Cortical Transformation of Wide-Field (Multiwhisker) Sensory Responses

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Hirata A, Castro-Alamancos MA. Cortical transformation of wide-field (multiwhisker) sensory responses. J Neurophysiol 100: 358–370, 2008. First published May 14, 2008; doi:10.1152/jn.90538.2008. In the barrel cortex of rodents, cells respond to a principal whisker (PW) and more weakly to several adjacent whiskers (AWs). Here we show that compared with PW responses, simultaneous wide-field stimulation of the PW and several AWs enhances short-latency responses and suppresses long-latency responses. Multiwhisker enhancement and suppression is first seen at the level of the cortex in layer 4 and in the ventroposterior medial thalamus. Within the cortex, enhancement is manifested as a reduction in spike latency in layer 4 but also as an increase in spike probability in layer 2/3. Intracellular recordings revealed that multiwhisker enhancement of short-latency responses is caused by synaptic summation that can be explained by synaptic cooperativity (i.e., convergence of synaptic inputs activated by different whiskers). Conversely, multiwhisker suppression of long-latency responses is due to increased recruitment of inhibition in cortical cells. Interestingly, the ability to differentiate multiwhisker and PW responses is lost during rapid sensory adaptation caused by high-frequency whisker stimulation. The results reveal that simultaneous and temporally dispersed wide-field sensory inputs are discriminated at the level of single cells in barrel cortex with high temporal resolution, but the ability to compute this difference is highly dynamic and dependent on the level of adaptation in the thalamocortical network.

INTRODUCTION

In the rodent vibrissa system (Bernardo and Woolsey 1987; Woolsey and Van der Loos 1970), thalamocortical cells located within the barreloids (Land et al. 1995) of the ventroposterior medial thalamus (VPM) receive tactile signals from the whiskers via primary sensory lemniscal afferents and relay those signals to the barrel cortex. Behaving animals use their whiskers to acquire tactile information through repetitive movements, called whisking, during which multiple whiskers contact objects simultaneously or in sequence (Carvell and Simons 1990; Kleinfeld et al. 2006; Welker 1964). Therefore it is important to understand the impact of multiwhisker stimulation on VPM and barrel cortex cells.

VPM cells respond primarily to a principal whisker (PW) but also respond to adjacent whiskers (AWs) (Armstrong-James and Callahan 1991; Brecht and Sakmann 2002b; Diamond et al. 1992; Minnery et al. 2003; Simons and Carvell 1989; Waite 1973) and the size of their receptive field is controlled by the level of arousal (Aguilar and Castro-Alamancos 2005; Friedberg et al. 1999; Hirata et al. 2006). Interestingly, the VPM response produced by simultaneous stimulation of the PW and several AWs is identical to the PW response, as if the AWs had not been stimulated, and this is the case during both quiescent and activated states (Aguilar and Castro-Alamancos 2005; Hirata et al. 2006). Thus although simultaneous multiwhisker stimulation does not significantly affect the effectiveness of VPM responses, an important question is whether simultaneous multiwhisker stimulation affects responses in the next processing stage, the barrel cortex.

A great wealth of knowledge exists about the characteristics of whisker responses in the barrel cortex (for reviews see Alonso and Swadlow 2005; Armstrong-James et al. 1995; Brecht 2007; Castro-Alamancos 2004b; Kleinfeld et al. 2006; Moore 2004; Simons et al. 1995). Single-unit studies have shown that cortical neurons respond maximally to deflection of the PW and more weakly to deflection of several AWs. At the subthreshold level, intracellular recordings reveal that cortical cells respond to many AWs, in addition to the PW (Brecht and Sakmann 2002a; Moore and Nelson 1998; Zhu and Connors 1999). It is also well known that stimulation of two whiskers in succession robustly suppresses the response to the second whisker (Simons 1983, 1985; Simons and Carvell 1989). However, less is known about the responses of cortical cells to simultaneous stimulation of the PW and several AWs because few studies have addressed this issue. Multiwhisker stimulation has been reported to produce either supralinear or infralinear excitatory cortical responses compared with the sum of single-whisker responses (Ghazanfar and Nicolelis 1997; Mirabella et al. 2001; Shimegi et al. 1999). Also, suppression of PW responses by prior AW stimulation was found to be greater when more AWs were stimulated (Brumberg et al. 1996; Simons 1985). In whisking animals, spontaneous neural activity within a barrel is enhanced when the AWs are trimmed, which suggests a net inhibitory effect of AWs on cortical barrels (Kelly et al. 1999).

In the present study, we evaluated the impact of single- and multiwhisker stimulation on cortical cells. The results show that multiwhisker responses are transformed between the thalamus and neocortex. In VPM, multiwhisker stimulation produces responses that resemble the PW response. In barrel cortex, short-latency responses are enhanced by multiwhisker stimulation, whereas long-latency responses are suppressed. Multiwhisker enhancement of cortical responses leads to a reduction in spike timing for both layer 4 and layer 2/3 cells, but also produces an increase in spike probability for layer 2/3 cells. Intracellular recordings revealed that multiwhisker enhancement of short-latency responses is caused by an increase in synaptic responses that can be explained by synaptic cooperativity—i.e., synaptic summation caused by convergence of synaptic inputs activated by different whiskers. Conversely,
multiwhisker suppression of long-latency responses is due to increased recruitment of inhibition in cortical cells.

METHODS

Surgery

Sixty-nine adult Spague–Dawley rats (300–350 g) were used in this study and cared for in accordance with National Institutes of Health guidelines for laboratory animal welfare. All experiments were approved by the Drexel University Institutional Animal Care and Use Committee. Rats were anesthetized with urethane (1.5 g/kg, administered intraperitoneally) and placed in a stereotaxic frame. All skin incisions and frame contacts with the skin were injected with lidocaine (2%). A unilateral craniotomy extended over a large area of the parietal cortex. Small incisions were made in the dura as necessary. Body temperature was automatically maintained constant with a heating pad at 37°C. The level of anesthesia was monitored with field recordings and limb-withdrawal reflexes and kept constant at about stage III/3 (i.e., slow large amplitude field potential [FP] cortical oscillations, absence of pinch withdrawal reflex, absence of whisker movements) using supplemental doses of urethane (Friedberg et al. 1999). For intracellular recording experiments, animals were subjected to the previous procedures but were also paralyzed with gallamine triethiodide (40 mg/kg supplemented every 2 h) and artificially ventilated through a tracheotomy.

Electrophysiology

In every experiment, a tungsten electrode was lowered into the depth of the barrel cortex (0.6–1 mm) to record FP and multunit activity. A second electrode was lowered into the vicinity (<300 μm laterally) to perform either single-unit or intracellular recordings from cells located in layer 2/3 (200–700 μm) or layer 4 (700–950 μm in depth). Single-unit recordings in barrel cortex were obtained, as previously described, using electrodes pulled from glass pipettes (10–30 MΩ) filled with saline. These electrodes generally record only a well-discernible single unit of very large amplitude. Every single unit included in this study corresponds to a recording in which there was only one discernible large-amplitude spike in the recording electrode. Intracellular recordings in barrel cortex were obtained using high-impedance (80–120 MΩ) sharp electrodes filled with K-acetate (2 M). All intracellular recordings included (n = 12) correspond to regular-spiking (RS) cells that had overshooting action potentials and were stable for >30 min. Based on depth, five were in layer 4 and seven in layer 2/3. Intracellular recordings during stimulus protocols were done without any injected current (DC = 0 nA) or with a small amount of negative current (DC = −0.2 nA) that was constant throughout the stimulus protocols. All responses shown are the average of 30 stimulus trials per condition. The average was calculated after a median filter (20 kHz acquisition rate; moving time window: 60 points) was applied to each trace to remove the action potentials. A median filter substitutes each value with the median value in the moving time window.

Whisker stimulation

Sensory stimulation consisted of independently deflecting six individual whiskers using six different whisker stimulators. Once the tungsten electrode was in the barrel cortex, the whiskers were trimmed to a length of about 15 mm and a handheld probe was used to identify the PW, the whisker evoking the strongest audible multunit response. The handheld mapping was then confirmed by placing a whisker stimulator on the PW and five other stimulators on AWs surrounding the PW. The PW always produced the most robust response: i.e., shortest-latency and largest-amplitude FP response. The five AWs were selected as those producing the most robust responses following the PW. Each of the selected whiskers was inserted into a glass micropipette (1/0.5 mm OD/ID) that was glued to the membrane of a miniature speaker. Each whisker was inserted into the micropipette for about 5 mm, leaving about 10 mm from the end of the micropipette to the skin. Application of a 1-ms square current pulse to the speaker deflected the micropipette and the whiskers inside. The resulting whisker deflection is a very low amplitude (~2°) and very high velocity (~1,000°/s) stimulus. The whisker stimulators were oriented in the preferred direction to produce the largest response as determined with the hand probe. Each of the six whisker stimulators was driven by counter/timer boards controlled with LabVIEW software (National Instruments, Austin, TX).

Whisker stimulation was delivered according to the following protocols. A trial consisted of an initial 2 s without whisker stimulation followed by stimulation delivered to each whisker at 2-s intervals (the order of whisker stimulation was randomly selected). The first whisker was stimulated 2 s after the trial began, the second whisker was stimulated 4 s after the trial started, and so on, so that the sixth (last) whisker stimulus was delivered 12 s after the start of the trial. Thus a single trial contained stimuli for all six whiskers and lasted a total of 14 s. Whisker stimulation at 10 Hz consisted of a train of 10 stimuli and the last stimulus in the train was used. When all whiskers were stimulated simultaneously (ALL) or at short interwhisker intervals (IWs), each trial lasted 5 s. Every trial was repeated ≥30 times to derive peristimulus time histograms (PSTHs) and to average FP and intracellular responses. In some experiments, protocols for individual whisker stimulation and simultaneous multiwhisker stimulation were combined in the same trial, so that stimulation of each individual whisker was followed (3 s after the last whisker) by stimulation of the six whiskers together in the same trial.

Data analysis

Spontaneous cell firing was computed by counting the number of spikes during the 2- to 3-s period at the beginning of each trial and for a minimum of 30 trials. Population data are presented as means ± SD. If the data were considered normally distributed, according to the Shapiro–Wilks normality test, we used parametric statistics. For two groups, we used the t-test (paired or independent). For more than two groups, we tested for a significant main effect using the repeated-measures ANOVA followed by comparisons with Bonferroni’s test (paired comparisons) or a one-way ANOVA followed by Tukey’s test (independent comparisons). If the data were considered not normally distributed, we used nonparametric statistics. For two groups, we used the Wilcoxon signed rank (paired comparisons) and the Mann–Whitney (nonpaired comparisons) tests. For more than two groups, we first tested for a significant main effect using the Friedman test (repeated measures) or the Kruskal–Wallis test (independent), followed by multiple comparisons with Wilcoxon and Mann–Whitney, respectively.

RESULTS

Single-unit data set and single-whisker responses

Data reported in this study are part of a larger unpublished data set consisting of single units and intracellular recordings from layers 2–4 of barrel cortex (200–950 μm). Cortical cells were classified according to cortical depth, spike width, and spontaneous firing rate. Classification in a cortical layer was based on the depth of the recording electrode from the pia. Layer 4 cells were between 700 and 950 μm and layer 2/3 cells were between 200 and 700 μm. Cells were also classified as regular spiking (RS) or fast spiking (FS) based on the width of the spike at half-maximal amplitude. RS cells had a spike width >0.3 ms (0.4 ± 0.07 ms; n = 33) and FS cells had a
width < 0.2 ms (0.18 ± 0.03 ms; n = 9). FS cells had a spontaneous firing rate > 2 Hz (4.4 ± 4 Hz; n = 9), whereas most RS cells (78%) generally had a spontaneous firing rate < 1 Hz (0.3 ± 0.4 Hz; n = 26 of 33). A number of RS cells (22%) presented significantly higher spontaneous firing rates (2.3 ± 0.8 Hz; n = 7 of 33). These fast RS cells were generally located in layer 4 (depth: 730 ± 180 μm for fast RS cells vs. 570 ± 148 μm slow RS cells; n = 7 vs. n = 26). The firing rate of all RS cells (fast and slow) taken together was significantly lower than that of FS cells (1.2 ± 2 vs. 4.4 ± 4 Hz; RS vs. FS cells; P < 0.001).

VPM and barrel cortex cells respond preferentially to stimulation of a PW. Thus stimulation of the PW triggers a notable response that is clearly differentiable (stronger and faster) from the responses evoked by AWs onto the same cells. Figure 1A shows average responses of thalamic VPM cells (n = 14), cortical FS (n = 9), cortical RS (n = 33), and cortical FPs (n = 49) produced by low-(0.2 Hz) or high-frequency (10 Hz) stimulation of the PW and of five AWs. Using a short-latency 5- to 15-ms peristimulus time window, we measured the responses evoked by each of the six whiskers and compared them to the spontaneous firing (noise) of each cell in the absence of whisker stimulation. For RS cells, during low-frequency stimulation (0.2 Hz), the PW (P < 0.001), AW1 (P < 0.01), and AW2 (P < 0.05) produced responses that were significantly stronger than the noise. During high-frequency stimulation (10 Hz), only the PW (P < 0.01) produced responses that were more salient than the noise in RS cells. For FS cells, during low-frequency stimulation (0.2 Hz), the PW (P < 0.001), AW1 (P < 0.001), AW2 (P < 0.01), AW3 (P < 0.05), and AW4 (P < 0.05) produced responses that were significantly stronger than the noise. During high-frequency stimulation (10 Hz), only the PW (P < 0.01) and AW1 (P < 0.05) produced responses that were more salient than the noise for FS cells. These results indicate that FS cells have receptive fields larger than those of RS cells. In addition, the response produced by stimulating the PW of FS cells (2.2 ± 0.8 spikes/stim; 5- to 15-ms window at 0.2 Hz) was significantly
stronger than the response produced by stimulating the PW of RS cells (0.6 ± 0.2; \(P < 0.001\)). Thus FS cells produce stronger responses, and from a larger number of whiskers, than do RS cells. These response properties are in agreement with previous reports that have characterized FS and RS cells in barrel cortex (e.g., Bruno and Simons 2002; Swadlow 1989).

**Cortical transformation of multiwhisker responses**

We next compared PW and multiwhisker responses. As previously reported for VPM cells (Aguilar and Castro-Alamancos 2005; Hirata et al. 2006), the response evoked by all six whiskers (i.e., the PW and five AWs) stimulated together (multiwhisker stimulation) is not significantly different from the PW response (see Fig. 1B, top panels). However, in barrel cortex, multiwhisker stimulation produces a different effect than that in VPM. Figure 1B overlays PW responses (black trace), multiwhisker responses (red trace), and the sum of the responses to each of the five AWs (gray trace). Whereas in VPM multiwhisker responses are similar to PW responses, in cortex multiwhisker responses are larger and faster than PW responses for both FS (\(n = 9\)) and RS cells (\(n = 33\)) located in layers 4 and 2/3. Moreover, this difference between PW and multiwhisker responses, which we term multiwhisker enhancement, is mostly apparent during low-frequency whisker stimulation (0.1 Hz) and not during high-frequency whisker stimulation (10 Hz). Figure 2 shows several measures from population data used to quantify the difference between PW and multiwhisker stimulation.

First, we measured the number of spikes (spike probability) evoked during either a short-latency (5- to 15-ms) or a long-latency (16- to 50-ms) time window after the whisker stimulus. The number of spikes evoked by low-frequency (0.2-Hz) multiwhisker stimulation during the short-latency time window was significantly larger than the number of spikes evoked by stimulation of the PW alone for both FS (\(P < 0.01\)) and RS cells (\(P < 0.001\); Fig. 2A). However, during high-frequency whisker stimulation (10 Hz), multiwhisker enhancement of short-latency spike probability was absent for both FS (\(P = 0.2\)) and RS (\(P = 0.4\)) cells (Fig. 2A). Thus multiwhisker enhancement measured as spike probability of short-latency responses (<16 ms) is present in both RS and FS cells but only during low-frequency whisker stimulation.

Second, the number of spikes evoked by low-frequency multiwhisker stimulation during the long-latency time window...
(16–50 ms) after the stimulus was significantly smaller than the spikes evoked by stimulation of the PW alone for RS cells ($P < 0.001$) and close to significant for FS cells ($P = 0.07$). Therefore in contrast to multiwhisker enhancement of short-latency responses, there is a multiwhisker suppression of long-latency responses that is most prominent in RS cells. Moreover, during high-frequency whisker stimulation, multiwhisker suppression of long-latency responses was absent for both FS ($P = 0.14$) and RS ($P = 0.1$) cells (Fig. 2B). Thus multiwhisker suppression of long-latency (16- to 50-ms) spike probability is present in RS cells, and to some extent in FS cells, but only during low-frequency whisker stimulation.

Third, multiwhisker enhancement of the short-latency response was obvious as a leftward shift in the time-to-peak of the response for either single units (spike timing) or FPs. This usually signifies an enhanced synaptic response that translates to a faster spike onset. To measure this spike-timing shift in single-unit recordings, we calculated for each cell the time from stimulus onset at which 30% of the spikes comprising the short-latency response (5–15 ms) occur (Fig. 3A and B) (see Fig. 3C). Multiwhisker stimulation produced a significant leftward shift in spike timing of short-latency responses during low-frequency stimulation, meaning that multiwhisker responses were significantly faster than PW responses for RS cells ($P < 0.001$). A reduction in spike timing was also close to significant for FS cells ($P = 0.06$) cells. However, this difference was not observed during high-frequency whisker stimulation for either RS ($P = 0.17$) or FS ($P = 0.15$) cells. Thus multiwhisker enhancement measured as a reduction in spike timing of short-latency responses (<16 ms) is present in RS cells, but only during low-frequency whisker stimulation.

Fourth, multiwhisker enhancement of the short-latency response during low-frequency stimulation may depend on the layer where the cells are located. Figure 3C plots the amount of multiwhisker enhancement of spike probability for the short-latency response as a function of the depth of the cortical cells. Cells located in layer 4 (700–1,000 μm) showed no significant multiwhisker spike probability enhancement of the short-latency response ($P = 0.8$; $n = 11$), whereas cells located in layer 2/3 showed significant enhancement ($P < 0.001$; $n = 31$). However, when the spike timing of the short-latency response was calculated, both layer 4 ($P < 0.01$) and layer 2/3 ($P < 0.001$) cells produced significantly faster responses during multiwhisker stimulation than during PW stimulation (Fig. 3D). Thus multiwhisker enhancement of low-frequency short-latency responses is expressed as an increase in spike proba-

![Figure 3](https://jhn.org/362/images/fig03A.png)

**Fig. 3.** Effect of multiwhisker stimulation on spike timing and on FP responses in barrel cortex. **A:** effect of multiwhisker and PW stimulation on spike timing in FS cells. Low-frequency multiwhisker stimulation decreases spike timing in FS cells. **B:** effect of multiwhisker and PW stimulation on spike timing in RS cells. Low-frequency multiwhisker stimulation decreases spike timing in RS cells. **C:** relationship between multiwhisker enhancement of spike probability (measured as in Fig. 2A) with the depth of the cortical cell (layer 4 vs. layer 2/3) and the cell type (RS vs. FS). Multiwhisker stimulation increases spike probability in layer 2/3 cells but not in layer 4 cells. **D:** effect of multiwhisker and PW stimulation on spike timing in layer 4 and layer 2/3 cells. Multiwhisker stimulation decreases spike timing in both layer 4 and layer 2/3 cells. **E:** effect of multiwhisker and PW stimulation on the peak amplitude of FP responses. Multiwhisker stimulation decreases the peak amplitude of the FP response ($P < 0.05$).
bility of layer 2/3 cells but also as a reduction in spike timing in both layer 4 and layer 2/3 cells. Among the recorded cells, we found that 6% of cells in layer 2/3 (2/31) and 36% of cells in layer 4 (4/11) showed no significant multiwhisker enhancement in both measures: spike probability and spike-timing shift. Interestingly, nonenhancing cells of layer 4 had spike latencies shorter than those of enhancing cells of layer 4 (8 ± 0.8 vs. 9.3 ± 1 ms). Therefore a significant number of cells in layer 4, which we call nonenhancing cells, behave in a manner similar to that of VPM cells, displaying no significant multiwhisker enhancement.

Regarding multiwhisker suppression of the long-latency response during low-frequency stimulation, we found that cells located in layer 2/3 showed significant suppression (P < 0.001), whereas those in layer 4 had less significant suppression (P = 0.07). Thus multiwhisker suppression of long-latency responses is most prominent in layer 2/3 cells.

Finally, we quantified the impact of multiwhisker stimulation on FP responses. Multiwhisker stimulation produced larger peak amplitudes and faster time-to-peak responses than did PW stimulation (Fig. 1B). Multiwhisker enhancement of FP peak amplitude was significant during both low-frequency (P < 0.001) and high-frequency (P < 0.01) whisker stimulation (Fig. 3E). Moreover, multiwhisker enhancement of FP time-to-peak was significant during low-frequency (P < 0.001) but not during high-frequency (P = 0.3) whisker stimulation (Fig. 3F). Thus multiwhisker enhancement is prominent in population responses of barrel cortex, particularly during low-frequency stimulation.

Taken together, these results show that there is a transformation of multiwhisker responses between the VPM thalamus and barrel cortex. In VPM, multiwhisker responses are similar to PW responses. In barrel cortex, short-latency multiwhisker responses are enhanced and long-latency multiwhisker responses are suppressed. Both multiwhisker enhancement and suppression are present during low-frequency whisker stimulation and not during high-frequency whisker stimulation. Multiwhisker enhancement measured as an increase in spike probability is present in both RS and FS cells located in layer 2/3 but not in layer 4. Multiwhisker enhancement measured as a reduction in spike timing is present mainly in RS cells located in both layer 4 and layer 2/3 cells. Moreover, a significant number of cells in layer 4 are nonenhancing and thus behave similar to VPM cells. Multiwhisker suppression of long-latency responses is primarily present in RS cells. Finally, multiwhisker enhancement is prominent in population FP responses of barrel cortex. In conclusion, there is a difference between thalamus and cortex in how cells contrast PW and multiwhisker stimuli that depends on the cortical layer, the cell type, and the stimulation frequency.

Intracellular correlates of multiwhisker enhancement

FP and single-unit recordings indicate that multiwhisker stimulation enhances the short-latency response and suppresses the long-latency response in cortex. To determine the subthreshold correlates of multiwhisker enhancement and suppression, we performed intracellular recordings from cortical cells located between 300 and 950 μm in depth (Fig. 4). Simultaneous FP recordings were conducted from an electrode placed adjacent to the intracellular electrode (700-μm depth; ~300 μm lateral; Fig. 4A). To reveal the subthreshold postsynaptic potentials (PSPs), the action potentials were eliminated from the intracellular recording using a median filter (Fig. 4A). To generate PSTHs, action potentials were detected from the intracellular records (Fig. 4A). A minimum of 30 whisker stimulation trials at 0.2 and 10 Hz were used to average responses.

An example of a layer 4 cell (880-μm depth) is shown in Fig. 4. During low-frequency (0.2 Hz) whisker stimulation, the PW produced a fast-rising excitatory postsynaptic potential (EPSP) response that peaked before 10 ms and very effectively triggered action potentials (Fig. 4, black traces). In contrast, stimulation of each of the AWs produced slower rising EPSPs that unreliably triggered action potentials. However, when all six whiskers were stimulated together (Fig. 4A, red traces), the EPSP rose faster than the PW EPSP. The steeper slope of multiwhisker EPSPs led to action potentials with a faster onset (see PSTH; Fig. 4A). The multiwhisker enhancement was also obvious in the simultaneously recorded FP response (see FP; Fig. 4A). During high-frequency (10 Hz) whisker stimulation, consistent with the rapid adaptation of cortical sensory responses, all intracellular responses were sharply suppressed and action potentials were rarely evoked in this cell (Fig. 4). At the subthreshold level, multiwhisker EPSPs were not more robust than PW EPSPs during high-frequency whisker stimulation. This is in accord with the lack of multiwhisker enhancement for single-unit responses during 10-Hz whisker stimulation described earlier.

If multiwhisker enhancement of low-frequency short-latency cortical responses is due to convergence of synaptic inputs driven by individual whiskers that sum to produce a faster rising EPSP, then summing the EPSPs produced by individual whiskers should yield an EPSP with a slope that is similar to the slope of the multiwhisker EPSP. Indeed, we found that for this cell summing the EPSPs evoked by each of the six whiskers stimulated alone produced an EPSP with a rising slope similar to the multiwhisker EPSP (Fig. 4B). This was also the case for the slope of the simultaneously recorded FP responses (Fig. 4C).

All the intracellular cells we measured were enhancing cells because they showed some degree of multiwhisker enhancement. Half of the cells we measured showed a “simple” behavior (6/12 cells), in which multiwhisker enhancement could be explained by convergence from individual whiskers. However, other cells (6/12) showed a more “complex” behavior. In complex cells, the multiwhisker EPSP occurred earlier than the summed EPSP, meaning that simple summation could not explain the entire enhancement. Figure 5A shows additional examples of simple cells (top row), in which the rising slope of the summed EPSP mostly overlaps the multiwhisker EPSP, and of complex cells (bottom row), in which the multiwhisker EPSP enhances earlier than can be explained by the summed EPSP. Therefore multiwhisker enhancement in complex cells engages something more than simple convergence. We propose that these cells reflect the enhancement that is being relayed from other cells that respond with shorter latency. If this is the case then simple cells should have EPSP onset latencies faster than those of complex cells. Indeed, all complex cells had multiwhisker response onset latencies >6 ms, whereas four of six of the simple cells had onset latencies

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<5.4 ms. In fact, when cells were considered together the onset latency of multiwhisker EPSPs was significantly faster (6.2 ± 0.9 ms) than that of PW EPSPs (6.57 ± 1 ms; P < 0.01; n = 12). However, when the cells were classified as simple and complex, the difference in onset latency between multiwhisker and PW EPSPs was present only for complex cells (6.6 ± 0.4 vs. 7.1 ± 0.6 ms; n = 6) and not for simple cells (5.8 ± 1 vs. 5.9 ± 1 ms; n = 6). According to depth, simple and complex cells were found in both layer 4 and layer 2/3. These results are consistent with the idea that complex cells are reflecting the enhancement occurring in faster-responding (simple) cells.

We obtained several additional measures of multiwhisker enhancement from the EPSPs of these cells (n = 12). First, we determined the time difference at which multiwhisker and PW EPSPs reached an amplitude that was half of the maximal PW EPSP amplitude for each cell. Using this measure, we found that all the cells showed an approximately 1-ms (0.95 ± 0.3 ms; range 0.5–1.5 ms) leftward time shift of the multiwhisker EPSP compared with the PW EPSP and that this time shift was not different between simple (0.9 ± 0.3 ms) and complex (1 ± 0.4 ms) cells. Thus multiwhisker stimulation enhanced the synaptic response of all the measured cells.

Second, we measured the rising slope of the EPSPs. Taking all the cells together, multiwhisker EPSPs had a significantly steeper slope than that of PW or AW EPSPs (P < 0.01; Fig. 5B). Moreover, PW EPSPs had a steeper slope than that of AW1 EPSPs (P < 0.05; Fig. 5B). However, the EPSP slope did not differ significantly between the AWs. Thus multiwhisker responses are significantly more robust than PW responses and these are significantly more robust than AW responses. Moreover, the increase in slope between multiwhisker and PW EPSPs is mostly attributable to simple cells (P < 0.01; n = 6) and not to complex cells (P = 0.09; n = 6). Thus although both simple and complex cells show a significant leftward shift of

**FIG. 4.** Example of an intracellular recording during multiwhisker stimulation. A: multiwhisker and single-whisker responses evoked from a cell located at a depth of 883 μm. The intracellular spikes were detected and used to derive a PSTH (top panels) and these spikes were eliminated using a median filter to reveal the subthreshold postsynaptic potentials (PSPs, middle panels). Also shown are the simultaneously recorded FP responses (bottom panels) for low-frequency (left panels) and high-frequency whisker stimulation (right panels). Multiwhisker stimulation (red traces) enhances the subthreshold excitatory postsynaptic potential (EPSP) and consequently reduces spike timing (red traces). B: in this cell, the rising slope of the multiwhisker EPSP overlays the rising slope of the summed EPSP calculated by adding the individual responses of each of the 6 whiskers (SUM). A similar effect can be observed for the FP response (C).
the EPSP during multiwhisker stimulation, the EPSP shift is attributable to a pure change in slope mostly in simple cells. In complex cells, the EPSP shift also involves a reduction in EPSP onset, which could easily mask any slope changes. These results indicate that convergence of synaptic inputs from multiple whiskers largely explains multiwhisker enhancement of low-frequency cortical responses in simple cells. In complex cells, multiwhisker enhancement seems to reflect enhancement already generated in other faster responding (simple) cells because the onset of multiwhisker EPSPs in complex cells is faster than the onset of PW EPSPs. However, differences in intrinsic integration properties (connectivity, synaptic dynamics, membrane properties, etc.) between simple and complex cells may also contribute to the differences observed between them.

Finally, there was no difference in slope between multiwhisker and PW EPSPs during high-frequency (10-Hz) whisker stimulation classified according to their depth as layer 4 \( (P = 0.9) \) and layer 2/3 \( (P = 0.9) \) cells, or as simple \( (P = 0.9) \) and complex cells \( (P = 0.9) \). This agrees with the lack of multiwhisker enhancement in single-unit responses during 10-Hz stimulation. Moreover, during 10-Hz whisker stimulation, AW responses were very strongly suppressed, which made slope measurements difficult. Thus the very slow rising slopes of the AW responses are unlikely to contribute significant converging summation. This may explain the lack of multiwhisker enhancement during 10-Hz stimulation.

**Correlates of multiwhisker suppression**

The previous results explain multiwhisker enhancement of short-latency responses due to synaptic summation caused by activation of convergent inputs that is relayed and amplified by successive cortical cells. However, multiwhisker stimulation also produces multiwhisker suppression of long-latency (15- to 50-ms) responses. Cortical responses evoked by thalamic or sensory stimulation consist of an EPSP, which is truncated a few milliseconds later by an inhibitory postsynaptic potential (IPSP) that lasts for tens of milliseconds (Gabernet et al. 2005; Higley and Contreras 2006; Porter et al. 2001; Sun et al. 2006); the time window for multiwhisker suppression coincides with the expression of the evoked IPSP. Thus one possibility is that multiwhisker stimulation triggers a stronger IPSP than single-whisker stimulation, leading to suppression of long-latency responses. Indeed, Fig. 4A shows that low-frequency multiwhisker stimulation produced a hyperpolarization that was much stronger than that produced by the PW. Figure 6A shows examples from two additional cells. In all the cells, the hyperpolarization produced by multiwhisker stimulation was larger than that produced by the PW. To determine whether stronger IPSPs may account for multiwhisker suppression of long-latency responses, we measured the peak hyperpolarization between 15 and 50 ms peristimulus with respect to the resting \( V_m \). Responses evoked by low-frequency multiwhisker stimulation produced a significantly stronger hyperpolarization be-

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**FIG. 5.** Intracellular correlates of multiwhisker enhancement. A: examples of EPSPs from simple cells and complex cells. In simple cells, the slope of the multiwhisker EPSP ("ALL 6 together") overlaps the slope of the sum of the EPSPs triggered by each of the 6 individual whiskers ("SUM"). In complex cells, the multiwhisker EPSP precedes the SUM EPSP. B: population data of the EPSP rising slope evoked by multiwhisker (ALL) and single whisker stimulation of the PW and 5 Aws for all the cells (*\( P < 0.05 \) vs. ALL).
the most robust measure of enhancement across layers and found that multiwhisker stimulation of AWs produces significantly faster spikes than the best AW (Aw1) during low-frequency stimulation ($P < 0.001$), but not during high-frequency stimulation ($P = 0.3$). For FP responses, the peak amplitude (Fig. 7C) was significantly different between multiwhisker stimulation of five AWs and stimulation of the best AW during low-frequency stimulation ($P < 0.001$), but also during high-frequency whisker stimulation ($P < 0.01$). Thus these results indicate that multiwhisker stimulation of AWs without the PW also produces enhancement of cortical responses.

**Effect of removing the PW on multiwhisker enhancement**

Multiwhisker enhancement may depend on the PW. To address this possibility, we tested the impact of removing the PW from the multiwhisker stimulus. If multiwhisker enhancement is due to convergence of synaptic inputs from multiple whiskers, it should still be manifested during multiwhisker stimulation of AWs without the PW. Indeed, Fig. 7 shows single-unit PSTHs ($n = 14$ cells) and FP ($n = 15$) responses evoked by multiwhisker stimulation of five AWs. We measured the spike timing of short-latency responses (Fig. 7B) as

The results indicate that multiwhisker responses are transformed between thalamus and cortex and that this transformation is frequency dependent. Thus although the VPM thalamus is unable to differentiate simultaneous multiwhisker stimulation from PW stimulation, the barrel cortex can distinguish
these responses by enhancing short-latency responses and by suppressing long-latency multiwhisker responses compared with those of the PW. Multiwhisker enhancement of short-latency responses is manifested in layer 2/3 as both an increase in spike probability and a decrease in spike timing. However, in layer 4, enhancement is manifested only as a decrease in spike timing in a group of enhancing cells and is not present in a group of nonenhancing cells that have shorter latencies. Enhancement is mostly the result of cooperative synaptic inputs driven by individual whiskers that converge to sum and produce a stronger synaptic response that leads to a faster spike onset. Moreover, this enhancement is then relayed to succes-

FIG. 7. Effect of multiwhisker stimulation of AWs. A: population single-unit PSTHs and averaged FPs recorded from the barrel cortex and evoked by multiwhisker stimulation of 5 AWs (excluding the PW), compared with the response evoked by the best AW (Aw1) at low frequency (0.2 Hz) and at high frequency (10 Hz). B: effect of multiwhisker stimulation of AWs on spike timing during low-frequency and high-frequency stimulation. The response evoked by the best AW is compared with the response evoked by 5 AWs together. C: effect of multiwhisker stimulation of AWs on FP peak amplitude during low-frequency and high-frequency stimulation. The response evoked by the best AW is compared with the response evoked by 5 AWs together (*P < 0.05).

FIG. 8. Effect of interwhisker time interval on multiwhisker enhancement. A: population single-unit PSTHs and averaged FPs recorded from the barrel cortex and evoked by multiwhisker stimulation of the PW and 5 AWs simultaneously (ALL together) or separated from each other by 1, 2, or 3 ms of interwhisker interval (IWI). The PW was always first. Note that multiwhisker enhancement occurs during simultaneous stimulation and somewhat during 1-ms ISI only but not during 2- or 3-ms IWIs. B: effect of multiwhisker stimulation at different IWIs on spike timing. Responses evoked with 2- and 3-ms IWI were significantly slower than those evoked by simultaneous stimulation. At 3-ms IWI the spike timing was equivalent to that of the PW alone (marked by the dashed line). C: effect of multiwhisker stimulation at different IWIs on FP peak amplitude. Responses evoked with 1-, 2-, and 3-ms IWI were significantly lower amplitude than those evoked by simultaneous stimulation. At 3-ms IWI the FP amplitude was equivalent to that of the PW alone (marked by the dashed line) (*P < 0.05).
sive responding cells. Multiwhisker suppression of long-latency responses is due to more effective recruitment of inhibition, which can be accounted for by multiwhisker enhancement of spike probability in FS cells. These results indicate that cortical cells can distinguish between multiwhisker, PW, and AW responses based on spike timing. However, this distinction is largely suppressed during rapid sensory adaptation caused by high-frequency stimulation.

At a functional level, behaving rats normally experience multiwhisker, not single-whisker, stimulation. During active sensation, the whiskers are activated in different orders and with varying degrees of whisker dispersion (IWIs) depending on the features of the stimulus. Our results show that simultaneous and dispersed wide-field sensory inputs are effectively discriminated at the level of single cells in barrel cortex with high temporal resolution, but the ability to compute this difference is highly dynamic and dependent on the level of adaptation in the thalamocortical network. Several previous studies have investigated the effect of simultaneously stimulating two or three whiskers on cortical responses (Ghazanfar and Nicolelis 1997; Mirabella et al. 2001; Shimge et al. 1999). Similar to our findings, an enhancement of spike probability was found in layer 2/3 cells during stimulation of pairs of whiskers with short IWIs (Shimge et al. 1999).

**Source of synaptic inputs for multiwhisker enhancement**

Robust PW responses in the barrel cortex are the result of strong convergence of thalamocortical inputs to a single barrel from cells in the homologous barreloid (Bruno and Sakmann 2006). A main finding of the present study is that multiwhisker stimulation leads to responses that are even stronger than PW responses, and this already happens in layer 4 cells. Multiwhisker enhancement can be explained by cooperativity between converging PW and AW synaptic inputs that sum to strengthen the PW response. Then the obvious question is: what are the origins of the AW subthreshold synaptic inputs that are being summed to enhance multiwhisker responses? Answering this question seems simple when considering multiwhisker enhancement in layer 2/3 cells. These upper layer cells simply integrate and sum synaptic inputs received from several barrels and also amplify the enhancement already present in layer 4.

But how is the enhancement generated in layer 4 cells? This question relates to the source of AW responses in layer 4. Interestingly, there are two views on this subject that have been largely derived based on data from suprathreshold (spikes) receptive fields. One view indicates that layer 4 AW responses originate in the homologous VPM barreloid (e.g., Bruno and Simons 2002; Kwegyir-Afful et al. 2005) and another view indicates that they are of cortical origin (e.g., Armstrong-James et al. 1991; Fox et al. 2003). Our study is not intended to address this important issue. However, our results show that layer 4 spikes show a highly significant approximately 1-ms spike-timing shift during multiwhisker stimulation. One possibility is that the cortical spike-timing shift is the reflection of a similar shift in VPM cells from the homologous barreloid. This would be reflected as a change in single-cell timing and/or cell population synchrony during multiwhisker stimulation in VPM (Pinto et al. 2000). However, our population PSTHs of VPM cell responses reveal that although thalamocortical cells in VPM have abundant AW responses (Aguilar and Castro-Alamancos 2005; Hirata et al. 2006), PW responses and multiwhisker responses do not differ. Although AW responses in thalamocortical cells are surely an important source of layer 4 AW responses (Bruno and Simons 2002; Kwegyir-Afful et al. 2005), these responses are unlikely to explain multiwhisker enhancement reported in our study. Another possibility worth mentioning is that cells in the medial sector of the posterior thalamus (POm) produce multiwhisker enhancement themselves and relay that to the barrel cortex. This option is worth exploring in future studies, but it seems unlikely based on the fact that POM cells respond with much longer latencies than VPM cells (i.e., they cannot account for short-latency enhancement) and do not project to layer 4 barrels (Diamond et al. 1992; Lu and Lin 1993).

The second possibility is that the spike-timing shift in layer 4 is due to convergent synapses from either cortical (adjacent barrels) or thalamic (nonhomologous barreloids) sources. Indeed, there is evidence that barrels can communicate between each other through horizontal excitatory connections that arise from excitatory cells located within the barrels (Brecht and Sakmann 2002a; Egger et al. 2008; Schubert et al. 2003). There is also evidence that whereas thalamocortical axons of VPM cells mostly project to single barrels, collaterals can also reach adjacent barrels (Arnold et al. 2001; Bernardo and Woolsey 1987; Jensen and Killackey 1987; Pierret et al. 2000). Even if these synaptic connections are very weak, they may suffice to explain the spike-timing shift of layer 4 cells during multiwhisker stimulation. Another potential source of converging inputs worth mentioning is found in thalamocortical recipient cells in layer 6 that respond to whisker stimulation and have intracortical collaterals reaching layer 4. The intracortical collaterals of these cells may reach several barrels, providing a source of convergence during multiwhisker stimulation.
In addition, an important finding in our study may serve to reconcile the two possibilities described earlier. We found that multiwhisker enhancement is largely absent during rapid sensory adaptation caused by 10-Hz whisker stimulation. This indicates that during sensory adaptation, the cooperativity from multiple whiskers is absent because responses are suppressed. Thus the AW responses from either adjacent barrels or non-homologous barreloids are so depressed that they can no longer cooperate to enhance the PW response. Interestingly, previous work agrees with this conclusion by showing that AW responses are more strongly depressed than PW responses during adaptation (Katz et al. 2006). Moreover, during forebrain activation or behavioral arousal the thalamocortical system is mostly in the adapted state (Castro-Alamancos 2004a) and AW responses are suppressed in barrel cortex (Castro-Alamancos 2002; Castro-Alamancos and Oldford 2002). Thus we suggest that the studies that find AW receptive fields of cortical origin are recording in the nonadapted state because they mostly use urethane anesthesia and stimulate at low frequency (Armstrong-James et al. 1991; Fox et al. 2003), whereas those studies that find little contribution of intracortical sources to AW receptive fields are recording in a more adapted state because they mostly use lightly narcotized animals (Bruno and Simons 2002; Kwegyir-Afful et al. 2005). Future work is needed to test this intriguing possibility and to determine whether multiwhisker and PW responses are distinct during different states of the thalamocortical network (Hirata et al. 2006).

If multiwhisker enhancement is generated by convergence of synapses from adjacent barrels, then there must be a population of cells in each barrel that serve as cross-barrel relays of the thalamic inputs from the homologous barreloid. These cells should not show any enhancement and have short latencies. Indeed, we found that nonenhancing cells are readily present in layer 4 and that they have the shortest latencies. Based on the results from our study, we propose the model shown in Fig. 9 to explain multiwhisker enhancement. Groups of nonenhancing cells in each barrel serve as interbarrel relays receiving inputs only from the homologous barreloid and sending fibers to adjacent barrels. Thus these cells behave similar to VPM cells but project to adjacent barrels and provide the source of the converging synapses that sum to produce enhancement in simple cells. Then the simple cells send converging synapses onto complex cells within a barrel and outside a barrel in upper layers.

**Long-latency multiwhisker suppression**

Previous work has shown that increasing the number of AWs that are stimulated increases the suppression of later PW responses (Brunberg et al. 1996). Accordingly, our results show that multiwhisker stimulation recruits stronger IPSPs. Thus any additional stimulus delivered during this strong IPSP will surely be inhibited. Another well-known consequence of inhibitory recruitment is to sharpen responses within a short time window after the stimulus (Gabernet et al. 2005; Higley and Contreras 2006; Porter et al. 2001; Pouille and Scanziani 2001; Sun et al. 2006). Since no spikes follow the initial short-latency spikes after the stimulus, multiwhisker stimulation provides a powerful sharpening effect. The source of the enhanced IPSPs during multiwhisker stimulation is either a more effective recruitment by excitation of inhibitory interneurons within the barrel of the PW and/or the recruitment of interneurons in other barrel columns that project to adjacent barrels (including the PW barrel), providing lateral inhibition between barrel columns. Evidence for interbarrel inhibitory projections is scant but cannot be ruled out. FS cells located in barrels provide strong feedforward inhibition and have extensive axonal arbors that are mostly restricted within the barrel (Porter et al. 2001; Sun et al. 2006) and are very effectively driven by thalamocortical activity in vivo (Bruno and Simons 2002; Swadlow 1989, 1995). FS and other interneurons located outside layer 4 have a variety of different axonal arborizations that can be recruited by multiwhisker stimulation providing widespread inhibition (Gupta et al. 2000; Porter et al. 2001). Interestingly, it was recently found in slices that inhibition increases disproportionately as excitation is increased in barrel cortex (Kapfer et al. 2007). Thus the stronger excitation caused by multiwhisker enhancement may recruit stronger inhibition. Indeed, our results show that FS cells per se recorded extracellularly show multiwhisker enhancement at the level of spike probability. Thus a more effective recruitment of FS cell output can account for the more robust inhibition during multiwhisker stimulation.

In conclusion, multiwhisker stimulation produces the most effective and sharpest sensory response in barrel cortex, which is clearly distinguishable from the PW response—traditionally considered the most effective. The ability to differentiate multiwhisker from PW responses is a new computation intrinsic to the barrel cortex because thalamocortical cells cannot differentiate them. Multiwhisker enhancement can be explained by converging inputs from multiple whiskers that cooperate to enhance synaptic responses. Intriguingly, the cortex loses the ability to differentiate PW and multiwhisker responses during rapid sensory adaptation, which suggests that this computation may be highly dynamic and dependent on behavioral state. At a functional level, multiwhisker enhancement allows the sensory cortex to discriminate, with high temporal resolution, between simultaneous and temporally dispersed wide-field sensory inputs. This computation may be useful during active sensation, when multiwhisker stimulation occurs.

**GRANTS**

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