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Physiological evidence that the 2-deoxyglucose method reveals orientation columns in cat visual cortex

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A method for measuring local rates of glucose uptake in mammalian brain tissue[1,2] has been used to study monkey cortex receiving visual stimulation through one eye[3]. The resulting pattern of glucose uptake resembled that of the ocular dominance columns, identified by several independent methods in the cortex[4-6]. In addition, the pattern of glucose uptake produced by binocular viewing of stripes of a single orientation[7-12] was consistent with the arrangement of orientation columns expected from the sequence of preferred orientations of single cells recorded in the visual cortex[4,13-16]. It was therefore assumed that the columns of glucose uptake revealed in the studies of orientation selectivity correspond to columns of cortical cells selective for the orientation viewed. Recent findings about the monkey visual cortex led us to question this assumption.[17-20] We have now investigated this question in the cat by making microelectrode recordings to determine preferred orientation of cortical units at known positions within the visual cortex and then stimulating the same animals with a full-field pattern of stripes to measure the rates of glucose uptake at the positions of each of the cortical units. As in previous studies, preferred orientation shifted gradually and progressively along electrode tracks parallel to the cortical surface[4,13-16] and a pattern of densely labelled columns was observed in the 2-deoxyglucose autoradiographs[7-12]. The centres of densely labelled cortical columns contained cells selective for the stimulated orientation. Preferred orientation shifted gradually away from the stimulated orientation at sites progressively more distant from the centres of the columns. Control experiments revealed no columns after stimulation with a pattern of changing orientation. Thus the columns revealed by the deoxyglucose method do correspond to the physiological orientation columns.

The experimental procedure consisted of two consecutive steps carried out on five normal adult cats. During the first step the preferred orientation of single cells in the medial bank of area 17 was determined by microelectrode recording; during the second step a striped pattern was presented to the animal after intravenous (i.v.) injection of deoxyglucose.

Initial surgery was carried out under barbiturate plus halothane anaesthesia. Venous and tracheal cannulae were inserted and a small skull opening made to expose the most posterior portion of one cortical hemisphere. On completion of surgery, the cats were paralysed (gallamine triethiodide, 10 mg per kg per h), and barbiturate anaesthesia was supplemented by artificial ventilation with 75% nitrous oxide–25% oxygen. End-tidal CO_2 was maintained at 4% and body temperature at 38 °C.

We then started a horizontal penetration into the medial bank of the lateral gyrus using a lacquered tungsten microelectrode[21]. The microelectrode shaft had been electropolished to <50 μm in diameter, allowing us to record from a long sequence of neurones in a single 10-mm penetration with minimal damage to the surrounding brain tissue.

The eyes were refracted and then aligned with a Risley prism by superimposing the receptive fields of a binocular unit. The areae centrales were plotted onto the tangent screen at 57 cm distance. Beginning 4–6 mm from the entry point, single cells from area 17 were recorded approximately every 100 μm. The preferred orientation of each cell was determined with a hand-held projector. An irregular sequence of electrolytic microlesions was made at ~1-mm intervals along the track by passing −4 μA for 4 s.

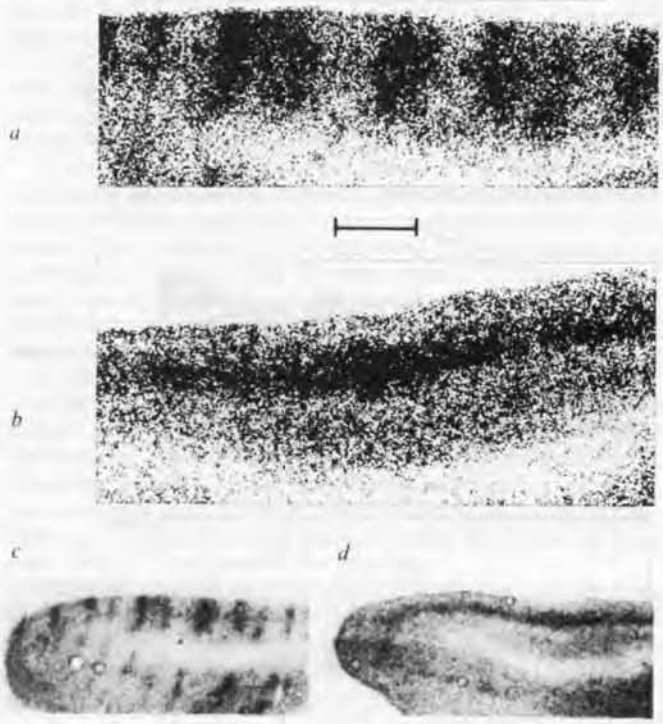

Fig. 1 Digitized radioactivity plots made from autoradiographs of parts of 20-μm horizontal sections of right visual cortex in two animals. *a*, Part of the medial bank of the lateral gyrus in area 17 of an animal that had viewed moving vertical stripes. Dark bands referred to as orientation columns run from white matter to cortical surface. *b*, Same area in a control animal that had viewed stripes of all orientations. No radial zones of increased radioactivity are visible. Up is medial, right posterior. *c*, Photograph of autoradiograph scanned in *a*. *d*, Photograph of autoradiograph scanned in *b*. Calibration bar is 1 mm for *a* and *b*, 3 mm for *c* and *d*.

The penetration was terminated after studying about 40 single units. With the microelectrode kept in its final position, we injected an i.v. pulse of 100 μCi per kg ^{14}C-2-deoxyglucose (Amersham, CFA.562) and exposed both eyes to a striped pattern moved perpendicular to the orientation of the stripes. This pattern consisted of white stripes 0.5–8.0° wide, separated by a similar spacing. It was swept in both directions at velocities increasing logarithmically from 0.3 to 20° s^{-1} during each 15-s period. We made sure that this pattern always covered at least the central 30° of the cat's visual field. In four of the animals, the pattern remained vertically oriented, while in one control animal the pattern was rotated between sweeps to each of 12 orientations, 15° apart.

After 45 min of exposure, the animals were given an overdose of barbiturate and were perfused with phosphate buffer followed by a buffered 4% solution of paraformaldehyde. The brain was then removed and blocks from cortical tissue cut and sunk slowly into Freon at −80 °C. The physiological experiment took 12–20 h; the whole procedure after killing the animal took 12–18 min.

Horizontal sections of the hemisphere containing the electrode track were cut at 20 μm in a cryostat, picked up on cover glasses, dried quickly on a hotplate at 70–80 °C and exposed along with a set of radioactive plastic standards (Amersham, 196363) to Kodak SB5 X-ray film for 10–15 days. The sections were then stained with cresyl violet for reconstruction of the electrode track.

A flatbed autodensitometer[22] measured optical density over areas of interest on autoradiographs containing the electrode tracks using a 32×32 μm spot moved in 32-μm steps. Either

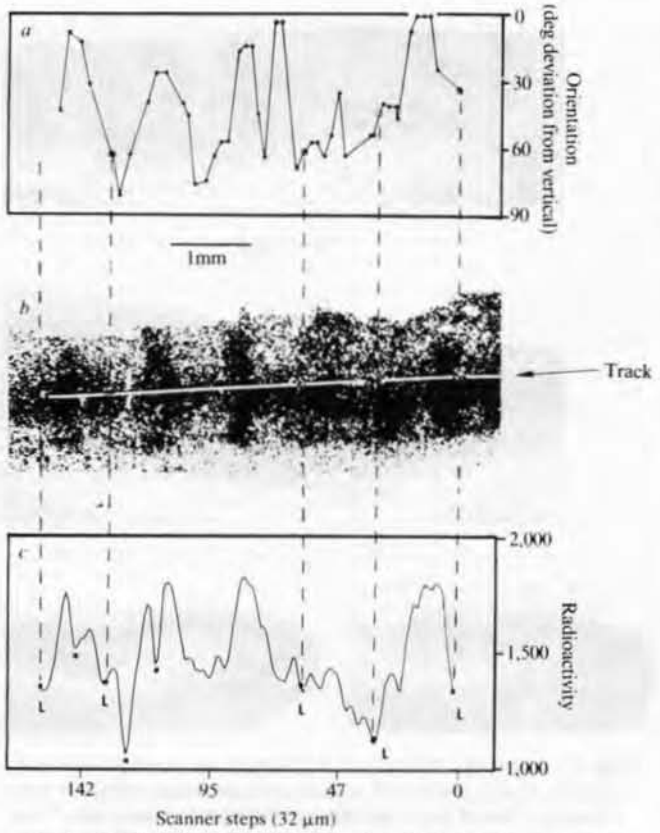

Fig. 2 Preferred orientation of single cells (*a*) and radioactivity (proportional to glucose uptake) (*c*) plotted as a function of distance (abscissa) along a microelectrode track (*b*) into area 17 of the visual cortex. Track starts at the right-hand side (arrow in *b*) and runs along the white line. *a*, Position of each dot gives the deviation of a single cell's preferred orientation from vertical, the orientation of the striped pattern used in the deoxyglucose experiment. Thus vertically oriented cells are represented by dots at the peaks of the graph, horizontally oriented cells at the troughs of the graph, and the two diagonals in between. *b*, Display of radioactivity made from autoradiographs of two adjacent 20 μm horizontal sections from area 17 of a cat's visual cortex. In the autoradiographs of these sections, the whole course of the penetration could be traced by means of microlesions seen at the interruptions of the white line. *c*, Radioactivity (in arbitrary units) along the electrode penetration. 'L' marks troughs corresponding to light areas in the autoradiograph caused by microlesions. '*' Marks troughs caused by fractures in the tissue. The vertical dashed lines connect positions of microlesions throughout the figure (open circles in *a*, interruptions of the white line in *b*, 'L' in *c*). It is apparent that peaks in the graph *a* (vertically oriented cells) match areas of high radioactivity levels (*b*, *c*) and vice versa.

1,000 or 10,000 digitized optical density values per mm^2 were stored for each section together with the optical densities of the radioactive standards. This method allowed us to convert the optical density at any position on the section into a radioactivity level. Such radioactivity levels are linearly proportional to the rates of glucose uptake[2]. Using a PDP11/23 computer we constructed a two-dimensional picture of the distribution of radioactivity which had produced each autoradiograph. Linear scans of such a picture made along the course (indicated by microlesions) of the electrode penetration quantitatively displayed the radioactivity as a function of electrode position.

Figure 1*a* shows a radioactivity scan of part of a horizontal section through area 17 from an animal that had viewed vertically oriented stripes. A pattern of dark and light bands extending mostly perpendicular to the brain surface is evident throughout all layers of grey matter. A similar scan taken from the brain of the control animal which had viewed stripes of all orientations is presented for comparison in Fig. 1*b*. No columnar variation in labelling density could be discerned, although differences in the labelling density between the laminae are evident. Autoradiographs from four additional control animals similarly disclosed no columnar variation in labelling density.

In most cases, the electrolytically marked locations in the brain were visible on the autoradiograph (as a reduction in the radioactivity over a distance of 50–150 μm along the track) as well as on the stained section. We were therefore able to locate recording sites by interpolation of the micrometre readings. Figure 2 shows examples of the finding that when a penetration crossed a labelled band, the preferred orientations of the cells recorded in this location were usually close to the vertical (the stimulated) orientation. On the other hand, lightly labelled portions of the electrode track usually matched recording sites of cells driven best by stimuli closer to the horizontal. A few cells

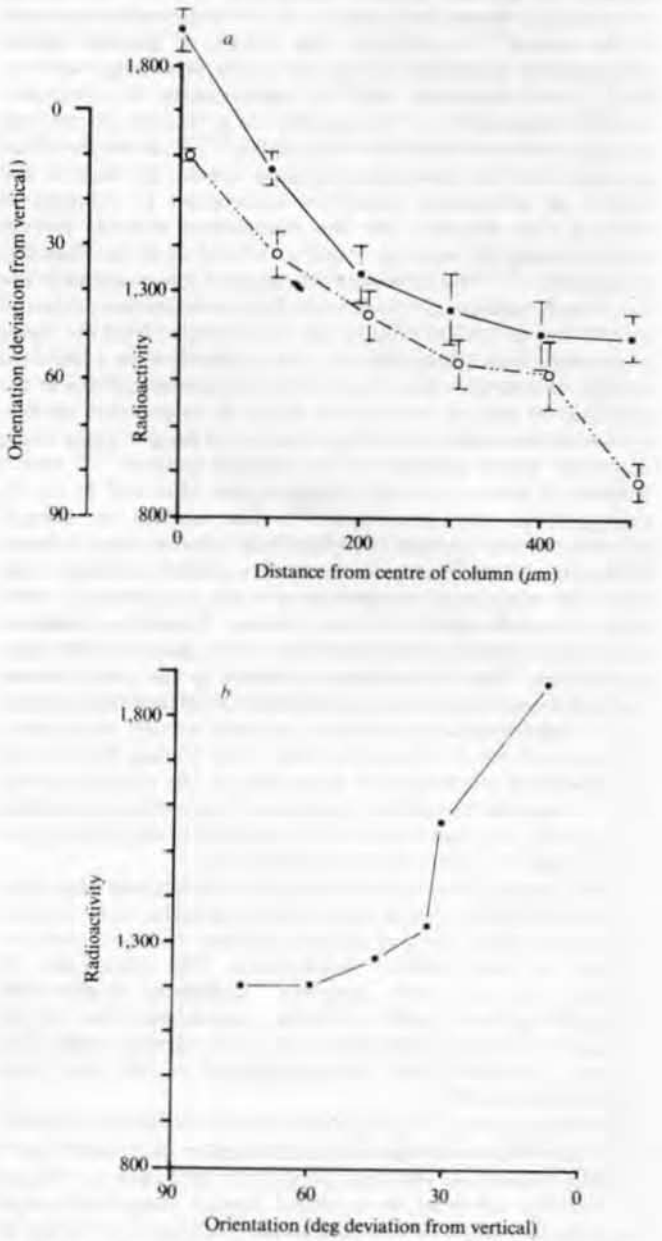

Fig. 3 *a*, Deviations of cells' preferred orientations from vertical ±s.e.m. (left ordinate, dashed line, ○) and radioactivity ±s.e.m. (right ordinate, solid line, ●) as functions of distance (abscissa) from the centres of 10 superimposed dark bands (orientation columns) in four experimental animals. *b*, Data from *a* plotted to show relationship between preferred orientation of recorded units and radioactivity (proportional to glucose uptake rate) at recording sites.

(such as the second and third cells to the left of the third lesion) were exceptions to this general pattern. Some exceptions might be due to extracellular microelectrodes recording from units whose cell bodies are located at some distance from the electrode tip, perhaps in another column. The following analysis demonstrates that, despite these exceptions, the relationship between preferred orientation and labelling density at recording sites is highly significant.

A total of 10 labelled columns, either 2 or 3 in each of the four experimental animals, were both cut in cross-section (the cortical surface lay normal to the plane of section) and traversed by our electrode tracks. For example, three of these columns are to the left of the third lesion in Fig. 2*b*. To the right of this lesion, the cortex is cut obliquely, as shown in a Nissl-stained section (not illustrated) and by the failure of the labelled deoxyglucose bands to extend all the way from layer II to the white matter.

Within the parts of the electrode penetrations that crossed these 10 labelled bands, a total of 65 units were recorded. Figure 3 shows the relationship between the preferred orientation of these cortical units and the deoxyglucose labelling densities at their recording sites. For this analysis, preferred orientations (dashed line in Fig. 3*a*) and radioactivity levels (solid line in Fig. 3*a*) at recording sites were plotted in groups according to the distance of each recording site from the centre of the nearest labelled band. In effect, the analysis of Fig. 3*a* first superimposes the deoxyglucose columns at their centres to form an average column. Average preferred orientation and average radioactivity level are then plotted as a function of distance from the centre of this average column. The analysis confirms the results of earlier studies[14–16]—preferred stimulus orientation changes linearly with distance from the centre of the orientation column ($R = 0.86$, Fig. 3*a*). Figure 3*a* also shows the decrease in average radioactivity level with distance from the centre of the column. The data from Fig. 3*a* are replotted in Fig. 3*b* to show that the radioactivity level is monotonically related to the preferred orientation ($R = 0.92$).

In the experiments using the 2-deoxyglucose method, labelled bands were seen in the visual cortex of cats that viewed vertical stripes. When animals viewed stripes of all orientations, no such bands were observed. Microelectrode recording showed that the labelled bands corresponded to vertical orientation columns.

These findings support the central assumption made in previous studies of orientation selectivity in cat visual cortex using the 2-deoxyglucose method[7–12]. They suggest that the deoxyglucose method should be useful for further studies of columnar systems in cat cortex. The present findings do not show whether orientation columns in the cat's visual cortex are discrete entities; the results are consistent with such an hypothesis but are also consistent with the notion that preferred stimulus orientation varies continuously across the cortical surface.

In the monkey's visual cortex, labelled columns have been observed[19] in conditions of visual stimulation similar to those which produced no labelled columns in our control cats. It has not yet been reported whether the labelled columns seen in the unstimulated monkey have the same range of densities as those seen in monkeys stimulated with patterns of single orientation. With this question unresolved, it would not be safe to extend the results of the present study to the monkey.

It will be of interest to determine to what extent the relationship between orientation specificity and the cortical metabolic pattern seen in the present study is based on a more general relationship: is neuronal firing the primary determinant of metabolic activity in the cortex? Experiments in progress, quantitatively comparing the neuronal discharge frequency elicited by visual stimuli with glucose uptake produced by these same stimuli,[23] will answer this question.

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1. Kennedy, C. *et al. Science* **187,** 850–852 (1975).
2. Sokoloff, L. *et al. J. Neurochem.* **28,** 897–916 (1977).
3. Kennedy, C. *et al. Proc. natn. Acad. Sci. U.S.A.* **73,** 4230–4234 (1976).
4. Hubel, D. H. & Wiesel, T. N. *J. Physiol., Lond.* **195,** 215–243 (1968).
5. Wiesel, T. N., Hubel, D. H. & Lam, D. M. K. *Brain Res.* **79,** 273–279 (1974).
6. LeVay, S., Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **159,** 559–576 (1975).
7. Hubel, D. H., Wiesel, T. N. & Stryker, M. P. *J. Comp. Neurol.* **177,** 361–380 (1978).
8. Stryker, M. P., Hubel, D. H. & Wiesel, T. N. *Abstr. Soc. Neurosci.* **3,** 1852 (1977).
9. Humphrey, A. L., Skeen, L. C. & Norton, T. T. *J. comp. Neurol.* **192,** 549–566 (1980).
10. Singer, W. *Expl Brain Res.* **41,** 199–215 (1981).
11. Albus, K. *Expl Brain Res.* **37,** 609–613 (1979).
12. Flood, D. G. & Coleman, P. D. *Brain Res.* **173,** 538–542 (1979).
13. Hubel, D. H. & Wiesel, T. N. *J. Physiol., Lond.* **165,** 559–568 (1963).
14. Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **158,** 267–294 (1974).
15. Albus, K. *Expl Brain Res.* **24,** 181–202 (1975).
16. Humphrey, A. L. & Norton, T. T. *J. comp. Neurol.* **192,** 531–547 (1980).
17. Hendrickson, A. E. & Wilson, J. R. *Brain Res.* **170,** 353–358 (1979).
18. Horton, J. C. & Hubel, D. H. *Abstr. Soc. Neurosci.* **6,** 315 (1980).
19. Humphrey, A. L. & Hendrickson, A. E. *Abstr. Soc. Neurosci.* **6,** 315 (1980).
20. Hendrickson, A. E., Hunt, S. & Wu, J. Y. *Abstr. Soc. Neurosci.* **6,** 671 (1980).
21. Hubel, D. H. *Science* **125,** 549–550 (1957).
22. Ross, M. J. & Stroud, R. M. *Acta crystallogr.* A **33,** 500–508 (1977).
23. Schoppmann, A. & Stryker, M. P. *Abstr. Soc. Neurosci.* **6,** 314 (1980).