Brain Research, 332 (1985) 143–149
Elsevier

BRE 20706

Short Communications

Identified axo-axonic cells are immunoreactive for GABA in the hippocampus and visual cortex of the cat

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(Accepted November 6th, 1984)

Key words: GABA-immunocytochemistry — visual cortex — hippocampus — axon initial segment — interneurons — axo-axonic cell — chandelier cells — epilepsy — Golgi-impregnation combined with GABA-immunocytochemistry

Chandelier or axo-axonic cells (AACs) are specialized interneurons terminating on the axon initial segments of pyramidal neurons. Two AACs have been localized by Golgi impregnation, one in the CA1 region of the hippocampus and one in the visual cortex of cat, for structural analysis and for the identification of their transmitter. They had 323 and 268 terminal bouton rows, respectively, probably making synapses with an equal number of initial segments. The distribution of the dendrites of the hippocampal cell was strikingly similar to that of pyramidal cells suggesting a similar input. Using an antiserum to GABA and postembedding GABA-immunocytochemistry, developed for Golgi-impregnated neurons, both cells were found to be GABA-immunoreactive. The strategic location of their synapses and the presence of GABA in AACs suggest that in normal cortical tissue they play a major role in GABA-mediated inhibition.

Chandelier[16] or axo-axonic cells[10] (AACs) are specialized interneurons that make multiple synaptic contacts exclusively with the axon initial segments (ISs) of pyramidal neurons in cortical areas[1,8,10,11]. They are thought to use GABA as a neurotransmitter since most boutons contacting the axon ISs of pyramidal cells have been found to be immunoreactive for glutamate decarboxylase (GAD) the enzyme responsible for GABA synthesis[9,12]. Considering the strategic location of their terminals, AACs could play a major role in GABA-mediated inhibitory processes. However, AACs and their terminals can be identified only when the characteristic axon arborization of the neuron is revealed in toto by Golgi impregnation or intracellular filling[2,8,10,16]. Thus, to prove that at least some of the GAD-positive boutons belong to AACs it was necessary to demonstrate GAD within the Golgi-impregnated boutons of one of these neurons in the visual cortex of cat[2]. Such direct evidence has not been available in the hippocampus where AACs were found only recently.

We studied further the structural and biochemical properties of these neurons with the following aims: (1) to obtain information about the dendritic arborization and possible input of AACs in the hippocampus where only a small proportion of the dendritic arbor of a single cell has been published[11]; (2) to strengthen the evidence that GABA is the transmitter of these interneurons by demonstrating GABA-immunoreactivity in AACs identified by Golgi impregnation, using a newly developed procedure[13]; and (3) the examination of AACs was also used to confirm the reliability of the method for GABA immunohistochemistry.

Two adult male cats were anaesthetized with chloral hydrate (350 mg/kg), fixed by transcardial perfu-

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sion, and blocks of the brain were processed for Golgi impregnation and gold toning as described previously[10,13]. The 90–150 μm thick Golgi sections containing the gold-toned neurons were embedded on slides in Durcupan ACM (Fluka) resin. One AAC from the hippocampus and one from the striate cortex were selected, drawn, photographed and re-embedded for sectioning on an ultramicrotome. Semithin sections (0.5 μm) were cut from the soma of both cells and processed for post-embedding GABA-immunocytochemistry[14] using the unlabelled antibody peroxidase–antiperoxidase method[15]. An antiserum to GABA (Code no. 7) was produced in rabbits; the characterization of this serum and the immunocytochemical procedure used in this study are described elsewhere[3,13].

Some sections of both cells were reacted with a control antiserum that had been pre-incubated with GABA coupled to polyacrylamide beads[3]. Part of the axon including identified boutons of the hippocampal AAC was serially sectioned for electron microscopy[2]. The specimens were contrasted with uranyl acetate en block and the sections were stained with lead citrate.

The hippocampal neuron was drawn from a section about 120 μm thick. The cell was situated in the stratum pyramidale of the CA1 region (Fig. 1). Its dendrites were oriented mainly parallel with the apical dendrites of pyramidal cells and formed an arborization that spanned all layers of the hippocampus from the alveus to the hippocampal fissure. The dendrites emitted occasional spines and had branches towards their ends. The axon originating from the base of the elongated soma descended to the lower stratum oriens (Fig. 1B). Its horizontal collaterals emitted the characteristic specialized terminal segments that climbed along the axon ISs of pyramidal cells. Many single boutons or irregularly placed horizontal groups of boutons were also encountered (Fig. 1). Altogether 323 terminal segments were observed; these probably represented an equal number of postsynaptic pyramidal cell axon ISs. The total number of pyramidal cells contacted was probably greater since some axon collaterals were cut at the surface of the section.

To confirm that this neuron was in fact an AAC, the postsynaptic targets were examined in electron microscopic serial sections. All boutons in stratum pyramidale including ones from 6 vertical terminal segments made type II synaptic contacts with axon ISs (Fig. 2E, F). The latter could be identified by their origin from the base of pyramidal neurons and the characteristic microtubule fascicles, electron-dense membrane undercoating and cisternal organelles (Fig. 2F). The identified boutons of the AAC established synapses with both the shaft and the spines of the axons as did numerous other boutons (Fig. 2E, F). These latter boutons were similar to the identified ones and probably most of them originated from other AACs converging onto the same pyramidal neuron.

One gold toned AAC was selected from the striate cortex of the cat for its exceptionally complete axon arborization that could be followed in three 90 μm thick consecutive sections. Only the main axon collaterals and the synaptic bouton rows are shown in Fig. 3A because the drawing of the axon collaterals connecting the vertical, terminal segments would have completely obscured the axon arbor. The axon arborization was within layers II and upper III and no descending collateral could be detected as with some of the previously published cells[2,6]. Some collaterals could not be followed to their ends, but even so 268 terminal bouton rows were observed (Fig. 3B). On the basis of previous EM studies[1,9,10] this probably represents the same number of post-synaptic pyramidal cells. AACs have been extensively documented in the visual cortex[1,2,9,10], thus, it was not necessary to confirm the specificity of this particular neuron. The dendritic arbor was only partially revealed, but most of the impregnated dendrites passed into layer I (Fig. 3A).

The postembedding unlabelled antibody enzyme method[15] was used to show whether AACs were GABA-immunoreactive. The general distribution of GABA-immunoreactivity in the cortex and hippocampus has been described previously[14]. In brief, the proportion, type and distribution of GABA-immunoreactive neuronal perikarya and terminals were in good agreement with results obtained for GAD[2,14]. Both the nucleus and the cytoplasm of the GABA-immunoreactive neurons stained but other neurons not thought to use GABA as a transmitter, such as pyramidal cells, were completely negative. Both the positive and the negative neurons were contacted by immunoreactive varicosities which were also present

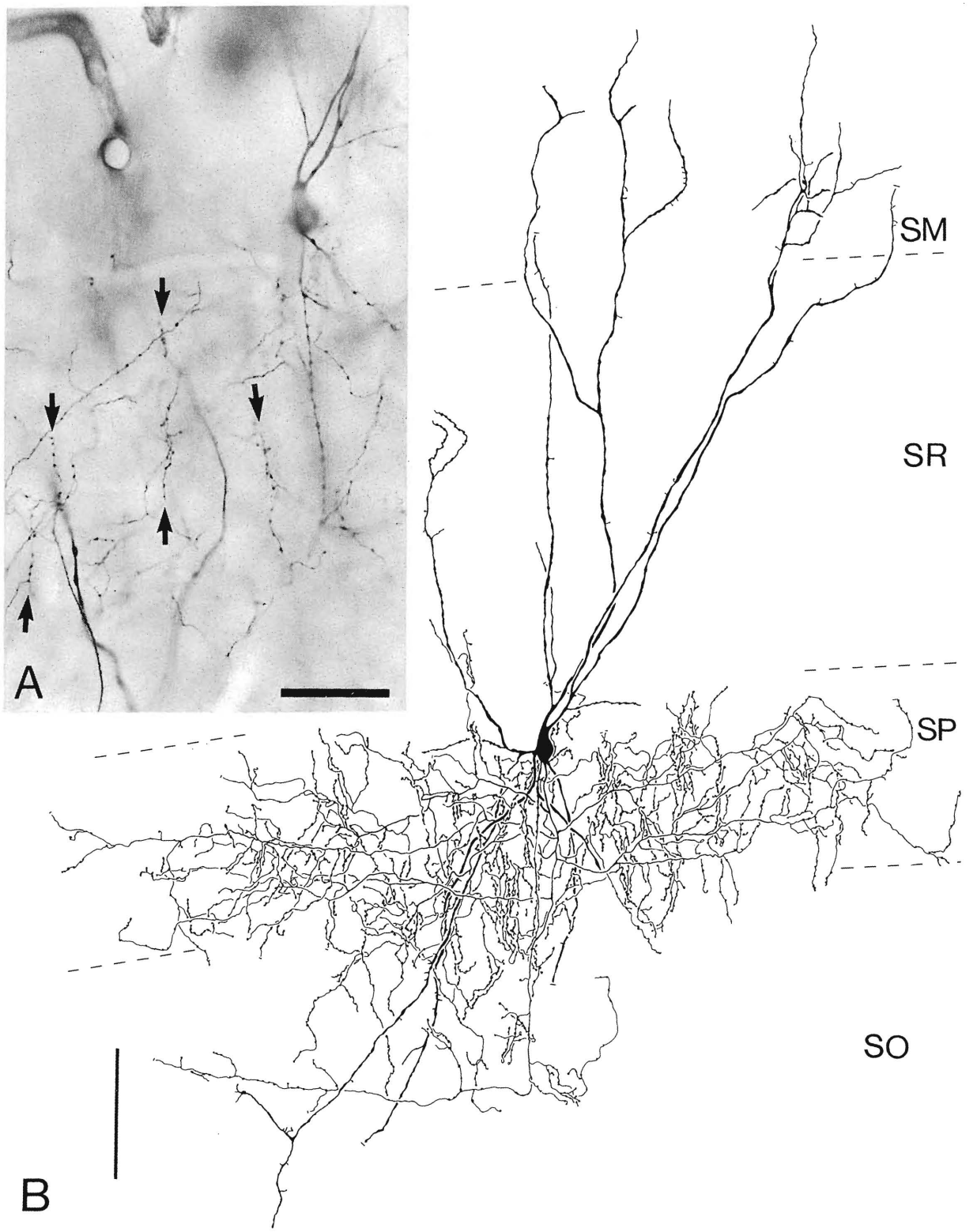

Fig. 1. Light micrograph (A) and drawing (B) of an axo-axonic cell in the CA1 region of the cat's hippocampus. The dendrites span all the layers (SM, stratum moleculare; SR, stratum radiatum; SP, stratum pyramidale; SO, stratum oriens) from the alveus to the hippocampal fissure. Note vertical terminal segments of the axon (arrows) aligned with the axon initial segments of pyramidal cells. Scales: A, 50 μm; B, 100 μm.

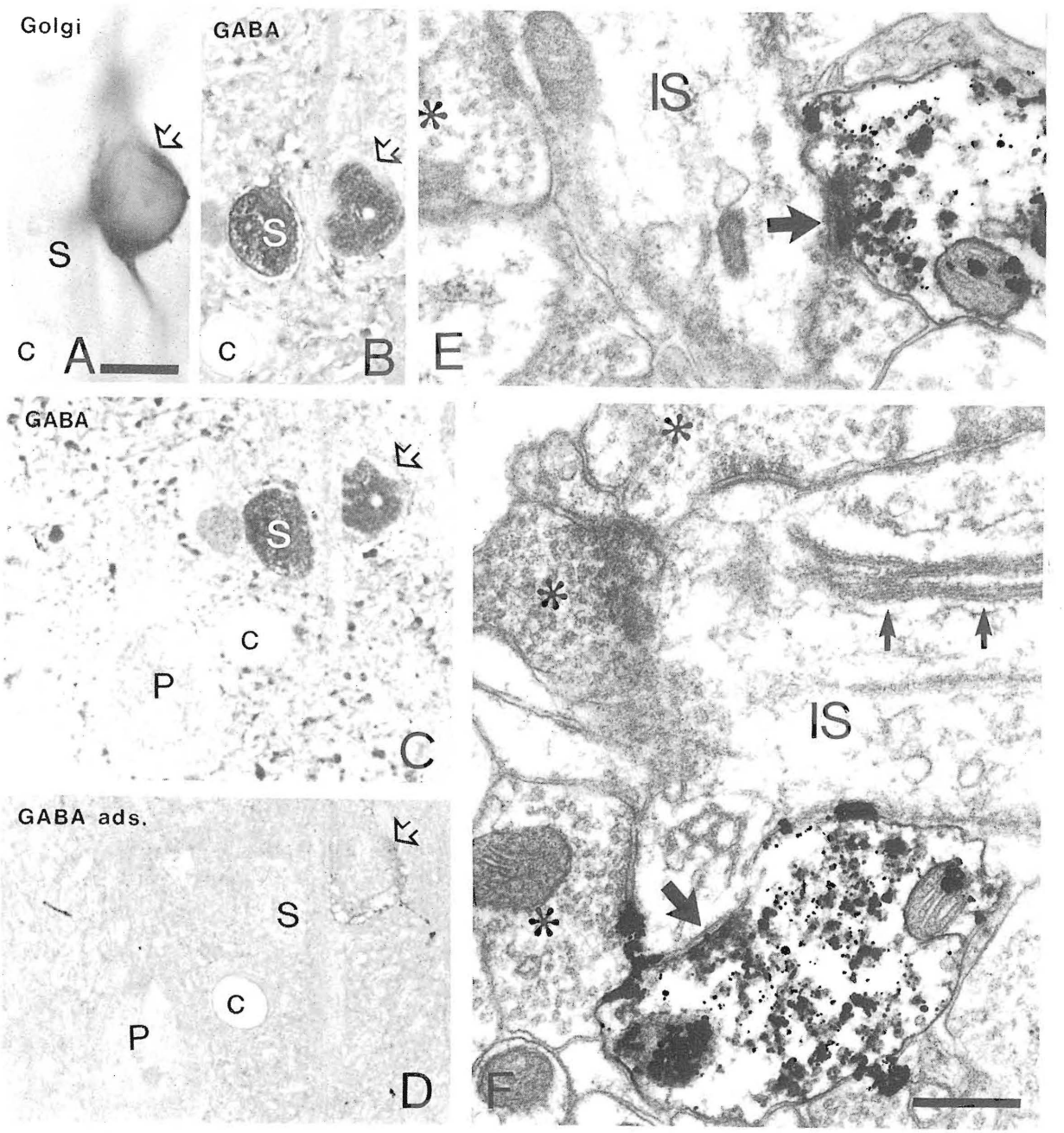

Fig. 2. A: light micrograph of the soma of the same cell as in Fig. 1, as seen in the thick Golgi section. B, C and D: semithin (0.5 μm) sections of the same cell reacted with antiserum to GABA (B and C) or with the same serum pre-incubated with GABA coupled to polyacrylamide beads (D). Note dark peroxidase reaction-endproduct showing GABA-immunoreactivity, in the axo-axonic cell (open arrow) and in a nearby non-pyramidal cell (S) as well as in terminals in the neuropile and surrounding a non-immunoreactive pyramidal cell (P). Capillary (c) serves as correlation mark. E and F: electron micrographs of gold toned boutons of the same axo-axonic cell forming type II synaptic contacts (thick arrows) with the shaft (E) or a spine (F) of pyramidal cell axon initial segments (IS). Similar non-impregnated boutons (asterisks) converge onto the ISs. A lamellar body (small arrows in F) is also present. Scales: A–D, same magnification, 10 μm; E and F, same magnification, 0.5 μm.

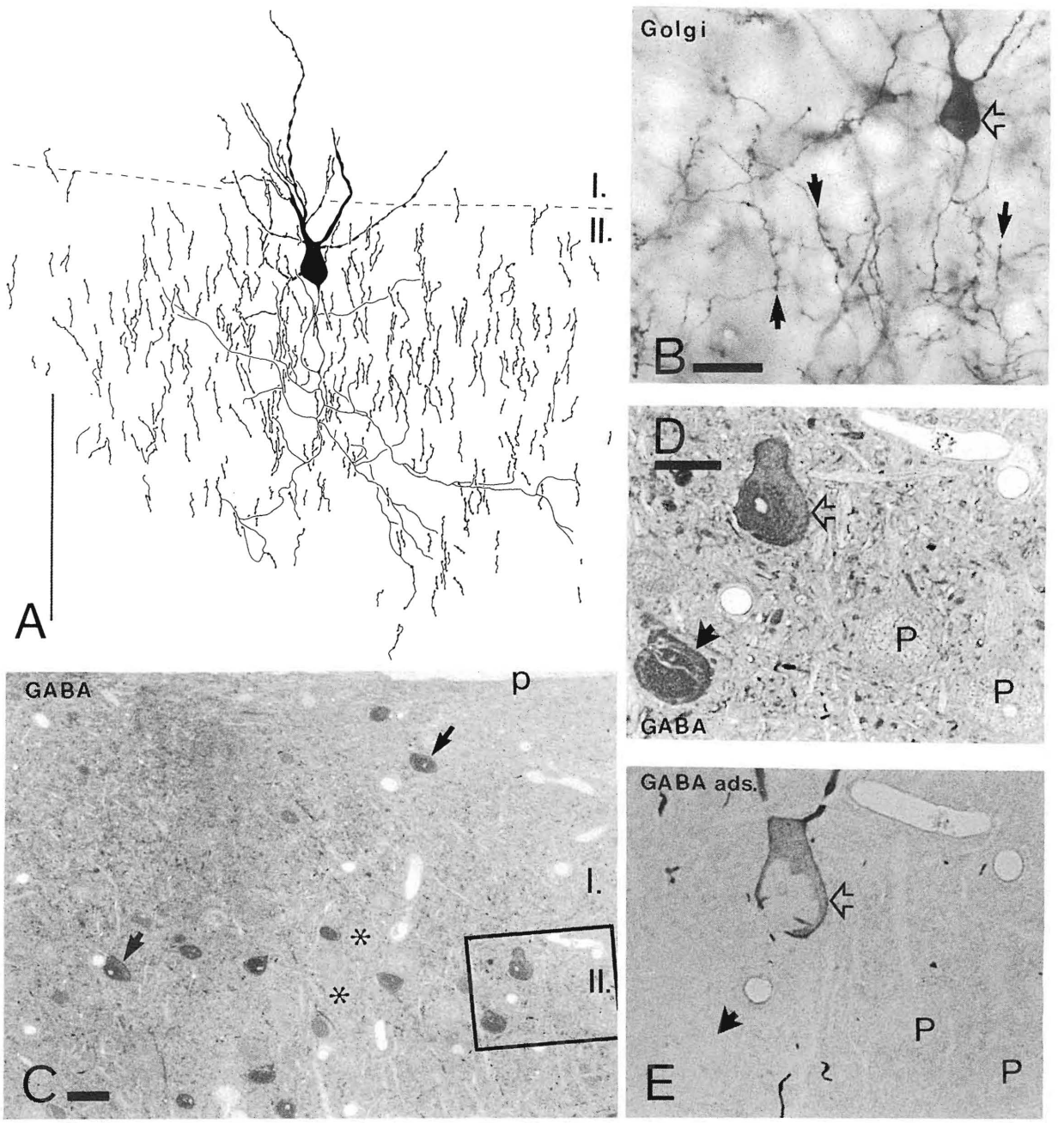

Fig. 3. A: drawing of an axo-axonic cell in layer II of the cat's striate cortex. For clarity only the main branches and the bouton-bearing specialized terminal segments of the axon are shown. B: light micrograph of the same cell (open arrow) with some of the terminal segments (arrows) indicated. C: semithin section (0.5 μm) of the same area reacted for GABA-immunoreactivity. GABA-positive neurons (e.g. arrows) including the axo-axonic cell (in framed area and also shown in D at higher magnification) are present in both layers I and II amongst non-immunoreactive cells (e.g. asterisks). D and E: serial sections (0.5 μm) showing the GABA-immunoreactive axo-axonic cell (open arrow), another GABA-positive neuron (solid arrow) and non-immunoreactive pyramidal cells (P). The latter are contacted by immunoreactive varicosities in D. E: pre-incubation of the antiserum with GABA coupled to a carrier prevented all immunostaining. The cytoplasm of the axo-axonic cell is dark due to the grey coloured gold deposit originating from the Golgi impregnation. Scales: A, 100 μm; B and C, 20 μm; D and E, 10 μm.

in the neuropile and have been shown to be axons and nerve terminals[13]. All immunoreactivity was abolished by the pre-incubation of the serum with GABA coupled to a solid-phase carrier (Figs. 2D and 3E). This, together with previous adsorption experiments using several amino acids, strongly suggests that the serum reveals immunoreactive GABA[3,14].

The somata of both the cortical and the hippocampal AAC were immunoreactive for GABA when reacted using semithin sections (Figs. 2B, C and 3C, D). Parts of the cytoplasm of the hippocampal neuron were GABA-negative due to the heavy deposition of metallic gold resulting from the Golgi impregnation (Fig. 2C). The cytoplasm of the cortical neuron was stained more weakly than that of non-impregnated cells in the same section. However, the dark brown immunoperoxidase reaction of the nuclei and the lighter brown staining of the cytoplasm in some of the sections makes the GABA-immunoreactivity of the cells certain (Figs.2B, C and 3C, D). This shows that the transmitter GABA is present in AACs as well as the enzyme responsible for its synthesis.

The immunocytochemical demonstration of the presumed transmitter in neurons that has been identified by Golgi-impregnation is the most direct approach to the neurochemical characterization of cells in an area where neurons with different connectivity may use the same transmitter. The reliability of the method is shown in this study by the demonstration, in two cortical areas, of immunoreactive GABA in AACs.

The extensive vertical distribution of the dendritic arbor of the hippocampal AAC deserves some attention because the different layers receive input from different afferents. In the neocortex the afferents are not segregated to such an extent and it has been impossible to predict what may activate AACs. The inescapable conclusion from the drawing of Fig. 1B is that hippocampal AACs have the same dendritic distribution as the pyramidal cells and are therefore likely to have access to all the input available to CA1 pyramidal neurons. The descending dendrites of the AAC correspond to the basal dendrites of the pyramidal neurons in the stratum oriens. The major ascending dendrites of the AAC correspond to the apical dendrite of the pyramidal cell; the only difference being that they are more numerous. Even the terminal tuft in stratum moleculare corresponds to similar branching at the end of apical dendrites of pyramidal cells. If AACs share the same input as pyramidal cells then the key to their function is not so much the source of the input but the timing of their activation relative to the activation of pyramidal cells which they could inhibit through the axon IS.

The results show that AACs contain immunoreactive GABA and this makes it likely that they use it as transmitter at their terminals on the axon ISs of pyramidal cells. This supports previous suggestions for the inhibitory role of AACs unique to cortical structures such as neocortex[1,2,6,8–10], hippocampus[11,12], dentate gyrus[4] and the amygdala[7]. Their presence in these areas which are susceptible to epileptic activity and the specificity of their termination lead to the suggestion that the abnormal function of these cells may be related to epilepsy[11]. In this respect, the finding of GABA in AACs may be relevant in view of the deficiency of the GABAergic system in epileptic areas[5]. The AACs more than any other GABAergic neuron are in a position to control the firing of hundreds of pyramidal neurons simultaneously. It remains to be established if there are differences in the structural and functional properties of AACs in normal tissue and in tissue exhibiting epileptic activity.

The authors are grateful to Mrs. C. Kassapidis, to Dr. Susan E. Rundle and to Miss S. Thomas for excellent technical assistance. The work was supported by the Neurosurgical Research Foundation of South Australia Inc., the Epilepsy Association of South Australia Inc., the Children's Medical Research Foundation of South Australia Inc., and the National Health and Medical Research Council of Australia.

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