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Functional mapping of cortical areas with optical imaging

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Sensory areas in mammalian cortex compute sensory inputs of different modalities in order to perceive the environment. Much is known about the anatomical pattern of inter-laminar connections, which form the basis of the computational process. Nevertheless, less is known about the functional relevance of these wiring patterns. We used intrinsic optical signals (IOSs) *in vitro* to investigate functional properties of inter-laminar connections in cortical brain slices of rat sensory cortex. By electrical stimulation in layer VI, a columnar-shaped IOS in all cortical areas was found. We detected different laminar patterns of activation in different cortical areas. In primary sensory areas, like primary visual cortex and primary somatosensory cortex, the peak intensity of IOSs occurred in layer IV, which receives the main thalamic input. In secondary sensory areas, like the secondary visual cortex or the secondary somatosensory cortex, the maximum of IOSs amplitude was shifted to layer II/III. In motor areas, IOS peak amplitude is located in layer II/III. In the hind limb area, considered as amalgam between sensory and motor function, a mixture of the activity patterns observed in primary sensory and a motor area occurred with a peak amplitude in layers II and IV. At different stimulation sites within one cortical area, the shape of columnar IOSs remained very similar, reflecting a canonical architecture of functional micro-circuitry. We conclude that both primary and secondary sensory cortical areas display their characteristic functional activation pattern, regardless of their sensory modalities.

Keywords: Intrinsic optical signals; Cortical micro-circuit; Column; Rat; Brain slice

Introduction

Whereas much is known about the anatomical connections and specific cytoarchitectonic properties in different cortical areas, our knowledge about the functionally relevant micro-circuitry in mammalian cortex is much more limited. In recent years, new insights in functional cortical circuitry have been revealed by optical techniques *in vitro* (Aizenman et al., 1996; Yuste et al., 1997) and *in vivo* (Bakin et al., 1996; Bonhoeffer and Grinvald, 1991; Ohki et al., 2005; Stosiek et al., 2003). In primary sensory cortex, the main thalamic input to cortical layer IV represents the first and most effective functional projection in the hierarchy of information processing (Bruno and Sakmann, 2006; Miller et al., 2001; Yuste et al., 1997). The functional projections into the secondary sensory areas have been studied less intensively.

The introduction of new optical methods, have greatly enhanced our ability to analyze the functional cortical micro-circuitry. Both membrane potential-sensitive and Ca^{2+}-sensitive fluorescent dyes confirmed the columnar shape of neuronal information processing units (Yuste et al., 1992, 1997) already discovered several decades before (Hubel and Wiesel, 1963; Powell and Mountcastle, 1959). The use of intrinsic optical signals (IOSs) *in vivo* led to a breakthrough in analyzing cortical information processing in sensory systems (Bonhoeffer and Grinvald, 1991; Malonek et al., 1994). *In vitro*, IOSs reflect activity-induced changes of extracellular space (ECS) volume (Fayuk et al., 2002; Holthoff and Witte, 1996, 1998; Witte et al., 2001). Nevertheless, several lines of evidence suggest that IOSs are strictly coupled to synaptic transmission. Firstly in cortical brain slices, the amplitude of optical signals correlates with the amplitude of extracellular field potentials, which reflect excitatory postsynaptic potentials (Holthoff et al., 1994). Secondly in hippocampus, IOSs occur only in layers in which synaptic transmission takes place (MacVicar and Hochman, 1991). And thirdly, the density of synaptic connections and the amplitude of activation determine the amplitude of IOSs (Dodt et al., 1993; Holthoff and Witte, 1996; MacVicar and Hochman, 1991).

Using IOSs we investigated the functional properties of different cytoarchitectonic cortical areas. Specifically, we asked the following questions:

- Do different cytoarchitectonic cortical areas display characteristic and therefore distinct activation columns?
- Are these patterns independent of the sensory modalities?
- Do the activation patterns change with intensity of activation?

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Materials and methods

Slice preparation

Experiments were done on brain slices of 14-day-old male Wistar rats. After ether anesthesia, animals were decapitated, and the brains were quickly removed and were cooled down to 4 °C in artificial cerebral spinal fluid (ACSF) [containing (in mM) NaCl 124, $NaHCO_3$ 26, KCl 3, $CaCl_2$ 2, $MgSO_4$ 2, NaH_2PO_4 1.25, and glucose 10, equilibrated with 5% CO_2 in O_2 to pH 7.4]. Coronal brain slices of 400-μm thickness were cut from occipital and parietal regions using a vibratome (Campden Instruments). To ensure that nerve fibers in the cortex ran parallel to the cutting plane, brain slices from parietal regions were cut with an angle of 20° to the coronal plane.

Imaging

Intrinsic optical signals (IOSs) were recorded as previously described (Holthoff and Witte, 1996). Brain slices were perfused submerged with warmed ACSF (32 °C) at 3 ml/min and equilibrated for at least 20 min before each experiment. Slices were illuminated in the dark-field configuration of an upright microscope (Axioskop FS, Zeiss) with near-infrared light (750±50 nm). IOSs were recorded at low optical magnification (Plan-Neofluar 2.5×, Zeiss) using a CCD-camera (C2400-77, Hamamatsu). Using the CCD-camera control device (Hamamatsu), the camera signal was contrast enhanced by a factor of 3. The shading correction unit of the camera control device was used to balance the overall brightness of the resulting image. Further image processing procedures were done using a video processing unit (DVS 3000, Hamamatsu, Herrsching). To detect IOSs, representing small changes in brightness of the dark-field illuminated slices, images were background subtracted and digitally enhanced. Therefore, the background intensity was measured before each stimulation by averaging 64 subsequent images. This background intensity image was subsequently subtracted from the current video signal. The resulting difference images were digitally enhanced by a factor of 4 to 8. The achieved video signal was stored on an S-VHS video recorder (Panasonic, Hamburg) and analyzed off-line with NIH-Image Software using a Macintosh Computer equipped with a frame-grabber card (Scion Corporation, Frederick MA).

Slices from occipital and parietal region were stimulated successively at different positions in layer VI at the border of the white matter. Each brain slice contained several different cortical areas, which were demarcated after the experiment (see below). The electrical stimulation was composed of a train (50 Hz for 2 s) of single stimuli, each 200 μs long. Beginning at the medial part of the slices, intrinsic optical signals were elicited successively every 500 μm moving laterally. The procedure was repeated going into the opposite direction from lateral to medial parts of the slices and stimulating interstitially of the positions of the first stimulation series. This procedure allowed us to get an estimate of the reproducibility of the optical signals in different cortical areas.

Cortical area demarcation

In a selected number of slices, the cortical areas were demarcated with two different methods. First, in both occipital ($n=4$) and parietal ($n=5$) slices cortical area boundaries were determined using the unprocessed microscopic dark-field images. Because the field of view did not cover the entire slices, only parts of the slices were recorded successively and the images of the whole slices were reconstructed off-line using Photoshop (Adobe). Using anatomical atlases (Paxinos and Watson, 1986; Zilles and Wree, 1995), cortical areas were identified by analyzing the relative thickness of the cortical layers and the characteristic appearance of different cortical and subcortical areas. An important criterion for cortical area demarcation was the relative thickness and appearance of layers IV and VI. In the parietal cortex, layer IV showed its largest width in area Par 1. In area Hl, layer IV was clearly identifiable but smaller than in Par 1. In the motor area Fr and the secondary sensory area Par 2, lamina IV was almost not detectable because of its small width. In parietal slices, the different appearance of layer VI in the dark-field images of different cortical areas was also used for demarcation. Area Par 1 could be distinguished from Par 2 and Hl because in this area layer VI appeared darker.

A similar procedure was used to demarcate cortical areas in occipital slices. The primary visual cortical area Oc 1 was delimited by its thick layer IV (Fig. 1). In contrast, layer IV was smaller in the secondary visual areas Oc 2L and Oc 2M. The granular (RSG) and agranular (RSA) retrosplenial cortical areas were easily demarcated from neocortical areas by their four-layered structure.

The results were verified in a second step by mapping the cortical areas in cresyl-violet-stained slices. In this case, the same slices were fixated, resliced with a kryotome in 50-μm steps, and stained with cresyl violet (Zilles and Wree, 1995). Both methods gave identical results with respect to the localization of cortical area boundaries (Figs. 1A and B). Therefore, in all other experiments only the dark-field images were used to demarcate cortical area boundaries. For better illustration, images of IOSs were smoothed (7×7 Gaussian filter, NIH-Image) and were color-coded. Red colors represent high optical signal amplitude, and blue colors represent low optical signal amplitude. Examples of unfiltered optical data of an occipital and a parietal region are shown in Figs. 4 and 5, respectively. All quantitative measurements were performed on unprocessed optical data.

Statistical values are reported as mean±standard deviation of the mean.

Results

All intrinsic optical signals (IOSs) elicited in 25 coronal brain slices of 17 animals showed a columnar shape. As described before (Holthoff and Witte, 1996), the IOS time course was slow. Signals peaked within the first 4 s after onset of stimulation and fell off to resting level during the following minute. After stimulating layer VI electrically, changes in IOS could be recorded in layers II to VI as shown before (Holthoff and Witte, 1996, 1998). Layer I was never involved and the signal stayed at pre-stimulus level. We found different laminar patterns of IOSs determined at peak amplitude depending on the cortical area, in which optical signals were elicited.

Primary sensory areas

In primary visual cortex, IOS amplitude was maximal in layer IV (Figs. 2 and 7A). The maximal IOS intensity in layer IV was clearly demarcated as a separate peak from adjacent layers. Intensity in layer II/III declined from the border of layer IV to layer I. At the edge to layer I, optical signals dropped suddenly to pre-stimulus levels.

IOSs in primary somatosensory area (Par 1) showed features similar to those in primary visual cortex (Figs. 2 and 7B). In Par 1,