Intrinsic signal optical imaging in the forepaw area of rat somatosensory cortex

PAUL M. GOCHIN*, PURVIS B Edenbaugh†, JACK J. GELFAND*, CHARLES G. GROSS‡, AND GEORGE L. GERSTEIN†

*Department of Psychology, Princeton University, Princeton, NJ 08544; and †Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104

Communicated by George A. Miller, June 2, 1992

ABSTRACT The responses of somatosensory cortex (S-I) to tactile stimulation of the forepaw were assessed by intrinsic signal optical imaging. The tips of digits two or five were alternately touched with mechanical tappers while video photographs were taken of S-I illuminated by an 800-nm light source. The resulting images showed two highlighted areas about 300 μm in diameter and 500 μm apart. Generation of these images required <1 hr. Electrode penetrations placed in the areas highlighted during stimulation provided multiunit recordings with receptive fields appropriate for the stimulated digit and not the other digit. Penetrations between the highlighted areas yielded receptive fields on intervening digits. These results demonstrate that intrinsic signal optical images are obtainable in S-I and confirm the functional somatotopy previously reported using electrical recording. Furthermore, the short time required to produce the images and the obtainable spatial resolution suggest that optical recording could be employed for the study of cortical reorganization in this brain region.

Optical imaging provides a means of studying the activity of regions of the cerebral cortex over time and space. The method produces a neural-activity-dependent image through the use of a video camera. Optical recording is similar to the 2-deoxyglucose method in that an image related to neural function is produced and in the spatial resolution of the image (1, 2). The major advantages with optical imaging are that an image may be obtained in about 1 hr and that many images may be recorded from the same subject under different experimental conditions. Consequently, the optical method may be used for studying the effects of experience or injury on the organization and reorganization of cortical areas.

Most optical recording studies reported to date have utilized voltage-sensitive dyes (1, 3, 4). More recently, it has been shown that there are intrinsic changes in reflection of light related to neural activity that may be detected without dyes (2, 5). There have, however, been very few published reports using intrinsic optical signal measurements without dyes, and most of those have been in primary visual cortex. It is therefore unknown whether intrinsic signal optical imaging is generally applicable to other systems.

Electrophysiological studies have revealed topographic organization of somatosensory cortex (S-I) in the rat (6–8). In this report we show that stimulation of the digits of the forepaw yields intrinsic optical signals in S-I that correspond to this somatotopic organization.

MATERIALS AND METHODS

Albino rats (≈350 g) were anesthetized with an intraperitoneal injection of 28 mg of ketamine and 3.1 mg of xylazine and maintained by continuous intravenous infusion of a solution of 18 ml of Ringer's lactate, 135 mg of ketamine, 13 mg of xylazine, and 0.026 mg of glycopyrrolate at 0.5–1.5 ml/hr. Temperature was maintained at 37°C. An opening in the skull was made over the forepaw cortex extending from approximately 2.5 mm anterior to 1.5 mm posterior to the bregma and from 2.5 mm to 5 mm lateral. The dura was left intact throughout the experiment. A well of dental acrylic was built around the opening. The well was filled with normal saline and capped with a glass coverslip, forming a sealed chamber by capillary action.

The hair on the left forelimb was clipped and the dorsum of the paw was glued with cyanoacrylate to a Plexiglas plate that was rigidly fixed to the table. The paw was firmly fixed so that there was little mechanical coupling of the somatosensory stimulus to sites distant from the point of contact.

Galvanometer motors were used to move small aluminum bars that tapped the last phalanx of digits two and five of the forepaw. The contact area was about 1.0–1.5 mm². The contact force was adjusted to ≈3 g. The tap pattern was 166 ms on and 166 ms off, starting 1 s prior to and maintained throughout the data-collection period. Each trial consisted of 11 taps over 3 s to one digit tip followed by a 14-s delay and 11 taps over 3 s to the other digit tip followed by a second 14-s delay. The number of trials was determined by the number of images that needed to be averaged to achieve an adequate optical signal-to-noise ratio.

The optical apparatus was similar to that of Ratzlaff and Grinvald (9). A tandem-lens imaging system was employed with a Photometrics series 200 Peltier-cooled charge coupled device (CCD) camera. The tandem-lens system employed a Nikon 50-mm f/1.8 lens closest to surface of the brain. The second lens was a Nikon 100-mm f/2.8 lens. An 800-nm wavelength, 10-nm full width at half maximum (FWHM) optical filter was placed in the collimated section formed by the tandem-lens system. The camera imaged a 2.8 mm × 3.5 mm area of cortex onto a Thomson TH-7863 CCD chip. This chip has an aluminum mask deposited on one half of the chip to facilitate a frame-shift exposure protocol. The sensitive area of the CCD chip was 288 × 384 pixels. A fiber-optic microscope ring illuminator mounted concentrically on the tandem-lens assembly directed the light from a Newport model 780 stabilized incandescent light source onto the brain. Light from the source was filtered by a 100-nm FWHM optical filter centered at 800 nm. The light intensity was adjusted so that the maximally illuminated CCD pixel collected slightly less light than its well capacity of ≈250,000 photoelectrons.

The exposures were accurately timed by shifting the collected image from an unmasked to a masked region of the CCD chip. Eight images were collected during the stimulation of each finger during each trial. Images collected during stimulation of one digit were subtracted from the images collected during the stimulation of the other digit. The final images were the sum of the differences between the re-

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‡To whom reprint requests should be addressed.
sponses to the stimulation of digits two and five over 24 pairs of trials convolved with $5 \times 5$ Gaussian kernel to further improve signal to noise.

Microelectrode recordings were obtained to confirm the patterns of observed optical activity. An image of the surface of the brain was made using a 546-nm filter to highlight the blood vessels. This image was overlaid on the 800-nm subtracted images, and the positions of active areas relative to the blood vessels were noted and used as a guide for placement of the microelectrode penetrations. A microelectrode with about 1-MΩ impedance was inserted to a depth of between 300 and 800 μm. Peri-stimulus time histograms were made from multiunit activity passed through a window discriminator.

RESULTS

Optical images of digit-specific activation of somatosensory cortex were obtained in six rats. Fig. 1 shows typical optical and extracellular recording data. Stimulus difference and vascular reference transdural images are shown. Because of the subtraction technique, two neurally activated areas in the stimulus photograph are evident—a light area corresponding to stimulation of digit two and a dark area corresponding to stimulation of digit five. The locations of the stimulation on digits two and five are shown in Fig. 1B. The regions of activation are $\approx 0.5$ mm in diameter and are separated by $\approx 1$ mm. The rms noise (relative to the total reflectance) for the data shown in Fig. 1 was 1:25,000. In this image the regions of peak activation correspond to signals that were 1:2000, relative to the total reflectance.

Peri-stimulus time histograms of multiunit recordings from the same optical recording experiment illustrated in Fig. 1A are shown in Fig. 1C. These data were obtained by penetrations at the locations of the arrows originating in the stimulus photograph. These locations are denoted as site A and site B in the labeling of the histograms in Fig. 1C. Cells at site A and site B were most responsive to digits two and five, respectively. Data in Fig. 1 show that activity recorded at locations within an activation zone for a particular digit was overwhelmingly associated with that digit. We see in Fig. 1 that while tapping on digit two and recording from site A, the electrical signal was large and correlated with the tapping time as marked by the arrows in the histogram. Alternatively, while tapping on digit two and recording from site B, the electrical response was no longer present. The opposite was true for tapping on digit two and recording from the same two sites. Activity in the intermediate zone was not strongly associated with either digit. Rather, the strongest multiunit activity for these intermediate zones was associated with the middle two digits that had not been used in producing the optical recordings.

DISCUSSION

These results show that intrinsic signals can be optically recorded in rat primary somatosensory cortex. We have furthermore confirmed that the extent and location of areas produced in the image correspond well with those determined by extracellular electrical recording. The relative positions of the optically determined digit representations are also consistent with anatomical observations (10, 11). The size of the activated regions was about 0.5 mm, suggesting that at least this much spatial resolution is attainable. The shape of regions was approximately circular. The relative location, size, and spacing of digit sites tended to be reasonably

![Fig. 1](image-url)
consistent between rats. We therefore have confirming evidence for functionally determined somatotopy in S-I, using an independent measure: optical imaging.

During the course of these experiments some effort was made to obtain signals from the hindpaw of the rat. Only weak and inconsistent signals could be obtained. Although these results are only preliminary, it appears that with our current methods we may be observing a difference in signal strength between the forepaw and hindpaw areas. We have also noted differences between the forepaw and hindpaw in electrical recordings. Although single unit recordings are easily obtained in the hindpaw region, forepaw recordings tend to be multiunit. Histological examination of these areas reveals that pyramidal cells in the forepaw representation are more densely packed than in the hindpaw representation (12). We also have been unable to detect acoustic activation of rat auditory cortex with our procedures. On the other hand, in cats we have replicated the results of others, demonstrating isoorientation patches in visual cortex (5). These observations may foreshadow limitations, or at least difficulties, that may be encountered in the use of particular optical imaging methods.

These results demonstrate that intrinsic signal recording is applicable outside of visual cortex. Furthermore, the ability to rapidly acquire images (in <1 hr) and spatial resolution of at least 0.5 mm suggests that, at least in some brain regions, this technique should prove valuable for the study of cortical reorganization after injury or experience.

We acknowledge the many helpful discussions with A. Grinvald and J. Lowrance. We also acknowledge Pedro Maldonado for help in the rat auditory cortex experiments. This work was supported by a grant from the McDonnell–Pew Program in Cognitive Neuroscience (P.M.G. and J.J.G.) and by National Institutes of Health Grants R37 MH-46428 (G.L.G.) and MH-19420 (C.G.G.).