We regard such spikes in both thalamic and cortical units as arising from neuronal somata rather than axons (see Simons and Carvell 1989). We distinguished two type of cortical neurons, FSUs and RSUs, based on previously published methods (Kyriazi et al. 1996a). The action-potential waveforms of the FSUs are approximately one-half the duration of RSUs (Mountcastle et al. 1969; Simons 1978).

Experiments were terminated with an overdose of pentobarbital sodium administered intravenously. The rats were perfused for HRP and cytochrome oxidase histochemistry. For cortical experiments the right hemisphere was sectioned tangential to the pial surface overlying the barrel field. Alternate sections were processed for either HRP or cytochrome oxidase histochemistry (Simons and...
Land 1987). Thalamic units were cut in the coronal plane. With the use of microdrive readings and HRP spots, electrode tracks were reconstructed. Data are reported only for units recorded in the cytochrome oxidase-rich barrel centers in cortical layer IV and lower layer III, or in VPM, where the barreloids reside. Because of the difficulty in visualizing the barreloids in standard section planes, no attempt was made to reconstruct individual electrode tracks with respect to individual barreloids.

Stimulus control and data acquisition

The principal whisker was determined by manually deflecting the whiskers and listening to an audio monitor; the whisker that elicited the strongest response was defined as the PW. Individual whiskers were deflected with the use of multielement electromechanical stimulators constructed from piezoelectric bimorph benders (Simons 1983). The stimulators are small enough to attach to several neighboring whiskers. One stimulator was placed on the PW (~10 mm from its base), and others were placed on each of the PW’s four immediately adjacent neighbors in the same row or arc. The stimulator array was controlled by a laboratory computer (LSI 11/73, Digital Equipment). The computer controlled the stimulation protocols (see below) performed bookkeeping for matching stimuli and their responses, and stored the spike data on disk. Sequential interspike intervals were measured with a resolution of 100 μs. Data were collected for 500 ms bracketing the stimulus, which lasted ~200 ms (see below). A graphics terminal provided online visual representations of receptive-field properties.

Stimulation protocols

The basic experimental procedure involves use of a condition-test paradigm to determine how a neuron’s response to deflection of one whisker is affected by concurrent stimulation of one or more of its neighbors. The test stimulus consists of a 1-mm ramp-and-hold deflection having onset and offset velocities of ~125 mm/s and a plateau duration of 200 ms. A single whisker is deflected in eight different directions, i.e., in 45° increments relative to the horizontal alignment of the whisker rows. Each stimulus is repeated 10 times, and direction parameters are randomized. Stimuli were delivered with interstimulus intervals of 2–3 s. A battery of such test stimuli were delivered alone or in conjunction with a conditioning stimulus, which consisted of low-amplitude vibrations applied to one or more nearby whiskers. The output of a random-noise generator was filtered to produce “pink” noise in a frequency range from 10 to 200 Hz. This signal was in turn amplified to produce a maximum peak-to-peak whisker movement of 1 mm, which was superimposed on a 0.5-mm ramp-and-hold deflection. Hereafter, this is referred to as the noise stimulus. As illustrated in Fig. 1, during a single condition-test trial, the conditioning whisker is vibrated for 1,500 ms; 1,300 ms after the onset of the noise, the test whisker is deflected with the use of the 200-μs ramp-and-hold stimulus. This time interval allows for the dissipation of any effect that might be evoked by the ramped onset of the conditioning stimulus, permitting the neural system to achieve a steady-state level of activation by the noise stimulus before delivery of the test deflection. Test alone and conditioned-test stimuli were randomly interleaved. For each stimulation set, there were 160 stimulus presentations, 80 with noise (8 directions × 10 trials) and 80 without.

Typically, the PW served as the test stimulus, and conditioning stimuli were applied to one to four adjacent whiskers. This was accomplished in three ways. In one, adjacent whiskers were recruited into the conditioning stimulus on the basis of the previously described inhibitory gradient. A complete condition-test battery was delivered wherein the noise was applied only to the caudally adjacent whisker, neuropil the most inhibitory one. A second battery was run including the rostral whisker, a third incorporated the ventral whisker, and a fourth included the dorsal. The second paradigm employed the same strategy but added the whiskers in the reverse order (dorsal, ventral, rostral, caudal). In a third protocol, the influence of each adjacent whisker on PW test responses was examined individually and then in combination (see 1st paradigm for order). Finally, for some neurons, the noise stimulus was applied to the PW, which served as the conditioning whisker, and the test stimulus was applied to the caudal whisker.

The conditioning stimulus was applied similarly to all conditioning whiskers and in one direction (dorsal). We chose to apply the noise in this fashion because of the logistical difficulties involved in generating a large number of independent noise waveforms and because the combinatorial possibilities of direction × number of conditioning whiskers constituted too large a data space to explore systematically.

Data analysis

Time intervals between spikes were measured with a resolution of 100 ms and stored sequentially on disk. The initial step in the data analysis was to convert the raw data, consisting of individual spike trains, into peristimulus time histograms (PSTHs) having 1-ms bins. The mean and variance of spike discharges during the ON response, OFF response and the background activity were computed from the individual spike trains. ON and OFF responses measure the average number of spikes that fall within 20-ms windows corresponding to the arrival of afferent volleys of activity in either the cortex or thalamus due to whisker deflection onset and offset. The maximal ON response (ON) is the number of spikes elicited an average by deflections of the PW in the preferred orientation for the neuron being studied. Because ON responses of most cells average <2.0 spikes/stimulus, interspike interval analysis is not performed, and spike counts per unit time is the metric used for the data analyses. The ON and OFF responses are the average ON or OFF responses for all eight deflection angles. Background activity is measured during a 100-ms period before the initial whisker deflection. Inhibition was measured with the use of condition-test ratios whereby the conditioned-test response was divided by the test alone response. Condition-test ratios <1.0 are indicative of inhibition, and ratios >1.0 are indicative of facilitation. Data were analyzed with a DEC LSI 11/73 and/or with a 486 PC running the data base management and statistics package from SPSS. Data were analyzed for three groups: RSUs, FSUs, and thalamocortical units (TCUs). Kruskal-Wallis tests of variance (K-W) were used for comparisons among them. Two-tailed Kolmogorov-Smirnov
(K-S) or paired t-tests were used for two-sample comparisons, e.g., between RSUs and FSUs or between RSU responses to caudal versus rostral whiskers. An alpha level of ≤0.05 was used as the criteria for statistical significance.

RESULTS

Figure 2 illustrates the nature of the data obtained in this study. It shows the effects of deflecting an RSU's PW in eight different directions alone (A) and in the presence of the noise vibrations applied to all four immediately adjacent whiskers (B). At most angles the unit responded briskly to the initial deflection of the whisker, i.e., the ON response, and less so when the whisker was returned to its neutral position, i.e., the OFF response. Application of the noise stimulus reduced the size of the ON and OFF responses while only slightly increasing the unit’s “background” activity. At the bottom of each panel, a graph shows the magnitude of the unit’s ON responses in polar coordinates (polar plot). In the presence of the adjacent whisker vibrations, the neuron’s response to stimulus onset is reduced by ~50%, but it displays greater selectivity for PW movements in a downward direction.

Figure 3 shows representative results obtained from the three types of units (RSUs, FSUs, and TCUs) recorded in this study. Each panel shows PSTHs accumulated over all eight deflection angles when the PW was stimulated alone and in the presence of noise vibrations applied to the four immediately adjacent whiskers. In the absence of the conditioning stimulus, the FSU is considerably more active than the RSU (compare the prestimulus firing levels in Fig. 3, A and B), and, unlike the latter, it responds almost equally well to stimulus onsets and offsets. When the adjacent whiskers are vibrated, the ON and OFF responses of the RSU are reduced, whereas those of the FSU are relatively unaffected. Interestingly, the background activity of the FSU, but not the RSU, was noticeably elevated by the conditioning stimulus. Figure 3C shows the response of a TCU, which like the FSU, displayed an increase in background activity in response to the noise stimulus. Also like the FSU and in contrast to the RSU, the thalamic neuron’s stimulus-evoked responses were only slightly decreased by the adjacent whisker vibrations.

Spatial summation of inhibition

Figure 4A shows the effect on PW ON responses of increasing the number of adjacent whiskers that are vibrated together. For each of the three unit types, an average condition-test ratio was computed, and this is plotted as a function of the number of adjacent whiskers stimulated. The latter were incorporated into the conditioning stimulus in the fol-
Following order: caudal, rostral, ventral, and dorsal. There is little effect of the conditioning stimulus on either the FSU or TCU populations. Average condition-test ratios were in the range of 0.80–1.10, and K-W tests of variance revealed no differences as a function of the number of whiskers stimulated ($P > 0.05$). Conversely, RSUs were more strongly inhibited, and the amount of inhibition depended on the number of conditioning whiskers (K-W test, $P = 0.03$). Two adjacent whiskers evoked more inhibition than one (0.79 vs. 0.66), but two did not differ from three or four (paired t-test, $P > 0.05$). Thus it appears that an asymptotic limit was reached wherein the PW response could not be inhibited further. Compared with both FSUs and TCUs, RSUs displayed more inhibition with one and four adjacent whiskers, the least and most inhibitory conditions, whereas the FSUs and TCUs did not differ from each other (K-S tests).

The three unit types also differed in terms of background activity and the effects of adjacent whisker vibrations on it (Fig. 4B). FSUs displayed the highest rates of spontaneous discharge (29.7 Hz), RSUs displayed the lowest (1.7 Hz),
and TCUs had an intermediate value (8.1 Hz). In response to adjacent whisker vibrations, background activity in FSUs and TCUs increased to a greater extent than activity in RSUs. K-W tests of variance revealed no significant effect in RSUs related to the number of adjacent whiskers vibrated (P > 0.05), whereas TCU activity was strongly affected (P < 0.001). Effects were not observed in FSUs either, but this could reflect the small sample size (n = 5) and large variance. On the other hand, the rate of increase (the slope in Fig. 4B) in background activity was greater for the FSU population (slope = 0.359) than for either the TCUs (0.299) or RSUs (0.030). Taken together, the findings are consistent with the idea that adjacent whisker-evoked excitatory responses in FSUs mediate the inhibition of PW-evoked RSU responses.

Inhibitory spatial gradients

As noted above, the previously described data were obtained by incorporating the adjacent whiskers into the conditioning stimulus in the sequence: caudal, rostral, ventral, and dorsal. We refer to this as the CR protocol. To examine whether the spatial sequence influences the summation of the whiskers' inhibitory effects, we compared data from Fig. 4A with data obtained when whiskers were incorporated in the opposite order, namely dorsal, ventral, rostral, and caudal. Hereafter, this sequence is referred to as the DV protocol. For TCUs, the same neurons (n = 51) were studied with both protocols. For RSUs, the data were obtained in different neurons in different experiments (CR, n = 47; DV, n = 37); only five units were studied with both protocols. No FSUs were encountered in experiments employing the DV protocol.

Figure 5A shows average condition-test ratios obtained with the CR and DV sequences. Both curves are similar in that inhibition appears to asymptote at the same level when three or four adjacent whiskers are vibrated. The curves differ, however, in how this asymptotic level is reached. In the CR protocol, the caudal whisker exerts a strong inhibitory effect by itself, and the addition of the rostral whisker brings the total inhibition to the asymptotic level. On the other hand, in the DV protocol the dorsal whisker by itself evokes relatively little inhibition, and this is greatly enhanced by the addition of the ventral whisker; even greater levels of inhibition are produced when the rostral whisker is subsequently engaged. The asymmetries in the two curves are consistent with the existence of an inhibitory gradient among the PW’s four adjacent neighbors. A cortical origin for it is demonstrated by the absence of any difference in the CR and DV curves obtained for thalamic neurons (Fig. 5B).

For 20 RSUs (from the DV protocol) we separately tested the inhibitory effect of each of the four adjacent whiskers. Data are plotted in Fig. 6. Consistent with the above findings, the caudal whisker evoked significantly more inhibition than the dorsal one (paired t-test, P < 0.001). Within the horizontal row of vibrissae, the caudal whisker is more inhibitory than the rostral one (paired t-test, n = 20, P = 0.046), and, within a vertical arc, the ventral is more inhibitory than the dorsal (paired t-test, n = 20, P = 0.045). No row or arc gradients were observed in the thalamic neurons (paired t-
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1.6 1 1
0 1 2 3 4
Number of Adjacent Whiskers Vibrated

FIG. 7. Effect on angular tuning as a function of the number of adjacent whiskers vibrated (CR protocol). Plotted are the means ± SE (n = 47). The tuning index is computed by dividing the maximum ON response by the ON8 response; a larger value denotes greater tuning.

Test, P's > 0.05: data not shown), and in fact each whisker produced equivalent effects on the PW response.

Angular tuning

Most thalamic and cortical neurons displayed some degree of angular sensitivity, as illustrated by the noncircular polar plots in Fig. 2. To quantify the tuning properties of each recorded unit, a tuning index was derived wherein the maximal ON response (in spikes/stimulus) was divided by the ON response averaged over all eight deflection angles (ON8). A value of 1.0 denotes a circular polar plot in which all stimulus angles are equally effective; a theoretical maximum of 8.0 denotes a response to only 1 deflection angle. Figure 7 plots the tuning index as a function of the number of adjacent whiskers vibrated using the CR protocol. As the number of adjacent whiskers stimulated increases, RSUs become more sharply tuned (K-W test, \( P = 0.001, n = 47 \)). Similar effects were observed with the DV protocol (K-W test, \( P = 0.002, n = 37 \), data not shown). By contrast, thalamic neurons studied with either the CR or the DV protocol exhibited no change in tuning regardless of the number of adjacent whiskers vibrated (K-W tests, P's > 0.05). Like TCUls, the five FSUs examined showed no increase in their tuning index.

Disproportionate affects of surround inhibition

Figure 8 plots the condition-test ratio of the RSU population for PW responses in addition to those evoked by stimulus onset. In decreasing order of average response magnitude, these are, the ON, OFF, ON8, and OFF8 responses (plotted from right to left). Data shown were obtained with the use of the DV protocol. In general, those responses that are initially largest are affected least. With the vibration of only one adjacent whisker, the maximal ON response, the largest initial response, is decreased by 9%, whereas the OFF8 response, the smallest initial response, is inhibited by 22%. The amount of inhibition increases as the number of adjacent whiskers vibrated increases, but the relative effects on the different components of the PW response remain constant. Similar results were seen with the use of the CR protocol (data not shown). TCU responses fail to show this disproportionate effect.

PW-evoked inhibition

Levels of inhibition were greatest when the PW served as the conditioning stimulus. This series of experiments looked at the effect of deflecting the caudally adjacent whisker in eight directions while the noise stimulus was applied to the PW. Adding the noise stimulus to the PW increased the firing rate of the RSUs from a basal level of 1.8 ± .07 (SE) Hz to 8.7 ± 2.5 Hz (n = 16). For FSUs the noise stimulus increased the average background firing rate from 12.9 ± 6.2 Hz to 32.1 ± 23.2 Hz (n = 4). Taken together with the data of Fig. 4B, the findings demonstrate that the noise stimulus more effectively engages barrel neurons when applied to the PW than when it is applied to adjacent whiskers.

The PW is the most effective excitatory and inhibitory stimulus for barrel neurons. Figure 9A, top, shows a neuron's response (RSU) when the PW was deflected by itself. It is considerably larger than the adjacent whisker response (middle). For this unit, application of the noise stimulus to the PW eliminated the adjacent whisker response (compare Fig. 9A, middle and bottom). Condition-test ratios for ON8 responses were computed for the same neurons when the caudal whisker was the conditioning stimulus and when the PW was the conditioning stimulus. When the PW was the conditioning stimulus, there was significantly more inhibition than in the case where the caudal whisker was the conditioning stimulus (paired t-test, \( P = 0.034, n = 16 \)).

![Condition-test ratios for different components of the PW response as a function of their test alone responses. For data points see text. The curves represent the effect of 1-4 adjacent whiskers (ADJ) on the PW responses. Plotted are the means from the DV protocol (n = 37). Note that for each adjacent whisker condition initially smaller responses (test-alone) are inhibited more.](image)
INHIBITORY SUMMATION IN WHISKER BARRELS

A RSU

PW Alone

ADJ Alone

PW Noise + ADJ

B TCUs

FIG. 9. Response of an RSU to its PW deflection (A, top), to the caudally adjacent whisker alone (A, middle) and to the caudally adjacent whisker when the noise stimulus is applied to the PW (A, bottom). One tick is equivalent to 1 spike for A. B is the summed response of 32 TCUs (top) and 33 in the middle and bottom panels; all have been scaled appropriately. PSTHs follow the same conventions as in Fig. 3. The absence of the adjacent whisker response in the bottom panel of A is unlikely to reflect the absence of such a response in the TCU population (bottom panel of B).

TCUs condition-test ratios for the ONS response increased to 1.59 ± 0.36 (n = 33), indicating that the PW vibration did not inhibit the adjacent whisker response but added to it (see, for example, Fig. 9B). To visualize the overall thalamic input to the barrel neurons, we constructed population PSTHs for stimuli in which the PW was deflected by itself (Fig. 9B, top, n = 32), when the adjacent whisker was deflected by itself (middle), and when the PW was the conditioning stimulus (bottom, data are based on 33 TCUs). Note that the adjacent whiskers response in TCUs is still present despite the application of the noise stimulus to the PW. This indicates that the strong effects evoked by PW stimulation on barrel RSUs are of cortical origin.

DISCUSSION

Inhibitory summation of inputs from multiple adjacent whiskers

The primary motivation for the development of the "noise" stimulus was to reduce the likelihood that inhibitory responses due to multiple adjacent whisker stimulation would saturate. The traditional ramp-and-hold deflection is a suprathreshold excitatory stimulus for most neurons in the whisker-to-barrel pathway, and such a strong stimulus may cause adjacent whisker-evoked inhibition to reach saturating levels with the deflection of only a single whisker. The noise stimulus allowed us to assess interactions between multiple adjacent whiskers. Before this study, one plausible hypothesis was that inhibitory effects of adjacent whiskers were antagonistic to each other, for example, the inhibitory effects of the rostral whisker could negate the inhibitory effects of the caudal whisker. The present findings rule out this hypothesis because inhibition evoked by adjacent whisker vibrations summate or remain at asymptotic levels.

The application of the noise stimulus to any one of the four whiskers adjacent to the PW caused a noticeable, non-saturating decrease in PW responsiveness. Depending on the combination of adjacent whiskers stimulated, asymptotic levels were reached with the application of noise to two or more adjacent whiskers. Operationally, adjacent whisker-evoked inhibition can perhaps be thought of as being described by a sigmoidal function. When one or two whiskers are vibrated, their summative effect lies on the linear portion of the curve, but stimulation of three or four whiskers places their cumulative effect on the cusp (asymptotic part) of the sigmoid. During whisking behavior, rats repetitively sweep their whiskers across a surface (Carvell and Simons 1990). These movements result in whisker perturbations that are of much smaller amplitude than those applied in the present experimental paradigm. Thus during normal whisking behavior, the resultant effect of four or more whiskers might summate entirely within the linear range.

The adjacent whisker-induced decreases in PW-evoked activity in the RSU population were mirrored by increases in the background levels of the FSUs. As described below, adjacent whisker-evoked activity in the latter is likely to mediate the observed inhibitory effects. Interestingly, increases in background activity in the FSUs also summate but, unlike the case of the RSUs, they did not appear to reach an asymptotic limit with the stimuli used here. Thus,
although the activity of the inhibitory barrel neurons did not saturate, their influence did.

**Inhibitory spatial gradients**

The data in Fig. 6 demonstrate that the inhibitory influence of an adjacent whisker depends on its spatial position relative to the PW. The caudal whisker induces the most inhibition, the dorsal whisker the least. Interestingly, the inverse relationships have been observed in FSUs with the use of ramp-and-hold stimuli, namely the caudal whisker excites FSUs more than the rostral whisker and the ventral more than the dorsal (Simons 1995). We were unable to examine this relationship in the present study because only a single FSU was encountered in experiments where we assessed the influence of adjacent whiskers individually. However, the fact that RSUs display an inhibitory gradient that appears to vary with the levels of FSU prestimulus activity allows us to infer that a similar relationship would hold true for the noise stimuli as well.

The mechanism underlying the spatial gradient is not clear. In the present study, and, in an earlier one using punctate stimuli (Simons and Carvell 1989), a spatial inhibitory gradient was observed in RSUs but not in TCUs. This argues that the gradient is either of cortical or thalamocortical origin. One possibility is that multiwhisker neurons within a barreloid are differentially excited by input from the periphery, and this pattern of excitation is transmitted to the inhibitory neurons in the cortex. However, increases in background TCU activity did not differ when adjacent whiskers were vibrated. This suggests that the inhibitory gradient observed in the cortex is not due to a gradient in brainstem-to-thalamus connections.

Another possibility is that there is a gradient of thalamocortical afferents from VPM, such that those neurons within the barreloid that receive input from the PW and the caudal whisker make more, or more effective, synapses on barrel neurons than barreloid neurons that receive input from the PW and the rostral whisker. The basis for this could be an internal topography within individual barreloids. It is known that VPM neurons receive excitatory input from multiple whiskers (Nicolelis et al. 1993; Simons and Carvell 1989; Sugitani et al. 1990), and it is quite possible that, within any given barreloid, neurons situated in its dorsal aspect receive input from both the PW and the caudal whisker, whereas neurons in the ventral aspect receive input from the PW and the rostral whisker. A similar topography is seen in the thalamocortical projection to the barrel, where the sides of the barrels tend to receive inputs from the homologous and neighboring barreloids, whereas the centers receive input mainly from the homologous barreloid (Land et al. 1995). This type of suborganization is suggested also by physiological data from Sugitani et al. (1990), which appear to show successively encountered neurons whose receptive fields alternate between multiwhisker and single-whisker at a period on the order of the dorsal/ventral width of a barreloid.

Along with this topography, the dorsal aspect of a barreloid could give rise to more, or more effective, thalamocortical afferents. Because the dorsal-ventral progression of barreloids in VPM corresponds to the caudal-rostral alignment of whiskers on the mystacial pad, the dorsal side of any barreloid would have a stronger influence on the barrel than the ventral side, thus exciting the FSUs more when the caudal whisker is vibrated.

Another, perhaps related, possibility is that sensory experience during development plays an important role in establishing the gradient. In experiments where the whiskers of rats were trimmed during the first 6 wk of life, there was overall less cortical inhibition, but the spatial gradient was still present (Simons et al. 1993). Thus experience does not appear to play a dominant role in the development of the spatial inhibitory gradient.

**Disproportionate effects of inhibition**

Inhibition in the barrel cortex serves as a contrast enhancement mechanism whereby responses that are initially the smallest are reduced the most. In the present study, for example, adjacent whisker vibrations caused greater reductions in responses to stimulus offsets than onsets and in responses evoked by nonpreferred versus preferred deflection angles. These results are strikingly similar to those obtained with microiontophoretically applied γ-aminobutyric acid (GABA) agonists and antagonists (Kyrizati et al. 1996a,b). In these studies it was found that GABA application disproportionately inhibited the same responses, e.g., OFF, that were most strongly diminished by the adjacent whisker vibrations used here. The converse was seen in those studies with application of the GABA A antagonist bicuculline methiodide (BMI). In the present study, several units were examined during BMI microiontophoresis, and the inhibition due to adjacent whisker vibrations were considerably reduced (unpublished data).

PW deflections evoke the strongest excitatory and inhibitory effects throughout the cortical column (Simons 1985). Comparable results were obtained here. When the noise stimulus was applied to the PW, the average ON response evoked by adjacent whisker deflections was reduced more than when the PW deflection was preceded by vibration of even the caudal whisker, the most inhibitory adjacent whisker. Similar findings have been made in both the cat visual cortex (Ferster 1986) and the primate somatosensory cortex (Laskin and Spencer 1979), where the optimal stimulus induces the most excitation and the most inhibition.

**Implications for thalamocortical and corticocortical circuitry**

According to our previously proposed model (Simons and Carvell 1989), spiny (RSU) and smooth (FSU) barrel neurons receive largely similar, convergent thalamic inputs but integrate them differently such that the receptive-field properties of smooth (inhibitory) neurons faithfully reflect the combination of their thalamic inputs, whereas the receptive fields of spiny (excitatory) neurons are more reflective of the responses that are most common among their thalamic inputs. RSUs and FSUs integrate these inputs differently because of differences in their intrinsic membrane properties (Connor and Gutnick 1990) and because of the location of thalamocortical synapses on them (for a review see, White 1989). FSUs respond more linearly than RSUs to their thalamic inputs. Anatomic studies have shown that thalamocortical axons preferentially synapse onto the soma and proximal dendrites of smooth cells. Such synapses are presumably
more effective (Rall 1967), than those on spiny cells that are made on spines scattered along the length of the dendrites. Consistent with these findings, FSUs in the present study responded much more vigorously than RSUs to the small adjacent whisker vibrations produced by the noise stimulus. TCUs were more strongly excited by adjacent whisker stimuli than RSUs. In this regard, FSU responses appear to reflect closely those of TCUs. Like TCUs but in contrast to RSUs, the angular tuning of FSUs was unaffected by adjacent whisker conditioning stimuli. Also, condition-test ratios were similar for TCUs and FSUs. Previous studies have demonstrated other similarities between FSUs and TCUs, on the one hand, and differences between FSUs and RSUs, on the other, in terms of their responses to stimulus offsets (Kyriazi et al. 1994). These results are paralleled by computer simulations demonstrating that, in response to a thalamic input signal having a slow rise time, activity in simulated RSUs is quickly suppressed while simulated FSU activity continues to reflect the nature of the thalamic input (Pinto et al. 1996).

RSUs display significantly more adjacent whisker-evoked inhibition than TCUs (and FSUs). Therefore the response suppression observed in the barrel is not simply a reflection of inhibition occurring at earlier stations in the afferent pathway. Rather, it reflects cortical processing. We have proposed previously that between-whisker inhibition is mediated within a barrel by its constituent FSUs (Kyriazi and Simons 1993; Simons 1995; Simons and Carvell 1989). The present findings are consistent with this inasmuch as the background activity of FSUs increased as a function of the number of adjacent whiskers stimulated; these changes appear to be somewhat reciprocal to the decrease in PW-evoked RSU activity. Moreover, application of the noise stimulus to the PW increased FSU background activity from 13 Hz to >30 Hz, whereas vibration of one adjacent whisker increased it to only 20 Hz. This greater increase in FSU background activity can account for the finding that, compared with adjacent whisker stimuli, PW deflections evoke more inhibition.

One premise underlying our model of barrel function is that multiple TCUs converge onto any single RSU (Kyriazi et al. 1994; Simons and Carvell 1989; Kyriazi and Simons 1993). Normally, RSUs are less selective for deflection angle than TCUs. Interestingly, when the noise stimulus was applied to all four adjacent whiskers, tuning ratios for the RSUs increased, becoming comparable with those of TCUs. This suggests that RSUs receive input from at least several TCUs having similar but not identical angular preferences. Similarly, because thalamic neurons have varied multi-whisker receptive fields, the total projection from a barreloid to its homologous cortical barrel conveys information about the (common) receptive-field center, i.e., the PW, plus adjacent whiskers. The present findings demonstrate that both receptive-field size and angular tuning are focused byafferent inhibition. Converse effects are observed after application of BMI (see above). Thus thalamic neurons with overlapping receptive fields can form the basis of the more focused RSU receptive fields observed in the layer IV barrel. A somewhat similar organization has been proposed for the geniculocortical system in visual cortex (Hubel and Wiesel 1968).

Behavioral implications

Surround inhibition appears to play a role in normal behavior, because when all adjacent whiskers (n = 8) are acutely trimmed in awake behaving rats, cortical activity increases (Kodger et al. 1995). When actively palpating an object, rats utilize the entire whisker array. Because many whiskers are simultaneously engaged, overall activity in all barrels is reduced (see McCasland and Woolsey 1988). Therefore only the most vigorous afferent response, the initial perturbation of the PW, will overcome the resultant cortical inhibition. Less vigorous responses, such as those associated with perturbations of noncolumnar whiskers and inputs from nonoptimal deflection angles, will be more strongly suppressed. Effectively, this acts to increase the "PW-ness" of the cortical column, providing a mechanism for contrast enhancement. For whiskers, i.e., those displaced from the PW by one row or arc, have been shown to decrease adjacent whisker-evoked inhibition when both the adjacent and far whiskers are simultaneously vibrated (Brumberg and Simons 1994). Thus global features of a complex stimulus could influence the way in which local, textural cues are integrated by inputs from immediately neighboring vibrissae.

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