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Optical imaging of rat somatosensory cortex reveals representational overlap as topographic principle

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WE measured reflectance changes by means of optical imaging of intrinsic signals to study the topography of the paw representations in rat somatosensory cortex. Following circumscribed tactile stimulation of single digits or pads, we found large and partially overlapping areas of reflectance changes (ΔR). The diamaters of their focal zones defined at 75% maximal ΔR were in the range of 150 μm and preserved all details of the underlying maps. Zones of overlap were in the range 15–25% measured at half-maximal ΔR. In contrast, we found sharp boundaries with no overlap between the fore- and hindpaw representations. The data suggest that large and overlapping cortical maps constitute a normal type of neural representation supporting the idea of a distributed neural processing scheme.

Key words: Somatosensory cortex; Optical imaging; Receptive fields; Representational maps; Forepaw/hindpaw; Representational borders; Cortical magnification; Point spread function; Rats; Distributed processing

Introduction

According to electrophysiological mapping studies using multiple microelectrode penetrations and receptive field measurements, maps of the rat paw representation in the somatosensory cortex (SI) are characterized by small, low-threshold, cutaneous receptive fields, located on single digits, pads or parts of the heel. Based on receptive field measurements, a representation of the paw skin surface over a total cortical area of about 1–2 mm^2 can be reconstructed, suggesting the existence of a fine-grained and highly ordered topography.[1–6] Here we report experiments in which details of such representational maps and their borders were obtained by optical recordings of intrinsic signals in the SI of adult rats. This method has the advantage of revealing the contribution of the distributed populations of cortical neurones, including their subthreshold activity.

While the usefulness of this method has been demonstrated in the analysis of the visual cortex,[7–11] maps of the somatosensory cortex with high spatial resolution are rare.[12,13] We therefore attempted to demonstrate that a mapping of reflectance changes in SI is possible that preserves and maintains details about the topography of the single pads and digits of the paw representations. Different degrees of functional topographic overlap were found to exist within the representation of the paws and between the fore- and hindpaw. The resulting large areas of reflectance changes were in line with the small size and distinct nature of receptive fields, when cortical magnification and receptive field overlap are additionally taken into account.

Materials and Methods

A total of 24 rats were anaesthetized with urethane (20% in Ringer solution, 1.5 mg kg^{-1}, i.p.) and held under urethane anaesthesia during the entire course of the experiment. For details of the experimental procedures see Refs 5, 6. Treatment of all animals was within the National Institutes of Health Guide for Care and Use of Laboratory Animals (Revised 1987). In brief, after unilateral opening of the skull, the dura was removed. The cortex was covered with silicon oil, which was held in place by a small wall of dental cement. Conventional electrophysiological controls were made by recording extracellularly action potentials from small clusters containing 2–4 neurones at depths of 600–800 μm using glass microelectrodes (1–2 MΩ) filled with 3 M NaCl. Alternatively, local field potentials (LFPs) of activity of the fore- and hindpaw evoked by tactile stimulation were recorded in cortical layer II/III and IV by multiple penetrations. The spatial distribution of activity in each layer was computed by interpolating the levels of the LFP amplitudes for 25, 50 and 75% of the maximal amplitude for each penetration site. The resulting LFP maps were colour-coded as described in the legend to Figure 3.

A mechanical stimulator was used to apply

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computer-controlled tactile stimuli of variable duration delivered at 3 Hz. Up to six skin locations could be stimulated independently with probes of 1–1.5 mm diameter. For comparison, a so-called whole-paw stimulation was employed by means of a specifically designed cushion that allowed simultaneous stimulation of the entire paws.

For optical measurements, we used a Lightstar II imaging and acquisition system (LaVision) with a 2 MHz A/D converter and a Peltier cooled, slow scan 12 bit digital CCD-camera. The CCD was controlled by a 486 PC with 64 MB RAM. Images were obtained with acquisition times of 80 ms duration. Averaging was achieved by adding intertrial sequences consisting of five images of 80 ms duration, which were averaged to six trials. By this, a considerable reduction of data acquisition time could be obtained. The cortex was illuminated with a cold light source using interference filters of 546 or 614 nm (HWB = 10 nm). Controls (non-stimulus conditions) were taken as blank images prior to each stimulus presentation. Images were computed by subtracting the stimulus from the non-stimulus condition. Data were stored on magneto-optic discs. Data analysis was performed on Sun workstations using custom made analysis software written in IDL™. The spatial distributions of reflectance changes were colour-coded and quantitatively computed in terms of cortical area for 25, 50 and 75% of the maximal reflectance changes.

Results

Following point-like tactile stimulation of the single digits or pads we found fairly broad distributions of optical signals with a substantial degree of overlap between them. Their main foci, however, could clearly be distinguished and separated revealing an overall distinct map of the different digits and pads.

In initial experiments we used a 546 and a 614 nm light source. As we did not observe systematic differences but rather identical spatial distributions, most of the presented data were obtained with the 546 nm light source which yields larger signals. On average, the amplitudes of the intrinsic signals, i.e. the relative reflectance changes ΔR, were in the range of 0.5–1% for the 546 nm light source. The mean size of the two-dimensional signal distributions measured at 75% maximal amplitude was 0.1 mm^2 for the pads and 0.08 mm^2 for the digits. At 50% maximal amplitude of the signals, these areas covered an area of 0.6–0.4 mm^2. Consequently, there was an overlap between the signals obtained following pad and digit stimulation in the range 15–25%. Including even lower amplitudes of 25% of maximal ΔR in the analysis revealed activation of large areas covering nearly the entire paw representation. The sizes of the representational areas differed remarkably for the different amplitude levels. The mean area found for the 75% level was only 5–10% that of the 25% level. This indicates that the

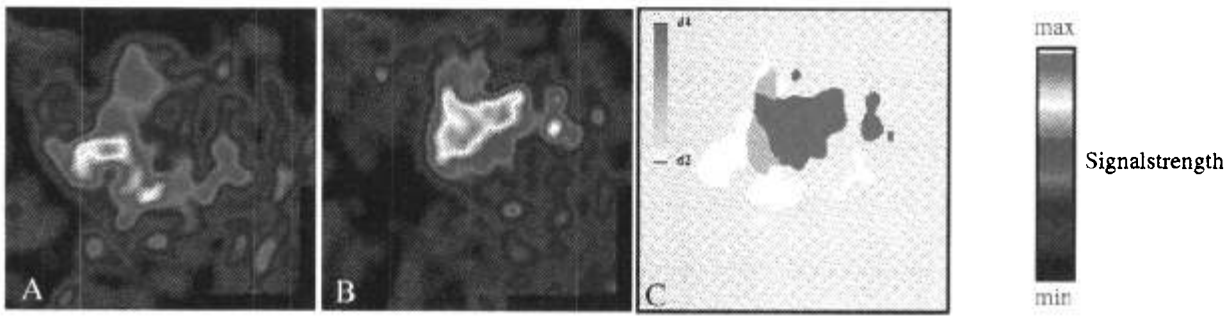

FIG. 1. Optical recordings of intrinsic signals following point-like stimulation of the distal aspects of digits D3 (**A**) and D4 (**B**). In (**C**) the resulting map is computed for 50% of maximal reflectance changes (D3 = yellow, D4 = red). Scale bars (coloured corners) are 1 mm. Each plot is oriented with caudal left and medial up. Colour-coding of intensity is shown on the right.

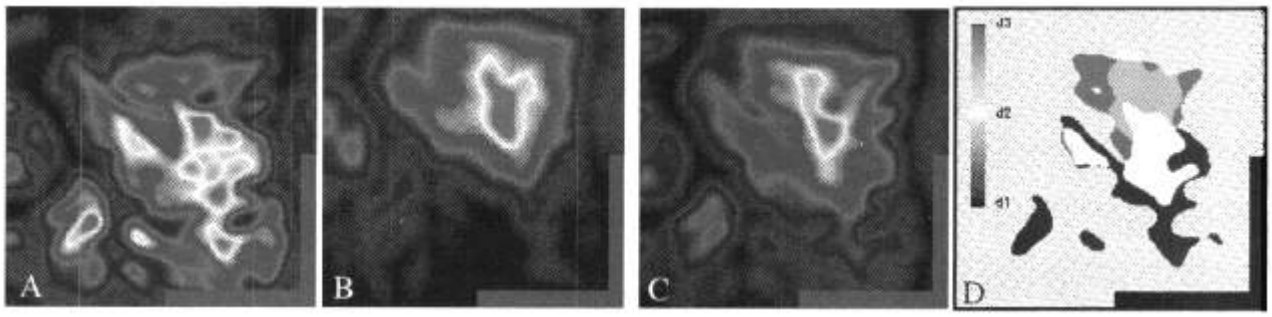

FIG. 2. Optical recordings of intrinsic signals following point-like stimulation of the distal aspects of digits D1 (**A**), D2 (**B**) and D3 (**C**). In (**D**) the resulting map is computed for 50% of maximal reflectance changes (D1 = blue, D2 = yellow, D3 = red). Scale bars (coloured corners) are 1 mm. Each plot is oriented with caudal left and medial up. For colour-coding of intensity see Fig. 1.

75% area of reflectance changes might correspond to the so-called spike based point spread function, which in the cat visual cortex has been reported to be 5% of the optically measured one.[14]

Generally, the optically obtained distributions of signals were non-homogeneous and often multi-peaked, particularly in the non-maximal parts of the signals, resulting in complex patchy patterns of activity (Figs 1, 2). The reliability of these measurements was quite high. In an independent study investigating cortical reorganizational changes, repeated measurements after several hours showed a fairly similar pattern of reflectance changes (Godde *et al*, unpublished). A similar observation of considerable robustness of optically obtained maps was reported by Bonhoeffer and Grinvald.[10]

While the analysis of so-called intrapaw representations of single digits and pads revealed a substantial overlap, a quite different result was observed when representations of the entire fore- and hindpaw were investigated. Using whole-paw stimulation we were able to measure simultaneously reflectance changes related to the stimulation of the entire fore- and hindpaw, respectively. In contrast to the above described intrapaw overlap, the representations of the hind- and forepaw were clearly separated with no overlap even at the 50% level of signal amplitudes, suggesting the existence of completely independent and separated representational zones of the different body parts. These distributions were also highly non-homogeneous. They were usually characterized by territories of enhanced activity interrupted by intermediate zones of less activity presumably corresponding to the transition from digits to pads (Fig. 3).

All of the reported features were studied by conventional local field potential (LFP) mappings using up to 70 penetrations in the same individual animal. These measurements revealed LFP maps of activity distributions that were in full accordance with the spatial reflectance distributions of the optical data imaged in the same animal (Fig. 3).

Discussion

Using optical measurements of intrinsic signals we were able to demonstrate the existence of a detailed, but considerably overlapping intrapaw representational topography consisting of separate foci of activity following stimulation of single digits and pads. We were also able to show that the representations of the fore- and hindpaw mapped side by side with no overlap, resulting in the existence of sharp, functional borders of representations of different body parts.

It should be noted that the method of imaging intrinsic signals not only contained and preserved topographic details, but allowed a rapid assessment of a cortical representation within a few minutes. This was possible by combining the usage of a fast CCD camera with a new data averaging scheme as described in Materials and Methods. In contrast, mapping procedures using 30–40 electrode penetrations require several hours and still provide only limited spatial resolution.

The differential degree of representational overlap found for different aspects of intra- and interpaw representations argues in favour of the reliability of optical imaging methods. Although many studies have shown a considerable capacity of spatial resolution of this method,[8,9] light scattering, non-specific factors due to blood flow and metabolism were raised as factors jeopardizing highly resolved spatial measurements. In order to provide additional and individual controls, we performed systematically local field potential (LFP) measurements in layers II/III and IV in the same animals. It can be assumed that LFPs reflect similar contributions of pre- and postsynaptic neural activity including subthreshold activity as do intrinsic signals. In any case, optical images and LFP maps provide estimates about the shape and dimension of cortical point spread functions. These measurements, specifically recordings in layers II and III, revealed a profound correlation of the spatial distribution of the optical and electrical signals (cf. Fig. 3).

How can the large and overlapping areas of reflectance changes, which are on first sight contradictory to the smallness and distinctness of receptive fields, be explained? In a recent study performed in monkey striate cortex using voltage-sensitive dyes, unusual large areas of excitation following photic stimulation were encountered which were several-fold

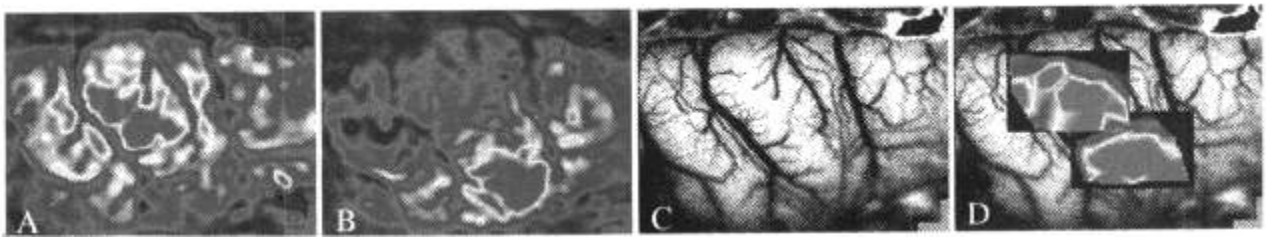

FIG. 3. Optical recordings of intrinsic signals following whole-paw stimulation of the forepaw (**A**) and the hindpaw (**B**). Videoimage of the cortical surface (**C**). In (**D**) local field potential (LFP) maps are shown obtained for stimulating of the fore- and hindpaw superimposed on the image of the brain surface (activity >50% of maximum is shown in red, penetrations without any activity are blue). Scale bars (coloured corners) are 1 mm. Each plot is oriented with caudal left and medial up. For colour-coding of intensity in the optical recordings see Fig. 1.

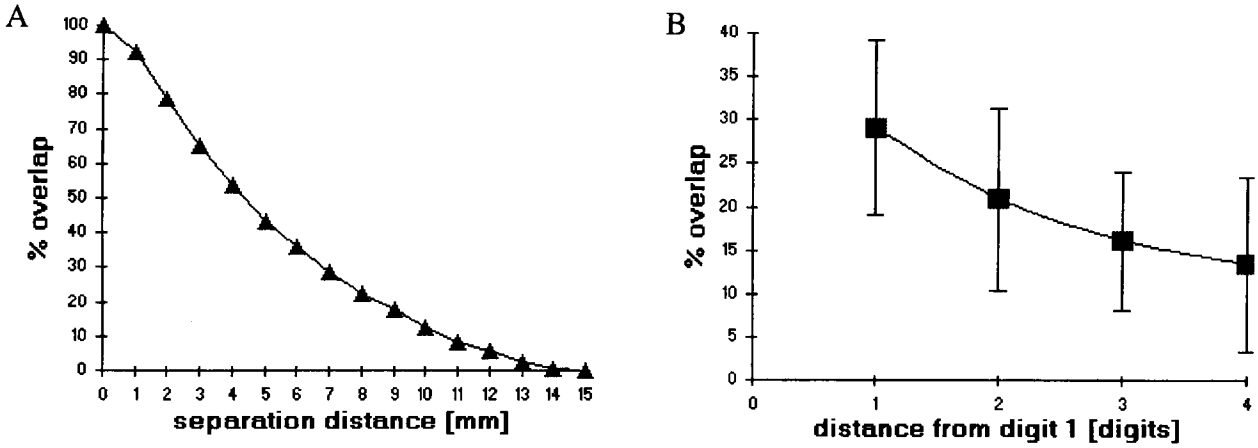

FIG. 4. Percentage overlap as a function of separation distance between two stimuli on the paw surface at 50% maximal response according to the theoretical calculations based on average receptive field size and magnification (**A**). Experimentally measured overlap as obtained for half-maximal reflectance changes for various separation distances of the stimuli used (**B**, mean values ± s.e.m. $n = 12$).

larger than would be predicted from retinotopic mapping data.[15] Using intrinsic signals to map rodent barrel cortex, fairly large response areas were described.[13] Imaging data of cat visual cortex indicated that the spike-based point spread function was only 5% of the optically measured.[14] Own unpublished data relating to recording of intrinsic signals in cat auditory cortex revealed large and overlapping areas following pure tone presentations. We will show that the mismatch between RF size on the one hand and the cortical map and overlap size on the other hand can be resolved, when cortical magnification and receptive field overlap are also taken into account.

From our electrophysiological recording studies,[5,6] some basic assumptions can be made about average RF size, RF scatter and average representational cortical area. Based on these data we assume that 500 μm cortex represents 10 mm skin surface. Assuming for simplicity elliptical RF shapes and ignoring the systematic gradient of RF size along the distal–proximal axis on the paw, the mean RF length as obtained from quantitative measurements of RF profiles was 8 mm skin defined at 50% maximal activity.[16] From the size of the rat paws it can be calculated that the tactile stimuli applied to the digits and pads were separated by about 7–11 mm on the skin. Based on these assumptions and constraints, an excitational area corresponding to the cortical point spread function and a distance-dependent overlap area can be calculated. Our calculations revealed a mean excitational area corresponding to an area of reflectance changes of about 0.4 mm^2 for the 50% level of maximal amplitude. For the separation distance of 7–11 mm, our calculations predicted an area of overlap of 30–10%. In comparison, our experimental data yielded 0.4–0.6 mm^2 and an overlap range of 25–15%. The slight difference for the pad area between optical data (0.6 mm^2) and the prediction (0.4 mm^2) is probably due to the fact that in this first attempt we did not consider the gradient of RF size along the distal–proximal extension of the paw. There is a striking agreement between the theoretically predicted data that incorporate RF size together with cortical magnification and the actually measured data which indicates that no further assumptions are needed to explain both the size of the point-spread function and their distance-dependent overlap. Combined, they provide an additional argument for the credibility of intrinsic reflectance measurements, and consequently for the interpretation of the data in terms of overlapping representational networks.

In order to explain the differential degree of representational overlap within the paw representations and across forepaw/hindpaw borders, specific properties of neural processing or of anatomical projectional patterns along these boundaries must be assumed. It is an interesting question how far such regions are characterized by anisotropic dendritic or axonal arborization or synaptic densities. On a functional level one could speculate about active suppression of existing connections. In any case, these findings bear interesting implication for the capacity of plastic changes that might act beyond such borders. For example, we have recently shown that the functional border between motor and somatosensory cortex can be reversibly relocated by nearly 1 mm.[6]

We can provide two-fold evidence that large cortical zones with a substantial degree of representational overlap can be regarded as a normal type of sensory representations. How can this view be reconciled with the notion of the rather sharply defined maps obtained following reconstructions of RF data? We argue that this contradiction does not arise because RF measurements give a wrong estimate

of cortical representations. Instead, the framework of maps *per se* cannot deal adequately with a concept of overlapping representations, as mapping procedures always implicitly make use of a point-to-point transformation.[17–19] When RFs are used to reconstruct the positional relationships between RFs into a cortical map, they are reduced to their centres, and their spatial extent, dimensions and overlap are not utilized. In our alternative view, each cortical location contains information not only about a corresponding point, but about an areal zone of the sensory field.

Conclusion

Using optical imaging of intrinsic signals, we addressed the problem of cortical representations and their overlap with specific emphasis of representational borders. We were able to demonstrate topographically ordered intrapaw representations of single digits and pads that are characterized by a substantial width of their point spread functions and by a substantial overlap between them. On the other hand, the fore- and hindpaw representations were clearly separated with no overlap, indicating that functional related representations are closely coupled by overlapping networks, while functionally independent but cortically neighbouring representations lack this overlap. We conclude that in contrast to the concept of maps which necessarily assume a point-to-point transformation, these overlapping networks in functionally related areas provide the substrate for distributed processing based on a point to area transformation.

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