Temporal Characteristics of Response Integration Evoked by Multiple Whisker Stimulations in the Barrel Cortex of Rats

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We investigated the responses of 114 cells in the barrel cortex of rats to describe the temporal characteristics of excitatory interactions among neurons serving two vibrissae. To examine these interactions, the principal whisker and one adjacent whisker in the same row were stimulated simultaneously or serially at various interstimulus intervals (ISIs). In 37% of the cells tested, combined stimulation of two whiskers exhibited response facilitation; the response to the combined stimulus was larger than the sum of the responses to stimulation of the individual whiskers. The occurrence and magnitude of the facilitation were strongly dependent on the ISI. The ISI capable of producing facilitation for a particular cell was tuned to a narrow range (mean $\pm$ SD, 5.3 $\pm$ 2.3 msec). The ISI that evoked the maximal facilitation was 1.3 $\pm$ 1.3, 3.4 $\pm$ 2.3, and 2.8 $\pm$ 4.5 msec for neurons in layers II/III, IV, and V/VI, respectively. These ISIs corresponded to the difference in latencies between the responses to the individual stimulations of the principal and adjacent whiskers. A significant response facilitation was observed in the regular-spiking cells but not in the fast-spiking cells. When the ISI was longer than the range that evoked facilitation, a suppression of the response to the second whisker stimulation was observed. Facilitation was observed predominantly in layer II/III cells (69%) and to a lesser extent in cells of layers IV (15%) and V/VI (24%). Our results suggest that, in the barrel cortex, the temporal relationships among tactile stimuli are coded by facilitatory and inhibitory interactions among neurons located in neighboring barrel columns.

Key words: somatosensory system; barrel cortex; response interaction; response facilitation; spatiotemporal; multiwhisker stimulation

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In rodents, the facial whiskers provide tactile information on space and nearby objects and motion of self relative to an object (Richter, 1957; Griffiths, 1960; Schiffman et al., 1970; Carvell and Simons, 1990). In the primary somatosensory cortex (SI), morphologically and functionally distinct modules called "barrels" are arranged topographically, thereby representing terminal fields of the thalamocortical inputs of individual whiskers (Woolsey and Van der Loos, 1970; Welker and Woolsey, 1974). Neuronal circuitry of the barrel cortex transforms inputs from thalamic neurons having receptive fields covering multiple whiskers with weak inhibitory surrounds so that individual cortical neurons display receptive fields predominantly representing single whiskers and having strong inhibitory surrounds. The result is a precise somatotopic map of the whiskers in the cortex (Simons and Carvell, 1989; Armstrong-James and Callahan, 1991; Nicolelis and Chapin, 1994).

Under natural conditions, tactile information about surrounding objects or motion of the rat itself is produced from the simultaneous or successive stimulation of several whiskers. Thus, the integration of the spatiotemporal patterns of inputs evoked by natural stimuli must be important for the processing of somatosensory information about the surrounding environment. Consequently, we presume that neurons in the barrel cortex respond differently when multiple whiskers are stimulated from when single whiskers are stimulated. Supporting this idea of important interactions among barrels, Simons (1985), Kyriazi et al. (1994), and Brumberg et al. (1996) reported that a response elicited by stimulation of a single whisker could be modified by including surrounding whiskers in the response. The interactions observed were predominantly inhibitory and would serve to enhance the spatial contrast between the principal and adjacent whiskers.

Furthermore, Armstrong-James and Fox (1987) and Armstrong-James et al. (1992) reported that excitation from layer IV was first relayed within a single barrel to the superficial layers and then to the superficial layers of adjacent columns. A recent intracellular recording study showed that a subthreshold input from a single whisker spreads to five rows and arcs of cortical barrel columns (Moore and Nelson, 1998). Such a divergence of excitation demonstrates that excitatory influences from neighboring barrels are available within any one barrel column. In support of this notion, Ghazanfar and Nicolelis (1997) found that simultaneous deflection of three whiskers evoked response facilitation in ventral posterior medial thalamic (VPM) neurons and layer V barrel cortex neurons.

Neurons in the barrel cortex often respond to deflection of a single whisker with a single spike, which suggests that excitatory influences have a short time course. Therefore, if neighboring whiskers are stimulated with the appropriate interstimulus interval (ISI), the excitations derived from nearby whiskers will facilitate the response of the principal whisker. To examine this possibility, we first measured separately the latencies of the responses evoked by deflection of the principal whisker and those of a neighboring whisker. Then, we combined the stimulation of the two whiskers with a time delay that adjusted for the latency difference. We confirmed our hypothesis that many cells in the barrel cortex exhibited a facilitatory interaction. In this report, we
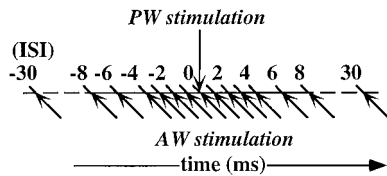
Figure 1. Schematic of the paradigm of combined whisker stimulation. The principal and adjacent whiskers (*PW* and *AW*), in the same row, were briefly deflected either in the rostral or caudal direction at intervals of 0, 1, 2, 3, 4, 6, 8, 10, 12, and 30 msec. The timing of whisker stimulations is indicated by *arrows*. The paired whiskers were deflected in the same direction under all stimulus conditions.

Table 1. Laminar distributions of cells

|	Cells (*n*)
II/III	39
IV	33
V/VI	42
Total	114

describe the electrophysiological properties of the facilitatory interaction between neurons in the barrel cortex elicited by the stimulation of two whiskers.

MATERIALS AND METHODS

Preparation. All efforts were made to minimize animal suffering and the number of animals used. Toward this end, the depth of anesthesia was carefully checked throughout the duration of the experiments, and every effort was made to collect as much data as possible from each animal
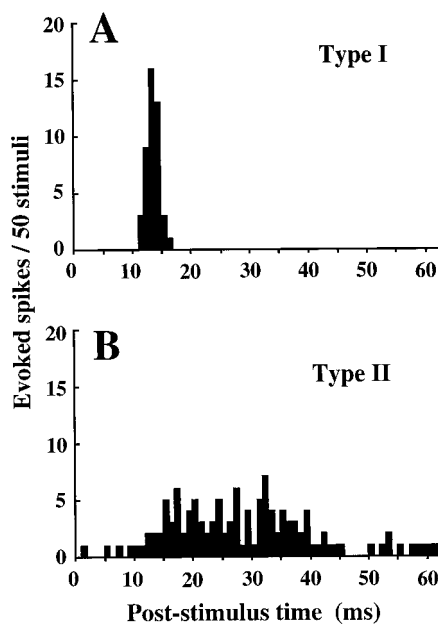

Figure 2. Typical response of barrel cortical cells to single whisker deflection. *A*, Type I response showing fixed latency with a spike aggregate within a short time window (2–5 msec) in the PSTH. The cell was located in layer II/III, and the E2 (PW) whisker was deflected in the caudal direction. *B*, Type II response showing variable latencies ranging 10–50 msec without distinct aggregates of spikes in the PSTH. The cell was located in layer V, and E1 (PW) whisker was deflected in the rostral direction. PSTHs were constructed with spikes accumulated over 50 repetitions of PW deflection. Bin widths, 1 msec.
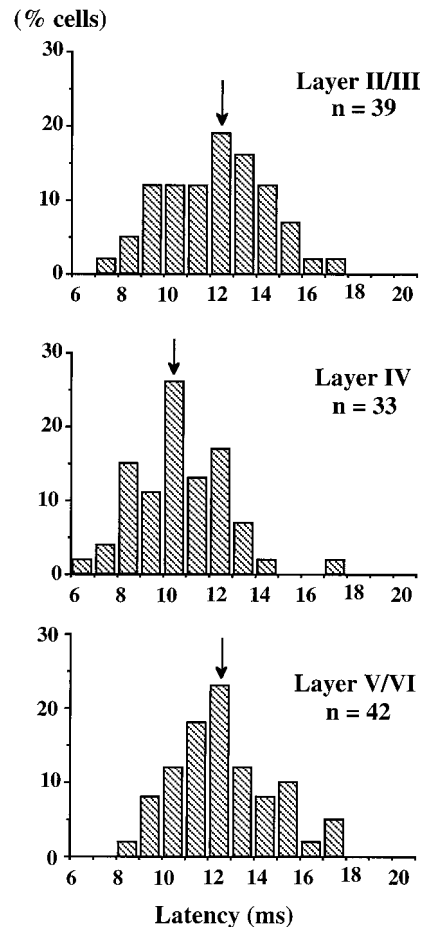
Figure 3. Histograms of the shortest latencies of responses to the PW deflection. *Top*, Layer II/III; *middle*, layer IV; *bottom*, deep layer. The average latency for layers II/III, IV, and V/VI is 10.0, 12.0, and 12.2, respectively (indicated with *arrow*).

after stable recording conditions were achieved. Because of the nature of this study, the use of alternatives to *in vivo* techniques was not possible. The surgical procedures used were all in accordance with the National Institutes of Health guidelines for the care of experimental animals (National Institute of Health, Committee on Care and Use of Laboratory Animals, 1985) and the regulations of the Animal Care Committee of the Osaka University Medical School. Fifty-three adult Sprague Dawley rats weighing between 200 and 450 gm were used in this study. Dexamethasone acetate (Decadron-A, Banyu) was injected (0.4 mg, i.m.) 12–24 hr before the start of the experiments. The animals were anesthetized with urethane (1.25 gm/kg, i.p.). The local anesthetic lidocaine was given at pressure points and around surgical wounds. After the initial surgery, the animal was placed on a stereotaxic headholder. The depth of the anesthesia was monitored throughout the duration of the experiment by testing reflexes and changes of heart rate to pinching of the tail. If the heart rate changed when the tail was pinched, urethane was added. It was ensured that respiration was regular (80–100 breaths/min) and spontaneous movements were absent. Rectal temperature was maintained at 37–38°C by a thermostatically controlled heating pad.

Device for whisker stimulation. Whiskers were stimulated mechanically using stimulating probes attached to a galvanometer (Ito et al., 1979; Ito, 1985). Three galvanometers were used to deflect up to three whiskers independently. Whiskers contralateral to the recorded barrel cortex were trimmed to a length of 15 mm and securely held with a wedge at the tip of the stimulating probe. The tip of the stimulating probe was positioned at a distance of 10 mm from the facial skin. The excursion of the tip of the probe was 1.1 mm over 10 msec, and the onset and offset velocity was 110 mm/sec at the tip without a hold phase in either direction. This velocity was sufficient to elicit a supramaximal response as reported by
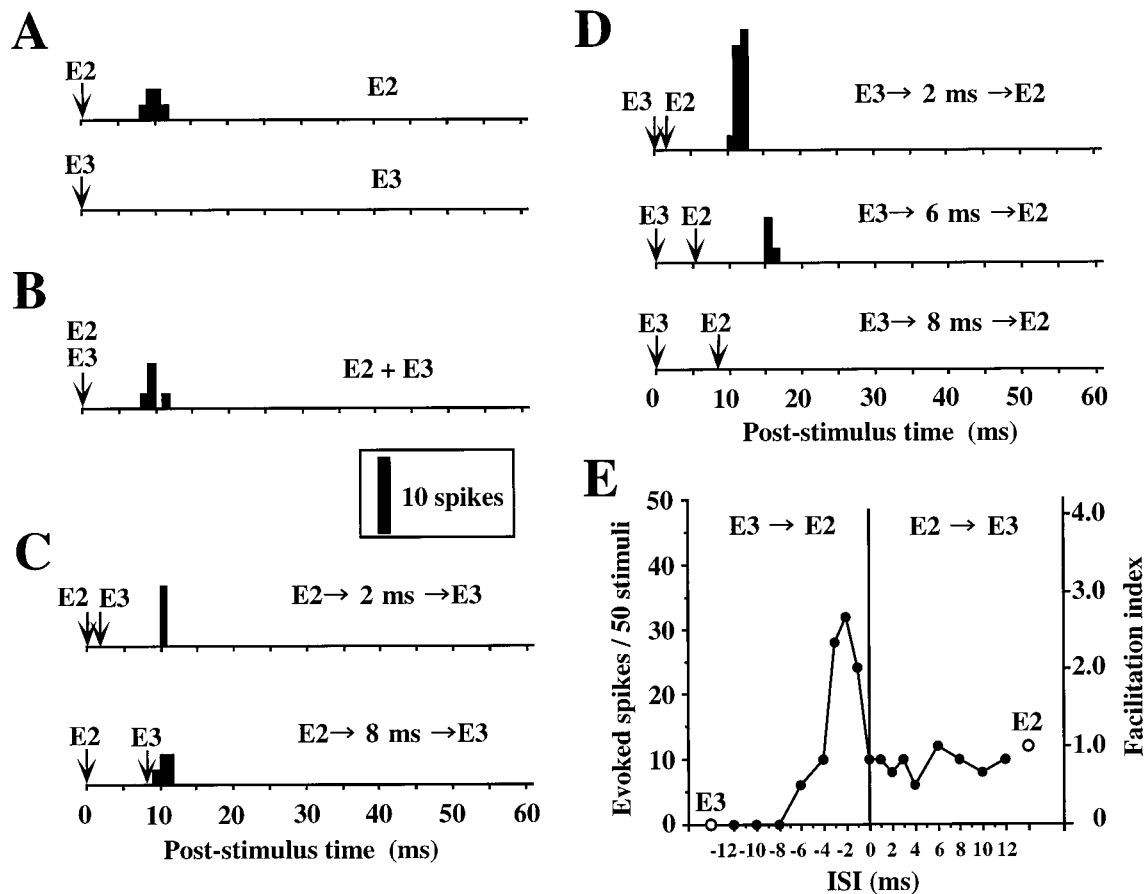
Figure 4. An example of response facilitation of a layer II/III cell. *A–D*, PSTHs of responses accumulated over 50 stimuli. *A*, Responses to deflection of the E2 (PW) (*top*), and E3 (AW) (*bottom*) whiskers. E3 stimulation did not evoke spikes. *B*, Response to simultaneous stimulation of E2 and E3 whiskers. *C*, Responses to successive stimulation of E2 and E3 whiskers. The PW (E2) stimulation was followed by AW (E3) stimulation with an ISI of 2 (*top*) or 8 (*bottom*) msec. The magnitude of the responses differed little from that to single stimulation of the E2 whisker (*A, top*). *D*, Responses to successive stimulation of E3 and E2 whiskers. Antecedent stimulation of E3 with an ISI of 2 msec induced response facilitation (*top*), but that with an ISI of 8 msec induced suppression (*bottom*). Whiskers were deflected from the rostral to the caudal position. The timing of the stimulations is indicated by *arrows*. Bin width, 1 msec. *E*, Relationship between response magnitude expressed as the number of spikes per 50 stimuli (*left ordinate*) or facilitation index (*right ordinate*) and ISI (*abscissa*). The two *open circles* indicate the responses to individual stimulation of the E2 or E3 whisker. *Filled circles* indicate the responses to combined stimulation. Note that the mode and magnitude of response interaction is strongly dependent on the ISI, and the response facilitation sharply tuned to a narrow range of intervals.

Ito (1979) and Ito (1985). The whisker was deflected either rostrally or caudally from its natural position.

Whisker stimulation. A schematic of the paradigm of combined whisker stimulation is illustrated in Figure 1. To examine how neurons code the sequence and timing of stimuli, two whiskers were stimulated either simultaneously or sequentially. For the sequential stimulation, the principal whisker (PW) was stimulated before or after the adjacent whisker (AW) at varying ISIs. Most units were tested with a full set of ISIs of 0, 1, 2, 3, 4, 6, 8, 10, 12, and 30 msec for a few different stimulation combinations of the two whiskers. In this report, a set of data tested with a full set of intervals for a particular whisker combination is called a "case." When the cell was lost before acquisition of a full set of results, the data were discarded. In some cases, longer ISIs of 60, 100, 200, 300, and 400 msec were also tested.

Electrophysiological recordings. A rectangular hole (3 × 4 mm) was made by removing the skull, dura, and arachnoid above the posteromedial barrel subfield of the SI cortex (PMBSF) (4–7 mm lateral to the midline and 0–4 mm posterior to bregma) for inserting the recording electrode. Single-pipette glass microelectrodes were used in this study to achieve the best isolation of single units and also for well-localized dye marking of the recording sites. The electrodes were filled with 0.5 M sodium acetate containing 4% Pontamine Sky Blue (Tokyo Kasei, Tokyo, Japan). The resistance of the electrodes ranged from 13 to 22 MΩ, as measured *in situ*. They were oriented vertical to the pial surface and advanced through the cortex by means of an electronically controlled

microdrive (SM21; Narishige, Tokyo, Japan). In most recordings, we could obtain well-isolated single cells that exhibited unitary spikes with the same waveform, amplitude, and time course.

When a single-unit activity was isolated, PW contralateral to the recorded cortex was assessed qualitatively by manually deflecting the whiskers. Then, electrically controlled stimulators were set along the whisker row. Multiwhisker stimulation, which is the combined stimulation of the PW and one of two AWs in the same row, was routinely tested for each cell. Recordings were restricted to cells in barrel columns of the caudal D-E rows, and γ, δ, and the majority of cells were located within the barrel columns "E1" and "E2". γ and δ whiskers were stimulated in combination with D1 or E1 whiskers.

For each ISI, responses to 50 or 25 stimuli at a frequency of 0.5 Hz were accumulated to construct peristimulus time histograms (PSTHs).

Analysis of whisker responses. The response magnitude of a given cell was defined on most occasions as the number of spikes evoked between 5 and 37 msec after the onset of the whisker stimulation. On rare occasions, when the late component of the responses fell beyond this time window, the window was expanded to include the late response. The spontaneous firing rate was subtracted from the response magnitude. The whisker that elicited the response with the shortest latency or the strongest magnitude was defined as the PW.

Facilitation index of whisker responses. To quantitatively assess the response facilitation by combined whisker stimulation, we calculated the facilitation index (FI) according to the following formula: FI = $R_{com}/$
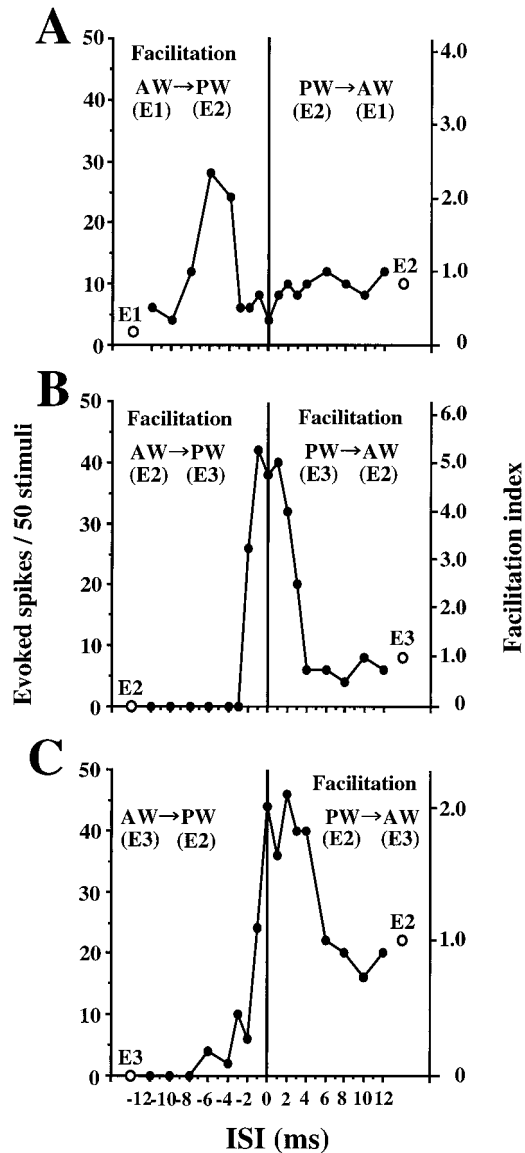
Figure 5. Three representative patterns of ISI tuning curves. *A*, Response facilitation was observed only when AW was stimulated before PW: a layer IV cell. *B*, Response facilitation is noted for both stimulation orders: a layer II/III cell. *C*, Response facilitation was observed only when PW was deflected antecedently: a layer V cell. The percentage for each category was 34, 55, and 11% for *A–C*, respectively. The notations are the same as those in Figure 4*E*.

R_{sum}, where R_{com} is the maximum number of spikes elicited by the combined stimulation of two whiskers, and R_{sum} is the sum of the spikes induced by a single stimulation of each whisker. A FI value <1.0 implies a suppressive interaction of the response to combined deflections of the two whiskers, whereas that >1.0 indicates an augmenting interaction of the response compared to a simple summation of the responses to two single whisker stimulations. In the present report, we defined a response interaction with a FI ≥1.25 as a significant facilitation, which will hereafter be referred to as "response facilitation."

To analyze the effective time range to obtain response facilitation, the effective range of ISI that induces response facilitation (ERI) was measured as the time width of ISIs at which FI was ≥1.25.

Histology. After each penetration, dye marking was produced at the recording sites by passing tip-negative currents (intensity, 5 μA; duration, 1 sec at 0.5 Hz; 200 pulses). After the recording session, the animals were deeply anesthetized with an overdose of anesthetics and perfused transcardially with PBS followed by 4% paraformaldehyde in phosphate

buffer (PB). The recorded cortical hemispheres were flattened and post-fixed in 4% paraformaldehyde/30% sucrose in PB for 4–12 hr. Sixty-micrometer-thick frozen tangential sections of the SI were cut out on a microtome and stored in PBS. The serial sections were histochemically stained for cytochrome oxidase (CO) (Wong-Riley, 1979). Then, the laminar locations of the recording sites and barrels in layer IV were identified by observation under a microscope. Because Nissl staining was not used, barrel territories were divided into two regions, the CO-dense centers (barrel hollow) and the CO-sparse septal regions between hollows (septa). Accordingly, "septa" in this report includes both barrel sides and septa.

RESULTS

A total of 153 cells were recorded from the PMBSF, and their response properties were characterized. Among them, 124 cells remained sufficiently stable for >2 hr to allow accomplishment of the combined stimulation tests with combinations of different whiskers and/or different directions of whisker deflection. The laminar distribution of the cells is summarized in Table 1.

### Response properties for single whisker stimulation

The responses of the recorded 124 cells were classified into three types according to the PSTH patterns for single whisker stimuli (50 or 25 repetitions); type I, response with fixed latency with a spike aggregate within a short time window (2–5 msec) in the PSTH (n = 110; Fig. 2*A*), type II, that with varying latency ranging from 10 to 50 msec without prominent peaks in the PSTH (n = 4; Fig. 2*B*), and type III, that with only a small number of spikes with fixed latency in response to single whisker stimulation, but responding with significant firing frequency to combined whisker stimulation (n = 4; data not shown). The remaining six cells did not exhibit spike responses to any single whisker stimulation. Because type II cells and nonresponsive cells were not suitable for the analysis, we only analyzed the remaining 114 type I and III cells in the present study.

Two types of firing units, regular-spiking units (RSUs) and fast-spiking units (FSUs), were distinguished based on the firing pattern and time course of action potentials: RSUs exhibited a spike frequency adaptation with a spike width approximately double that of FSU (Simons, 1978). The proportion of RSUs and FSUs was 84.2% (n = 96) and 15.8% (n = 18), respectively. RSUs and FSUs are thought to correspond to spiny and smooth neurons, respectively (McCormick et al., 1985). Consistent with previous studies (Simons, 1978; Simons and Woolsey, 1979, 1984), FSUs were observed mainly in layer IV (66%) and rarely in other layers (22% in layer II/III and 11% in V/VI). Such a bias was, however, not observed for RSUs (36, 22, and 42% in layers II/III, IV, and V/VI, respectively).

The latency histograms of the responses to PW stimulation are shown in Figure 3. The average latencies (indicated by arrows) are significantly shorter for the cells in layer IV (10 msec) than for those in other layers (12.0 msec for layer II/III and 12.2 msec for V/VI cells) (p < 0.01; one-way ANOVA followed by Scheffe's *post hoc* test). The mean difference in latencies between the responses to PW and AW stimulation was 1.6, 4.0, and 3.0 msec for the cells of layers II/III, IV, and V/VI, respectively.

Typical patterns of response interaction

A representative facilitatory interaction of responses to combined multiwhisker stimulation is shown in Figure 4. This cell was an RSU type, located in layer II/III. The PSTHs illustrate the responses to the individual stimulation of whisker E2 or E3 (Fig. 4*A*) and to combined deflection of the two (Fig. 4*B–D*). This cell responded to a single stimulation of E2 (PW) but not of E3 (AW)
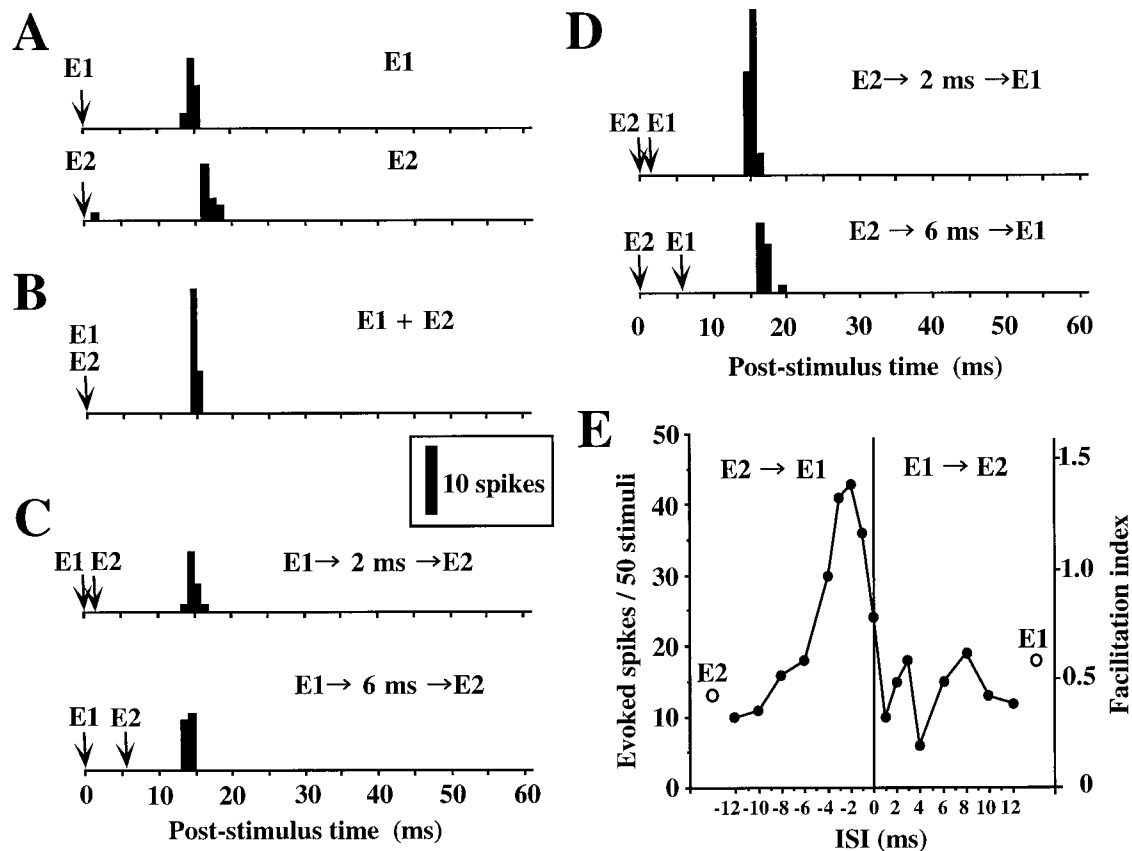
Figure 6. Response facilitation observed when AW was stimulated before PW. *A–D*, PSTHs of responses to deflections of E1 (PW) and E2 (AW) (*A*), and to simultaneous (*B*) and successive (*C, D*) deflections of the two. *E*, ISI tuning curve. Other notations, as in Figure 4. Note that the latency difference between the two whiskers was 3 msec, and response facilitation was observed when E2 stimulation preceded E1 stimulation by a few milliseconds, where two excitatory responses evoked by individual whisker stimulation would be expected to coincide in the cell. This neuron was recorded in layer II/III.

whisker (Fig. 4*A*). In combined stimulation trials, the magnitude of response varied depending on the ISI. When E3 was stimulated 2 msec before E2, a remarkable response facilitation (~270% of the sum of the responses to the individual whisker stimulations) was observed (Fig. 4*D, top PSTH*). The response-tuning curve to ISI of the cell is shown in Figure 4*E*. The response facilitation was observed when the stimulation of E3 preceded that of E2 by 1–3 msec. Moreover, when E3 stimulation preceded E2 stimulation by >8 msec, the E2 response was completely suppressed (Fig. 4*D, bottom PSTH, E*). On the other hand, when E2 was stimulated simultaneously with (Fig. 4*B*) or before E3 (Fig. 4*C*), no modulatory effect was observed. These results demonstrate that the mode and strength of these interactions clearly depend on the order of stimulation and the time interval between the two whisker deflections. Furthermore, stimulation of the E3 whisker evoked a subthreshold level of excitation with a peak latency of 12–13 msec, which was followed by an inhibitory response starting at 15–16 msec.

Response facilitation was observed in 37% (42 of 114) of cells and 22% (56 of 250) of cases analyzed in the present study. In 63% of these cases, the facilitation was observed when the PW stimulation was combined with an AW stimulation, which by itself did not evoke a spike response. This implies that the subthreshold excitatory response to AW stimulation would contribute, to a considerable extent, to the spike response to multiwhisker stimulation.

The response-tuning profile of the ISI varied from cell to cell

depending on the stimulus conditions, such as the combination and deflection direction of the whiskers even for the same cell (see Figs. 4–7). Hence, we grouped the tuning curves according to the three patterns based on the relative timing of the PW and AW stimulation that evoked response facilitation (Fig. 5); in the first pattern, response facilitation was observed only when the AW deflection preceded that of PW (*A*), in the second, stimulation in both orders evoked response facilitation (*B*), and in the third, response facilitation was observed only when PW stimulation preceded that of AW (*C*). Fifty-five percent of the tuning curves were categorized into pattern B, 34% into A, and 11% into C.

The variety of tuning profiles is considered to be attributable to the diversity of temporal dynamics of the individual whisker responses. In other words, the tuning profile was closely related to the time course of the individual excitatory responses. A typical example of a type A case is shown in Figure 6. This cell was an RSU type recorded in layer II/III and responded to stimulation of E1 (PW) and E2 (AW) with 18 and 13 spikes per 50 stimuli, respectively (Fig. 6*A*). The shortest latency of responses to a single stimulation of either E1 or E2 was 13 and 16 msec, respectively. When these whiskers were deflected simultaneously, a response similar to that elicited by single stimulation of the E1 whisker was evoked (Fig. 6*B*). If two responses to a single stimulation of the E1 and E2 whiskers are summative, the stimulus protocol to induce the maximal response facilitation is expected to be stimulation of E2 followed by that of E1 at an interval of ~3 msec, which is the difference in the latency of responses to the
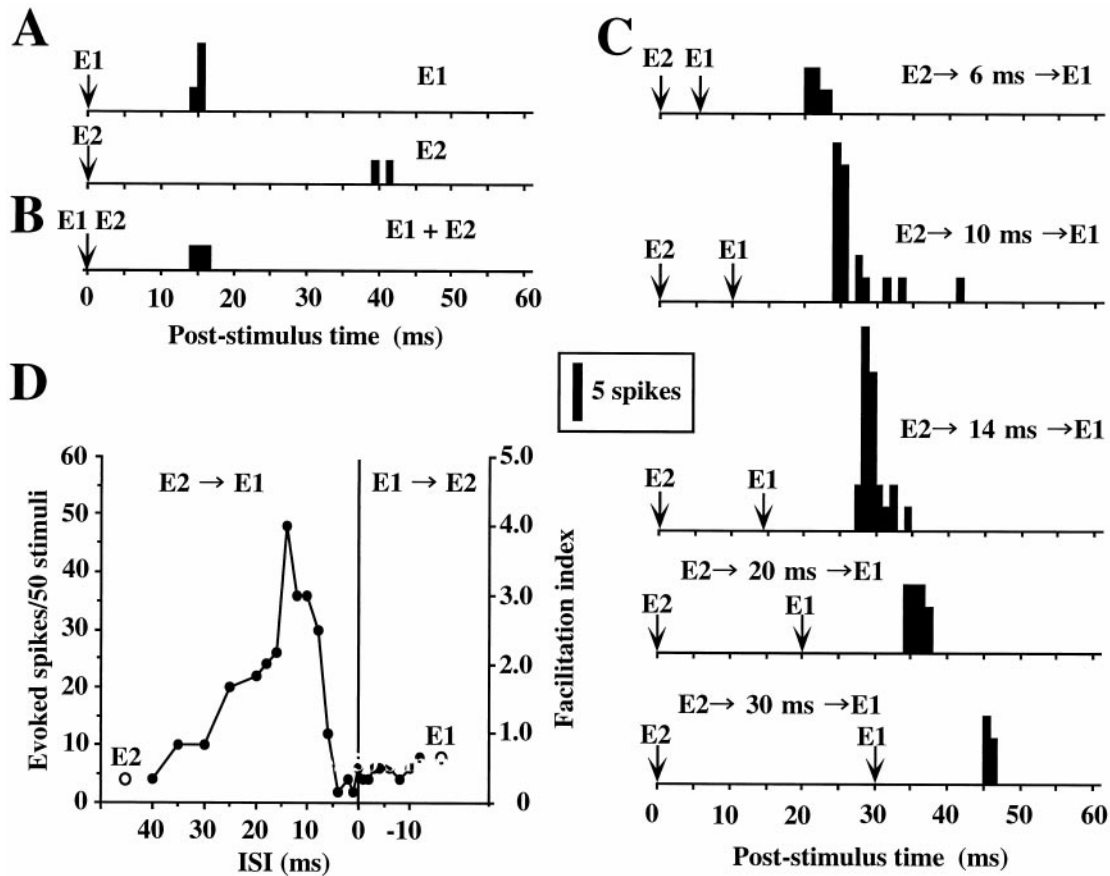
Figure 7. An example of a layer V cell showing facilitatory interaction with a broad tuning profile. *A–C*, PSTHs of responses to separate deflections of E1 (PW) and E2 (AW) (*A*), and to simultaneous (*B*) and successive (*C*) deflections of the two. *D*, ISI tuning curve. In *C*, AW was stimulated antecedently to PW. Other notations, as in Figure 4. Facilitation was observed over a wide range of ISIs when E2 stimulation preceded E1.

individual whisker stimulations. This prediction was confirmed as shown in the top PSTH in Figure 6, *D* and *E*. Thus, the response interaction seems to depend on the relation between the ISI and the latency difference between the responses to the single whisker stimulations.

It should be noted that, in the cell shown in Figure 6, when the ISI was longer than that effective to induce response facilitation, the response to the second whisker deflection was completely suppressed (Fig. 6*C*, *PSTH*, *E2*; *D*, *bottom PSTH, E1*). This type of response interaction, that is, facilitation with a shorter ISI and suppression with a longer ISI, was commonly observed. It seems to be related to the time course of response to the preceding whisker stimulation, that is, rapid excitation and a subsequent long-lasting suppression. Another example supporting this notion is shown in Figure 7. This cell was a layer V cell of the RSU type. It showed very broad tuning profile to ISI for response facilitation, and this type of tuning curve was exclusively observed in layer V cells. As shown in *D*, such response facilitation was observed when the stimulation of the E2 whisker preceded that of the E1 whisker by 6–30 msec. This suggests that the single stimulation of the E2 whisker evoked a prolonged subthreshold excitatory response with an onset latency ~6 msec longer than that of the response to E1 stimulation.

Four RSUs exhibited facilitatory interaction with burst-like firing in response to multiwhisker stimulation, but they responded with only a single spike to single whisker stimulation (data not shown). Three of them were located in layer V and one in the

superficial layer. Regarding neuronal properties, including low spontaneous activity (<1 Hz), spike width and laminar localization, they were apparently unlike FSUs and resembled the intrinsically bursting (IB) neuron, a second type of pyramidal neurons (McCormick et al., 1985; Agmon and Connors, 1989; Chagnac-Amitai et al., 1990).

The response interaction of FSUs exhibited patterns different from those of RSUs. A typical example of a response interaction of an FSU is shown in Figure 8. This cell was located in layer IV and responded vigorously to deflections of either D1 or D2 with a firing rate of approximately two spikes per stimulus (*A*). As shown in *B* and *E*, when two whiskers were deflected simultaneously by which two excitatory inputs would arrive at the cell within a very short period, the magnitude of the response was almost equal to that elicited in response to single stimulation of either D1 or D2. On increasing the ISI, regardless of the order of whisker deflection, the magnitude of the response began to increase and, with an ISI of longer than 8 msec, reached the level of a simple sum of the responses to the two individual whisker stimulation (*C–E*). The basic pattern of the ISI tuning curve was the same for all of the 18 cells that were identified as FSUs, and no facilitation was observed for any of them.

Laminar variation of the incidence of response facilitation

A conspicuous laminar difference was observed in the proportion and degree of response facilitation with an FI ≥1.25. Facilitation was most frequently observed in layer II/III cells and occurred in
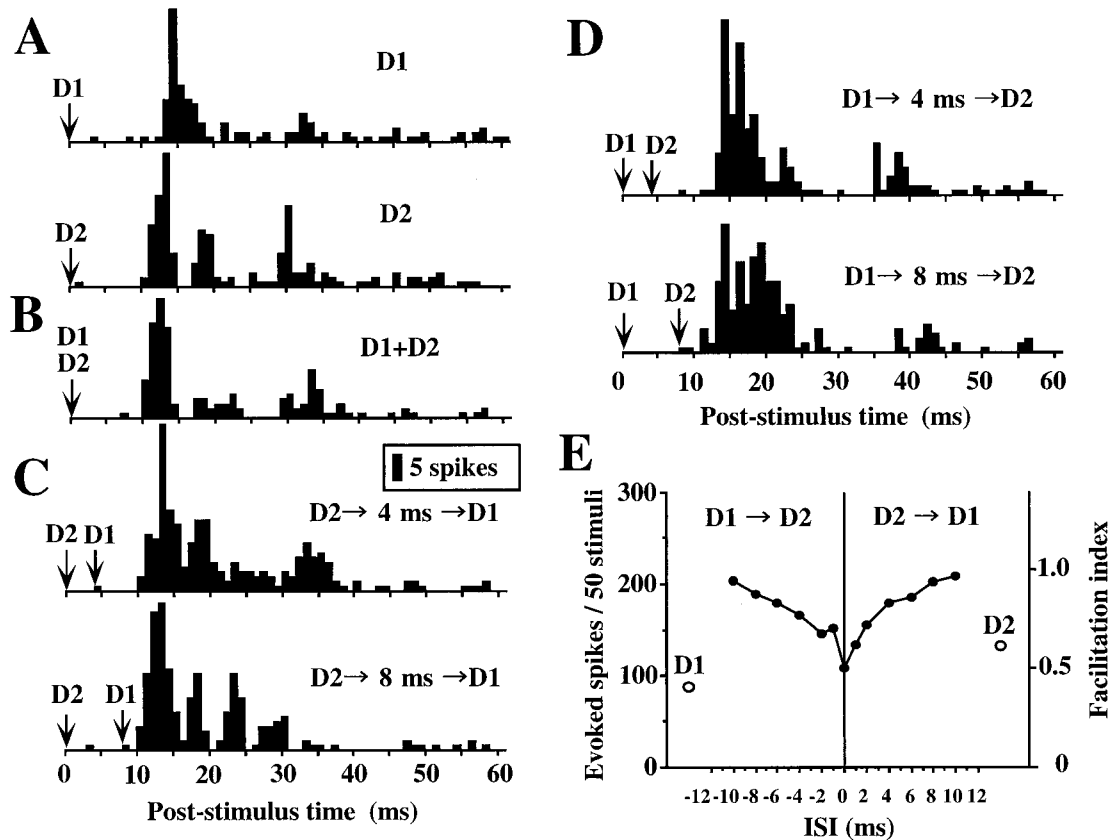
Figure 8. A typical example of the response interaction of an FS cell. *A–D*, PSTHs of responses to separate deflections of D2 (PW) and D1 (AW) (*A*) and to simultaneous (*B*) and successive (*C, D*) deflections of the two. *E*, ISI tuning curve. Other notations, as in Figure 4. When the two whiskers were stimulated simultaneously, the magnitude of the response was almost equal to that to single stimulation of either D1 or D2. The response interaction was simply additive for longer ISIs. This neuron was recorded in layer IV.

69% of the cells analyzed. In contrast, only 15 and 24% of cells in layer IV and infragranular layer, respectively, exhibited response facilitation. Figure 9*A* shows the histogram of the facilitation index values for all the cases analyzed. It clearly demonstrates that a higher degree of facilitation was observed in the superficial layer cells. The difference was particularly clear at FIs of 1.25 ≤ FI < 2.0 and 2.8 ≤ FI. The percentage of cases that did not show response facilitation is indicated in *B*.

ISI tuning

The optimal ISI of whisker stimulation and also the width of the effective ISI are good measures for an estimation of the input mechanism of the response facilitation. Therefore, we measured the ERI, which was defined as the time width of the effective ISI for facilitation with an FI ≥1.25 for each case. An example of such a measurement of ERI in the tuning curve of the cell (same as shown in Fig. 4*E*) is indicated in Figure 10*A*. The distribution histogram of ERI for each layer is shown in Figure 10*B*. The averaged ERI of each layer was 4.5 ± 2.4, 5.5 ± 1.3, and 6.9 ± 6.4 (mean ± SD) msec, for layers II/III, IV, and V/VI, respectively. The mode of overall distribution was 3 < ISI < 4 msec, and layer II/III cells showed a clear unimodal distribution with a peak at 3 < ISI < 4 msec. Only in a few cases, layer IV cells had an averaged ERI slightly longer than that of layer II/III cells. The ERI of cases of layer V/VI cells was distributed in a wide range without any prominent peak.

We also examined the preferred ISI for the induction of re-

sponse facilitation. First, we measured the optimal ISI of whisker stimulation that elicited maximal facilitation. The average value of each layer was 1.3 ± 1.3, 3.4 ± 2.3, and 2.8 ± 4.5 msec, for layers II/III, IV, and V/VI, respectively. Thus, the cells tended to prefer an ISI of only a few milliseconds, and these values corresponded well with the differences in the latencies of responses to the stimulation of PW and AW for each layer (1.6, 4.0, and 3.0 msec for layers II/III, IV, and V/VI, respectively). This result supports the contention that the optimal ISI is determined by the relative timing of the excitatory responses evoked by the stimulation of individual whiskers. Second, the incidence of response facilitation was calculated for the tested ISIs for each layer. The percentage of cases with significant response facilitation as a function of the ISI is shown in Figure 11. Curves for the cases of layers II/III and V/VI have a peak at the ISI of 1 msec, which covers both sides of 0 msec (simultaneous stimulation). This kind of tuning pattern implies that the cells exhibited facilitatory interaction of responses to stimulation of both deflection orders of the PW and AW. Although cells seemed to exhibit facilitation slightly more often when AW was deflected first, stimulation in the opposite order was also effective for the cases outside of layer IV, suggesting a convergence of temporally overlapping two excitations. In contrast, the tuning curve of layer IV cells had a peak clearly displaced by a few milliseconds to the side where the AW stimulation was followed by the PW stimulation (Fig. 11, *filled circles*). This suggests that in layer IV cells, the onset of
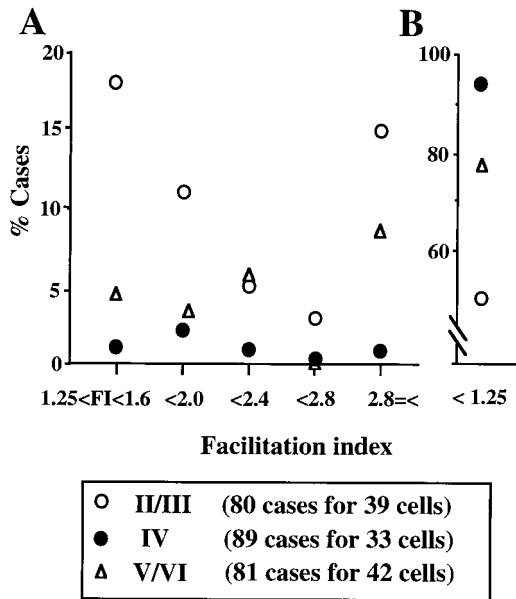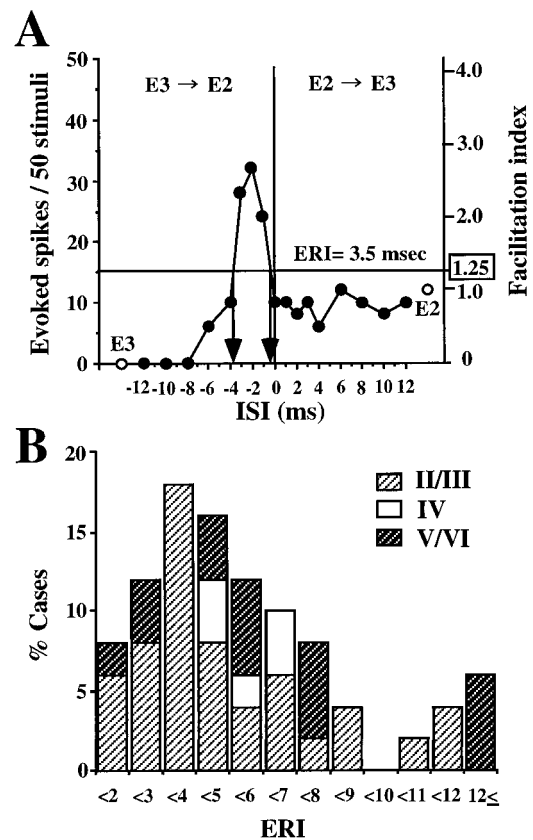
Figure 9. Laminar difference in the facilitation Index (FI). For calculating the FI, the maximal response to combined whisker stimulation was divided by the simple sum of the responses to individual whisker stimulation. *A*, Percentage of cases which exhibited significant (1.25 ≤ FI) response facilitation. The averaged number of cases per cell in each layer was 2.2 ± 1.1, 2.6 ± 1.2 and 2.1 ± 1.1 for layer II/III, IV, and V/VI, respectively. *B*, Percentage of cases that did not exhibit significant facilitation.


Figure 10. ERI. *A*, Schematic illustration of ERI measurement. Two ISIs with FIs of 1.25 were determined, then the time width between the two was measured as ERI. In this example, ERI was 3.5 msec. *B*, Distribution of the percentage of cases that exhibited response facilitation plotted against the ERI. The mode and average of ERI for all layers are 4.0 and 5.3 msec, respectively. Averaged ERI and SD are 4.5 ± 2.4 (*n* = 31 cases), 5.5 ± 1.3 (*n* = 5 cases), and 6.9 ± 6.4 (*n* = 14 cases) msec, for layers II/III, IV, and V/VI, respectively.

excitation evoked by the AW stimulation does not overlap with the time course of excitation evoked by the PW stimulation. In layer V/VI cells, there seemed to be two populations of cases contributing two peaks; the first population showed ISI tuning with a peak over zero, and the second exhibited facilitation with an optimal ISI of a few tens of milliseconds contributing to the second and wide peak largely shifted toward the adjacent-first side of the tuning curve (Fig. 7, and *open squares* in Fig. 11). These results suggest that there are two groups of cells in layer V/VI, one that receives excitatory inputs with a latency difference of >5 msec between the PW response and AW response, and another that receives excitatory inputs with a small latency difference similar to layer II/III cells. Therefore, cells in layer II/III should respond best to coincident deflection of whiskers, and those in layer IV to sequential deflection, and layer V/VI seemed to consist of two groups of cells that exhibit response facilitation to either coincident or successive deflection.

Long ISI

The last issue we address in this report is the effects of whisker stimulation with a much longer ISI (30–400 msec) on response to subsequent stimulation. The representative pattern of interaction we observed in this test (*n* = 21) was the monotonous suppression of response to the second stimulation. A typical example of response interaction with longer ISIs is shown in Figure 12. This cell was recorded from layer II/III and exhibited response facilitation on simultaneous stimulation of E1 and E2 whiskers (*B*). When E2 stimulation was delayed by >3 msec, the response to the E2 stimulation began to be suppressed. The E2 response was completely suppressed by a preceding E1 stimulation at an ISI of 30 msec (*D, top PSTH, F*), and such a suppressive effect lasted >100 msec (*F*).

Figure 13 indicates the suppressive effects of a preceding stim-

ulation of AW at an ISI of 30 msec in 21 layer II/III cells. Eighteen of the twenty-one neurons showed suppression (average reduction, 88.4%) which often lasted up to 100 msec. As a whole, the pattern of the time course of the effects of preceding whisker stimulation was that the stimulus-evoked early component of both the suprathreshold spike response and subthreshold excitation contributing to facilitatory interaction was followed by a long-lasting suppression. On the other hand, a suppressive effect was also occasionally observed without preceding excitation in all layers.

DISCUSSION

We have examined the interaction of responses to combined stimulation of two neighboring whiskers in barrel cortex neurons and have characterized the facilitatory interaction of the response. A large population of neurons (37%) showed a facilitatory interaction of response, that is, the response was greater than the linear sum of the responses to individual whisker stimulation. There was a clear laminar difference, and most cases that exhibited facilitation were of layer II/III neurons. Also, the response facilitation was observed in RSUs but not in the FSUs. The incidence and magnitude of facilitation were strongly dependent on the ISI.
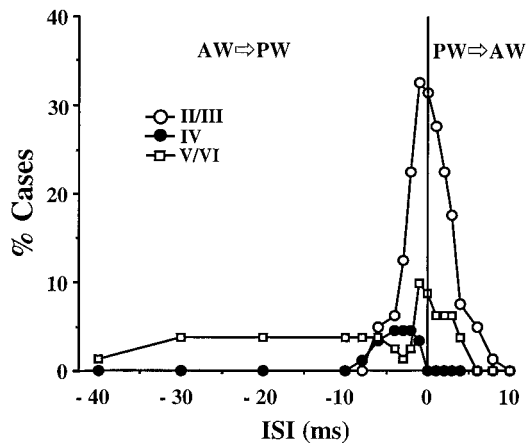
Figure 11. The relationship between the incidence of response facilitation and the ISI. The percentage of cases that showed significant response facilitation was plotted against the ISI. The percentage of cases for layer II/III cells (n = 37 cases for 27 cells) (*open circles*) that prominently peaked at −1 msec. The curve covered deflection orders of both the PW and the AW, suggesting that the two excitations overlapped temporally. In contrast, the peak of the tuning curve of layer IV cells (*filled circles*, n = 5 cases for five cells) was clearly displaced by a few milliseconds to the left side when AW was stimulated first. There were two types of case populations in layer V/VI cells (n = 14 cases for 10 cells) (*open squares*). Note that there are two peaks for the cases of layer V/VI cells, a peak over zero, and another broad peak displaced by more than a few tens of milliseconds.

Facilitatory interaction of response to multiwhisker stimulation

Previous single-unit recording and optical imaging studies have demonstrated that sequential stimulation of two whiskers evoked primarily inhibitory interactions, that is, an excitatory response elicited by PW stimulation was suppressed if an AW was antecedently displaced (Simons, 1985; Kleinfeld and Delaney, 1996; Goldreich et al., 1998). These findings lead to the notion that the cortical barrel column works as a single-whisker processing unit, and its function would be secure from any interference from AWs by inhibitory interaction. On the other hand, a recent study (Ghazanfar and Nicolelis, 1997) has indicated that nonlinear summation in response to the simultaneous deflection of three whiskers was observed in both cortical layer V and thalamic neurons. The present study also suggested that integration of tactile information derived from multiwhisker displacements would occur, at least in part, in the barrel cortex.

Anesthesia

There is an argument that urethane anesthesia selectively augments responses to AW stimulation (Simons et al., 1992). This raises the possibility that the response facilitation observed in the present study was caused by this urethane effect. This, however, seems unlikely because the facilitatory interaction of response of layer V cells to multiwhisker stimulation was also reported in animals anesthetized with pentobarbital (Ghazanfar and Nicolelis, 1997), suggesting that the facilitatory response interaction is not peculiar to urethane-anesthetized animals.

Physiological cell types

The RSUs and FSUs showed different types of interaction of response to multiwhisker stimulation. When two whiskers were stimulated coincidentally, a summation of the two excitations occurred in RSUs, whereas only one of the two excitations appeared in FSUs. There seems to be much room for response summation in RSUs that enables the spatiotemporal integration of information derived from different whiskers. On the other hand, the response of FSUs seems to be nearly saturated with the single whisker input, with little room for multiple whisker interaction. This could be partly attributable to a property of thalamocortical inputs that are sufficiently strong to drive layer IV cells without additional cortical inputs (Stratford et al., 1996).

Locus of input convergence

There are two dominant hypotheses for the region of convergence of excitations related to the surrounding whiskers. The first attributes it to subcortical interaction (Simons and Carvell, 1989) and the second to an intracortical mechanism (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991). Ghazanfar and Nicolelis (1997) reported that multiwhisker stimulation evoked a nonlinear summation of excitatory responses in both the VPM and layer V neurons of the SI, suggesting that reverberating activity of the thalamocortical loop is responsible for the spatial propagation of the multiwhisker response. In our results, response facilitation was observed predominantly in layer II/III neurons (69%) and to a lesser extent in neurons of layers IV (15%) and V/VI (24%). If convergence of excitations for facilitation took place mainly at the subcortical level, we should have observed a facilitatory response interaction more frequently in the layers IV and V which are known to be direct targets of thalamic afferents. Therefore, our results seem to favor the idea that an intracortical mechanism is responsible for the facilitatory response interaction. However, we cannot exclude the possibility of response facilitation at the subcortical level.

Laminar difference in response interaction

There are a few possible explanations for the laminar difference in facilitatory interaction. First, the difference in cell population between layer IV and other layers might be one of the causes. In our results, all the cells that exhibited response facilitation were RSUs that were preferentially located in extragranular layers, and none of the 18 FSUs whose laminar distribution was biased to the granular layer exhibited facilitation.

Second, because layer II/III cells receive their main inputs from thalamic afferents disynaptically via layer IV (Bernardo et al., 1990; Armstrong-James et al., 1992) in addition to monosynaptically to lower layer III (Jensen and Killackey, 1987), the excitatory response of these cells to either PW or AW stimulation would be more synchronized with a narrow time course than that of layer V/VI cells, which mainly receive input from layer II/III cells (Chapin et al., 1987; Bernardo et al., 1990), except for layer Vb, which also receives direct input from the thalamus (White, 1978). In support of this notion, both the effective ERI and ISI for facilitation were more narrowly tuned in layer II/III cells than in layer V/VI cells (Figs. 10, 11).

Third, there are laminar differences in the neural connection supplying the surrounding-whisker-related inputs to the cells. The excitatory connections of the horizontally projecting axon collaterals of pyramidal cells (horizontal connections) are particularly abundant in layer II/III (Bernardo et al., 1990; Hoeflinger et al., 1995), which can provide surrounding whisker-related excitations primarily produced within neighboring barrel columns with a short delay (1.6 msec, in our results) compared with that of the principal whisker-related excitation (Armstrong-James and Fox, 1987; Armstrong-James et al., 1992; Kleinfeld and Delaney, 1996; Welker et al., 1993). In contrast, the lateral connections
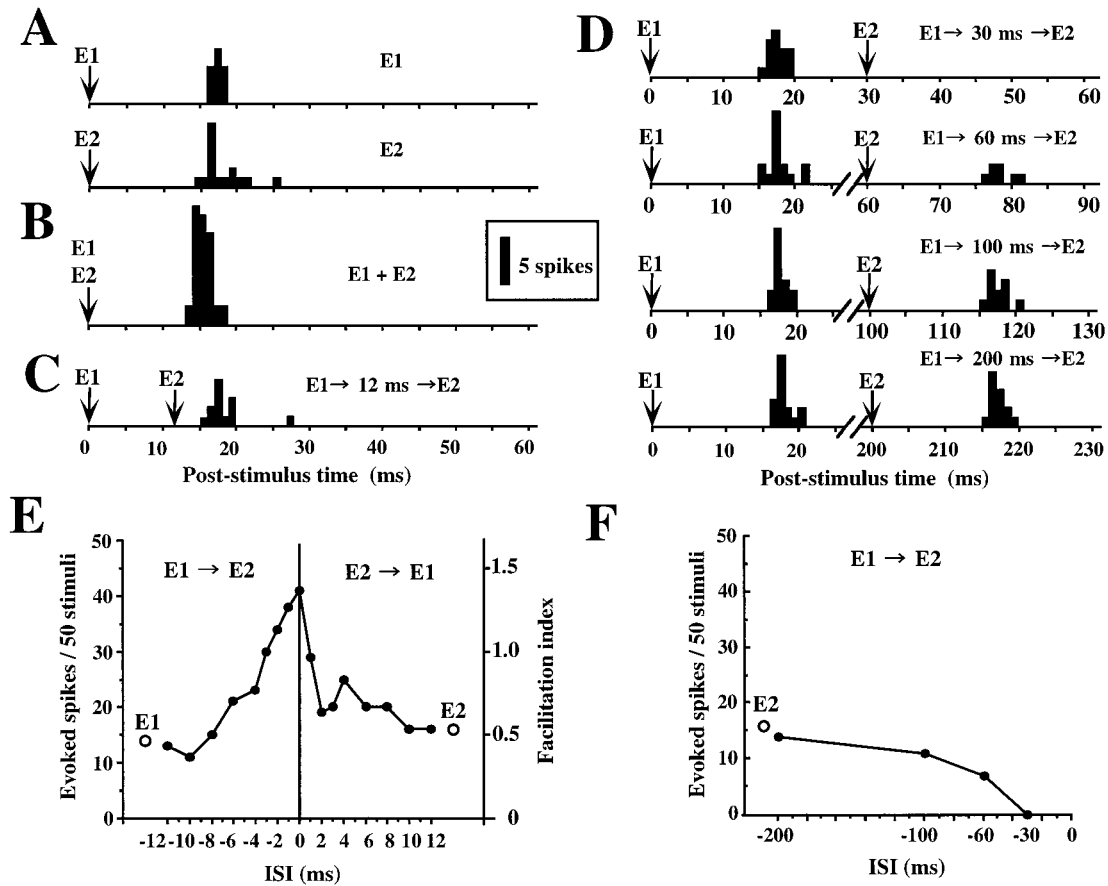
Figure 12. Long-lasting inhibitory interaction of response. *A–D*, PSTHs of responses to separate deflections of E2 (PW) and E1 (AW) (*A*), and to simultaneous (*B*) and successive (*C, D*) deflections of the two whiskers. *E*, ISI tuning curve. *F*, Response magnitude of E2 stimulation preceded by E1 stimulation at ISIs of >30 msec. Other notations, as in Figure 4. This neuron was recorded in layer II/III.

among barrels within layer IV are sparse (Woolsey et al., 1975; Hoeflinger et al., 1995; Yuste et al., 1997), and excitatory inputs related to the surrounding whiskers are supposedly provided via superficial layers with a substantially large delay (4.0 msec, in our results) (Armstrong-James and Fox, 1987; Armstrong-James et al., 1992; Welker et al., 1993), which would be less effective for facilitatory interaction with the PW response.

## Characteristics of the facilitatory interaction

The observed facilitatory interaction of response to multiwhisker stimulation was surprisingly well tuned to a narrow range of ISIs (Fig. 11). This would reflect a short time course of membrane excitation of the barrel cortex neurons evoked by individual whisker stimulation. According to our analysis of ERI (Fig. 10), the duration of the membrane excitation for response summation was estimated in most cases to be <9 msec. This small time window for the facilitatory response interaction should give neurons in the barrel cortex very fine temporal resolution.

The maximal response facilitation occurs when the peaks of two excitations overlap, i.e., to induce maximal facilitation, the AW should be stimulated before the PW so as to compensate for the difference in peak latency between the two excitations. With extracellular recordings, we cannot directly determine the difference in peak latencies between the two excitations elicited by PW and AW stimulation. However, we can make an approximation. The latency difference in spike response for cells of layers II/III, IV, and V/VI was 1.6, 4.0, and 3.0 msec, respectively, and these

values corresponded to the optimal ISIs for the facilitation in each layer (Fig. 11). This suggests that the two excitations elicited by PW and AW stimulation arrive at the cortex basically independently and are summated in cortical cells.
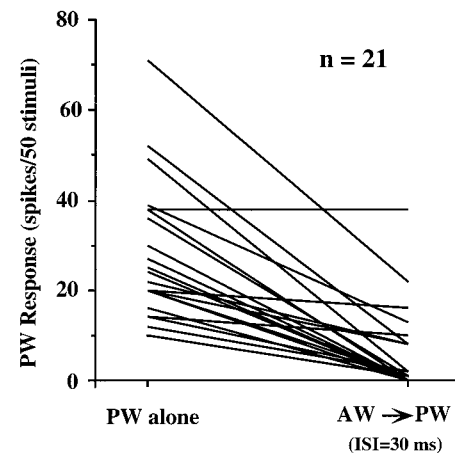
Figure 13. Suppressive effects of antecedent AW stimulation (ISI = 30 msec) on PW response in layer II/III cells. The magnitudes of the PW response were compared between control response (PW alone) and response with preceding AW stimulation at an ISI of 30 msec (AW→PW) for 21 cells. Eighteen of 21 neurons measured were clearly suppressed by the preceding AW stimulation.
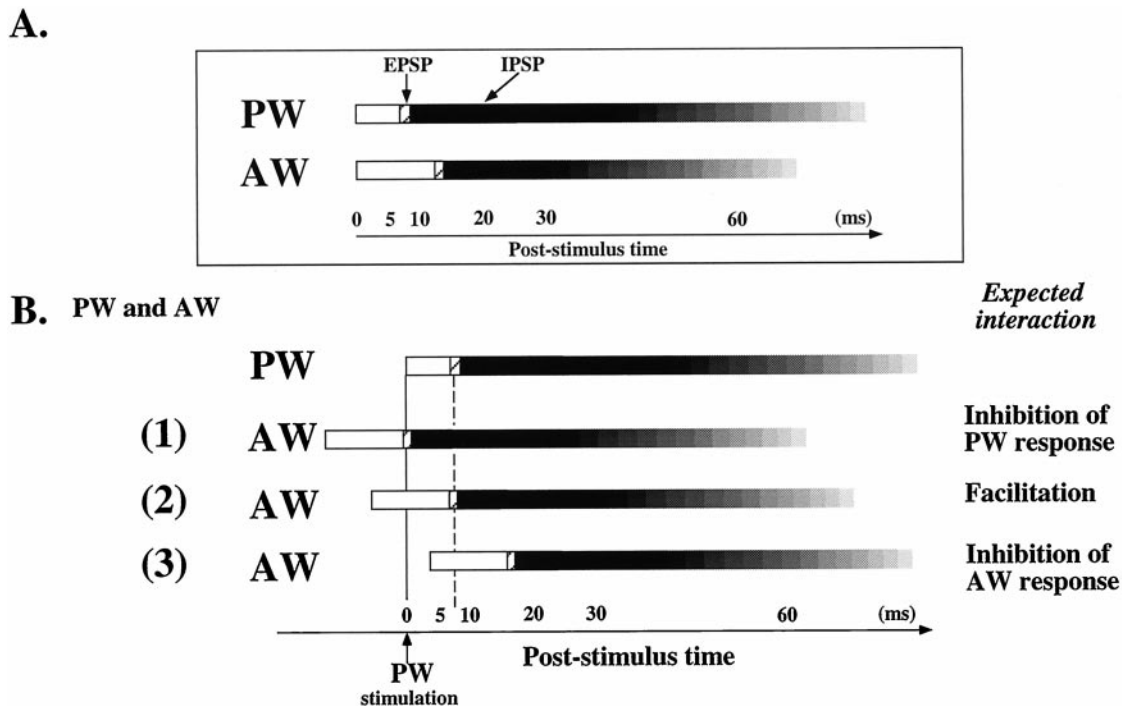
Figure 14. Proposed model of ISI-dependent response interaction. *A*, The response patterns of barrel cortex neurons to single PW or AW deflection, based on an intracellular recording study (Carvell and Simons, 1988; Moore and Nelson, 1998). PW deflection produces initial EPSPs (*hatched bars*) followed by longer-latency IPSPs (*filled bars*) of 50–100 msec duration. The time course of the AW response is similar to that of the PW response, but the fast excitatory component of the former is smaller and has a longer latency than that of the PW response. *B*, Expected patterns of response interaction to combined deflection of two model whiskers. *B2*, When two whiskers are stimulated to induce synchronized excitation in the cell, facilitatory interaction occurs as a result of nonlinear summation. *B1, B3*, When two excitations evoked by multiwhisker stimulation do not coincide with each other, only the preceding excitatory response is recorded, and the subsequent excitation is inhibited.

Possible mechanism of response interaction to multiwhisker stimulation

The mechanism presumed to underlie the ISI-dependent response interaction is schematically illustrated in Figure 14. In intracellular recording studies in the rat barrel cortex (Carvell and Simons, 1988; Moore and Nelson, 1998), whisker deflection of either PW or AW basically evoked initial EPSPs followed by long-lasting (50–100 msec) IPSPs, whereas the excitation elicited by PW stimulation was greater in amplitude and shorter in latency than that by the AW stimulation. Moore and Nelson (1998) never observed IPSPs without EPSPs. Therefore, we assumed that the PW stimulation elicits a response with a fast excitatory component of short duration and a subsequent long-lasting inhibitory component (Fig. 14*A*, *PW*), and that the AW stimulation also elicits a response with a first excitatory component with a slightly longer latency than that evoked by PW stimulation, also followed by a long-lasting inhibitory component (Fig. 14*A*, *AW*). When two whiskers are stimulated with an ISI appropriate to induce synchronized excitation in cortical neurons, facilitatory interaction occurs as a result of summation (Fig. 14*B*, *2*). When the two excitations do not coincide with each other, only the preceding excitatory response is recorded, and subsequent excitation is diminished because of interaction with the inhibitory component of the preceding response (Fig. 14*B*, *1*, *3*). Such a simple mechanism of temporal cooperation between response facilitation and inhibition in barrel cortex neurons would provide a neuronal basis of stimulus coincidence detection with a magnificent temporal resolution.

The role of facilitatory and inhibitory interaction of response in a behavioral context

Most facilitation occurred only when the two whiskers were stimulated within several milliseconds of each other. This strongly suggests that response facilitation serves as a detection mechanism for the coincidence of two-whisker stimulation. Moreover, sequential stimulation with a larger ISI resulted in an extinction of response to the latter stimulation by the inhibitory interaction, which might have the function of enhancing the spatial contrast between the stimulated and nonstimulated whiskers (Simons, 1985; Simons and Carvell, 1989). Therefore, it seems likely that the facilitatory interaction of response caused by coincident whisker stimulation and the inhibitory interaction elicited by other stimulation accentuate the difference in temporal patterns among the responses to various stimuli.

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