Immunocytochemical localization of glutamic acid decarboxylase in monkey striate cortex

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Glutamcyclc acid decarboxylase (GAD) functions as an inhibitory transmitter in the mammalian cerebral cortex including visual cortex. We have localized the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) in monkey striate cortex and mapped the distribution of neuronal cell bodies and p[secitotic terminals positive for GAD (GAD+). Primate striate cortex has the advantage of being precisely laminated and has well characterized inputs and outputs. Standard light microscopic immunochemistry techniques were used, with an antiserum to mammalian GAD which has been extensively characterized. We have found that both cortical layers contain both neurons and synaptic terminals which are GAD+, but in

different quantities. No variation in the distribution or number of GAD⁺ terminals was found in layer IVC which would correspond to the ocular dominance columns, but in layers II and III a 375-μm periodic repeat of GAD⁺ dots arranged in rows was found. When the same brains were histochromically stained for the mitochondrial enzyme cytochrome oxidase, the laminar density and distribution of cytochrome oxidase staining was the same as for GAD and an identical pattern of dots in rows was found in layers II and III.

**Experimental procedure**

Primary visual or striate cortex from four normal Macaca fascicularis monkeys was studied. In one animal the left eye was injected with 3 nCi of a mixture of 3H-proline and fucose 14 days before the animal was killed, to label the cortical ocular dominance columns. In two animals the striate cortex received 1 μl injections of colchicine (10 μg ml⁻¹) 1 day before they were killed to increase the GAD content of the neuronal cell bodies. GAD immunocytochemistry and cytochrome oxidase staining methods are described in the legends of Figs 1 and 2.

In sections cut perpendicular to the cortical surface (Fig. 1b), the layers were strikingly outlined in the anti-GAD-immunoperoxidase sections; sections incubated without specific anti-GAD serum showed no staining (Fig. 1a). All layers contained GAD staining but the intensity of labelling varied with the layer. The shifts in density corresponded to the laminar boundaries identified in thionine-stained sections. Under higher power (Fig. 2), the GAD staining was localized to cell bodies, beaded processes and small round densities which resembled synaptic boutons. Ribak has shown in electron micrographs of GAD-stained rat visual cortex that these densities are synaptic terminals synthesizing cell bodies and dendrites. Because our preliminary electron microscopy has confirmed this finding for the monkey, we shall provisionally call these small densities terminals. There was no difference in laminar staining of central to mid-peripheral retinal representation in cortex; far peripheral cortex was not examined.

![Image of a section cut parallel to the cortical surface passing through layers II and III which has been reacted in specific anti-GAD serum. An orderly pattern of dots aligned into rows can be seen (arrows).](image)

![Schematic representation of the laminar distribution of GAD⁺ neuronal cell bodies; this drawing is based on many different sections from colchicine pretreated cortex.](image)

![Fig. 3](image)
Distribution of GAD⁺ terminals

As judged by both eye and photodensitometry, the upper halves of layer I, IVA, and IVC were the most heavily labelled, II, III, and VI slightly less so, and lower I, IVB, and V were quite lightly labelled (Fig. 1a-c). The labelling in layer I was uniformly distributed and consisted of small, round GAD⁺ terminals in no obvious pattern. In all the other layers the labelling showed two patterns: GAD⁺ heads processes run parallel or perpendicular to the cortical surface, and GAD⁺ terminals encircle both unstained and GAD⁺ cell bodies (Fig. 2). Most of the GAD⁺ terminals are ~1 μm in size, but occasionally there are clusters of much larger, 3–5 μm terminals. GAD⁺ terminals continue at least 50 μm into the white matter below the most inferior layer VI neurones.

Some GAD-reacted sections from the ‘H-proline and fusocse eye-injected monkey were coated for autoradiography, using Kodak NTB2 emulsion and 3–4-month exposures. Ocular dominance columns were apparent in layer IVC, but no identical pattern of dots in rows was found. More than 80% of the GAD⁺ dots superimposed on the cytochrome oxidase dots in an adjacent section. When cortex sections from the ‘H-proline/fusocse eye-injected monkey were first stained for GAD or cytochrome oxidase and then processed for trans-synaptic autoradiography, the rows of dots in layer III ran parallel to the centres of the ocular dominance columns in layer IVC (Fig. 4).

Distribution of GAD⁺ cell bodies

Neuronal cell bodies have been considered to be specifically stained if they show an unstained nucleus with neuronal characteristics and a heavily stained cytoplasm with a sharp cellular outline (Fig. 2N). In the two brains which received no colchicine, there were very few GAD⁺ cell bodies in layers I–IV, but in layers V and especially VI, regularly spaced GAD⁺ neurones 15–20 μm in diameter were found. Small GAD⁺ cell bodies 8–10 μm in diameter lay in the lower edge of VI or in the white matter just under VI. In the two brains receiving colchicine injections, there was a striking increase in the number of GAD⁺ cell bodies, especially in layers I–IV. Even if our material is an underestimate because of the limitations inherent in these in vitro colchicine injections, our experiments indicate that monkey striate cortex contains many GAD⁺ neurones ranging in size from 9 to 30 μm. GAD⁺ neurones occur in every cortical layer and most appear to be various types of stellate neurone. Their distribution is schematically presented in Fig. 1d. In all layers some GAD⁺ neurones receive GAD⁺ terminals onto their cell bodies or dendrites. Most unstained cell bodies are outlined by GAD⁺ terminals in all layers.

Conclusions

Three findings emerge from this study. (1) The striate cortex of Macaca monkeys is very rich in both GAD⁺ cell bodies and synaptic terminals. Unlike rat visual cortex, there are clear laminar differences in the distribution of both GAD⁺ cell bodies and synapses. (2) There is a striking similarity between GAD distribution and cytochrome oxidase staining. Wong-Riley first pointed out the good correlation between cytochrome oxidase staining, GABA or GAD content and iron concentration in regions like cerebellum and basal ganglia. She suggested that inhibitory GABAergic cells are mitochondria-rich, essential components of the intrinsic neuropil, especially heavily for the haem enzyme cytochrome oxidase. This agrees with an earlier study where the nerve terminals which became labelled after exposure of rat brain homogenates to ‘H-GABA were characterised by a high density of mitochondria within their cytoplasm. Our data support these suggestions and provide more direct evidence that cytochrome oxidase staining may be largely localized to GABAergic neurones. (3) The cytochrome oxidase and GAD⁺ pattern of rows of dots in layers II and III is identical to that shown in Macaca striate cortex using 2-deoxyglucose labelling in which similar rows of dots are parallel to the ocular dominance columns. In this study, sections doubly processed for GAD or cytochrome oxidase and trans-synaptic autoradiography also reveal that each row of dots is aligned parallel to the centre of each ocular dominance column. It therefore appears that there are rows of GAD⁺ inhibitory neurones in supragranular Macaca striate cortex which may be preferentially related to each eye.

This work was supported by NIH grants EY 01208 and NS 13224. A.H. was supported by a Wellcome Research Travel Grant. The helpful criticism of L. Iversen and P. Emson, and the technical assistance of J. White, are gratefully acknowledged.