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Retrograde transport of γ-amino[3H]butyric acid reveals specific interlaminar connections in the striate cortex of monkey

cortical inhibition/GABA uptake/uptake inhibition/Golgi impregnation/nonpyramidal neuron

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ABSTRACT Several lines of evidence suggest that γ-amino- butyric acid is an inhibitory neurotransmitter in the cerebral cortex. To study the intracortical projection of neurons that selectively accumulate this amino acid, we injected radioactive γ- amino butyric acid into the upper layers of the striate cortex of monkeys along tracks at an oblique angle to the pia. Sections from the injected area were then processed by a combination of autoradiography and Golgi impregnation to reveal the distribution of labeled neurons and their morphological characteristics. Labeled neurons always occurred around the injection site in each layer. In addition, a consistent radial pattern of perikaryal labeling was observed in layers IVc–VI below the injection track in layers I–IVa. The closer the injection track was to the pia the deeper the peak density of labeled cells appeared. After injection in layers IVa and the lower part of III, the highest number of labeled neurons was in layer IVc; after injection in the upper part of layer III, most labeled neurons were in layer V; and, after injection in layers I and II, the proportion of labeled neurons increased in the lower part of layer V in layer VI. All these neurons in the infragranular layers are presumably labeled by retrograde axonal transport via the labeled fiber bundles that extended from upper to lower layers. Thirty-four Golgi-stained neurons of various types were also examined for retrograde labeling. Two were labeled, and both were aspiny stellate cells in layer V. The arrangement of these putative GABAergic neurons, with axons that ascend from lower to upper layers in a regular pattern and arborize locally, would enable them to mediate inhibition within cortical columns and between neighboring columns.

There is substantial evidence that γ-aminobutyric acid (GABA) is a neurotransmitter in the cerebral cortex (1–5). In particular, it has been shown that GABA-mediated inhibition enhances the specialized receptive field properties of many neurons in the visual cortex (6–12). Two methods have been used to study the distribution and structure of neurons responsible for GABAergic inhibition in the cortex; (i) autoradiographic demonstration of selective high-affinity uptake of [3H]GABA and (ii) immunohistochemical localization of the GABA-synthesizing enzyme glutamic acid decarboxylase (GluDCase) (13–20). Both methods reveal neuronal perikarya and nerve terminals but not their linking axons; i.e., we do not know which cell bodies in any lamina give rise to nerve terminals mediating GABAergic inhibition in that or any other lamina. Immunohistochemical demonstration of GluDCase reveals a regularly patchy nonuniform horizontal distribution in the monkey cortex (17), but it is also important to understand the connections of GABAergic neurons across and between layers because neurons whose receptive field properties are influenced by GABA-mediated interactions are organized in columns orthogonal to the layers and such that neurons in a column have similar properties (21). Recently, by exploiting the selective uptake and retrograde transport of [3H]GABA (22, 23) after its injection into the deep layers of the monkey striate cortex, we found a population of neurons in cortical layer II and the upper part of layer III that sends projections vertically through all layers of the cortex (18). Here, we report the laminar distribution of GABA-accumulating neurons after injection of [3H]GABA into the upper layers and provide evidence for the nonpyramidal nature of some of these neurons.

MATERIALS AND METHODS

Treatment of Animals. Two adult male monkeys (one Macaca mulatta, one Macaca fascicularis) were used. One of them (A) had been used in behavioral tests of memory and its fornix had been sectioned surgically 6 months before the present experiment. The other (B) had a high titer of antibody to Herpes simiae and could be used only in acute experiments. There was no reason to assume that their visual cortices were abnormal. They were sedated with ketamine hydrochloride (intramuscularly at 10 mg/kg; Ketalar, Parke, Davis) and anesthetized with sodium pentabarbitone (intravenously, Sagatal; May and Baker, Dagenham, England). Then, the occipital lobe was exposed, and the striate cortex of both hemispheres was injected at several sites with [3H]GABA (0.33 mM; 60 Ci/mmol; 1 Ci = 37 GBq; Radiochemical Centre) in Krebs bicarbonate solution. Injections were delivered using glass micropipettes (tip diameter, 30–50 μm) penetrating at various oblique angles to the surface. Three injections parallel with the lunate sulcus were analyzed in the present study. They involved cortex corresponding to an eccentricity of 2–4° from the fovea. The pipette was advanced 7 mm from the pia and either 0.1 μl (2 μCi; injection 1, animal A) or 0.05 μl (1 μCi; injections 2 and 3, animal B) of [3H]GABA was injected at each of 6 (no. 1) or 10 (nos. 2 and 3) sites, 1 or 0.5 mm apart, respectively, as the capillary was gradually withdrawn.

One injection track (no. 2) ended in layer IVc, and the other two passed obliquely from the pia to the bottom of layer III. Two further injections, one in each animal, were made using the same GABA solution together with the competitive GABA uptake inhibitor cis-1,3-aminocyclohexanecarboxylic acid (100 mM; AHC). After various survival times (injection 1, 35 min; injection 2, 50 min; injection 3, 30 min), the animals were perfused with fixative (18).

Abbreviations: GABA, γ-aminobutyric acid; GluDCase, glutamic acid decarboxylase; AHC, cis-1,3-aminocyclohexanecarboxylic acid.

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**RESULTS**

Labeled neurons occurred around the injection track in each layer from I to IVc when the layer was injected (Figs. 1 and 2). They could be seen using bright-field illumination. The proportion of neurons that were labeled was the same irrespective of the amount of radioactivity around the track but, in sections having higher radioactivity, the area containing labeled cells was larger. The inclusion of ACHCh in the \( ^{3}H \)GABA solution either completely prevented perikaryal labeling or confined it to the immediate vicinity of the capillary track, where all cells were labeled to the same degree as the neuropil.

In addition to the neurons around the injection track, a regular pattern of perikaryal labeling was observed radially below and above the injection track and this pattern depended on the laminar position of the injection site (Fig. 2). Groups of neurons accumulating \( ^{3}H \)GABA lay outside the area of the spread of the isotope in layers I–VI when the labeled GABA had been injected into layers I–IVa. These neurons were presumably labeled by retrograde transport after the selective uptake of \( ^{3}H \)GABA at their terminals because the closer the injection was to the pia the deeper the peak density of labeled cells in the cortex beneath (Fig. 2). In addition, densely labeled and vertically disposed fiber bundles, probably representing the axons of these neurons, were also present (Fig. 3 A and B). The border between layers V and IVc was sharply delineated by neuropil labeling in addition to that of cell bodies (Fig. 3A). The lateral extent of the area containing labeled neurons in each layer also showed a regular pattern. The most widespread labeling was in the top of layer V, with neurons present up to 350 \( \mu \)m laterally from a point below the injection track. The band of labeled neurons in layers IVc and VI was slightly narrower, about 250 \( \mu \)m laterally in layer IVc (Fig. 3A). In layers IVb and VI, labeled neurons were present only directly below the injection track. When layer IVc was injected, the distinct group of labeled neurons in layer II described earlier (19) appeared directly above the injection track (Fig. 2A). When layer II was injected with spread to layer I, a very wide labeling pattern was found in these layers (Fig. 2F) but, as this injection site was very near the point where the capillary penetrated the pia, the widespread labeling may reflect spread of \( ^{3}H \)GABA on the pial surface. Apart from this latter case, the lateral distribution of labeled perikarya probably reflects the tangential spread of the axons of labeled neurons in the upper layers where the injection was delivered.

 Autoradiography itself gives little information about the type of neuron being labeled. We therefore processed for autoradiography Golgi-stained gold-toned neurons of identified types. A total of 3 spiny stellate cells in layer IVc, 14 nonpyramidal cells with smooth dendrites in layers IVC–VI, and 17 pyramidal cells of various types in layers V and VI, all from the area where perikaryal labeling occurred below the track, were impregnated and examined for uptake of \( ^{3}H \)GABA. The two cells that were labeled were aspiny stellate neurons, both in the upper part of layer V. As the labeling of the neuropil is strong in layer V (Fig. 3A), labeled perikarya were identified by comparison with nearby nonlabeled perikarya, not by comparing them with the neuropil (Fig. 3C).

**DISCUSSION**

The specificity in the pattern of interlaminar connections revealed by \( ^{3}H \)GABA labeling (Fig. 4) raises the question whether the neurons identified this way use GABA as a transmitter and therefore whether these interlaminar connections are probably inhibitory. The answer really depends on the reliability of uptake of exogenous \( ^{3}H \)GABA solely or chiefly by neurons that use it as a transmitter. The results obtained with the competitive GABA uptake inhibitor ACHCh show that the perikaryal labeling in our experiments occurs by an active uptake process. ACHCh also prevented labeling of the perikarya by \( ^{3}H \)GABA in a study of rat neostriatum (25). Further evidence for the specificity of \( ^{3}H \)GABA uptake comes from separate experiments in the cerebellum (13, 26, 28) and in the olfactory bulb (29, 30) showing that the labeling of neuron that accumulates \( ^{3}H \)GABA also contains GABA.

In the cortex, GluDCase has been localized only in nonpyramidal cells (14, 17) and only nonpyramidal neurons have
been found to accumulate $[^3]H$GABA (16). In the present study, two of the labeled neurons in layer V were nonpyramidal cells with smooth dendrites similar to those reported in rat cortex (19). However, it would be difficult to prove that all our labeled cells in the different layers are of this type.

Evidence for ascending intracortical projections comes from
FIG. 3. Laminar distribution of labeled neurons after injection of \(^{3}H\)GABA into layer III of the striate cortex through a capillary that was nearly parallel with the pia (p). (A) Dark-field micrograph showing the injection track (asterisk), numerous labeled neurons (arrows) in layers IVc and V, and labeled vertical fiber bundles (double arrows). In addition to perikarya labeled in the top of layer V, the neuropil is also strongly labeled, delineating this layer sharply from layer IV. (B) Bright-field micrograph showing layers IVc and V (asterisk) marked by numerous labeled neurons (arrows). Unlabeled neurons (open arrows) and labeled vertical fiber bundles (double arrows) are also present. (C and D) The perikarya of Golgi-impregnated gold-toned stellate neurons (SN) in layer V show accumulation of \(^{3}H\)GABA; unlabeled neurons (open arrows), capillaries (c), and labeled neurons (arrows) are indicated. The stellate nonpyramidal neuron with smooth dendrites seen in D is also shown in the drawing (E) and the light micrograph (F) taken before processing for autoradiography. (Bars: A, 100 \(\mu\)m; B and E, 50 \(\mu\)m; C, 10 \(\mu\)m; F, 20 \(\mu\)m).
neurons are partially determined by the putative GABAergic connections shown here. Nevertheless, the precision of the ascending vertical projections suggests that they can contribute to the physiological columnar organization. It is significant that layer IVc, which receives the bulk of the geniculate input, contains a high proportion of putative GABAergic neurons, which project to upper layers where there is less direct geniculate input. Thus it is possible that this projection mediates feed-forward inhibition if activated directly by specific afferents, parallel with the presumed excitatory pathway thought to be mediated by spiny stellate cells.

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