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Segregation of on- and off-center afferents in mink visual cortex

(area 17/kainic acid/electrophysiology/ocular dominance)

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ABSTRACT **In the lateral geniculate nucleus of the mink, on-center and off-center neurons occupy separate layers [LeVay, S. & McConnell, S. K. (1982) *Nature (London)* 300, 350–351]. To study the mode of termination of geniculate afferents in area 17, we recorded from their terminal arborizations in layer IV after the destruction of cortical neurons by injection of kainic acid. At the majority of recording sites, multifiber responses were entirely or predominantly of one type: on-center or off-center. Responses obtained during perpendicular penetrations showed the same preferred sign of contrast throughout the thickness of layer IV. During tangential penetrations through the layer, we encountered sequences of on- and off-center activity separated by stretches of mixed responses. We conclude that on- and off-center afferents terminate in separate, alternating patches that occupy the full thickness of layer IV. These coexist with another set of patches in which the same afferents are segregated by eye of origin.**

Most retinal ganglion cells have on-center or off-center receptive fields. An on-center cell is excited by an increase in light intensity in the center of its receptive field; an off-center cell responds to a decrease in intensity (1). Neurons in the lateral geniculate nucleus, the main target of the optic nerve, also have on-center or off-center receptive fields; there appears to be little convergence of on- and off-center inputs at this level (2–4). Geniculate neurons project in turn to the visual cortex. How cortical receptive fields are elaborated from geniculate inputs is not yet clear, but there is evidence for at least partial convergence of the on and off channels onto single cells (5–8).

Recent work has revealed a surprising degree of anatomical order in the on/off system. In the retinas of those species studied, on-center and off-center ganglion cell bodies are intermingled, but their dendrites ramify at different levels in the inner plexiform layer (9, 10). In the lateral geniculate nucleus, the arrangement of on-center and off-center cells varies among species. In the cat, for example, on and off cells are intermixed within single layers of the nucleus. In the mink, another carnivore, on- and off-center cells are found in separate layers: there is an on and an off layer for each eye (11). At least partial segregation of on and off cells in the lateral geniculate nucleus has been reported for ferret (12), monkey (13), and tree shrew (14), but the sequence of the layers varies.

Neuroanatomical studies in various species have shown that cells in different geniculate layers can project differentially to the visual cortex, their axons terminating in either separate columns (15) or layers (16). We have therefore examined whether on-center and off-center inputs are anatomically segregated in the visual cortex of the mink. To do this, we have recorded directly from geniculate terminals in area 17 after silencing the activity of cortical cells in the vicinity with injections of kainic acid, a neurotoxin that selectively destroys cell bodies and spares axons (17). The kainic acid technique was developed by Helen Sherk in this laboratory.

METHODS

Recordings were made from the primary visual cortex (area 17) of 14 adult male minks (*Mustela vison*) of the dark ranch variety (Berkshire Fur Farms, Hinsdale, MA). Minks were anesthetized with a mixture of ketamine hydrochloride (Ketaset, Bristol Laboratories, 50 mg/kg) and xylazine (Rompun, Bayvet, 3 mg/kg i.m.). Supplemental doses were given as required during the experiment. Rectal temperature was maintained at 38°C. In early experiments, atropine and phenylephrine were applied to the corneas; in later experiments, these drugs were not used. Minks were fitted with contact lenses matching the corneal curvature (3.6-mm radius). Although the animals were not paralyzed, eye position remained stable over the course of the experiment (as judged, for example, by the recording of identical receptive field positions during lowering and withdrawal of the electrode). Kainic acid (0.2% in saline) was first injected into area 17 from a micropipette. The drug was injected during withdrawal of the pipette, at a rate of 0.13 μl per mm of cortex traversed. Recordings were then made with a tungsten microelectrode (10–20 MΩ at 1 kHz) advanced parallel to the micropipette track at a distance of 200–500 μm from it. Responses to visual stimulation were tested at 25-μm intervals; stimuli were stationary flashing light spots of 0.5°- to 5°-diameter. We did not attempt to distinguish between X-like and Y-like responses. Small electrolytic lesions (1–1.5 μA for 3 sec, electrode positive) were made at various points of interest over the course of the penetration. Up to four penetrations were made in the vicinity of a single kainic acid injection. The amplitude of responses began to decrease $\approx$3 hr after the injection of kainic acid, at which time we either moved to the other hemisphere or terminated the experiment. Minks were perfused with 10% formol saline; brains were cut in frozen sections at 20 μm and stained with cresyl violet for reconstruction of electrode tracks.

RESULTS

Kainic acid silenced cortical neuronal activity, greatly enhancing recordings from the terminal arborizations of geniculate afferents. Outside of layers IV and VI, no neuronal activity, whether visually evoked or spontaneous, could be recorded. (The only exception was that we occasionally recorded faint responses in layer V.) In layer IV, and to a lesser extent in layer VI, rich multiunit activity consisting of fast low-amplitude spikes could be recorded at virtually every recording site. We believe for several reasons that this activity arose from geniculate afferents. First, it was encountered only in the layers where geniculate afferents terminate (18). Second, the aggregate activity often included several recognizable units. These units were monocular, nonoriented, and spontaneously active; they had center-surround receptive

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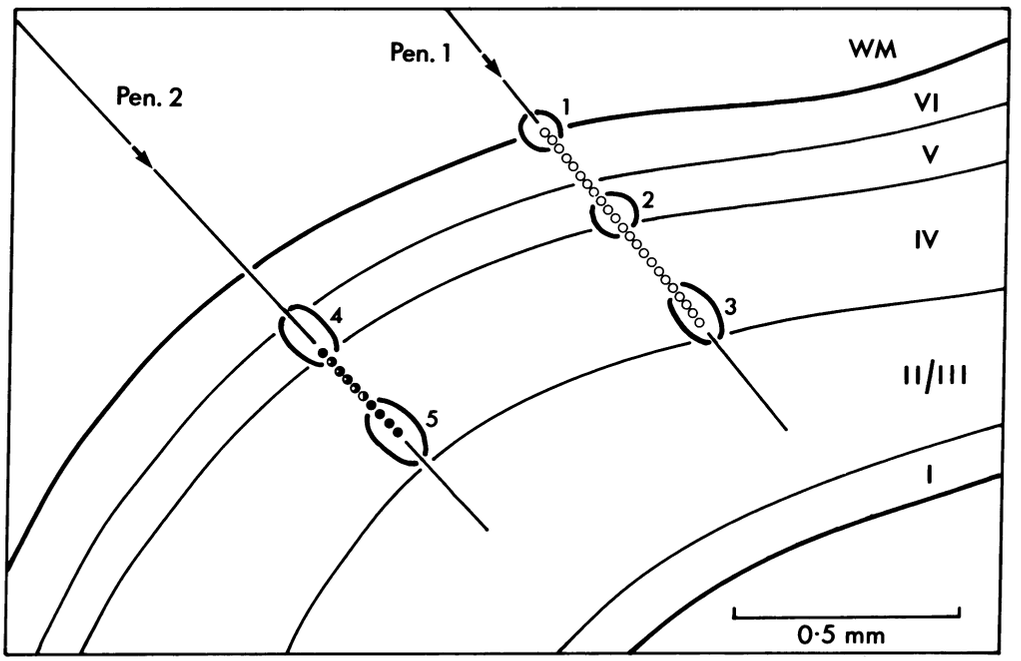

FIG. 1. Histological reconstruction of two electrode penetrations made nearly perpendicular to the cortical layers in the tentorial region of area 17. Parasagittal section, dorsal up and posterior to the right. The penetrations enter the cortex from the white matter (WM). The type of multifiber response obtained at each recording site is represented by the following symbols: circles, contralateral eye; squares, ipsilateral eye (Figs. 2 and 3 only); open symbols, pure on-center; quarter-filled, predominantly on-center; half-filled, mixed; three-quarter-filled, predominantly off-center; filled, pure off-center. Electrolytic lesions are outlined and numbered. In penetration 1, weak on-center activity was recorded as soon as the electrode entered layer VI (lesion 1). As the electrode advanced into layer IV, the activity greatly increased in strength but remained pure on-center (lesion 2). Activity ceased as the electrode left layer IV (lesion 3). In penetration 2, 700 μm away from penetration 1, responses were obtained only in layer IV (lesions 4 and 5) and were predominantly off-center. Only contralateral eye responses were obtained in both penetrations.

field organization resembling that of units described in the lateral geniculate nucleus of the mink (11). The optimal stimulus for the unresolved multiunit activity was also a small spot (1–3°) centered in the aggregate receptive field; increasing the spot diameter caused a decrement in response.

We commonly found that multiunit activity at a given recording site was exclusively or predominantly on-center or off-center, suggesting that these two types of afferents are not completely intermingled in the cortex. Nevertheless, at many sites roughly equal mixtures of on- and off-center units were recorded. Of 1502 recording sites, 22% gave pure on responses, 10% were on-dominated, 37% were about equally mixed, 13% were off-dominated, and 18% gave pure off responses.

In two animals, five electrode penetrations were made at an angle perpendicular to the cortical layers. In each of these penetrations, similar responses were obtained throughout the thickness of layer IV. For example, Fig. 1 shows two penetrations made 700 μm apart in the same animal. In one penetration, responses were exclusively on-center; in the other, responses were predominantly off-center or off-dominated. Of the remaining three penetrations (not shown), two were similar to those described above, and in the third, a mixture of on- and off-center responses was found throughout layer IV. These results suggest that on and off afferents are segregated into patches that extend through the entire thickness of layer IV, and perhaps through layer VI as well (Pen. 1). In addition, the activity encountered during perpendicular penetrations was driven solely by one or the other eye.

During tangential penetrations, on the other hand, we observed sequences of on- and off-center activity in layer IV, and the preferred sign of contrast changed at irregular intervals. Fig. 2 shows a reconstruction of a penetration that passed tangentially through layer IV for 1.5 mm, in the course of which three major functional regions could be identified: an off sequence of about 275 μm, an on sequence of about 400 μm, and finally an on-dominated sequence of about 125 μm. These regions were separated by regions of mixed activity ≈150 μm long. While throughout most of the penetration responses were obtained only through the contralateral eye, there were two short stretches in which activity could be driven through both eyes. (More typically, contralateral eye responses dropped out as the electrode traversed an ipsilateral eye column.) During the course of this penetration, aggregate receptive fields progressed across the visual field in an orderly fashion (Fig. 2). At some sites where both on and off activity were recorded, the on-center and off-center receptive fields were separate from one another or only partially overlapping (not shown).

We attempted to delineate the on and off patches further by making multiple parallel tangential penetrations. Fig. 3 shows a reconstruction of three such penetrations spaced 150 μm apart. The most striking feature of this reconstruction is a patch of on-center activity in the ipsilateral eye, 200 μm wide and extending the full thickness of layer IV. The match between the three penetrations is not as good in other regions, probably because the penetrations do not lie in a plane exactly orthogonal to the layers.

In total, 36 mm of layer IV were studied during tangential penetrations (45 penetrations in 12 minks). There were 101 stretches of on-dominated activity, 93 stretches of off-dominated activity, and 136 stretches of mixed responses. The on and off stretches ranged from 25 μm (i.e., one recording site) to 875 μm long, with a median of 125 μm (after correction for the obliquity of the penetration to the plane of layer IV). Similarly, mixed stretches ranged in length from 25 to 850 μm, but the median length was only 75 μm. The size of ocu-

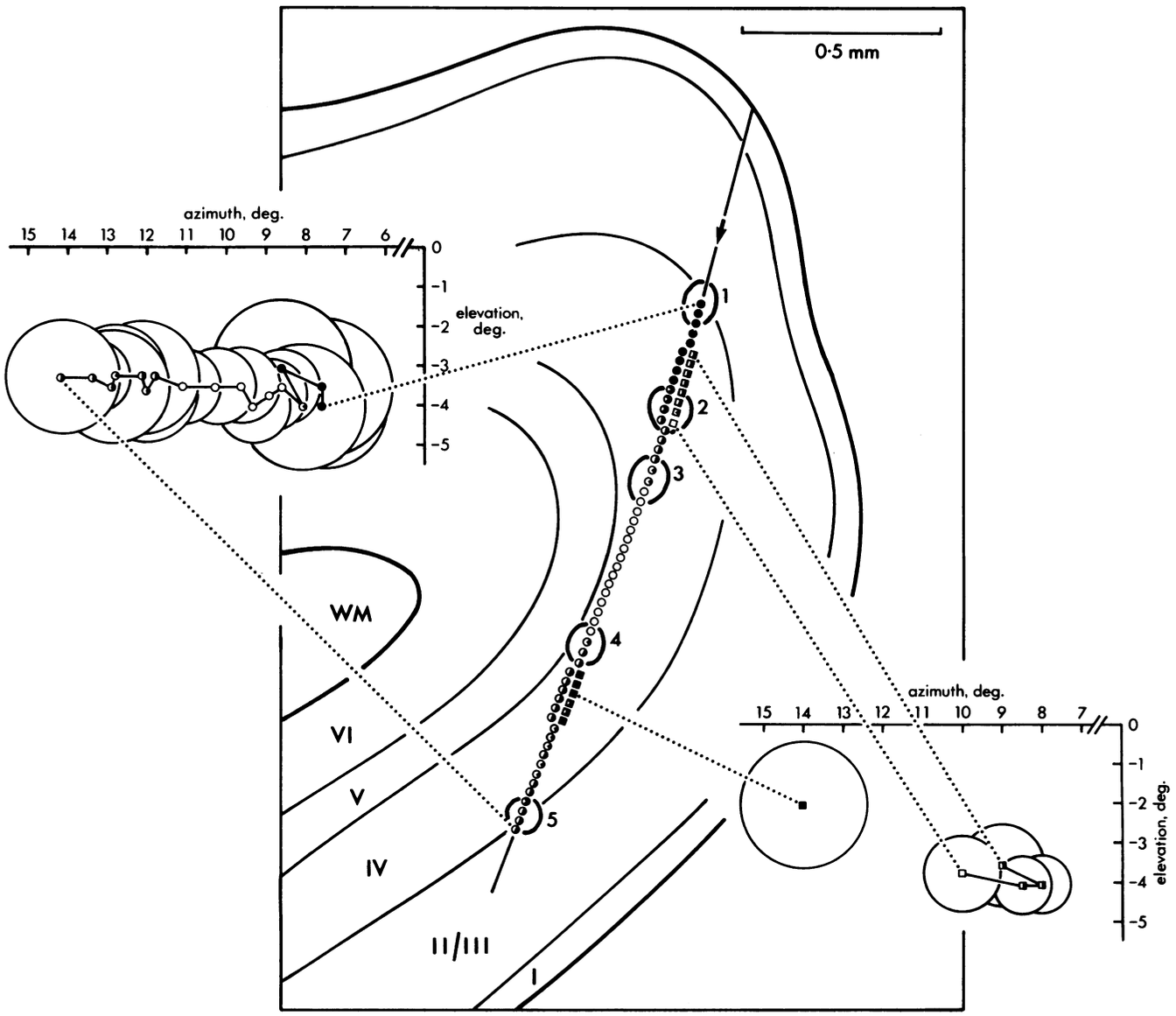

FIG. 2. Reconstruction of a tangential electrode penetration through layer IV in the right visual cortex. For explanation of symbols, see Fig. 1. Between the sites marked by lesions 1 and 2, off responses were obtained by stimulation of the contralateral eye. Mixed responses from the ipsilateral eye were also obtained during the latter part of this stretch. Lesions 2 and 3 bracket a short segment of mixed activity in the contralateral eye that was followed by a 450-μm stretch of on responses also in the contralateral eye (lesions 3 and 4). After lesion 4, there was a stretch of off responses in the ipsilateral eye and mixed responses in the contralateral eye; this was followed by an on-dominated and then a mixed stretch in the contralateral eye. Lesion 5 marks the point of exit from the layer. (*Upper inset*) The progression of receptive fields in the contralateral eye. (*Lower inset*) Progression of receptive fields in the ipsilateral eye. Only a small portion of the left lower quadrant of the visual field is shown in each case. Symbols show the geometric centers of the aggregate receptive fields, and large circles show the extent of receptive field centers as mapped with small spots; surrounds are not shown. Shifts in receptive field position were not detected at every recording site. Note that aggregate receptive fields were smaller for sites in the middle of the penetration, deep in layer IV, than for more superficial sites.

lar dominance columns in the mink as determined by these recordings is somewhat larger than that of the on and off patches. Contralateral eye columns are about 350 μm and ipsilateral eye columns are about 225 μm across. We have also confirmed the presence of these ocular dominance columns by transneuronal autoradiography (unpublished observations).

The borders of the ocular dominance columns and the on and off patches appeared to be independent of one another. When changes in ocular dominance occurred, they were sometimes changes in on and off responses and sometimes not. At sites where responses to both eyes were recorded simultaneously, there was a tendency for the preferred sign of contrast to be similar in the two eyes (see for example Fig. 3).

DISCUSSION

This study of mink visual cortex has shown that on- and off-center lateral geniculate afferents terminate in separate patches in layer IV. The patches extend the full thickness of layer IV and have a median width of 125 μm. The afferents are also segregated on the basis of eye of origin, but there appeared not to be any regular spatial relationship between the two types of patches.

We are confident for reasons stated in *Results* that the responses obtained in kainic acid-treated cortex are from geniculate afferents to area 17. Less clear is whether responses were obtained only from synaptic regions or from myelinated preterminal fibers as well. This latter possibility, as well as the likelihood that activity can be recorded at some dis-

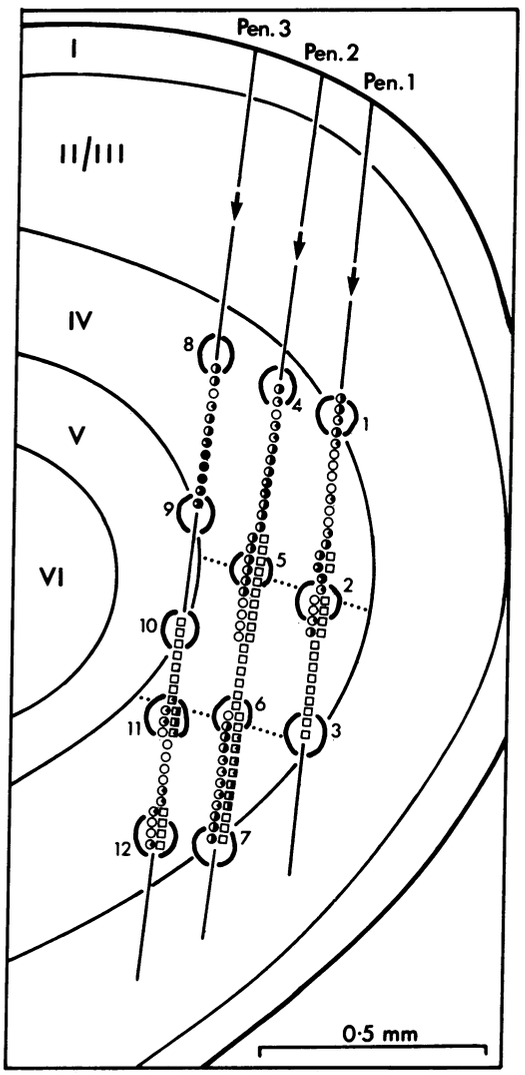

FIG. 3. Reconstruction of three parallel electrode penetrations made 150 μm apart in layer IV. For explanation of symbols, see Fig. 1. The most notable feature of these penetrations is a zone of on-center activity in the ipsilateral eye delineated by lesions 2 and 5 above, and by lesions 3, 6, and 11 below. This on zone spans the entire thickness of layer IV in the region bounded by the dotted lines.

tance from the electrode tip, makes it conceivable that the borders between on and off patches are even more precise than they appeared in our recordings. Microelectrode recordings are poorly suited to determining the exact size and shape of functional divisions of the cortex, so our measure of the width of the on and off patches (a median value of 125 μm) must be considered tentative. It would be useful in this respect to show the patches anatomically, either by the injection of tracers into single geniculate layers or by using the 2-deoxyglucose method. At any rate, it seems that the on and off patches are somewhat smaller than ocular dominance columns.

Thus our results show that in the mink, information from on- and off-center retinal ganglion cells is conveyed in anatomically separate channels through the lateral geniculate nucleus and into layer IV of visual cortex. This pattern is similar to that described for eye preference (15). In the latter case, the segregation of geniculate terminals forms the basis for the functional organization of the cortex into ocular dominance columns. The significance of the patchy arrangement of on and off afferents for the elaboration of cortical response properties is as yet unknown. Previous studies in cat and monkey have stressed the convergence of on- and off-center inputs onto single cortical cells (5–8). Considerable convergence may occur in the mink too, because there are areas in which on and off afferents overlap extensively, and because the dendrites of cortical neurons may span more than one patch. Nevertheless, our findings raise the possibility that in the mink a functional organization analogous to that for ocular dominance might be generated in which groups of cells receive predominantly on- or off-center inputs.

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