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Voltage-sensitive dye imaging of intervibrissal fur-evoked activity in the rat somatosensory cortex

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Abstract

The intervibrissal fur-evoked activity in the rat somatosensory cortex was investigated using high-resolution optical imaging with a voltage-sensitive dye. The optical imaging revealed that the intervibrissal fur representation forms a U-shaped band around the borders of the posteromedial barrel subfield (PMBSF), and that this representation is characterized by a rostral-to-caudal somatotopic organization. When $GABA_A$-mediated inhibition was partially suppressed by treatment with bicuculline, stimulation of the intervibrissal fur elicited spreading of an excitation wave in an area outside the PMBSF. The spreading wave propagated in both directions along the aforementioned U-shaped band of cortex, but barely invaded the center of the PMBSF. These imaging results suggest a distinct subdivision of cortex adjacent to, but outside, the PMBSF in the rat somatosensory cortex; this region receives input from intervibrissal fur, and seems to process its sensory information through well-developed local horizontal connections.

Keywords: Vibrissae; Intervibrissal fur; Barrel cortex; PMBSF; Optical imaging; Voltage-sensitive dye

The anatomical representations of the large mystacial vibrissae are topographically organized in the posteromedial barrel subfield (PMBSF) of layer IV primary somatosensory (SI) cortex in rat. Neurons above, below, and within individual barrels are most responsive to input from a particular vibrissa [8]. Conversely, little is known about the cortical projections of intervibrissal fur in rodents. It had been thought that projections from intervibrissal fur would be somatotopically interspersed among those of the mystacial vibrissae within the PMBSF. However, most electrophysiological studies have failed to locate fur-evoked activity within the PMBSF, and the barrel field of the rat SI cortex is remarkably unresponsive to stimulation of intervibrissal fur [2,5,16]. In two electrophysiological studies, intervibrissal fur-evoked responses were elicited close to the anterior border of the PMBSF in rat [11], and along the lateral and medial borders of the PMBSF in mouse [9]. Studies in which 2-deoxyglucose (2DG) was used to map cortical response locations in rat produced similar results [12,13]. Each of these studies suggested that the representation of intervibrissal fur lies outside the PMBSF and appears instead to be localized to the borders of the PMBSF. In the present study, we used optical imaging of a voltage-sensitive dye to map the representation of intervibrissal fur in rat SI cortex with a greater degree of spatial resolution than has been the case in previous studies.

Eight male Wistar rats (180–220 g) were anesthetized with ketamine (80 mg/kg i.p.) and xylazine (8.8 mg/kg i.p.). Supplemental injections were used to maintain a constant level of anesthesia as assessed by respiration rate, heart rate, rectal temperature, corneal reflex, and the color of the extremities. Each animal was positioned in a stereotaxic frame and a craniotomy (5 mm × 5 mm) was performed over the left somatosensory cortex. The dura was removed after a well of dental acrylic had been built around the exposed cortex. The surface of the cortex was exposed for ∼1 h to 0.6 mg/ml RH-795 (Molecular Probes, Eugene, OR) that had been dissolved in artificial cerebrospinal fluid (ACSF). RH-795 is a fast response probe that transforms changes in excitable membrane

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potential into optical signals with a submillisecond delay [3]. After the staining, the cortex was rinsed thoroughly and the well was filled with ACSF. The composition of ACSF was (mM): NaCl 125, KCl 5, $CaCl_2$ 2, $MgSO_4$ 1.25, NaH_2PO_4 1.25, $NaHCO_3$ 22, glucose 10.

All of the mystacial whiskers on the right side of the snout were carefully plucked under a microscope. We did this time consuming procedure 1 day before the experiment, assuming that no reorganization of the barrel cortex would occur in the short time before the optical experiments. An electromechanical actuator (P1105m, TDK) was used to stimulate the intervibrissal fur. A fine cosmetic brush (3 mm wide) was attached to the actuator probe and a single driving pulse to the actuator displaced the brush by ∼2 mm for 10 ms, which swept the intervibrissal fur on the snout over an area of ∼6 mm^2. We carefully positioned the brush not to touch the rat's skin; therefore the brushing stimulation was effective for the intervibrissal fur, but not for the whisker pads.

Optical signals from the exposed cortex were recorded using a tandem-type epifluorescence microscope and a MOS-based monolithic array camera (64 × 64 pixels), both of which were developed in our laboratory [4,14]. Light from a tungsten–halogen lamp was filtered (535 nm) before being reflected down onto the brain surface by a dichroic mirror (580 nm), and fluorescence from the cortex was projected to a sensor through a long-pass filter (600 nm). Fluorescence images were acquired at a rate of 1.2 ms/frame and covered an area of cortex of ∼4.2 mm × 4.2 mm. The camera was positioned to focus 250 μm below the surface of the brain. Image acquisition was triggered by an electrocardiogram using a stimulus/non-stimulus subtraction method [10,15]. Data for eight trials were averaged.

In the first experiment, intervibrissal fur-evoked responses were mapped and somatotopy was assessed in the normal condition. Then, GABAergic inhibition was partially suppressed using bicuculline. Bicuculline methiodide (5 μM) was dissolved in ACSF and applied to the SI cortex by placing a cotton swab that had been soaked with the bicuculline solution onto the surface of the cortex. After 5 min, the cotton swab was removed and the cortex was rinsed thoroughly with ACSF. In the second experiment, the bicuculline-enhanced spread of fur-evoked activity was monitored. During the experiments, we recorded epicortical potentials with a silver ball electrode that was positioned in close proximity to the area of cortex from which we recorded optical signals. No spontaneous epileptiform activity was observed in any animal.

After the experiments, the cortex was lesioned and processed for cytochrome oxidase (CO) histochemistry. Lesions (at least 3) were made at distinct points on the cortical surface vasculature, such as bifurcations, and a bipolar stimulating electrode was used to produce a small lesion by passing dc current through it. The animals were killed with an overdose of pentobarbital (Nembutal). A block of brain that included the area of cortex that had been imaged was removed, fixed overnight at 4 °C in a 4% paraformaldehyde solution, and then sectioned tangentially at a thickness of 80 μm. Tissue sections were exposed to CO to assess oxidative metabolic activity, and the pattern of the barrels in the SI cortex was reconstructed using two or three CO-stained sections from layer IV. The barrel pattern was superimposed on the optical imaging results by comparing the lesions in CO sections with the vasculature of the cortex surface.

Fig. 1 shows the optically mapped cortical representation of intervibrissal fur. We stimulated the intervibrissal fur in five areas on the rat snout: (1) dorsocaudal, (2) dorsorostral, (3) rostral, (4) ventrorostral, and (5) ventrocaudal. Panels 1–5 show the cortical maps obtained after stimulating the fur in each of these regions, respectively. Optical responses in the cortex were always observed around the borders of the PMBSF. Examination of such maps revealed that the intervibrissal fur in the dorsal (ventral) region of the snout projected ventrally (dorsally) to the PMBSF, while the caudal (rostral) fur projected caudally (rostrally).

In the next experiment, we examined the role of GABAergic inhibition in shaping the intervibrissal fur-evoked responses in the surrounding cortex of the PMBSF. Fig. 2 shows spreading waves of excitation that were elicited by stimulating the intervibrissal fur, after $GABA_A$-mediated inhibition was partially suppressed. The spreading wave propagated along a U-shaped band around the PMBSF, and the U-shaped band included the receptive fields of intervibrissal fur that were mapped in Fig. 1. When the fur in the dorsocaudal area of the snout was stimulated (Fig. 2A), the resultant cortical activity was initiated from the region ventrocaudal to the PMBSF, propagated in a band lateral to the row A barrels from caudal to rostral, via the anterior border of the PMBSF, and then in a band medial to the row E barrels from rostral to caudal; a clockwise direction in the figure. By contrast, activity was propagated in the opposite direction (i.e., counterclockwise in the figure) when the fur in the ventrocaudal area was stimulated (Fig. 2B). Some activity was also observed in the anterolateral barrel subfield. The optical signals in response to stimulation of the intervibrissal fur before and after the application of bicuculline are shown in Fig. 2C. The traces on the left and right correspond to Fig. 2A and B, respectively. The onset of the fluorescence signals was ∼10 ms after stimulation in the area where the cortical response was initiated. After the application of bicuculline, the propagation of the spreading wave shown in Fig. 2 was monitored in every animal. The velocity of propagation was essentially constant along the U-shaped pathway in both directions and was estimated to be 0.28–0.33 m/s.

We next analyzed the extent of fur-evoked activity across the borders of the PMBSF (Fig. 3). The borders were defined by the circumscribed curves placed on the medial edges of the row E barrels (as shown in Fig. 3A) and the lateral edges of the row A barrels. Pixels were chosen at positions along the line perpendicular to the PMBSF border, and the maximum amplitude of the optical signal was evaluated for each pixel. Example records in response to fur stimulation in the ventrocaudal area before and after bicuculline are indicated