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LETTERS TO NATURE

Regular patchy distribution of cytochrome oxidase staining in primary visual cortex of macaque monkey

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In two preliminary studies, normal macaque[1] and squirrel-monkey[2,3] striate cortex cut parallel to the surface and stained for cytochrome oxidase (a mitochondrial enzyme) showed a striking pattern of regularly spaced patches. This was surprising, since until then no physiological or anatomical studies had suggested such a patchy organization. In the present study in the macaque we found that the patches were arranged in rows spaced about 350 μm apart. When one eye was injected with tritiated proline the rows of patches in layers II and III lay in register with the ocular-dominance bands seen autoradiographically in layer IVc. Removing one eye caused the patches in every other row to shrink and blanch. The rows of patches are therefore centred on the ocular dominance columns. Regions labelled by 2-deoxyglucose autoradiography after stimulating one eye with black-and-white stripes in all orientations consisted of rows of patches that lay in register with the cytochrome oxidase patches in every other row. On stimulating monkeys with stripes of a single orientation, the deoxyglucose-labelled regions formed a lattice that included the cytochrome oxidase patches but was more extensive. Thus either the deoxyglucose is not labelling the orientation columns at all, or the orientation columns coalesce in the areas marked by the cytochrome oxidase stain.

Wong-Riley has used a stain for cytochrome oxidase to demonstrate a pattern of alternating light and dark bands in layer IV of a monocularly deprived kitten, the lighter bands presumably reflecting a lowered metabolic activity in the set of columns belonging to the closed eye[4]. Our original purpose was to examine cytochrome oxidase activity in the different layers of the striate cortex of the normal macaque monkey. Sections (50 μm) from glutaraldehyde–paraformaldehyde fixed tissue were cut on a freezing microtome and processed for cytochrome oxidase activity following a procedure modified from Seligman *et al.*[5]. In sections cut perpendicular to the cortical surface, cytochrome oxidase staining was darkest in layers IVa and IVc, which receive the major direct projections from the lateral geniculate body[6]. Layers II and III were lightly stained but showed periodic fluctuations in density. To examine the pattern more closely we sectioned the cortex tangentially. Cytochrome oxidase staining showed an array of dark oval patches about 150 × 200 μm which were most obvious in Layers II and III but were also present, though faint, in layer VI. Although varying to some extent from animal to animal, the patches were generally aligned in rows spaced ~350 μm apart, with the long axis of the patches parallel to the rows. Within a row the patches were ~550 μm apart, but in some places became confluent. Occasionally, patches in neighbouring rows seemed to be aligned, forming a square or hexagonal array over small regions of cortex.

The rows of cytochrome oxidase patches resembled ocular dominance columns in their spacing and in intersecting the 17–18 border at right angles (Fig 1*a*). It was obviously important to learn whether these rows were really related to the ocular dominance columns, and, if so, whether the patches lay along the dominance columns or straddled the borders between them. We therefore removed one eye of a macaque monkey, killed the animal 10 days later and stained the visual cortex for cytochrome oxidase. Sections through layer IVc showed ocular dominance columns visable as alternating light and dark bands (Fig. 1*b*), in striking contrast to the uniformly deep staining of this layer for cytochrome oxidase in normal monkeys. In sections passing through layers II and III there were parallel

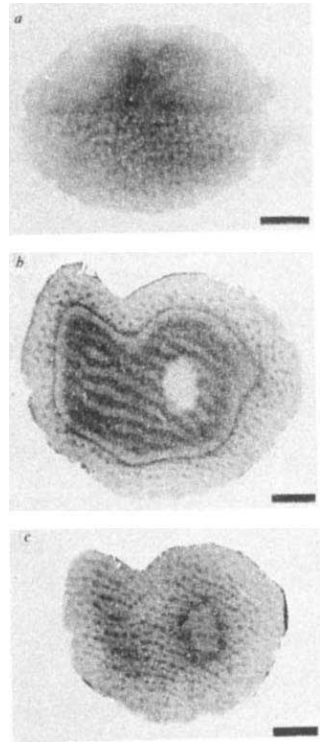

Fig. 1 *a*, Tangential section through the superficial layers of the visual cortex of the normal macaque monkey; cytochrome oxidase stain. The section passes through the 17–18 border, which runs horizontally in the figure with area 17 below and 18 above. The patches in 17 are aligned in rows perpendicular to the 17–18 border. *b*, Cytochrome oxidase section tangential to area 17 in a macaque monkey that had its right eye removed 10 days previously. Section grazes layer V, which forms a pale oval near the centre, surrounded from within out by layers IVc (dark stripes), IVb (pale and narrow), IVa (dark, very narrow) and II, III (containing patches). Dark and pale alternating stripes in layer IVc are typical of ocular dominance columns, in their regularity, direction and spacing. *c*, Same monkey as in *b*. This section is more superficial, grazing layer IVb. Cytochrome oxidase patches lie in rows that are alternately dark and pale: the pale rows lie over the light stripes in layer IVc corresponding to the injured eye. Scale bar, 2 mm.

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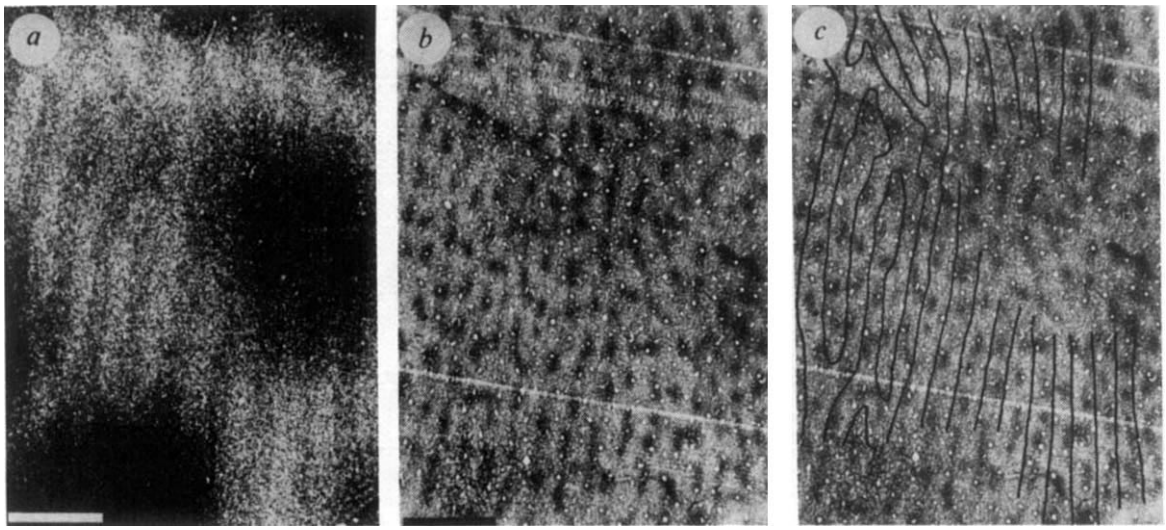

Fig. 2 *a*, Dark-field autoradiograph of striate cortex in a normal monkey whose right eye was injected with ^{3}H-proline 2 weeks earlier. The section is tangential, grazing layer V (dark ovals) but passing mainly through layer IVc. Here typical ocular dominance columns are seen as light bands of label corresponding to the injected eye separated by darker gaps. *b*, More superficial section from the same block as *a*, cytochrome oxidase stain. The rows of cytochrome oxidase patches in the superficial layers follow the pattern of the ocular dominance patches. This is shown in *c* by drawing the borders of the columns from *a* directly onto *b*. Scale bar, 1 mm.

rows of patches as in normal monkeys, but in every other row the patches were paler and smaller (Fig. 1*c*). When one of these sections was aligned with a section through layer IVc, using as a guide small blood vessels (which generally run normal to the cortex through its full thickness), the alternating rows of dark and light patches fell into precise register with the dark and light bands in layer IVc. This indicated that the rows of patches in normal monkeys lie centred on the ocular dominance columns rather than along the borders separating them. All the cells in any given patch are thus likely to be strongly dominated by the same eye.

In a second monkey we injected one eye with 2 mCi ^{3}H-proline to label the ocular dominance columns in layer IVc by transneuronal autoradiography. Figure 2*a*, a dark-field autoradiograph of a section tangential to layer IVc, shows typical ocular dominance columns in which the light bands represent the injected eye. A more superficial section stained for cytochrome oxidase shows the expected array of patches (Fig. 2*b*). When aligned with the autoradiograph, again using radial blood vessels as a guide, the rows of patches follow the course of the ocular dominance columns and are centred on them (Fig. 2*c*).

In a third monkey we used the 2-deoxyglucose method to label regions of cortex activated by stimulation of one eye. We anaesthetized the animal with sodium thiopental injected ^{14}C-2-deoxyglucose (100 μCi per kg), and stimulated the visual field of the right eye with a set of black-and-white stripes of irregular width and spacing, moved slowly back and forth and steadily rotated so as to expose the animal to all orientations about once every minute. After 45 min the monkey was killed, the brain perfused, frozen and sectioned and the dried sections pressed against X-ray film. Alternate sections were stained for cytochrome oxidase. A pattern representing ocular dominance columns was visible in the deoxyglucose autoradiographs of tangential sections through layer IVc. An adjacent section stained for cytochrome oxidase showed no trace of ocular dominance columns in layer IVc, indicating that the brief period of monocular stimulation was insufficient to affect cytochrome oxidase levels. When a cytochrome oxidase section through layers II and III (Fig. 3*a*) was superimposed on a deoxyglucose autoradiographs of layer IVc, the rows of cytochrome oxidase patches were again centred over the ocular dominance columns, confirming the results of the eye removal and transneuronal autoradiography experiments.

Deoxyglucose sections adjacent to Fig. 3*a* showed an array of patches of increased deoxyglucose uptake (Fig. 3*b*) which lay

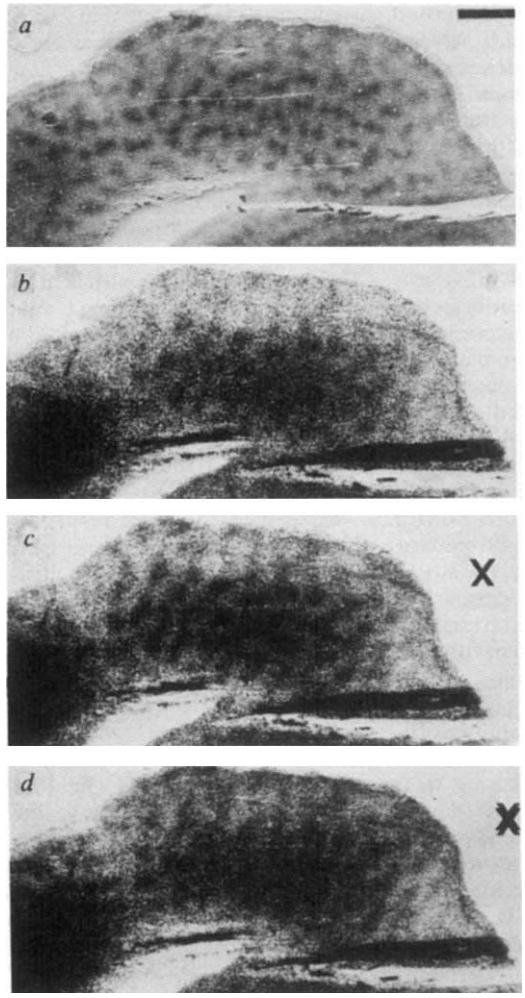

Fig. 3 *a*, An array of cytochrome oxidase patches in layers II, III of striate cortex of a normal macaque monkey. *b*, This 2-deoxyglucose autoradiograph of an adjacent section shows patches of uptake of label after stimulation of one eye with black-and-white stripes in all orientations. *c*, When *a* and *b*, photographed on film, are overlapped in precise register the two patterns reinforce. *d*. When shifted out of alignment by 150 μm (see Xs) the patterns tend to cancel. Scale bar, 1 mm.

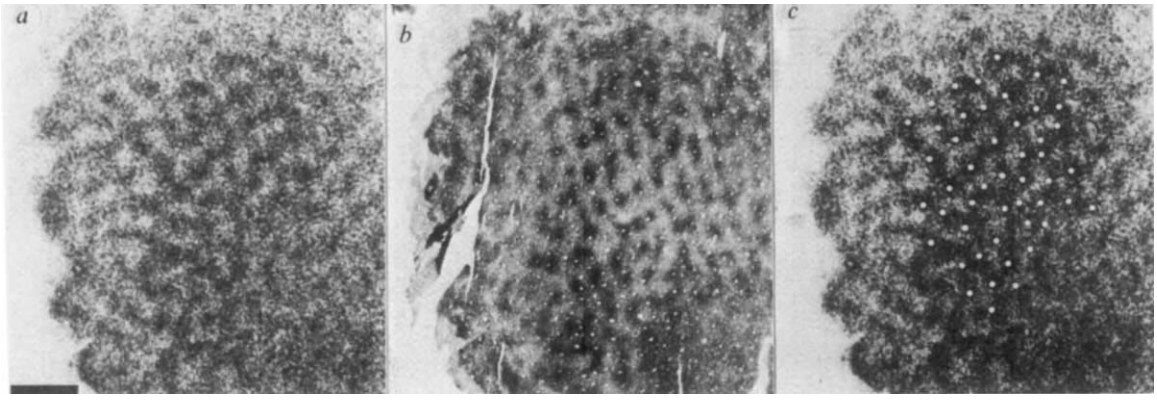

Fig. 4 *a*, Complex pattern of ^{14}C-2-deoxyglucose label in layers II, III of macaque striate cortex after stimulation of both eyes with vertical stripes. *b*, An adjacent section stained for cytochrome oxidase shows an array of patches. *c*, The cytochrome oxidase patches fall within the lattice of deoxyglucose label, as shown by representing each patch in *b*, with a small white dot, and placing them directly on *a*. Scale bar, 1 mm.

over the deoxyglucose-labelled ocular dominance columns in layer IVc. A similar patchy pattern from stimulating one eye with all orientations has been observed by Kennedy *et al.*[7] and by Hendrickson and Wilson[8]. The deoxyglucose pattern resembled the cytochrome oxidase pattern, but the patches were larger and the rows more widely spaced. When Figs 3*a* and 3*b* were superimposed, in and out of register (Fig. 3*c*, *d*), the patches of increased deoxyglucose label matched the cytochrome patches lying in every other row.

Attempts to learn whether there was any relationship between the cytochrome oxidase patches and orientation columns produced unexpected results. In two experiments we stimulated with vertical black-and-white stripes after injecting 2-deoxyglucose. As shown in Fig 4*a*, the pattern in tangential sections through layers II and III was periodic and yet highly complex, forming a lattice of stripes, rosettes and patches, as reported previously[9]. The patches seen in adjacent sections stained for cytochrome oxidase (Fig. 4*b*) fell along the lattice of deoxyglucose label (Fig. 4*c*). Where the deoxyglucose pattern appeared patchy, the patches tended to coincide with the cytochrome oxidase patches; the deoxyglucose pattern thus included the cytochrome patches but seemed to extend beyond and between them. We first thought that the cytochrome oxidase patches might have some special relationship to vertical orientation columns[2], but two similar experiments using deoxyglucose and horizontal stripes gave the same result, with the cytochrome oxidase patches lying along the lattice formed by the deoxyglucose label. Thus all orientations of line stimuli probably activate cortex in the regions of the patches, as indeed is suggested by the deoxyglucose experiment in which one eye was stimulated with all orientations. A combination of physiological recordings and a double-label deoxyglucose technique[10] will probably help clarify the relationship between the patches and the orientation columns.

In squirrel monkeys Humphrey and Hendrickson[3] have shown that the deoxyglucose pattern obtained when both eyes are stimulated with all orientations is identical to the cytochrome oxidase pattern. Similarly, we have found in two squirrel monkeys a strong correlation between the cytochrome oxidase patches and the pattern after stimulation with horizontal stripes in both eyes. In the human and the galago, a prosimian primate, the striate cortex likewise shows cytochrome oxidase patches, we find that the cat and tree shrew lack such patches. A patchy distribution of cytochrome oxidase staining may reflect a system of inputs, outputs and intrinsic connections unique to the visual cortex of primates.

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Effect of chemical environment on levels of substance P and somatostatin in cultured sensory neurones

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The neurotransmitter produced by autonomic neurones is influenced by the environment in which they develop[1]. For example, Le Douarin[2] and her colleagues have shown that regions of the neural crest which normally give rise to cholinergic ganglia can be induced to develop into adrenergic cells by transplanting them into a region of the crest which normally gives rise to adrenergic ganglia. Similarly, sympathetic neurones grown in culture express either adrenergic or cholinergic properties depending on whether they are grown in the absence or presence of certain types of non-neuronal cells[1,3–5]. Patterson *et al.*[6,7] demonstrated that this is not due to selective survival of a population of neurones but that the transmitter choice of individual neurones can be altered by soluble factors produced by non-neuronal cells. Like autonomic neurones, sensory neurones derive from the neural crest and it therefore seems likely that the type of transmitter they produce could also be influenced by the environment in which they develop. Here I demonstrate that when sensory neurones from embryonic chick dorsal root ganglia are grown together with ganglionic non-neuronal cells or with medium 'conditioned' by incubation with such cells, they produce increased amounts of somatostatin (SOM). This increase is neither accompanied by an increase in substance P (SP) content nor a detectable change in neuronal survival and thus differs from the effect of nerve growth factor (NGF), which increases survival of sensory neurones without affecting the relative levels of SOM and SP.

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