Optical Imaging and Electrophysiology of Rat Barrel Cortex. II. Responses to Paired-vibrissa Deflections

A study was undertaken to investigate the response of the rodent somatosensory barrel cortex to paired-whisker stimuli. Cortical responses to controlled whisker deflections were recorded by (i) conventional multi-unit extracellular recording within the cytochrome oxidase rich barrels centers, and (ii) intrinsic signal optical imaging, a technique that measures an optical correlate of neuronal activity thought to be related to the deoxygenation of hemoglobin in activated regions. Stimuli were applied to two whiskers in sequence, at temporal separations ranging from 0 to 60 ms. Over intervals of 10–40 ms, the primary effect of paired-whisker stimulation was suppressive. We suggest that paired-whisker inhibition results from the activation of layer IV fast-spike units within the principle whisker’s barrel, by excitatory input arriving from a surround-whisker. Paired-whisker stimulation produces inhibition in intrinsic images, because it results in a net reduction in layer II/III and/or layer IV metabolism. Intra-cortical inhibition may serve to convert the sequence of inputs from the whisker array into a barrel cortex magnitude code that can be read by higher cortical areas.

Introduction
Natural stimuli encountered by behaving animals are spatio-temporally complex, and hence a key issue in the study of sensory processing is the effect that stimulation outside a receptive field center has on simultaneous or near-simultaneous stimulation within the center receptive field. In primary visual cortex, contextual stimuli have been reported variously to inhibit (Nelson and Frost, 1978; Gulyas et al., 1987; Gilbert and Wiesel, 1990; Kapadia et al., 1995) or facilitate (Nelson and Frost, 1985; Gilbert and Wiesel, 1990; Kapadia et al., 1995) neuronal activity. In cat primary somatosensory cortex, simultaneous stimulation of a receptive field center and multi-points within its surround evoked greater responses than those evoked by stimulating the receptive field center alone (Gardner and Spencer, 1972). Similarly, in layer V of rat barrel cortex, multiple whisker stimulation was reported to produce a response greater than the arithmetic sum of the responses obtained from stimulation of each whisker alone (Ghazanfar et al., 1996). On the other hand, conditioning stimuli applied at various time intervals with respect to center-field stimulation were reported to produce inhibitory effects in both cat (Laskin and Spencer, 1979b) and rat (Simons, 1985; Simons and Carvell, 1989) somatosensory cortex.

We have undertaken a combined extracellular recording and intrinsic signal optical imaging study, in order to characterize the response of rat barrel cortex to paired-whisker stimulation. While neurons within a barrel-column respond maximally to deflection of the homologous whisker (principal whisker, PW), they also receive weaker excitatory input from surrounding (SW) whiskers (Simons, 1978; Armstrong-James and Fox, 1987; Simons and Carvell, 1989; Armstrong-James et al., 1991, 1992). As behaving rodents palpate objects with their whiskers to gain information about their surroundings, groups of whiskers are deflected either in sequence or in unison, and SW–PW interactions are likely. Armstrong-James and Fox (1987) have proposed that SW stimulation might facilitate the response to subsequent PW stimulation. These authors suggest that SW facilitation could serve to prime cortical units to imminent stimulation of their PW during active whisking behavior. However, the single-unit studies of Simons (1985) and Simons and Carvell (1989) suggest that the predominant effect of SW stimulation on the response to subsequent stimulation of the PW is inhibitory.

The results of the present study suggest that inhibition resulting from adjacent whisker deflection plays a prominent role in barrel cortical processing. These results are in substantial agreement with the single-unit studies of Simons (1985) and Simons and Carvell (1989). Based on the spatial activation patterns observed in cortex upon paired-whisker stimulation, we propose that the barrel cortex uses fast inhibitory mechanisms to convert the temporal sequence of inputs from the whisker array into a magnitude code, in which earlier-deflected whiskers are identified by the greater activation of their corresponding barrels. Some of these data have been presented in preliminary form (Peterson et al., 1993).

Materials and Methods
This study reports data recorded from eight female Sprague–Dawley rats (220–260 g, Banton-Kingman and Simonsen). Surgical preparation was as described previously (Goldreich et al., 1996).

Mapping
Physiological recordings were made using either 10 μm carbon fiber electrodes (see Armstrong-James and Millar, 1979) or tungsten micro-electrodes (Microprobe, 1–2 MΩ at 1 kHz) which were inserted orthogonally to the pial surface to a depth of 580 μm, with a hydraulic microdrive. The neural signals were bandpass-filtered around 1 kHz, amplified ×10 000, displayed on an oscilloscope and connected to an audio monitor. All recordings were of multi-unit activity. Initially, a map was made of the receptive fields at various locations throughout the barrel cortex. The location of penetration sites were marked using a computerized mapping program (MAP – Peterson and Merzenich, 1995; see http://senselab.med.yale.edu/people/peterson/Software.html#MAP) on a digitized image of the cortical surface. The receptive field at each penetration site was subjectively characterized by lightly tapping on each whisker with a hand-held fine-glass probe, and noting the whisker that evoked the largest audible response. Following the mapping, imaging and quantitative electrophysiology were performed in turn. The order of these varied from experiment to experiment.

Imaging
The imaging technique has been described (Peterson and Goldreich, 1994; see also the companion paper: Peterson et al., 1998). The protocol for a typical imaging experiment is shown in Figure 1. Stimuli were delivered to two whiskers on the right side of the face using piezoelectric bimorphs (Telense Systems, Inc; see Corey and Hudspeth, 1980; Simons, 1985). Bimorphs were placed up against the caudal aspect of two

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whiskers, typically D1 and γ, so that they held the vibrissae slightly rostral to their resting position. Stimulation frequency was 4 Hz at a 50% on/50% off duty cycle (125 ms on, 125 ms off square wave), delivered in the rostro-caudal axis (normally with rostral on, caudal off) at ~20 mm from the mystacial pad. Stimulus amplitudes were ~200 µm (0.6°). The inter-whisker stimulation intervals (ISIs) used in these experiments varied from 0 to 60 ms. A relatively high stimulation frequency was chosen in order to efficiently excite cortex, so that the time needed to acquire images could be kept within reasonable limits. We elected not to apply stimuli at >60 ms ISI, in order to avoid potential complications due to inhibition or facilitation of the response to the ‘first-stimulated’ whisker by the ‘second-stimulated’ whisker, such as might occur at ISIs greater than one-quarter of the square wave period (i.e. 62.5 ms). Four stimulus paradigms were employed throughout the experiment, resulting in images obtained for the stimulation of each of the two vibrissae alone, and each temporal sequence of paired stimulation (e.g. D1 stimulation followed by γ stimulation 10 ms later, and γ stimulation followed by D1 stimulation 10 ms later). In this paper, we use the notation W1 → W2 to indicate that the second whisker (W2) was deflected at some stated ISI after the first whisker (W1) was deflected.

An automated procedure was used to outline images with best-fit ellipses, as described (Peterson et al., 1998). Each best-fit ellipse outlines the response to a PW, the whisker whose deflection produced the image outlined by that ellipse. Other stimulated whiskers are referred to as SWs, with respect to that ellipse. Ellipses were first defined for cases of PW stimulation only. For instance, the images resulting from D1 stimulation alone and from γ stimulation alone were separately fit with ellipses. These same ellipses were then applied to the paired-stimulation images to obtain the average intensity value for the defined areas (Fig. 2A). The intensity of the D1 area was measured in both the D1 → γ image and in the γ → D1 image; the intensity of the γ area was similarly measured in both images. An optical condition test (CT-O) ratio was then calculated for each whisker’s image as the ratio of the intensity within its ellipse when it was stimulated second to when it was stimulated first (Fig. 2B). The CT-O ratio for D1 was:

\[
\text{D1 ellipse intensity in } γ \rightarrow \text{D1 image} \\
\text{D1 ellipse intensity in } D1 \rightarrow γ \text{ image}
\]

The condition-test ratio for γ was defined from the same set of images as:

\[
\text{γ ellipse intensity in } D1 \rightarrow γ \text{ image} \\
\text{γ ellipse intensity in } γ \rightarrow D1 \text{ image}
\]

The CT-O ratio compares the response evoked by deflection of two whiskers in a particular sequence at a particular ISI to that evoked by deflection of the same two whiskers at the same ISI but in the opposite sequence. This procedure ensures that the overall level of activation of the tissue is similar under the two conditions, so that any detected difference can be attributed with confidence to a difference in stimulus sequence. CT ratios >1 indicate a facilitatory effect of SW stimulation; ratios <1 inhibition; and ratios of 1 no effect of SW stimulation on the subsequent response to the PW.

In three experiments, a variation from the normal protocol was used in which a sequence of ISIs was applied, and in the interest of time only PW → SW and SW → PW images were collected during each stimulus battery. The PW and SW alone images were collected before the beginning of the paired-whisker batteries in these cases. In one of these three experiments, the PW ellipse was defined from a PW → SW image, because the PW alone image was taken with different microscope settings.)

As mentioned previously (Peterson and Goldreich, 1994), image quality typically improved as an experiment progressed. The improvement was usually abrupt: while images often showed little or no obvious signal for several hours following dural removal, within a 1 h time period, they typically appeared in clear form, and remained so for the remainder of the experiment. Hence, an initial selection of images was accomplished by simple visual inspection. In an attempt to formalize a basis for subsequent image selection, we applied the following criteria:

1. An image was discarded if ellipse-fitting on the PW whisker alone image failed (i.e. the ellipse was in an inappropriate topographic location as judged by the electrophysiologically derived map, as might happen when the image signal was weak, causing the ellipse-fitting algorithm to focus entirely on vascular artifacts).

2. An image was discarded if the ellipse could be appropriately defined but the image intensity within this region was greater for SW stimulation alone than it was for PW stimulation alone (indicating, for instance, a vein draining the SW image that flowed over the PW region, or perhaps a very weak PW-evoked image).

Five out of six experiments taken during our final year of imaging, when the imaging technique had been satisfactorily worked out) yielded images which survived these selection criteria. In total, 19 PW ellipse fits were obtained from eight animals. Eight PW ellipses were fitted to D1 images (four from experiments that paired D1 and γ, two that paired D1 and D2, and two that paired D1 and D3), seven were fitted to γ images (six paired with D1, one paired with C1), two to D2 images (paired with D1), one to a D3 image (paired with D1), and one to a C1 image (paired with γ). The number of CT ratios obtained at each ISI were 8, 9, 8, and 5 for ISIs of 10, 20, 40 and 60 ms respectively. Most of the images were obtained several hours after imaging commenced.

Vascular images are a potential problem with any technique that is based on a blood-related signal, particularly if spatial analyses are performed. Finding vascular-sparse zones of the barrel cortex overlying two neighboring barrels is nearly impossible. Accordingly, in this study, we did not measure the spatial structure of images (beyond outlining images with best-fit ellipses, a procedure that is relatively unaffected by vascular patterns); rather, we made measurements relating to image intensity only. As the optical absorption properties of large veins change in response to activation of nearby cortical tissue, we consider that the
inclusion of images with noticeable vascular signal (see Fig. 2C) is justified, provided that only intensity, and not spatial, measures are taken.

**Electrophysiology**
The bimorph stimuli used during multi-unit micro-electrode recordings were identical to those used during the imaging experiments (4 Hz, 125 ms on, 125 ms off, square waves), but the stimulus battery included ISIs of -20, -10, 0, 10, 20, 40 and 60 ms delivered at each penetration site. (Negative ISIs indicate that PW stimulation preceded SW stimulation.)

Spikes were obtained by thresholding traces at 5–8 times the measured standard deviation of the potentials obtained when no stimulus was applied (the noise level of the recordings). A computer algorithm was employed to measure the standard deviation of the recordings from intervals during which there was no spiking, so spontaneous activity did not usually affect the threshold. Spikes were binned over multiple trials to form peri-stimulus time histograms. If penetration sites within an experiment were classified as having the same PW, the spikes from these sites were binned together.

The neuronal onset response was defined as the number of spikes occurring in the interval from 2 to 18 ms following PW stimulus onset. An electrophysiology condition test (CT–E) ratio was calculated by dividing the spike counts in this interval during paired-whisker stimulation by the corresponding spike counts during PW stimulation alone. For instance, when recording in the D1 barrel, the CT–E ratio reflecting the response to γ → D1 stimulation was

\[
\text{D1 onset response during } \gamma \rightarrow \text{D1 stimulation} / \text{D1 onset response to D1 alone}
\]

**Results**

**The Optical Response to Paired-whisker Stimulation**
The primary question addressed by this study was whether the stimulation of a SW facilitates, suppresses or does not affect the response to subsequent stimulation of the PW. Figure 2A,C shows intrinsic optical images from two experiments in which stimuli were applied to whisker D1 alone, whisker γ alone, D1 followed by γ (D1 → γ), and γ followed by D1 (γ → D1).

Clearly, the images evoked by stimulation pair γ → D1 typically resemble more closely the images evoked by γ stimulation alone than they do those evoked by D1 stimulation. Similarly, the images evoked by stimulation pair D1 → γ tend to resemble images evoked by D1 more closely than they do those evoked by γ. Thus, response suppression is indicated by the observation that paired-whisker stimuli tend to produce images similar to those produced by stimulation of the earlier-deflected whisker alone.

**Dependence of the Optical Response on ISI**
To quantify the effects of surround-whisker stimulation, we computed an optical condition test (CT-O) ratio for each collection of images (see Materials and Methods). This procedure is illustrated in Figure 2B for the images shown in Figure 2A. The CT-O ratio compares the average intensity within the PW ellipse when the PW was stimulated second to the intensity when it was stimulated first. A plot of the CT-O ratio against inter-whisker stimulation interval (ISI) for all experiments that passed criterion (see Materials and Methods) is shown in Figure 3A. Values <1 indicate that suppression, not excitation, is the primary effect of paired-whisker stimulation. The statistical significance of the data shown in Figure 3A was calculated from paired t-tests comparing the intensity of the PW ellipse under the two conditions. These comparisons are shown in Figure 3B for all images at each ISI. Suppression was statistically significant at ISIs of 10, 20 and 40 ms, but was not significant when deflection of the SW preceded deflection of the PW by 60 ms.

![Figure 2](image-url)  
**Figure 2.** Paired-stimuli images. (A) The images obtained in response to paired-stimulation of D1 and γ, at 10 ms ISI, are shown. The response to the γ → D1 sequence (lower left) most closely resembles the response to γ alone (upper left), while the response to D1 → γ (lower right) resembles the response to D1 alone (upper right). These observations suggest that a pronounced effect of paired-stimulation is suppression rather than simple summation or facilitation. Best-fit ellipses determined from the upper two images are shown. (B) This graphic illustrates the technique for computation of the CT ratio, using D1 as the PW. The percentage change in absorption within the D1 ellipse in the γ → D1 paradigm is divided by its percentage change in D1 → γ paradigm. A corresponding technique would yield the CT ratio for PW γ. (C) Images resulting from paired stimuli applied to γ and D1 from an additional animal, at 20 ms ISI. Scale bar at lower left: 1.0 mm; color scale at lower right: percentage absorption increase.

**The Electrophysiological Response to Paired-whisker Stimulation**
Multi-unit responses recorded within the D1 barrel to paired stimulation of whiskers D1 and γ are shown in Figure 4. The response to stimulation of γ alone (the SW) is shown in the upper left. It is relatively weak, with an onset response of only 0.86
spikes/stimulus. By contrast, the response to the PW, D1, is strong, with an onset response of 2.3 spikes/stimulus. When γ and the principal whisker, D1, were stimulated in sequence, a dramatic decrease in the response to D1 was observed, due to the powerful suppressive effect of γ stimulation (left column).

For instance, at a separation of 20 ms, γ stimulation decreased the response to D1 from 2.3 to 0.36 spikes/stimulus, a reduction of 84%. The suppressive effect of γ stimulation on the subsequent response to D1 stimulation is not evident at ISIs of 60 ms. Sequential stimulation of D1 followed by γ (right column), while abolishing the normally weak response to γ within the D1 barrel, elicits no obvious suppression of the D1 response.

**Dependence of the Electrophysiological Response on ISI**

The effect of paired-whisker stimulation on multi-unit activity is quantified in Figure 5A. This figure plots CT-E ratios (see Materials and Methods) as a function of ISI. Once again, values <1 indicate that suppression, not excitation, is the primary effect of paired-whisker stimuli. The statistical significance of the data shown in Figure 5A was calculated from paired t-tests comparing the number of spikes evoked by PW onset under the two conditions. These comparisons are shown in Figure 5B for each ISI employed. Statistically significant suppression was observed over ISIs of 10–40 ms. No suppression was observed at 60 ms ISI, nor was suppression observed when PW deflection occurred simultaneously with or preceded SW deflection.

**Comparison of Caudal → Rostral with Rostral → Caudal Sequences**

A secondary question of interest was whether the relative positions of the first and second stimulated whiskers on the face influenced the degree of evoked suppression. Figure 6 plots CT ratios obtained when the SW was rostral to the PW against those obtained when the SW was caudal to the PW. Each data point is derived from two images or multi-unit recordings obtained from stimulating the same pair of whiskers in both possible sequences. For instance, each open square compares the CT-O ratio derived from a W1 → W2 sequence (measured on the W2 ellipse) to that derived from the W2 → W1 sequence (measured on the W1 ellipse from the same stimulus battery). Each closed diamond compares the CT-E ratio derived from a W1 → W2
sequence (measured from a multi-unit recording in the W2 barrel) to that derived from the W2 → W1 sequence (measured from a recording in the W1 barrel of the same animal). Neither the imaging data nor the electrophysiological data indicate a statistically significant difference between these two sequences ($P > 0.05$, two-tailed t-tests).

**Comparison of Metabolic with Electrophysiological Responses**

Comparison of Figures 3 and 5 reveals that CT-E ratios are lower than CT-O ratios at each ISI which yielded statistically significant suppression. The effect of measurement technique on CT ratio was statistically significant ($P < 0.01$, two-way ANOVA, ISI × technique, $F = 10.54$).

**Discussion**

We have investigated the response of the rodent somatosensory barrel cortex to paired-whisker stimuli. Cortical responses to controlled whisker deflections were recorded by a combination of conventional multi-unit extracellular recordings, and intrinsic signal optical imaging, a technique that provides a spatial view of cortical activation thought to be related to the deoxygenation of hemoglobin in activated tissue. We have found by both techniques that statistically significant suppression is present at inter-whisker stimulation intervals of 10, 20, and 40 ms. Statistically significant suppression was not observed at ISIs of 60 ms, nor was it observed when PW stimulation occurred simultaneously with or preceded SW stimulation by 10 or 20 ms (electrophysiology data only). No statistically significant differences were observed between the suppression exerted by caudal SWs on rostral PWs and rostral SWs on caudal PWs, as measured from optical images or multi-unit records. CT ratios derived from optical images were found to be greater than those derived from multi-unit records at each ISI that yielded statistically significant suppression. These results are discussed below.

**The Response to Paired-whisker Stimulation**

Broadly speaking, there are three possible responses to paired-whisker stimuli such as those we have applied: (i) the response to the PW could be unaffected by prior deflection of an SW; (ii) the responses could summate in a facilitatory (nonlinear) fashion, such that the response to deflection of the PW is enhanced by preceding SW deflection; or (iii) the response to the PW could be suppressed by preceding SW deflection. Our
results clearly indicate that suppression caused by preceding SW deflection is the strongest of these processes.

We found that the effect of two-whisker stimulation was to produce an image (in the PW representation) that was of lesser intensity when the PW was stimulated second than when it was stimulated first. This result suggests that the sequence of stimulation determines the metabolic response, with later-stimulated whiskers being relatively suppressed by earlier-stimulated ones. Multi-unit extracellular recordings, which allow for temporal analyses but lack the spatial view provided by the imaging technique, indicate that the onset response to PW stimulation is smaller when preceded by SW stimulation than when delivered alone. This result is consistent with single-unit recordings from another laboratory (Simons, 1995), and corroborates the suppression seen in the images. Similar response suppression has been reported by laboratories utilizing the voltage-sensitive dye imaging technique (Orbach et al., 1985; Kleinfeld and Delaney, 1996).

Despite the differences in measurement and analysis techniques, both the electrophysiologically derived CT ratios and those derived from images show response suppression in the range 0.3–0.7 for ISIs ranging from 10 to 40 ms; both indicate maximal suppression at 20 ms ISI; and neither finds statistically significant suppression at 60 ms, although we cannot rule out the possibility that with larger sample sizes a small degree of suppression might be detected at this interval. The most salient difference between the optical and electrophysiological data is that the CT-O ratios are higher than the corresponding CT-E ratios at all ISIs associated with statistically significant suppression. It is likely that this difference arises at least in part from the difference in stimulation protocols employed. Since the CT-O ratio compares the intensity within the PW ellipse during SW → PW stimulation to the intensity within the same ellipse during PW → SW stimulation, this ratio will be augmented by excitatory effects of SW stimulation. The physiological CT ratio, on the other hand, compares the response to the PW during SW → PW stimulation to the response to the PW alone; this one-whisker-to-one-whisker comparison is unaffected by SW excitation. For example, if SW stimulation reduces a PW onset response from 2 to 0.5 spikes per stimulus, while evoking a 1 spike/stimulus SW excitation, then the CT-E ratio would equal 0.25 (i.e. 0.5/2). The CT-O ratio, on the other hand, assuming identical responses measured in metabolic (optical absorption) units, would equal 0.75 (i.e. 1.5/2), the difference reflecting SW excitation. [This argument assumes for simplicity that the SW excitation is eradicated by preceding PW stimulation (see Fig. 4). However, the CT-O ratio would exceed the CT-E ratio even without this assumption; for the example given, its minimum value would equal 1.5/3, or 0.5.]

Neither the imaging nor the electrophysiological data indicate a difference in the degree of suppression evoked by caudal whisker → rostral whisker vs. rostral whisker → caudal whisker sequences. These data should be interpreted cautiously, since owing to a relatively small sample size the probability of detecting a difference in CT ratio of 0.2 between these conditions was estimated by power analysis to be only 70%. McCasland et al. (1991) report that suppression detected with single-unit recording is more pronounced when the SW is caudal or ventral to the PW. These authors propose that this asymmetry of suppression underlies the decreasing caudo-ventral to rostro-dorsal activity gradients seen in 2-deoxyglucose images of barrel cortex when the radiolabel is injected prior to a period of active whisking behavior. Most of our data come from the straddler, γ, and the arc 1 whisker, D1. Since γ is both caudal and dorsal to D1, no prediction of asymmetry with respect to suppression can be readily drawn from the report of McCasland et al. Further studies concentrating on the deflection of pairs such as c1 and γ will be needed to determine whether suppressive gradients such as those described will apply to straddlers.

The Origin of Response Suppression

It may be argued that the reduction in cortical responsiveness to principal whisker stimulation following stimulation of a surround whisker reflects inhibition at a subcortical rather than a cortical level. Apparent inhibitory effects detected in cortex might reflect a reduction in afferent excitation consequent to subcortical inhibition. We consider this possibility to be unlikely for several reasons. Firstly, there is now substantial evidence that GABAergic inhibition shapes receptive fields in the barrel cortex.
(Orbach et al., 1985; Lamour et al., 1988; Simons, 1995) as it does in the somatosensory cortices of other species (Alloway and Burton, 1986; Dykes et al., 1984; Juliano et al., 1989). Excitatory surround receptive fields are dramatically enhanced (Orbach et al., 1985; Lamour et al., 1988), and inhibitory surrounds of layer IV cells dramatically reduced, by bicuculline iontophoresis (Simons, 1995). CT ratios in layer IV are changed from 0.5 to 0.8 by bicuculline iontophoresis (Kyriazi et al., 1995). Secondly, surround whisker inhibition is only rarely encountered in the VPM nucleus (Simons, 1995). Thirdly, the proportion of rapidly adapting to slowly adapting neurons increases progressively and dramatically in a rostral progression up the rodent somatosensory neuraxis, presumably reflecting the cumulative effects of lateral inhibition (Lichtenstein et al., 1990). For these reasons, we consider it likely that the response suppression observed in the experiments reported here reflects inhibition at the cortical level.

Within layer IV, however, the circuit that mediates SW inhibition is unresolved. In particular, it is unclear whether the SW input arrives via inter-barrel connections, or directly from the VPM nucleus. Anatomical studies (Aroniadou et al., 1995; Gottlieb et al., 1995) reveal a paucity of long-range (>1 mm) axonal connections within layer IV of barrel cortex. Simons and Carvell (1989) propose that multi-whisker thalamocortical input from a VPM barreloid provides the surround receptive fields of cells in the corresponding cortical barrel; intra-barrel disynaptic inhibition mediated by smooth stellate cells accounts for the inhibition observed upon SW → PW deflection. Connections from thalamic barreloids to non-homologous barrels could also contribute to surround receptive fields in barrel cortex (Land et al., 1995).

A barrel lesion study performed by Armstrong-James et al. (1991), however, suggests that inter-barrel connections mediate SW excitation. Furthermore, based on analyses of response latencies in VPM and cortex, it is argued that the surround receptive fields of barreloid cells could not produce the surround receptive fields of barrel cells (Armstrong-James and Callahan, 1991). These authors envision an inter-barrel origin of surround receptive fields. The anesthetic types used by Armstrong-James et al. and Simons and Carvell differ. Simons and Carvell (1992) have shown that urethane, the anesthetic used by Armstrong-James et al., enhances the long-latency excitatory surround receptive field component to a level above that seen in the awake animal. Armstrong-James et al.’s data may thus be based on polysynaptic and/or slow-EPSP-mediated inter-barrel connections that are artificially enhanced by urethane anesthesia. Preliminary lesion experiments performed on fentanyl-sedated rats suggest that SW excitation and inhibition are not mediated by surround barrels (D. Goldreich HT Kyriazi and D.J. Simons, personal communication).

Whatever the source of the SW afferent input to the PW barrel, it seems reasonable to propose, first, that the input is excitatory; and, second, that its effect is to engage disynaptic inhibitory circuitry within the barrel. A double-labeling study involving retrograde tracers and glutamic acid decarboxylase (GAD) immunohistochemistry (Aroniadou et al., 1995) indicates that the vast majority of axonal collaterals from inhibitory cells in barrel cortex occupy less than a 200 µm radius horizontal to the cell body, a distance too short to mediate inter-columnar connections. Furthermore, the timecourse of post-stimulus suppression below spontaneous firing rates following PW stimulation (see Peterson et al., 1998; see also Carvell and Simons, 1988) is similar to the timecourse of inter-whisker inhibition evoked by SW deflection (Figs 3 and 5; see also Simons and Carvell, 1989), consistent with an intra-barrel origin for inhibition in both cases. A schematic of the circuitry that may underlie SW inhibition is shown in Figure 7.

### A Behavioral Role for Inhibition?

Inhibition as a result of time delay has been suggested from psychophysical studies performed on human skin (Von Bekesy, 1967; Maskin and Spencer, 1979b). This form of inhibition may play an important role in the ability of mammals to interpret tactile inputs. The data reported here suggest the hypothesis that during behavior, inhibition serves to diminish responses to whiskers that are deflected at short latency following their neighbors.

Support for the behavioral importance of inhibition in barrel cortex comes from the observation that rats are incapable of distinguishing between textured surfaces with 1.00 and 1.06 mm inter-groove spacing, unless at least two adjacent whiskers palpate these surfaces. Furthermore, rats whose whiskers are trimmed to the skin surface from birth to age 45 days are severely impaired in this discrimination task after their whiskers regrow (Simons, 1995); such animals have abnormally large excitatory receptive fields and abnormally weak or absent inhibitory fields (Simons and Land, 1987, and unpublished observations). By contrast, both neonatally trimmed rats and those using only one whisker are capable of distinguishing between a smooth surface and one milled with 30 µm deep grooves spaced at intervals of 50 µm. We propose that this latter task is more a detection than a discrimination task, i.e. the fractional difference in textural dimensions is very large in this task, but very small (6%) in the task that requires two whiskers. Even very fine detections may be adequately accomplished with a single whisker, owing to the sensitivity of the barrel cortex to low-amplitude whisker deflections (see Peterson et al., 1998).

Fine discriminations, on the other hand, may require paired- or multi-whisker inhibitory interactions.

Connor and Johnson (1992) propose two means by which textures with spatial periods of the order of millimeters could be perceived by the primate using its hand (see also Hsiao et al., 1993). These two mechanisms, by means of which surface roughness would be coded by the average firing rates of central neurons, are as follows (adapted to the whisker system): (i) temporal mechanism — the variation in spiking activity arriving from any one whisker could be assessed over time; or (ii) spatial mechanism — at any point in time, the variation in spiking activity could be assessed across whiskers. It is noted that each of these proposed mechanisms could be accomplished through cortical circuits involving dual excitation and inhibition. Only the first hypothesis, however, predicts that roughness could be perceived using a single sensory receptor. Psychophysical experiments involving roughness estimates of different textures, along with neurophysiological recordings from primary afferents, lead Connor and Johnson (1992) to favor the second hypothesis. As mentioned, Simons (1995) found that rats require two whiskers for fine texture discriminations, a fact that also supports the second hypothesis.

We consider it unlikely, however, that a purely spatial mechanism could subserve millimeter-order texture discrimination in the rodent whisker-to-barrel system. The reason for this is that the whiskers move so rapidly that they travel significant distances over the time period during which inhibition acts (10–40 ms post-stimulus); inhibition cannot be considered to act instantaneously during whisking behavior, an assumption
implicit in the model proposed by Connor and Johnson (1992). At an average whisker protraction velocity of 580°/s during object scanning (Carvell and Simons, 1990), the contact point of a whisker with a scanned surface moves on the order of 1 mm/10 ms (Simons, 1995). During the 30 ms or more over which inhibition is strongly active, the PW itself could undergo multiple deflections, inhibiting its own responses. Thus, it is not possible to separate spatial from temporal mechanisms of inhibition in this system, and we suggest that both play a role in shaping the cortical response to a scanned surface.

**Imaging of Inhibition**

Imaging techniques which measure metabolism or blood flow, such as 2-deoxyglucose (2-DG) autoradiography, positron emission tomography (PET), single photon emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI) and intrinsic imaging, suffer from the criticism that the measured signals may reflect the activity of inhibitory as well as of excitatory neurons. Hence, it is considered difficult to draw conclusions regarding neurophysiological processes from data obtained using these techniques alone. The images obtained in the present study, in agreement with the electrophysiology, indicate inhibitory interactions. This result is somewhat surprising in light of the fact that barrel fast-spike units (FSUs), the presumptive GABAergic smooth stellate cells, are more sensitive to peripheral stimulation than are regular-spike units (RSUs) (Simons and Carvell, 1989). Furthermore, GAD-positive barrel cortex neurons are more densely labeled by 2-DG than are GAD-neurons in the behaving rodent (McCasland et al., 1992).

The simplest hypothesis that would account for the reduction of optical signal during inhibition is that the signal originates primarily from excitatory cells. Paus et al. (1995) have proposed that the majority of stimulus-evoked blood flow originates from excitatory transmission. Excitatory transmission, they argue, leads to an intracellular Ca²⁺-induced increase in nitric oxide synthesis, with consequent nitric oxide release and vasodilatation. The relevance of this argument to our measurements is uncertain, however. Although blood flow to activated barrels increases upon single-whisker stimulation (Cox et al., 1993), it is likely that the intrinsic signals that we measure are due to increases in blood deoxyhemoglobin content rather than blood volume (see Peterson and Goldreich, 1994).

A second explanation is that the optical signal reflects predominantly layer II/III responses, and in these layers the response reduction results primarily from a reduction in driving excitatory input arriving from layer IV, consequent to inhibition in that layer, rather than from inhibition within layers II/III themselves. We suspect that the optical signal originates primarily from blood within superficial cortex, as the penetration of incident light decays with depth. Perhaps the metabolic demand of the inhibitory layer IV cells is not observed, as the deoxygenation caused by these cells occurs too far beneath the pial surface to contribute substantially to the optical signals that we image superficially. A bicuculline iontophoresis study (Kyrizai et al., 1995, and personal communication) provides direct evidence that the apparent response inhibition observed in layer II/III cells with extracellular electrodes actually results from inhibition occurring within layer IV. Iontophoresis of this GABAₐ receptor antagonist onto layer II/III cells increases their CT ratios only slightly, from 0.4 to 0.5, as predicted if the primary mechanism of paired-whisker inhibition in this layer is a reduction in afferent excitatory drive originating from layer IV, rather than the action of inhibitory synapses onto the recorded cell. By contrast, bicuculline iontophoresis increases the CT ratio of layer IV RSUs from 0.5 to 0.8, suggesting the influence of direct inhibitory contacts on these cells.

Finally, it is possible that layer IV does contribute substantially to our optical measurements, via deoxygenated blood that is taken from that layer into superficial draining veins. If this is the case, then it may be that within layer IV itself, spiking activity averaged over the barrel — including inhibitory as well as excitatory neurons — is lower during the SW → PW paradigm than it is during the PW → SW paradigm. While PW → SW stimulation effectively eradicates the SW response, thereby evoking about as many spikes as does PW stimulation alone, SW → PW stimulation may evoke fewer spikes, as a result of the relatively weak excitation but profound inhibition elicited by SW stimulation on the subsequent PW response. As noted, SW stimulation activates FSUs more strongly than RSUs (Simons and Carvell, 1989), presumably owing in part to the intrinsic membrane properties of the FSUs (McCormick et al., 1985; Connors and Gutnick, 1990; Amitai and Connors, 1995), and in part to the presence of thalamocortical synapses on the somata of smooth cells, but not of spiny cells (White et al., 1984; Benshalom and White, 1986). The GAD-positive cell population, while constituting only 13–15% of the layer IV cells within a barrel (Lin et al., 1985), apparently exerts potent inhibition on the spiny population, via inhibitory contacts on the somata of the spiny cells (Benshalom and White, 1986; see Fig. 7). As a result, SW stimulation might be expected to evoke relatively few spikes from the barrel neuron population as a whole, yet severely reduce the population’s subsequent response to PW deflection. Just such a result is indicated by the multi-unit PSTHs (Fig. 4).

Although we cannot claim that the multi-unit recordings derive from a representative sample of the barrel cell population, if SW → PW stimulation indeed evokes fewer spikes within the barrel than does PW → SW stimulation, then total barrel metabolism would presumably be lower in the former condition.

**Notes**

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