then passaged sufficiently (not more than four passages) to produce a precipitating antigen[13]. The number of blood samples that yielded isolates and the antigenic typing of these isolates are given in Tabie 2. The antigenic typing used was only able to distinguish between the presence of attenuated virus alone (absence of A antigen) and the presence of virulent virus either alone or in mixture with attenuated virus (presence of A antigen). No correlation was noted between the ability to re-isolate virus and the presence or absence of antibody of either type. From these results it was concluded, first, that the existence of the attenuated vaccine virus in the circulation of chicks for 14 weeks did not result in its reversion to the original type (that is, the A antigen remained absent), and, second, that its presence in the circulation did not prevent the establishment of virulent virus in the circulation when inoculated.

Table 2. RE-ISOLATION OF VIRUS IN EXPERIMENT 4, 14 WEEKS AFTER VACCINATION AND 10 WEEKS AFTER CHALLENGE

Group	Virus isolations	Isolates with A antigen
A	6/7*	5/6†
B	4/7	1/3
D	5/7	5/5
G	19/24	0/5

* Number of chickens yielding virus isolates over the number tested.
† Number of isolates with A antigen over the number tested.

Type of Protection

These results confirm our previously reported observations on the lack of pathogenicity of the passaged virus, and demonstrate a particularly high level of resistance induced in chickens by the inoculation of an attenuated virus that persists in the circulation of the chicken. The attenuated virus produced significant protection even when inoculated at 1 day of age. Whether the protection against virulent challenge was mediated by circulating antibody, cellular immunity, interference or interferon was not determined. It was, however, clearly demonstrated that precipitating humoral antibody production was stimulated by the vaccination procedure. Nevertheless, the induction of such antibody did not prevent the establishment of virulent virus in the circulation. This may be because the virus is cell associated and is thus protected from the direct effect of antibody. If humoral antibody played a part in immunity in these experiments, it was in a role other than that of preventing infection with virulent virus. It was considered unlikely that any interferon production that might be induced by attenuated virus was responsible for the protection, for it was not possible to demonstrate its production *in vitro* using the inhibition of virulent virus as a test of its presence (our unpublished observations).

Before the use of such an attenuated virus in the field can be advocated, it will be necessary to determine what the long term effects, if any, will be of the persistence of the virus in the vaccinated birds, and whether it will provide protection in field conditions.

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Anatomical Demonstration of Columns in the Monkey Striate Cortex

by

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Anatomical investigation of the monkey striate cortex supports the physiological concept that the cortex is subdivided vertically into columnar aggregates of cells. Here the shape and size of two independent and overlapping column systems are described.

THE cortical column is a relatively recent physiological concept, for which no anatomical basis has yet been found. The mammalian cerebral cortex, a sheet of cells some 1·5 mm thick and many sq. cm in surface area, contains millions of nerve cells unevenly distributed in six to eight layers. These fairly conspicuous horizontal subdivisions have long been known to anatomists. Vertically, there are no comparable anatomically obvious subdivisions, except for separations between certain specific cortical regions such as visual areas 17 and 18. In 1957, however, Mountcastle[1] made the surprising discovery that the somatosensory cortex is subdivided by a system of vertical columns, extending from surface to white matter, having cross-sectional widths of the order of 0·5 mm. Since then single-cell recordings have revealed analogous systems in both cat and monkey[2,3]: in the primary visual cortex of the macaque monkey, for example, we have described two overlapping and independent systems of columns, one based on responses of groups of cells to specific line orientations, the other on eye preference. We describe here an independent anatomical demonstration of the eye-preference columns.

In higher mammals, impulses from the two eyes pass back to the lateral geniculate bodies and from there are relayed to the striate cortex. Here for the first time the two paths meet, and signals from the two eyes converge on single cells[2,4], presumably making possible the fusion of the two visual images. The convergence does not take place immediately, however. Fibres arriving from the lateral geniculate body terminate chiefly on cells of

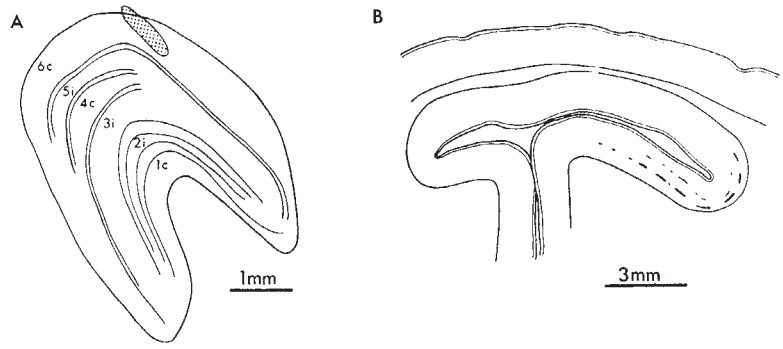

Fig. 1. *A*, Tracing of a coronal section through the left lateral geniculate body of a rhesus monkey showing the position of a lesion in layer six. c, Contralateral eye; i, ipsilateral eye. *B*, Parasagittal section through part of the occipital cortex. Tracing shows areas of terminal degeneration in the anterior part of the mushroom-shaped buried segments of the calcarine fissure. Note the patchy nature of the projections, and the more superficial, thinner areas of degeneration.

cortical layer four, which lies midway between surface and white matter. Microelectrode recordings show that some cells in this layer respond to stimulation of the left eye, others to the right, but few if any respond to both eyes[3]. Moreover, it is clear that the two types of cells are not scattered at random along the fourth layer, but are grouped together in small regions which extend through the layer's entire thickness. Viewed from the surface, the regions form a mosaic, like a patchwork quilt of two kinds of material.

Conventional anatomical methods such as the Nissl or Golgi give no hint of any eye-dominance groupings. Besides confirming the physiological results, an anatomical demonstration of the regions would be useful in indicating their size and shape, especially as this information is not easy to obtain with microelectrode techniques. Up to now, for example, it has not been possible to tell whether the left-eye, right-eye regions form a checkerboard, a series of alternating stripes, or islands of one type lying in a matrix of the other.

We have used the Nauta method[5] as modified by Fink and Heimer[6] to demonstrate the eye-preference regions. When an axon is severed from its cell body, degeneration occurs peripheral to the cut over a period of several days. The Nauta–Gygax method selectively stains these degenerating axons, making it possible to trace them to their destination. The Fink–Heimer modification improves greatly the chances of staining axon terminals[7] and is especially useful when there is a high density of degenerating terminals.

A great difficulty with silver-degeneration techniques is in placing the lesion precisely, especially deep within the brain, and in making the area of destruction small and select. By passing current through metallic microelectrodes[8] a lesion can be made as small as a few hundred microns in diameter, and can be accurately placed by recording from and identifying single cells beforehand. In the work described here we have made lesions in single layers of the lateral geniculate body, and have observed what are almost certainly degenerated terminals (boutons) in the striate cortex.

The monkey lateral geniculate body, which receives its input direct from the two eyes, has six layers, each layer consisting of a plate of cells on which the contralateral field of vision is mapped in an orderly way. The six maps are in register, one above the other. All of the cells in a given layer receive their input from one eye only, so that if the layers are numbered from below upward, layers one, four and six receive input from the contralateral eye, and two, three and five from the ipsilateral. A lesion, confined, for example, to a small part of the sixth (most dorsal) layer, should produce terminal degeneration only in patches of cortical layer four that are connected with the contralateral eye. The mosaic ought then to be obvious. A lesion in geniculate layer six that extends down to include the part of five just below ought to give confluent degeneration in the cortex, with no patches.

Our procedure was to insert stereotaxically a tungsten microelectrode into the lateral geniculate body of an anaesthetized rhesus monkey (*Macaca mulatta*), advancing the electrode until we recorded from cells in the most dorsal layer of the lateral geniculate body. The electrode path was in the coronal plane 45° to the vertical. Cells were identified by their responses to small-spot stimulation of the visual field of the contralateral eye, and by the position of the receptive fields with respect to the fovea. The electrode could then be advanced from layer to layer, each transition (except four five) being evident from the shift from one eye to the other. A lesion was made in one layer by passing 5–15 μA (electrode negative) for 5–15 s. For larger lesions in a single layer one could repeat the procedure several times, each time advancing the electrode until new cell activity was encountered, and stopping only when a shift in eyes indicated that the electrode tip had crossed to the next layer. That the path of destruction so made was a continuous one was verified by recording continuously on withdrawal of the electrode and by later histological study. The animal was allowed to recover, and 5 days later the brain was perfused,

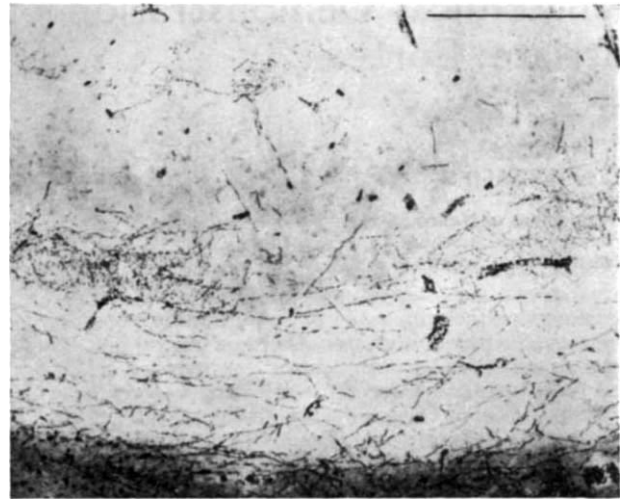

Fig. 2. Photomicrograph of striate cortex stained by the Fink–Heimer method. Same monkey as in Fig. 1. From the densely staining white matter near the bottom of the figure degenerating fibres ascend obliquely. About halfway up, to the left and right of the micrograph, two broad strips of terminal degeneration can be seen as a mass of dark granules. These two patches are separated in the middle of the figure by a terminal-free portion containing horizontal and oblique degenerating fibres. Other fibres can be seen ascending to a second, more superficial, thinner layer of terminal degeneration near the top of the figure. Scale, 200 microns.

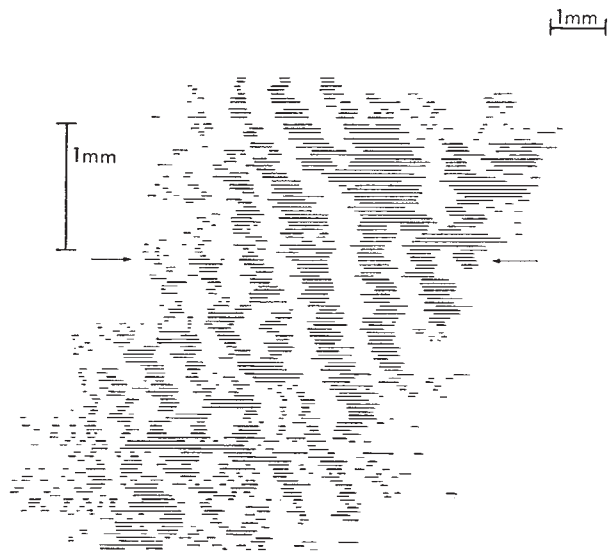

Fig. 3. Reconstructed map of regions of fourth layer degeneration as they would appear if seen from above. Same monkey as in Figs. 1 and 2. Each horizontal line represents areas of terminal degeneration from one parasagittal section; the degeneration illustrated in Fig. 1*B*, for example, is represented between the two arrows. Shaded areas thus represent those parts of layer four receiving input from the contralateral eye; unshaded areas, ipsilateral eye.

frozen-sectioned and silver-stained according to a slightly modified Fink–Heimer method.

The site of a typical lesion is shown in Fig. 1*A*. The electrode entered the left lateral geniculate body at an oblique angle, so that it was possible to produce destruction over a long narrow area confined to the most dorsal layer. The receptive fields of the first cells recorded were in the left eye, 9° below the horizontal meridian of the visual field, and 10° to the left of the vertical midline. The subsequent degeneration was in the corresponding part of the striate cortex, deep in the calcarine fissure, as illustrated in the parasagittal section of Fig. 1*B*. A much lighter degeneration was scattered for about 1 cm along the stem of the calcarine fissure. No degeneration was seen outside the striate cortex, confirming the findings of Wilson[9].

In the main area of degeneration there were dense patches of stained terminals, presumably boutons, deep in layer four. These patches alternated with areas of about equal horizontal extent that were virtually free of terminals. As shown in Fig. 2, degenerating fibres entered the cortex from the white matter in a relatively even, non-patchy fashion, but then coursed obliquely through the sixth and fifth layers, to arborize profusely in the patches of layer four while largely avoiding areas free of terminal degeneration. A few fibres also ran horizontally through the terminal-free regions, crossing from one terminal-rich patch to the other.

A small number of fibres ascended vertically or obliquely through the fourth layer to an upper tier deep in the third layer, where a second set of thinner patches of terminal degeneration lay directly over the lower set. A fibre rarely ascended to higher levels, but no obvious areas of terminal degeneration were seen there. This bipartite distribution of cortical terminals from the geniculate has not to our knowledge been described before; its physiological significance is not yet clear. A similar distribution is seen after a lesion in layer five. At present we are studying the projections from the middle and ventral pairs of layers, to see whether there are differences in the levels at which they terminate.

To obtain a map of the patches of degeneration as viewed from the surface of the cortex, a reconstruction was made from serial sections through the focus of degeneration. A photographic enlargement was made of each slide, and the regions of terminal degeneration were marked on each photograph. By tracing the regions from successive photographs on to closely spaced parallel straight lines, it was possible to reconstruct the mosaic as it would appear if the cortex were flattened out. Fig. 3 shows the resulting map, made from 125 30 micron thick serial sections. The mosaic consists not of a checkerboard or set of islands but of a series of roughly parallel stripes. The shaded parts of the figure thus represent regions of cortex receiving input from the right eye; the unshaded portions, the left eye. The diagram is for technical reasons distorted by the unequal scales in the vertical and horizontal directions. In this experiment about ten columns of each type are represented. The periodicity (after correcting for the unequal scales) is about 1 mm. The figure suggests that the stripes are not everywhere perfectly regular; a stripe is occasionally interrupted when the two neighbouring stripes on either side run together. Four monkeys, three with lesions in the sixth layer and one with a lesion confined to the fifth, have given almost identical results, with alternating stripes of about the same periodicity. Because the field positions of cells in the geniculates of the four monkeys ranged from about 3° to 16° from the area centralis, this suggests a surprising constancy in size over the striate cortex. If one assumes such a constancy, the order of magnitude of the number of eye-preference stripes in the entire striate cortex would be about seventy-five for each eye, or a total of 150. With further experience it may be possible to learn whether there is any systematic relationship between field position and direction of stripes.

In a control experiment a lesion similar to that of Fig. 1 was extended to invade both the sixth and fifth layers for roughly equal distances (Fig. 4*A*). The results are illustrated in Figs. 4, 5 and 6. Over much of the area of

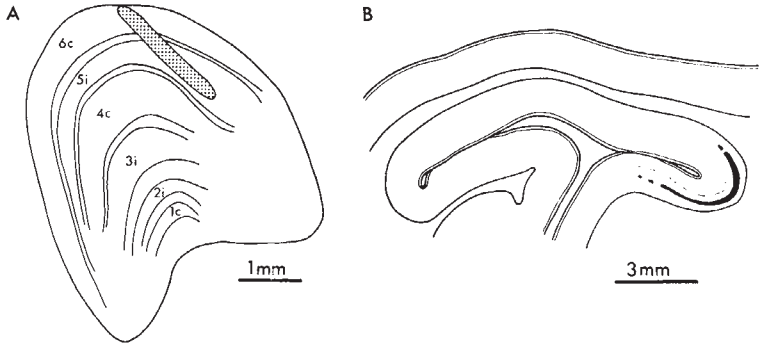

Fig. 4. Tracings analogous to those of Fig. 1 for a control monkey in which the lesion involved layers 5 and 6. *A*, Coronal section through left lateral geniculate body. *B*, Parasagittal section through occipital cortex. Except at the ends, the area of terminal degeneration forms one continuous strip.

projection to layer four there was a continuous band of degenerating terminals. In the reconstructed map there is, as expected, a large central area of solid degeneration. In addition there are hints of columns to the upper left and lower right, in width and direction very similar to the columns of Fig. 2. These presumably represent the initial and terminal portions of the elongated geniculate lesion, where one layer was involved but the corresponding region in the neighbouring layer spared.

In all lesions there has been a fairly extensive region of thinly scattered degeneration to one side of the main focus. This is assumed to be caused by destruction of passing fibres originating in cells of the lower geniculate layers, penetrating the upper layers on their way to the cortex. Fortunately, these fibres do not proceed outwards in radial fashion, but project upwards and backwards along the shortest route. If they projected radially, a lesion in one layer would interrupt axons from the cells of all the deeper layers, and these experiments would have failed.

Recordings from striate cortex indicate that a higher-order cell, lying in a layer superficial or deep to the fourth, receives its chief input from the fourth layer patch immediately below or above, and a subsidiary input from adjacent patches. Such a cell can thus be influenced from the two eyes, but responds more strongly to the eye that corresponds to the nearest fourth layer patch. Hence the cells of a fourth layer patch, together with all cells directly above and below, constitute a group dominated by one eye. The cortex can therefore be thought of as subdivided into columns or slabs with walls perpendicular to the surface and long narrow cross-sections determined by the fourth layer mosaic. All, or nearly all, cells in a given column thus respond more actively to one eye than to the other, reflecting the patchiness of layer four plus the preponderance of vertical over diagonal and horizontal connexions in the superficial and deep layers.

Superimposed on the eye-preference columns there exists a second completely independent set of columns in which cells with common stimulus orientation preference are grouped together[2,3]. Several kinds of evidence, including surface mapping with microelectrodes[10], suggest that these orientation columns also have the shape of parallel sheets, the thickness of which is probably much less than that of the eye-preference columns. We still have

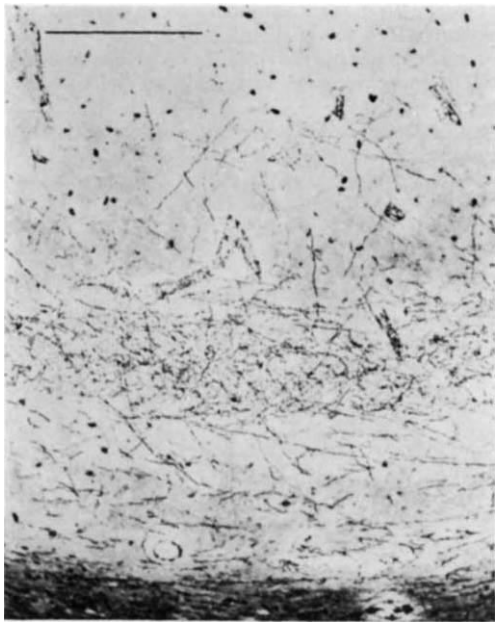

Fig. 5. Photomicrograph showing non-patchy fourth layer degeneration in the monkey of Fig. 4. The more superficial strip of degeneration deep in layer three is seen near top of figure. Scale, 200 microns.

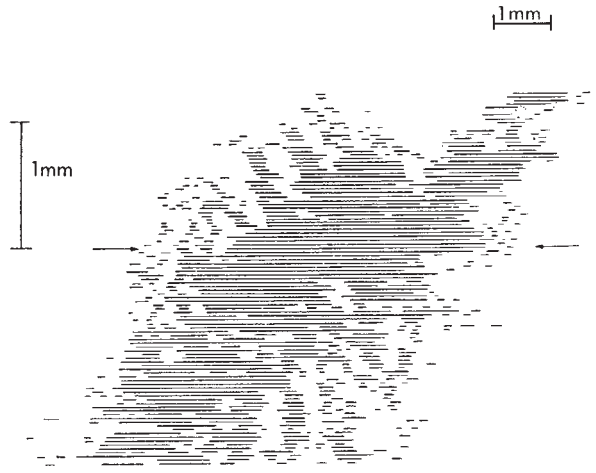

Fig. 6. Map of degenerated regions of layer four from monkey of Figs. 4 and 5. Note regions of confluent degeneration, with suggestion of parallel stripes to the upper left and lower right.

no means of demonstrating the orientation columns anatomically, and the relationship between the two systems—whether, for example, the two sets of slabs are orthogonal or parallel—remains to be determined.

The eye-preference columns resemble in several ways the columns described by Mountcastle in cat somatic sensory cortex[1]. In that system the columns are also determined by the afferents to the cortex, and are also of just two types, concerned with superficial and deep somatic sensation. Poggio and Mountcastle[11] have shown that the ventrobasal thalamus, where the somatosensory cortical afferents originate, is similarly subdivided into clusters of cells, analogous perhaps to the geniculate layers, one type of cluster containing only cells responsive to skin stimulation; the other, cells responsive to stimulation of deep receptors, such as those around joints. It may therefore be possible to demonstrate somatosensory cortical columns anatomically by these methods.

The existence of the striate cortex of two independent and overlapping column systems at once suggests the possibility of still others. Indeed, in the striate cortex there is a strong suggestion of regional aggregations of colour coded cells, and also of cells with directional selectivity[3]. Evidence that these groupings are also columnar is still lacking, although that seems a likely possibility. We have suggested elsewhere[3] that columnar systems may represent a solution to the problem of plotting more variables than two on a two-dimensional surface. For area seventeen, position in the visual field is translated into the two surface coordinates in the cortex, while the cortical layering is concerned with hierarchical levels of integration, such as receptive-field complexity and mixing of the two eye inputs. Analysis of other stimulus parameters, including eye-dominance, stimulus orientation and possibly also direction of movement and colour coding, requires a breaking up of the surface into a number of independent and overlapping systems of mosaics. We have presented here a direct anatomical demonstration of one of these systems.

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