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DOES THE VESTIBULAR APPARATUS PLAY A ROLE IN THE DEVELOPMENT OF THE VISUAL SYSTEM?

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SUMMARY

1. The receptive field properties of visual cortical neurones were investigated in kittens that had been subjected to either unilateral or bilateral labyrinthectomy shortly after birth.

2. Two kittens were reared in a normal visual environment. Another two were reared in the dark with recurrent exposures to vertically oriented black and white stripes, which in normal kittens is known to bias the distribution of receptive field orientations.

3. For both normally reared and stripe-reared labyrinthectomized kittens, no differences were detected in cell types, preferred orientations, binocularity, columnar organization, or any other neuronal properties, compared with similarly reared intact kittens.

4. The failure to detect deficits in visual development after labyrinthectomy is discussed in relation to other reports of vestibular influences on the visual system of the adult cat.

INTRODUCTION

Neuronal activity in the visual system of the cat has been shown to be under a modulating influence from the vestibular system. This modulation has been demonstrated at the level of the optic tract (Marchiafava & Pompeiano, 1966), at the lateral geniculate nucleus (Papaioannou, 1973*a*, *b*), as well as at the cortical level (Jung, Kornhuber & da Fonseca, 1963). If one assumes that the neuronal circuitry mediating these interactions is present at the time of birth, it would appear likely that, during ontogeny, vestibular signals play a role in determining the properties of visual neurones.

Neurophysiological studies (e.g. Hubel & Wiesel, 1962) have shown that cells in the cortex possess far more complicated properties (e.g. binocularity, orientation selectivity, directional selectivity, etc.) than those at lower levels in the visual pathway. Consequently, at the visual cortex there are more parameters for which one might detect changes following labyrinthine sensory deprivation. Another reason for choosing the visual cortex as the locus of our investigations is the remarkable plasticity of cortical neurones. It has been shown, for example, that it is possible to bias the orientation selectivity of cells in the visual cortex by selective visual stimulation during early development (Hirsch & Spinelli, 1970, 1971; Blakemore & Cooper, 1970; Blakemore, 1973).

In the present study, neurones in the visual cortex were investigated in kittens that had been subjected to unilateral or bilateral labyrinthectomy shortly after birth. Two kittens were reared in a normal laboratory environment. Another two kittens were reared in total darkness, but were exposed daily to vertically oriented black and white stripes; in a normal kitten this procedure leads to dramatic modification of the cortex such that all cells adopt vertical, or near vertical, as their preferred orientation. Data from two intact kittens were used as controls.

METHODS

Labyrinthectomy

Four kittens were labyrinthectomized on the seventh post-natal day, before eyelid opening, when their weights were between 175 and 250 g. Kittens I and III sustained unilateral labyrinthectomy on the left side, kittens II and IV bilateral labyrinthectomy. Kittens at this age are very sensitive to barbiturates, so a dose of only 21 $\mathrm{mg.kg^{-1}}$ of sodium pentobarbitone (Nembutal) was mixed with two volumes of physiological saline and injected I.P. Supplementary doses of a volatile anaesthetic (Halothane) were administered as necessary.

Surgical procedures were performed under sterile conditions. The skin was incised behind and below the pinna, the parotid gland reflected and the muscle overlying the auditory bulla retracted. In the kitten, the bulla is located much closer to the mid line of the basal surface of the skull than it is in the adult cat; consequently, care has to be taken to avoid damaging the external carotid artery and auricular vein during the above procedures. The bulla, which is cartilaginous at this age, was then cut away to expose the lateral surface of the petrous part of the temporal bone. A dental drill was used to destroy the posterior part of the petrous bone containing the vestibular organs, while endeavouring to leave the anterior portion of the petrous bone, containing the cochlea, intact. The hole produced by the drill was filled with an absorbable gelatin matrix (Sterispon, Allen & Hanbury), which had been dipped in powdered thrombin. The skin wound was sutured with absorbable catgut sutures. Finally, the kitten was given an intramuscular injection of 30000 units of a long-lasting antibiotic (Penidural, Wyeth).

Rearing conditions

Labyrinthectomized animals. Kittens I and II (litter-mates) were reared in a normal laboratory environment, at first with their mother in a cage and then loose in a colony until the time of neurophysiological experiments during the 22nd post-natal week.

Kittens III and IV (not litter-mates) were housed, immediately after the labyrinthectomy, in a totally dark room with their mothers. In this dark room they experienced only a few seconds of dim red light each day during feeding and cleaning. In addition they received periods of selective exposure to vertical stripes in the manner of Blakemore & Cooper (1970). Each kitten, wearing a neck ruff to prevent it from seeing its own body, was placed for about 2 hr each day on a glass platform in a tall cylindrical chamber, the inside of which was covered with high-contrast black and white vertical stripes.

Kittens III and IV were not exposed to this environment until the gross postural effects of labyrinthectomy had disappeared. Kitten III (unilateral labyrinthectomy) was exposed for a total of 71 hr from 8½ to 13 weeks of age, when the physiological experiment was performed. Kitten IV (bilateral) had 70 hr of exposure from 6 to 13½ weeks, when the recordings were taken.

Control animals. One other animal, kitten V, was reared under normal conditions like those for kittens I and II, but it was not labyrinthectomized. Recordings were taken from the visual cortex at 12 weeks of age. Finally, kitten VI, also not operated, was treated like kittens III and IV, being exposed to vertical stripes for a total of 51 hr from 10 to 13½ weeks; recordings were taken at 20 weeks.

Final behavioural assessments of vestibular function

Immediately before preparing the animals for recording, we tested the vestibular and auditory reflexes of the labyrinthectomized kittens in order to assess the long-term consequences. In every case there was a deficit in tonic labyrinthine forelimb reflexes and in the righting reflexes during free fall, without a concomitant loss in the ability to orient to sounds with the eyes covered.

Neurophysiology

The cats were prepared under ultra-short-acting Brietal sodium anaesthesia. During recording they were paralysed by continuous I.V. infusion of Flaxedil in dextrose solution 7·5 mg/kg.hr, and anaesthesia was maintained by artificial ventilation with 80% N_2O, 19% O_2, 1% CO_2. Expired CO_2 was measured and the maximum P_{CO_2} kept at 3·5–4%. E.e.g. and e.c.g. were monitored continuously as well as body temperature, which was maintained at 37° C. Glass-insulated tungsten micro-electrodes (Levick, 1972) were introduced by a hydraulic advancer through a sealed chamber into the visual cortex, in the region of Horsley-Clarke coronal plane 0–2 mm, very close to the mid line. Amplification of action potentials was conventional and analysis was performed entirely by listening to the responses on an audio monitor.

The slit-shaped pupils were photographed before preparation and after paralysis in order to assess any rotation of the eyes and the arrays of receptive fields were corrected accordingly. The pupils were then dilated with homatropine and phenylephrine (Evans), the corneae were covered with contact lenses and 3 mm artificial pupils were used. The refractive state of the eyes, assessed by ophthalmoscopy, was corrected for viewing a translucent screen 57 cm from the cat, with spectacle lenses in front of the eyes. A reversible ophthalmoscope was used to plot the projections of the areae centrales on the screen. The images of spots, slits, bars and edges were back-projected on to the screen by moving cut-out shapes on the stage of an overhead projector. A reflector cast identical images on to sheets of paper for plotting receptive fields.

Each recording experiment lasted for at least 1 day and the number of units recorded from each animal ranged from 23 to 45. The total number of neurones from all six cats was 171, of which seven are not included in our analysis since they were monocularly driven, were generally recorded in the white matter, had fibre-like action potential wave forms and had centre-surround receptive fields typical of cells in the lateral geniculate nucleus: we assume that they were afferent geniculate fibres.

For the purpose of histological construction, at least three electrolytic lesions were made along each electrode penetration. To produce these lesions between 5 and 10 μA (d.c., electrode negative) were passed for 5–10 sec.

Histology

After recording, the animals were perfused through the heart with physiological saline, followed by a 10% solution of buffered formalin. The brains were removed, blocked, frozen and sectioned at 40 μm. The sections were stained with cresyl violet and sometimes counterstained with Luxol fast blue, in order to reconstruct the electrode penetrations through the visual cortex (see Fig. 2).

The temporal bones, attached to each other by the basi-occipital plate, were decalcified and embedded in paraffin. Serial sections, 10 μm thick, were cut in the horizontal plane and every tenth section was mounted. The mounted sections were stained with one of the following three stains: Masson's trichrome, Haematoxylin-orange G, Haematoxylin-picro Ponceau S. These sections were examined to assess the extent of the intended labyrinthine damage. In only one case (on the right side in kitten IV) were the cochlea and cochlear nerve involved in the lesion. In every case the intended total labyrinthine lesion was confirmed, except that in the left side of kitten IV the damage was limited to the utricular macula. Fig. 1*a* shows some of the damage to the vestibular apparatus on the left side, in one of the animals (kitten I), while Fig. 1*b* shows the left cochlea undamaged in the same animal.

RESULTS

All the neurones in this study had receptive fields within 10° of the area centralis. Histological reconstruction showed that most of our recordings were taken from area 17, but some of the units were certainly from area 18, particularly at the beginning of the most anterior penetrations. We found no obvious differences in the consequences of labyrinthectomy for cells in area 18, as opposed to those in area 17. For the purposes of this analysis all units therefore are considered together.

For each neurone the receptive field was plotted separately through each eye and the cell classified into one of the following four classes:

1. *Orientation-selective neurones*
   (*a*) *Simple.*
   (*b*) *Complex.*
   (*c*) *Hypercomplex.*

We followed the criteria of Hubel & Wiesel (1963, 1965) as summarized by Blakemore, Fiorentini & Maffei (1972) in defining these classes.

2. *Pure direction-selective neurones*

In the normal adult cat there is a small percentage of cortical units that respond to movement of any stimulus in a particular direction, the response being just as vigorous for a small spot as for an elongated edge

(C. Blakemore & S. Anstis, in preparation). In view of Barlow & Pettigrew's (1971) description of this kind of direction-selectivity without orientation-selectivity in the cortex of the visually inexperienced kitten, we took great care to distinguish between these two characteristics.

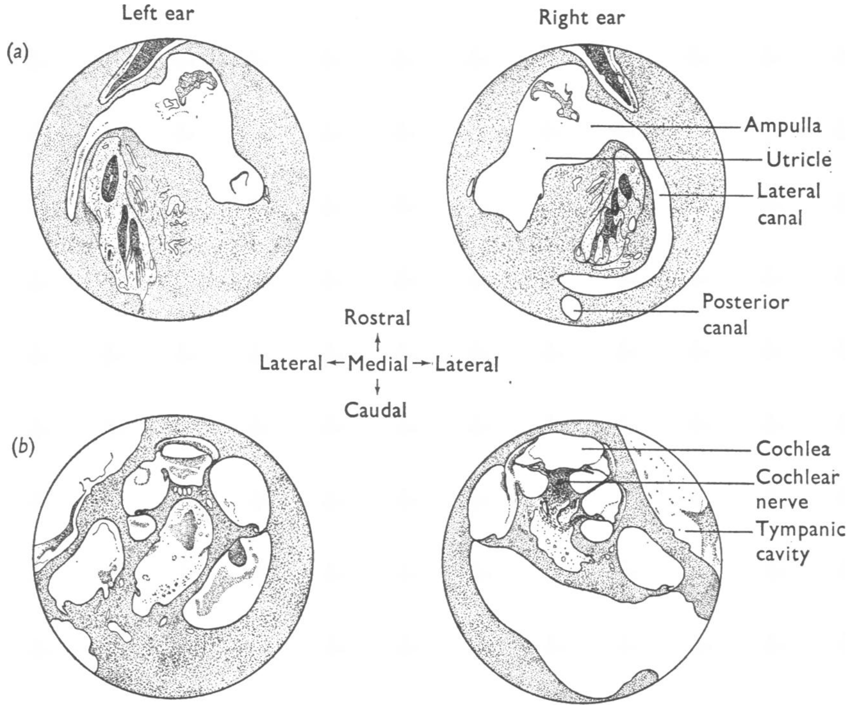

Fig. 1*a*. Horizontal sections through both labyrinths of kitten I. The normally developed lateral and posterior semicircular canals are seen in the right (unoperated) labyrinth, while on the left (operated) side the lateral semicircular canal is malformed and filled with debris and the posterior canal is entirely absent. × 6.
b, As above but at a more ventral level, showing the cochlea and cochlear nerve normally developed on both sides. There is a certain amount of disruption of the more delicate tissues, but since it is present equally on both sides (and in the cochleae of all animals in this series) it is presumably a histological artifact. × 6.

3. *Non-oriented neurones*

While very few cells in the adult cortex show no selectivity for orientation or direction of movement, such units are common in the visually inexperienced kitten (Hubel & Wiesel, 1963; Barlow & Pettigrew, 1971) and they are also found in kittens reared in selective visual environments (C. Blakemore, in preparation). These non-oriented neurones generally respond to spots, as well as edges, moving in any direction. They tend to respond more sluggishly than orientation-selective and directional cells.

4. *Visually unresponsive neurones*

These cells were spontaneously active, although some of them had very low discharge rates and consequently were easy to miss. They did not respond to any of the visual stimuli that we used. Such cells are common in the visually inexperienced kitten cortex (Hubel & Wiesel, 1963; Barlow & Pettigrew, 1971).

Kittens with normal visual experience

Fig. 2 shows a micro-electrode penetration through area 17 of the right hemisphere in kitten II (bilateral labyrinthectomy). On the right is a reconstruction of the penetration: each short line on the penetration shows the position at which an orientation-selective cell was recorded and indicates its preferred orientation. There is a clear region in the middle of the penetration where rather little activity was encountered, but inspection showed that this portion corresponded closely with the passage of the electrode through white matter. In fact, in none of the three animals with normal visual experience did we come across any inactive or 'silent' grey matter.

Fig. 2 further shows that, just as in the intact, normally reared cat, neighbouring cells prefer the same orientation (Hubel & Wiesel, 1962). Thus the organization of cortical neurones in orientation columns does not seem to be disrupted by deprivation of vestibular input during early development.

We also found no obvious changes in the binocularity of cortical cells for the labyrinthectomized kittens. The neurones were classified into the seven ocular dominance groups proposed by Hubel & Wiesel (1962). The upper part of Fig. 3 shows eye dominance histograms for kittens V (intact), I and II (labyrinthectomized). The lower part of Fig. 3 shows the preferred orientations for the orientation-selective neurones from the same three animals: there is no clear difference between them.

In these experiments we also examined many other response characteristics such as the size of the receptive fields, the proportions of simple, complex and hypercomplex cells, the general responsiveness to visual stimuli and the fineness of orientational 'tuning'. We could find no difference in any of these characteristics between the intact and labyrinthectomized animals. Of course, there is so much variability in these properties for normal cats that it would be difficult to detect subtle changes.

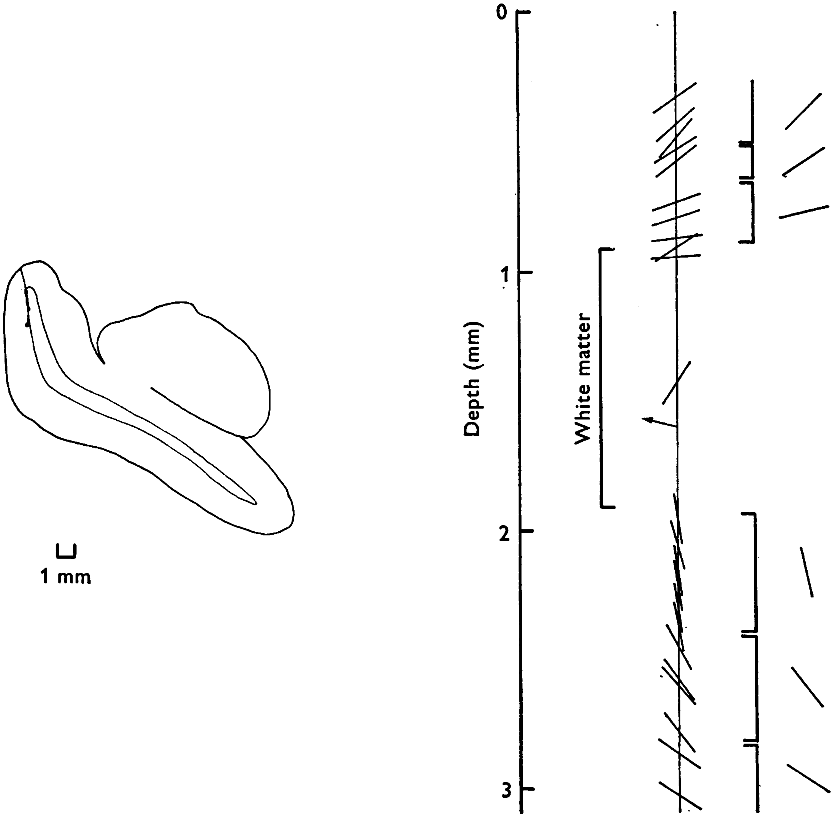

Fig. 2. A reconstructed penetration in kitten II, which was bilaterally labyrinthectomized and reared in a normal environment. The coronal section of the right visual cortex, on the left, shows the penetration through area 17, down the medial bank of the postlateral gyrus. The three small circles along the penetration are electrolytic lesions used to identify the track.

The same penetration is shown diagrammatically on the right where each short line marks the preferred orientation of a neurone recorded at that position in the penetration. The region marked *white matter* is the part of the penetration that passed through the fibre layers under the cortex, as seen in the section. In this region rather few units with the characteristics of cortical cells were recorded, and the unresolved background activity had the properties of afferent geniculate fibres. The short arrow in the middle of the white matter shows the position and preferred direction of the only pure direction-selective unit recorded in this animal.

Successively recorded units tended to have very similar preferred orientations and each columnar group of cells is marked by a bracket with the average optimal orientation for the column next to it.

Kittens with selective visual experience

Previous experiments have shown that orientation selectivity is modified by exposure to stripes at any time between 3 weeks and 3 months of age (Blakemore, 1973). Although the three kittens in this study were exposed for slightly different durations and at somewhat different ages, there was certainly always adequate exposure within this sensitive period to ensure modification in the normal kitten.

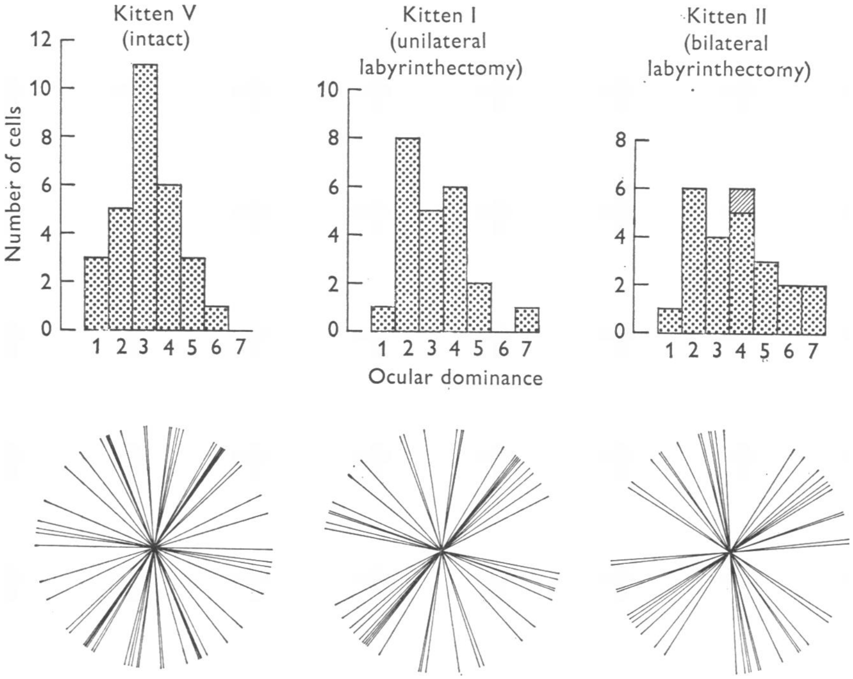

Fig. 3. The histograms above show the ocular dominance of neurones, using the seven groups of Hubel & Wiesel (1962): (groups 1 and 7 are monocular units driven exclusively by the contralateral and ipsilateral eyes respectively; group 4 cells are equally influenced by both; groups 3 to 1 represent increasing dominance by the contralateral eye, groups 5 to 7 increasing dominance by the ipsilateral eye). The stippled blocks are orientation-selective units, the cross-hatched block a pure direction-selective unit. The results are shown separately for kittens V, I and II and below each histogram is a polar diagram representing the preferred orientations for all the orientation-selective units from that animal. Each line is drawn at the optimal orientation for one unit.

Fig. 4 shows reconstructions of penetrations in both left and right hemispheres of kitten III (unilateral labyrinthectomy). Again there is an absence of 'silent' cortex and there is clear columnar grouping of

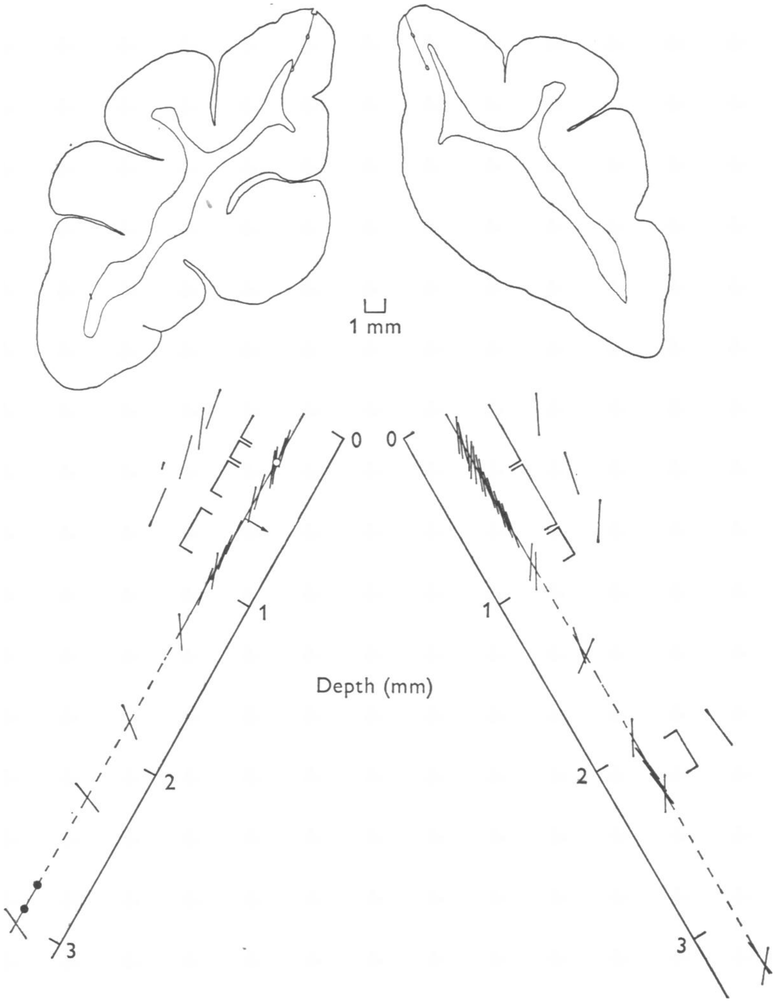

Fig. 4. Penetration reconstructions, like those of Fig. 2, for tracks through the right hemisphere (on the right) and the left hemisphere (on the left) of kitten III which was unilaterally labyrinthectomized and reared in an environment of vertical stripes. The symbols are the same as in Fig. 2; the filled circles represent non-oriented units, and the empty dot a visually unresponsive unit (in the left hemisphere). The reconstructed penetrations below are drawn at approximately the angles of the tracks, seen in the sections above. The short lines show the preferred orientations, relative to the vertical axis of the page. The interrupted parts of each diagrammatic penetration are regions in which the micro-electrode was purposely advanced, without stopping, for about 0·5 or 1·0 mm, so that a greater total region of cortex could be sampled during the penetration.

orientation-selective cells. However, all neurones preferred orientations close to vertical, as is the case for intact animals with similar visual experience.

Fig. 5 shows the categories of units, their binocularity and preferred orientations for kittens VI, III and IV. Although some visually unresponsive and non-oriented cells were found in the labyrinthectomized kittens, they were also present in the control animal. It is clear that the binocularity and the modification of orientation selectivity, as well as all the other response characteristics that we examined, were not influenced by early labyrinthectomy.

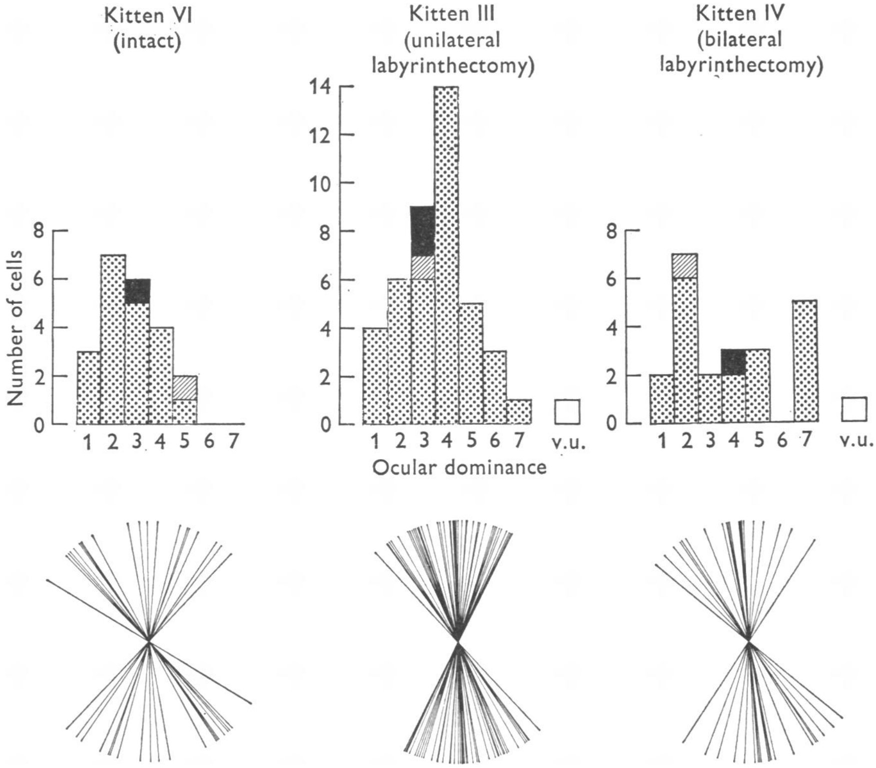

Fig. 5. Ocular dominance histograms and distributions of preferred orientations, as in Fig. 3, for kittens VI, III and IV, all reared in environments of vertical stripes. The symbols are the same as for Fig. 3; the filled blocks represent non-oriented units and the empty blocks are visually unresponsive units (v.u.). For kitten III, as shown in Fig. 4, there were no obvious differences for the two hemispheres, so all the units are pooled for this diagram.

DISCUSSION

There are certainly both genetic and environmental influences in the developing nervous system (for discussion see Jacobson, 1969). In the visual cortex, particularly, manipulations of the animal's experience have demonstrated a great degree of plasticity. For instance, monocular deprivation (Wiesel & Hubel, 1965), disrupted of congruent binocular input (Hirsch & Spinelli, 1971) and selective visual exposure (Blakemore & Cooper, 1970) all cause dramatic changes in cortical organization. However, all these experiments involved manipulation of the *visual* input to the visual cortex.

In the adult cat the activity of visual cortical cells has been shown to be affected by auditory (Morrell, 1972; Fishman & Michael, 1973), somatic (Horn, 1965) and vestibular inputs (Jung *et al.* 1973). In fact some cells of the visual cortex have been shown to change their properties during bodily tilt, which stimulates the vestibular apparatus (Horn, Stechler & Hill, 1972; Denney & Adorjani, 1972; Spinelli, 1970). Consequently, one might reasonably ask whether manipulating these *non-visual* inputs early in development could also alter the organization of the visual cortex.

We have deprived kittens, unilaterally and bilaterally, of input from the vestibular system and yet have failed to detect changes in any of the visual properties of cortical cells. Labyrinthectomy does not even interfere with the modification of receptive field properties caused by selective visual exposure (Fig. 5). We found no difference between labyrinthectomized and intact kittens, not even between the two hemispheres in an animal with a unilateral labyrinthine lesion (Fig. 4). Although we made no detailed examination of the histological structure of the visual cortex after labyrinthectomy, cursory inspection showed no deviation from the normal. This correlates with the absence of any large regions of unresponsive cells in our physiological experiments.

These experiments rule out the possibility that vestibular input plays a major part in the development of the visual cortex. However, even in the normal adult cat, the percentage of visual cortical cells clearly influenced by bodily tilt is extremely small (Horn *et al.* 1972). Therefore it is possible, if unlikely, that we simply failed to sample those cells most likely to be affected by vestibular deprivation. Finally, we have examined the properties of visual cortical cells under static conditions. It may be that normally any vestibular influence on the visual system operates mainly under conditions of dynamic stimulation of the labyrinths. If this is the case our experiments would, of course, fail to detect any deficits which might result from labyrinthectomy.

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