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Quantitative Comparison Between Functional Imaging and Single-Unit Spiking in Rat Somatosensory Cortex

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Masino, Susan A. Quantitative comparison between functional imaging and single-unit spiking in rat somatosensory cortex. *J Neurophysiol* 89: 1702–1712, 2003. First published December 18, 2002; 10.1152/jn.00860.2002. The profile of activity across rat somatosensory cortex on stimulation of a single whisker was examined using both intrinsic signal imaging and electrophysiological recording. In the same animals, under sodium pentobarbital anesthesia, the intrinsic signal response to a 5-Hz stimulation of whisker C2 was recorded through a thinned skull. Subsequently, the thinned skull was removed, and individual cortical neurons were recorded at multiple locations and in all cortical layers in response to the same whisker stimulation paradigm. The amplitude of the evoked response obtained with both techniques was quantified across the cortical surface with respect to distance (≤ 1.6 mm) from the peak intrinsic signal activity. Cortical neurons were rated as having a significant or nonsignificant whisker-evoked response as compared with a baseline period of spontaneous firing; a minority of neurons exhibited a small but significant increase in neuronal spiking even at long distances (>1.6 mm) from the optically determined peak of activity. Overall, this analysis shows a significant correlation between the two techniques in terms of the profile of evoked activity across the cortical surface. Furthermore, this data set affords a detailed and quantitative comparison between the two activity-dependent techniques—one measuring an intrinsic decrease in light reflectance based largely on metabolic changes and one measuring neuronal firing patterns. Studies such as this, comparing directly between imaging and detailed electrophysiology, may influence the interpretation of the extent of the activated area as assessed with *in vivo* functional imaging techniques.

INTRODUCTION

There has been an enormous increase in the use of noninvasive *in vivo* imaging techniques to study the function of the brain in general and the cerebral cortex in particular. Imaging studies reveal defined areas exhibiting increased activity during complex cognitive tasks, such as language, calculation, and attention (Caplan et al. 2002; Simon et al. 2002). Constant improvements in imaging technologies and increasingly widespread availability of noninvasive functional imaging virtually guarantee future relevance for investigating brain function. However, as most of these imaging techniques are based on measuring metabolic changes that are related indirectly to synaptic transmission [e.g., positron emission tomography (PET), functional magnetic resonance imaging (fMRI), intrinsic

signal optical imaging (ISI)], the neuronal activity underlying functional images is not obvious. Specifically, the location of peak alterations in activity can be identified with imaging technologies, but the area surrounding the peak of activity that exhibits significantly altered neuronal firing has not been explored.

Thus far in every cortical area investigated, electrophysiological recordings verified stimulus-evoked activity at the location corresponding to the peak of activity detected with an imaging technique (for example, fMRI: Logothetis et al. 2001; ISI: Bakin et al. 1996; Brett-Green et al. 2001; Hodge et al. 1997; Masino et al. 1993) and across a limited region of the cortical surface (Disbrow et al. 2000; Logothetis et al. 2001; Peterson et al. 1998; Sheth et al. 1998). As yet, though, stimulus-evoked suprathreshold activity has not been characterized across a large region of the cortical surface and has not been compared with imaging techniques. Quantifying evoked neuronal activity and comparing it to functional imaging is of the utmost importance; a small but significant increase in specifically timed neuronal firing is critical in terms of information processing and may be more relevant than the overall number of action potentials (Panzeri et al. 2001). Focusing solely on the location of peak activity observed with functional imaging risks misinterpreting the areal extent of activity or even the cortical field(s) deemed as active. Therefore a major unresolved issue is the relationship between the size and amplitude of the activated area as assessed by imaging and the size and amplitude of the activated area as assessed with single-unit recordings that quantify neuronal firing patterns.

The posteromedial barrel subfield (PMBSF) of the rodent somatosensory cortex—“barrel cortex”—contains a somatotopic representation of the vibrissae, or whiskers, and provides an ideal system to investigate the relationship between optically and electrophysiologically detected neuronal activation. An individual whisker can be stimulated precisely and independently, and a number of groups have employed this model system to image cortical activity *in vivo* (Hess et al. 2000; Kleinfeld and Delaney 1996; Masino et al. 1993; Orbach et al. 1985; Peterson et al. 1998; Sheth et al. 1998; Yang et al. 1996). In general, the areal extent of cortical activation assessed *in vivo* with imaging techniques is larger than expected from single-unit recordings in barrel cortex (Armstrong-James et al.

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1992). Whether the large spread of cortical activation observed during imaging best reflects neuronal spiking, subthreshold synaptic activity, or metabolic sources detected by imaging, such as blood flow, has not been well resolved.

Several studies have compared ISI to either sub- or supra-threshold synaptic activity across a limited region of barrel cortex. Peterson et al. (1998) compared ISI to single-unit firing patterns in rat barrel cortex for a range of minimal stimulus amplitudes and found a nearly perfect correlation. However, their data are restricted to within one adjacent barrel column away from the optical center and do not address the outer regions of the activation where a discrepancy is more likely. Brett-Green et al. (2001) recorded neuronal spiking within a large area of activity observed with ISI (≤ 1.26 mm from the optical peak) when activating whiskers represented peripherally in the barrel field but did not quantify or examine the profile of activity across cortex in detail. Researchers using *in vivo* whole cell recordings observe large subthreshold receptive fields (≤ 16 whiskers) (Moore and Nelson 1998; Zhu and Connors 1999), suggesting that the spread of excitation influences the majority of barrel cortex when a single whisker is stimulated. However, the overall extent of suprathreshold neuronal activity as compared with the extent of intrinsic signal imaging has not yet been quantified.

To address this issue directly, the activity in rat barrel cortex on stimulating a single whisker was examined with both ISI and single-unit recordings in the same animals. The amplitude of the evoked activity across the cortical surface was measured with both techniques. Using this detailed and quantitative comparison, electrophysiology and functional imaging were significantly correlated. A subset of neurons exhibit significant evoked spiking on stimulation of a single whisker even at the outer regions of the optically detected activity and, unexpectedly, within all layers of the cortex. These results aid in establishing a clearer connection between the two methods and consequently enhance the ability to interpret imaging data in somatosensory cortex. In addition, this data set provides basic information regarding the characteristics and extent of suprathreshold activity across a large region of barrel cortex in response to stimulation of a single whisker. Some of these results appeared previously in abstract form (Frostig et al. 1994, Masino and Frostig 1999).

METHODS

General methods

SUBJECTS. Ten adult male Sprague-Dawley rats (355–670 g) were studied. Each subject underwent both intrinsic signal imaging and subsequent electrophysiological recording in the left barrel cortex. Each subject was anesthetized initially using sodium pentobarbital (Nembutal, 50 mg/kg *ip*) and maintained in an areflexic state during the experiment with a constant intraperitoneal infusion (Nembutal, 0.1–0.4 ml/h) and additional supplements if necessary. The subject remained in a stereotax throughout the experiment. Vital signs such as temperature, oxygen saturation, and heart rate were monitored constantly; body temperature was maintained with a heating blanket.

WHISKER STIMULATION. Identical whisker stimulation was used during both intrinsic signal imaging and electrophysio-

logical recordings. Whisker C2 on the right snout was deflected 0.5 mm rostrally using a computer-controlled whisker stimulator positioned 15 mm from the snout. This whisker stimulus does not cause detectable movement of any other whiskers, and the stimulator did not touch the whisker except during the stimulation. Within each trial of data collection, whisker C2 was deflected at 5 Hz for 1 s.

Data collection

INTRINSIC SIGNAL IMAGING. Images of the functional representation of whisker C2 were collected as previously described (Chen-Bee et al. 1996; Masino and Frostig 1996; Masino et al. 1993). Briefly, an 8 (rostral-caudal) \times 6-mm (medial-lateral) area of skull overlying the left somatosensory cortex was thinned with a drill bit to ~ 100 μ m. A wall of petroleum jelly was built over the thinned area, filled with silicon oil, and sealed with a coverglass. This arrangement is noninvasive to the cortical tissue, as the skull remains intact, but it allows visualization of the major cortical vasculature. A slow-scan charge-coupled device camera (Photometrics, Tuscon, AZ) was positioned over the PMBSF and focused on the blood vessel pattern overlying the cortical surface. Images of the cortical vasculature later served as a reference to compare the location of the intrinsic signal responses with the location of the electrophysiological recordings. The camera was then defocused 300 μ m for data collection.

Light reflectance values were collected over a 6.9 \times 4.7-mm cortical area in a 192 \times 144-pixel array. The functional representation of whisker C2 was always positioned within this window (its location relative to the vascular pattern is known from previous experiments). Data were collected in nine consecutive 500-ms frames. Two frames (1 s) of baseline light reflectance values were collected. The computer-controlled, 5 Hz for 1 s stimulation of whisker C2 was synchronized to start at the beginning of frame three. Between each 4.5-s trial of continuous data collection there was a 15-s intertrial interval. Each data session consisted of a block of 32 such trials randomly interlaced with eight control trials (no whisker stimulation delivered). Images were created from the intrinsic signal data to verify that there were no artifacts (bubbles, movement of the wall and cover glass, etc.) and to determine if the functional representation of whisker C2 could be visualized reliably. At this point, imaging was terminated and electrophysiological recording of single-unit responses began on the same rat.

SINGLE-UNIT RECORDING. After collecting the array of intrinsic signal changes in barrel cortex in response to stimulation of whisker C2, the thinned skull and underlying dura mater were removed carefully. Teflon-coated tungsten microelectrodes (1.1 ± 0.2 Ω , Microprobe, Clarksburg, MD) were used to record neuronal activity at multiple locations in response to stimulation of whisker C2. Amplifier gain was set at 1,000 (DAM-80, World Precision Instruments, Sarasota, FL), and band-pass filtering was applied between 0.5 and 3 kHz. Cortical recording locations were selected in random order and covered a wide area (>3.0 mm) of barrel cortex. At each cortical location, the surface of the cortex was noted with an audio monitor, and recordings were made at three sites—one recording was performed in the middle of each of the supragranular, granular, and infragranular layers. On average, two

cells were recorded at each site, for a total of six cells per location. Across all 10 animals, a total of 461 cells were analyzed (22–62/animal) from 221 layer-specific sites (10–31/animal) at 83 different cortical locations (5–10/animal).

Single-unit responses were isolated from the multiunit clusters using a template-matching program (Multispikes Detector; Yissum, Israel) and collected into individual data buffers using HIST software (Spike Systems, New York). Single-unit waveforms were selected and isolated during both spontaneous activity and stimulation of whisker C2. Although it was possible to record from three cells simultaneously, usually one or two distinct neuronal templates were identified reliably at each recording site. Prior to data collection, the accuracy of each template was evaluated strictly during both spontaneous and evoked activity to ensure that there was no contamination either with evoked potentials or with another template.

During single-unit recording, each trial consisted of 2 s of data collection. One second of spontaneous activity was followed by 1 s containing the 5-Hz rostrocaudal deflection of whisker C2 using the identical computer-controlled stimulus as during the prior imaging session. At each recording site, 32 trials of whisker stimulation were presented with a 15-s interval between each trial.

Data analysis

INTRINSIC SIGNAL IMAGING. After the experiment, the amplitude of the evoked intrinsic signal was calculated at each cortical location where single-unit responses were recorded. A circular polygon (37 pixels) encompassing 0.05 mm² was positioned over the location of each electrophysiological penetration (example of an intrinsic signal array and locations of electrophysiological penetrations shown in Fig. 2). This allowed a direct comparison of the evoked intrinsic signal with the evoked neuronal response. (The intrinsic signals extracted from polygons and corresponding electrophysiological responses are illustrated in Fig. 3). The amplitude of the intrinsic signal within each polygon was calculated from the raw intrinsic signal rather than from the image of intrinsic signal activity as images can be created with a variety of different algorithms (Bonhoeffer and Grinvald 1996; Chen-Bee et al. 1996, 2000) and bias the assessment of the amplitude of the activation.

The amplitude of the intrinsic signal response was calculated by first extrapolating the baseline of the intrinsic light reflectance from frames 2 to 9. As there are ongoing large, slow changes in the intrinsic reflectance of cortical tissue that are unrelated to the specific whisker stimulation (Chen-Bee et al. 1996; Masino et al. 1993), establishing a baseline within each trial period avoids incorporating these nonspecific, global reflectance changes and biasing the signal amplitude. The distance from this baseline to the frame that was the greatest distance from the baseline (highest amplitude) was recorded as the amplitude of the evoked intrinsic signal response. The peak data frame was most often frame 6, which contained the data collected 1.5–2.0 s after the onset of whisker stimulation.

SINGLE-UNIT RECORDING. The amplitude of any stimulus-related spiking activity was analyzed for each cell. First, the 2 s of spiking activity accumulated during the 32 trials were divided into 50-ms bins. Next, the level of spontaneous activity for each cell (S) was determined by averaging the baseline activity prior to any whisker stimulation. The response evoked

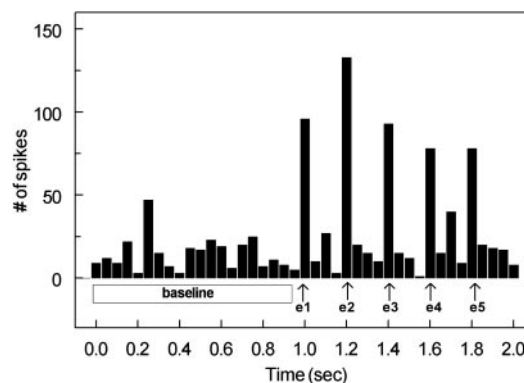


FIG. 1. Data analysis and example of a single-unit responding to deflection of whisker C2. The peristimulus time histogram (PSTH) contains 32 trials. The 2 s of activity (1 s baseline, 1 s with whisker stimulation) were divided into 50-ms bins for a quantitative analysis. The average number of spikes collected during a bin of baseline spontaneous activity (S ; average of the 1st 20 bins indicated by baseline bar), and the average number of spikes in the 5 bins following the 5-Hz deflection of whisker C2 [E ; ($e1 + e2 + e3 + e4 + e5$)/5] were calculated. The amplitude of the evoked response for each cell was defined as $(E - S)/S$. In addition, a Poisson distribution was created out of the baseline spontaneous activity for each individual cell. A whisker-evoked response was considered significant if the probability of the number of spikes in any evoked bin ($e1$, $e2$, $e3$, $e4$, or $e5$) was calculated as $P < 0.01$ based on the Poisson.

by whisker stimulation (E) was determined by averaging the responses collected in the bin following each of the five deflections of whisker C2. The amplitude of the evoked response was calculated as $(E - S)/S$ (Fig. 1).

A Poisson distribution was created out of the spontaneous activity to determine the level of evoked response that would be significant relative to the level of spontaneous activity. Significance was established as $P < 0.01$. In this way, the response in the 50-ms bin immediately following any of the five whisker deflections ($e1$, $e2$, $e3$, $e4$, $e5$; see Fig. 1) was evaluated as significantly different or not different from the level of spontaneous activity established for each cell. If any of the five bins following the whisker deflection showed a significant evoked response, the cell was rated as significant. Some cells only responded to the first deflection but with enormous response amplitude (often in the granular layer near the optical peak). Rarely, a cell responded significantly (as determined by the Poisson) only to a later bin (see Fig. 4D); but in all cases, the response amplitude averaged across the five bins for such cells was $>150\%$ of the spontaneous activity and the cell was considered significant. This calculation of significance determined the percentage of cells exhibiting significant evoked activity for each cortical distance (Fig. 8). All other quantifications, as well as the correlation between the evoked intrinsic signals and neuronal responses, consider the calculated amplitudes of both significant and nonsignificant cells, which reflects the averaged response across the five deflections.

COMPARISON BETWEEN INTRINSIC SIGNALS AND SINGLE-UNIT RESPONSES. To compare between the intrinsic signal responses and single-unit responses at different cortical locations across multiple animals, a standard mapping system was devised. Cortical locations were rated with respect to their distance away from the location of the peak intrinsic signal response as determined by imaging. Previous experiments have shown that the largest whisker-evoked single-unit responses are obtained from the center of that whisker's functional rep-

resentation as visualized with intrinsic signals (Masino et al. 1993). To compare responses between subjects at different cortical locations, this optical peak location was considered the “zero” point, and all locations were coded according to their radial distance from this site. This mapping system normalizes the electrophysiological and intrinsic signal responses from different animals with respect to their distance from the optical peak. Radial distances from the peak were binned and analyzed in 200- μm increments, with locations between the peak and 200 μm from the peak coded as distance 1, from 200 to 400 μm from the peak coded as distance 2, and so on. Cortical “distance units” referred to in the text and figures are based on these 200- μm increments. All graphs and quantitative comparisons presented here represent the distribution of intrinsic signal and neuronal responses obtained up to distance 8 (1.6 mm from the optical peak). However, a limited number of additional locations were sampled, up to distance 15 (3.0 mm from the optical peak).

As all electrophysiological mapping was performed blind to the exact location of the optical peak, sometimes more than one penetration occurred at the same incremental distance from the optical peak but at a different radial position. In these cases, two or more sites within the same increment were averaged such that each animal had one number for the intrinsic signal amplitude and one number for the neuronal response amplitude at each distance. Thus both types of data contained one number per distance per animal to allow comparison between similar data sets. The correlation between the intrinsic signals and the single-unit responses with respect to cortical location was calculated using the Pearson Product moment. Data for each distance were pooled between animals, and amplitudes were normalized according to the highest value within each animal prior to the correlation analysis.

RESULTS

When assessed using identical sensory stimulation in the same animal, optically and electrophysiologically measured activities show a similar profile across rat barrel cortex. The area with the highest amplitude intrinsic signal activity also exhibited the highest amplitude evoked single-unit responses. Both measures also decreased reliably with increasing distance from the center of the optical activity. Surprisingly, a subset of neurons responded with significant spiking on whisker stimulation even at long distances (≥ 1.6 mm) from the center of the optical activity.

Figure 2 shows an example of the intrinsic signal array and the distribution of cortical locations where both single units and intrinsic signals were sampled from this individual animal. First, the intrinsic signals were collected through a thinned skull (Fig. 2A), and then the thinned skull and dura mater were removed to perform single-unit recordings at the locations shown in Fig. 2B. Four of the nine locations where electrophysiology was performed (indicated by white squares) are labeled with their respective distance units from the optical peak, indicated by the cross (see METHODS). For example, 4 indicates 600–800 μm from the optical peak and 15 indicates 2,800–3,000 μm from the optical peak. The underlying intrinsic signals and neuronal responses at these specifically labeled locations are further examined in Fig. 3. In this example, the single-unit recording locations were widely dispersed; in other

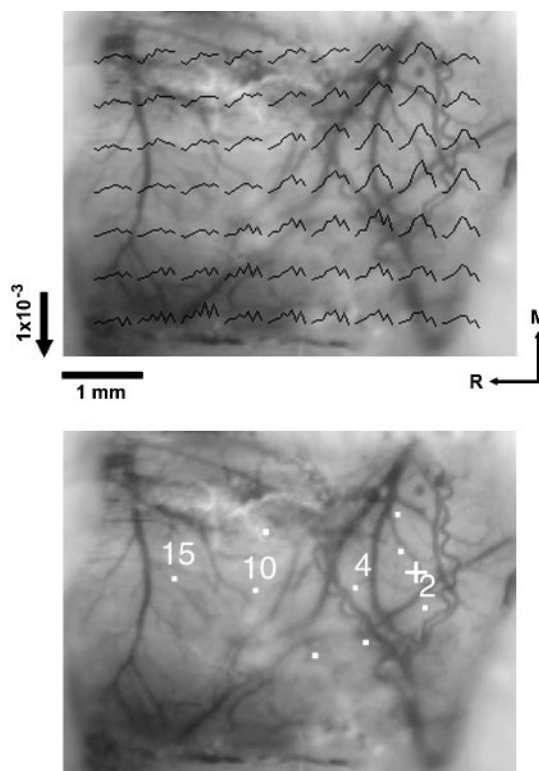


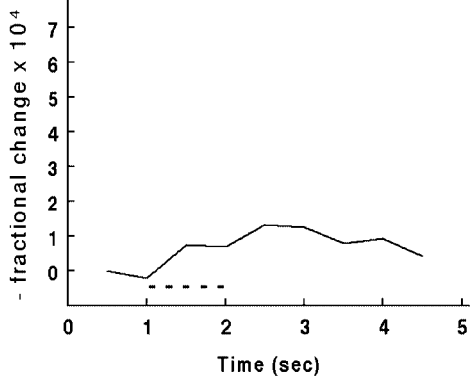
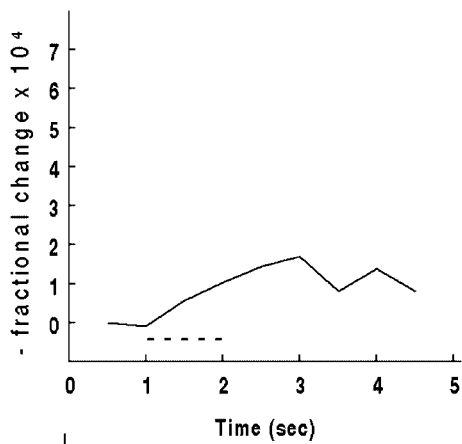
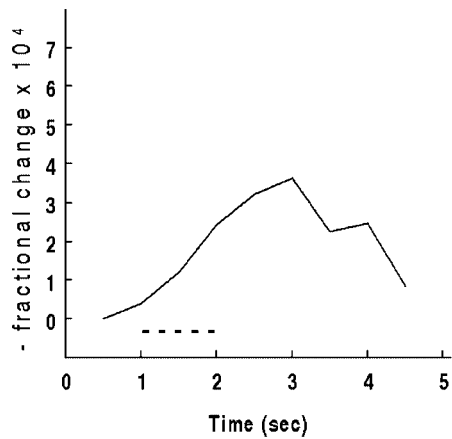
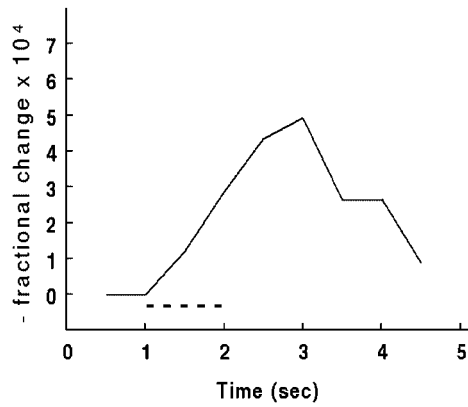
FIG. 2. An example of an intrinsic signal array and specific cortical locations sampled in an individual animal. *A*: the array of raw intrinsic signals superimposed over the cortical vasculature. Each trace represents the average intrinsic signal change for the area below it (20×20 pixels) during the 4.5-s trial period. An upward deflection of the intrinsic signal trace represents an increase in activity; the location exhibiting the largest deflection represents the center of the functional representation of whisker C2; this optical peak is indicated with a cross in *B*. Horizontal and vertical scale bars indicate 1.0 mm and the fractional change in light reflectance, respectively. Arrows point to medial (M) and rostral (R). *B*: each white square superimposed on the cortical vasculature represents a cortical location where single units were sampled. The points positioned across the center are labeled with their respective distance (according to 200- μm increments; see METHODS) from the optical peak, indicated by the cross. The underlying electrophysiology and intrinsic signal response at the numbered locations are examined further in Fig. 3.

experiments, they proceeded more radially across the field; but in all cases, the electrophysiological penetrations were done in random order.

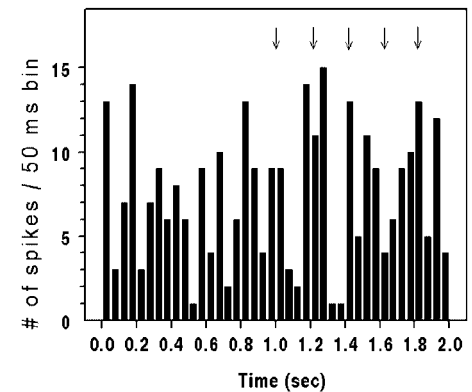
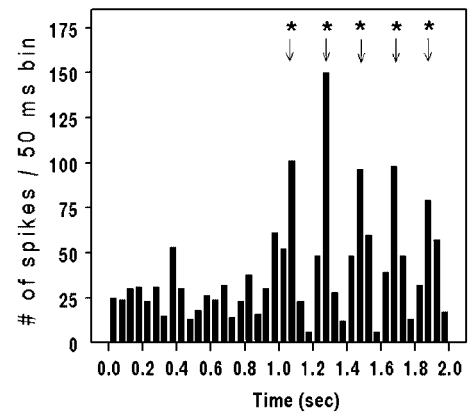
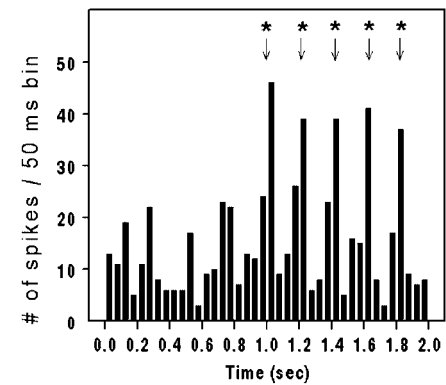
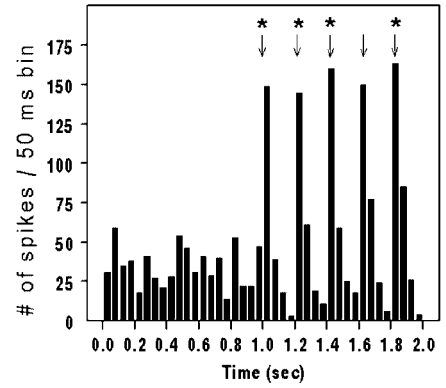
The specific intrinsic signal and single unit responses sampled at the locations indicated in Fig. 2B are shown in Fig. 3. The vertical columns are arranged from top to bottom in terms of increasing distance from the optical peak. In *A*, note the progressive decrease in the amplitude of the whisker-evoked intrinsic signal activity as distance from the optical peak increases. Each intrinsic signal trace was extracted from a small polygon placed over the site of the electrophysiological recording (see METHODS). In *B*, there is also a progressive decrease in the amplitude of single-unit responses. Note that it was possible to record evoked at distance 10 (1,800–2,000 μm) from the optical peak where a subset of neurons displayed a significant response. The evoked response here occurs one bin later than responses recorded closer to the optical peak, indicating that the latency to a significant evoked response is longer (50–100 ms) at this distance. The neuron illustrated here recorded at distance 15 did not display a significant evoked response (indicated in *inset*, n.s.).

A Intrinsic Signals

**Distance from
Optical Peak (μm)**



B Single Unit Spiking



To further examine the variety of neuronal responses at a given distance from the optical peak, a number of cells recorded at distance 5 (800–1,000 μm from the peak) are illustrated in Fig. 4. All the cells were recorded from the granular layer, and examples from three different animals and four different locations within these animals are shown. Across all animals, 23 granular cells were recorded from 13 locations at distance 5 from the peak. The peristimulus time histograms (PSTHs) show examples of high and low spontaneous and evoked activity (Fig. 4, *A* and *B*, respectively) and additional aspects of whisker-evoked activity. Detailed features of evoked single-unit activity observed in this study include afferent inhibition, a post excitatory period of decreased activity that is thought to reflect postsynaptic cortical inhibition (illustrated here by the activity depressed below spontaneous levels after each whisker deflection in Fig. 4, *C* and *D*) (Carvell and Simons 1988), and an offset response that occurred every time the whisker returned to its original position (Fig. 4*E*). A small number of cells responded only to the stimulus offset but were not included in the current analysis. Except for Fig. 4*F*, all of the cells shown in Fig. 4 displayed significant whisker-evoked activity. Overall, 7/23 granular layer cells did not show any significant evoked response.

Additional examples of intrinsic signal and single-unit responses from individual animals are shown in Figs. 5 and 6. Figure 5 illustrates intrinsic signal responses at distances 2, 5, and 14 (Fig. 5*A*) and significant whisker-evoked single unit responses recorded at each of these distances in the same animal (Fig. 5, *B–D*, respectively). Figure 6 illustrates intrinsic signal responses at distances 8 and 13 (Fig. 6*A*) and shows both significant (Fig. 6*B*) and nonsignificant (Fig. 6, *C* and *D*) neuronal responses from this animal at these distances.

Figure 7 illustrates the overall profile of intrinsic signal amplitude (Fig. 7*A*) and single-unit response amplitude (Fig. 7, *B* and *C*) with increasing distance from the optical peak. Note that both measures decrease reliably over distance. Single-unit response amplitude shows a sharp drop over distances 1 and 2, $\leq 400 \mu\text{m}$ from the optical peak (Fig. 7*B*). When examined on a smaller scale to exclude the high values at distances 1 and 2, the profile of the drop in single-unit amplitude farther from the peak becomes more apparent (Fig. 7*C*). There was no systematic change in the level of spontaneous activity across the cortical surface (data not shown).

In addition to a decrease in the amplitude of evoked activity, a smaller percentage of neurons exhibited significant evoked spiking as the distance from the center increased (Fig. 8). Interestingly, there is a drop in the number of significant responders at distance 6. Note that even at the farthest distance quantified here (distance 8; 1,400–1,600 μm from the optical peak) there is still a subset of cells (34%) which display a significant evoked response. As quantified in Fig. 7, *B* and *C*, the amplitude of this evoked spiking is relatively small. Al-

though the number of locations sampled beyond distance 8 (up to distance 15) was too limited to include in the summary, a subset (20–30%) of neurons at these long distances displayed a small but significant evoked response on stimulation of whisker C2 (see Figs. 3 and 5 for examples).

The profile of the intrinsic signal and single-unit response amplitudes across barrel cortex were highly correlated. The correlation was significant ($P < 0.05$) if it included only cells that were significant responders (data not shown) or if it included all cells, both significant and nonsignificant responders; the correlation was higher if it included all cells (Table 1). This is not surprising, given that the intrinsic signal represents the summation of all cells, significant and nonsignificant, over this large cortical area. In addition, the correlation was significant for each of the supragranular, granular, and infragranular layers, all of which displayed small but significant evoked spiking at long distances from the optical center. The correlation was highest in the supragranular layer (see Table 1).

DISCUSSION

The present study describes the profile of neuronal and intrinsic signal responses across rat barrel cortex in response to stimulation of a single whisker. It provides further validation that the peak activity measured with intrinsic signal optical imaging corresponds to the center of the whisker's functional representation as determined by the highest amplitude single-unit responses. These results show a good correlation between neuronal spiking and functional imaging in terms of both the relative amplitude of activity and the areal extent of the activated cortical surface. Although other sources such as subthreshold activity and/or blood flow contribute to intrinsic signals, across the neuronal population there remains a significant relationship between stimulus-evoked intrinsic signal activity and single-unit spiking in rat barrel cortex.

Intracellular recordings in barrel cortex reveal a large subthreshold response area as assessed by the multi-whisker receptive field size of single cortical neurons (Moore and Nelson 1998; Zhu and Connors 1999). On average, a single neuron responds to >10 vibrissae, but the amplitude of the response drops significantly with increasing distance from the principal vibrissae (Armstrong-James et al. 1992; Zhu and Connors 1999). The present findings obtained using extracellular single-unit recordings are in agreement regarding the drop in response amplitude as distance from the principal vibrissae increases but provide new evidence that a spiking component exists within the large subthreshold response area revealed by intracellular recording techniques.

The large extent of barrel cortex that responded with significant neuronal spiking is surprising and likely influenced by the type of stimulus delivered, the quantification of neuronal responses, and the experimental design. With respect to experi-

FIG. 3. Comparison of intrinsic signals vs. single-unit responses at a range of distances from the optical peak. The intrinsic signal traces and representative single units shown are sampled from an individual animal. Both the intrinsic signals and the single-unit histograms are an average of 32 trials. *A*: the raw intrinsic signals obtained in response to deflection of whisker C2 at each of the distances labeled in Fig. 2*B*. The traces represent the signal obtained from a 0.05 mm^2 area underlying each point. The 5-Hz whisker deflection is indicated by the dotted line along the *x* axis between 1.0 and 2.0 s. *B*: PSTHs are shown that include spontaneous and stimulus-evoked activity of representative cells sampled at the locations of the intrinsic signals shown in *A*. Spontaneous activity for each cell is obtained for 1 s, and the 5-Hz whisker deflection is again indicated by \downarrow between 1.0 and 2.0 s. *, significant responses ($P < 0.01$). Note scale differences in *y* axes between different PSTHs. All single units shown were sampled from the supragranular layers.

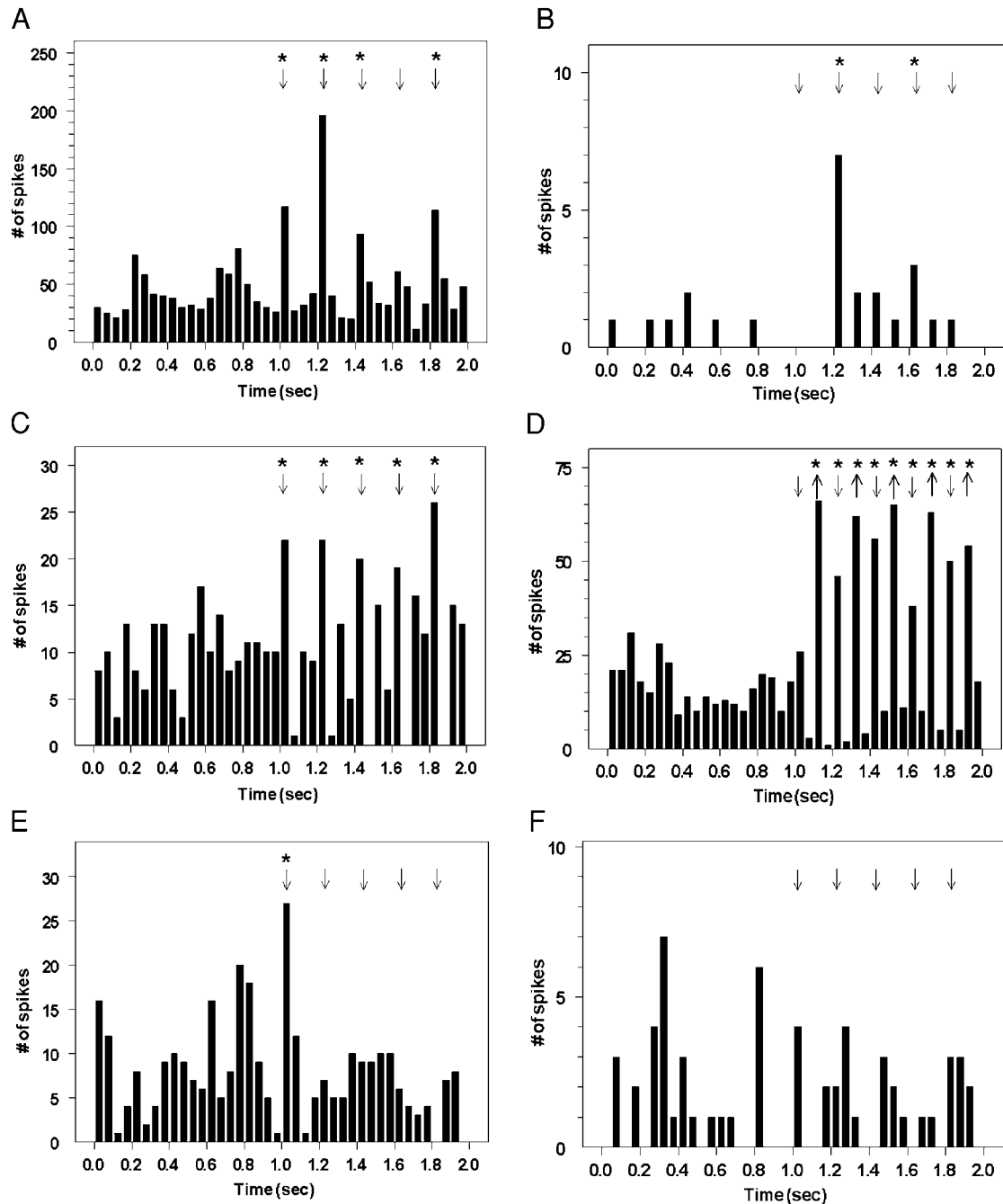


FIG. 4. Examples of PSTHs of cells recorded from 3 different animals (*A*, *B* and *D*, and *C*, *E*, and *F*; the neuronal response illustrated in *C* was recorded from the same animal as neurons *E* and *F* but at a different radial location). All neurons were recorded in the granular layer at distance 5, which contains the increment spanning 800–1,000 μm from the peak of intrinsic signal activity. Note scale differences in y axes between different PSTHs. *A*: a cell with high spontaneous activity and a significant whisker-evoked response. \downarrow , the bins following each whisker deflection; *, significant evoked responses. *B*: a cell with very low spontaneous activity but which still exhibited a significant whisker-evoked response to 2 of the 5 whisker deflections. This cell was recorded simultaneously with the cell shown in *D*. *C*: a cell with significant whisker-evoked responses (*) and obvious postexcitatory afferent inhibition (Carvell and Simons 1988) following each evoked response, as evidenced by the bin following each whisker deflection containing little or no spiking activity. This cell was recorded from the same animal as cells shown in *E* and *F* but at a different granular layer site also 800–1,000 μm from the peak. *D*: a cell which exhibited a significant response to the onset of every whisker deflection except for the first (\downarrow and *), and an even more prominent response to the offset of each deflection (\uparrow and *) when the whisker returned to the starting position. This cell was recorded at the same site and simultaneously with the cell shown in *B*. *E*: a cell that responded significantly to the first but not to the 4 subsequent deflections of the 5-Hz stimulation. This cell was recorded from the same animal as *C* and *F* and from the same recording site simultaneously with the cell shown in *F*. *F*: an example of a cell that did not display any significant whisker-evoked spiking. This cell was recorded simultaneously from the same site as the cell in *E* and from the same animal but at a different recording site as the cell shown in *C*.

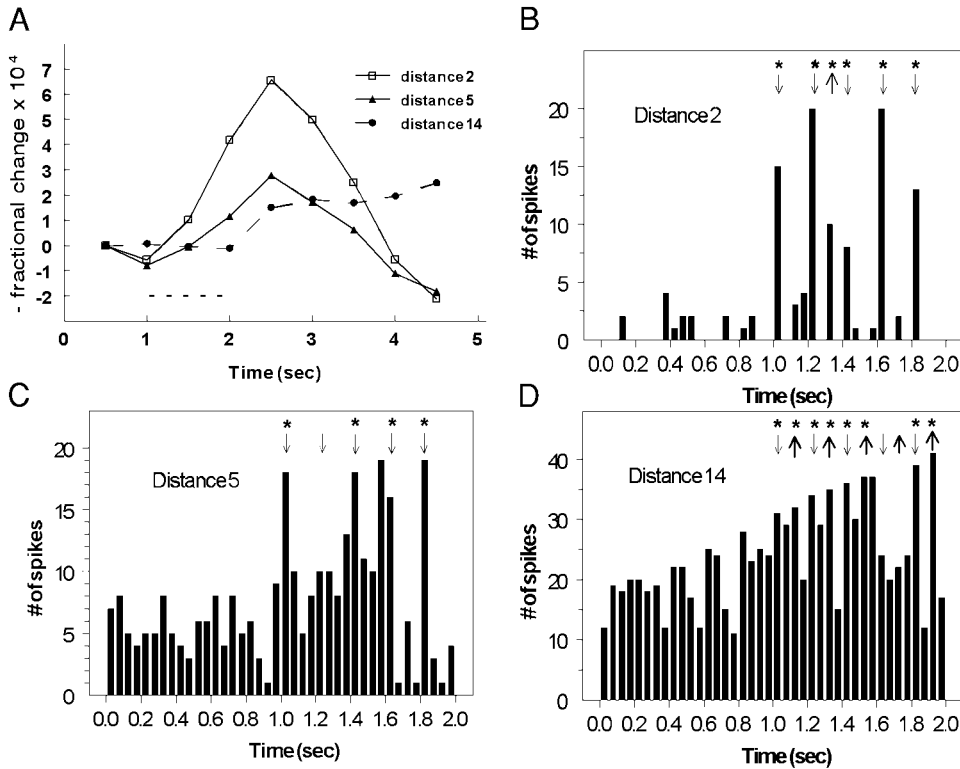


FIG. 5. Examples of intrinsic signals and single-unit responses from an individual animal at distances 2, 5, and 14. *A*: the intrinsic signals were extracted from polygons at electrophysiological penetration locations at distances 2, 5, and 14 from the optical peak. Note that the intrinsic signal sampled at distances 2 and 5 shows a characteristic rise and fall related to the whisker stimulation, superimposed on a downgoing baseline, whereas at distance 14, there is little stimulus-evoked change. *B*: a response from a neuron in the supragranular layer sampled at distance 2. This neuron gave a significant evoked response to each deflection (\downarrow and $*$), and showed 1 significant offset response (\uparrow with $*$). *C*: an example of granular layer cell at distance 5 from the optical peak in this same animal. *D*: a neuron sampled from the supragranular layer at distance 14. Although the amplitude of the response was much smaller than that recorded at distance 2 or distance 5, this cell showed a significant evoked response to the majority of the whisker deflections, and also displayed significant offset responses (\uparrow). The average evoked response in the evoked bins (5 onset responses only) was 64% higher than the spontaneous activity.

mental design, most previous studies have not looked in a detailed way at the total extent of spiking activity in cortex on stimulation of a single whisker. Most often, studies have focused on receptive fields, quantified as how many whiskers an individual neuron responds to. Cells were selected here without bias as to their response to the whisker stimulation, and this may have served to identify a population of small but signifi-

cant responses overlooked in experiments specifically aimed at studying whisker-evoked responses. The PSTHs shown in Figs. 3–6 underscore the wide variety of responses observed among cells in the cortex, and both significant and nonsignificant neuronal responses were found at every distance from the optical peak.

Regarding the details of the whisker stimulation and quan-

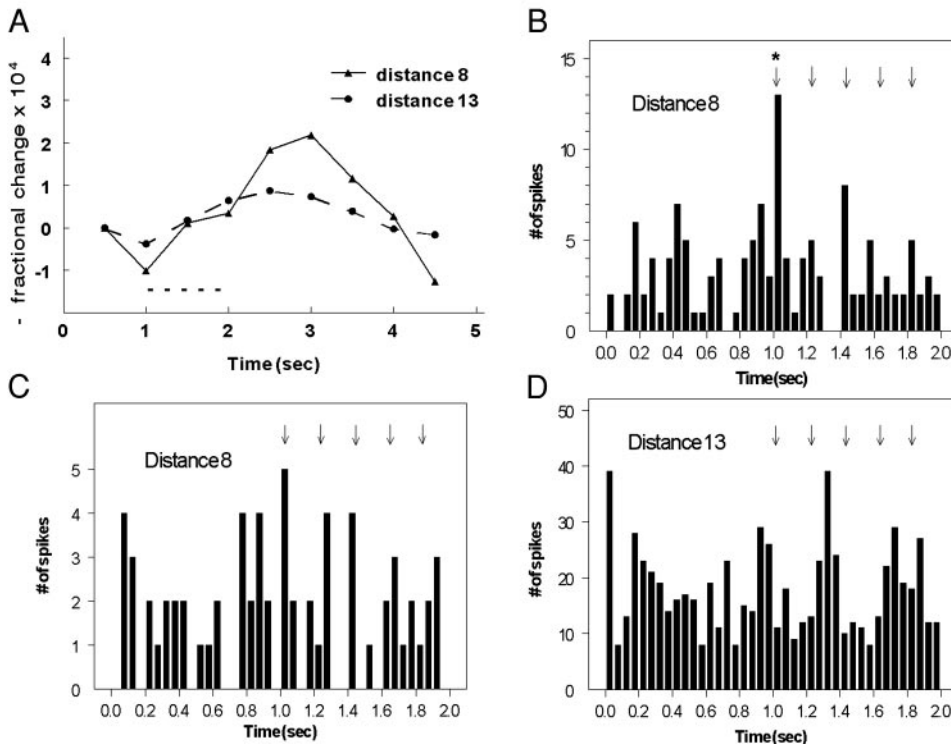


FIG. 6. Examples of intrinsic signals and single-unit responses from an individual animal at distances 8 and 13. *A*: intrinsic signals sampled from sites of electrophysiological penetrations at distances 8 and 13. Both distances show an evoked intrinsic signal, with a smaller amplitude observed at distance 13. *B*: a cell recorded from the infragranular layer distance 8 that showed a small evoked response to the first deflection. *C*: a cell recorded from the granular layer at distance 8 that did not display a significant evoked response. *D*: a cell recorded from the infragranular layer at distance 13 that did not show a significant evoked response.

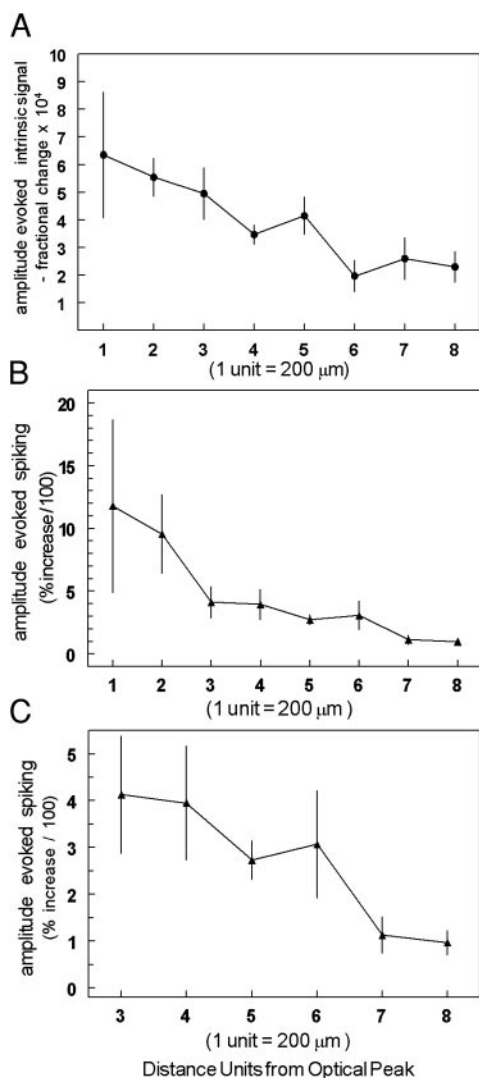


FIG. 7. Response profiles of the average intrinsic signal amplitude vs. average evoked neuronal response amplitude. *A*: change in intrinsic signal amplitude with increasing distance from the optical peak. Intrinsic signal amplitudes were calculated (see METHODS) for each location for each animal and averaged for this composite graph. *B*: change in the amplitude of evoked single-unit activity with increasing distance from the optical peak. The response amplitude was calculated for each cell (see METHODS and Fig. 1). All cells at a given distance away from the peak were averaged within each animal and included in this composite graph. *C*: a closer examination of the change in single unit response amplitude at distances beyond 400 μm from the optical peak ($>$ distance 2). Eliminating the large amplitudes of the initial distances from this graph reveals the profile of the decrease in evoked spiking with increasing distance. There was no systematic change in spontaneous activity with increasing distance from the optical peak (data not shown).

tification of neuronal responses, in this study identical whisker stimulation was used during both the optical and single unit recording. Thus because the intrinsic signal reflects five whisker deflections within each trial (5 Hz stimulation for 1.0 s), a cell was counted as significant if it responded significantly to any of the five deflections (see Fig. 4, *B* and *E*, for examples). Previous single-unit studies that delivered only a single whisker deflection may have missed a subset of the neurons identified here during the five deflections. In general, if the response from each cell is not quantified with respect to an adequate sample of its own spontaneous firing rate, allowing for an appropriately long latency after the whisker stimulation,

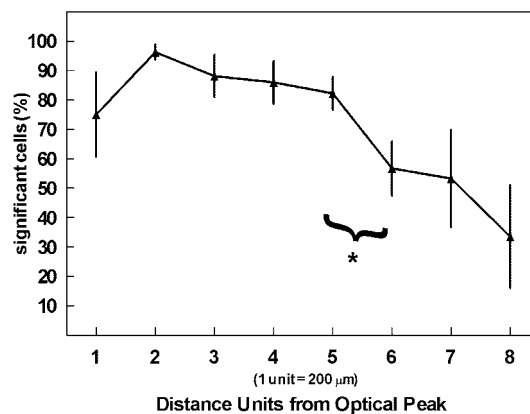


FIG. 8. Profile of significant single-unit responses across the cortical surface. The percentage of sampled cells that demonstrated a significant whisker-evoked response is plotted with respect to distance from the optical peak. A significant evoked response was defined by a Poisson distribution of the spontaneous activity for each cell (see METHODS and Fig. 1). The percentage of cells showing a significant evoked response was calculated for each distance for each animal before compiling into this overall average. The high variability (and seemingly lower percentage) at distance 1 is due to reduced sampling (a lower n) within the very limited spatial area $\leq 200 \mu\text{m}$ from the peak of the optical response. The electrophysiological recordings were performed without knowing the location of the peak, and the small sample size at this distance included several unresponsive cells. However, the large amplitude of the evoked single-unit response at this distance, even when including the nonresponders (Fig. 7*B*) as well as the high intrinsic signal amplitude (Fig. 7*A*) confirms the robust stimulus-related activity at this distance. This graph illustrates a significant drop in the number of cells that displayed a significant evoked response between distances 5 and 6, indicated by bracket and asterisk. Prior to distance 5, $>80\%$ of cells displayed a significant evoked response ($82.3 \pm 5.6\%$ for distance 5 and $85.5 \pm 3.5\%$ for distances 1–5 combined), whereas after distance 5, the percentage dropped to $<60\%$ ($56.7 \pm 9.3\%$ for distance 6, and $47.8 \pm 7.2\%$ for distance 6–8 combined; $P < 0.05$ for distance 5 vs. distance 6). Even with the continued decline in significant cells as distance from the optical peak increased, there remained a subset of cells (30%) that were significant responders at 1.6 mm from the optical peak (distance 8) and beyond; limited sampling was performed up to 3.0 mm from the optical peak (summary data beyond distance 8 not shown, but see Fig. 3*B* for an example of a significant evoked response at distance 10, and Fig. 5*D* for a significant response at distance 14).

many cells with a small but significant evoked response are easily missed. Additionally, the stimulator used here delivered a small, high-velocity deflection. Initial characterization of response properties of individual barrel cortex neurons in an unanesthetized rat noted that $\sim 30\%$ of cells respond only to a high-velocity stimulus (Simons 1978). Finally, in this study the stimulator did not touch the whisker unless it was actively deflecting it. To obtain accurate data on the latency from the

TABLE 1. Correlation analysis between the intrinsic signal (IS) amplitude and single-unit (SU) response amplitude across the cortex upon whisker stimulation

Correlation Between IS and SU	r	P
All cortical layers	0.87	0.005*
Supragranular	0.93	<0.001 **
Granular	0.83	0.01*
Infragranular	0.83	0.01*

The profile of the amplitude of the evoked intrinsic signal response and the evoked single-unit response were correlated (r) across the cortical surface, ≤ 1.6 mm from the optically determined peak. Overall, the two measures are significantly correlated ($P < 0.05$), with the highest correlation between the evoked intrinsic signals and the supragranular layer responses. * $P < 0.05$, ** $P < 0.001$.

stimulus to the neuronal response, most studies of the whisker-to-barrel system position the whisker such that it touches the stimulator slightly even when it is not being actively deflected. In the present study, the ability to measure latencies was sacrificed so as to avoid any habituation that might occur by chronically placing a stimulator against the exquisitely sensitive whisker. Using the present stimulation paradigm, significant evoked responses and detailed features of whisker-evoked activity, such as afferent inhibition (Carvell and Simons 1988) (see Fig. 4, C and D), were clearly detected.

An interesting feature revealed in this data set is the apparent drop off in cells with a significant evoked response at ~1.0 mm from the optical peak (between distances 5 and 6; see Fig. 8). Up to this distance, the great majority (>80%) of the cells recorded exhibited a significant evoked response to stimulation of the single whisker C2, whereas at distance 6 and beyond the percentage of cells is significantly lower. Perhaps this reflects a decrease in the efficacy of intracortical connections and information relay across the cortex. Anatomical evidence supports this, as tracers injected into a single barrel spread ~1.0 mm in all directions (Hoeftinger et al. 1995) and individual supragranular neurons can spread even farther (Gottlieb and Keller 1997). A suggestive drop in the amplitude of both the intrinsic signal and the neuronal response amplitude is observed at a similar distance as the decrease in the percentage of significant cells, neither of these changes was significant. Unexpectedly, small but significant evoked activity was recorded in all cortical layers at long distances from the optical peak. However, spiking in the supragranular layer showed the highest correlation with the intrinsic signal.

Other studies have compared intrinsic signals and neuronal activity in several cortical areas. In cat visual cortex, Das and Gilbert (1995) recorded receptive fields and found that the area in the visual field that evoked a change in the intrinsic signal was much larger than the area that evoked neuronal spiking. Using intrinsic signal optical imaging in the primary auditory cortex of the rat, combined with postimaging multiple- and single-unit recordings, Bakin et al. (1996) demonstrated a good correspondence between activity measured with the two techniques. Spitzer et al. (2001) found a less robust correspondence in the primary auditory field of the cat. However, these studies did not quantify the amplitude or profile of the neuronal spiking across the cortex, and in most cases used images and not the raw intrinsic signals for analysis. Both of these quantification issues clearly influence any comparison.

The differences among the preceding studies comparing the intrinsic signal area to the spiking area may also reflect inherent features of different cortices—functional architecture, lateral inhibition, or cortical vasculature (discussed in Spitzer et al. 2001). Knowledge of these differences between cortices is critical to enable accurate interpretation of imaging data with respect to underlying neuronal activity. In addition to potential inherent cortical differences, however, previous studies performed a less detailed evaluation of single-unit responses. In a limited number of animals, they addressed solely whether evoked spiking activity could be detected (often qualitatively) at various locations and recorded and analyzed relatively few single neurons from few locations and depths in the cortex. Such an approach is not an optimal correlate of the intrinsic signal, which represents the sum of the simultaneous activation of thousands of functionally heterogeneous neurons over a large

three-dimensional volume of cortical tissue. Many neurons from many locations need to be sampled and analyzed quantitatively to establish a profile of neuronal activity comparable with the intrinsic signal response across the cortical surface. Previous studies in barrel cortex reported a good correspondence between intrinsic signal activity and spiking neurons (Frostig et al. 1994; Masino and Frostig 1999; Peterson et al. 1998; Polley et al. 1999). The present work confirms and extends those findings.

One issue of clinical import is the interpretation of functional imaging data (fMRI) (Heeger and Ress 2002). fMRI, used widely to assess and interpret brain activity, relies on similar underlying metabolic changes as intrinsic signal imaging. Although fMRI lacks the spatial resolution of intrinsic signal imaging (Grinvald et al. 1986), its ability to be completely noninvasive is an obvious advantage. Recently Logothetis et al. (2001) combined fMRI and electrophysiology in monkey visual cortex. Focusing primarily on temporal aspects of evoked activity, they found that local field potentials correlated best with fMRI within a 1.0 mm² area. Irrespective of the relative contribution of subthreshold versus spiking activity to the fMRI signal, the present study agrees entirely with their conclusion that the spatial extent of activation in functional imaging experiments may often be underestimated significantly.

It is likely that the majority of cells in barrel cortex do display subthreshold responses on stimulation of a single whisker as subthreshold receptive fields can be very large (Zhu and Connors 1999). Accordingly, the profile of either subthreshold activity or local field potentials may correlate best with intrinsic signal activity across the cortical surface. However, this study establishes the surprising finding that a subset of cells across barrel cortex exhibit significant spiking activity on stimulation of a single whisker. Thus the intrinsic signal response represents a summation of a large number of subthreshold and a small but potentially important subset of suprathreshold responses at long distances (>1.6 mm) from the center of a whisker's functional representation.

By evaluating the response to identical whisker stimulation with both intrinsic signal imaging and single-unit electrophysiology in the same animals, this study provides a framework for interpreting results obtained using intrinsic signal imaging in rat barrel cortex. It demonstrates that the amplitude of spiking activity across barrel cortex is significantly correlated with intrinsic signal activity, and the areal extent of spiking neurons is well-represented by the areal extent of the intrinsic signals. Finally, these findings suggest that when one considers spiking activity across the cortex, the area activated by a variety of stimuli may be quite large, and perhaps the spread of diminishing activity is an important aspect of information processing in the cerebral cortex.

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