Fine-Scale Organization of SI (Area 3b) in the Squirrel Monkey Revealed With Intrinsic Optical Imaging

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Chen, Li Min, Robert M. Friedman, Benjamin M. Ramsden, Robert H. LaMotte, and Anna Wang Roe. Fine-scale organization of SI (Area 3b) in the squirrel monkey revealed with intrinsic optical imaging. J Neurophysiol 86: 3011–3029, 2001. Optical imaging of intrinsic cortical activity was used to study the somatotopic map and the representation of pressure, flutter, and vibration in area 3b of the squirrel monkey (Saimiri sciureus) cortex under pentothal or isoflurane anesthesia. The representation of the fingerpads in primary somatosensory cortex was investigated by stimulating the glabrous skin of distal fingerpads (D1–D5) with Teflon probes (3-mm diam) attached through an armature to force feedback-controlled torque motors. Under pentothal anesthesia, intrinsic signal maps in area 3b obtained in response to stimulation (trapezoidal indentation) of individual fingerpads showed focal activations. These activations (ranging from 0.5 to 1.0 mm) were discrete and exhibited minimal overlap between adjacent fingerpad representations. Consistent with previously published maps, a somatotopic organization of the fingerpads was observed with an orderly medial to lateral progression from the D5 to D1 fingerpads. Under isoflurane anesthesia, general topography was still maintained, but the representation of fingerpads on adjacent fingers had higher degrees of overlap than with pentothal anesthesia. Multi- and single-unit recordings in the activation zones confirmed the somatotopic maps. To examine preferential inputs from slowly adapting type I (SA) and rapidly adapting type I (RA) and type II (PC) mechanoreceptors, we applied stimuli consisting of sinusoidal indentations that produce sensations of pressure (1 Hz), flutter (30 Hz), and vibration (200 Hz). Under pentothal anesthesia, activation patterns to these different stimuli were focal and coincided on the cortex. Under isoflurane, activation zones from pressure, flutter, and vibratory stimuli differed in size and shape and often contained multiple foci, although overall topography was maintained. Subtraction and vector maps revealed cortical areas (approximate 250-μm diam) that were preferentially activated by the sensations of pressure, flutter, and vibration. Multi- and single-unit recordings aided in the interpretation of the imaging maps. In conclusion, the cortical signals observed with intrinsic signal optical imaging delineated a somatotopic organization of area 3b and revealed different topographical cortical activation patterns for pressure, flutter, and vibratory stimuli. These patterns were dependent on anesthesia type. Possible relationships of these anesthesia effects to somatosensory cortical plasticity are discussed.

INTRODUCTION

Intrinsic optical imaging has proven to be a useful tool for studying the cortical processing of sensory information because of its spatial resolution and ability to obtain multiple images from the same cortical region in response to different sensory stimuli. It has been instrumental in furthering the idea, especially in the visual system, that initial cortical processing involves the separation of sensory information into distinct topographical maps. Such maps comprise concurrent feature-specific modular and columnar organizations within each cortical area. For instance, in the first visual area (V1), optical imaging reveals multiple functional maps that delineate the relative alignments of ocular dominance, orientation, spatial frequency, and color representations for given retinotopic locations (Bartfeld and Grinvald 1992; Blasdel 1992a,b; Bonhoefer and Grinvald 1991; Crair et al. 1997; Ts’o et al. 1990). In the second visual area (V2), distinct functional maps for color, form, and disparity representations also have been described (Roe and Ts’o 1995, 1999).

In somatosensory cortex, the topographic map of the body surface has been well established with single- and multiunit recordings (e.g., Nelson et al. 1980; Pons et al. 1985, 1987; Sur et al. 1982; Woolsey et al. 1942). However, in contrast to primary visual cortex, there is only limited evidence as to whether different tactile features form multiple functional domains within the somatotopic maps of the four Brodmann areas (3a, 3b, 1, and 2) of primary somatosensory cortex (SI). Brodmann areas 3b and 1 receive input primarily from cutaneous afferents where areas 3a and 2 receive input primarily from deep afferents (muscle spindles and joints); however, only a few studies describe modular topographic domains within primary SI. The best evidence for functional domains within SI comes from electrophysiological mapping studies that observe islands of hand dorsum or hairy skin representations in the middle of the cortical representation of hand glabrous skin (Paul et al. 1972; Pons et al. 1987; Sur et al. 1982), zones of neurons with slowly adapting type I (SA) or rapidly adapting type I (RA) and II (PC) mechanoreceptor responses within Brodmann area 3b (Paul et al. 1972; Sretavan and Dykes 1983; Sur et al. 1981, 1984), and zones of neurons in Brodmann area 2 with either deep or cutaneous receptive field properties (Pons et al. 1985).

Another prominent characteristic of somatosensory cortex is its dynamic receptive field organization. Somatosensory maps
in SI undergo significant reorganization in response to peripheral nerve lesions (Merzenich et al. 1983) as well as in response to training in behavioral tasks (Recanzone et al. 1992b). Dynamic changes in cortical representation have also been reported in response to repetitive stimulation of the periphery (Lee and Whitsett 1992), peripheral nerves (Schroeder et al. 1995) and cortical loci (Recanzone et al. 1992b). Dynamic receptive field changes have also been reported in response to changes in anesthesia (e.g., Duncan et al. 1982) and to other pharmacological manipulations (Alloway and Burton 1991; Whitsett et al. 1999). These issues regarding the plasticity or stability of somatosensory cortical maps have significant implications for our understanding of both the columnar and tangential dimensions of functional organization in SI.

To build on this body of knowledge, we applied optical imaging methods to study the topography, modality-specific mapping, and stability of responses within area 3b of the squirrel monkey. While the basic somatotopic organization of area 3b is well established, the organization, size, convergence, and shape of cortical activity induced by fingertip stimulation still needs further investigation (Favorov and Kelly 1996; Godde et al. 1995; Tommerdahl and Whitsett 1996; Tommerdahl et al. 1998, 1999). In this paper, we first establish that optical imaging methods can be used to detect cortical activity in somatosensory cortex (under pentothal anesthesia) by showing the well-established topographical organization of the fingertips. We then show that the maps are different under isoflurane anesthesia and support this with electrophysiological mapping. Finally, we obtain optical imaging evidence suggesting an organization for pressure, The advantage of force control versus displacement control are that the rate of adaptation of mechanoreceptors is less and the potential of skin creep and skin stress relaxation is less under force than under displacement mode (Pubols and Benkisch 1986). Two such motors were available, and we were thus able to stimulate two fingertips in any single block of trials. Within any single block, somatosensory stimuli (e.g., digits A and B), and null stimulus (blank) conditions were presented in a randomly interleaved fashion.

We used four different tactile waveforms known to elicit the different percepts of indentation, pressure, flutter, and vibration. Indentation stimulus comprised of a single trapezoidal vibrotactile waveform [Fig. 1C, ramp rates: 150 g/s; amplitude: 30 g (306 mN), total duration: 4 s; baseline offset: 2 g (20.4 mN), 2nd trace]. This indentation is known to predominantly stimulate SA mechanoreceptors, although RA and PC mechanoreceptor recruitment will occur (Cohen and Vierck 1993). The other three vibrotactile waveforms (Fig. 1C) consisted of different sinusoidal stimuli (2-s duration) that produce sensations of: pressure [1 Hz, 20 g (204 mN), orange trace], flutter [30 Hz, 6 g (61.2 mN), red trace], or vibration [200 Hz, 1.6 g (16.3 mN), blue trace]. In humans, these sensations reflect the activation of the RA, SA, and PC mechanoreceptors, respectively (Johansson et al. 1982; Talbot et al. 1968). The 30- and 200-Hz vibrotactile frequencies were chosen because RA and PC mechanoreceptors show the lowest threshold for activation at these frequencies, respectively (LaMotte and Mountcastle 1975; Mountcastle et al. 1972); with low-frequency stimulation (1 Hz), SA mechanoreceptors respond vigorously, whereas RA and PC mechanoreceptors do not (Johansson et al. 1982; Talbot et al. 1968). Thus each stimulus was designed to preferentially activate one receptor type over the other two (Table 1).

In control experiments, the trapezoid and 1-, 30-, and 200-Hz stimuli were found at these force values to produce baseline to peak fingertip displacement amplitudes of 790, 450, 65, and 12 μm, respectively. The sinusoidal waveforms were presented with the probe already loading the skin with a baseline indentation of 20 g (204 mN, blank condition).

**Optical imaging and analysis**

**PREIMAGING ELECTROPHYSIOLOGY.** A brief electrophysiological mapping procedure was used to locate the fingertip region of Brodmann area 3b prior to imaging. The responsive skin area of the unit activity was identified by initially palpating areas on the contralateral arm and hand while listening to the audio amplifier for spike activity. Then if the unit activity was located on the hand, the skin was lightly tapped with a 2-mm-diam probe or stroked with a cotton wisp. Area 3b units were discriminated on the basis of small receptive field size, and we were thus able to stimulate two fingerpads in any single block (Pichlmayr et al. 1984). In this state, humans and monkeys have lost consciousness, have reduced reflex activity, have no reaction to painful stimuli, and undergo skin incision, skin clips, skin retraction, endotracheal tube placement, and other invasive surgical procedures without changes in EEG activity.
chamber was cemented over the craniotomy, filled with lightweight silicone oil, and sealed with a cover glass (Fig. 1A). The cortical surface was illuminated through the chamber window with 630-nm wavelength light provided by optic fiber light guides. Images of reflectance change (intrinsic hemodynamic signals) were acquired by an eight-bit video CCD camera using an Imager 2000 system (Optical Imaging, Germantown, NY). Technical details and a discussion of the issues involved with intrinsic imaging have been presented previously (Bonhoeffer et al. 1996; Grinvald et al. 1988; Ts’o et al. 1990).

Vibrotactile stimuli were presented in a randomly interleaved manner in blocks consisting of 10 trials per stimulus type. In a typical session, four to five blocks of trials (40–50 trials) were collected per stimulus condition. Intrinsic signal maps were collected at 5 image frames per sec for 3 s starting 200 ms prior to stimulus onset (Fig. 1C, top). Inter-stimulus intervals were 6–8 s. Between vibrotactile stimulus presentations and during blank conditions, the stimulus probe remained in contact with the skin with a contact force of either 2 g during trapezoidal stimulation blocks or 20 g for sinusoidal stimuli trials.

**TABLE 1.** Stimulus waveforms

<table>
<thead>
<tr>
<th>Waveform</th>
<th>Peripheral Receptor</th>
<th>Activated</th>
<th>Sensation</th>
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<tbody>
<tr>
<td>Trapezoid</td>
<td>SA</td>
<td>Indentation</td>
<td></td>
</tr>
<tr>
<td>1-Hz sinusoid</td>
<td>SA</td>
<td>Pressure</td>
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<td>30-Hz sinusoid</td>
<td>RA</td>
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<tr>
<td>200-Hz sinusoid</td>
<td>PC</td>
<td>Vibration</td>
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**FIG. 1.** Optical imaging and stimulation methodology. *A:* lateral view of imaging area. Craniotomy was positioned over the anterior parietal cortex exposing the central sulcus and Sylvian fissure near the fingerpad region. Optical chamber was implanted over the exposed area, filled with lightweight silicone oil, and sealed to dampen cortical pulsation. This image was obtained over the fingerpad region of area 3b; same case as shown in Figs. 2 and 3. Scale bar: 4 mm. (Drawing of squirrel monkey brain adapted from Sur et al. 1982.) *B:* stimulator setup. The glabrous skin of either 1 or 2 distal fingerpads (D1–D5) was stimulated by a Teflon probe (3-mm diam) attached to a force feedback controlled torque motor. *C:* stimulus waveforms. Trapezoidal shaped indentations (purple trace, ramp rate: 150 g/s, amplitude: 30 g, approximately 0.79 mm, total duration: 4 s) were used to map the somatotopic organization of somatosensory cortex. Sinusoidal vibrotactile stimuli producing sensations of pressure (yellow trace, 1 Hz, amplitude 20 g, 0.45 mm), flutter (red trace, 30 Hz, 6 g, 65 μm), and vibration (blue trace, 200 Hz, 1.6 g, 12 μm). Total stimulus duration 2 s. Image acquisition began 200 ms before stimulus onset and lasted for 3 s. *D:* standard image analysis procedure. Original image was high- and low-pass filtered, then thresholded at the 15% level (colored orange). The outline determined from the orange color-coded area was overlaid with original unprocessed image, with blood vessel map, and with filtered images for comparison.

**IMAGE ANALYSIS.** For each stimulus condition, image frames were summed to maximize signal-to-noise ratio. Because the fingerpad representation was located near the central sulcus, blood vessel artifact was at times substantial, leading to low signal-to-noise ratios. We attempted to minimize blood vessel artifact in several ways: by synchronizing image acquisition with respiratory and heart cycles to minimize movement noise associated with respiration and heartbeat, by blank subtraction where the “blank” reference is either a 3 s image acquired during no stimulus (Blank condition) presentation or the first frame of each 3 s image acquisition, see following text, and by masking artifacts from large blood vessels to exclude contaminated pixels from analysis.

To ensure that the imaged signal was not contaminated by occasional high-amplitude noise, we examined images block-by-block to remove those that contained excessive noise (usually due to significant blood vessel artifact). Usually all blocks were used, but occasionally a bad block was not included in subsequent analyses. We also compared images obtained by summing different blocks of trials. Presence of sporadic high-amplitude noise would produce different half-trial maps, whereas consistent nonartifactual signal would produce similar maps. These methods were used to confirm that the imaged signals from area 3b were consistent and repeatable and were not due to noise signals occurring in one or a few of the trials.

Two types of image analyses were conducted: single-condition analyses and two-condition subtraction analyses. “Single-condition”
maps were obtained by subtracting the blank image from those images obtained from each given vibratocile stimulus. Single-condition maps indicate the response magnitude at each location in the image for a particular stimulus condition. Such blank subtractions not only measure change from baseline but also reduce blood vessel artifact and minimize effects of uneven illumination. Dark pixels in single-condition maps indicate a response to that stimulus condition greater than that of the blank condition image, gray pixels indicate a response not different from blank, and lighter pixels could indicate some level of activation less than that in blank.

Whereas single-condition maps reveal the presence of a response to a particular stimulus, stimulus subtraction reveals preference for one stimulus over another. “Two-condition subtraction” maps were obtained by subtracting the sum of images obtained for one stimulus condition (e.g., pressure) from that obtained under another (e.g., flutter). In this case, dark pixels indicate preference for one condition, light pixels a preference for the other condition, and gray pixels equal preference for both. Single-condition maps provide the most reliable indication of stimulus-specific activations, but this may occur at the expense of signal-to-noise ratio. Difference maps yield better overall signal quality by accentuating the preference for one condition over another, but activations common to both stimulus conditions are eliminated. Thus single-condition and subtracted maps may or may not be similar in organizational structure (e.g., see Fig. 6).

In some cases, custom-written software was used to perform an image “vector” analysis that simultaneously compared intrinsic response to SA, RA, and PC stimulus conditions. This analysis produces color-coded maps that signify the relative preference for one stimulus over another and has been used successfully in optical imaging of visual cortex (e.g., orientation, Bonhoeffer and Grinvald 1991; T’so et al. 1990). This method combines multiple images pixel by pixel (in an n-vector space), where the preferred response of each pixel is determined by the degree to which a dominant pixel value is greater in magnitude than the others (the direction and length of the vector sum). This response preference is mapped in a color space where preferences for pressure, flutter, and vibration are color coded by red, yellow/green, and blue, respectively. Magnitude of response is indicated by color saturation. Pixels that do not respond to any vibratocile stimuli are indicated by dark gray, and pixels that respond equally well to all stimuli are indicated by light gray or white.

**Histological procedures**

At the end of data collection, animals were given a lethal dose of pentothal (40 mg/kg iv) and perfused through the heart with saline followed by 4% paraformaldehyde. After extraction of the brain, the desired cortical region was removed, flattened, and immersed in a 30% sucrose solution. The receptive fields (RFs) of multiple or single units were outlined through a series of indentations with a 2-mm-diam hand-held probe. Then, isolated or multiple units were classified as either having response properties similar to SA and RA based on their dynamic responses to static indentation produced by the probe placed in the center of their receptive field. SAs characteristically show a sustained response to static indentation while RAs only respond to the onset and offset of the stimulus (Mountcastle et al. 1969; Talbot et al. 1968). To evaluate whether the unit activity had PC activity (highly vibration sensitive Pacinian response like properties), we determined whether the unit had a relatively large RF and responded to indirect distant vibrations (tapping on the table). For a subset of units with RFs near the center of the fingerpad, we stimulated with the same stimuli we used for the intrinsic imaging. We found that cortical units with cutaneous RFs on the middle or proximal fingerpads did not respond to these stimuli, confirming that our imaged responses were due specifically to distal fingerpad stimulation.

Conventional techniques were used to amplify, filter, and display unit activity. To collect, store, and analyze records of neuronal spike trains at 0.02-ms temporal resolution, we used Spike 2 hardware and software (Cambridge Electronic Design, UK). During the recording session, electrolytic lesions were made at sites of particular interest by passing current pulses (±7 μA for 7 s) through the electrode tip.
anesthesia despite maintenance of similar anesthesia levels (see METHODS). Our results with pentothal and isoflurane anesthesia are thus presented separately.

We studied eight hemispheres in six monkeys. Of these (see Table 2), three hemispheres in three monkeys were studied with pentothal anesthesia, one in which all five fingers were mapped and two in which fingerpads D3/D4, and D2/D4, D3/D4 were mapped. Under isoflurane anesthesia, five hemispheres in three monkeys were studied, two in which two pairs of digits were mapped (D1/D3, D2/D4 and D2/D5, D1/D3) and two in which one pair of digits (D2/D4) was mapped.

Optical response in area 3b

As shown in Fig. 2, stimulation of single fingerpads typically led to foci of dense cortical activations. Similar to signals from visual cortex, stimulated optical signals typically peak within 1- to 3-s poststimulus onset and have magnitudes ranging from 0.1 to 1.0% change in reflectance (cf. Chen et al. 2000; Masino et al. 1993; Tommerdahl et al. 1999). This was true for both pentothal (Fig. 2A) and isoflurane (Fig. 2B) anesthesia, although isoflurane signals tended to be smaller in magnitude.

One way of checking whether signals are contaminated by occasional high-amplitude noise is to divide the blocks of trials into two halves and compare the resulting half-trial images. Occurrence of high-amplitude sporadic noise on some trials should likely produce different half-trial maps, whereas consistent nonartifactual signal should produce similar maps. Figure 2C illustrates images obtained for summing different blocks of trials from a case using pentothal anesthesia (top) and isoflurane anesthesia (bottom). In each case, images resulting from the sum of half the trials, either first or latter half, produced very similar optical maps. By examining both the temporal development and consistency of these maps, we were able to confirm that the imaged signals from area 3b were consistent and repeatable and were not due to sporadic noise signals occurring in one or a few of the trials.

The most prominent source of noise in our images was typically blood vessel artifact, arising from the central sulcus vessel near area 3b. Large vessel artifacts are a common contaminant of optical images and remain because of significant signal change caused by pulsation and changes in oximetry accompanying such pulsation. In addition to synchronization of image acquisition with heartbeat, to further reduce contribution from vessel artifact, we often used “first-frame subtraction.” First-frame subtraction involves subtracting the first frame (200-ms prestimulus blank) of each trial from all subsequent frames prior to any subsequent analysis. While effectively reducing blood vessel artifact, first-frame subtraction produces relatively vessel-free maps with a somewhat grainy appearance (e.g., Fig. 2A). Other constant baseline noise sources (such as slow oscillation, roughly 0.1 Hz) (Mayhew et al. 1996) were in part removed by blank subtraction. Further, because our stimuli were randomly interleaved and were averaged (40–50 trials), it is unlikely that such background oscillations would contribute in a significant and consistent way to our images. Thus whatever spontaneous levels of reflectance present were largely eliminated from the resulting images.

Topography of the fingerpads under pentothal anesthesia

For all three monkeys studied under pentothal anesthesia, the presence of an orderly topographical map of the fingerpads within area 3b was observed with optical imaging methods. In the case shown in Fig. 3, focal activations were obtained for trapezoidal stimulation of each of the five fingerpads studied. Activation foci were located in area 3b, in the region anterior to and near the central sulcus (large vertical blood vessel in Fig. 3B). These 0.5- to 1-mm sized activations were either round or oval in shape and were topographically organized, with D1–D5 activations aligned in an orderly lateral to medial progression (Fig. 3, A and B). The activations obtained for D2–D4 fingerpads were comparable in strength and in size; D1 stimulation exhibited the weakest signal. D5 activation appeared smallest in size (<0.5 mm) but may extend into the central sulcus. Both the size and progression of digit representation revealed by these optical signals were consistent with prior electrophysiological maps (e.g., compare Fig. 3, B and C) of the representations of the body surface in the somatosensory area 3b of squirrel monkeys (Merzenich et al. 1987; Sur et al. 1982).

Sample electrophysiological recordings also supported these images. For example, in the case shown in Fig. 3, we recorded units with small RFs that were limited to single digits (Fig. 3B). Small RFs are a characteristic feature of area 3b and serve to distinguish it from area 1 (located posterior) and from the deep receptor responses commonly found in area 3a (located anterior).

Histological methods further corroborated the location of our optical recordings. We used superficial Nissl-stained tangential sections of tissue from the imaged area that revealed a clear pattern of surface vasculature (Fig. 3D) to precisely align the vasculature of the tissue with the vasculature of the imaged

<table>
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<th>TABLE 2. Case summary of topography and submodality studies</th>
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<tr>
<td><strong>Anesthesia</strong></td>
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<td>Pentothal</td>
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area (cf. Roe et al. 1995, 1999). Using these methods, adjacent CO-stained histological sections were aligned with the optical images (Fig. 3). Comparison of Fig. 3, D and E (blue outline) revealed that intrinsic signals evoked by fingerpad stimulation were localized to regions of relatively dark CO staining that is characteristic of Brodmann area 3b in the primates and is distinguishable from surrounding lighter stained areas (e.g., area anterior to 3b) (cf. Jain et al. 1998; Tootell et al. 1985).

The lateral to medial organization of the D1–D5 digit representation was consistently observed in each of the other experiments in which the representation of single pairs of fingerpads was examined (see Table 2) (also Chen et al. 2000). For example, under pentothal anesthesia, activation areas due to D4 stimulation were always medial to D3 stimulation and medial to D2 stimulation.

**Comparison of topography under pentothal and isoflurane anesthesia**

When we imaged area 3b under isoflurane anesthesia, we found topography was less well defined than during pentothal anesthesia, but general relative topographies were maintained (see Figs. 7 and 8). Consistently, we found that topography was easier to interpret with pentothal than with isoflurane. In pentothal experiments \((n = 3)\), the activation spots were oval or round in shape and had diameters ranging from 0.5 to 1.0 mm (Figs. 3 and 4), and overlap between activation zones of adjacent fingers was minimal (estimated to be 10% or less as determined by comparing the area of single condition activation with the area after subtraction of the adjacent digit activation). In isoflurane experiments \((n = 5)\), the activation zones were less punctate, more irregular in shape, and often extended.

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**FIG. 2. Development and reproducibility of the optical signal.** A series of images are presented showing the temporal development of intrinsic cortical response to stimulation of the fingerpad. A: indentation of D2 under pentothal anesthesia. B: 1-Hz sinusoid stimulation on D4 under isoflurane anesthesia. Fifteen 200-ms consecutive frames are shown. Stimulus onset occurs during the 2nd frame (at 200 ms). Each image represents 200-ms summation of optical responses. The blood vessel maps of imaged area are shown at bottom right. C: 3 sets of maps obtained by summing the 1st 20 (left), 2nd 20 (middle), and all 40 (right) trials under either pentothal (top row) or isoflurane (bottom row) anesthesia. The red outlines on all 3 maps indicate the activation regions obtained from total map (summation of all 40). Scale bar: 1 mm. A, anterior. M, medial.
over larger areas with a more complex structure (Figs. 5, 7, and 8). We observed that primary zones of strong activation were often accompanied by secondary zones of weaker activation. This resulted in apparent overlap (estimated to be about 30–40%) in the signals between adjacent fingerpads.

**Examination of submodality within area 3b**

A submodal functional segregation within area 3b of monkeys has been previously suggested using electrophysiological mapping methods, based on the irregular band-like segregation of SA and RA inputs in the middle layers of cortex (Sur et al. 1984). To address whether SA and RA inputs are segregated also in superficial layers, we examined both single-condition and subtraction maps obtained in response to pressure, flutter, and vibration stimulation of single digits.

**DETERMINATION OF STIMULUS PARAMETERS.** We first attempted to maximize the preferential stimulation of each of the SA, RA, and PC receptor populations by using stimuli of minimal intensity in the appropriate frequency ranges (1 Hz, 4 g for SA; 30 Hz, 3 g for RA; and 200 Hz, 1 g for PC) (Mountcastle et al. 1972). However, this yielded no detectable activation patterns in our intrinsic signal images with pentothal anesthesia. We thus increased the intensity (amplitude of modulation) of our stimuli. Stimulation at 20 g (0.45-mm displacement), 6 g (0.065-mm displacement), and 1.6 g (0.012-mm displacement) for pressure, flutter, and vibration stimuli, respectively, resulted in reliable and readily detectable imaged activation. The data presented in this paper were collected with the higher intensity stimuli.

Psychophysically, at these higher intensities, the 1-, 30-, and 200-Hz sinusoidal stimuli are still perceived as pressure, flutter, and vibration (Bolanowski et al. 1988; Lamotte and Mountcastle 1975; Talbot et al. 1968). However, due to the intensity of the stimulation, it was likely that, in addition to the targeted mechanoreceptors, the others were also activated to some extent (Talbot et al. 1968). Despite the fact that at these intensities the specificity of our stimulation was likely to be compromised, differences between activation patterns may still (by subtraction) be attributable to preference for one stimulus condition over another. We will, throughout the remainder of the paper, use the terms pressure, flutter, and vibration to refer to the stimulation frequencies used that are known to best activate each of the SA, RA, and PC mechanoreceptor populations (Fig. 1C, Table 1).

**Similar pressure/flutter/vibration activations under pentothal anesthesia**

Figure 4 illustrates two cases in which we examined submodality organization in area 3b using pentothal anesthesia. In case 1 (Fig. 4, left), images were obtained following pressure, flutter, and vibration stimulation of fingerpad D3 in one animal (same case as shown in Fig. 3). For case 2 (Fig. 4, right) images were obtained from stimulation of a D3 fingerpad in another animal with the three types of vibrotactile stimuli. In each case, the activation regions obtained in response to pressure (Fig. 4A), flutter (Fig. 4B), and vibration (Fig. 4C) stimulation are shown on the left, and the strongest activation regions indicated with colored outlines on the right (Fig. 4F, yellow; Fig. 4G, red; Fig. 4H, blue). To illustrate how the outlines were obtained, the low-passed images are shown for one case (case 1, Fig. 4, F–H). Note that regions containing blood vessel artifact are masked in case 1 (Fig. 4, F–H).

Comparison of activation outlines in both cases (shown in Fig. 4 of cases 1 and 2) did not show evidence of any obvious topographical differences in the location of the cortical activ-

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**FIG. 3.** Optical imaging of fingerpad somatotopy in area 3b under pentothal anesthesia. A: intrinsic cortical activities were obtained with single-indentation stimuli presented to the contralateral D1–D5 distal fingerpads. The series of cortical activations were organized in an orderly lateral to medial progression from D1 to D5 (shown from left to right). B: blood vessel map of imaged area. Yellow box indicates imaged region shown in A. D1–D5 activations (indicated by the orange circles) lie along and anterior to the central sulcus. Multi- and single-unit activities recorded from sample penetrations (green dots) were consistent with the imaged somatotopy. Blue box indicates region of histological alignment shown in 3b. C: from Sur et al. (1982), digit topography in area 3b. D: Nissl stain of very superficial tangential tissue section reveals blood vessel pattern that allows alignment of adjacent sections with blood vessel map. E: cytochrome oxidase (CO) staining of histological sections confirmed that the intrinsic signals were from Brodmann area 3b. Scale bar: 1 mm. P, posterior. M, medial.
ity. Each of these activations were quite similar to those obtained with single-indentation stimuli (compare Fig. 4, A–C with Fig. 3A, panel D3). Although none of our stimulation parameters fully isolated SA, RA, and PC afferent contributions, they were designed to preferentially activate one receptor population over the other two. We considered the possibility that some condition-specific preference existed within the activated regions. To examine this possibility, we subtracted images obtained from two different stimulation conditions. The results of such subtractions reflected preference maps for one condition over another. As shown in Fig. 5, D (flutter minus vibration) and E (pressure minus flutter), these subtraction maps were fairly uniformly gray, suggesting the lack of preference for any one condition over another within the activation zones and reinforcing the similarity of the maps.

**Differential pressure/flutter/vibration activations under isoflurane anesthesia**

To illustrate the responses under isoflurane anesthesia, we show in detail three of the seven cases studied. Two are shown in Fig. 5 and the third in Fig. 8. The other four cases (not shown, see Table 2) also exhibited different activations to pressure, flutter, and vibration and are similar to what is described in the following text.

In **case 1** (Fig. 5, left), the cortical images obtained during presentation of our three vibrotactile waveforms to fingerpad D3 produced different activation patterns to pressure (Fig. 5, A and D, yellow), flutter (Fig. 5, B and E, red), and vibration (Fig. 5, C and F, blue). As shown in Fig. 5G, pressure and flutter activations demonstrated similar regions of activation. In contrast, vibration (Fig. 5, C and F, blue) elicited only a few weak foci of activation.
(Fig. 5, C and F, blue) that showed very little overlap with the strongest activation areas induced by pressure and flutter. However, a correspondence between secondary activation regions (weaker activations outside thresholded areas) of the pressure, flutter, and vibration maps was observed (Fig. 5, A–C).

Case 2 (Fig. 5, right) also exhibited differential pressure, flutter, and vibration activation. Strong D2 activation was largely confined to a 1- to 2-mm area (see outlines in Fig. 5, D–F), although less intense activity is observed lateral to this region of activation (Fig. 5, A–C). When pressure, flutter, and vibration activation were compared, different patterns were obtained, although approximately the same region of area 3b was activated (Fig. 5G). Pressure stimulation (Fig. 5A; outlined in D) revealed a single strong focus of activation, roughly 1–1.5 mm in size, surrounded by a broader region, roughly 2.5 mm, of less intense activation. In contrast, flutter stimulation (Fig. 5B; outlined in E), resulted in an elongated region of activity consisting possibly of two to three patches (0.5–0.75 mm in size) of activation. Vibration stimulation (Fig. 5C; outlined in F) produced two to three patches similar in size (about 0.5 mm) to that observed for flutter but not identical in location (compare red and blue outlines in Fig. 5, E and F). Thus there were differences between the pressure, flutter, and vibration activation patterns with a suggestion of submodal patchiness.

Comparison of submodality representation under pentothal and isoflurane anesthesia

It was apparent from the single condition maps obtained under both pentothal and isoflurane anesthesia that there were similar areas of activation induced by pressure, flutter, and vibration and that single cortical locations were responsive to some extent to each of the three conditions. To examine whether there was preference for one condition over another at single locations, we compared the relative activation by two-condition subtraction analysis and by vector analysis. Figure 6A illustrates the result of subtracting two stimulation conditions where preference for the first condition is indicated by darker pixels and that for the second condition by lighter pixels and equal preference for both conditions by gray pixels. In contrast, to the relatively flat gray subtraction maps obtained under pentothal anesthesia (right, same as case 1 in Fig. 4), subtraction of isoflurane maps (left, same as case 2 in Fig. 5) revealed alternating dark and light zones. Under isoflurane, the flutter minus vibration image (Fig. 6C) revealed alternating zones of preference, although with a different periodicity than (Fig. 6A) either pressure-flutter or pressure-vibration (Fig. 6B) images. The presence of these dark and light patches suggested that there exist different zones of preference for pressure, flutter, and vibration. Such patchy zones were never seen under pentothal anesthesia.

Although subtraction maps revealed relative dominance of either of two conditions, it was difficult to discern the relative contributions of two or more conditions at any single location. To examine the relative contributions of each of the three pressure, flutter, and vibration conditions at each cortical location, we performed a vector analysis (commonly used to generate visual orientation preference maps, see METHODS). In a color space that ranges from red to blue, preference for pressure, flutter, and vibration were color coded by red, yellow/
green, and blue, respectively. Magnitude of response was indicated by color saturation. Thus for example, a location with saturated red indicates a high preference for pressure, while a location coded orange indicates comparable preference for pressure and flutter. Equal preference for all three would appear on a gray scale (black indicating poor response to all 3, white indicating strong response to all 3). As shown in Fig. 6 (E–I, bottom left), the vector map obtained from the pentothal case showed a single locus which was dominated by vibration (blue) activation. A weak ring of color ranging through pink, yellow, orange, and blue haloed this center. In contrast, when we examined vector maps of pressure, flutter, and vibration conditions obtained under isoflurane anesthesia (Fig. 6, E–I, bottom right), these revealed significant substructure reminiscent of visual orientation maps. As shown in Fig. 6I, under isoflurane anesthesia, this vector analysis revealed roughly 250-μm-sized activation preference zones. Although this suggested substructure was not seen uniformly throughout all areas of activation in this case, it was present in the zone of strongest fingerpad activation and suggested some degree of
organization in the representation of pressure, flutter, and vibration. The scale of this organization was consistent with that suggested by previous electrophysiological studies (Sur et al. 1981, 1984) with some indication of possible submodal banding.

In all seven cases (see Table 2) studied using isoflurane anesthesia, we observed patterns suggesting different zones of preference for pressure, flutter, and vibration for fingerpads D2 (Fig. 5, case 2), D3 (Fig. 5, case 1), D4, and D5 (cases not shown) stimulation. These patterns were never observed with pentothal anesthesia in terms of extent of activations, differences in activations, or possible organization of submodality preference. In sum, these results, obtained under isoflurane anesthesia, suggested that within each digit’s representation, there were zones of pressure, flutter, and vibration preference. Our data suggested that each of these modalities was not represented in single, continuous zones, but rather exhibit patchy (roughly 250-μm-sized) fluctuations in dominance.

Electrophysiological examination of optical maps

To examine the functional bases of these maps, we studied single- and multiunit responses of the imaged area with multiple electrode penetrations (Table 3). We wanted to provide further confirmation of optically imaged topography as well as attempt to discern the relationship of single- and multiunit responses to the imaged pressure, flutter, and vibration activations in area 3b. Because of the unexpected results we found under isoflurane anesthesia, we focused our electrophysiological recording efforts on these cases. In Figs. 7 and 8, we illustrate two cases with relatively extensive mapping, obtained under isoflurane anesthesia. In comparison with previously published maps (Nelson et al. 1980; Pons et al. 1985; Sur et al. 1982), electrophysiological mapping revealed a similar topography. This topography generally concurred with the optical maps, although the correlation was better in one case (Fig. 7) than the other (Fig. 8).

Figure 7 illustrates the physiology accompanying the images for case 2 shown in Fig. 5. Images obtained during D2 fingerpad stimulation are shown on the left and those during D4 stimulation are shown on the right. D4 activations, though weaker than those for D2, were spatially distinct (medial and posterior) to those of D2. To maximize correlation with optical images, cells were recorded primarily from the superficial layers (0–600 μm) of cortex. Responses to tapping or brushing of the fingerpad were determined by listening to the audio amplifier. Typically, we recorded two to three cells per penetration. In a subset of cells recorded, spike trains were collected and peristimulus time histograms generated. Once the responsive digit was identified, cells were classified as SA if the response to fingerpad indentation gave a sustained response (e.g., Fig. 7I). RA cells (Fig. 7J) gave a transient response at the onset and at times at the offset of indentation. In some recording sites, both SA and RA cells were found (Fig. 7, square). PC responses (Fig. 7H) are characterized by high sensitivity to high-frequency vibration (Mountcastle et al. 1969). The pressure, flutter, and vibration stimuli we used for optical imaging preferentially elicited responses from the SA, RA, and PC units, respectively.

The correlation between the electrophysiology and imaging suggesting an orderly topography in area 3b in this case was reasonably good. In an anterolateral to posteromedial direction, we encountered cells responsive to distal finger tips D1 (blue dots), D2 (red dots), D3 (green dots), and D4 (yellow dots) stimulation (Fig. 7A). Sites responsive to middle (M), proximal phalanges (P), palm (gray dots), and hairy skin (H) stimulation were found primarily laterally and posterior to digit tip representations (T), consistent with previously published observations (Sur et al. 1982). We saw evidence of some nontopographic responses. For example, in a few penetrations we recorded responses to both D1 and D2 (red and blue dots) and both D2 and D3 (yellow and green dots). Within the general topography, individual D2 and D4 multiunit responsive sites were generally organized in a lateral-to-medial progression. However, they were not in complete agreement with optically imaged activation zones (compare red symbols with dark zones in Fig. 7, B, C, and G; compare yellow symbols with dark zones in Fig. 7, E–G). Because a majority of the electrode penetrations were not optimally placed over regions of optical signal (due either to density of blood vessels or to lack of optical map during electrophysiology), we cannot fully evaluate the relationship between the optical and neurophysiological response. Differences in the topography between the electrophysiology and images suggest that the properties of individual neurons do not always reflect the activity of the cortical population in somatosensory cortex.

The adaptive properties of neurons found in the electrode penetrations agreed in some cases, but not in others, with the different activation zones observed in our functional image maps. Of the sites in which we recorded cells responsive to distal or middle D2 stimulation (Fig. 7A, red dots, n = 4), all contained both SA and RA cells (Fig. 7, B–D, red squares), consistent with the fact that these penetrations were located in activation zones of both the flutter image (Fig. 7B) as well as the pressure image (Fig. 7C). Of the D4 responsive locations (yellow symbols, n = 12), 9 of the 12 penetrations showing units with RA responses were located in flutter activation zones (see Fig. 7F, 4 square and 5 triangle). Only four of these penetrations contained SA cells (Fig. 7E, yellow squares); these were located in zones activated by both flutter and pressure (Fig. 7, E and F, squares). Thus most penetrations that we recorded exhibited mixed responses, suggesting that SA and RA responses are not strongly segregated in the superficial layers.

Figure 8 illustrates another case in which we mapped the fingerpad representation under isoflurane anesthesia. Electrophysiological recordings revealed a map consistent with the general lateral-to-medial progression of D1–D5 topography (Fig. 8C), although the mapping was less organized in this case. Optical images revealed D2 activation (Fig. 8A, red circle over most prominent activation region) lateral to that of D4 activation (Fig. 8B, yellow circle over most prominent activation region). However, only two of the six penetrations with D2

<table>
<thead>
<tr>
<th>Case</th>
<th>Total No. of Penetrations</th>
<th>SA</th>
<th>RA</th>
<th>PC</th>
<th>Mixed (SA/RA)</th>
<th>Hairy Skin</th>
<th>Others</th>
</tr>
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<tr>
<td>990914</td>
<td>70</td>
<td>0</td>
<td>35</td>
<td>1</td>
<td>9</td>
<td>11</td>
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<td>990923</td>
<td>34</td>
<td>1</td>
<td>15</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>12</td>
</tr>
</tbody>
</table>

SA, slowly adapting type I; RA and PC, rapidly adapting type I and II, respectively.

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response fell in the D2 activation zone, and five of eight penetrations with D4 response fell in the D4 activation zone. D3 responses tended to fall in between these two zones. When we imaged this region for D2 response to pressure, flutter, and vibration, we again saw distinct patterns of activation in the same cortical area (compare Fig. 8, D–F). Of the two D2 penetrations that lay within the imaged D2 activation zone, one contained mixed SA and RA responses and the other contained RA responses. Thus as suggested by the imaging, single cortical locations can contain a mixture of modality-specific responses. Such mixed responses within a single activation area suggest that the optical signal results from biases in SA versus RA contribution to the local response. Because a number of the D2 electrode penetrations fall outside of the region of optical

**FIG. 7.** Electrophysiological examination of optical maps obtained with isoflurane anesthesia. Same case as in Fig. 5, case 2. A: all of the electrode penetrations from area 3b are shown. The locations of the receptive fields recorded are indicated by color-coded dots (D1–D4, hairy skin and palm). The electrophysiological map is generally consistent with topography shown in optical images (compare D2 images as shown in B–D and D4 images in D–G) and with published reports (e.g., Sur et al. 1982). B–D: images obtained during flutter, pressure, and vibration stimulation, respectively, of D2. Red symbols indicate penetrations responsive to D2 fingertip stimulation; these penetrations contained neurons with both slowly and rapidly adapting type I (SA and RA) response properties (square) and overlie regions of both flutter (B) and pressure (C) activation. E–G: images obtained during flutter, pressure, and vibration stimulation, respectively, of D4. Penetrations in which only RA units were recorded (triangles) were over flutter activation region (E). Penetrations in which both RA and SA units were recorded (squares) were over both flutter (E) and pressure (F) activation regions. H: example of a rapidly adapting type II (PC) unit. I: example of a SA unit. J: example of a RA unit. Scale bar: 1 mm.
activity, this case further suggests that the response properties of individual neurons do not always reflect the area of activity of cortical population.

In sum, the electrophysiological recordings provided insights into the basis of the optical images, one that underscores the distinction between the population response and the single-unit response. The optical signal is an average population signal, whereas the electrophysiology reveals samples from single cortical sites. Thus it is not surprising that optical and electrophysiological samples are not in complete agreement. With respect to topography, the optical image maps were generally consistent with the electrophysiology. With respect to modality, we were not able to obtain a sufficient density of penetrations to electrophysiologically determine the submodality organization within a fingerpad representation. The fact that in most penetrations there was a mixture of units with SA and RA responses is consistent with the coincident activation between the single-condition SA and RA maps. It also suggests the existence of local modality-specific biases within single-digit representations. We suggest the optical images reveal an organization of submodality within area 3b that is difficult to discern with electrophysiology alone (cf. Sretavan and Dykes 1983; Sur et al. 1981, 1984).

DISCUSSION

We used intrinsic signal imaging methods to examine the functional topography of somatosensory cortex (area 3b) in squirrel monkeys. In this study, we focused on a small region of the somatosensory cortex representing only the distal fingerpads. This permitted us to closely examine both the topographic organization of digit representation and possible substructure within single fingerpad domains. Under pentothal anesthesia, optical imaging revealed orderly focal (0.5–1.0 mm) representations to indentation of the distal fingerpads within area 3b. Under isoflurane anesthesia, pressure, flutter, or vibration stimulation of a single fingerpad revealed a complex overlay of domains composed of multiple foci spanning 2–4 mm. These features of the intrinsic images suggested some degree of segregation of preference for pressure, flutter, and vibration sensation that may parallel the relative dominance of SA, RA, and PC input distribution in area 3b.
Topographic organization of the fingerpad representation within area 3b

Our results support as well as extend those of previous studies on the somatotopic organization of primate somatosensory cortex. Under pentothal, we find that stimulation of single fingerpads produce focal activations in area 3b and an orderly medial to lateral topography of D5 to D1 representation of the fingerpads, in keeping with the presence of small RFs limited to a single digit. Both the imaged size of fingerpad representations and topography are consistent with previous electrophysiological studies on somatosensory cortical representation (Nelson et al. 1980; Paul et al. 1972; Pons et al. 1987; Sur et al. 1982). Tommerdahl and associates (Tommerdahl and Whitsett 1996; Tommerdahl et al. 1998, 1999), also using optical imaging methods to study area 3b, demonstrated a topographic representation of the body map (radial interdigital pad, D2 fingertip, and similar sites on the leg and foot) consistent with the somatotopic map described by Sur and associates (1982). Thus imaging studies provide a direct two-dimensional understanding of cortical organization that were previously inferred from multiple-electrode penetrations.

Such direct visualization can offer added understanding of sensory cortical representation. In previous studies, boundaries of fingerpad representation were inferred and delineated schematically from few electrode penetrations. Optical imaging, in contrast, provides high spatial sampling (e.g., a few micrometers per pixel, allowing a functional resolution of 50 μm or better), permitting a more detailed map of the extent and shape of activation zones. These have revealed round or oval fingerpad representations under pentothal anesthesia. In addition, it provides a distribution map of the graded population response.

Imaging under isoflurane has revealed a significant degree of overlap between adjacent fingerpad representations. This overlap may reflect latent secondary cortical inputs that suppressed under pentothal. Thus as in visual cortex, where, for example, imaged ocular dominance columns indicate the predominance of left versus right eye input, somatosensory representation is also based on a population response. Somatosensory cortical representation is not discrete. In fact, studies show evidence for significant representational overlap and input convergence (Ghazanfar et al. 2000; Godde et al. 1995; Masino et al. 1993; Moore and Nelson 1998; Nicolelis et al. 1998; Petersen and Diamond 2000; Zhu and Conners 1999).

Different maps under pentothal and isoflurane anesthesia

We observed that different intrinsic imaging maps were obtained under different anesthetic conditions. In response to mechanical probe indentation of the fingerpad, the imaged activation obtained under pentothal anesthesia was small and oval, whereas that obtained under isoflurane anesthesia was larger and less uniform, containing regions of strong activation as well as broader regions of weaker activation. Under pentothal, recorded topography was very consistent images and published maps; under isoflurane, topography was less organized, consistent with the isoflurane images. Thus the imaged signal reflected underlying cortical activity. Although we did not image the same cortical area under both anesthetic conditions, we believe that the anesthetic was the primary determinant of imaged differences in activation. We never observed focal activation with isoflurane as the anesthetic. Parameters such as method of optical chamber preparation and recording and maintenance of animal’s physiological and anesthetic state were similar across sessions. Indeed, when we reimaged the same cortical area with the same anesthetic (isoflurane) in two different sessions separated by 2 mo, we obtained qualitatively similar images (not shown). Therefore the differences observed between images obtained under pentothal and isoflurane were not primarily due to variability in procedure or to differences in depth of anesthesia.

Anesthesia dependence of optical maps has also been observed in other studies. In cat auditory cortex, relatively poor optical maps are obtained with pentothal anesthesia, whereas topographically organized tonotopic maps are present with ketamine anesthesia (Spitzer et al. 2001). In area V4 of the primate, orientation and color maps are more easily obtained with sufentanil than with pentothal, probably because pentothal has greater suppressive effects in this area (Ghose and Ts’o 1997). In area 3b of the primate, the size of the intrinsic signals observed under halothane is reduced by low doses of ketamine (Tommerdahl and Whitsett 1996). Thus different anesthetics in each cortical area may have different effects on the imaged signal.

The basis for such anesthetic-dependent effects is suggested from electrophysiological and pharmacological studies. In most studies of area 3b under general anesthesia, an orderly representation of the hand is present; and neurons have small RFs that approach the size of the receptive fields of the low-threshold mecanoreceptors (Nelson et al. 1980; Paul et al. 1972; Pons et al. 1987; Stryker et al. 1987; Sur et al. 1982). Other studies emphasize the diversity of somatosensory cortical responses and have shown significant variability in RF size and topography depending on level of alertness/anesthesia, on attentional state, and on activation of local neurotransmitters (especially GABAergic) systems (Alloway and Burton 1991; Dykes et al. 1984; Iwamura et al. 1985, 1993; Juliano and Whitsett 1985, 1987).

While the actions of general anesthetics are not under entirely understood, isoflurane and other gas anesthetics are thought to work as a direct agonist of the GABA(A) receptor (e.g., Antkowiak 1999). A model of the lamina-dependent effects of GABA activation in somatosensory cortex has been developed for the barrel fields (Kyrizai et al. 1998). There it was proposed that GABA-A activation is involved in the local circuitry present in layer IV barrels that is involved in surround inhibition. Yet layer IV’s output to other nonlayer IV neurons was relatively resistant to GABA-A activity. Similarly, we propose in the primate that isoflurane increases the local circuitry involved in the surround inhibition of layer IV neurons, while having minimal effects on layer IV projection neurons. Consequently, the cortico-cortical and secondary thalamo-cortical projections areas are still active under isoflurane anesthe sia as we revealed with our intrinsic optical images.

The pharmacological actions of pentothal are less clear, but it has been reported to augment GABA responses and decrease those to glutamate (Prichard and Ransom 1995). In the rat somatosensory cortex, glutamate was most effective in uncovering new RFs and enhancing preexisting somatic responses in layers II/III (Lamour et al. 1988). We propose that in the primate pentothal acts by increasing GABA-mediated inhibition and decreasing glutamate excitation of neurons in layers...
II/III. As a result, because of the more suppressive actions of pentothal has on cortical activity, only the core areas of the thalamocortical projections activate layers II/III, resulting in the restricted domains of cortical activity that we observed with our intrinsic optical images. It has also been shown that whereas the effectiveness of isoflurane is blocked with bicuculline application, pentothal (which has been reported to augment GABA inhibition and decrease responses to glutamate) is resistant to bicuculline effects (Antkowiak 1999; Dutar and Nicoll 1988; Prichard and Ransom 1995). Taken together, these studies suggest that isoflurane and pentothal do influence receptor systems that have been shown to shape cortical RFs and that the actions of isoflurane and pentothal are not the same. Such differences may lead to differential effects on cortical activation patterns.

Modular domains in the cortical processing of vibrotactile stimulation

Our intrinsic imaging revealed evidence for the presence of domains with differential responsiveness to types of vibrotactile stimulation. Under isoflurane anesthesia, different cortical locations were responsive to some or all three pressure, flutter, and vibration conditions. These representations were not single continuous zones but rather exhibited patchy submillimeter fluctuations in dominance.

As suggested by both psychophysical and neurophysiological findings, the presence of the different spatial activation patterns for our different vibrotactile stimuli is not an unexpected finding. When the glabrous skin is indented with vibratory stimuli, three different sensations can be felt. A local flutter sensation is evoked by low-frequency stimulation (2–40 Hz). A deep radiating hum of vibration is evoked by higher frequencies (40–200 Hz). And pressure is felt for stimuli below 2 Hz. Numerous psychophysical studies show that independent sensory channels mediate these three sensations, suggesting that there is some degree of separation in their central projections to somatosensory cortex (Bolanowski et al. 1988; Gescheider et al. 1985; Labs et al. 1978; Talbot et al. 1968; Verrillo 1965). Additionally, microneurography experiments provide direct evidence that different low-threshold mechanoreceptor afferents elicit distinct sensations of pressure, tapping/flutter and vibration (Ochoa and Torebjork 1983; Torebjork and Ochoa 1980). For example, stimulation of an SA afferent alone evokes the sensation of pressure while stimulation of RA afferents evokes sensations that have been described as either tapping, flutter, buzzing, or vibration as the frequency of stimulation was increased. Thus the distinct psychophysical sensations evoked by vibrotactile stimuli suggest some degree of segregation in the processing of pressure, flutter, and vibration in somatosensory cortex.

Such segregation within primary somatosensory cortex for SA and RA mechanoreceptive input has also been previously suggested (Paul et al. 1972; Sretavan and Dykes 1983; Sur et al. 1984). Using microelectrode mapping techniques in ketamine-anesthetized squirrel monkey area 3b, Sur et al. (1984) observed the segregation of SA and RA responses within middle cortical layers that formed alternating domains of activity elongated in a rostrocaudal direction. Similarly with intrinsic imaging, Tommerdahl and associates (1999) observed similar activation patterns for stimuli that preferentially activate RA and PC mechanoreceptors. In our study, with intrinsic imaging, we were able to map the location of cortical domains responding to the different vibrotactile stimuli independent of the adaptation properties of the neurons. Therefore under isoflurane anesthesia, we were able to discern different patterns of cortical activity in the upper layers of cortex that were compatible with the SA and RA domains reported in prior electrophysiological studies for Layer IV.

Correspondence between images and electrophysiology

A number of studies report reasonably good correspondence between imaged response and suprathreshold spiking response. This has been demonstrated by good correlation between electrophysiological examination of imaged maps such as those for visual orientation, ocular dominance, color, and spatial frequency in cats and primates (e.g., Issa et al. 2000; Roe and Ts’o 1995) as well as that of somatosensory barrel cortex in rodents (Peterson et al. 1998; Polley et al. 1999; Sheth et al. 1998). Furthermore, imaging of visual point stimulation produces highly focal activations in primate V1 (Roe and Ts’o 1994; Slavov et al. 2000).

Our imaging maps showed a population bias of one stimulus preference over another. Most of our penetrations had mixed SA and RA or solely RA responses that did not always correlate with the expected properties of neurons that would code the vibrotactile stimuli. Therefore our data suggest that the adaptation properties of individual neurons do not entirely reflect functional cortical response. In other words, each activation zone does not reflect pure SA or RA response but rather results from a sum of population of activity elicited by the vibrotactile stimulus.

Studies in the cats and ferrets suggest that there is considerable contribution from subthreshold sources such that a point stimulus produces activation sizes many times that predicted by spiking activity (Das and Gilbert 1995; cf. Toth et al. 1996). Thus at least in principle, optical imaging is able under some circumstances to detect subthreshold activation. This leads to the possibility that at least some of the activation detected under isoflurane anesthesia is due to subthreshold activity. The broad, diffuse activation we observe reflects subthreshold inputs from distant thalamocortical arbor inputs to layer IV or from horizontal connections in the superficial layers. Some of the influence from SA, RA, and PC inputs to area 3b may also be subthreshold. The presence of cortical domains that are preferentially dominated by SA, RA, or PC response also raises the possibility that these subthreshold influences are not uniformly distributed in area 3b (cf. Hickmott and Merzenich 1998, 1999; Juliano et al. 1990).

Columnar organization in primate somatosensory cortex

While there is a strong body of evidence to support the columnar organization in primary visual cortex, the degree of columnar organization in somatomotor areas is still debated (cf. Ashe and Georgopoulos 1994; Favarov and Kelly 1996; McKenna et al. 1982; Moran and Schwartz 1999; Todorov 2000). Although we have not done the experiments to examine the columnar organization of the fingerpad representation in area 3b (cf. Sur et al. 1985), we contend that our optical imaging evidence is suggestive of some degree of columnar
organization in area 3b. Because the intrinsic optical imaging method detects reflectance from the superficial 400–600 μm of cortex, it is unable to directly detect activity from the middle layers (Grinvald et al. 1988). However, the previously electrophysiologically mapped organization of SA and RA responses in the middle layers of cortex (Sur et al. 1982) is similar in geometry to that reported here. Although this similarity does not rule out the possibility that very different functional organizations exist in the middle and superficial layers, we find such a view unparsimonious and inconsistent with previous electrophysiological reports on columnar organization (Sur et al. 1985). We emphasize that columnar organization does not necessarily imply uniformity of response, but rather an overall bias in response as measured by imaging methods. Indeed, even in visual cortical areas, it is well known that superficial layers of V1 are largely binocular and yet ocular dominance columns are readily imaged. Thus we believe that the imaged organizations reflect to some extent the organizations present in the middle layers and that our functional maps provide support for a columnar view of somatosensory cortical organization.

Possible relationship to somatosensory plasticity: a model

The precedence of cortical plasticity in the adult somatosensory cortex has been well established (Elbert et al. 1995; Garraghty and Kaas 1991; Merzenich et al. 1983; Recanzone et al. 1992a,b). Changes in functional organization can occur both in response to peripheral or central injury or to somatosensory experience. Rapid shifts in RF organization have also been observed (e.g., Schroeder et al. 1995). Our finding that activations may range from single small foci to relatively extensive multiple foci depending on stimulus or type of anesthetic underscores the dynamic nature of functional organization in somatosensory areas. These anesthesia- and stimulus-influenced responses further suggest that plastic changes in somatosensory areas are not solely dependent on long-term anatomical changes.

As described by Garraghty and Sur (1990), single thalamocortical axons innervating area 3b have arbor extents from 350 to 800 μm, at times giving rise to several arbors spanning 2.5–3.0 mm of cortex. Their proposal that different parts of the input arbor may have different influences on cortical cell activation parallels well our findings that pentothal and isoflu-
rane differentially affect primary and secondary sites of cortical activation. Based on this, we suggest a model for our anesthesia-dependent results. As depicted schematically in Fig. 9, fibers subserving pressure (red, SA), flutter (green, RA), and vibration (blue, PC) each have multiple terminal arbors spanning an extent of 2–3 mm. However, under conditions (such as pentothal anesthesia) where cortical activity in layers II/III is suppressed, only the central arbor (heavy lines) is strongly activated, leading to a focal imaged activation zone. With less suppressive anesthetics (such as isoflurane), the activity of surrounding input arbors are revealed, resulting in the imaging of broader secondary activation zones under isoflurane. Modulation of activity within secondary arbor zones may further reveal differential SA, RA, and PC inputs to each zone, resulting in the imaged patterns of interdigitated stimulus preference domains.

Thus this anatomical substrate combined with our functional imaging results provides a basis for rapid and multi-focal functional modulations of cortical response. We hypothesize that the focal activation represents a primary response region and that additional zones of activation reflect secondary (modulatory/subthreshold) response regions. The modulations of these secondary regions may comprise the zones in which reported “reorganizations” occur, either due to experience, lesions, or other active processes which reveal dynamic changes in cortical organization. In the alert animal, these modulations are expected to be even more prominent and dynamic. Optical imaging may prove to be a method by which plastic and/or dynamic responses to cutaneous stimulation may be studied.

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