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Autoradiographic demonstration of ocular-dominance columns in the monkey striate cortex by means of transneuronal transport

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In the past few years the technique of mapping pathways in the central nervous system by anterograde axoplasmic transport of radioactive molecules has come into wide use and is now an important supplement to Nauta degeneration methods[2,6,10,13]. Several investigators[1,3,7,9,12] have noted radioactive substances in the postsynaptic cells, which suggests that these cells take up labeled material released from the terminals[1,3,7]. Grafstein[4] was the first to explore the possibility of tracing a pathway beyond the terminals of the initially labeled cells, by examining also the projections of the recipient postsynaptic neurons. By radiochemical measurements and by autoradiography, it was shown that after injection of [^{3}H]proline and [^{3}H]fucose into one eye of a mouse the contralateral striate cortex was more heavily labeled than the ipsilateral, and that the label was concentrated in layer IV[5,14].

In the macaque monkey the geniculostriate pathway terminates in a very dense, highly localized manner, mainly in layer IV C. Furthermore, projections from the 2 eyes end in a characteristic alternating stripe-like pattern of ocular-dominance columns[8]. It occurred to us that if radioactive substances were transported transneuronally, injection of labeled material into one eye followed by autoradiography of the cortex might reveal the entire system of ocular-dominance columns.

For the autoradiographic study of transneuronal transport in the primate visual system, 50 μl of a saline solution containing L-[6-^{3}H]fucose (2.5 mCi/ml, 13.4 Ci/mmole) and L-[^{3}H]proline (7.5 mCi/ml, generally labeled, 6.8 Ci/mmole) was injected into the vitreous of the left eye of a 3 kg normal Rhesus macaque. This injection was repeated 5 times at 12 h intervals (total dose 3.0 mCi). The animal was perfused with 10% formalin 3 weeks after the initial injection. The lateral geniculate nucleus was cut into 20 μm frozen sections, and the striate cortex was embedded in paraffin and cut into 15 μm sections. The sections were coated with Ilford K5 emulsion, left in the dark for 2–4 months and developed in Dektol. Sections were counter-stained with thionin.

In the lateral geniculate nucleus all layers receiving projections from the injected eye were strongly labeled (Fig. 1A and B). The other layers showed grain counts higher than background, perhaps partly because of fibers of passage from the retina,

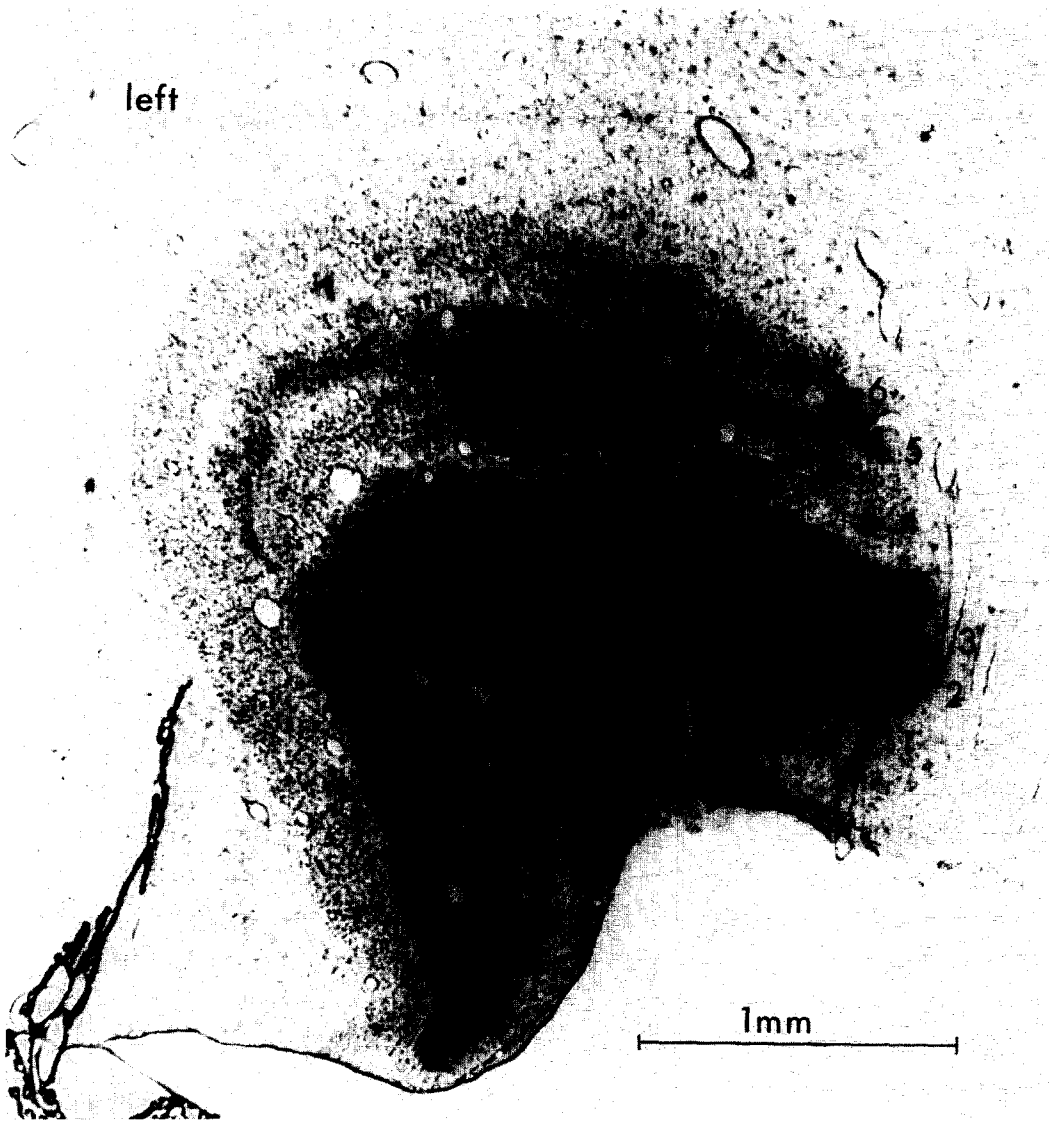
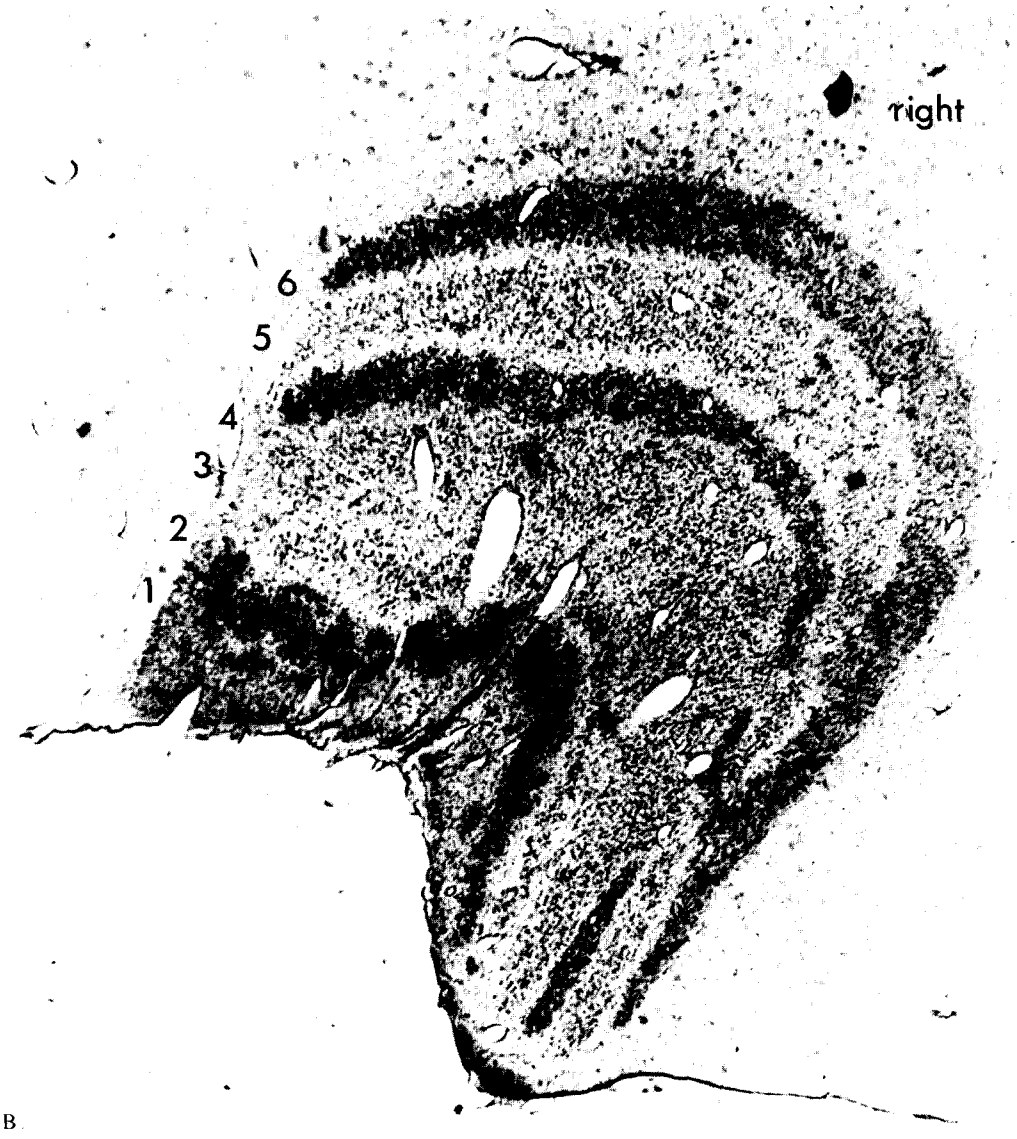


Fig. 1. Autoradiograph of left (A) and right (B) lateral geniculate bodies; coronal sections. Nissl counterstained, bright-field illumination. 1–6, the layers are numbered 1–6 from ventral to dorsal.

but also, probably, because of diffusion of radioactive material from recipient layers to adjacent ones.

In the striate cortex an accumulation of silver grains in layer IV was most easily visualized under dark-field illumination. A typical section is shown in Fig. 2. Deposition of granules can be seen in layer IV C and as a thin 'upper tier' in layer IV A. This distribution agrees well with that seen in Fink–Heimer stained material after a geniculate lesion[8]. An apparent difference between the two methods was a lack of any obvious concentration of radioactive material in layer I, which receives a very sparse input in Nauta stained material.



B

Fig. 1B.

The accumulation of silver grains in layer IV was interrupted by conspicuous gaps in which the density of grains was considerably less, although it was higher than that of the background (Fig. 2). The widths of the dark and light bands were about equal, measuring 0.3–0.5 mm. Fig. 3 shows a montage reconstruction of a section through cortex in the calcarine fissure. The plane of section is almost tangential to the cortex, cutting through layer IV C and grazing layer V. The alternating bands of IV C thus form an oval surrounding layer V. In the upper part of the reconstruction 5 or 6 columns are cut tangentially and appear as parallel stripes. The pattern revealed by Figs. 2 and 3 is identical to that seen with the Fink–Heimer modification of the Nauta method after a lesion confined to one layer of the lateral geniculate nucleus,

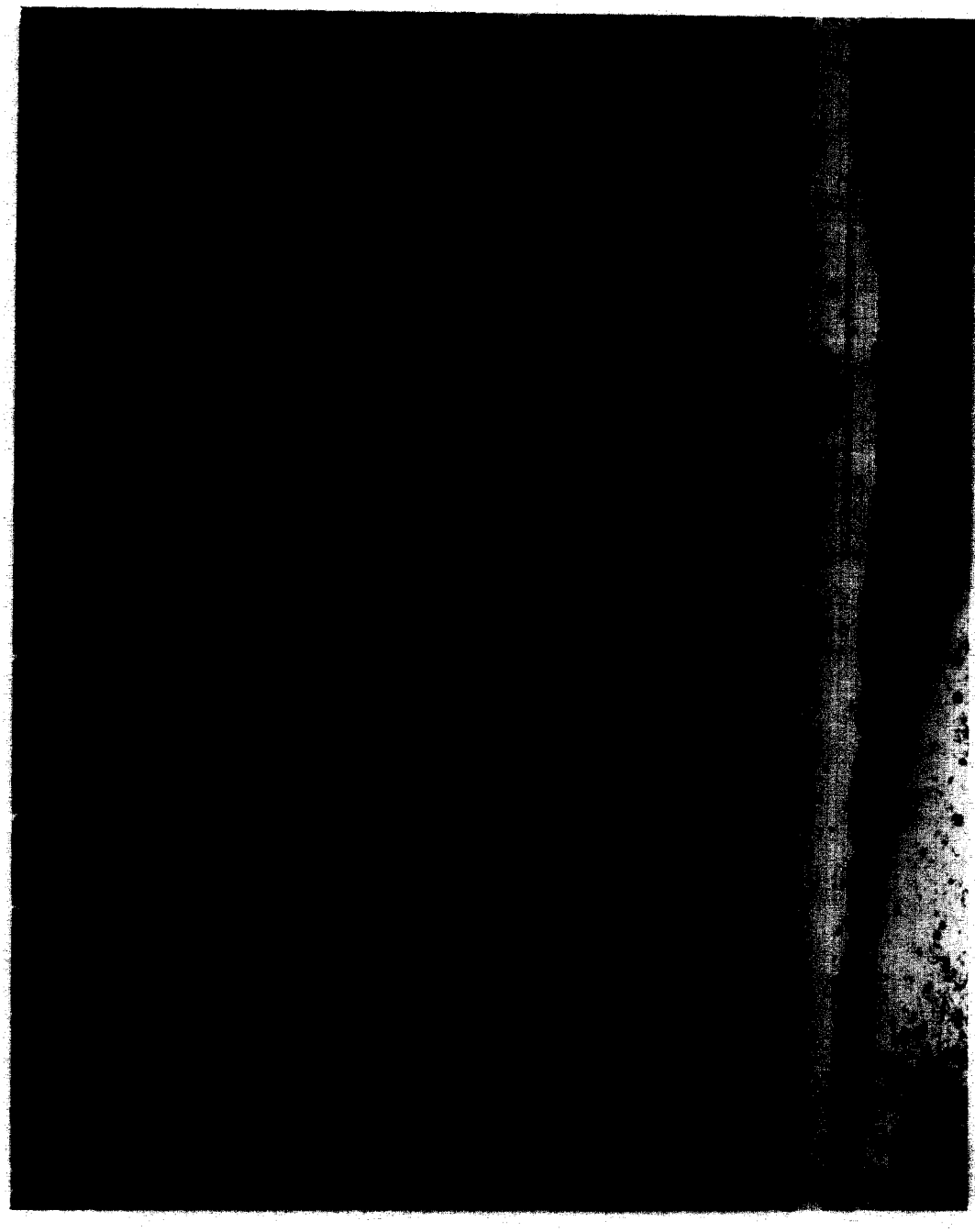


Fig. 2. Autoradiograph of a section through calcarine cortex (area 17), photographed under dark-field illumination. Bright bands of silver-granule accumulations appear in layer IV C at regular intervals, separated by gaps of equal width in which grains are fewer, although still more numerous than in the layers immediately above and below. Similar bands, much fainter and thinner, form an upper tier in layer IV A.

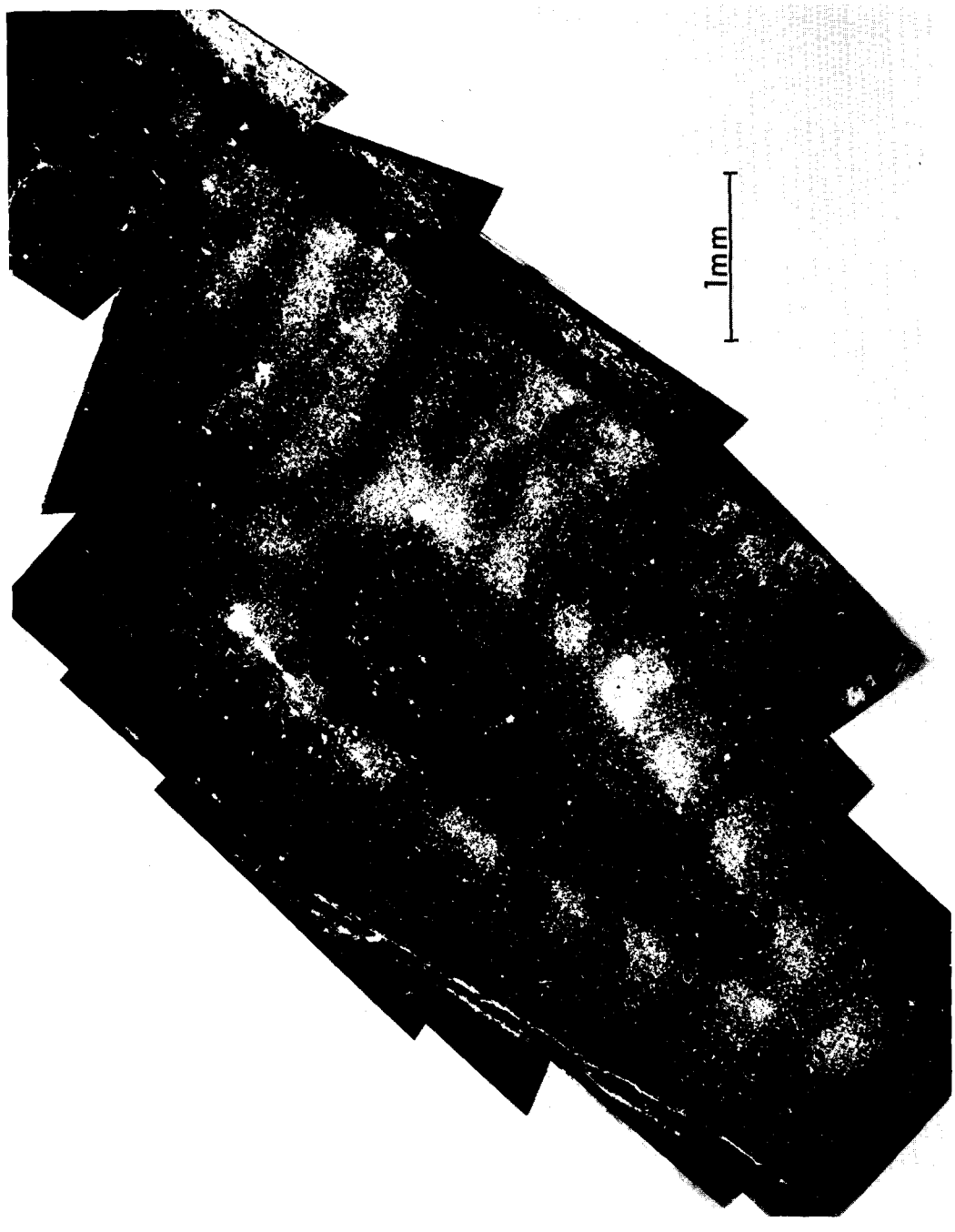


Fig. 3. Montage of a set of dark-field microphotographs from a section through calcarine cortex. The section passes almost tangentially through a dome-shaped region of cortex, cutting through layer IV to form an oval ring of alternating light and dark patches. The oval encloses a part of layer V which has been grazed by the plane of section. Above, 5–6 of the layer IV C patches are cut tangentially and form a set of parallel stripes. Two-thirds of the way from pia to layer IV C, one can see a suggestion of the thin layer IV A 'upper tier'. Below, the thin pial surface is visible; together with the pial surface of adjacent cortex it forms parallel lines like train tracks.

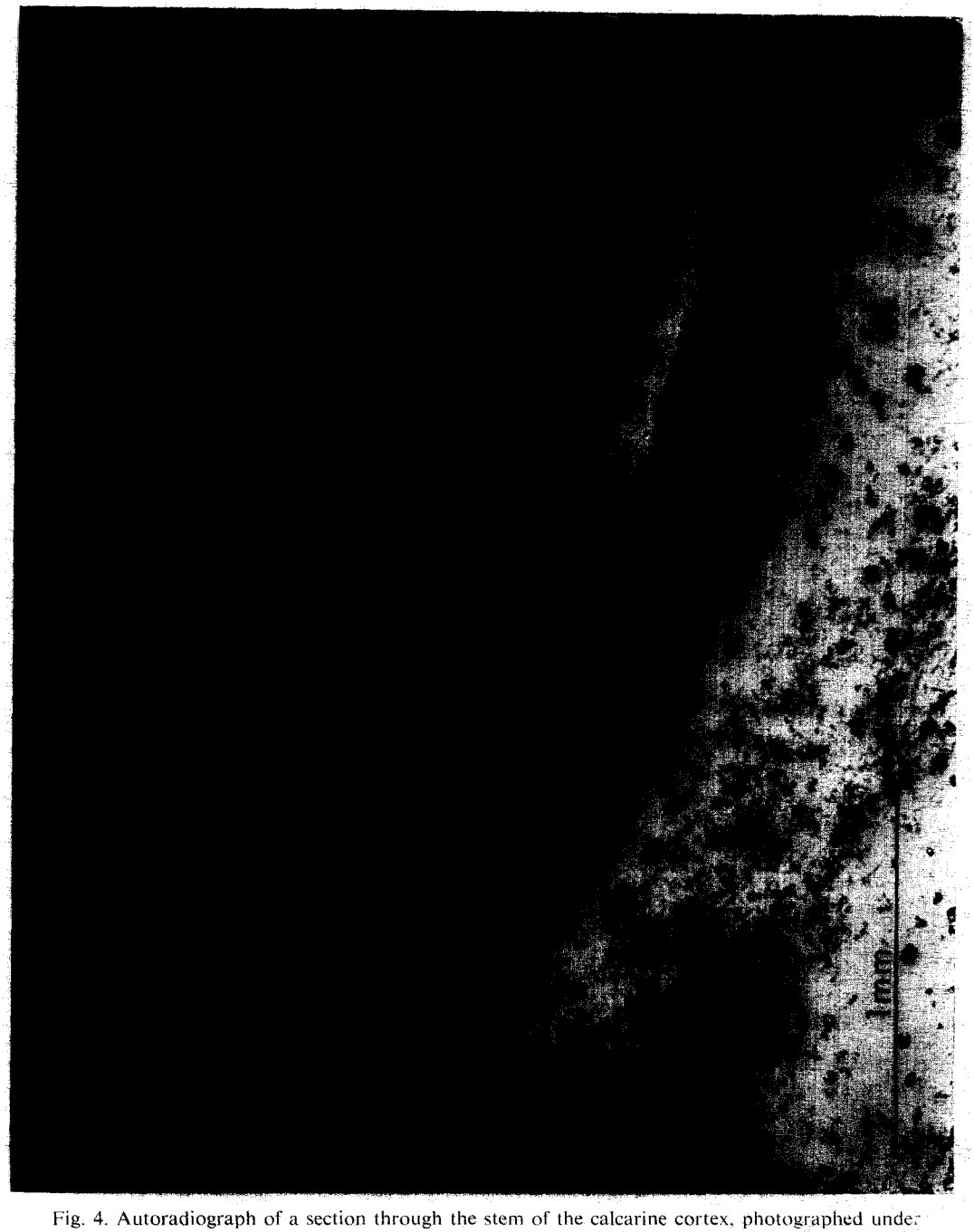


Fig. 4. Autoradiograph of a section through the stem of the calcarine cortex, photographed under dark-field illumination. Same section as in Fig. 2, but shows the temporal-crescent representation. Since the ipsilateral eye has no input to this region, the granules form a continuous band in layer IV C, with a sparse accumulation in IV A.

and certainly represents the morphological equivalent of the physiologically defined ocular-dominance columns. In the monocular segment of the cortex, contralateral to the injected eye, there were, as expected, no gaps, but a continuous band of silver grains in the IVth layer (Fig. 4). No attempt was made to reconstruct the columns in the entire occipital lobe, since we have subsequently found a simpler method of demonstrating ocular-dominance columns in normal material[11].

The presence of labeled material within the IVth-layer gaps may be due to fibers of passage from the geniculate layers representing the injected eye. It may also be the result of diffusion within the geniculate, from the 3 layers on each side receiving input from the injected eye, to their neighbors.

In summary, this study confirms Grafstein's original findings of transneuronal transport in the mammalian visual system and also provides an additional independent demonstration of the ocular-dominance columns in the striate cortex.

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1 ALVAREZ, J., AND PÜSCHEL, M., Transfer of material from efferent axons to sensory epithelium in the goldfish vestibular system, *Brain Research*, 37 (1972) 265–278.
2 COWAN, W. M., GOTTLIEB, D. I., HENDRICKSON, A. E., PRICE, J. L., AND WOOLSEY, T. A., The autoradiographic demonstration of axonal connections in the central nervous system, *Brain Research*, 37 (1972) 21–51.
3 DROZ, B., KOENIG, H. L., AND DI GIAMBERARDINO, L., Axonal migration of protein and glycoprotein to nerve endings. I. Radioautographic analysis of the renewal of protein in nerve endings of chicken ciliary ganglion after intracerebral injection of [^{3}H]lysine, *Brain Research*, 60 (1973) 93–127.
4 GRAFSTEIN, B., Transneuronal transfer of radioactivity in the central nervous system, *Science*, 172 (1971) 177–179.
5 GRAFSTEIN, B., AND LAURENO, R., Transport of radioactivity from eye to visual cortex in the mouse, *Exp. Neurol.*, 39 (1973) 44–57.
6 HENDRICKSON, A. E., Electron microscopic distribution of axoplasmic transport, *J. comp. Neurol.*, 144 (1972) 381–397.
7 HENDRICKSON, A., Electron microscopic radioautography: identification of origin of synaptic terminals in normal nervous tissue, *Science*, 165 (1969) 194–196.
8 HUBEL, D. H., AND WIESEL, T. N., Laminar and columnar distribution of geniculocortical fibers in the macaque monkey, *J. comp. Neurol.*, 146 (1972) 421–450.
9 KORR, I. M., WILKINSON, P. N., AND CHORNOCK, F. W., Axonal delivery of neuroplasmic components to muscle cells, *Science*, 155 (1967) 342–345.
10 LASEK, R., JOSEPH, B. S., AND WHITLOCK, D. G., Evaluation of radioautographic neuroanatomical tracing method, *Brain Research*, 8 (1968) 319–336.
11 LEVAY, S., HUBEL, D. H., AND WIESEL, T. N., The pattern of ocular dominance columns in macaque visual cortex revealed by a reduced silver stain, (1974), in preparation.
12 MIANI, N., Transport of S-100 protein in mammalian nerve fibers and transneuronal signals, *Acta neuropath. (Berl.)*, 6, Suppl. V (1971) 104–108.
13 MOORE, R. Y., AND LENN, N. J., A retinohypothalamic projection in the rat, *J. comp. Neurol.*, 146 (1972) 1–14.
14 SPECHT, S., AND GRAFSTEIN, B., Accumulation of radioactive protein in mouse cerebral cortex after injection of ^{3}H-fucose into the eye, *Exp. Neurol.*, 41 (1973) 705–722.