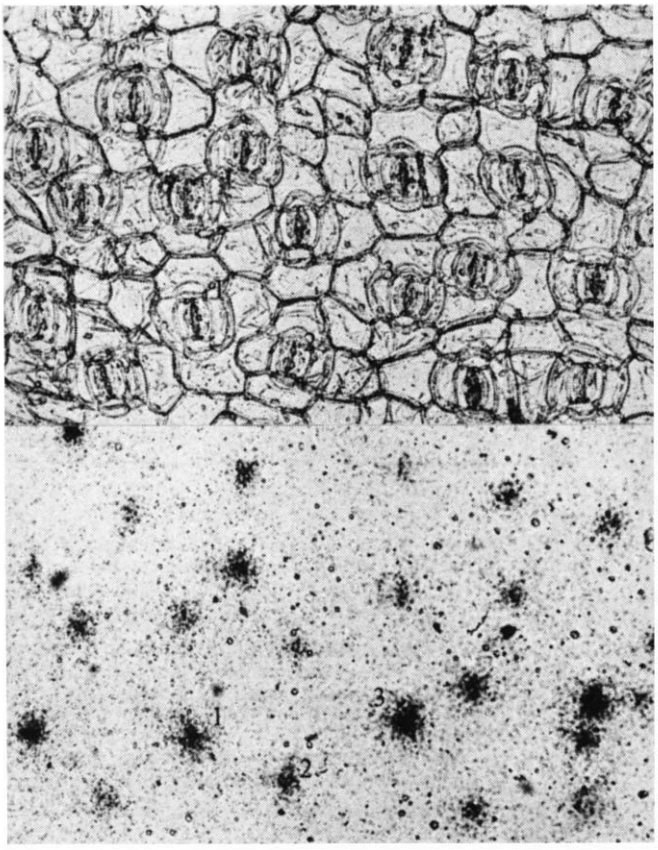

Fig. 3 Microautoradiograph of an epidermal strip of *C. communis* exposed to darkness and normal air for 2 h to close the stomata and after treatment as described in the legend to Fig. 2. Blackened areas located predominantly over the stomatal complexes indicate ^{14}C label in starch. (Stomata 1, 2 and 3 correspond to the numbered, blackened areas.) The dried epidermis was placed on a glass slide and a piece of Ilford Pan F film firmly taped on top with the emulsion in contact with the tissue. On top of the film was placed another slide and the whole was clamped together with screw-clips. After exposure for 29 d the piece of film was detached from the tape and tissue and developed in Kodak DX-80 developer.

and starch disappearance[6] have been observed with stomatal opening. We have also found high levels of starch in guard cells of *C. communis* when stomata were closed; this level decreased as stomata opened although starch could still be detected at wide stomatal apertures. This suggests that there is a dynamic flux of carbon between malic acid and starch with the direction of starch production outpacing its breakdown when stomata are closing. Results of an investigation of pathways for malic acid–starch interconversions will be published elsewhere.

In conclusion we have established that malic acid is a precursor of starch synthesis in guard cells of *C. communis*. But, the mechanism of the control of guard cell CO_2 fixation and hence malic acid synthesis, and the control of the production of starch from malic acid in relation to stomatal movements, remain obscure.

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Orientation columns in macaque monkey visual cortex demonstrated by the 2-deoxyglucose autoradiographic technique

In the past fifteen years physiological studies of the primary visual cortex in higher mammals have provided evidence for two independent systems of functional subdivisions, ocular dominance columns and orientation columns[1]. These two systems are closely related to two important functions of visual cortex: combining at a single-cell level the information that originates in the two eyes, and rearranging the spatial information from the lateral geniculate body so that cells after the initial stage of visual processing come to respond to specifically oriented lines in the visual field.

The ocular dominance columns have been demonstrated anatomically by three different staining techniques[3–6]. We describe here the use of a new method[7] which makes possible the anatomical demonstration of the orientation columns.

The original evidence that cells are aggregated according to their response characteristics came from microelectrode recordings[1]. In a penetration perpendicular to the cortical surface all cells are dominated by the same eye and all give optimal responses to the same stimulus orientation, whereas in an oblique or tangential penetration there is an alternation of the dominant eye, from left to right and back, and, at the same time, a series of regular changes in optimal stimulus orientation in steps of 10° or less, clockwise or counterclockwise. Reversals in direction of rotation occur sporadically, on the average every few mm, and the orderly sequences of small orientation shifts are occasionally broken by abrupt large shifts of up to 90°. A full cycle of either type, one eye and then the other or a rotation through 180°, generally requires a horizontal movement along the cortex of 1 mm or less[2]. The constancy of eye dominance and of optimal stimulus orientation during perpendicular penetrations indicates that the two sets of subdivisions are arranged perpendicular to the surface and the layers. Because of their cross-sectional shape in brain sections perpendicular to the surface, the subdivisions have been called 'columns', and a complete set of columns (left plus right eyes, or a full 180° rotation) is called a hypercolumn.

Inspection of cortical sections stained by conventional methods gives no hint of these vertical subdivisions. The only obvious segregation of cells is the horizontal system of layers, and this segregation has certain physiological correlates. Layer IVc, at about mid-cortical thickness, is the site of termination of the geniculate afferents and contains cells that differ in their physiological properties from cells in the other layers in two respects: like the geniculate afferents, they have no orientation preference; and they are almost all strictly monocular. In contrast, cells in the layers above and below IVc almost all show clear orientation specificity and about half are binocular, though any given cell is likely to respond best to one or the other eye.

In the last few years considerable progress has been made in working out the geometry of the columnar subdivisions. Three independent anatomical methods have made it possible to see the ocular dominance columns in layer IVc[3–6] where they form a set of parallel bands which are for the most part straight, but in places form loops and whorls and occasionally show bifurcations and blind endings. The columns must therefore have the form of parallel sheets rather than being pillar-like. For the orientation columns,

microelectrode recordings, especially multiple parallel penetrations, likewise suggest an arrangement in parallel sheets, and the frequent reversals in direction of rotation would be compatible with a swirling pattern.

Until very recently no method has been available for demonstrating the orientation columns anatomically. Several years ago Sokoloff and his group[7] developed a procedure for labelling active brain tissue. The method depends on the fact that brain cells, which use mainly glucose as a source of energy, take up 2-deoxyglucose as though it were glucose and metabolise it as far as 2-deoxyglucose-6-phosphate, but no further. This compound cannot easily pass out of the cell, and tends to accumulate. Physiologically active regions of brain may then be identified by the use of radioactively labelled deoxyglucose and autoradiography. This method was used by Sokoloff's group in monkeys in which one eye was stimulated during a 45-min period immediately following intravenous injection of deoxyglucose[8]. (The other eye was occluded in some monkeys and had been previously removed in others.) The result, a striking demonstration of the ocular dominance columns in the striate cortex, differed from the results obtained by the other anatomical techniques in demonstrating the columns through the full cortical thickness, rather than showing just the parts in layer IVc.

We have used the deoxyglucose method to reveal the orientation columns. Our procedure is based on that of Sokoloff and his group, to whom we are indebted for first-hand instruction in the method. We injected a lightly anaesthetised juvenile macaque monkey with ^{14}C-2-deoxyglucose, 150 μCi kg^{-1} in 100 μCi ml^{-1} saline, and then stimulated for 45 min by moving back and forth in front of the animal a black screen on which were pasted a set of irregularly spaced vertical white stripes. Stripe widths were 0.5–1°, and movement was 2–4° s^{-1}. Both eyes were held open, protected by contact lenses, refracted at the screen distance (1 m) and aligned with a variable prism over one eye so that the foveas were superimposed. To be sure of the alignment and as a check on the state of the animal we also recorded from single binocular cells in striate cortex and superimposed the receptive fields.

At the end of the stimulus period the animal was given an additional dose of anaesthesia followed by a lethal dose of intravenous KCl. It was then decapitated, and the skull was cleaned of skin, gradually immersed over a 4-min period in liquid Freon-22 at −125 °C, and stored at −80° C. Small blocks of brain were later sectioned at 20 μm in a cryostat at −26 °C, and the sections were picked up on a cover slip and immediately dried at 98 °C. These sections were then pressed against X-ray film for 2–3 weeks and the film was finally developed. Some of the sections used for autoradiography were also later stained for Nissl substance.

An autoradiograph made from a section perpendicular to the striate cortex is shown in Fig. 1. Vertical regions of dense label can be seen extending through the full thickness of cortex. About midway through the thickness is a dense uniformly labelled horizontal band. By comparing the autoradiographs with neighbouring sections stained for Nissl substance it can be shown that this band corresponds to layer IVc. This agrees with the observation from microelectrode recordings, that cells in layer IVc show no orientation specificity. No such uniform labelling was seen in IVc in the results of Sokoloff's group[8] or in our experiments, when ocular dominance columns were labelled by this method.

The vertical labelled regions in Fig. 1 are on the average about 0.6 mm apart and occupy a considerable fraction of the repeat distance, as expected from the fact that each cell responds not just to a single orientation but to a range of orientations to either side of the optimum, with some cells highly selective and others less so. For example, in a sharply tuned cell the orientation that evokes a half-maximal response may be 10–15° from the optimal. The widths of the labelled regions must depend on many factors including the sharpness of tuning curves (response plotted against stimulus orientation), and the relationship between cell activity and deoxyglucose uptake, and between uptake and the grain density of the autoradiographs.

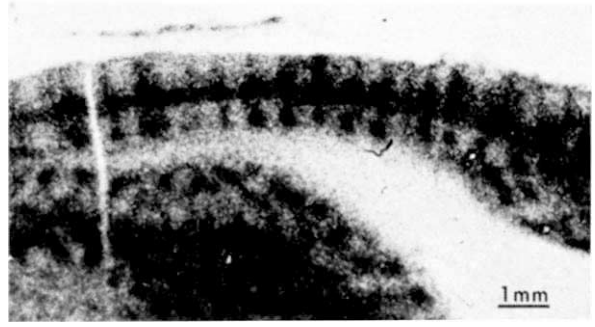

Fig. 1 Autoradiograph of a ^{14}C-2-deoxyglucose section through monkey striate cortex, perpendicular to the surface. The stimulus consisted of moving vertical, irregularly spaced white stripes presented to the entire visual field of both eyes for 45 min. Labelled regions are vertical in cross section, about 0.6 mm apart, and extend through the full cortical thickness. Layer IVc, situated at about mid-depth and identified from neighbouring Nissl-stained sections, is uniformly labelled, as expected from the absence of orientation specific cells in that layer.

A tangenital section through the dorsolateral surface of the occipital lobe is shown in Fig. 2. The white matter is grazed in two places which appear as pale ovals. These are surrounded by layers VI and V, cut almost tangentially, and here the labelled regions can be seen face-on, forming a complex pattern of rings, loops, and branching stripes. Their separation is strikingly constant, averaging roughly 570 μm. Only in a few places is there a suggestion of parallel stripes. Surrounding this area is layer IVc, which is again uniformly labelled, and just outside IVc is IVb, where the aggregations of label are particularly dense. Layers II and III are more lightly labelled but also show distinct aggregations. (The dense bands at the extreme left, perpendicular to the surface, are microtome knife artefacts.)

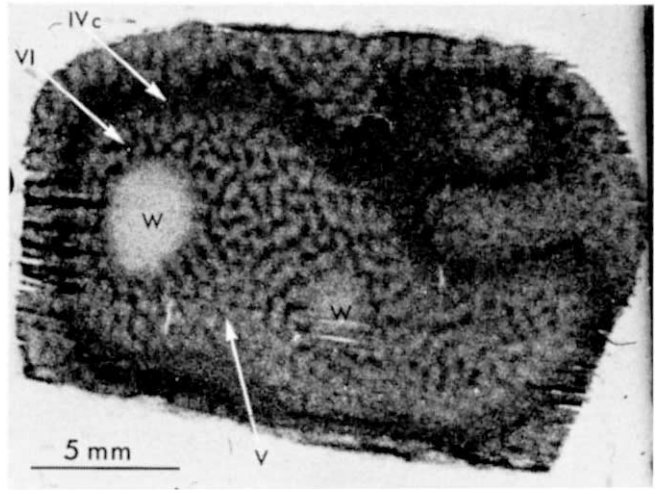

Fig. 2 Autoradiograph of a tangential section through the dorsolateral surface of the occipital lobe (striate cortex) of the same monkey. Dark areas are radioactively labelled. The section grazes white matter (W) in two places, which are seen as pale ovals. Surrounding these are densely labelled sets of orientation columns in layer VI. Outside this is layer V, lightly labelled, and then IV c, which is uniformly labelled with no hint of columnar subdivisions. The upper layers again show densities related to columns. (Dense bands perpendicular to the surface at the extreme left are artefacts produced by the microtome knife.)

More superficial sections from this block, tangent to the upper layers (II–III) rather than to white matter, show an almost identical pattern, as expected from the fact that the labelled regions are perpendicular to the surface.

So far we have examined too few brains to be sure that the pattern of the orientation columns is always as complex in form as that shown here. Ocular dominance columns were examined in the same region as that shown in Fig. 2, by transneuronal autoradiography following an injection of ^{3}H-proline into one eye two weeks before the deoxyglucose experiment. These showed a more regular pattern of stripes, with a spacing only slightly coarser than that of the orientation hypercolumns (770 μm compared with 570 μm). There was no obvious tendency for the two sets of columns to be related in any simple way: they were not consistently orthogonal, and were certainly not parallel. This result will be published separately[9].

The anatomical demonstration of the orientation columns provides still another verification of the columnar organisation of the striate cortex. It is reassuring to find such agreement between morphology and physiology, and unusual to find the physiology actually leading the way to a structural description.

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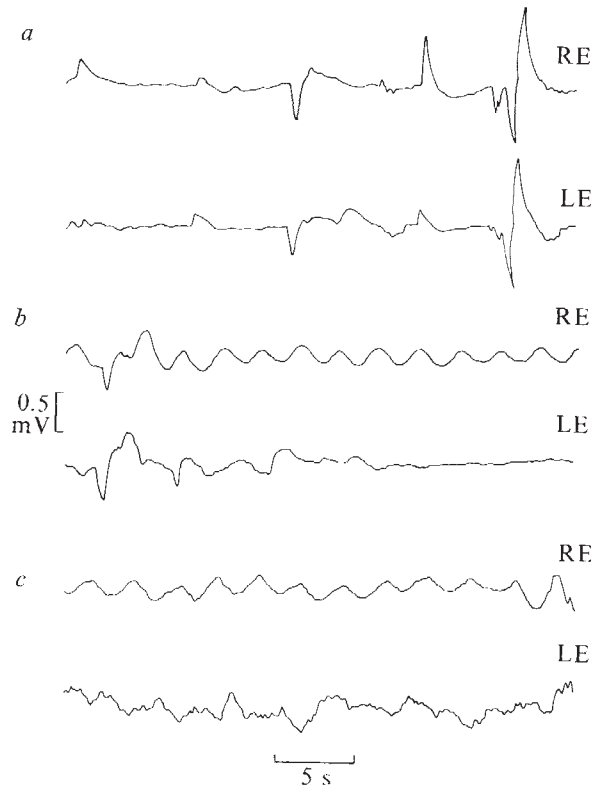

Fig. 1 *a*, Electro-oculographic a.c. recordings of horizontal movements of the right eye (RE) and left eye (LE) of a normal cat in the dark. *b*, The same, recorded 1 d after section of the ophthalmic branch of the right Vth nerve and *c*, 1 week after section of the ophthalmic branch of the left Vth nerve. Upward trace deflections indicate rotation of the eyes to the right, downward deflections, rotations to the left. The vertical bar corresponds to 5° horizontal eye rotation. Band pass: 0.1–20 Hz.

Instability of the eye in the dark and proprioception

THE extraocular muscles of most mammals contain stretch receptors which are not necessarily in the form of muscle spindles. The cat, for example, has no muscle spindles, but the oculomotor muscles contain structures, with spiral endings, that are in parallel with the muscle fibres and respond to stretch[1]. Most proprioceptive fibres run extraorbitally in the ophthalmic branch of the Vth nerve[2,3]. The cell bodies of these fibres seem to be located in the mesencephalic root of the Vth nerve[4]. There is electrophysiological evidence of proprioceptive projections to the cerebellum[5,6], superior colliculus[7,8] and visual cortex[9]. The function of ocular proprioception is uncertain, although there is evidence that in man it may be an indicator of eye position in the dark[10]. In the cat it seems to have a role in maintaining the binocularity of single neurones in the visual cortex[11,12]. Because most proprioceptive fibres run into the ophthalmic branch of the Vth nerve, we have investigated eye movements in the alert animal before and after section of this branch. We report here that, in the absence of visual and vestibular stimuli, the eye ipsilateral to the section becomes unstable and performs slow pendular oscillations. Asymmetries are observed in the horizontal vestibular nystagmus recorded in total darkness from the two eyes after unilateral section.

Cats chosen for their docility were anaesthetised with pentobarbital. A plastic cylinder was fixed to the skull and Ag–AgCl electrodes were implanted for recording horizontal movements of both eyes. The cat was trained to stay in a comfortable box with its head fixed by the plastic cylinder to a holder. The holder could either prevent any head motion or allow head rotations about a vertical axis. The cats were trained, over several days, to perform horizontal eye movements, tracking a visual target with the head either fixed or free to rotate horizontally. Eye movements were calibrated in each session by setting two pointers 30 cm from the eyes and displacing them 15° on either side of the cat's medial plane. The cat's attention was then called to either pointer while an experimenter checked the correctness of fixation, and the corresponding position of the recording trace was marked.

The cat box and the head holder were fixed to a table that could be oscillated sinusoidally about a vertical axis at various amplitudes and velocities.

When sufficient eye movements were recorded, the animal was again anaesthetised and the ophthalmic branch of the right Vth nerve was cut at the exit from the orbit or at the entrance into the semilunar ganglion. A small aperture was made into the parieto-temporal bone and the brain was lifted gently to reveal the three branches of the Vth nerve. Care was taken not to touch the oculomotor nerves. The operation was repeated on the left side after 2 or 3 weeks. After the experiments the animal was killed and the completeness of the section was checked.

We present here only results concerning eye movements in the dark, either spontaneous or elicited by head or body oscillations. The records of horizontal eye movements of normal cats that are awake in darkness contain periods with frequent spontaneous saccades, and periods with very few saccades, but with slow drifts mainly conjugate in the two eyes.

After section of the ophthalmic branch of the Vth nerve