Cortical Organization in the Etruscan Shrew (Suncus etruscus)

Claudia Roth-Alpermann, Farzana Anjum, Robert Naumann, and Michael Brecht

Bernstein Center for Computational Neuroscience, Humboldt University, Berlin, Germany

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Cortical organization in the Etruscan shrew is of comparative interest because of its small size and because the Etruscan shrew is an amazing tactile hunter. Here we investigated cortical organization in Etruscan shrews by electrophysiological mapping. We developed an anesthesia protocol for this very small mammal in which we combined massive application of local anesthesia, very slow induction of general anesthesia, and passive cooling. Under this anesthesia regime, we characterized auditory, visual, and somatosensory cortical responses. We found that large parts of shrew cortex respond to such stimuli. Of the responsive sites, a small fraction (~14%) responded to visual stimuli in a caudally located region. Another small fraction of sites (~11%) responded to auditory stimuli in a centrally located region. The majority of sites (~75%) responded to tactile stimuli. We identified two topographically organized somatosensory areas with small receptive fields referred to as putative primary somatosensory cortex and putative secondary somatosensory cortex. In a posterior-lateral region that partially overlaps with piriform cortex, we observed large somatosensory receptive fields and often polysensory responses. In an anterior-lateral region that partially overlaps with piriform cortex, we observed large unimodal somatosensory receptive fields. Our findings demonstrate a remarkable degree of tactile specialization in Etruscan shrew cortex.

INTRODUCTION

A fundamental principle of cortical organization is the ordered representation of information in maps (Racik and Singer 1987). Electrophysiological mapping approaches like electrical stimulation (Fritsch and Hitzig 1870), field potential recording (Woolsey 1958), and unit recordings (Hubel and Wiesel 1959) have led to the discovery of a multitude of such cortical maps, which in most but not all cases were shown to correspond to anatomically identified cortical areas. A striking result from such mapping studies is the observation that cortical maps differ grossly from species to species: in visual animals like cats (Tusa et al. 1981) and monkeys (Allman and Kaas 1971), a large number of cortical areas were identified that respond to different aspects of visual stimuli, whereas in auditory animals like bats ordered maps for sound features were discovered (Riquimaroux et al. 1991).

The rich diversity of cortical organization in mammals calls for comparative analysis. Recognizing similarities and differences in the representation of sensory information across the cortices of different mammals might help us understand principles of cortical processing. For any comparative analysis, extreme cases are of particular interest. In this study, we analyzed the cortical organization in the Etruscan shrew, Suncus etruscus, which in many respects is an extreme mammal.

The Etruscan shrew is the smallest (or one of the smallest) mammals. The small body size and low body weight (2 g) of Etruscan shrews goes along with a very high metabolic rate (Fons and Sicart 1976; Schmidt-Nielsen 1984). Etruscan shrews are predominantly nocturnal and have a large whisker array but small (<1 mm) eyes and prey (among other animals) on crickets that are almost as big as the predator itself. Not surprisingly, shrews are amazing hunters that recognize their prey by tactile Gestalt cues. They succeed in overwhelming and killing their fast moving prey by high-speed/high-precision attacks (Anjum et al. 2006). The neurobiology of Etruscan shrews is of interest both because of their small size and their remarkable behavioral capacities. The small brain size of Etruscan shrews—and in particular their very thin cortex of only 400 μm thickness—is ideal for two-photon-microscopy based approaches to brain function.

Work on related shrew species has demonstrated that these shrews have few sensory cortical areas, which include a large primary and secondary somatosensory cortical area and a primary visual and auditory cortex (Catania et al. 1999). The large size of somatosensory areas is in line with the numerous specializations of insectivores for somatosensation (Catania 2005). In earlier work (Catania et al. 1999), it was also noted that—due to problems associated with anesthesia—it was difficult or even impossible to perform electrophysiological mapping in the small shrew species.

The goal of our study was therefore twofold. We intended to develop an anesthesia protocol that would make it possible to perform long-duration electrophysiological mapping experiments in the Etruscan shrew. We intended to obtain sensory maps as complete as possible of the cortex of the Etruscan shrew.

We describe an experimental protocol that allowed for extended electrophysiological experiments in Etruscan shrews. We found that large parts of the Etruscan shrew cortex responded to touch, sound, or light. Based on response properties, we delineated an auditory region, a visual region, a putative primary somatosensory cortex S1, a putative secondary somatosensory cortex S2, and two lateral somatosensory regions.

METHODS

In agreement with previous work on other small shrew species (Catania et al. 1999), we found it difficult to perform long-duration mapping experiments with Etruscan shrews and, accordingly, initial experiments failed. We therefore adjusted the anesthesia protocol to make it more suitable for these small animals, and these modifications are described in the first part of METHODS. In the second part of METHODS, we described the standard recording and histology techniques we used in our mapping experiments.
Anesthesia protocol for Etruscan shrews

The anesthesia protocol we used was modified from the procedures of earlier investigators (Catania et al. 1999). These modifications aimed at minimizing the amount of general anesthetic required, while still guaranteeing a sufficient level of anesthesia and analgesia.

Systemic anesthesia

Urethan anesthesia was used in all mapping experiments reported here. To minimize the stress associated with the intraperitoneal urethan injection, animals were first lightly anesthetized by isoflurane inhalation. Specifically we injected 0.045 ml of 4% urethan solution in water for an adult ~2.5 g shrew. This corresponds to a dose of ~0.72 mg urethan/g body weight. We also tested combinations of urethan and ketamine-supplements (as in Catania et al. 1999), ketamine/xylazine mixtures, or a gas anesthesia with isoflurane 2%, N2O: O2 (2:1). In our experience, however, the pure urethan anesthesia led to the best results with respect to survival, and all data reported here were collected under urethan anesthesia combined with passive cooling (see following text).

Slow application of general anesthesia

In our experiments, it was essential to increase the level of anesthesia very slowly and gradually. If such precautions were not taken, shrews tended to be either too lightly anesthetized or died of an overdose. In our most successful final protocols, we gave supplementary urethan anesthesia (15–25% of the initial urethan dose) only 40 min after the initial injection. Further injections were given no earlier than 60 min after the initial dose. This paced application of general anesthesia delayed the onset of surgery considerably, often for more than an hour, and thereby might have also increased the effectiveness of our other measures (local anesthesia, cooling see following text). The disadvantage of the delayed onset of the physiological experiment was outweighed by long survival of shrews under this treatment regime.

Epicutaneous and subcutaneous local anesthesia

To minimize the level of general anesthesia required, we found it to be essential to reduce the aversive effects of surgery by massive local anesthesia. To this end, we applied the local anesthetic ointment Emla (Astra Zeneca, Wedel, Germany) at the site of the future skin incision and covered it with a piece of parafilm. In addition we injected 20× diluted 2% lidocaine solution subcutaneously below the skin covering the skull. Local anesthesia was applied during the initial isoflurane anesthesia (see preceding text), and incisions were performed no earlier than 20 min after the initial application of the local anesthetics.

Supplementing oxygen

In a subset of experiments, we supplemented oxygen through an O2 tubing positioned in front of the animal. In some experiments, the animal’s state seemed to improve after adding the O2 supply.

Passive cooling

In initial experiments, we made efforts to keep the shrews at their normothermic body temperature of around 35 to 36°C (Frey 1979) by placing the animals on a heating pad. In subsequent experiments, however, we found that shrews were easier to maintain and were less responsive to painful stimuli if we omitted the warming and allowed them to cool down. Entering a torpid state with reduced body temperature is a physiological reaction of shrews to cold environments and food deprivation (Frey and Vogel 1979). In a subset of animals (n = 2), the body temperature was determined at the very end of the experiment and was found to be 27 and 29°C, respectively.

Because all hand-mapping experiments were carried out in hypothermic animals, we tested how body temperature affects neuronal responses in Etruscan shrews. To address this issue, we performed a series of experiments in which we varied the temperature of our animals systematically and then applied air puff (data not shown) or piezoelectric stimuli to the whiskers (Fig. 1). In all cases neural responses were preserved over the whole range of 24 to 36°C body temperature. While the “on” response did not change much at different temperatures, the “off” response was very pronounced at higher temperatures but decreased with lower temperatures. At 36°C, the response latency was between 5 and 10 ms and thus similar to rat and mice (Armstrong-James et al., 1992). For lower body temperatures, latencies became gradually longer (Fig. 1). In summary, our anesthesia protocol preserved cortical responses and did not affect RF size in our measurements (also see results, Fig. 8).

With all of the preceding measures combined, we obtained recordings in >50% of experiments. The average duration of an experiment with a shrew anesthetized with urethan as described in the preceding text was 3.6 h (217 ± 185 min).

Recording and preparation

ANIMALS (N = 44). Capture, handling, and maintenance of Etruscan shrews have been described in detail before (Anjum et al. 2006). Data presented here refer to 44 animals (about half of which were wild captures, half of which were captive born in our colony). Etruscan shrews were captured under a permit of the local government (permit No. N 6085/T-A31, Firenze, Italy) in the provinces Firenze and Livorno, and all our procedures complied with German regulations on animal welfare and were approved by an ethics committee.

RECORDING. Shrews were anesthetized and handled as described in the preceding text. At the onset of surgery, the eyes were covered with eye ointment, and the skull was exposed by carefully removing the temporal muscles and pushing them away laterally and posteriorly. The skull overlaying the right cortical hemisphere was cleaned with Ringer solution followed by 1% H2O2 solution, followed by 70% ethanol and was glued with superglue to a bent 19-gauge needle. After drilling, the bone overlaying the left cortical hemisphere was removed. Standard extracellular multi-unit recordings were performed at a depth of 300 ± 50 μm, which roughly corresponds to cortical layer V in Etruscan shrews. We used commercial tungsten electrodes (R = 0.5 MΩ, World Precision Instrument, No. TM33A05KT) for recording. The neuronal signal was amplified and filtered between 10 Hz and 2 kHz by an extracellular amplifier (NPI electronics) sampled at 25 kHz by a Power1401 data-acquisition interface under the control of Spike2 software (CED, Cambridge, UK). Spike counts were generated from the filtered signal by setting a threshold two times above noise level using Spike2 software.

Etruscan shrews have small neurons resulting in small extracellular signals in recordings. We therefore made major efforts to optimize artifacts and took a couple of measures like groundning the experimenter, shielding electrode, using a metal probe for mapping. At the same time, we maximized signals by using strong optimized handled stimuli. Control procedures like moving the somatosensory mapping probe close to the electrode in the air instead of deflecting the whiskers or applying pseudolight stimuli without the torch being turned on were used at every recording site.
FIG. 1. Whisker responses at different shrew body temperatures. Peristimulus time histograms (PSTHs, 1 ms bin size) of multi-unit responses to piezoelectric stimulation (~10° deflection, ~1 ms rise time) of a single whisker (n = 20 trials per temperature). Neuronal responses were obtained at the same recording site in putative area S1 while the body temperature of the shrew varied from 36°C (top) to 24°C (bottom). Stimulus onset at time point 0, duration of the stimulus 200 ms, as indicated below. Neuronal response to stimulus offset was most pronounced at higher temperatures and decreased with lower temperatures. Right: same data as left, but zoomed into the time of stimulus onset. Dashed line, the time of first response at 36°C. Note that the response latency was longer for lower temperatures.
Mapping procedure

DOCUMENTATION OF THE RECORDING SITES AND STEREOTAXIC ALIGNMENT. Directly after removing the bone plate from the cortex we took a digital photograph of the cortical surface, on which we indicated the position of each recording site. Penetrations were made with an anterior-posterior or medial-lateral spacing of 250 μm (in most cases) or 500 μm. All penetrations were aligned to a reference point midline in front of the olfactory bulbs. This point could be easily recognized in all animals by the medial bone suture and two large blood veins, which run from lateral to medial in front of the olfactory bulbs and join midline. The anterior-posterior and medial-lateral coordinates of each recording site relative to this reference point were taken, and this information was also used for aligning and averaging across response maps.

HAND-MAPPING OF RECEPTIVE FIELDS. At each recording site, we tested for the presence of visual responses with a small flashlight with which we shone light from all directions into the animal’s eyes. We tested for the presence of auditory responses with a variety of auditory stimuli (clicks, claps) the most effective of which was invariably ringing with keys. The strong selectivity for metallic clanging suggests that high-frequency hearing predominates in Etruscan shrews. Finally the entire contralateral body surface was mapped with light rapid strokes with a thin metal stick. For sites responsive to whiskers, a special attempt was made to determine the exact extent of the receptive field and the number of whiskers that evoked responses. At some but not all sites the ipsilateral body surface was also mapped; most whisker receptive fields were bilateral.

COMPUTER-CONTROLLED MAPPING OF RECEPTIVE FIELDS. In a separate set of experiments, either auditory or visual cortex or whisker receptive field was mapped using computer-controlled stimuli (Figs. 6–8). Auditory and visual cortex were mapped at a body temperature of 30°C, whisker receptive field at a body temperature of 36°C. The auditory stimulus consisted of a 20 kHz tone of 200 ms duration presented to the contralateral ear with a loudspeaker;
the visual stimulus was a stationary white LED flashing onto the animal’s contralateral eye for 200 ms. Whisker receptive field size was assessed with piezoelectric stimulation of single whiskers at the contralateral side of the snout.

ILLUSTRATION AND QUANTIFICATION OF MAPS. Recordings were filtered and amplified and multi-unit signals were listened to on an audio monitor. For the computer-controlled mapping, spike trains were recorded and peristimulus time histograms (PSTHs) calculated post hoc. In hand-mapping experiments, the presence and strength of responses was judged subjectively from the audio signal. For all sites, we illustrate the most effective response and posterior-lateral somatosensory region respectively in areas S1, S2, the anterior-lateral somatosensory W, X, Y, Z.

Histology

Tissue Preparation and Histochemistry. In a subset of experiments, electrolytic lesions were placed at selected recording sites. On the completion of physiological recordings animals were perfused transcardially (after an additional dose of anesthetic if required) with 0.1 M phosphate-buffered saline (PBS) at pH 7.2 followed by a solution of 4% paraformaldehyde. The brain was removed from the skull and immersed in fixative. Brains were embedded and sectioned in coronal sections. For histological analysis, Nissl, hematoxylin or CO stains were performed, in some cases on alternating sections (5 μm thickness for hematoxylin or 30 μm thickness for Nissl and cytochromoxidase staining).

LIGHT MICROSCOPY AND ANATOMICAL RECONSTRUCTION. Processed sections were viewed with StereoInvestigator software (MBF Bioscience) employing a Olympus BX51 microscope (Olympus) with a MBFCX9000 camera (MBF Bioscience) mounted on the microscope. StereoInvestigator software was used for acquiring

FIG. 3. Results of a further mapping experiment. A: outline of the Etruscan shrew brain with major blood vessel pattern (opaque) and numbered penetration sites. Responses of from different cortical regions/modalities are color-coded. B: physiological map of sensory cortical regions. Dashed line, preliminary border between putative areas S1 and S2. C: whisker receptive fields of penetrations in putative primary somatosensory cortex S1. D: body and limb receptive fields of penetrations in putative area S1. E: whisker receptive field of penetrations in: putative secondary somatosensory cortex S2 (top, bluish), in the posterior-lateral somatosensory region (middle, greenish) in the anterior-lateral somatosensory region (bottom, reddish). F: body and limb receptive fields of penetrations in different regions. Responses at sites 4, 6, 14, 42, and 79 had poorly defined receptive fields, which are not illustrated. Scale bar applies to A and B.
whole-field images or composite micrographs. Digitized images were processed with Adobe Photoshop (Adobe Systems, San Jose, CA). In some cases, brain sections were cut out to isolate them from the surrounding embedding material. If brightness or contrast was adjusted, these adjustments were applied to the entire image.

Cortical area borders were detected by laminar and cell density changes in the sections that have been processed for Nissl substance and cytochrome oxidase (CO).

RESULTS

Our assessment of the physiological organization of Etruscan shrew cortex is based on 12 mapping experiments, seven of which resulted in relatively complete maps (with 38–98 penetrations per experiment) and five of which resulted in partial maps (with 6–16 penetrations per experiment). We aimed for mapping the Etruscan shrew cortex as completely as possible and therefore mapped sites also included allo-cortex, i.e., sites in piriform and entorhinal cortices. We placed electrolytic lesions and applied histological analysis to verify the location of recording sites. However, a detailed cytoarchiteconic analysis is beyond the scope of our study.

To verify the location of recording sites. However, a detailed cytoarchiteconic analysis is beyond the scope of our study. A comprehensive description of the cytoarchitecture, areas and neuron numbers in Etruscan shrew cortex is in preparation (Naumann R, Anjum F, Roth-Alpermann C, Brecht M, unpublished data).

Cortical response regions

Based on response properties, we distinguish a number of cortical regions in shrew cortex. We grouped and color-coded responses throughout the paper according to the cortical region they putatively stemmed from. In gray, we display the putative somatosensory cortical area S1 and in blue, the putative somatosensory cortical area S2. In green, we present the posterior-lateral somatosensory/polysensory region, red is used for the anterior-lateral somatosensory region, violet for the auditory region, and orange for the visual region. Assigning responses to cortical regions is an interpretation and the evidence supporting our present partitioning scheme is summarized at the end of the result section.

Individual hand-mapping experiments

CASE 1. The results of a single representative mapping experiment are shown in Fig. 2, which illustrates the best response modality for each penetration site. In this case, we performed many penetrations and achieved a relatively complete coverage of the cortical surface (Fig. 2A). The anterior parts of the mapped area were largely but not completely unresponsive (Fig. 2A, gray numbers), whereas the remainder of the exposed cortex responded to touch, light or sound. Of the responsive sites only a small fraction (7%) of sites responded best to visual

FIG. 4. Results of a mapping experiment with electrolytic lesions. A, outline of the Etruscan shrew brain with major blood vessel pattern (opaque) and numbered penetration sites. Responses of from different cortical regions/modalities are color-coded. At the two encircled sites, electrolytic lesions were placed. B: physiological map of sensory cortical regions. Dashed line, the preliminary border between putative areas S1 and S2. C: whisker receptive fields of penetrations in putative primary somatosensory cortex S1. D: body and limb receptive fields of penetrations in putative secondary somatosensory cortex S2 (top, bluish) and in the polysensory/somatosensory region (bottom, greenish). E: body and limb receptive fields of penetrations in putative secondary somatosensory cortex S2 (top, bluish) and in the polysensory/somatosensory region (green). Responses at sites 27, 33, 40, and 43 had poorly defined receptive fields, which are not illustrated. Scale bar applies to A and B.
stimuli (Fig. 2A, orange numbers). Some of the visually responsive sites were clustered at a posterior/medial location. A larger fraction (17%) of the responsive sites showed auditory responses (Fig. 2A, violet numbers), and these responses were clustered slightly posterior and lateral from the center of the cortical exposure. By far most of the responsive sites (76%) responded best to touch.

In the putative primary somatosensory cortex S1 (black letters Fig. 2A, gray area in B) we detected mainly whisker responses in this animal. The relatively small whisker receptive fields are illustrated in Fig. 2C. We observed only one putative S1 paw receptive field (Fig. 2D). Whisker responses that originated from the putative secondary somatosensory cortical area S2 had somewhat larger receptive fields than in cortical area S1 (Fig. 2E, blue color). We observed even larger whisker receptive fields in a posterior-lateral region (Fig. 2E, green color) and an anterior-lateral region (Fig. 2E, red color). While somatosensory responses predominated in the posterior-lateral region, we also observed polysensory responses in this region. At two sites, we observed strong polysensory responses to touch, sound, and light (Fig. 2A). We also detected responses to paw and body stimulation in putative cortical area S2, the posterior-lateral region and the anterior-lateral region (Fig. 2F).

CASE 2. The results of a further mapping experiment are shown in Fig. 3; in this case, the mapping did not extend as far laterally as in the previous experiment (A). Again we illustrate the best response modality for each penetration site. Some anterior-lateral and some very medial sites of the mapped area were unresponsive (Fig. 3A, gray numbers). Of the responsive sites, only a small fraction (13%) of sites responded best to visual stimuli (Fig. 3A, orange numbers), and most of these sites formed a stripe at posterior locations. Thirteen percent of the responsive sites showed auditory responses (Fig. 3A, violet numbers), and these responses were clustered slightly posterior and lateral from the center of the cortical exposure. Similar to the example shown in Fig. 2, by far most of the responsive sites (74%) responded best to touch. As before we color-coded somatosensory responses according to the cortical region they putatively stemmed from; Fig. 3B shows an overview of putative cortical regions as identified in this experiment. In putative S1 we detected whisker responses with small receptive fields (C) and a number of paw and body receptive fields (D). Whisker receptive fields were larger in putative cortical areas S2, the posterior-lateral region and the anterior-lateral region (E). In the posterior-lateral region and the anterior-lateral region, nonwhisker somatosensory receptive fields often encompassed large parts of the body (F).

CASE 3. The results of a third mapping experiment are shown in Fig. 4. In this case, we placed electrolytic lesions at two penetration sites (Fig. 4A). We illustrate the best response modality for each penetration site. Some anterior and some medial sites of the mapped area were unresponsive (Fig. 4A, gray numbers). Of the responsive sites, only a small fraction (12%) of sites responded best to visual stimuli (Fig. 4A, orange numbers) and many of these sites formed a stripe at posterior locations. Eleven percent of the responsive sites showed auditory responses (Fig. 4A, violet numbers), and these responses were clustered slightly posterior and lateral from the center of the cortical exposure. Similar to the example shown in Fig. 2, by far most of the responsive sites (77%) responded best to
touch. As before we color-coded somatosensory responses according to the cortical region they putatively stemmed from. Figure 4B shows an overview of putative cortical regions as identified in this experiment. In putative S1, we detected whisker responses with small receptive fields (Fig. 4C) and a number of paw and body receptive fields (Fig. 4D). Whisker receptive fields were larger in putative cortical areas S2 and the posterior-lateral region (Fig. 4E). Only one penetration was
made in this experiment in the anterior-lateral region, and we observed a weak and poorly defined somatosensory response. In the posterior-lateral region, nonwhisker somatosensory receptive fields often encompassed the entire body (Fig. 4F).

To understand how response regions relate to cytoarchitectonic subdivisions of the shrew cortex, we used the electrolytic lesions as a reference to align response maps with cytoarchitectonic boundaries (Fig. 5A). There was a good correspondence between response properties and cytoarchitectonic subdivisions. Visual responses were found in a cortical region that was thin and had a narrow but dense layer II (Fig. 5, B and C). Laterally adjacent to the visual responses was a cortical region of greater thickness in which we observed somatosensory responses. Far laterally at the border of piriform cortex (Fig. 5B) or in entorhinal cortex (C) we observed polymodal responses.

**Topological organization**

The results shown in Figs. 2–4 provide evidence that Etruscan shrews possess specialized cortical regions with a topological organization: 1) across different animals, responses to a specific sensory modality clustered in similar locations. 2) Putative somatosensory area S1 showed a somatotopy with the body/paw representation at the medial-anterior border of S1, the macrovibrissae represented in the center of S1, and the lower jaw represented at the lateral border of S1. It must be added, however, that the somatotopic representation was not equally clear in all experiments. 3) Putative S2 also showed clear signs of coarse somatotopy with macrovibrissae represented in the center of S2 and the body/paw representation at the lateral-posterior border of S2. This layout mirrors the somatotopy of S1. 4) In putative S1, we also observed sites that followed a fine somatotopy. For example, in the body/paw representation shown in Fig. 3, A and D, we observed a systematic progression along the anterior-posterior axis. Interestingly, the direction of movement along this axis was reversed comparing brain sites and their respective body receptive field, i.e., neurons at a posterior electrode position would respond to an anterior body part, whereas responses at anterior recording sites would be triggered by stimulating posterior body parts. Specifically the most anterior recording site (80, Fig. 3A) responded to tail stimulation (receptive field 80, D), posterior to it recording site 69 responded to hind limb stimulation, further posterior to it site 68 responded to the hind part of the body, and the far posterior recording site (50) responded to forelimb stimulation. It must be added, however, that while we locally observed a strict topographical order, there were many deviations from a systematic progression of receptive fields, and we did not observe a fine grain overall topography in our mapping experiments. The lack of a fine grain overall topography also made it difficult to exactly determine the S1/S2 border. Therefore the division of putative S1 and putative S2 should be regarded as preliminary. 5) The posterior-lateral region and the anterior-lateral region differed from S1 and S2 with respect to receptive field size and the presence of polymodal responses; these differences will be discussed in the following text.

A comparison of Figs. 2B, 3B, and 4B demonstrates features consistent across animals as well as individual differences of the cortical maps. The relative location of cortical areas to one another was highly consistent: in all animals we observed a medial-anterior located putative S1, a medial-posterior located putative S2, a very posterior visual region, a central-laterally located auditory region, and a mediolaterally located somatosensory region with large RFs and a posterior-lateral somatosensory/polymodal region. Also the coarse topography within putative S1 and S2 was similar across animals. However, the size and exact shape of cortical areas differed across animals.

**Advantages and limitations of hand-mapping**

Hand-mapping is a qualitative experimental approach with the important advantage that different sensory stimuli can be presented rapidly one after the other. This is of special importance in such animals as the Etruscan shrew where experimental time is limited due to the difficult anesthesia. Using hand-mapping, it is thus possible to test a large number of recording sites for different sensory modalities in a given experiment, i.e., comprehensive cortical maps can be obtained for individual cases. However, hand-mapping is nonquantitative and relies on the subjective judgment of the experimenter. For that reason, we carried out separate mapping experiments where each sensory modality (auditory, visual, or somatosensory) was tested with computer-controlled stimulation. Simultaneous multi-unit recordings of neuronal responses allowed to assess cortical responsiveness in a quantitative fashion. These computer-controlled mapping experiments were combined with extensive histological lesion analysis.

**Individual computer-controlled mapping experiments**

**COMPUTER-CONTROLLED AUDITORY STIMULATION.** Auditory stimulation consisted of a 20 kHz tone presented to the contralateral ear with a loud speaker. Neurons that responded to auditory stimulation were located in a region posterior and lateral to the center of the cortex (Fig. 6A). Location and size of auditory cortex mapped by computer-controlled stimulation corresponded to the results of hand-mapping experiments. Electrolytic lesions were placed in both responsive and nonresponsive...
parts of cortex (encircled numbers in Fig. 6A), and we were able to retrieve all lesion sites by post hoc histological analysis in horizontal brain sections stained for Nissl or cytochrome oxidase. Auditory responses were observed in an area that stained darkly for CO. Sections for three selected locations along the anterior-posterior axis are shown in Fig. 6, B–D: lesion sites are marked by encircled numbers and the arrowheads denote the cytoarchitectonic boundaries of visual, auditory, and somatosensory cortex. PSTHs of neuronal responses at different recording sites are shown in Fig. 6, E–G. The location of the

\[ X = \text{visual response} \]
\[ X = \text{no visual response} \]
\[ \bigcirc = \text{lesion site} \]
different recording sites is indicated on the small inset brain and corresponds to the lesioned sites shown in the histological panels above. There was a good correspondence between cytoarchitectonic subdivisions and neuronal response properties. Recording/lesion site 1 in the middle of the CO intense area we defined as auditory cortex (Fig. 6, C, bottom) showed strong auditory responses as well as the neighboring site 21 (Fig. 6F). At recording/lesion site 11, there was no response to auditory stimulation (Fig. 6, E, bottom), whereas neurons at recording site 4, located ca. 250 μm more medial in the CO intense patch, showed auditory responses (Fig. 6, E and B).

Computer-controlled visual stimulation

Visual stimulation was applied by a stationary white LED flashing onto the contralateral eye of the shrew. Neuronal responses to visual stimulation were recorded in a posterior stretch of cortex extending from anterior-medial to posterior-lateral (Fig. 7A). Location and size of visual cortex mapped by computer-controlled stimulation corresponded to the results of hand-mapping experiments. Lesions were placed in both responsive and nonresponsive parts of cortex (encircled numbers in Fig. 7A), and we were able to retrieve all lesion sites in post hoc histological analysis (Fig. 7, B–D). Visual responses were observed in an area that stained darkly for CO. There was a good correspondence between cytoarchitectonic subdivisions and neuronal response properties, as already documented for the computer-controlled auditory experiment. Neurons at the recording site 13, just within the borders of visual cortex, showed a clear response to visual stimulation. At recording/lesion site 14 however, around 250 μm more lateral, neurons did not respond (Fig. 7, F and C).

Computer-controlled stimulation of single whiskers

Whisker receptive field (RF) size was assessed by computer-controlled piezoelectric stimulation of single whiskers and a representative example is shown in Fig. 8. Figure 8A shows a schematic of the Etruscan shrew whisker pad with the tested whiskers marked in black, the PSTHs of three different whiskers are shown in B. The computer-controlled experiments showed that RFs in medial shrew cortex (putative S1) comprise around 10 whiskers (Fig. 8C), which is similar to RF size assessed in hand-mapping (Fig. 11C). Furthermore, we assessed RF size at different shrew body temperatures from 24 to 36°C and found no temperature-dependent difference (data not shown). The electrolytic lesion placed at the recording site (Fig. 8A) confirmed that the electrode was placed in the middle of a dark area with intense CO staining, the somatosensory cortex. Furthermore, in the Nissl-stained brain section (Fig. 8E, top) a blood-filled electrode track 250 μm medial to the lesion can be seen clearly (marked with an arrow). This was the site of a second penetration, where we also observed neuronal responses to hand-held and piezoelectric whisker stimulation.

To summarize, the size and location of auditory and visual cortex and the size of whisker RFs measured with computer-controlled sensory stimulation corresponded to the data obtained in hand-mapping experiments.

Averaged maps

METHODOLOGICAL CONSIDERATIONS. In the remainder of the paper, we will illustrate data averaged from the 12 successful hand-mapping experiments, but before doing so we would like to add some methodological considerations.

LIMITATIONS. Using electrophysiological mapping experiments, it has been shown that cortical areas can differ between individuals (Merzenich et al. 1987) and therefore averaging may result in an artificial map that was not observed in any one animal. For the same reason, averaging will blur borders of cortical representations, and the resulting averaged map can show gradual transitions, even though in every single experiment sharp borders were observed. To average we aligned maps to an anterior-medial reference point (see METHODS). With increasing distance from this reference point (i.e., the more lateral and posterior the coordinates), the scatter of our averaged maps is likely to increase. For aligning and mapping, we used a two-dimensional (anterior-posterior, medial-lateral) coordinate grid and ignored the brain curvature; the strongly curved far lateral parts of cortex will be underrepresented in our data. It is important to understand that in our vertical mapping approach, this means lateral points (on a curved brain) are less accurately placed than midline points (drawn on a flat photograph) and that the 300 μm depth was likely not reaching the same cortical layers in all penetrations. All of these factors limit the usefulness of averaged maps. We nevertheless show averaged maps because they allow pooling data across animals and quantifying aspects of cortical organization across animals.

AVERAGING PROCEDURE. In maps averaged across different animals, we were interested in what fraction of shrew cortex showed a particular response characteristic. Because in our experiments we did not map all parts of shrew cortex equally often, we averaged for each coordinate the frequency of each response type.

Averaged maps for response modalities

Averaged maps for response modalities are shown in Fig. 9. Penetrations were made with a spacing of 250 μm and the way our averaging grid superimposes on a shrew brain is illustrated in Fig. 9A. Somatosensory responses predominated in the...
shrew cortex (Fig. 9B) and made up 75% of responsive cortical locations. Auditory responses clustered at a single location slightly posterior and lateral from the center of the cortex (Fig. 9C) and made up 11% of responsive cortical locations. Visual responses were found in a stripe at posterior locations and made up 14% of responsive cortical locations (Fig. 9D). Polysensory responses, which we defined here as sites that responded roughly equally well and strongly to multiple modalities, were found at far lateral and far posterior locations (Fig. 9E), and we thus refer to this part of the cortex as the...
posterior-lateral region. Polysensory responses did not consistently occur elsewhere in the shrew cortex. However, even at far lateral and far posterior locations, polysensory responses made up only a fraction of all responses, and a comparison of Fig. 9, B and E, shows that the posterior-lateral region is dominated by somatosensory responses. As seen in Fig. 9F only a small proportion of shrew cortex located anterior did not respond to light, sound, or touch. In particular, the lateral parts of shrew cortex responded in almost all experiments. This observation suggests that the anesthesia protocol introduced here preserved cortical responsiveness very well.

**Averaged maps of somatosensory responses**

Averaged maps for somatosensory response properties are shown in Fig. 10 along with a shrew brain (A). Macrovibrissa responses predominated in shrew cortex and made up 42% of all responsive cortical locations (Fig. 10B). Macrovibrissa responses were particularly common in anterior-lateral parts of cortex. In putative areas S1 and S2 macrovibrissa responses were most common at the border of S1 and S2, suggesting that these areas were joined together at their macrovibrissa representation. Responses to lower jaw stimulation were found in a
rather restricted area (5% of all responsive cortical locations, Fig. 10C). This value is likely to be an underestimate of the true extent of the lower jaw representation because of the poor accessibility of the lower jaw region for stimulation. The averaged map of body/paw responses fell in several hot spots. It seems plausible that these the body/paw representations correspond to putative area S1 and S2 and potential further somatosensory representations as indicated in Fig. 10D.

**Averaged maps and cytoarchitectonic boundaries**

How are averaged maps of response properties related to the mean position of cytoarchitectonic boundaries? This issue is addressed in Fig. 11. We plot the position of two major cytoarchitectonic boundaries relative to the blood vessel pattern and a map of whisker receptive field size. As shown in two example brains in Fig. 11A, the medial border of piriform cortex (squared line) often coincides with the lateral branch of the medial cerebral artery. A few hundred micrometers more medially is another prominent cytoarchitectonic boundary (dashed line) which corresponds most likely to the lateral border of putative area S1. Figure 11B shows a systematic progression of RF positions in putative S1, whereas lateral from S1 the progression discontinues and RF size increases. This single experiment observation is corroborated by the analysis of population data. Figure 11C illustrates the average position of the lateral border of putative S1 and the medial border of piriform cortex in six shrew brains, relative to an averaged map of receptive field size of whisker responses (obtained in 12 shrews). Whisker receptive fields were small in the medial parts of shrew cortex (~10 whiskers), which correspond to putative areas S1 and S2. Lateral from putative S1 receptive were significantly larger (Fig. 11C). The largest whisker receptive fields were observed in the anterior-lateral region.

Comparing the position of the medial border of piriform cortex plotted in Fig. 11 to the average extent of responses as mapped in Fig. 9 shows that regions corresponding Etruscan shrew piriform cortex often showed (for the most part with a probability >80%, Fig. 9B) somatosensory responses. In contrast piriform cortex only rarely showed auditory (Fig. 9C) or visual (D) responses. Thus in our experiments tactile specialization of shrew cortex even extended into piriform cortex, an area classically thought to be olfactory. The somatosensory RFs in piriform cortex were very large (Fig. 11C).

**Synopsis of cortical regions in Etruscan shrew cortex**

The results presented here led to the physiological identification of at least six cortical regions. The mapping of these regions onto the shrew cortex is illustrated in Fig. 12.

First, we identified a small visual region. This region forms a stripe at the posterior end of the cortex. We observed unimodal visual or bimodal visual (Figs. 2A and 4A)/somatosensory responses in this area. It is unclear, if these bimodal responses reflect polysensory characteristics of this area or if somatosensory responses simply come about because—due to the stripe-like shape of the area—all recording sites are close to somatosensory area S2.

Second, we identified a small auditory cortical region. We observed unimodal auditory responses in this region.

Third, we identified a large somatosensory cortical area we refer to as putative S1. We observed predominantly unimodal somatosensory responses and a reproducible somatotopy in this area. Somatosensory receptive fields were small in this area.
Fourth, we identified a further somatosensory cortical area we refer to as putative S2. We observed unimodal somatosensory responses and a reproducible somatotopy, which mirrors the one of putative S1. Somatosensory receptive fields were small in this area. Although we observed in some of the experiments larger receptive fields in putative S2 than in putative S1, this difference was not robust across experiments and the average receptive field size was not different between S1 and S2 (Fig. 11, B and C).

Fifth, in a posterior-lateral position, we identified a third somatosensory region. In addition to the dominating somatosensory responses, we often observed polysensory responses here (Fig. 9E). Somatosensory receptive fields were larger in this area than in putative areas S1 and S2 (Fig. 11, B and C). The somatotopic organization in this region remains to be clarified. This region seems to encompass several cortical areas and the lateral regions overlap with piriform and entorhinal cortex.

Sixth, in an anterior-lateral position, we identified a fourth somatosensory region. In this area we observed mainly unimodal whisker responses with very large receptive fields. Accordingly, we observed in all experiments a marked and abrupt increase in receptive field size when moving the electrode laterally out from putative S1 into the anterior-lateral region. The somatotopic organization in this region remains to be clarified. This region seems to encompass several cortical areas and the lateral regions overlap with piriform cortex.

DISCUSSION

In this article, we describe a protocol for the anesthesia and the mapping of the Etruscan shrew cortex. We found that large parts of the shrew cortex represented somatosensory stimuli. Somatosensory responses were represented in multiple regions, while auditory and visual stimuli activated only restricted parts of shrew cortex.
Methodology of our mapping approach

Consistent with previous reports on the anesthesia of small shrew species (Catania et al. 1999), our initial attempts to anesthetize Etruscan shrews failed. The anesthesia protocol developed here, however, overcame these problems and allowed us to perform acute experiments with Etruscan shrews with success rates >50%. The key elements of this mapping approach were the massive application of local anesthesia, the very slow induction of general anesthesia, and the passive cooling of the animal. The passive cooling led to a considerable drop in the animal’s body temperature without, however, interfering with the cortical responsiveness. We consider the possibility that such massive hypothermia could distort and in particular degrade sensory responses, but our experiments demonstrated preserved cortical responsiveness even at body temperatures as low as 24°C (Fig. 1). At the same time, we think it is unlikely that the cooling could alter maps by inducing responses that would otherwise not be there. We reason that the Etruscan shrew’s capacity to preserve cortical responsiveness during hypothermia might be related to their ability to enter “torpor,” a physiological state of hypothermia and reduced behavioral responsiveness (Frey and Vogel 1979).

The averaged maps of Etruscan shrew cortex presented here were obtained in hand-mapping experiments which had the advantage that a large number of recording sites—close to 100 in our best case—could be tested with different sensory stimuli. Thereby we were able to obtain comprehensive physiological maps for individual cases even in limited experimental time. A separate set of experiments with computer-controlled auditory, visual, or whisker stimulation confirmed size and location of sensory cortical areas.

Two further restrictions of our mapping of sensory responses need to be considered. First we did not use any olfactory and gustatory stimuli. Second the accessibility of body parts may have distorted our somatosensory maps. For example we did not detect any receptive fields within the shrew mouth and reckon that this is a false negative result.

Organization of Etruscan shrew cortex

The cortical maps derived here provide insights into the information processing architecture of this tiny mammal. The first observation was that large parts (7.3 mm² of ~12 mm² total neocortical surface i.e., ~60%) of Etruscan shrew cortex responded to sensory stimuli. The true fraction of sensory cortex in Etruscan shrews is probably substantially higher because we mapped only three quarters of the cortical sheet and because we did not test for olfactory and gustatory responses. The second observation was that Etruscan shrew cortex was dominated by somatosensory responses. This finding is in good agreement with the analysis of Etruscan shrew behavior. Behavioral analysis of Etruscan shrew prey capture demonstrated that these animals heavily rely on tactile cues and that tactile shape cues suffice to trigger attacks on plastic prey “dummies” even in the absence of the correct olfactory, gustatory, auditory, and prey motion cues (Anjum et al. 2006). The third observation was that large parts of the somatosensory areas responded to macrovibrissae stimuli, and again this finding is in line with the conclusions from behavioral analysis (Anjum et al. 2006).

Tactile processing in the Etruscan shrews appears to occur in multiple cortical regions. As summarized in the preceding text, evidence for multiple specialized areas came from the analysis of topography, response modality, polysensory responses, and receptive field size. We used the terminology putative S1 and putative S2 for two large somatosensory areas because these areas appear to be homologous to the S1 and S2 areas identified by Catania and colleagues in closely related shrew species (Catania et al. 1999). The distinction of these two areas is also supported by histological analysis, in particular their cytochrome oxidase staining pattern (Naumann R, Anjum F, Roth-Alpermann C, Brecht M, unpublished results). It must be noted, however, that a clear physiological border between these two areas could often not be established and that our evidence on the division of these two areas is preliminary. Our mapping approach was not suited to determine in depth the functional
characteristics of these areas. A few preliminary conclusions can be drawn. Putative S1 and S2 appear to be “early/low level” stages of cortical processing topographically organized with relatively small receptive fields. The receptive field size of ~10 whiskers per multi-unit recording site was similar or slightly larger than what has been measured in other shrew species (Catania et al. 1999). RFs in the Etruscan shrew seemed to be larger than most of the receptive field sizes that have been reported in rodent S1 (Simons 1978). In this context, it should be noted, however, that most of our recordings were done in the deep cortical layers, where receptive fields are large in rodent somatosensory cortex (Manns et al. 2004; Simons 1995). In contrast, the posterior-lateral region appears to be a “higher-order” somatosensory region involved in polysensory integration. The anterior-lateral somatosensory region also seems to be a higher-order somatosensory region that mainly represents macrovibrissal information in very large receptive fields.

Comparative considerations

The pattern of cortical organization described by us for the Etruscan shrew has a number of similarities to patterns of cortical organization as they have been described in related shrew species.

In particular Catania and colleagues investigated Northern-American shrew species and described an arrangement of areas S1 and S2 that is very similar to our account: 1) the overall position of these areas in the cortical sheet, 2) the antero-medial position of S1 relative to S2, 3) the relative size of these areas, 4) a similar overall topography of S1 with a medially located paw and body region, 5) a similar overall topography of S2 with a posteriorly located paw and body region, and 6) a mirror-image-like arrangement of these areas.

In the Catania et al. (1999) study, the position and size of areas A1 and V1 were similar to auditory and visual region of the Etruscan shrew. The most obvious difference between the Etruscan shrew and the species studied by Catania is larger overall extent of sensory cortex in Etruscan shrews and the fact that the posterior-lateral region and anterior-lateral somatosensory regions were not described in the former study.

If one compares the Etruscan shrew cortex to that of other mammals studied so far, it is clear that this animal is one of the most extreme tactile specialists studied to date. Only few animals like the star-nosed mole (Catania and Kaas 1997) and the naked mole-rat (Catania and Remple 2002) devote a similar fraction of their neocortex to somatosensory representations. It appears likely that putative areas S1 and S2 of the Etruscan shrew are homologous to these respective areas described in shrews and to areas S1 and S2 as they have been described in rodents. The homology between shrew, rodent, and primate appears likely that putative areas S1 and S2 of the Etruscan shrew is not a purely olfactory region but that it also shows somatosensory and polysensory responses (Fig. 11).

Perspectives

The cortical maps described here will provide a basis for further investigating how the Etruscan shrew’s sensory world is generated. Beyond just delineating cortical maps it will be fascinating to explore the functional characteristics of the areas identified here. In addition it seems most promising to direct two-photon microscopy to the shrew cortex (Stosiek et al. 2003). This application can greatly benefit from the small brain size of the Etruscan shrew and will allow visualizing cortical network in unprecedented completeness.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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