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Vertical organization of neurones accumulating ^{3}H-GABA in visual cortex of rhesus monkey

P. Somogyi*, A. Cowey†, N. Halász‡ & T. F. Freund*

* 1st Department of Anatomy, Semmelweis Medical School, 1450 Budapest, Tuzoltó u. 58, Hungary
† Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, UK
‡ Institute of Biophysics, Biological Research Centre, Szeged, Hungary

Electrophysiological and pharmacological studies[1-6] indicate that the specific responses of most visual cortical neurones depend on intracortical γ-aminobutyric acid (GABA)-mediated inhibitory processes. GABAergic interneurones have been visualized in all layers of the mammalian cerebral cortex by immunocytochemical methods[7,8] and by high-affinity uptake of exogenous ^{3}H-GABA[9-11]. It is recognized that GABA is synthesized and specifically accumulated by aspinous and sparsely spinous stellate cells, but there is no evidence available to indicate whether the laminar distribution of these cells and their axonal projections are related to the known role of GABAergic inhibitory processes in the generation of responses in visual cortical cells. It would therefore be of value to delineate the intracortical projection of the axons of different types of GABA-releasing neurones in regions of cortex where the receptive field properties of the neurones, and their modification by GABA antagonists, are well known. The selective high-affinity uptake of labelled GABA has been useful in delineating GABAergic systems[12]; recently, it has been shown that exogenous ^{3}H-GABA is specifically taken up and transported retrogradely by axons of neurones thought to be GABAergic[13,14]. Using microinjections of ^{3}H-GABA into different layers of the monkey visual cortex, we have examined the pattern of labelled neurones. We report here a bimodal distribution of GABA-accumulating neurones after injection into layers V and VI, with one group of neurones around the injection site and the other directly above, in layers II and III. We provide evidence that the latter neurones are non-pyramidal cells, probably labelled by retrograde axonal transport from the deep layers.

Two adolescent male macaque monkeys were used. One had taken part in behavioural tests of memory and had received surgical section of the fornix 6 months before the present study. The other had a high antibody titre to *Herpes simiae* and could therefore not be used in long-term experiments. There was no reason to suppose that the visual cortex of either monkey was abnormal. The animal was sedated with an intramuscular injection of ketamine hydrochloride (10 mg per kg; Ketalar, Parke-Davis) and then deeply anaesthetized with an intravenous injection of sodium pentobarbitone (Sagatal, May and Baker). After exposing the lateral surface of one occipital lobe, the cortex was injected with ^{3}H-GABA (0.33 mM, 60 Ci mmol^{-1}; Radiochemical Centre) dissolved in Krebs bicarbonate[15]. All the injections were delivered using glass micropipettes (30–50 μm tip diameter) penetrating at a very oblique angle to the surface of the cortex. The pipette was advanced 7–9 mm from the pia and 0.1 μl (2 μCi) ^{3}H-GABA injected by pressure[16] at each of four to six sites at 1–2 mm intervals along the injection track as the capillary was gradually withdrawn. In the first monkey the GABA injection track (no. 1) in area 17 passed obliquely through layers V and VI and ended in the white matter. In the other monkey one injection track (no. 2) in area 17 advanced obliquely from layer I to layer IVA, and another injection track (no. 3) in area 18 (posterior lip of the lunate sulcus) included all layers and the white matter as the capillary proceeded gradually deeper. After a post-injection survival time (no. 1, 40 min; no. 2, 55 min; no. 3, 35 min) the animals were perfused with fixative and slices of the injected cortex processed for Golgi staining and gold toning, as described previously[16]. Semi-thin (1 μm) sections perpendicular to the

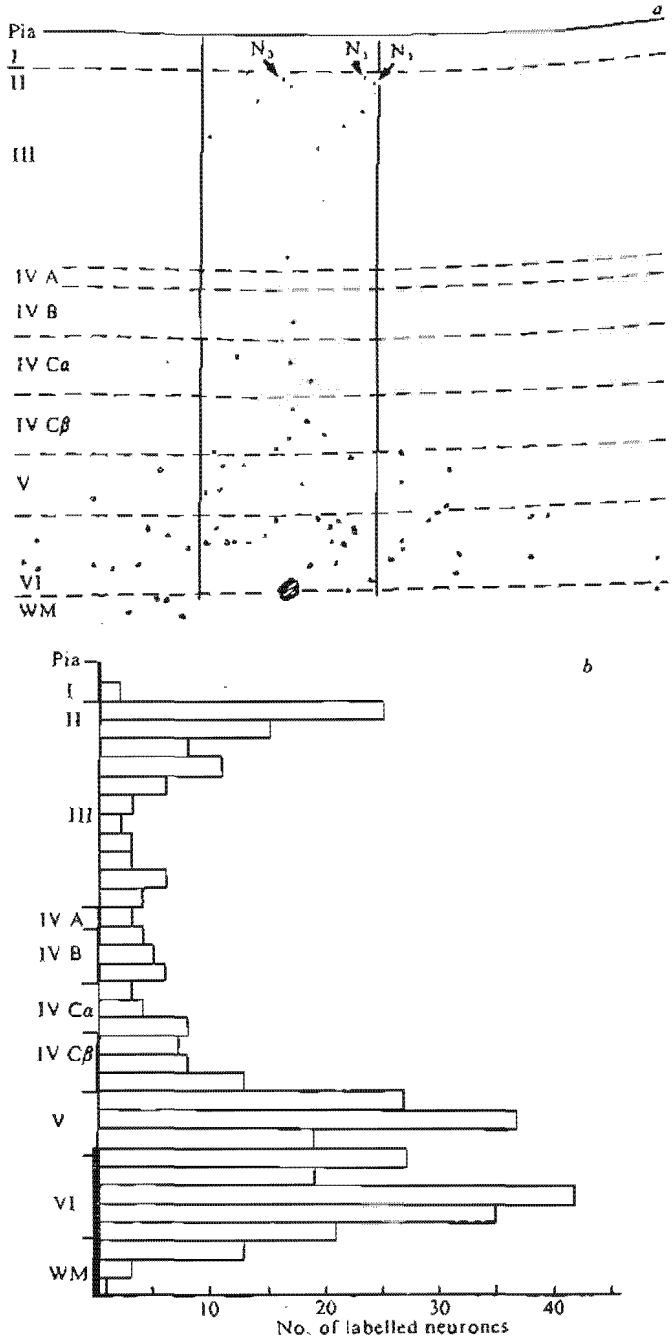

Fig. 1 *a*, Location of labelled neuronal perikarya (dots) in a single section of monkey striate cortex after ^{3}H-GABA injection at the border of layer VI and the white matter. The drawing is of a section perpendicular to the pial surface; the injection was made obliquely at an angle of almost 90° to the plane of the section. Besides the large labelled neurones around the injection track (shaded area) and a few neurones in layer IV, there is a group of small labelled neurones in layer II and upper layer III. N_1–N_3 are shown on light micrographs in Fig. 2. Note the vertical alignment of labelled neurones above the injection track. The vertical lines enclose a 500 μm column in which cells were counted for *b*. *b*, Distribution of 393 ^{3}H-GABA-labelled cells in all layers (I–VI) of the striate cortex, counted in 13 semi-thin sections cut perpendicular to the injection track at different levels of injection no. 1. Thick vertical line represents the levels where ^{3}H-GABA was injected. WM, white matter.

injection track were cut from the Golgi sections and processed for autoradiography[11]. Golgi staining was used to reveal the processes of some of the GABA-accumulating cells, but because of the unreliability of the staining, no unequivocal identification of the cell type of neurones labelled in the upper layers has yet been obtained.

Neurones in each of layers I–VI in both areas 17 and 18 became labelled when the layer was injected with ^{3}H-GABA. The number of labelled cells gradually decreased in all directions from the injection track, but labelled cells could always be found above the capillary track up to and including layer II. A distinct, bimodal pattern of labelled neurones and fibres was observed when the injection track passed through lower layer V, layer VI and the superficial white matter (injections 1 and 3; Fig. 1*a*). Large labelled neurones were seen around the injection site with scattered neurones as far as 1–1.5 mm from the injection site in layers V and VI. Occasionally, labelled neurones were found in layer IV and lower layer III, always above the injection track. In addition, another group of strongly labelled small, mainly fusiform cells was observed in layers II and upper III (Fig. 2*a*, *b*). The quantitative distribution of the neurones labelled by one oblique penetration in area 17 is shown in Fig. 1*b*.

It is unlikely that the neurones in the upper group were labelled by GABA taken up from the extracellular space in the upper layers, because there were very few labelled neurones in lower layer III and layer IV, although these layers contain a large number of GABA-accumulating neurones when injected directly. A more likely explanation is that the neurones in layers II and upper III were labelled by retrograde axonal transport of ^{3}H-GABA from the injection site in the deep layers. This is supported by the observation that strongly labelled fibre bundles pass through layers IV and lower III (Fig. 2*c*).

What type of neurones in the upper layers accumulate GABA from the deeper layers? There is a very strong projection from layer II to layer V in area 17 of the monkey, mediated by small pyramidal cells[17]. Thus the terminals of these neurones could take up the injected GABA. As pyramidal and stellate neurones can be differentiated on the basis of fine structural criteria, we studied the characteristics of six labelled neurones from the upper layers. After localizing the labelled neurones in semi-thin sections, a series of 84 and 132 ultrathin sections, respectively, were cut from two blocks containing the remainder of the perikarya of the labelled neurones. Using electron microscopy we found that labelled neurones had an indented eccentric nucleus with dense clumps of chromatin. There were many free polyribosomes in the cytoplasm, making the cell conspicuously electrodense. The thin dendrites emerging from the perikarya contained densely packed microtubules (Fig. 2*d*) and some of the dendrites were beaded, receiving predominantly asymmetric synapses. The perikarya received few, mainly symmetrical synapses from boutons containing pleomorphic vesicles, but occasional asymmetric synapses established by boutons containing round synaptic vesicles were also observed (Fig. 2*e*). All these features are characteristic of small stellate cells in the monkey primary sensory cortex[18,19]. For comparison we also studied two Golgi-stained, gold-toned small pyramidal cells in layer II. These neurones differed from labelled cells in that the nucleus was large, round and relatively transparent and there were few free ribosomes. Thick dendrites tapered gradually from the perikaryon and an apical dendrite was observed. The perikarya and proximal dendrites received only symmetrical synapses, confirming previous findings[20,21].

These data taken together provide evidence for a GABA-accumulating stellate neurone system with perikarya situated in layer II and upper layer III, and with axons descending vertically in bundles into layer V. One stellate neurone, the double-bouquet cell with tight vertical axon bundles described in previous studies[22–25], corresponds very well to the above characteristics but more detailed information is required before we can conclude definitely that GABA-accumulating neurones of the upper layers are, or include, double-bouquet cells. It was shown recently[25] that this neurone makes symmetrical synaptic contacts, similar to those which contain glutamic acid decarboxylase (GAD) in the monkey[26], with dendritic shafts and spines. Although the axons of double-bouquet cells do not enter layer VI the fact that an injection here produces labelled neurones in layers II and III is easily explained by diffusion of the GABA across the V–VI boundary.

The presence of a GABAergic vertical neurone system passing throughout layers II to V of the visual cortex and capable of influencing neurones in layer VI with dendrites in layer V is significant in view of the functional, columnar organization of cortical neurones[27]. The distribution of GAD immunoreactivity in the plane of the cortical surface was recently shown to follow a pattern resembling ocular dominance columns[7]. On the other hand, it has been shown in area 17 that cortical neurones in a radial column are maximally excited by a bar of light or grating

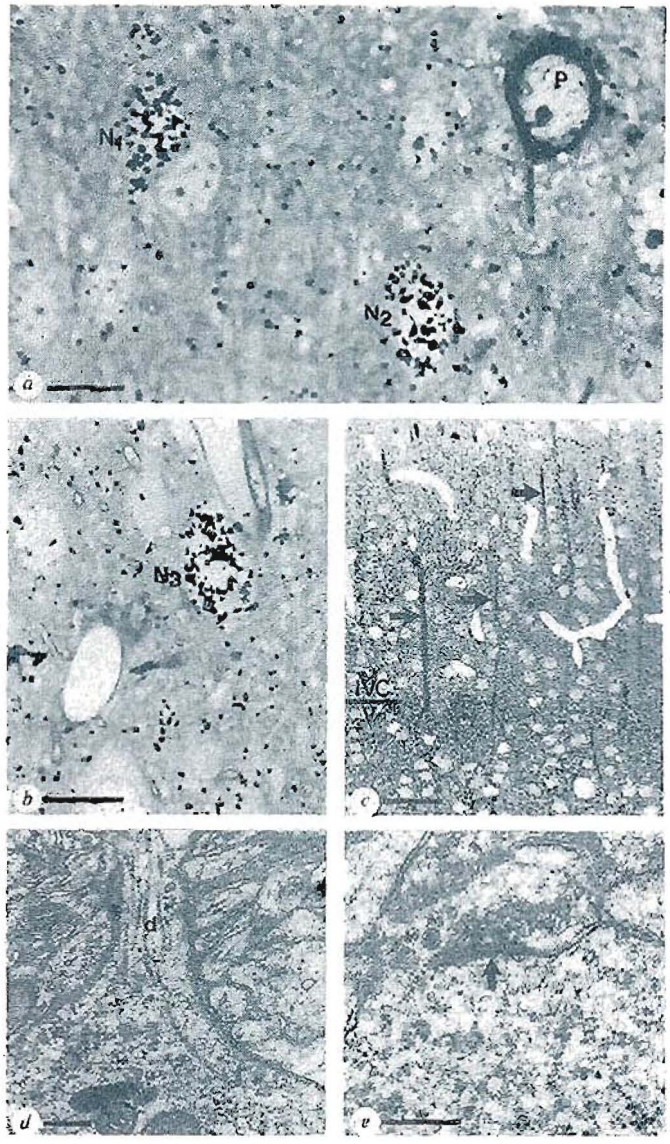

Fig. 2 Light (*a*–*c*) and electron microscopic (*d*, *e*) details from the area shown in Fig. 1*a*. *a*, *b*, Semi-thin sections of ^{3}H-GABA-labelled neurones (N_1–N_3) in layer II, which were also serially sectioned for electron microscopy. One Golgi-stained pyramidal cell (P) is also present. *c*, Vertical labelled fibre bundles (arrows) passing through layer IVC. *d*, Origin of a small dendrite (d) from the neurone N_3 directed towards the pia. *e*, Asymmetric synapse on the perikaryon of the labelled neurone N_2. *a*–*c*, Ilford K5 emulsion; 30 days exposure. Scale bars: *a*, *b*, 10 μm; *c*, 50 μm; *d*, *e*, 0.2 μm.

of a particular orientation, and that this orientation specificity is temporarily abolished by the GABA antagonist bicuculline[2–6]. The columnar system of GABA-accumulating axons described here may have an important role in this specificity, as the axon bundles are highly restricted in lateral extent (20–50 μm in diameter) and could have a powerful effect locally.

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