Structural-Metabolic Organization of Field 4 of the Cat Brain in Normal Conditions and after Unilateral Enucleation of the Eye

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Comparative data on the structural-metabolic organization of field 4 of the cat brain in normal conditions and after unilateral enucleation of the eye are presented. Cytochrome oxidase was detected histochemically. Data were processed by a computerized method using an original video capture system. Data were obtained demonstrating the uneven distribution of enzyme along sublayer IIIb of field 4 in animals with unilateral enucleation. A hypothesis based on published data is suggested whereby the alternation of high- and low-reactive areas is evidence for the ordering of the retinal representations of the right and left eyes in the sensorimotor cortex.

KEY WORDS: sensorimotor cortex, field 4, structural-metabolic organization, unilateral enucleation of the eye, cytochrome oxidase.

The thalamofrontal associative system plays a special role in the organization of behavioral responses to sensory stimuli; in carnivores, this system includes the sensorimotor cortex (SMC), including field 4 [2]. One of the tasks of sensorimotor interactions at the level of the SMC is the organization of visually controlled movements. A key point in the development of our understanding of the performance of these movements consists of studies of the structure of visual inputs into the SMC [4, 7, 8]. Observation of the visual inputs to the SMC by traditional neuromorphological methods of assessing connections is extremely difficult because there is a series of cortical and thalamic centers which can mediate the transmission of visual information to this area of the cortex; at the same time, most of these structures are multimodal, i.e., can transmit information from several sensory systems [1, 9, 10]. The obvious interaction of the functional and metabolic needs of nervous tissue means that this question can be addressed by studying the dynamics of metabolism after sensory deprivation. Data on the metabolic and immunochemical labeling of the brain, summarized in Toporova's review [5] provide evidence that cytochrome oxidase (CO), an endogenous marker of functional activity in neurons, allows the spatial ordering of the representation zone of a receptor surface to be observed at different levels of the brain both in intact animals (barrels, barreloids in the somatosensory system of rodents) and after lesioning or stimulation. In particular, unilateral enucleation of the eye and unilateral suturing of the eyelids in monkeys and cats are followed by morphofunctional rearrangements, detectable by staining for CO, in all structures which are targets for inputs from the operated eye [5, 6, 15]. However, sensory inputs, as shown by physiological studies, are also characteristic of the SMC, where their role is to provide sensory support for movement [2, 4, 7].

Given that the level of enzyme activity can easily be altered by experimental treatments and is independent of the number of relay points between the receptor surface and the structure being studied, the aim of the present work was to undertake histochemical studies of CO activity in field 4 of the SMC in cats in conditions of unilateral enucleation of the eye. The literature contains no data on CO activity in the SMC as assessed in experiments of this type. Unilateral enucleation of an eye should change the sensory influx to the SMC, thus removing pools of heterogeneous CO activity reflecting the receptor representation.

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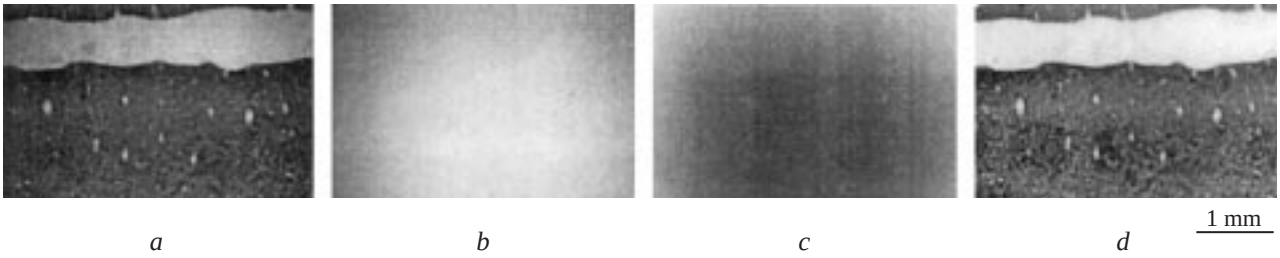

Fig. 1. Sequence of processes for ensuring illumination uniformity with the image capture system. *a*) Image of preparation; *b*) image of an empty microscope field in the same illumination conditions; *c*) image of the empty field after inversion; *d*) image obtained by summing the images of the preparation and the inverted empty field.

MATERIALS AND METHODS

Studies were performed on five adult male cats: three underwent unilateral enucleation and two were intact. Enucleation surgery was performed in collaborative experiments between the laboratory of Neurohistology, I. P. Pavlov Physiological Institute, and the laboratory for the Physiology of Sensorimotor Systems, A. A. Ukhtomskii Science Research Institute of Physiology, St. Petersburg State University. The surgical method has been described by Toporova et al. [6]. Under deep Nembutal anesthesia, animals' brains were perfused with isotonic sodium chloride solution followed by a fixative mixture containing 0.4% paraformaldehye and 1.25% glutaraldehyde in 0.1 M phosphate buffer pH 7.4. Brains were then removed from skulls and blocks were cut including the cortex of the anterior and posterior sigmoid gyrus; these were placed in serial sucrose solutions in phosphate buffer, with concentrations increasing from 10% to 30%, and frontal or sagittal cryostat sections of thickness 30 μm were cut. Each third section was incubated with reaction mix for detection of CO activity using the Wong-Riley method [6, 15]. Computer images of preparations were obtained using an instrument consisting of a Biolam-I microscope, a Mustek 300W camera, and a personal computer. The camera had an image resolution of 640 × 480 pixels and recorded data on the color of each pixel with six color bits.

A total of 25 sections from each cerebral hemisphere from intact and experimental were analyzed.

Image processing methods for histological preparations were adapted, and the GIMP image processing program suite (http://www.gimp.org) was used. Frequency proportions in images were altered by simultaneously using Fourier (http://www.geocities.com/SiliconValley/Way/1484).

Given that the investigation and photographing of preparations were performed at low microscope magnifications (objective ×2.5) while study areas were relatively large and differences in staining intensity were small, it was difficult to obtain uniform illumination over the whole field. The Kjeller method would not allow absolutely uniform illumination, so the following method was used. Before imaging, the optical densities of the darkest and lightest areas of the preparation were determined. The illumination brightness was selected such that the darkest transparent area (for example, the most clearly stained cell) was about 15% lighter than the absolutely opaque areas (for example, precipitates of stain), using the program to determine the optical density of image areas of interest. This was followed by image capture (Fig. 1*a*), followed by imaging of an empty microscope field (without preparation) in the same illumination conditions (see Fig. 1*b*); the image of the empty field was then inverted (see Fig. 1*c*). The two images were then summed point by point by using the program to create two layers – the upper was the image of the preparation, and the lower was the inverted image of the empty field. The resulting image was as close as possible to an image collected under conditions of ideally uniform illumination (see Fig. 1*d*).

After preliminary processing, the mean optical density of the preparation was measured, and the results were used for constructing plots.

RESULTS

Cytoarchitectonic layers of field 4 in frontal and sagittal brain sections of intact animals differed in terms of the staining intensity of the neuropil and neuron bodies. Cell bodies were clearly visible only in layer V; layers I, II, III, and VI had different levels of neuropil CO activity and therefore had different optical densities (Fig. 2*a*). Layer I had a low CO activity level, with a highly active narrow part of this layer directly beneath the pial surface. Staining along the whole layer was uniform. Layer II was characterized by high neuropil CO activity. Staining was uniform along the whole layer. Layer III could be divided into two sublayers with different staining intensities: an upper layer, which was the thinner, had moderate CO activity, and corresponded to sublayer IIIa, and a lower, thicker, highly active layer corresponding to layer IIIb. Staining along both layers was

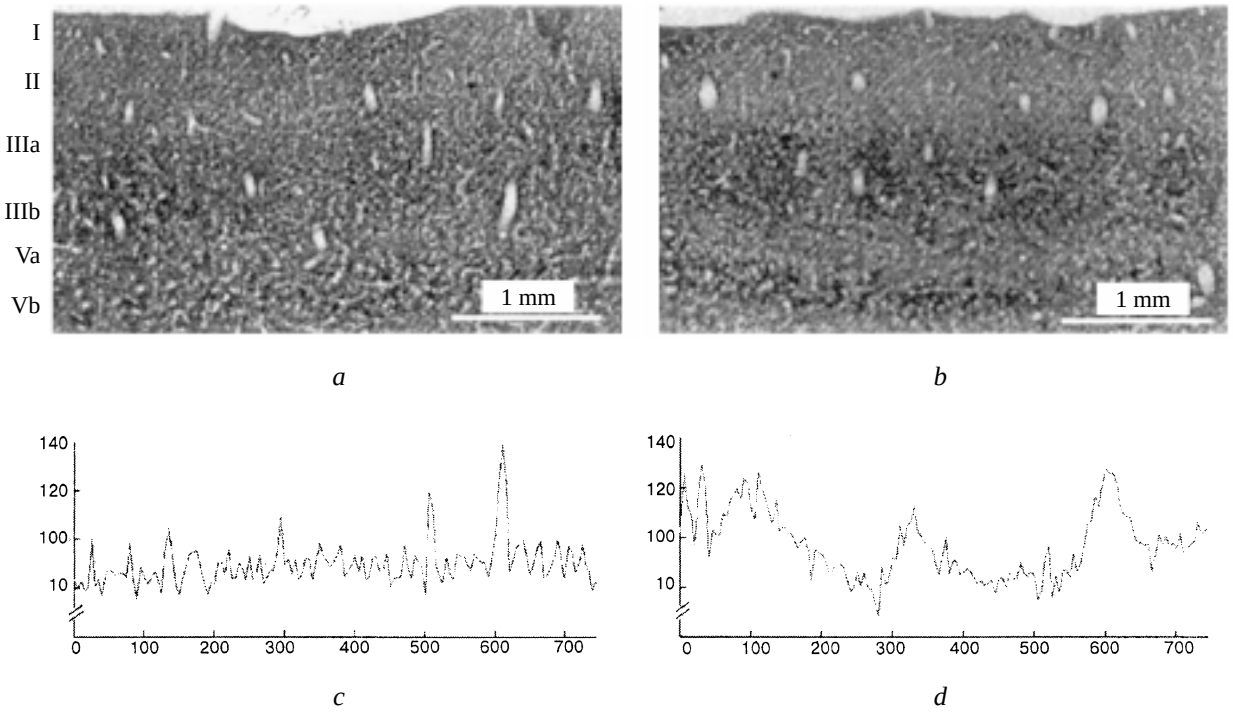

Fig. 2. Distribution of cytochrome oxidase (*a*, *b*) by layers and plots of the optical density (*c*, *d*) of sublayer IIIa of subfield 4γ in the brains of intact (*a*, *c*) and unilaterally enucleated (*b*, *d*) cats.

uniform. Layer V had low neuropil CO activity and could be divided into two sublayers: sublayer Va, in which a small number of intermediate and large intensely stained pyramidal neurons were clearly distinguishable, and sublayer Vb, consisting of groups of large pyramidal neurons with high CO activity. Neurons in this layer were clearly seen on a lighter background. Layer VI had uniformly low neuropil CO activity. The underlying white matter was characterized by very low optical density.

In enucleated animals, field 4 of the ipsi- and contralateral hemispheres also showed the layerwise distribution of enzyme activity seen in intact animals. However, unlike the situation in intact animals, layer III in enucleated animals showed non-uniform staining: intensely staining areas were separated by weakly staining areas. These regularly repeating spots with higher and lower levels of CO activity were most clearly seen in subfield 4γ of the posterior sigmoid gyrus of the contralateral hemisphere (see Fig. 2*c*). However, the diffuse boundaries of spots with smooth transitions from intense staining to weak staining made it difficult to measure them. Therefore, the mean optical densities along each layer and along cortical cross-sections were initially measured on computer images of subfield 4γ of the brains of intact and enucleated animals, after which plots of these values were made (see Fig. 2*b*, *d*). This processing supported visual observations of different CO-active layers of the cortex of subfield 4γ and the uniformity of enzyme distribution along the layers in an intact animal (see Fig. 2*b*). Plots of the optical density of layer III of field 4 in experimental animals provided evidence of the regular ordering of areas of high and low CO activity in sublayer IIIb, which was clearest in subfield 4γ (see Fig. 2*d*). Measurement of the areas with different levels of CO activity using these plots established the sizes of areas with different levels of CO activity, which ranged from 800 to 1200 μm. Study of the optical density plots in the cortex section showed that the main changes in CO activity occurred in sublayer IIIb.

A graphical demonstration of the non-uniformity of layer III was obtained after the proportion of optical frequencies in the image was altered. This processing gave the best visualization of repeating structures with different levels of CO activity in sublayer IIIb of field 4γ in unilaterally enucleated animals (Fig. 3*a*, *b*).

DISCUSSION

The results obtained from this histochemical study of CO activity followed by computer processing of video images of preparations led to the conclusion that in field 4 of the sensorimotor cortex of the cat brain, unilateral enucleation of the eye leads to changes in metabolic activity as compared with intact animals, with the appearance of alter-

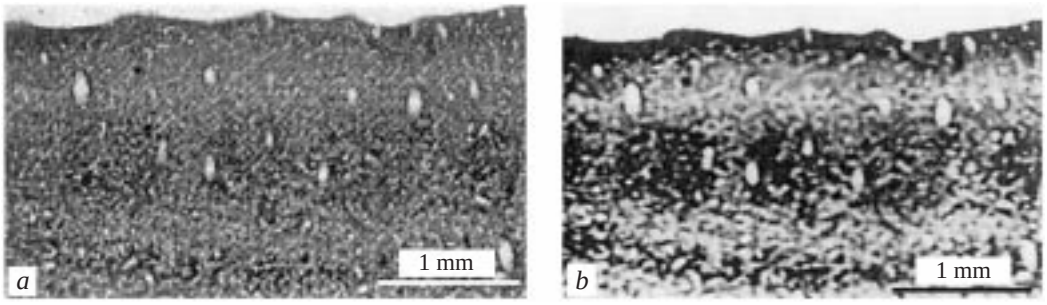

Fig. 3. Non-uniformity of cytochrome oxidase activity on a frontal section of subfield 4γ before (*a*) and after (*b*) changing the frequency composition of the images.

nating spots of increased and decreased enzyme activity located in sublayer IIIb. The alternation of these high- and low-activity areas probably provides evidence that the ordering of the retinal representations of the right and left eyes is characteristic not only for sense-specific brain centers [5, 14], but also for the SMC.

Analysis of published data on the organization of the afferent connections of field 4 leads to the conclusion that visual information to this field can arrive via thalamic, corticocortical intrahemispheric, and callosal projection fibers. Thus, corticocortical connections allow visual information to arrive from the associative cortical fields 7, 5a, and 5b [14].

The SMC receives myo- and somatotopically ordered inputs from several nuclei of the lateral thalamus [1, 3, 9, 10, 13]. All nuclei of the ventrobasal complex project to field 4 moderately weakly in cats. The delimiting zone [VL–VPL(bz)] (VL = ventrolateral nucleus; VPL = ventral posterolateral nucleus; bz = delimiting zone; VA = ventral anterior nucleus) is characterized by overlapping spinothalamic and cerebellothalamic terminals and has strong projections to field 4. The ventromedial nucleus and the thalamic transitional zone (VA–VL), whose afferent fibers come from the posterior interpositus nucleus of the cerebellum and the substantia nigra, have moderately poor projections to this field. In addition, field 4 receives inputs from the associative mediodorsal nucleus [1, 2].

The most powerful input to field 4 comes from the VL, as indicated by many studies [1, 3, 9, 10, 13]. The thalamic input from the VL nucleus of the lateral thalamus mediates inputs from the dentate and interpositus nuclei of the cerebellum. A characteristic feature of the terminal of the projection of this nucleus to field 4 is its strict relationship with sublayer IIIb, while projections from the other thalamic nuclei are more diffusely seeded into layers III, V, and VI [11, 12]. Terminals of the corticocortical connections are associated with sublayer IIIa and layer II [1].

Thus, the greatest changes in CO activity in enucleated animals were seen in sublayer IIIb, as shown by optical density plots, and this is an indirect indicator that the visual inputs to field 4 are mediated via the thalamic VL nucleus, a result which supports previous suggestions [7]. Evidence supporting this hypothesis is also provided by the sizes of areas with differing CO activity, which correspond to the branching diameters of single thalamocortical fibers running from the VL, which is 800–1000 μm [10].

Summarizing the current results and analysis of published data leads to the conclusion that changes in the metabolic activity of individual neuron pools in field 4 of the SMC in cats occur after enucleation because of disturbances in the sensory afferent influx. This fact is probably structural evidence for the arrival of visual information in the SMC and explains the ability of neurons of this area of the cortex to execute programs for visually controlled movements.

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