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Spatiotemporal Characteristics of Neuronal Sensory Integration in the Barrel Cortex of the Rat

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Ego-Stengel, Valérie, Tadeu Mello e Souza, Vincent Jacob, and Daniel E. Shulz. Spatiotemporal characteristics of neuronal sensory integration in the barrel cortex of the rat. J Neurophysiol 93: 1450–1467, 2005. First published October 20, 2004; doi:10.1152/jn.00912.2004. In primary sensory cortices, neuronal responses to a stimulus presented as part of a rapid sequence often differ from responses to an isolated stimulus. It has been reported that sequential stimulation of two whiskers produces facilitatory modulations of barrel cortex neuronal responses. These results are at odds with the well-known suppressive interaction that has been usually described. Herein, we have examined the dependency of response modulation on the spatiotemporal pattern of stimulation by varying the spatial arrangement of the deflected vibrissae, the temporal frequency of stimulation, and the time interval between whisker deflections. Extracellular recordings were made from primary somatosensory cortex of anesthetized rats. Two contralateral whiskers were stimulated at 0.5 and 8 Hz at intervals ranging from 0 to ±30 ms. Response interactions were assessed during stimulation of the principal and adjacent whiskers, first from the same row and second from the same arc. When tested at 0.5 Hz, 59% of single units showed a statistically significant suppressive interaction, whereas response facilitation was found in only 6% of cells. In contrast, at 8 Hz, a significant supralinear summation was observed in 19% of the cells, particularly for stimulations along an arc rather than along a row. Multi-unit recordings showed similar results. These observations indicate that most of the interactions in the barrel cortex during two-whisker stimulation are suppressive. However, facilitation can be revealed when stimuli are applied at a physiological frequency and could be the basis for internal representations of the spatiotemporal pattern of the stimulus.

INTRODUCTION

The vibrissal system of rodents has become one of the dominant models for investigating the mechanisms of sensory information processing. During exploration of their environment, rats contact objects with multiple whiskers in a complex spatio-temporal pattern. Thus previous studies have aimed at understanding the integration of information from deflections of several whiskers. These investigations have mostly reported a suppressive effect of the stimulation of one whisker on the response to stimulation of another whisker, using both electrophysiological recordings (Brunberg et al. 1996; Carvell and Simons 1988; Mirabella et al. 2001; Simons 1985; Simons and Carvell 1989) and optical imaging techniques (Goldreich et al. 1995; Kleinfield and Delaney 1996). However, a facilitatory interaction has also been described by several laboratories (Erchova et al. 2003; Ghazanfar and Nicolelis 1997; Ghazanfar et al. 2000; Shimegi et al. 1999, 2000). These apparently conflicting results could arise from several differences in the experimental protocols used, namely the range of interstimulus intervals (ISIs), the laminar location of the recordings, and the type of recorded neurons.

Whiskers are arranged on the mystacial pad obeying a precise geometrical pattern in caudorostral rows and dorsoventral arcs. During exploratory behaviors, rats move their vibrissae caudorostrally. This movement creates a functional asymmetry between rows and arcs: whiskers in the same row will either contact the object near simultaneously or might not contact the object at all. Anatomical studies have revealed a bias toward within-row connectivity in the intracortical circuitry (Bernardo et al. 1990a,b; Hoeflinger et al. 1995; Kim and Ebner 1999). Additionally, unit recording (Armstrong-James and Fox 1987; Simons 1978) and optical imaging (Kleinfield and Delaney 1996) studies have reported activity patterns in the barrel cortex following single-whisker deflections elongated along rows. In line with those results, electrophysiological investigations have mainly tested multi-whisker integration along rows (Carvell and Simons 1988; Shimegi et al. 1999, 2000; Simons 1985; Simons and Carvell 1989; but see also Mirabella et al. 2001). However, a supralinear interaction most prominent for whiskers located in the same arc was recently described (Ghazanfar and Nicolelis 1997), suggesting that modulatory influences could exist both in the rostrocaudal and dorsoventral directions. Because the effect of the deflection of one whisker on the response to the deflection of another whisker depends on the precise ISI (Shimegi et al. 1999, 2000), it becomes critical to test multi-whisker integration both along rows and arcs with a range of ISIs.

In natural conditions, rats perform whisking at frequencies from 2 to 20 Hz (Carvell and Simons 1990; Gao et al. 2001; Welker 1964). Thus the low-frequency stimulation (typically 0.5 Hz) usually applied in experiments reflects the state the rat is in while resting. Therefore we explored the response interactions of barrel cortex units both at low frequency and for a frequency of stimulation relevant to a behaviorally active state.

We recorded the extracellular activity of single and multi-units in the barrel cortex of the anesthetized rat and studied the response to deflections of the principal whisker (PW) and an adjacent whisker (AW), either in the same row or in the same arc, for a range of ISIs and both at low- (0.5 Hz) and
high-frequency stimulation (8 Hz). Part of this work has been reported in abstract form (Shulz et al. 2001).

**METHODS**

**Animal preparation**

Twenty-two adult male Wistar albino rats weighing 300 ± 40 (SD) g, obtained from the Animal Breeding Unit of our Institute, were used for these experiments. Maintenance, manipulations, and surgery were performed in conformity with National (JO 87–848) and European legislation (86/609/CEE) on animal experimentation and met the American National Institute of Health standards. The animals were anesthetized with urethane (1.5 g/kg, ip) and received an injection of atropine methyl nitrate (0.3 mg/kg, im) to reduce secretions in the respiratory path. Supplementary doses of urethane (0.15 g/kg, ip) were administered when necessary throughout the experiment to maintain an adequate level of anesthesia, indicated by the absence of eye blink reflex, response to hind paw pinch, and vibrissae movement. The electrocardiogram was monitored throughout the experiment. Body temperature was maintained at 37°C.

After the animal was mounted in a stereotaxic frame, the left scalp and temporal muscle were resected. A local anesthetic (xylocaine, 1%) was injected subcutaneously before skin incision. A 4 × 4-mm craniotomy was made to expose the left postero-medial barrel subfield (PMBSF; P0-4, L4-8 from bregma; Chapin and Lin 1984). The dura craniotomy was made to expose the left postero-medial barrel subfield. Lysis of the surrounding connective tissue was performed with iris scissors. A small piece of bone measuring 4-mm in thickness was removed and a bone flap was saved for the end of recording at a given site, the electrode was advanced by 1-ms bin width) were constructed, the onset latencies of the responses were discriminated, multi-unit data were collected, either by defining a template encompassing several waveforms or by amplitude sorting. At this stage, whenever the identity of the PW was ambiguous, it was further investigated by computer-controlled stimulation of the reverse order. Stimuli were applied in trains of 3 s each at a given frequency. One complete protocol was performed either at 0.5 or 8 Hz and consisted of 20 trains at 0.5 Hz and either 2 or 20 trains at 8 Hz for each different ISI, carried out in a pseudorandom order every 4 s (Fig. 1B). During completing the protocol for one whisker combination (row or arc), a different AW was selected, and the protocol was performed again for the second whisker combination. For 28 single and multi-units, protocols for arc and row stimulation were carried out both at 0.5 and 8 Hz. For the remaining units, only one frequency was tested (0.5 Hz: 48 units; 8 Hz: 146 units).

**Electrophysiological recording**

Neural activity was recorded extracellularly by tungsten electrodes (FHC, 2–10 MOhm at 1 kHz) that were lowered in the cortex using an electronically controlled microdrive (MO-81, Narishige). Signals were amplified (gain 10,000) and filtered for spike activity (0.3–3 kHz; MCP Plus 8, Alpha-Omega). For each recording site, up to three single units were isolated using a template-matching spike sorter (MSD, Alpha-Omega). The shape of action potentials was continuously inspected to ensure that the same neurons were recorded throughout the protocols. When action potential waveforms could not be discriminated, multi-unit data were collected, either by defining a template encompassing several waveforms or by amplitude sorting. At the end of recording at a given site, the electrode was advanced by ≥100 μm before the next site to avoid recording the same units. The electrode signal was stored on a Digital Tape Recorder (sampling frequency 12 or 24 kHz; DTR 1801, Biologic). Spike time acquisition at 4 kHz and data processing were done with a custom-made software (Elphy, G. Sadoc, CNRS-UNIC).

**Whisker stimulation**

Once units were isolated, vibrissae were at first manually deflected while monitoring the extracellular signal. This allowed us to estimate the location of the PW. At this stage, whenever the identity of the PW was ambiguous, it was further investigated by computer-controlled stimulation of all putative PWs. Peristimulus time histograms (PSTH; 1-ms bin width) were constructed, the onset latencies of the responses were visualized on-line, and spike counts over a 80-ms window after the stimulus were quantified. The PW was defined as the whisker eliciting the maximal neuronal response with the shortest onset latency. In all cases, the identity of the PW was later checked off-line by calculating the onset latency of the response to the different whiskers. This value was measured on each PSTH as the first bin exceeding an activity threshold (spontaneous activity + 3 SD) and for which the sum of the responses at that bin and the following one exceeded twice the threshold. Also, in the same manner, an end-of-response latency was calculated as the time of the last bin meeting the same requirements. The off-line latency analysis confirmed the online determination of the PW in 97% of cases (216/222 single and multi-units). In the remaining six cases, the identity of the PW was corrected off-line; in all these instances, the latencies of response for the investigated whiskers were in fact identical and only the spike counts differed slightly.

Once the PW was determined on-line, that whisker and one AW in the same row or in the same arc were chosen for the experimental protocol. Typically, two whisker combinations were tested at each recording site (1 along a row and 1 along an arc; Fig. 1A). The majority of our recordings were made from barrels corresponding to whisker rows in Fig. 1, B–D, and the AW was equally chosen from dorsal, ventral, anterior, and posterior positions (see RESULTS). After the length of 1 cm, we inserted the selected whiskers in short polypropylene tubings glued on piezoelectric bimorphs (Polytec-P). Mechanical stimulation of each whisker was controlled by the data acquisition software driving an amplifier (Polytec-P) and consisted of pulses of 5-ms rise time, 10-ms hold phase, and 5-ms fall time. The input signal was filtered by an RC circuit with a constant time of 2.2 ms to reduce mechanical ringing of the actuator. The amplitude of the residual ringing is 1.5% of the full displacement of the piezo (unseen at the scale of Fig. 1B). The strobo-caudal deflection was 430 μm in amplitude (5°) at ~5 mm from the follicle of the deflected whisker with maximal velocity of 67 mm/s (Fig. 1B).

During one trial, each of the two whiskers (PW or AW) was stimulated alone, or both whiskers were stimulated together at a given ISI. ISIs ranged from 0 to 30 ms (0, 1, 2, 3, 4, 6, 8, 10, 12, and 30 ms) for the two temporal orders (PW before or after AW; Fig. 1, B and C). Positive ISIs in text and figures correspond to the PW being stimulated before the AW, and negative ISIs correspond to the reverse order. Stimuli were applied in trains of 3 s each at a given frequency. One complete protocol was performed either at 0.5 or 8 Hz and consisted of 20 trains at 0.5 Hz and either 2 or 20 trains at 8 Hz for each different ISI, carried out in a pseudorandom order every 4 s (Fig. 1B); in addition to the stimuli for the different ISIs, trains of stimulation of the AW alone or the PW alone were also performed during the pseudorandom protocol, although not represented in the figure). This yielded a total of 40 deflections at 0.5 Hz and 48 or 480 deflections at 8 Hz for each ISI. After completing the protocol for one whisker combination (row or arc), a different AW was selected, and the protocol was performed again for the second whisker combination. For 28 single and multi-units, protocols for arc and row stimulation were carried out both at 0.5 and 8 Hz. For the remaining units, only one frequency was tested (0.5 Hz: 48 units; 8 Hz: 146 units).

**Data analysis**

The spontaneous activity for each trial was calculated on a 300-ms window prior to the stimulation. The response of a unit to whisker stimulation was defined as the spike count in a specific temporal window (typically 5–40 ms) after subtraction of the spontaneous activity. This counting window started 5 ms after the onset of the stimulus and ended 5 ms after the end time of the response for that particular unit, defined as the maximal end-of-response latency across all PW stimulation protocols. It was systematically verified on all the individual PSTHs for that unit that the response for any stimulus configuration (frequency, whiskers, ISI) was indeed contained in the defined counting window, so that facilitation in a part of the response not present initially was never missed.

The use of a specific time window for each unit, calculated from its response kinetics, enabled us to evaluate facilitatory and suppressive
effects using ratios of neuronal activity defined below. The alternative choice of using an identical temporal window for all units has the disadvantage of rendering those methods insensitive either for units with low evoked responses (if the window is large) or for units with long duration responses (if the window is short), and thus would have lead to missing some of the effects.

For protocols carried out at 8 Hz, the response during stimulation trains was composed of an initial adapting phase during the first 500 ms followed by a steady-state response (see RESULTS, Fig. 2, and Ahissar et al. 2000; Ego-Stengel et al. 2001). Since we were interested in comparing the steady-state regimen at 8 Hz with responses at 0.5 Hz, only deflections between 500 and 3,000 ms of each 8-Hz train were included in the quantification at that frequency. PSTHs with 1-ms bins were constructed for each type of stimulation (whiskers, ISI, and frequency) by averaging the instantaneous firing rate of each unit relative to the onset of deflection of the vibrissae.

We used two different indexes to assess response summation due to multi-whisker deflections. First, we calculated the facilitation index (FI) proposed by Shimegi et al. (1999, 2000). The FI is the ratio between the observed response to the combined deflection of whiskers and the sum of responses to individual deflections calculated on the same time window. FI values above 1 indicate a facilitatory effect, whereas FI values below 1 reveal a suppressive interaction. This ratio could be calculated only for units with a measurable level of activity (sum of responses to deflections of the PW alone and the AW alone larger than 0.05 action potentials per stimulus (a.p./stim.), corresponding to 1 a.p. every 20 stimuli). Second, we quantified whisker interactions with a condition-test ratio (CTR), which has been previously used in the study of sensory integration (Gardner and Costanzo 1980a; Goldreich et al. 1998; Simons 1985; Simons and Carvell 1989). This ratio specifically measures the effect of the first deflection (the conditioning stimulus) on the response of the unit to the second deflection (the test stimulus). For each ISI, the observed mean spike count, evoked in a time window starting 5 ms after the second stimulus, was divided by the mean spike count expected if the first stimulus had no effect, i.e., the response to deflection of the second whisker alone summed with the appropriate late part of the response to the first whisker deflection (estimated from responses to deflections of that whisker alone) (Simons and Carvell 1989). As for the FI, a linear summation of responses yielded a ratio equal to 1; sublinear and supralinear summations resulted in ratios <1 and >1, respectively. Again, the CTR could be calculated only for units with a measurable level of activity (responses to the 2nd deflection larger than 0.05 a.p./stim.). The sublinear and the supralinear summations are referred to in the text as suppression and facilitation, respectively. It should be emphasized that these nonlinear interactions are relative to the steady-state response within an 8-Hz train and not to the response evoked at low frequency of stimulation (i.e., a nonadapted state).

These two methods rely on the comparison between an observed response and an expected response under a linear summation hypothesis. Thus for both the FI and the CTR, statistical significance of facilitatory and suppressive effects could be determined by comparing, for each ISI, the set of observed spike counts to the set of expected spike counts with a two-tailed t-test, as proposed by Simon and Carvell (1989) for the CTR. The significance level was set at 0.01. Note that this statistical test could be performed even when the low level of activity precluded calculation of the ratios, so that all units are included in the statistical analysis. To compare our results directly to the studies by Shimegi and collaborators, we also evaluated the occurrence of facilitation using their ad hoc criterion, i.e., an FI > 1.25.

FI and CTR estimators of whisker interactions are not equivalent. First, the activity of the unit between the first and second deflections in one trial is included both in the numerator and denominator of the FI ratio but not of the CTR ratio. As this activity cannot be affected by the upcoming stimulation, the CTR is more sensitive to changes in the response to the second deflection than the FI. Second, to calculate the FI on a constant time window for all ISIs, we had to systematically lengthen this window by 30 ms (the duration of the longest ISIs) compared with the actual neuronal response duration. In cases of low response levels and/or high spontaneous activity, this was found to increase the variability of the measure. In contrast, short integration

FIG. 1. Experimental protocol. A: after determination of the principal whisker (PW), this whisker and 1 of the adjacent whiskers (AWs) in a row and/or an arc were selected for stimulation. B: entire protocol at either 0.5 or 8 Hz consisted of a pseudorandom sequence of trains with different interstimulus intervals (ISIs; see METHODS). Stimulation trains of only 1 whisker deflected (PW or AW) were also included in the sequence, although not represented on this scheme (see METHODS). Whiskers were deflected in trains of 3 s, with a 1-s interval between 2 successive trains. Input waveform for each deflection consisted of a 5-ms rostrocaudal movement followed by a 10-ms plateau and an opposite ramp back to the rest position of the whisker (filled trace). This signal was filtered by an RC circuit with a time constant of 2.2 ms, avoiding mechanical ringing of the actuator. When this filtered signal is fed into the actuator, it induces a movement (given by the superimposed dashed line) with a maximal amplitude of 430 μm. C: PW and AW were deflected with an ISI among the positive and negative values indicated from −30 to 30 ms.
time windows adapted to the response of phasic units could be used for the CTR. For these reasons, we chose to focus on results obtained from the CTR analysis.

Average values are displayed as means ± SE unless indicated otherwise. For comparisons among populations, statistical significance was assessed using a commercial software (Statistica, Statsoft) with a probability threshold at 0.05. To avoid sphericity assumptions, MANOVAs were used instead of ANOVAs wherever relevant (Zar 1999). T-tests are unpaired unless indicated otherwise.

**Histology**

At the end of eight experiments, two to three small electrolytic lesions (30 pulses of 200 ms and 10 μA delivered at 0.3 Hz) were made at known depths, 500 μm apart. The animal was given a lethal dose of thiopentone and perfused transcardially with saline followed by a fixative solution (8% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). Coronal sections (80 μm) were cut through the right PMBSF and stained with cresyl violet to visualize cortical layers.
Forty-one single units and 23 multi-units having this direct histological evidence of lamina location were classified as supragranular (layers I–III), granular (layer IV), and infragranular (layers V–VI). In the other 14 experiments, we estimated the lamina position of the units by establishing a correspondence between the depth of the electrode penetration and the cortical layers. This relation was based on the depths of cortical layers determined by microscopic inspection of stained brain sections from 10 experiments (including the 8 above) in which electrolytic lesions at known depths were done. Based on this relationship, layer IV extended from 444 to 862 μm. Seventy-one single units and 87 multi-units were classified as supragranular, granular, and infragranular according to the recording depth with respect to layer IV.

RESULTS

The response interaction for two-whisker stimulation was studied in 112 single units and 110 multi-units recorded from the somatosensory cortex of 22 rats. Four hundred forty-four protocols were completed on these single and multi-units and were retained for statistical analysis.

Response characteristics to single-whisker deflection

The spontaneous activity of the recorded single units was on average 2.7 ± 0.4 a.p./s (averaged over 40 stimulations on all cells stimulated at 0.5 Hz, n = 34). The mean response to the deflection of the PW, excluding the spontaneous activity, was 0.7 ± 0.1 a.p./stim (n = 34). These values are within the range of spontaneous and evoked activity levels already reported in the barrel cortex (e.g., Ego-Stengel et al. 2001; Simons and Carvell 1989). The spontaneous and stimulus-evoked activities for the multi-unit population were significantly higher than for the single unit population (spontaneous activity = 5.5 ± 0.6 a.p./s, evoked activity = 2.1 ± 0.2 a.p./stim., n = 42, 1-tailed t-test; P < 0.0002 and P < 1.10^-11, respectively).

The stimulated whisker was mechanically deflected at frequencies of 0.5 and 8 Hz. Cells responded with a phasic increase of activity after each deflection. The PSTHs displayed in Fig. 2A1 show the average response of the single units (n = 148 protocols on 88 single units) to a 3-s train of stimulation of the PW (top) and the AW (middle) at 8 Hz. The response to each deflection was quantified by the number of action potentials in a temporal window adapted to each cell (see METHODS). By averaging this spike count across trains and across cells, the kinetics of the discharge and the response at 0.5 Hz. The response to the first whisker deflection in an 8-Hz train was obtained for the PW and the AWs across trains and across cells, the kinetics of the discharge and the response at 0.5 Hz. The response to each deflection was quantified by the PSTHs displaced in Fig. 2A1 show the average response of the single units (n = 34). The mean response to the deflection of the PW, excluding the spontaneous activity, was 0.7 ± 0.1 a.p./stim (n = 34). These values are within the range of spontaneous and evoked activity levels already reported in the barrel cortex (e.g., Ego-Stengel et al. 2001; Simons and Carvell 1989). The spontaneous and stimulus-evoked activities for the multi-unit population were significantly higher than for the single unit population (spontaneous activity = 5.5 ± 0.6 a.p./s, evoked activity = 2.1 ± 0.2 a.p./stim., n = 42, 1-tailed t-test; P < 0.0002 and P < 1.10^-11, respectively).

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Temporal profiles of suppressive effects in response to stimulation of two whisksers

In response to the combined deflection of two whisksers presented repetitively at 0.5 and 8 Hz, we observed in most cases a suppressive effect of the stimulation of the first whisker on the response to the stimulation of the second whisker. Figure 3 shows the PSTHs of response of a single unit submitted to the deflection of whiskers C3 or D3 alone or to the combined deflection of the two whisksers at different ISIs. Both individual whisker movements elicited transient responses in the time window of 5–20 ms (C3: 0.15 a.p./stim.; D3: 0.13 a.p./stim.; Fig. 3B), and the average spike count during the simultaneous deflection of the two whisksers (C3 + D3) was not significantly different from the sum of individual responses (0.3 a.p./stim.; Fig. 3B, bottom). In contrast, stimulation of the two whisksers at different intervals produced a general suppression of the response (e.g., ISIs 6 and 8 ms in Fig. 3C).

The interaction between the responses to the deflected whisksers was quantified using the FI and the CTR (see METHODS) as a function of ISI. The resulting ISI tuning curves are depicted in Fig. 3D, showing a significant suppressive effect when the two whisksers were stimulated at ISIs of −30, −12, −8, 1, and 2 ms. This effect was best revealed by the CTR quantification (Fig. 3D, bottom).

Among the cases showing a suppressive two-whisker interaction, we observed different profiles of the ISI tuning curves. In one type of profile, of which Fig. 3 shows one example, the suppression was found for long ISIs for both orders of the whisksers, whereas at short ISIs, there was either less or no suppression. Figure 4 shows an example of the opposite profile, i.e., a pronounced suppression at short ISIs, whereas responses sum almost linearly at long ISIs. The movement of whisker C4 alone elicited a strong response in the time window of 5–65 ms (C4: 1.42 a.p./stim.; C3: 0.27 a.p./stim.; Fig. 4B). In contrast, the average spike count following the simultaneous deflection of the two whisksers (C3 + C4) was very low (0.42 a.p./stim.), indicating a strong suppressive effect when both whisksers were stimulated together. This suppression was also seen when the two whisksers were stimulated within a short time interval (1–12 ms) for both temporal orders (Fig. 4C), but was less or not present for longer ISIs (30 ms). Examination of the FI and CTR tuning curves confirms a significant suppressive effect when the two whisksers were stimulated in close temporal contiguity (−12 to 4 ms).

Asymmetric profiles of suppression were also observed in our sample, as shown in Fig. 5 (see also Fig. 4D). For this infragranular single unit, the deflection of whisker B3 eliminated the response to the subsequent deflection of whisker B4, whereas the reverse order resulted only in a partial suppression. This asymmetry is best seen by comparing the PSTHs of...
FIG. 3. Suppressive interaction at long ISIs. A: for this single unit recorded at a depth of 506 μm (granular layer), whisker C3 was the PW and whisker D3 was chosen as the AW. In all PSTHs shown, the histogram was constructed from 40 deflections, the bin size is 1 ms, and the 2 different dashed lines indicate onset of deflections for the 2 whiskers. C: PSTHs of response to successive stimulation of the AW and the PW (left) or the PW and the AW (right) for all ISIs. D: facilitation index (FI; top) and condition-test ratio (CTR; bottom) ± SE as a function of the ISI. Response scale in action potentials per stimulus was added on the left of the FI graph, as it is proportional to the FI. ○, response values for the deflection of the PW and AW alone on this scale; ⋆, statistically significant nonlinear interactions (suppressive or facilitatory; 2-tailed unpaired t-test, P < 0.01).

FIG. 4. Suppressive interaction at short ISIs. A: for this single unit recorded at a depth of 264 μm (supragranular layer), whisker C4 was the PW and whisker C3 was chosen as the AW. B–D: same conventions as in Fig. 3. Significant sublinealities were found, particularly for short negative ISIs (e.g., −1 ms).
response for ISIs of 10 and 12 ms for both orders of stimulation (Fig. 5C, lines 2 and 3): when whisker B3 is deflected first, only the peak of activity evoked by B3 is observed, whereas when whisker B4 is deflected first, the PSTH exhibits two peaks. As a result, the CTR values for negative ISIs are higher than the CTR values for positive ISIs, and the CTR tuning curve has an overall step shape. Note that the CTR is more sensitive to changes in the response to the second deflection than the FI.

Finally, for a fourth category of units, a generalized suppressive interaction was observed for all ISIs tested. Although we did not attempt a systematic classification, our data suggest that overall, the majority of ISI tuning curves profiles could be classified in these four families, namely tuned order-independent suppressions for long ISIs, tuned order-independent suppressions for short ISIs, tuned order-dependent suppressions, and untuned broad-band suppressions.

**Influence of the frequency of stimulation on two-whisker interactions**

The predominant interaction between deflections repeated at 0.5 Hz was suppressive. For 20 of 34 single units (59%), a statistically significant suppressive effect of the deflection of one whisker on the response to the deflection of the second whisker was found for at least one ISI and one whisker combination (2-tailed t-test on the CTR, P < 0.01; Table 1). In contrast, a statistically significant facilitatory interaction was observed in only two occasions and only for one ISI. The predominance of suppressive interactions at 0.5 Hz was even stronger on the multi-unit recordings (40 of 42), with only one case showing a supralinear interaction.

To facilitate the comparison with previous works, we also evaluated response facilitation with the criterion used by Shimegi and colleagues (FI > 1.25 for at least 1 ISI). Supra-

![Fig. 5. Suppressive interaction selective for the order of stimulation. A: for this single unit recorded at a depth of 1,195 μm (infragranular layer), whisker B3 was the PW and whisker B4 was chosen as the AW. B–D: same conventions as in Fig. 3. Whisker stimulation was applied at 0.5 Hz. When whisker B3 was stimulated 1st, activity evoked by whisker B4 was totally suppressed. Reverse order resulted only in a partial suppression.](image-url)
linear summation assessed by this method was observed in 50% of the units tested at 0.5 Hz (n = 34 single units, see Table 1) in contrast with the observation that very little facilitation was found at 0.5 Hz using more stringent statistical criteria. Moreover, inspection of the PSTHs and tuning curves suggested that many FI values were within the observed variability in our sample of units.

For 88 single units and 86 multi-units, we tested the response to two-whisker deflections presented at a high-frequency of stimulation (8 Hz). Previous studies have shown that barrel cortex cells respond to trains of deflections at 5 Hz or more with an initial adapting phase during the first 500 ms, followed by a steady-state response (Ahissar et al. 2000; Ego-Stengel et al. 2001; Simons 1978). We have confirmed this observation here (see Fig. 2), and consequently, only deflections after 500 ms were included for quantification of the steady-state regimen in this work. Thus the term “facilitation” is used here to describe a supralinear two-whisker interaction relative to the linear prediction during the steady state of an 8-Hz train and not relative to the linear prediction during low-frequency stimulation.

Although suppressive interactions were again dominant, facilitatory interactions were revealed when stimulating at 8 Hz. For the single unit of Fig. 6A, movement of either whisker C1 or B1 evoked a small response (C1: 0.23 a.p./stim.; B1: 0.12 a.p./stim.). In contrast, the stimulation of whisker B1 followed 8 ms later by the stimulation of whisker C1 evoked 0.74 a.p./stim., which was significantly more than predicted by the sum of individual responses. This facilitation of the response to two-whisker deflections was more prominent for short ISIs and when the AW was stimulated before the PW. Accordingly, the tuning curves for this unit exhibit a one-sided facilitatory peak.

A more specific case of supralinear summation is shown in Fig. 6B. For this single unit with very low responses to the stimulation of the PW (0.07 a.p./stim) and none to the AW, there is a significant supralinearity when both whiskers are stimulated 1 ms apart.
We tested the response to two-whisker deflections both at 0.5 and 8 Hz in 10 single units (19 protocols) and 18 multi-units (34 protocols). The effect of the stimulation frequency could thus be studied on individual units. Figure 7 shows the PSTHs of response for a multi-unit recording after the stimulation of whiskers C2 (PW) and B2 (AW) at both frequencies. When two-whisker deflections were presented repeatedly at 8 Hz, a maximal significant facilitatory effect was revealed for a 3-ms ISI between the PW and the AW stimulation (Fig. 7, A3 and A4). In contrast, the facilitatory effect was not present when the whiskers were deflected at 0.5 Hz (Fig. 7, B3 and B4), indicating that the supralinear summation occurred only for high frequencies of stimulation.

For the whole population of single units tested at 8 Hz, a significant facilitatory interaction was observed on 19% of the units (17 of 88; see Table 1, quantification based on CTR values). The number of protocols showing facilitation at 8 Hz (19 of 148) was significantly larger than at 0.5 Hz (2 of 63, Fisher’s exact test, \( P < 0.05 \)); this result was even stronger in the multi-unit data (38/154 vs. 1/79, Fisher’s exact test, \( P < 0.00001 \)). Despite the occurrence of supralinear summation, suppressive effects were still dominant at 8 Hz and were observed on 42% of the single units (see Table 1).

To directly compare the effects at 0.5- and 8-Hz stimulation, we averaged the CTR tuning curves for all the protocols performed at each frequency (Fig. 8 A1 and B1). For 0.5-Hz stimulation, all mean CTR values were well below 1, yielding relatively flat curves. This confirmed that the dominant interaction was a sublinear summation and showed that interactions affected all ISIs. In contrast, the tuning curves for 8-Hz stimulation were closer to the linearity value for single units and above 1 for positive ISIs in multi-unit recordings (Fig. 8B1). Consequently, the mean CTR values across ISIs were significantly greater for 8 Hz than for 0.5 Hz stimulation (ANOVA, main effect of frequency, \( F_{(1,162)} = 5.05, P = 0.026 \) for single units and \( F_{(1,162)} = 27.1, P = 0.00001 \) for multi-
Note that the mean CTR value for each ISI results from the combination of facilitatory and suppressive effects in the population of tested units for that time interval. The proportions of significant cases of facilitation and suppression as a function of ISI confirmed the difference between 8- and 0.5-Hz stimulation (compare Fig. 8, A2 vs. A3 and B2 vs. B3). Facilitatory interactions were found almost exclusively for 8-Hz stimulation. Suppressive interactions were observed for both frequencies and distributed among all ISIs, although the number of suppression cases was smaller at 8 (46 over 148 protocols) than at 0.5 Hz (30 over 63 protocols, Fisher’s exact test, \( P < 0.03 \) for single units; 66/154 vs. 68/79 for multi-units, \( P < 0.00001 \)).

To confirm that the facilitatory effects revealed by the statistical comparison of observed and predicted spike counts are indeed significant, and not merely due to a high variability of activity in our sample, we performed the same analysis on a set of control data having the same intrinsic variability. For each protocol, the predicted responses, obtained under the linearity assumption, were randomly separated in two sets that were compared using the same statistical tests as for the observed/predicted comparison. On this control data set, which could be generated for all the ISIs, although the number of suppression cases was smaller at 8 (46 over 148 protocols) than at 0.5 Hz (30 over 63 protocols, Fisher’s exact test, \( P < 0.03 \) for single units; 66/154 vs. 68/79 for multi-units, \( P < 0.00001 \)).

Characteristics of the facilitatory interactions revealed at 8 Hz

As shown above, during the steady-state response to an 8 Hz train of stimulation, the two-vibrissa interactions more likely showed facilitation than at 0.5 Hz. This facilitation was relative to the linear prediction based on the steady-state responses to the separate stimulation of the PW and the AW at the same frequency (i.e., 8 Hz). This facilitation, however, was still sublinear relative to the summation predicted by the individual responses at low-frequency stimulation (i.e., 0.5 Hz). This point is exemplified in Fig. 7 where the facilitated spiking responses at 8 Hz did not reach the level of the individual response at 0.5 Hz. To further clarify the multi-vibrissa facilitation effect at high-frequency stimulation, we calculated the mean PSTH of the response to an 8-Hz stimulation train by averaging individual PSTHs for all the ISIs that showed a significant steady-state facilitation (Fig. 9A, average of 78 PSTHs from 17 single units, with the corresponding spike counts below) and those that showed no change (Fig. 9B, average of 2,332 PSTHs from 88 single units) or were suppressive (Fig. 9C, average of 402 PSTHs from 37 single units).
predominance of suppressive effects at low stimulation frequencies. However, the adaptation profiles were different in these three groups. In the case of facilitatory effects, the mean expected PSTH exhibited a strong adaptation during the train (Fig. 9A), whereas a weak adaptation was observed in the suppressive case (Fig. 9C), indicating that the subpopulations of cells showing supralinear and sublinear interactions have different transient kinetics in response to high frequencies of stimulation. Moreover, the facilitatory effect may be described as a strong reduction in the amplitude of the adaptation.

The specific integration time windows defined to quantify the evoked activity of each cell (see METHODS) correspond to response durations to the deflection of the whiskers. The distribution of response durations was bimodal with modes at 5 and 25 ms. Based on the bimodal distribution, we have classified the single units into phasic (<17-ms duration, n = 16) and tonic (>17-ms duration, n = 47). There was no statistical significant relationship between the duration of the response and the likelihood of facilitation at 8 Hz quantified on the number of protocols (1 over 29 protocols for phasic cells and 11 over 80 protocols for tonic cells, Fisher’s exact test, P = 0.18) or on the number of cells (1 over 16 phasic cells and 9 over 47 tonic cells, Fisher’s exact test, P = 0.43). No difference was found either on the CTR values (ANOVA, F(1,156) = 0.49, P = 0.49). Conversely, the proportion of suppressive interactions was higher for tonic neurons (6 over 29 protocols for phasic cells and 38 over 80 protocols for tonic cells, Fisher’s exact test, P = 0.015 and 5 over 16 phasic cells and 30 over 47 tonic cells, Fisher’s exact test, P = 0.04).

**Response interaction along arcs versus rows**

One of the main objectives of this study was to compare whisker interactions when the two whiskers belong to the same row versus to the same arc. Thus the response of a unit was tested for two combinations of whiskers, one along a row and one along an arc. The AWs were equally sampled around the PW (21% dorsal, 27% ventral, 28% anterior, and 23% posterior of the 211 protocols applied on single units). Figure 10 shows the results for a single unit showing facilitatory effects for both combinations of whiskers tested at 8 Hz. The response to two-whisker deflections, particularly when the AW stimulation preceded the PW stimulation, was significantly greater than the sum of responses to single-whisker deflections, and this for arc stimulation (Fig. 10A) as well as row stimulation (Fig. 10B).

In other cases, there was a clear dependency of the facilitatory effect on the whisker combination. For the cortical single unit in Fig. 11, a supralinear summation was observed only for stimulation of whiskers in an arc (significant for all but 1 ISI from −12 to +12 ms; Fig. 11A). Stimulation of two whiskers in a row did not evoke a significant supralinear interaction for any ISI but rather a general suppressive effect (Fig. 11B).

We compared the ISI tuning curves averaged over arc and row combinations of whiskers for all the single and multi-units tested at 8 Hz (Fig. 12A). In the case of arc stimulation, a mean supralinear summation was observed in both populations for several ISIs around 0 ms, as indicated by CTR values above 1. This was not the case for the combinations of whiskers along rows, for which the whole ISI tuning curves were below 1. The mean CTR values across ISIs were greater for arc stimulation.
than for row stimulation (ANOVA, main effect of whisker combination, $F_{(1,162)} = 9.27, P < 0.003$ for single units and $F_{(1,162)} = 11.65, P < 0.0009$ for multi-units).

In agreement with these results, we observed more significant facilitatory interactions when the stimulated whiskers belonged to the same arc (14 over 68 protocols) than to the same row (5 over 80 protocols, Fisher’s exact test, $P = 0.012$ for single units). Conversely, there were more significant cases of suppressions for row stimulation (27 over 80 protocols) than for arc stimulation (19 over 68 protocols), although this difference did not reach statistical significance (Fisher’s exact test, $P = 0.48$ for single units). The proportions of significant cases of facilitations and suppressions as a function of the ISI are shown in Fig. 12 for single units, separately for the four AWs surrounding the PW. Interactions between vibrissae on the same row (both anterior and posterior) are dominated by suppressions. The number of protocols showing a significant suppression for at least one ISI was higher than the number of protocols showing a significant facilitation (Fisher’s exact test, $P = 0.48$ for single units). The proportions of significant cases of facilitations and suppressions as a function of the ISI are shown in Fig. 12B for single units, separately for the four AWs surrounding the PW. Interactions between vibrissae on the same row (both anterior and posterior) are dominated by suppressions. The number of protocols showing a significant suppression for at least one ISI was higher than the number of protocols showing a significant facilitation (Fisher’s exact test, $P = 0.007, n = 48$ protocols for an anterior AW and $P = 0.0009, n = 32$ protocols for a posterior AW). Stimulation of whiskers belonging to the same arc produced a more equilibrated number of suppressions and facilitations (Fisher’s exact test, $P = 0.4, n = 34$ protocols for a dorsal AW, $P = 1.0, n = 34$ protocols for a ventral AW). Figure 12C summarizes the average percentage of cases showing facilitation and suppression for the different PW-AW configurations. The stimulation of the PW generates an anisotropic suppressive effect that is maximal on the response to the anterior AW and a maximal facilitatory effect on the response to the dorsal AW (see Fig. 12C, right). A similar profile is generated by the stimulation of the AWs on the response to the PW, with an additional facilitatory effect for the anterior AW.

**Influence of the neuron’s laminar position**

We estimated the cortical layer of each recording site from the depth of the electrode penetration and histological examination (see METHODS). We have grouped the units into supra-granular (27 single units and 30 multi-units), granular (31 single units and 34 multi-units), and infragranular (54 single units and 46 multi-units) divisions.

Facilitatory and suppressive effects were found throughout the thickness of the cortex, but the pattern of integration of multi-vibrissa information depended on the laminar position of the cortical cells. Figure 13 summarizes the distribution of facilitation and suppression effects at an 8-Hz stimulation frequency across supra-granular, granular, and infragranular divisions. The percentage of protocols showing a suppressive effect while stimulated at 8 Hz increased significantly from superficial to deeper cortical layers ($\chi^2, P < 0.001, n = 148$ protocols for the single units, Fig. 13A1 and $P < 0.03, n = 154$ protocols for the multi-units, Fig. 13A2). The relationship with layers was less pronounced for the facilitatory interactions,
although there was a trend in single and multi-units for layer IV showing more facilitations than the other cortical layers (Fig. 13, A1 and A2). This trend reached statistical significance for the multi-units ($/H9273^2$, $/H11005^0.04$, $/H11005^n154$). The more detailed examination of the effects as a function of ISI (Fig. 13, B and C, for single units) confirmed that different combinations of supra- and sublinear interactions exist in the different cortical layers.

The observation that more significant facilitatory interactions were found when the stimulated whiskers belong to the same arc than to the same row was not generalized to all cortical layers. The number of facilitation cases was significantly higher for arc than row stimulation in single units of layers V–VI ($/H11005^6/35$ vs. $/H11005^0/37$ protocols, Fisher’s exact test, $/H11005^P0.01$), but not in layers I–III ($/H11005^3/14$ vs. $/H11005^1/18$ protocols, $/H11005^P0.29$) or IV ($/H11005^5/19$ vs. $/H11005^4/25$ protocols, $/H11005^P0.47$). No difference was found in the number of suppression cases for arc and row stimulation across layers. The mean CTR values across ISIs confirmed that the arc-row difference was significant for single units in layers V–VI ($F_{1,154}^5.34$, $/H11005^P0.022$) as well as in layer IV ($F_{1,154}^5.78$, $/H11005^P0.017$), but not in layers I–III ($F_{1,154}^1.45$, $/H11005^P0.5$). Similar results were observed in the multi-unit population, confirming that the arc-row bias is particularly strong in layers V–VI.

**Discussion**

In the barrel cortex, the integration of the afferent activity originating from multiple whiskers produces heterogeneous effects such as sublinear and supralinear summations. Our results indicate that at a low frequency of stimulation (0.5 Hz), the deflection of one whisker results in either no change or in a suppression of the response to the deflection of a second whisker. When deflections are applied at a higher frequency (8 Hz), facilitatory effects are revealed such that the response to two-whisker deflections is statistically larger than expected if there was no interaction. The supralinear summation was most prominent when the two whiskers were aligned on an arc rather than on a row.

**Predominance of suppressive interactions**

The effect of two sensory stimulations displaced in time or space on the cortical response has been studied in several sensory systems and has led to the general result that suppressive effects strongly dominate for two successive stimuli: see Nelson (1991) for the visual system, Gardner and Costanzo (1980a,b) for the somatosensory system, and Brosch and Schreiner (1997) for the auditory system. This inhibitory effect was described also in the barrel cortex of the rat (Mirabella et al. 2001; Simons 1985; Simons and Carvell 1989). Our results agree with this general finding: following the deflection of one whisker at low frequency, we observed a suppression of the response to the deflection of a second whisker in the majority of the cases (59% of the neurons). However, the temporal window of these suppressive effects varied widely among the
recorded neurons. Individual tuning curves of selectivity to the ISI ranged from bell-shaped to U-shaped profiles. This is in contrast to the results of Simons (1985) and Simons and Carvell (1989), who consistently reported maximal suppressive effects for 10–20 ms (this would correspond to W-shaped ISI tuning curves). In agreement with these authors, we found that the order in which whiskers are deflected influences the interaction, so that both symmetrical and asymmetrical ISI tuning curves exist. Also, the geometry of the whisker combination in the mystacial pad is an important factor. We extend this last result, previously described for row stimulation, to the case of stimulation along an arc.

**FIG. 12.** Facilitatory and suppressive interactions depend on the whisker combination. A: mean CTR ± SE as a function of ISI averaged over all tests at 8 Hz for single units (left) and multi-units (right) along a row (black symbols; 80 single units and 81 multi-units) and along an arc (white symbols; 68 single units and 73 multi-units). In both cases, mean CTR values across ISIs were significantly greater for arc than for row stimulation (ANOVA, $F_{1,163} = 9.27$, $P = 0.003$ for single units and $F_{1,163} = 11.65$, $P = 0.0008$ for multi-units). B: percentage of cases for which a significant facilitatory (white bars) or suppressive (black bars) effect was found as a function of the ISI. The 4 panels represent dorsal ($n = 34$ cells, top left) or ventral ($n = 34$ cells, bottom left) whisker stimulation along an arc and anterior ($n = 48$ cells, top right) or posterior ($n = 32$ cells, bottom right) whisker stimulation along a row. C: average percentage of total facilitated (white plot) and suppressed (black plot) cases were computed from the histograms in B and depicted as polar plots. Positive ISIs (the PW preceding the AW stimulation) and negative ISIs (the AW preceding the PW stimulation) were separated in the right and left, respectively.

Incidence of supralinear interactions for stimulation at a low frequency

Despite the predominance of suppressive interactions that we have observed, previous studies on the spatiotemporal sensory integration performed by barrel cortex neurons have reported the presence of facilitatory interactions between the responses for different whiskers (Ghazanfar and Nicolelis 1997; Ghazanfar et al. 2000; Shimegi et al. 1999, 2000). Notably, the work by Shimegi and colleagues, using very similar procedures to those applied here (anesthesia, unit recording, frequency of stimulation, choice of ISIs), has described a much higher incidence of supralinear summation for 0.5-Hz stimulation than what we have
observed. Specifically, they reported 22% of facilitatory interactions (which concerned 37% of the cells; each cell could be tested for several combinations of whiskers), whereas, using the same index, we found no facilitation among the 63 protocols completed on 34 neurons.

The source of the discrepancy between these results is not clear. One possibility is that the percentage of facilitatory cases in the study by Shimegi and colleagues has been overestimated. Indeed, these authors did not use a statistical criterion, but placed an ad hoc threshold of 1.25 on the FI, above which they considered that a significant supralinear summation was taking place. When we used this same criterion on our data, we found 30% of facilitation cases (that concerned 50% of the cells), which is close to the percentage found by Shimegi and colleagues. However, none of these cases were significant when we compared the observed spike counts and those predicted by the linearity assumption with a statistical test.

The two studies also differ by their baseline level of activity for stimulation at low frequency. The recordings by Shimegi et al. (1999, 2000) show, in general, a lower level of both spontaneous and evoked activity than what we typically observed. Thus in our sample, the responses at 0.5 Hz might have been already close to a maximal firing rate for the units, so that no facilitation could be induced. It is worth noting however that higher levels of evoked responses have been reported under similar experimental conditions in urethane-anesthetized animals (Armstrong-James and Fox 1987); thus most probably maximal firing rates were not attained in our sample. In fact, the level of the evoked response we have observed (0.7 a.p./stim) is well within the range of responses that have been reported recently both in urethane-anesthetized animals (Celenk et al. 2004; 0.66 a.p./stim for layer IV cells and 0.39 a.p./stim for layer II-III cells) and in fentanyl-sedated animals (see Fig. 3A in Lee and Simons 2004). Thus a very low level of activity such as that observed in Shimegi et al. (1999, 2000) might be necessary for the emergence of supralinear summation.

Finally, most of the facilitatory effects found by Shimegi et al. (2000) were on units recorded in the septae. We have not quantified the proportion of our recordings made from barrels and septae. However, from the latency of responses and the ratio between the responses to the PW and the AW, we estimate that some of the 34 single units stimulated at 0.5 Hz could have been recorded from septae. None of the 34 cells showed a response facilitation based on the FI used by Shimegi et al. This point needs, nonetheless, further inquiry.

**Influence of the frequency of whisker deflections**

Significant supralinear interactions were found only at high-frequency stimulation (8 Hz). Interestingly, 8 Hz belongs to the range of frequencies naturally used during whisking, and the results obtained in these conditions might thus be more repre-
sentative of the multi-whisker integration during active behavior than those obtained at a low frequency.

The repetitive deflection of one or several whiskers modifies profoundly the activity level of the cortical network. As we (Ahissar et al. 2000; Ego-Stengel et al. 2001) and others (Simons 1978) have previously described, responses of barrel cortex neurons submitted to a train of deflections exhibit a rapid adaptation of response. In the steady-state regimen, the evoked spike count due to one deflection is thus lower than the spike count for an isolated deflection (or deflections applied at a low frequency). As mentioned above, this reduced level of activity in the network might be required to reveal increases in response and could thus explain the fact that we did not observe supralinear interactions at a low frequency of stimulation but only at 8 Hz. Also, our data suggest that facilitatory interactions are revealed mostly in cells adapting strongly to single-whisker deflections; that is, the cells whose activity underlies the overall modified state of the cortical network at high frequencies of stimulation. By reducing the adaptation rate in some of these cells, the nearly simultaneous deflection of two whiskers at a high frequency would create an activity pattern in the barrel cortex specific to the spatiotemporal combination of whiskers stimulated. It has been proposed (Moore 2004) that cortical adaptation may be also involved in the reduction of the spatial spread of SI activation (Sheth et al. 1998) and in shaping the temporal properties of neuronal responses (Garabedian et al. 2003) observed at the whisking frequency bandwidth.

Influence of the cortical layer

We have observed a heterogeneous combination of facilitatory and suppressive two-whisker interactions across layers. Sublinear summations were found mostly in infragranular layers and also in layer IV, whereas supralinear summations occurred in all layers but preferentially in layer IV. This is at odds with previous work, which suggested that the laminar distribution of neurons exhibiting selectivity to the spatiotemporal pattern of stimulation avoids layer IV. For example, a peak of supralinear summation was found in layers II/III (Shimegi et al. 1999), and facilitatory interactions were also found in layer V (Ghazanfar and Nicolelis 1997). In parallel, anatomical data have confirmed the lack of intracortical horizontal connections between barrels in layer IV and their predominance in nongranular layers, where they could be the basis for multi-whisker integration (Gottlieb and Keller 1997; Ito 1985; Lübke et al. 2000; Petersen and Sakmann 2000). These last observations, as well as electrophysiological recordings and optical imaging studies investigating functional circuitry (Goldreich et al. 1999; Laaris and Keller 2002; Petersen and Sakmann 2001; Petersen et al. 2001), have contributed massively to the idea that barrels in layer IV function independently of one another, i.e., activity from one barrel does not spread directly to an adjacent barrel.

Several considerations might help explain how, in this scheme, whisker facilitatory and suppressive interactions could still arise in layer IV. First, even though barrels themselves have no direct connectivity between them, long-range anatomical connections in layer IV have been described in the septae (Höflinger et al. 1995; Kim and Ehner 1999) and could participate in the integration of activity from several whiskers. Second, the integration of the activity from two whiskers could use the thalamocortical and intrab barrel circuitry. Indeed, a wealth of thalamic recordings (Diamond et al. 1992; Simons and Carvell 1989), intracellular cortical recordings (Moore and Nelson 1998; Zhu and Connors 1999), and extracellular layer IV recordings (Armstrong-James and Fox 1987; Armstrong-James and George 1988; Simons et al. 1992) showed that layer IV neurons receive robust multi-vibrissa input that is both subthreshold and suprathreshold. This input is contributed to by feed-forward connections from the multi-vibrissa receptive fields in the thalamus and probably by disynaptic and polysynaptic intracortical connections from neighboring barrels (Armstrong-James et al. 1991). The contribution of these different input lines is not fixed. The receptive fields in the medial division of the ventral posterior nucleus of the thalamus shrink at deep anesthesia (Friedberg et al. 1999), leading to a very weak surround receptive field. In those conditions, the stronger multi-vibrissa input is the cortico-cortical. Following single-whisker deflections, the excitatory drive from AWs is often too weak to trigger reliable firing because of the massive inhibition that rapidly takes over (Miller et al. 2001). However, when several whiskers are deflected nearly simultaneously, the thalamic and/or intracortical drive could become strong and synchronous enough to overcome the inhibition and add supralinearly.

Mechanisms of the facilitatory interaction

The facilitatory interaction was revealed under conditions of low evoked activity and high adaptation during repetitive deflections of the whiskers. The study of the mechanisms underlying the adaptation of cortical responses has revealed that the thalamocortical postsynaptic potentials decrease during a train of single-whisker deflections (Chung et al. 2002) and that the intrab barrel excitation is lower (Petersen 2002). Also, the propagation of activity from one barrel column to adjacent barrel columns in supra- and infragranular layers is reduced by the activation of the lateral GABAergic inhibition at these frequencies (Contreras and Llinas 2001; Petersen and Sakmann 2001; Sheth et al. 1998; see also Moore et al. 1999). This modified balance between excitation and inhibition in the cortical network could be an important factor in the occurrence of facilitatory interactions at 8 Hz (Moore et al. 1999). Specifically, the evoked activities produced by the deflection of two different whiskers could interact already in first-order cortical neurons by nonlinear summation of thalamocortical postsynaptic potentials. Divergent thalamocortical projections have been described (Arnold et al. 2001), which may provide an anatomical substrate for multi-whisker interactions. At low frequencies, the large excitatory postsynaptic potentials (EPSPs) might saturate the postsynaptic voltage changes and add sublinearly, whereas at a higher frequency, the EPSPs are small and a supralinear summation could take place. Alternatively, the facilitatory interaction might involve the intrab barrel circuitry, and for supra- and infragranular neurons, the connectivity between layers and between barrel columns. More complex changes involving the corticothalamic loop cannot be excluded. Determining if excitatory and inhibitory neurons in each layer display similar effects could provide critical clues in respect to these speculative hypotheses.
Influence of the spatiotemporal pattern of whisker stimulation

We show herein that supralinear summation is observed more often when the two stimulated whiskers belong to the same arc than when they belong to the same row. This finding agrees with the study by Ghazanfar and Nicolelis (1997), who showed more supralinear responses for multi-whisker deflections along an arc than along a row. We extend this result, obtained for simultaneous deflections at a low frequency of whisker stimulation (1 Hz), by testing a range of ISIs from −30 to 30 ms and using a stimulation frequency in the range of those used during whisking. Interestingly, this asymmetry in the incidence of facilitatory interactions is matched by an asymmetry between rows and arcs as the mystacial fan contacts an object. Because of the caudorostral movement of the whiskers during behavior, whiskers in a same row tend to be deflected successively by an object, so that the identity of the stimulated whiskers does not provide information about the location of the object along the rostrocaudal axis (but see Brecht et al. 1997). In contrast, only whiskers of an arc with the appropriate vertical position are deflected, so that the identity of the stimulated whiskers inside the arcs contains information about the dorsoventral position of the object. Thus rows and arcs are not functionally equivalent (Ahissar and Arieli 2001). The supralinear interaction that we observed predominantly for stimulation of whiskers in an arc could be a means of encoding spatial information in the cortex.

As Ghazanfar and Nicolelis (1997) already discussed, the finding that more facilitatory effects are observed for whiskers along arcs is intriguing in regard to the anatomical cortical connectivity bias along rows (Bernardo et al. 1990a,b; Hoeflinger et al. 1995; Kim and Ebner 1999). Corticothalamic projections, on the contrary, could provide a substrate for a bias toward arc facilitatory interactions. Indeed, excitatory projections from infragranular layers terminate along arcs in the thalamus, whereas disynaptic inhibitory influences are elongated along rows (Bourassa et al. 1995; Hoogland et al. 1987). Furthermore, we observed that the arc-row bias in the occurrence of the facilitatory interaction was strongest in the infragranular layers, a finding that is in agreement with the study by Ghazanfar and Nicolelis (1997), who recorded only in layer V. Thus, although facilitation was most prominent in layer IV, it might be necessary to involve the whole thalamocortical loop to explain our findings.

Interestingly, in the few cases where supralinear interactions were found for whiskers in a row, facilitation occurred for the rostrocaudal deflection of the adjacent rostral whisker first followed by deflection of the PW, which agrees with the spatiotemporal combination of stimulation expected for contact with an object during a protraction (Sachdev et al. 2001). However, it is difficult to relate this to the anatomical bias of axonal horizontal connections toward barrel columns situated more rostral (Bernardo et al. 1990a; Hoeflinger et al. 1995), which would more easily explain the influence of a caudal AW deflected before the PW.

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