Neuroscience and Behavioral Physiology, Vol. 35, No. 4, 2005

Neuronal Connection of the Cortex and Reconstruction of the Visual Space

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Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 89, No. 10, pp. 1281–1290, October, 2003. Original article submitted April 30, 2003, revised version received May 28, 2003.

The distributions of retrograde labeled cells in fields 17 and 18 and the fields 17/18 transitional zone were studied in both hemispheres of cats after microiontophoretic administration of horseradish peroxidase into individual cortical columns in fields 17, 18, 19, and 21a. The clustered organization of the internal connections of the cortical fields, the asymmetrical locations of labeled callosal cells relative to the injected columns, and the defined distribution of labeled cells in layers A of the lateral geniculate body suggested that eye-specific neuronal connections support "binding" of the visual hemifields separately for each eye. Application of marker to columns in fields 19 or 21a demonstrated disparate inputs from fields 17 and 18 and the fields 17/18 transitional zone. It is suggested that these connections may support the extraction of loci and stereoscopic boundaries located in the central sectors of the visual space.

KEY WORDS: cat, fields 17 and 18, cortical column, internal, interhemisphere, and disparate connections, horseradish peroxidase.

The visible space is reflected on the retinas according to the laws of geometrical optics. The point-by-point projection of the retina creates a screen representation of the visual picture in the primary projection fields of the cortex, where its reconstruction starts. However, the representation of the visual space in the cortex and the neuronal connections supporting its reconstruction depend on the anatomical organization of the visual system.

In ungulates and fish, the two eyes pointing to different sides support panoramic vision. The scheme of visual projections to the cortex in animals with laterally located eyes is shown in Fig. 1*a*. The visual space contains five objects, identified by numbers, but only one of these (object 3) is seen by the animal with both eyes. The complete overlapping of fibers in the visual chiasma has the result that the left hemisphere receives inputs only from the right eye, while the right hemisphere receives inputs only from the left eye. Thus, one half of the field of vision is represented in each hemisphere, and via only one eye. Reconstruction of the integrity of objects projected in both hemispheres requires interhemisphere connections. Assessment of depth in the surroundings of object 3 is also only possible with interhemisphere connections between the inputs from the two eyes.

Complete overlapping of fibers in the optic chiasma is seen in some animals with frontally positioned eyes, such as Siamese cats (Fig. 1*b*). In this case, each hemisphere of the brain receives inputs from only one eye, though they cover the entire field of vision. Since the fields of vision of the eyes overlap, these animals are capable of stereoscopic vision. This requires the formation of binocular neurons receiving inputs from both eyes, and this is only possible with interhemisphere connections. This is known to occur not in fields 17 or 18, where neurons have small receptive fields, but in the associative fields of the cortex, which are characterized by large receptive fields [13, 26].

In higher mammals (particularly predators and humans), the frontal positioning of the eyes is associated with partial overlapping of fibers in the visual chiasma (Fig. 1*c*). Fibers from the nasal half of the retina pass to the contralateral hemisphere, while those from the temporal half pass to the ipsilateral hemisphere. The result of this distribution of pathways is that each hemisphere of the brain contains the representation of only the contralateral half of the field of vision, though from both eyes. It is apparent that

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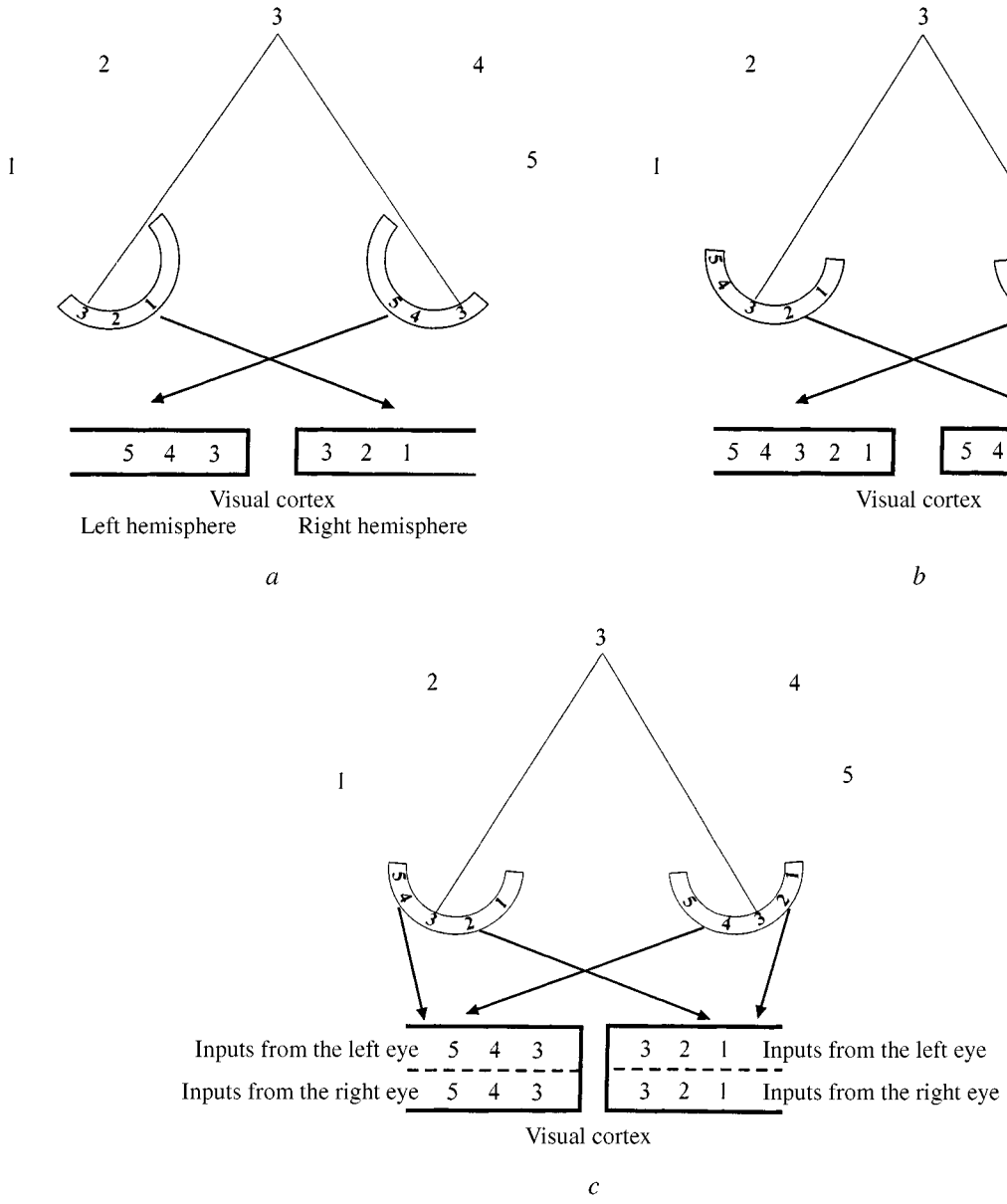

Fig. 1. Diagram of the projections of objects 1–5 in the visual cortex of the left and right hemispheres in animals with laterally positioned eyes and complete crossing of fibers in the optic chiasma (*a*), with frontally located eyes and complete crossing of fibers in the optic chiasma (*b*), and frontally positioned eyes with partial crossing of fibers in the optic chiasma (*c*).

combination of the inputs from the two eyes, i.e., the formation of binocular neurons, can in this case occur by means of intrahemisphere connections. However, the problem of connecting the two halves of the field of vision arises, as they are located in different hemispheres. As the boundary at which the halves of the field of vision are joined passes through the zone of maximal visual acuity, interhemisphere "binding" must be reliable and accurate. Thus, the main task which needs to be resolved for us to understand the mechanism of reconstruction of the visual space in the cortex of these animals is identification of the structure of the connections between the four visual pathways connecting the two retinas to the two hemispheres.

METHODS

Studies were performed on 30 adult cats weighing 3.0–3.5 kg. Connections were studied using horseradish peroxidase (HRP), which is subject to retrograde transport. HRP was applied to individual columns of neurons in fields 17, 18, 19, and 21a of the cortex. The HRP application zone

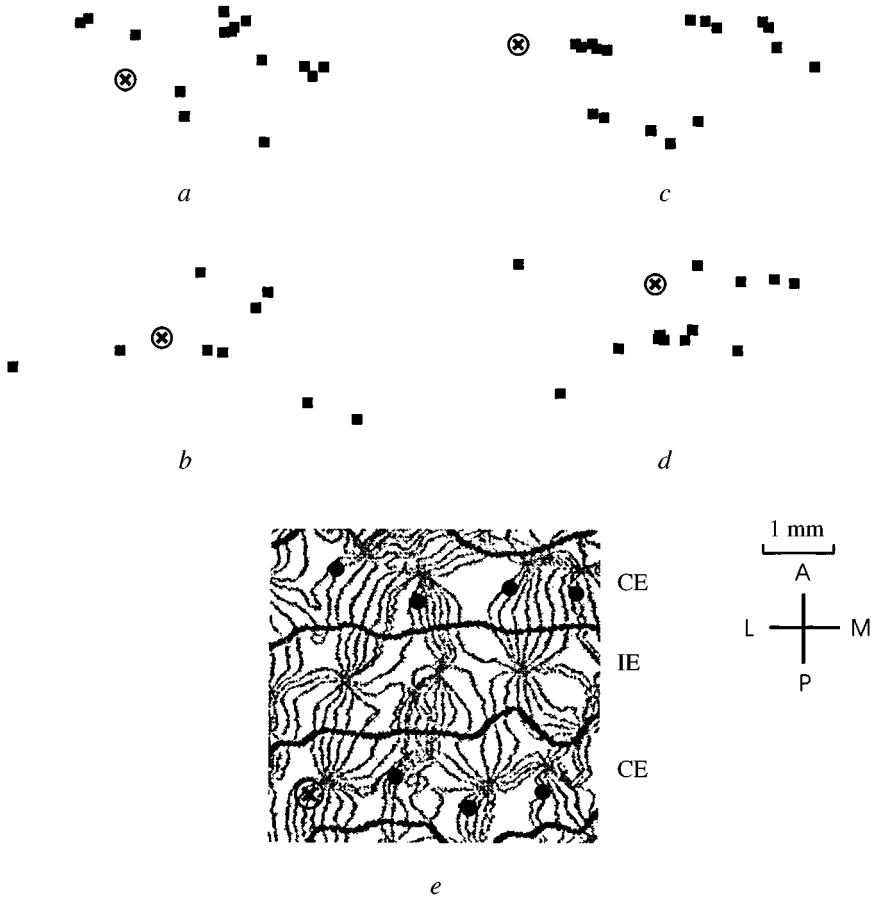

Fig. 2. Grouping of field 17 cells sensing axons to an individual cortical columns in field 17 in a cat. *a*–*d*) Plan view of the cortical surface onto which the cells in surface layers are projected; crosses show the injected column; *e*) suggested locations of clusters on a map of cortical neurons in terms of orientational preference and eye dominance [15]. Lines running vertically show isoorientation areas of the cortex; CE and IE show zones dominated by the contra- and ipsilateral eyes respectively.

diameter was up to 200–300 μm, which corresponds to the size of one or two orientation columns; in any case, zones were within a single eye-dominance column [9, 12]. Peroxidase was applied microionophoretically to the whole depth of the cortex, perpendicular to its surface (Fig. 3*a*, *b*). The skull was trepanned, the dura mater was opened, and microiontophoresis were performed under anesthesia (Calypsol or Nembutal, 40 mg/kg, i.m.).

Peroxidase (Boehringer) was used as an 8% solution in potassium phosphate buffer (pH 6.2) containing 0.1 M NaCl. An enzyme-filled glass microelectrode was oriented perpendicularly to the cortical surface and was inserted with a stepper to a depth of 1200–1500 μm. The microelectrode was then retracted by 200–300 μm and iontophoresis was performed with a constant current of +0.5 μA for 20 min (the reference electrode was attached over the other hemisphere). After the current was switched off, the microelectrode was retracted and kept for 10 min at a depth of 600–800 μm. After 36–48 h, cats were subjected to deep Nembutal anesthesia (100 mg/kg) and brains were fixed by perfusion. The following day, a cryomicrotome was used to cut continuous series of frontal brain sections of thickness 50 μm. Peroxidase was detected as described by Mesulam [14], after which sections were counterstained with safranin. The boundary of fields 17 and 18 was identified by staining several sections close to the application zone with toluidine blue as described by Nissl.

The continuous series of brain sections was then used to reconstruct the locations of labeled cells in fields 17 and 18 relative to the column treated with peroxidase. We addressed the distribution of cells in the tangential plane parallel to the cortical surface, corresponding to the projection of the field of vision. In addition, the numbers of labeled cells in the eye-specific layers A and A1 of the dorsal nucleus of the lateral geniculate body were identified.

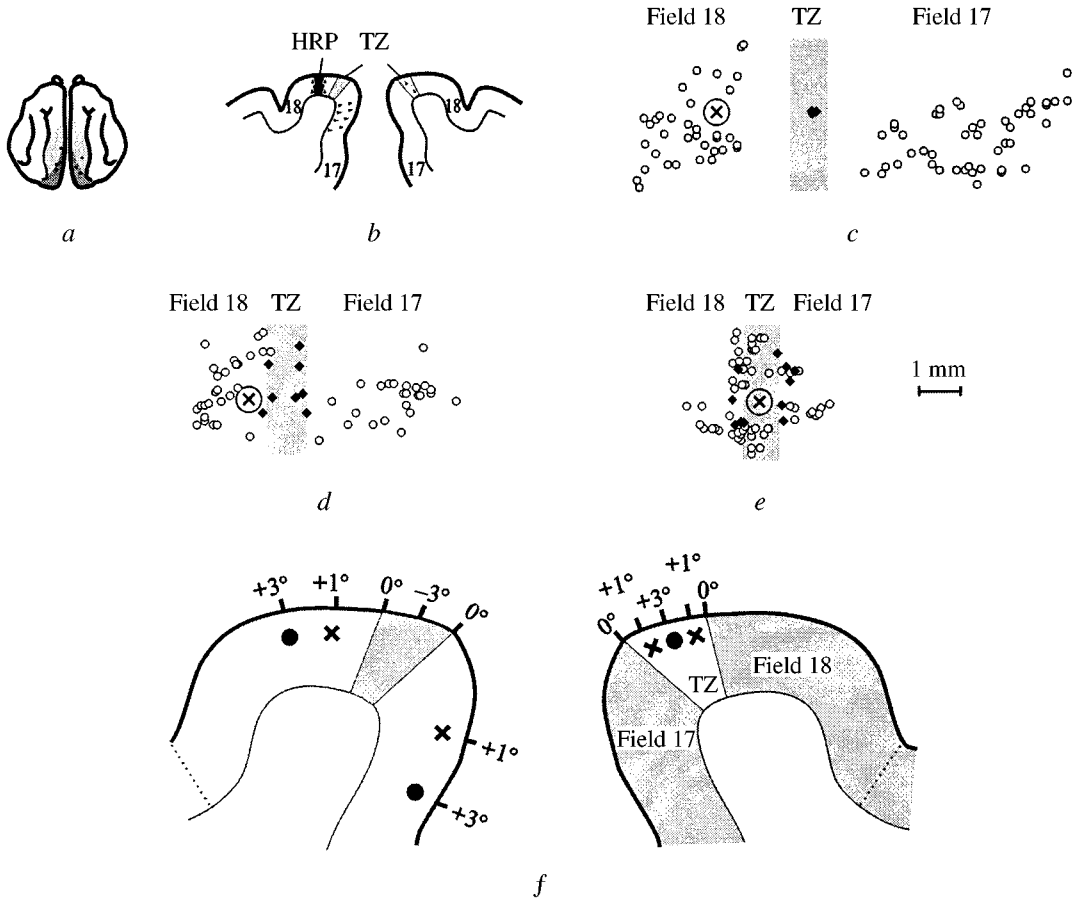

Fig. 3. Distribution of labeled cells in fields 17 and 18 of the cat cortex after application of horseradish peroxidase (HRP) to a column of neurons. *a*) Location of the study columns in fields 17 and 18; *b*) injected column in field 18 and labeled cells of both hemispheres on a frontal section of the brain; *c, d, e*) plan view of the cortical surface on which labeled cells of all layers are projected (the distribution of labeled cells in the opposite hemisphere are superimposed on the distribution of labeled cells in the injected hemisphere). White circles show labeled cells in the injected hemisphere; black diamonds show labeled cells in the opposite hemisphere; crosses show the position of the injected column; TZ = fields 17/18 transitional zone; *f*) diagram showing the relationship between the retinotopic coordinates of zones of fields 17 and 18 in one hemisphere (left) and the transitional zone of fields 17/18 of the opposite hemisphere (right).

RESULTS AND DISCUSSION

The internal, interhemisphere, and efferent connections of fields 17 and 18 were studied after application of horseradish peroxidase to 47 columns in fields 17, 18, 19, and 21a, representing different parts of the visual field (from –40° to +10°).

Internal Connections of Fields 17 and 18. Connections between cells in one cortical field formed by axons not entering the white matter were regarded as internal [20]. Previous studies [1] showed that the extent of axons from cells supporting the horizontal connections of fields 17 and 18 depend on the direction in which they travel. In field 17, the longest axons ran in the direction coinciding with the projection of the horizontal meridian of the field of vision, while in field 18, the longest axons accompanied the projection of the vertical meridian. This corresponds to the anisotropy of the magnification factors of these cortical fields for different meridians of the visual field, which in turn are determined by the distributions of the retinogeniculate inputs [24]. Thus, the microtopography of horizontal connections supporting the integration of information within the visual hemifield is concordant with the macrotopography of these fields.

With the aim of identifying the functional properties of connected cortical cells, the internal structure of the zones containing labeled cells was studied. Cells sending axons to individual columns of fields are non-uniformly distributed within the cortical field. Grouping of cells was most clearly evident in the upper layers of the cortex (Fig. 2*a–d*). Each plot is a plan view of the flattened surface of field 17 onto which the bodies of labeled upper-layer cells are projected. Groups of cells (clusters) formed two parallel rows, sepa-

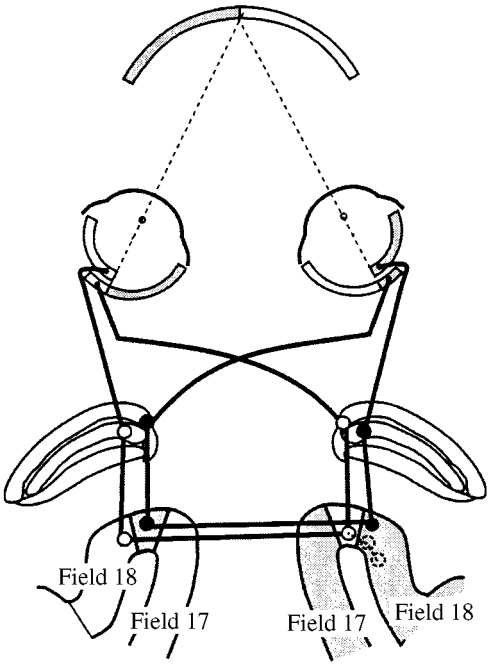

Fig. 4. Diagram of interhemisphere connections of fields 18 of the cat cortex. The similarly organized connections of field 17 are not shown. Internal horizontal connections of field 18 are shown by dotted circles only in the right hemisphere.

rated by a distance of about 1200 μm; the injected columns of neurons were located in one of these rows. The rows were oriented perpendicularly to the boundary of fields 17/18 regardless of the retinotopic coordinates of the application site. Clusters forming rows were located at smaller distances, i.e., about 800 μm.

Optical visualization of neuronal activity demonstrated [5, 6, 11, 15, 23] that orientation and eye-dominance columns form regular and periodically repeating patterns on the cortical surface, these being formed by the isoorientation and eye-dominant bands which intersect at a right angle. Our data were compared with the figure presented in [15] (Fig. 2*e*) which showed the distribution of orientation and eye-dominance columns. This comparison provides grounds for suggesting that horizontal connections link cells preferring the same orientation and having the same eye dominance. Similarity of cells in terms of only one of these features is an insufficient condition for the formation of horizontal connections between them. Our data (Fig. 2) indicate that an individual cortical column receives inputs from groups of neurons from 6–8 different hypercolumns [9]. These connections may support the integration of local information into more global percepts, such as the outlines at a specified level of contrast. Visuotopic maps [24] show that central columns may support integration over several degrees of the field of vision, while peripheral columns may cover more than 10°.

The suggestion that some proportion of the cells located between clusters are also connected to neurons in the columns studied should be noted. However, since these connections are not detected with peroxidase, which is not transported trans-synaptically, these connections are not direct but must be mediated by intercalated interneurons. Interneurons can be activated by known [18] inhibitory interactions between cells preferring different orientations or by cells receiving inputs from different eyes. This may be the basis of image segmentation.

Interhemisphere Connections. The anatomical and functional continuity between the hemispheres and, thus, between the projections of the visual hemifields, is supported by connections via the corpus callosum. Early morphological and neurophysiological studies [10, 21, 25] showed that callosal cells and callosal recipient cells are located close to the boundary between fields 17 and 18 and that their receptive fields intersect the vertical meridian or the areas immediately adjacent to it. More recent studies have demonstrated a transitional zone between fields 17 and 18, in which part of the ipsilateral visual hemifield is represented [19]. Local application of fluorescent stains and analysis of the locations of cells retrogradely labeled in the contralateral hemisphere showed [16] that callosal fibers link mirror-non-symmetrical areas of the cortex in the two hemispheres, but nonetheless representing the projections of one and the same part of the visual field.

We have also observed this asymmetry in the locations of areas of the two hemispheres linked by interhemisphere connections after application of peroxidase to individual columns of neurons [2, 4]. After application to a column in field 17 or 18, the only cells labeled in the contralateral hemisphere were in the transitional zone between fields 17 and 18 (Fig. 3*b–d*). After application of peroxidase to columns in the transitional zone, cells in fields 17 and 18 of the contralateral hemisphere were labeled (Fig. 3*e*). Depending on the distance of the injected column from the transitional zone, there were changes in the positions of labeled cells in the transitional zone of the contralateral hemisphere. Columns located far from the transitional zone received inputs from groups of cells in the center of the transitional zone (Fig. 3*c*). Columns located close to the transitional zone received inputs from two groups of cells in the transitional zone (Fig. 3*e*). This labeling pattern could be seen when 1) cells connected by interhemisphere pathways represented the same area of the field of vision, and 2) the transitional zone contained two mirror-symmetrical representations of the ipsilateral visual hemifield related to fields 17 and 18. This is shown in detail in Fig. 3*f*.

However, about a third of columns located in the callosal zone of fields 17 and 18 did not have interhemisphere connections. In the transitional zone between fields 17 and 18, all columns had interhemisphere connections. This raises the question of which eye sends inputs to columns with

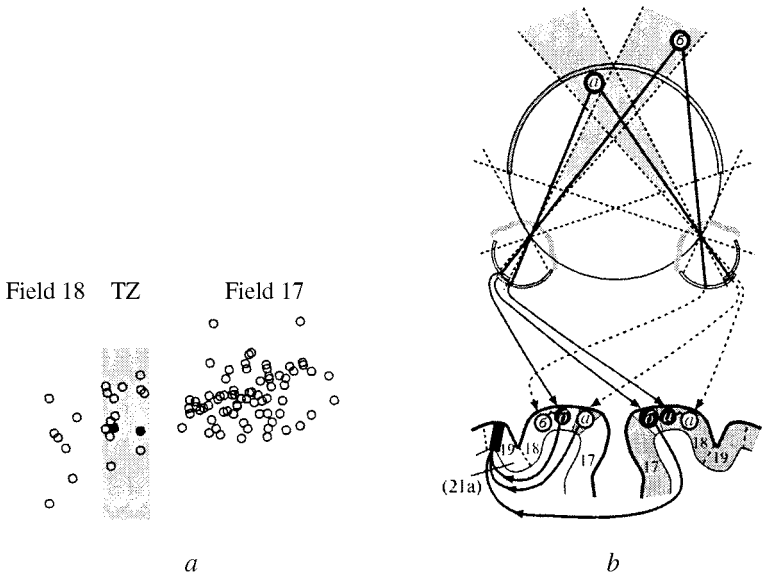

Fig. 5. Disparate inputs of columns in fields 19 and 21a. *a*) Distribution of labeled cells in fields 17 and 18 and the fields 17/18 transitional zone in a cat after detection of horseradish peroxidase in a column of neurons in field 21a. For further details see caption to Fig. 3*c–e*; *b*) diagram showing the connections of a column of field 19 (21a) with field 18 and the fields 17/18 transitional zone. (The similarly organized connections with field 17 are not shown.) The upper part of the diagram shows the projections to field 18 of two loci (*a*, *b*) of the visual space which may be represented in this column. White circles show cells receiving an input from the right eye labeled on a frontal section of the brain; the black rectangle shows a connection mediated by an interneuron.

interhemisphere connections and which sends input to columns without interhemisphere connections.

Neurons of the main input layer IV of columns are known to be monocular [9]. The numbers of labeled cells in the eye-specific layers A and A1 of the lateral geniculate body were measured for both types of column in field 17 and 18. This showed that columns without interhemisphere connections produced more labeled cells in layer A than in layer A1 (the A/A1 ratio was 2.0 ± 0.63). Thus, these columns received inputs preferentially from the contralateral eye. The A/A1 ratio for columns with interhemisphere connections was 0.60 ± 0.12, showing that these columns received inputs preferentially from the ipsilateral eye.

Olavarria [17], working at the same time as ourselves, showed that callosal neurons in fields 17 and 18 (outside the transitional zone) were located mainly in the dominance columns of the ipsilateral eye, while callosal neurons of the transitional zone of fields 17/18 were located in the dominance columns of the contralateral eye. Thus, our and Olavarria's data lead to the conclusion that callosal cells and cells receiving callosal inputs in the transitional zone of fields 17/18 are associated with visual pathways crossing in the chiasma and that these cells in fields 17 and 18 (outside the transitional zone) are associated with non-overlapping pathways.

The organization of interhemisphere connections as identified here is shown in Fig. 4. Eye-specific interhemisphere connections extend to those parts of the cortex in the two hemispheres which receive inputs from the zones of the nasotemporal overlap on the retina. In cats, this zone is a vertical strip in the temporal part of the retina, with a width of 25 ganglion cells, these projecting to the different hemispheres [18, 22]. Interhemisphere connections "bind" the projections of the left visual hemifield of the right eye located in the two hemispheres, as well as the projections of the right visual hemifield of the left eye, again located in the two hemispheres. Thus, the hemispheres are combined. However, these connections do not link the projections of the left and right visual hemifields from each of the eyes.

The internal horizontal connections of the fields are, firstly, eye-specific, and, secondly, extend to the transitional zone of fields 17/18, where the other visual hemifield is represented. They can therefore provide further "binding" of the projections of the left and right visual hemifields of each eye into a single whole (these connections are shown as dotted lines only in field 18 of the right hemisphere in Fig. 4).

Since no subcortical input from the ipsilateral eye to the transitional zone of fields 17/18 is observed [8], it can be concluded that in the right hemisphere, combination of the hemispheres and combination of the visual hemifields is mediated by cells receiving inputs from the left eye, while

these combining operations in the left hemisphere are performed by cells receiving inputs from the right eye.

Connections Supporting Stereoscopic Perception. At the level of the primary visual cortex, these connections are those forming binocular neurons tuned to different loci in the visual space. All known types of binocular neurons in the primary visual cortex show both excitatory and inhibitory interactions between the inputs from the two eyes [7], i.e., binocular connections are mediated by inhibitory interneurons. These di- and polysynaptic connections cannot be detected with the marker used here.

However, studies of the distribution of efferent cells in fields 17 and 18 sending axons to columns in fields 19 and 21a showed disparate inputs. In this case, labeled cells were located in fields 17 and 18 of the ipsilateral hemisphere, with a separate group of cells in the transitional zone between these fields, as well as in some columns in the transitional zone of the opposite hemisphere (Fig. 5*a*). The inputs of columns located in the transitional zones of fields 17/18 of the ipsilateral hemisphere in relation to the other identified inputs represent the other visual hemifield (Fig. 5*b*). We suggest that these input cells are initially connected to the opposite eye. Otherwise, the monocular receptive field of neurons in fields 19 and 21a would consist of two widely separated zones, a situation not supported by neurophysiological data.

There are two possible interpretation of these data. 1. If cells converging in a column in field 19 or 21a are monocular, then the receiving cells in columns would be binocular and, as shown in Fig. 5*b*, would be tuned to loci within the Weiss–Muller area (convergent disparity). 2. If cells converging on a column are binocular, i.e., tuned to loci in the visual space, then parts of a stereoscopic surface would be represented in columns as a result of the integration of information relating to several loci of the visual space. This raises a question – which visual space sector loci can be represented in those parts of fields 17 and 18 containing input cells for columns in field 19 and 21a? These loci were found to be located in the central sectors of the space around the Weiss–Muller zone. These sectors are shaded in Fig. 5*b*. A more detailed analysis of the projections of the loci of different sectors of the space in the cortex is presented in [3].

Thus, the initial stages of the reconstruction of the visual space in the cortex can be identified on the basis of data on the topography of direct connections between individual cortical columns.

This study was supported by the Russian Fund for Basic Research (Grants Nos. 00-04-49289 and 03-04-48258).

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