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A Type of Aspiny Neuron in the Rat Neostriatum Accumulates [^{3}H]γ-Aminobutyric Acid: Combination of Golgi-Staining, Autoradiography, and Electron Microscopy

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ABSTRACT

Light microscopic autoradiography was used to identify cells in the neostriatum that became labelled after the local injection of [^{3}H]γ-aminobutyrate (GABA). The GABA-accumulating cells comprised up to 15% of the total population of neurons. Thirty-seven of these cells were examined in the electron microscope and it was found that they all had similar cytological characteristics, i.e., prominent nuclear indentations, a moderate volume of cytoplasm, rich in organelles, and sparse synaptic input to the perikaryon. Nine of the cells that had accumulated GABA were also impregnated following Golgi staining. These Golgi-impregnated neurons were of medium size and all had dendrites that were aspiny, often varicose, and that occasionally followed a recurving path. After gold toning, the Golgi-impregnated, GABA-accumulating neurons were examined in the electron microscope and were found to receive boutons forming symmetrical or asymmetrical synaptic contacts on their somata and dendrites; the symmetrical synapses were most common on the cell body and proximal dendrites, while the distal dendrites mainly received boutons forming asymmetrical contacts.

We conclude that one type of GABAergic neuron in the neostriatum is a type of medium-sized aspiny neuron and that this neuron is likely to receive synaptic input both from neurons within the striatum and from neurons in distant brain regions. We suggest that this neuron is a local circuit neuron in the neostriatum since its morphological features are quite distinct from those of identified projecting neurons.

There are many biochemical, electrophysiological, and pharmacological studies consistent with the view that γ-aminobutyric acid (GABA) is one of the transmitters in the striatonigral pathway (see reviews by Dray, '79; Fonnum and Walaas, '79; Di Chiara and Gessa, '81). However, several lines of evidence suggest that GABA may also act as a transmitter within the neostriatum itself. Thus the results of biochemical studies (Hattori et al., '73; McGeer and McGeer, '75) have shown that sectioning of the rat brain at a level that interrupts the pallidal and nigral efferents of the striatum results in only a 15% decrease in striatal glutamate decarboxylase (GAD) activity, and morphological studies have shown that GAD immunoreactivity is present within axons and terminals in the striatum (Ribak et al., '79). Depolarization with excess potassium ions releases GABA from the striatum both in vivo (Van der Heyden et al., '80) and in vitro (Reubi, '80); high-affinity GABA-binding sites are present (De Feudis, '80) and have been demonstrated morphologically (Palacios et al., '81). Electrophysiological studies have shown that striatal neurons respond to administered GABA (Bernardi et al., '75; Spehlmann et al., '77; Misgeld et al., '82) and that evoked inhibitory postsynaptic potentials may be blocked by GABA antagonists (Spehlmann et al., '77; Park et al., '80; Misgeld et al., '82). Modulation of striatal GABAergic systems affects other transmitter systems. Thus

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administration of GABA mimetic compounds causes an increase in striatal acetylcholine levels, presumably due to an inhibition of its release (Scatton and Bartholini, '79, '80a). This effect is susceptible to picrotoxin and independent of the nigrostriatal dopaminergic system although partially dependent on the integrity of the corticostriatal pathway (Scatton and Bathiolini, '79, '80a,b). These results are supported by the observation that GABA inhibits the potassium ion-evoked release of newly synthesized acetylcholine in the striatum; this effect, however, is dependent on the integrity of striatal catecholamine systems (Stoof et al., '79). The effect of GABA on dopamine release is the opposite; thus in vitro studies using striatal slices have shown that the effect of GABA is to enhance both spontaneous and stimulated release of dopamine (Stoof and Mulder, '77; Giorguieff et al., '78; Giorguieff-Chesselet et al., '79; Stoof et al., '79; Starr, '79). A potentiation by GABA of the release of met-enkephalin has also been observed (Sawynok and LaBella, '81).

Finally, a functional role for GABA within the striatum is suggested by the results of behavioral studies. Thus the local administration of picrotoxin in rats produces contralateral choreoform movements (McKenzie et al., '72; McKenzie and Viik, '75) or profound local myoclonus (Tarsy et al., '78; Costall et al., '76); both effects can be overcome by the infusion of GABA. The unilateral elevation of striatal GABA levels in the mouse (Horton and Pycock, '77) or local injection of GABA in the cat striatum (Cools and Janssen, '76) causes ipsilateral turning, while bilateral infusion of low doses of muscimol into the ventral-intermediate region of the neostriatum (Scheel-Krüger et al., '80) but not other areas (Di Chiara et al., '79) produces catalepsy. Finally, infusion of GABA into the striatum blocks electrically induced head turning (Malick and Goldstein, '76), contralateral head-turning in cats induced by unilateral injection of dopamine (Cools and Janssen, '76), antagonizes apomorphine-induced stereotyped behavior in rats, and has a biphasic (enhancement/inhibitory) effect on haloperidol-induced catalepsy in rats (Scheel-Krüger et al., '80). The question arises, which neuron type or types mediate these GABAergic effects within the striatum?

Although there is good evidence for a GABAergic striatonigral pathway, it is not known which type of neuron in the striatum gives rise to this GABAergic projection. Two types of striatonigral neuron have so far been found: the medium-size spiny neuron (Somogyi and Smith, '79; Somogyi et al., '79; Kitai, '81) and a neuron with long smooth dendrites (Bolam et al., '81a). It has been speculated that the medium-size spiny neuron may use GABA as a transmitter (Ribak et al., '79; Somogyi and Smith, '79) and some of the medium-size neurons that project to the substantia nigra have been shown to have local axon collaterals in the striatum (Somogyi et al., '81a; Kitai, '81). It is possible, therefore, that the release of GABA within the striatum could originate from the local axon collaterals of striatonigral neurons. Alternatively, or in addition, it is possible that GABAergic local circuit neurons may be present in the striatum. One way of identifying neurons that might use GABA as a transmitter is to use autoradiography to see if they take up [^{3}H]GABA (Ehinger, '70; Hökfelt and Ljungdahl, '70; Iversen, '78; Sotelo, '75). We have used autoradiography in the light microscope to reveal cells that take up [^{3}H]GABA after its injection directly into the striatum and we have examined these identified cells by Golgi staining and by electron microscopy, following a similar approach to that applied to the cerebral cortex (Somogyi et al., '81c). The morphological and cytological characteristics of these putative GABAergic neurons have been compared with those of other characterized neurons in the neostriatum. Preliminary accounts of some of the results have been published (Bolam et al., '82; Clarke et al., '82).

METHODS

All experiments were carried out on female albino Wistar rats (150–180 gm) with the aid of the stereotaxic atlas of König and Klippel ('63). The animals were anesthetized with chloral hydrate (350 mg/kg IP in 0.9% w/v NaCl) and held in a stereotaxic frame. The experimental procedures were similar to those described earlier for the combination of Golgi staining and autoradiography in the neocortex (Somogyi et al., '81b). Aliquots of a solution of [^{3}H]GABA (50–60 Ci/mM, Amersham International) were dried under a stream of N_2 and redissolved in Krebs' bicarbonate solution to a concentration of 20 or 50 Ci/l (0.3–0.75 mM GABA). Injections were made (0.2 μl) through a glass micropipette (tip diameter 30–80 μm) with a gas pressure system at the coordinates given below. The period of the injection varied from 1 to 20 minutes (usually 15 minutes). Twenty to 40 minutes after the beginning of the injection the chest wall was opened and the animal was perfused through the heart for 1.5 minutes with saline (ca. 20 ml) followed by fixative (ca. 200 ml) consisting of 0.5% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The brain was then removed and postfixed in fresh fixative for 3–12 hours. The neostriatum was then dissected out and cut into 1-mm-thick horizontal slices which were washed several times in the phosphate buffer. The slices were then postfixed in osmium tetroxide (1% in the phosphate buffer), Golgi-stained, and 80-μm tissue chopper sections were gold toned, essentially as described previously (Somogyi et al., '79; Somogyi et al., '81c). The sections were dehydrated and mounted on microscope slides in resin (Durcupan ACM, Fluka).

Golgi-stained neurons close to the site of injection of [^{3}H]GABA were drawn and photographed and then reembedded in blocks of resin suitable for further sectioning. Semithin sections (1 μm) were taken from the perikarya of the impregnated neurons and mounted on gelatin-coated slides. Three sections were usually obtained from each perikaryon, which allowed three different exposure times and left a significant proportion of the cell body in the block, so that it could be examined in the electron microscope. The slides were then dipped in Ilford K5 or G5 autoradiographic emulsion (diluted 50:50 with distilled water and containing 1% glycerol), dried, and stored in slide boxes at 4°C for 1–30 days. The slides were developed using Kodak D19B and counterstained with a mixture of toluidine blue and Azur II. Non-Golgi-stained neuronal perikarya and Golgi-stained neurons, identified as radiolabelled in the light microscope, were then examined in the electron microscope by cutting ultrathin sections of the parts of the neurons that remained in the block of resin.

Ultrathin sections were collected on formvar-coated single slot grids and examined in a Philips 201C electron microscope using an objective aperture of 30 μm. The sections were contrasted using lead citrate solution (Reynolds, '63).

The results of studies on Golgi-stained and radiolabelled neurons are from three rats. Rat 1 received 0.2 μl of a 50

Ci/l solution of [^{3}H]GABA at stereotaxic coordinates: A, 7.6; L, 2.4; V, 4.9 mm; and with a survival time of 23 minutes after the start of the injection. Rat 2 received 0.2 μl of a 20 Ci/liter solution at the same anterior and lateral coordinates but 0.1 μl was given at V, 4.9 mm and 0.1 μl at 3.5 mm; the animal survived for 20 minutes. Rat 3 received 0.2 μl of a 20 Ci/liter solution at coordinates A, 7.2; L, 2.6; V, 5.0 mm (0.1 μl) and 3.9 mm (0.1 μl); it survived for 22 minutes.

In one experiment, in addition to receiving injections of [^{3}H]GABA, another three rats received injections of 0.2 μl [^{3}H]GABA solution containing 100 mM *cis*-1,3-aminocyclohexane carboxylic acid (ACHC), either immediately before or after the injection of [^{3}H]GABA alone at another site. The injections of the mixture of [^{3}H]GABA and ACHC were located at least 1.5 mm away from the sites where [^{3}H]GABA had been injected on its own. The intervals between the start of the intrastriatal injections and death ranged from 20 to 40 minutes.

RESULTS

Light microscopic autoradiography

Examination of autoradiograms of 1-μm sections revealed that there was a selective accumulation of silver grains over the perikarya of a proportion of neurons around the site where [^{3}H]GABA had been injected (Fig. 1). These neurons will be called "GABA-accumulating neurons." The intensity of labelling in different sections varied greatly and was dependent on many factors. The intensity of labelling within an individual section was, however, dependent on the closeness of the cells to the injection site; thus the closer the neuron, the greater the number of silver grains. Near to the injection site, neuropil labelling was high and the labelling of glia was also observed. In such heavily labelled areas, positively labelled neurons were identified by comparison with unlabelled neurons (Figs. 1, 4B, 5B,E). Labelled neurons more distant from the injection site showed accumulations of silver grains to a

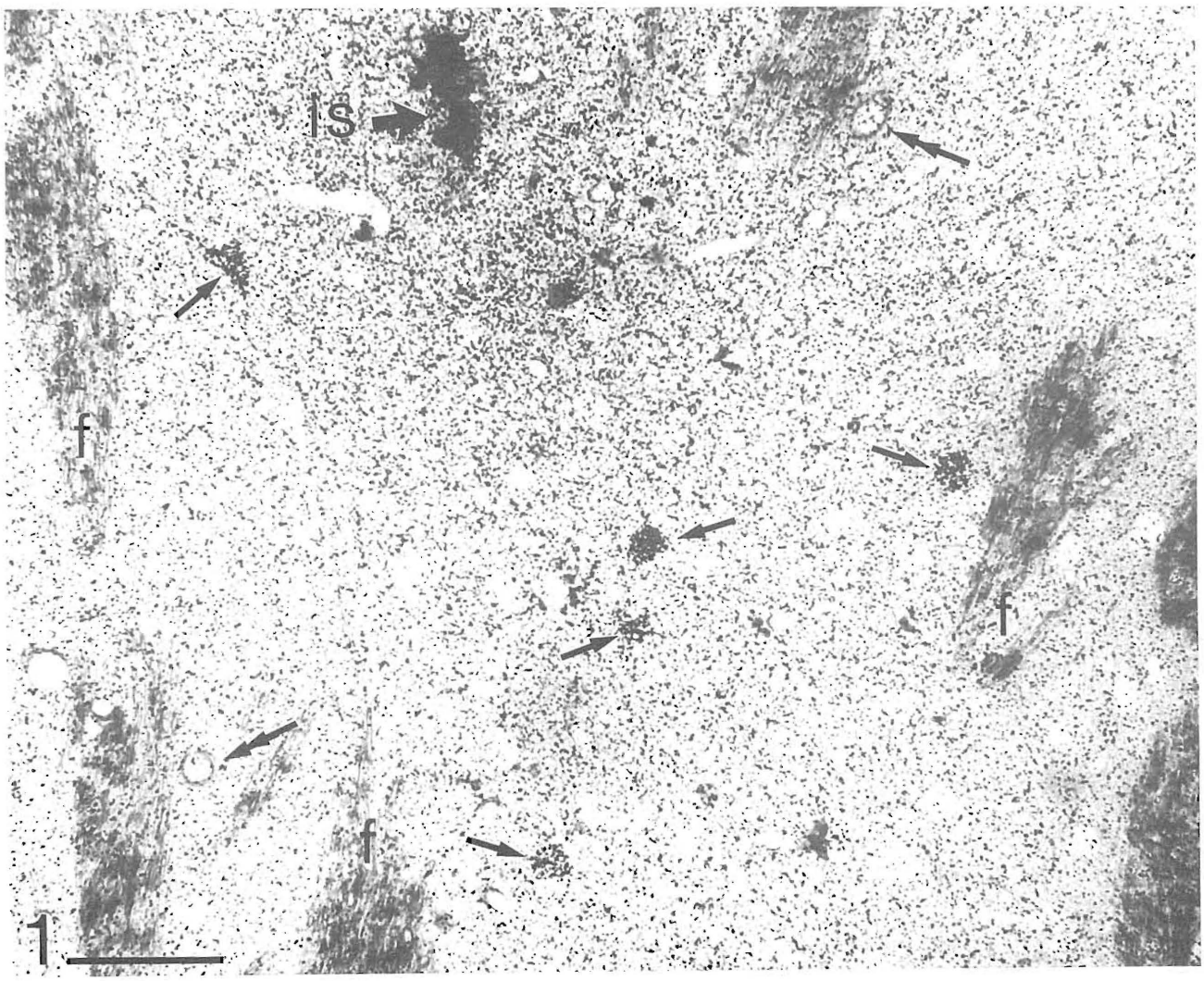

Fig. 1. Autoradiogram of a 1-μm plastic section from the rat neostriatum at the site of injection of [^{3}H]GABA. The injection site (is) appears black due to the high concentration of silver grains. The other dark areas, some of which are labelled (f), are fiber bundles. Unlabelled neurons appear as light areas. Neurons that have selectively accumulated the radiolabel are indicated by arrows. Two Golgi-stained neurons (double arrow) also appear in this section but have not accumulated the [^{3}H]GABA. Scale: 50 μm.

greater extent than either the neuropil or unlabelled neurons (Figs. 1, 2A, 6B). As has been pointed out previously (Somogyi et al., '81c) in Golgi-stained neurons, the most intense labelling occurred in sections which included the nucleus of the neuron. This is presumably related to two factors: first, the [^{3}H]GABA, or a metabolite, might be incorporated into the nucleus and, second, the nuclei are not impregnated with the Golgi deposit and therefore at the level of the nucleus less quenching of the radioactivity occurs. It can be seen that the accumulation of silver grains over neuronal perikarya was not due to the reagents or to the Golgi deposits, because the accumulation of grains was selective and because the majority of Golgi-impregnated neurons within the area of accumulation were not overlain with grains.

The proportion of neurons around the site of injection of the [^{3}H]GABA that accumulated the radiolabel varied from only one or two per 1-μm section to as many as 15. These were generally found within a radius of 400 μm from the injection track but occasionally labelled neurons were observed as distant as 600 μm. In the sections with a large number of GABA-accumulating cells, they represented as much as 10–15% of the total population of neurons within the area where accumulation occurred.

The administration of ACHC, a selective inhibitor of the neuronal uptake of GABA (Bowery et al., '76), together with [^{3}H]GABA, prevented the labelling of neurons even though in the same animals selective labelling occurred at sites where the [^{3}H]GABA was injected on its own. Some animals received an injection of an inhibitor of GABA transaminase (amino-oxyacetic acid, 10 mg/kg IP) 30 minutes prior to the intrastriatal administration of labelled GABA. The same pattern of selective labelling of neurons was found as in untreated animals.

[^{3}H]GABA accumulation in Golgi-stained neurons

Thirty selected Golgi-stained and gold-toned neurons that were within about 1 mm of the center of the injection track were first of all drawn and photographed from the 80 μm sections: all of these had smooth dendrites. In the same sections, we found 88 medium-size densely spiny neurons. All the Golgi-impregnated neurons were then reembedded and 1-μm sections were examined by autoradiography. Of the medium-size densely spiny neurons, none showed a selective accumulation of the radiolabel (examples are shown in Figs. 5A–C, 6A–C). Of the smooth dendrite neurons a total of nine neurons from three rats showed a positive accumulation of silver grains (Fig. 3), i.e., had a greater density of silver grains above the perikaryon than was present over the local neuropil (Figs. 5B, 6B) and/or over unlabelled neurons in the immediate vicinity (Table 1, Figs. 4B, 5B,E, 6B). The GABA-accumulating neurons had from six to 19 times as many silver grains above their perikarya as other neurons in the surrounding area (Table 1). On two occasions, Golgi-impregnated medium-size densely spiny neurons abutted against Golgi-stained neurons of other types (neurons 5 and 7 in Fig. 3) and so it was possible to compare their abilities to accumulate [^{3}H]GABA (Figs. 5A–C, 6).

Because of the capricious nature of the Golgi procedure, some of the neurons that accumulated the [^{3}H]GABA were only partially impregnated (neurons 1, 3, and 6 in Fig. 3 and one neuron which is not presented), while others were better impregnated (neurons 2, 4, 5, 7, and 8 in Fig. 3). This must be borne in mind when assessing the Golgi characteristics of these neurons. The Golgi-impregnated GABA-accumulating neurons have round or oval cell bodies of medium size (12–18 μm). Up to four primary dendrites of variable thickness emerge from the cell body and branch, usually within 10 μm. Secondary and tertiary dendrites also branch within a short distance from their origin and they branch frequently, either dividing equally or giving off characteristically thin, usually short (10–30 μm), dendrites at an angle of about 90° (Figs. 4A, 6A). These branches are occasionally longer (> 50 μm) and, because of the angle of emergence, follow a recurving path (neurons 5 and 7 in Fig. 3); they may also emerge from primary dendrites (neuron 8 in Fig. 3). The dendrites were essentially smooth (Figs. 3, 4A, 5D, 6A) and had only occasional spines. The secondary dendrites often had a marked varicose profile (neurons 4, 5, and 8 in Fig. 3; see also Fig. 4A, top left). The dendritic radius is usually in the order of 100–150 μm, but occasionally dendrites run for as far as 200–250 μm (see neurons 4 and 8 in Fig. 3). Initial segments of axons have been seen to emerge from the cell body or from a thick primary dendrite.

Electron microscopy of [^{3}H]GABA-accumulating neurons

Nine Golgi-stained and gold-toned GABA-accumulating neurons were examined in the electron microscope as well as 28 neurons (from four rats) that took up [^{3}H]GABA, but were not Golgi stained. The ultrastructural features of all these neurons were very similar. The most characteristic feature of the neurons was the nucleus, which had prominent indentations (Figs. 2C, 4C, 5C, 5F, 7). Occasional

TABLE 1. Number of Silver Grains Over GABA-Accumulating Golgi-Impregnated Neurons and "Unlabelled" Neurons in Rat Neostriatum[1]

Cell number (see Fig. 3)	Number silver grains (A)	Number grains over 'unlabelled' neurons mean of 5 (B)	Ratio $\frac{A}{B}$
1	127	12.2	10.4
2	205	17.2	11.9
3	125	19.8	6.3
4	111	17.4	6.3
5	92	7.4	12.4
7	44	5.2	8.4
8	134	9	18.8

[1]Grain counts were performed using a × 100 oil-immersion objective. The number of grains of neuron 6 were too high to count.

Fig. 2. A. Autoradiogram of a 1-μm plastic section of the neostriatum, close to the site of injection of [^{3}H]GABA. The open arrow indicates a neuron that has selectively accumulated the radiolabel. The same neuron is shown in electron micrograph B (open arrow). Other structures common to the two micrographs include the capillary (c) and fiber bundle (f). C. Higher-power electron micrograph of the same neuron; note the nuclear indentations (arrows). go, Golgi apparatus; er, rough endoplasmic reticulum. Synapses on the perikarya of identified [^{3}H]GABA-accumulating striatal neurons are shown in D–H. D. A large bouton makes symmetrical synaptic contact with [^{3}H]GABA-accumulating perikaryon (P). E. A large bouton makes symmetrical synaptic contact (arrow) with ^{3}H-GABA accumulating perikaryon (P) and with a nonidentified dendrite (d) (arrow); the dendrite also receives input from a bouton that forms an asymmetrical membrane contact (arrow). F, G. Small boutons make asymmetrical synaptic contact (arrows) with [^{3}H]GABA-accumulating perikarya (P). H. A [^{3}H]GABA-accumulating perikaryon (P) gives rise to a spine (S) that receives a symmetrical synaptic input (arrow). Scales: A, 10 μm; B, 5 μm; D, 0.2 μm. Magnifications of E–H are the same as for D.

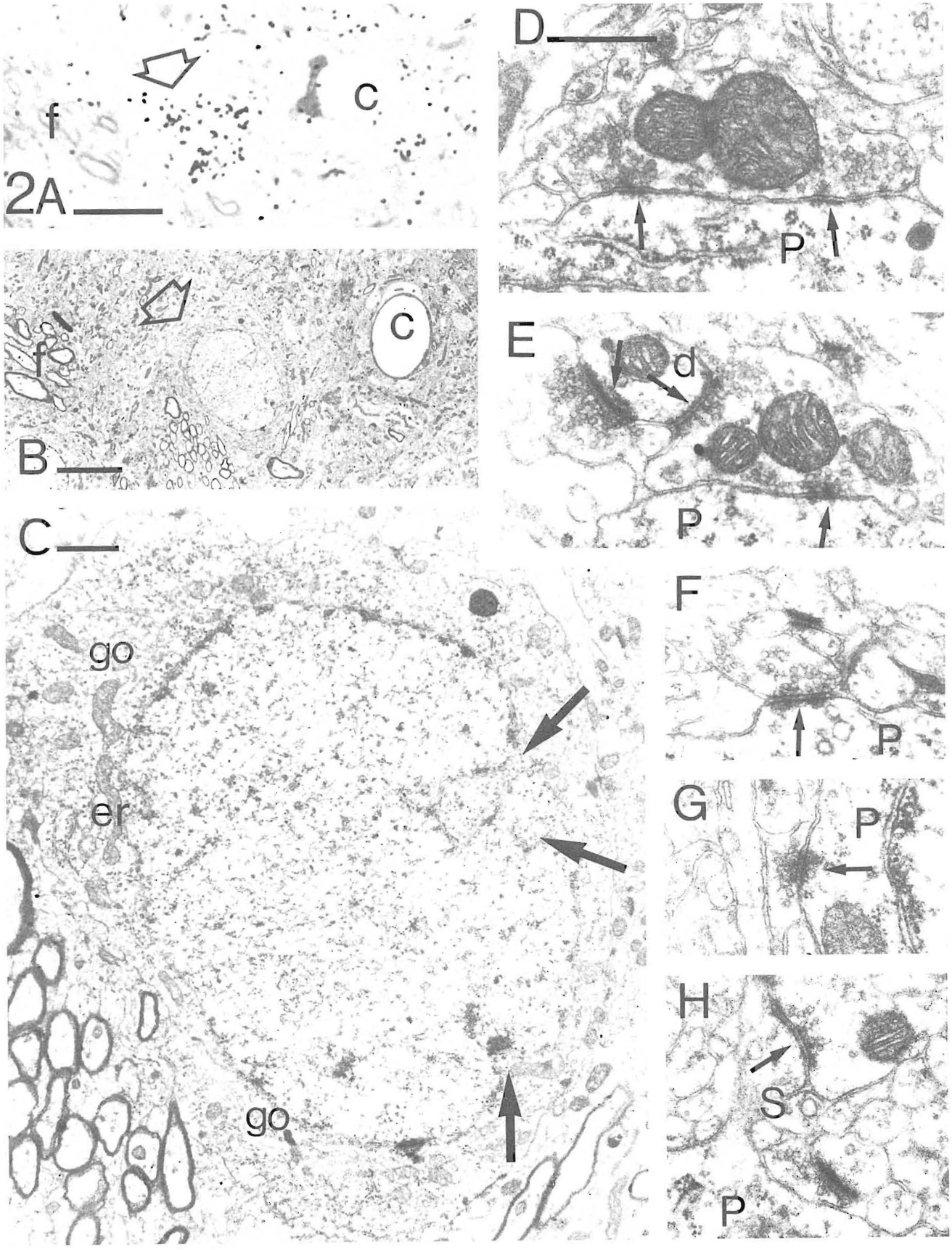

Figure 2

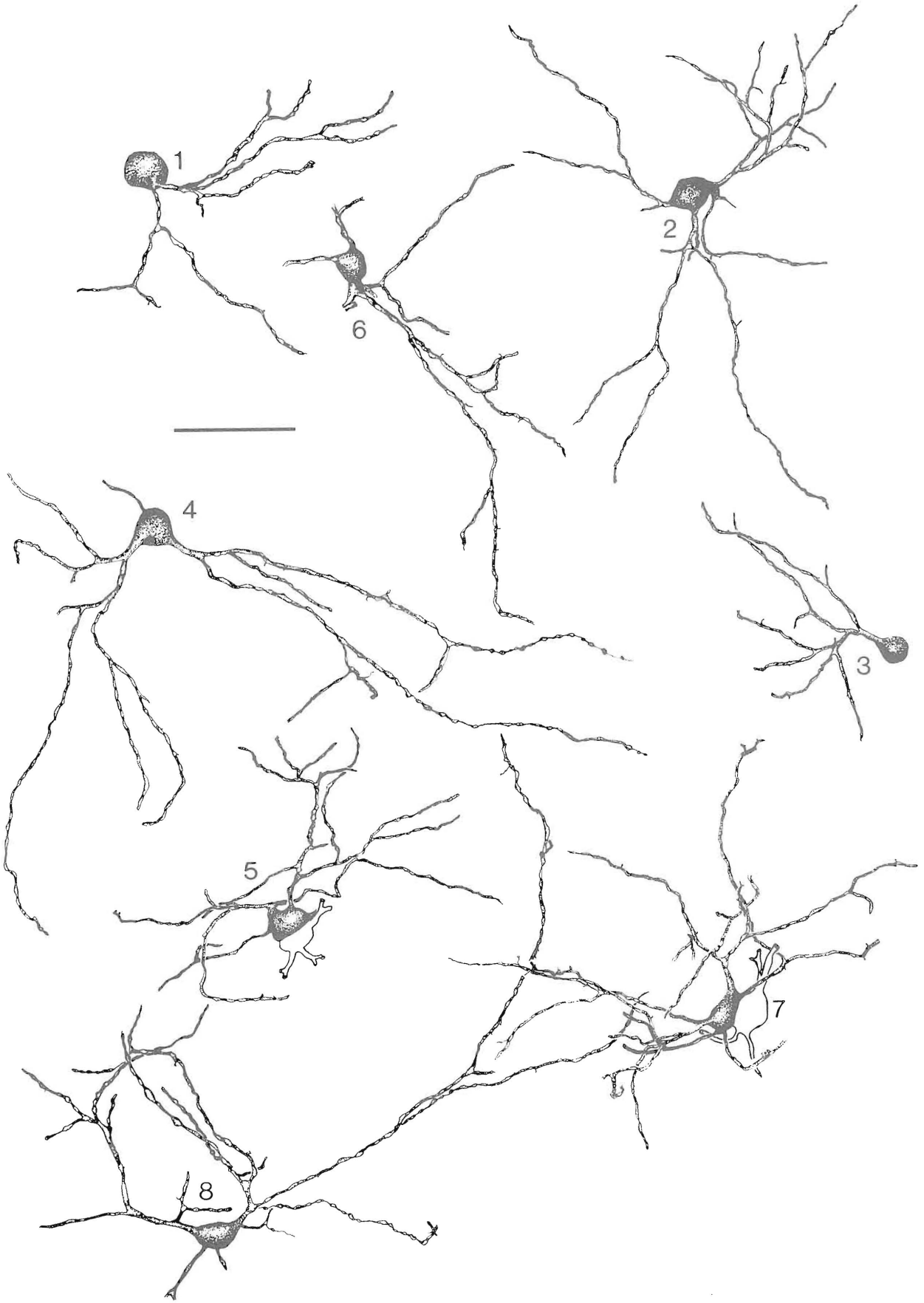

Fig. 3. Camera lucida drawing of Golgi-stained and gold-toned neostriatal neurons that accumulated locally administered [^{3}H]GABA. The neurons shown in outline next to neurons N5 and N7 are Golgi-stained medium-size densely spiny neurons that failed to accumulate the [^{3}H]GABA. Neurons 1–5 were from rat 1; neurons 6 and 7 from rat 2, and neuron 8 from rat 3. Scale: 50 μm.

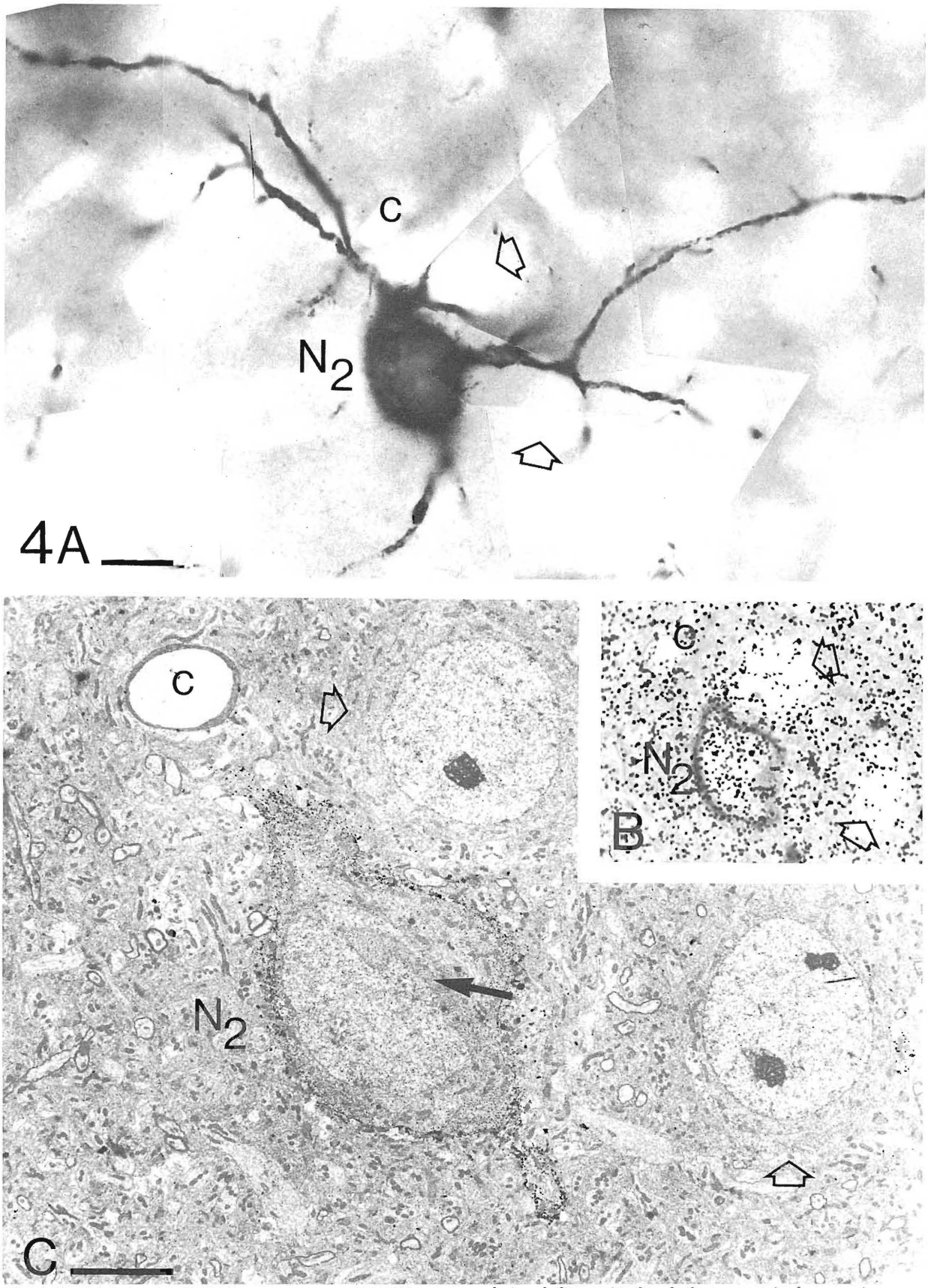

Fig. 4. A. Partial photomontage of Golgi-stained and gold-toned GABA-accumulating neuron number 2 in Figure 3. (The montage has been rotated through 90° in an anticlockwise direction with respect to the drawing in Fig. 3.) Note the frequent branching of the dendrites, the absence of spines, and the occasional varicose profile. Two unstained neurons (open arrows) and a capillary (c) are used for correlation and are also labelled in the micrographs B and C. B. Autoradiogram of a 1-μm section of the perikaryon of the same neuron (N_2). The Golgi-stained neuron shows a greater accumulation of silver grains than do the two unstained neurons (open arrows). C. Low-power electron micrograph of the same neuron (N). Note the darker appearance of the Golgi-stained neuron, due to the secondary Golgi deposit. A deep nuclear indentation is indicated by a filled arrow. The two unstained neurons (open arrows) and the capillary (c) are indicated so that the correlation can be made with the light micrographs in A and B. Scales: A and B, 10 μm; C, 5 μm.

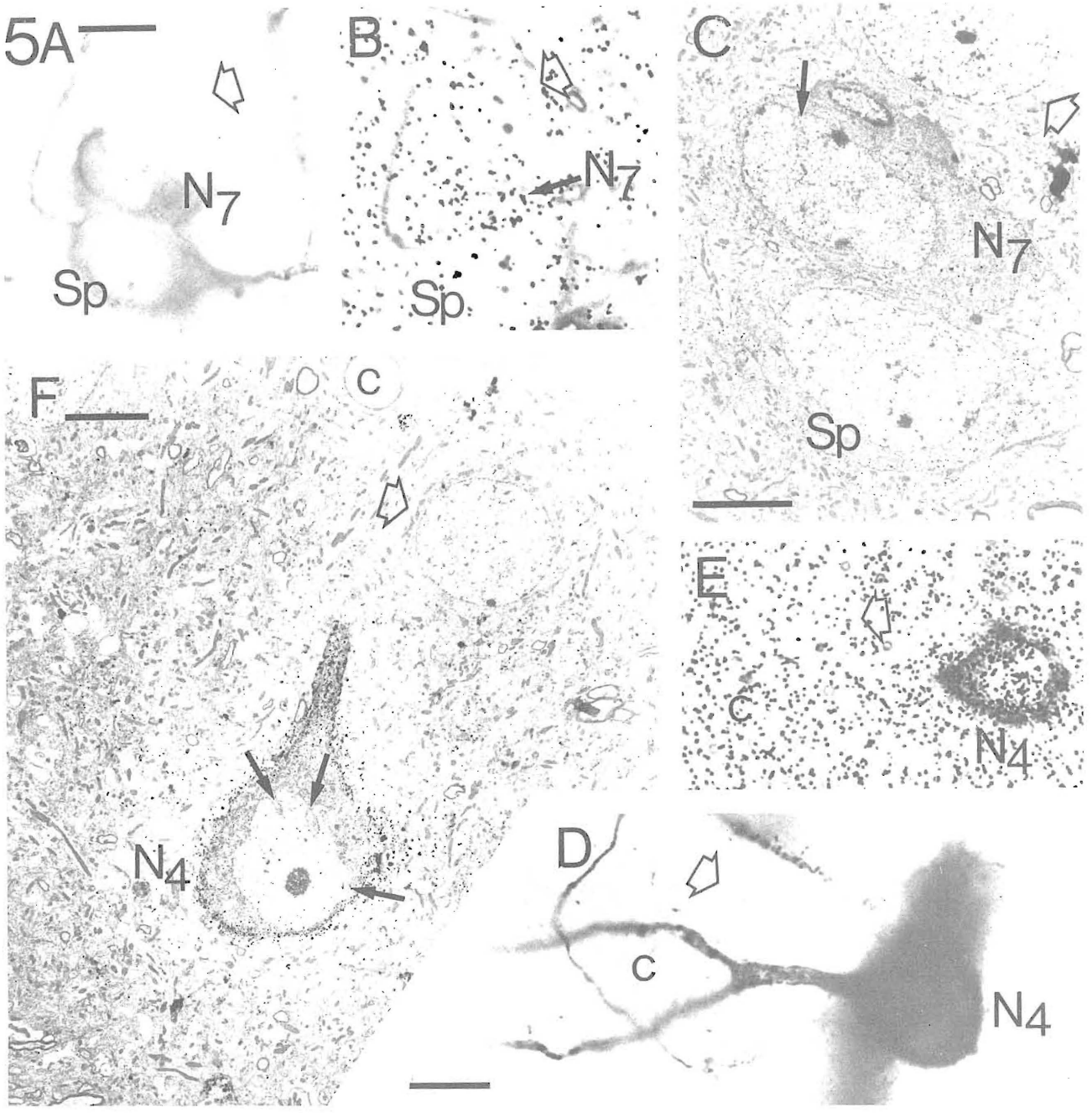

Fig. 5. A. Light micrograph of the perikaryon of Golgi-stained, gold-toned, GABA-accumulating neuron number 7 in Figure 3. The Golgi-stained neuronal perikaryon labelled Sp is a medium-size densely spiny neuron. The open arrow indicates an unstained neuron. B. Autoradiogram of a 1-μm section of the same three perikarya. The smooth dendrite neuron N7 is labelled by a greater number of silver grains than either the medium-size densely spiny neuron (Sp) or the unstained neuron (open arrow). C. Low-power electron micrograph of the same three neurons. Note the characteristic smooth nuclear membrane of the spiny neuron (Sp) and the indented nucleus (arrow) of the smooth dendrite GABA-accumulating neuron (N_7). D. Light micrograph of the perikaryon and a proximal dendrite of GABA-accumulating neuron number 4 in Figure 3. Note the smooth secondary dendrites. A capillary (c) and unstained neuron (open arrow) are indicated for correlation. E. Autoradiogram of a 1-μm section of the same neuron. Note greater accumulation of silver grains over neuron N_4 than over the unstained neuron (open arrow). F. Low-power electron micrograph of the same neurons. Note the nuclear indentations (arrows) of neuron N_4. Scales: A and B, 10 μm; C, 5 μm; D and E, 10 μm; F, 5 μm.

Fig. 6. A. Partial photomontage of GABA-accumulating neuron number 5 in Figure 3 and a Golgi-stained medium-size densely spiny neuron (Sp) that abutted against it (rotated through 180°). A capillary (c) and an unstained neuron (open arrow) are labelled for correlation. B. Autoradiogram of a 1-μm section of the same three perikarya. Note the greater accumulation of silver grains over neuron N_5 than over the medium-size densely spiny neuron or the unstained neuron. C. Low-power electron micrograph of the same area shown in B. D,E. Two boutons forming asymmetrical synaptic contacts (arrows) with dendrites of neuron N_5 (d). The bouton in E also makes asymmetrical synaptic contact with a nonidentified spine. Scales: A and B, 10 μm; C, 5 μm; D and E, 0.2 μm.

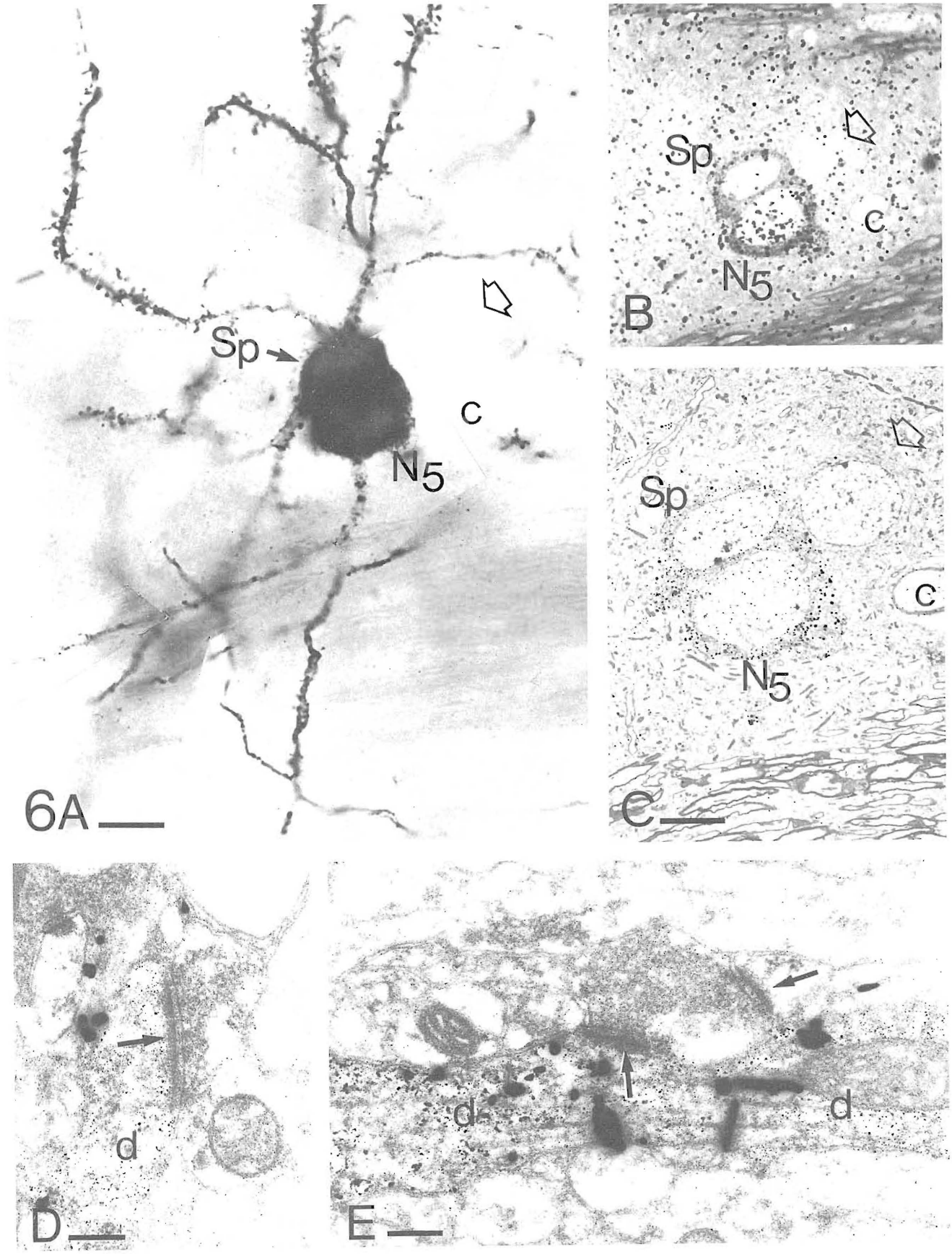

Figure 6

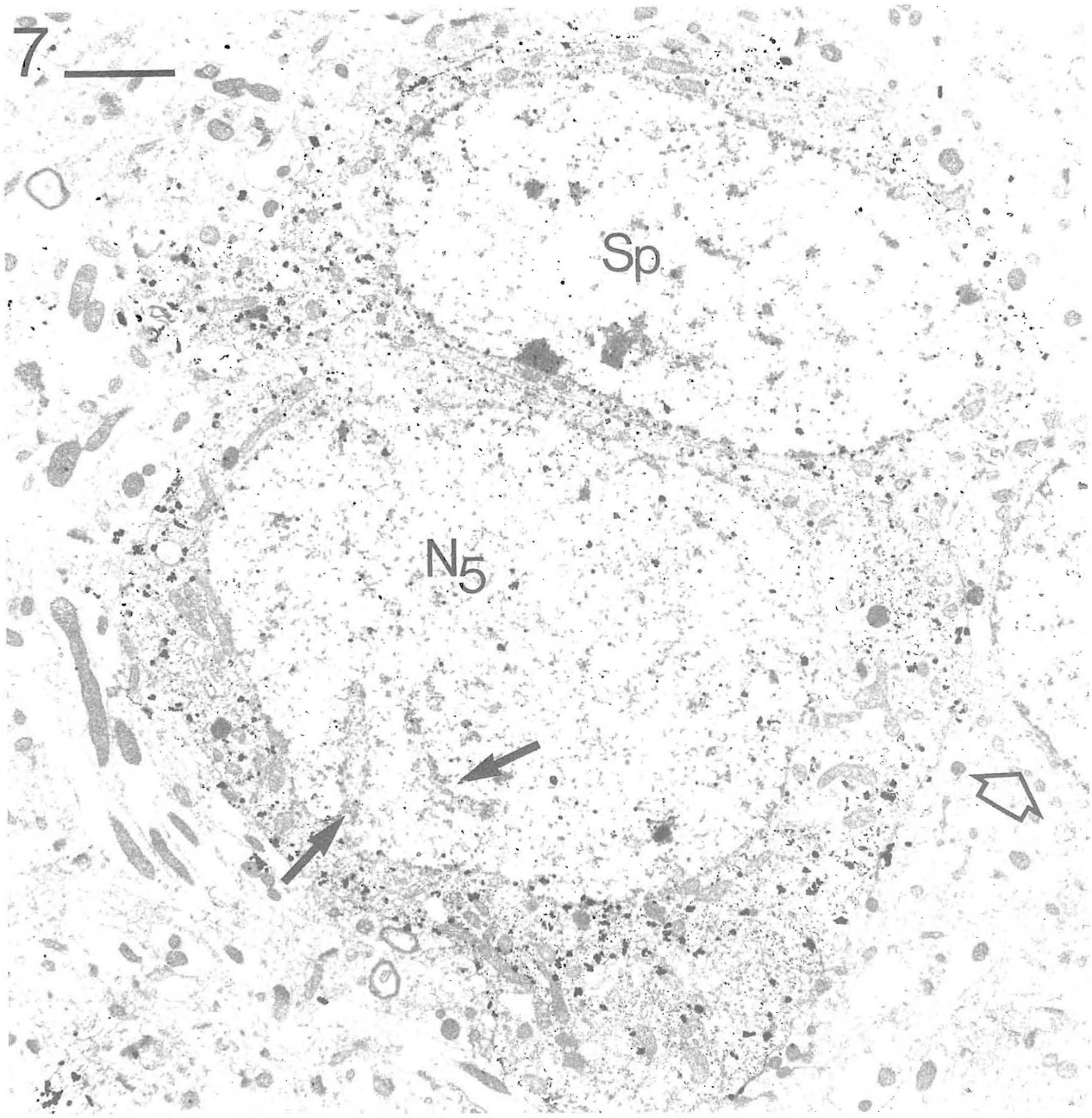

Fig. 7. Higher-power electron micrograph of GABA-accumulating neuron (N_5) and its "companion" medium-size densely spiny neuron (Sp). The open arrow indicates the unstained neuron also shown in Figure 6A, B, and C. Note the smooth nuclear envelope and thin rim of cytoplasm of the spiny neuron and the indented nucleus (arrows) and relatively large area of cytoplasm of neuron N_5. Scale: 2 μm.

filamentous nuclear inclusions were observed. The moderate area of cytoplasm around the nucleus was rich in organelles, including numerous mitochondria (up to 40 per section), four to seven regions of Golgi apparatus per section, numerous ribosomes (mainly arranged as polysomes), and up to nine dense bodies per section (Figs. 2C, 7). Rough endoplasmic reticulum usually occurred as isolated strands but occasionally two or more strands were arranged in stacks. On one occasion a somatic spine was observed (Fig. 2H).

The most proximal regions of the dendrites, as observed in both Golgi-impregnated and nonimpregnated GABA-accumulating neurons, also contained numerous cytoplasmic organelles. In Golgi-impregnated GABA-accumulating neurons the more distal primary and subsequent branches contained fewer organelles but were still rich in mitochondria, ribosomes, and microtubules. The varicose nature of the dendrites was confirmed and occasional spines were observed.

Synaptic input to identified [^{3}H]GABA-accumulating neurons

The axosomatic synaptic input to GABA-accumulating neurons was sparse and consisted of boutons forming both symmetrical (Fig. 2D, E, H) and asymmetrical membrane specializations (Fig. 2F, G). The boutons forming symmetrical synaptic contacts were generally large (up to about 0.7 μm in width), contained one or more mitochondria (Fig. 2D, E), and sometimes had more than one active site (2D). The boutons forming asymmetric contacts occurred less frequently and were much smaller (0.1–0.2 μm in width) (Fig. 2F, G). The proximal dendrites also received boutons that formed symmetrical synaptic contacts but these became less frequent distally. Distal dendrites received synaptic input from numerous boutons forming predominantly asymmetrical synaptic contacts (Fig. 6D, E) but these boutons were larger than those in synaptic contact with the perikarya.

DISCUSSION

There are several lines of evidence which lead us to the view that the neurons we have identified, which become radiolabelled after intrastriatal injection of [^{3}H]GABA, are likely to use GABA as their transmitter. The labelling of these neurons was probably due to an active uptake process (Fagg and Lane, '79), since we could only detect the labelling in one type of neuron and no labelling occurred in the presence of the GABA uptake inhibitor ACHC. The labelling is likely to be the result of the uptake of [^{3}H]GABA rather than a metabolite because animals pretreated with an inhibitor of GABA transaminase showed the same selective labelling of neurons. In other parts of the central nervous system there is evidence that the active uptake and accumulation of radiolabelled GABA into neurons is restricted to those that use GABA as a transmitter. Thus the uptake only occurs into specific populations of neurons (Chronwall and Wolff, '80; Halasz et al., '79; Hökfelt and Ljungdahl, '71, '72; Hösli and Hösli, '78; Iversen and Schon, '73; Iversen, '78; Somogyi et al., '81c) and the morphology of radiolabelled neurons is similar to the morphology of neurons immunostained for GAD (Brandon et al., '80; Saito et al., '74); and, finally, the labelled GABA taken up can be released in a calcium-dependent manner upon stimulation (Frankhuyzen and Mulder, '77).

Comparison of the GABA-accumulating striatal neuron with neurons in other brain areas that take up GABA

When the morphology of the striatal [^{3}H]GABA-accumulating neurons is compared with that of neurons in other brain regions that can take up [^{3}H]GABA under similar experimental conditions, certain common features are observed. Stellate cells in the cerebellum (Hökfelt and Ljungdahl, '71; Schon and Iversen, '72) share the characteristics of medium size, an indented nucleus, a moderate amount of cytoplasm, and receive only a few synapses on their soma. Similar cytological features occur in neurons which accumulate GABA in the olfactory bulb (Halasz et al., '79), lateral geniculate nucleus (Sterling and Davis, '80), and neocortex (Somogyi et al., '81c).

Comparison of the GABA-accumulating neuron with other types of Golgi-stained neurons in the striatum

A striking finding was the selectivity of the neuronal labelling after the intrastriatal injection of [^{3}H]GABA. All 37 neurons that we studied had a very similar ultrastructure, with a characteristic indented nucleus, and all nine of the neurons that were Golgi stained had the same type of dendritic morphology.

Several distinct morphological classes of neurons can be distinguished in the Golgi-impregnated neostriatum (for review see Pasik et al., '79; see also Danner and Pfister '79, '81a,b; Dimova et al., '80; Tanaka, '80). The different types are classified according to the size of the perikarya; thus there are medium-sized, giant, and small neurons, and they are further divided according to the presence or absence of dendritic spines. All the studies describe the medium-size densely spiny neuron; this type is the most frequently impregnated (Kemp and Powell, '71a) and probably does represent a high proportion of striatal neurons (Bolam et al., '81b). Other medium-size spine-bearing neurons have been described (Danner and Pfister, '81a) which may be different types altogether but which could be subpopulations of the medium-sized, densely spiny type (Chang et al., '81; Bishop et al., '82). The other major types are the giant neurons and the medium-sized, smooth dendrite neurons which have again been subdivided into several subclasses on the basis of dendritic morphology. The Golgi-impregnated GABA-accumulating neuron clearly belongs to one of the medium-size smooth dendrite class of neurons and shows some similarities to the aspiny type I in the dog (Tanaka, '80) and the aspiny types I and III in the monkey (Di Figlia et al., '80), except that our neurons may have longer dendrites. They are also similar to the spidery neurons of Fox et al. ('71/'72), the medium-sized smooth cell of Kemp and Powell ('71a) and the spidery neuron described by Danner and Pfister ('81b). The best correlation is with neurons of types II and III in the rat striatum as described by Dimova et al. ('80). These neurons have several primary dendrites which branch frequently, possess only occasional spines, and are often varicose (type III). The results of electron microscopy agree well with this conclusion, since the ultrastructural characteristics (e.g., indented nuclei) of our neurons and their types II and III are similar.

The GABA-accumulating neurons can readily be distinguished from Golgi-impregnated, medium-sized, densely spiny neurons (Figs. 3, 5A–C, 6, 7), some of which have been characterized as striatonigral neurons by the retrograde transport of horseradish peroxidase (Somogyi and Smith, '79; Somogyi et al., '81a), and from the large smooth dendrite neuron (striatonigral type 2) that also projects to the substantia nigra (Bolam et al., '81a). There are also clear differences between the GABA-accumulating neuron and the giant neuron of the striatum and other neurons that have been chemically characterized by immunocytochemistry including those reactive for substance P (unpublished observations), enkephalin (Pickel et al., '80; Di Figlia et al., '82), and somatostatin (Takagi, Somogyi, Somogyi and Smith, unpublished observations).

Other studies on putative GABAergic neurons in the striatum

There have been two previous studies on neuronal labelling following exposure of striatal tissue to [^{3}H]GABA. Hattori et al. ('73) observed accumulations of silver grains over neuronal perikarya following the incubation of striatal slices with [^{3}H]GABA, although these neurons were not further characterized. Iversen and Schon (1973) carried out electron microscopic autoradiography on the subependymal part of the striatum following intraventric-

ular injection of [^{3}H]GABA and described labelled cells which "had elliptical profiles, a large nucleus with distinct nuclear invagination, and relatively little endoplasmic reticulum in their cytoplasm." These authors suggested that such neurons might be local circuit neurons. The neuron illustrated (Fig. 7A Iversen and Schon, '73:) shows several similarities to the neurons described here, most notably the indented nucleus.

Our findings can be compared with the results obtained by immunocytochemical methods for the synthetic enzyme for GABA, glutamic acid decarboxylase (GAD). Thus, Ribak et al. ('79) and Ribak ('81) described neurons within the neostriatum that are immunoreactive for GAD; these authors suggested, on the basis of electron microscopic examination of immunoreactive perikarya and the presence of immunoreactive dendritic spines, that the neurons were of the medium-sized, densely spiny class that project to the substantia nigra. However, as we have pointed out previously (Somogyi et al., '81a), the neurons whose cell bodies were illustrated (Fig. 18: Ribak et al., '79; Fig. 3: Ribak '81) are clearly not medium-sized, densely spiny neurons. In fact they show several ultrastructural similarities to the GABA-accumulating neurons presented here. GAD-immunoreactive striatal neurons have also been studied in tissue culture (Panula et al., '81) and were found to have characteristically indented nuclei and a relatively small volume of cytoplasm with few organelles. These neurons are the most common type observed in tissue cultures and it has been suggested that they are the medium-sized densely spiny neurons (Panula et al., '79). However, as shown in several studies where light microscopically identified medium-size spiny neurons have been studied in the electron microscope (Somogyi and Smith, '79; Somogyi et al., '79; DiFiglia et al., '80; Dimova et al., '80; Wilson and Groves, '80; Frotscher et al., '81; Bishop et al., '82), such neurons do not have a markedly indented nucleus. It is possible that tissue culture of neostriatal neurons leads either to selective survival of certain types of neuron or to changes in the ultrastructure or transmitter characteristics, which makes it difficult to compare such studies with those on neurons in the intact brain.

Are the GABA-accumulating neurons local circuit neurons or projection neurons?

Are the GABA-accumulating aspiny neurons that we have described the source of the striatopallidal and striatonigral GABAergic pathways? Projection neurons make up a high proportion of the neurons in the striatum (Bolam et al., '81b; Woolf and Butcher, '81) whereas our GABAergic aspiny neuron is likely to comprise a smaller proportion of striatal neurons. The characteristic cytology of the GABAergic neuron is quite different from that of striatonigral neurons that have been studied in the electron microscope (Bak et al., '78; Bolam et al., '81a; Henderson, '81; Somogyi and Smith, '79) and its dendritic morphology is also different from that of striatonigral neurons that have been Golgi stained (Somogyi and Smith, '79; Somogyi et al., '79; Somogyi et al., '81a) and from that of striatonigral and striatopallidal neurons injected with horseradish peroxidase (Chang et al., '81; Kitai, '81). Thus it is unlikely that the GABA-accumulating neuron we have described is a projection neuron, although we cannot rule out that it might project to a region so far not identified as a target of striatal efferents. It is probable, therefore, that the aspiny GABAergic neuron is a local circuit neuron. None of our Golgi-stained GABA-accumulating neurons had a sufficiently well-impregnated axon for us to study axonal morphology; however, in other Golgi material we have found striatal neurons with a very similar morphology that do possess the extensive local axonal arborizations characteristic of local circuit neurons (unpublished observations).

Since one of the transmitters in the striatonigral pathway is likely to be GABA and two types of striatonigral neurons have been characterized (Bolam et al., '81a), the question may be asked: Why did neither of these neuron types accumulate the locally administered [^{3}H]GABA? A possible explanation for this is that [^{3}H]GABA labelling of the cell body might occur following uptake by axon terminals and retrograde transport to the cell body (Streit, '80; Somogyi et al., '81b,c). Although the medium-sized, spiny neurons that project from the striatum do have local axon collaterals (Preston et al., '80; Chang et al., '81; Somogyi et al., '81a; Kitai, '81), it is possible that insufficient [^{3}H]GABA is taken up by the local terminals in the striatum for the radioactivity to be detected in the cell bodies relative to the high background of radioactivity in the neuropil. It is noteworthy that neurons in the striatum could be retrogradely labelled following injection of a large dose of [^{3}H]GABA into the substantia nigra (Streit, '80). A similar problem has arisen in studies on the cerebellar cortex, where the projecting Purkinje cells that are by other criteria GABAergic do not take up [^{3}H]GABA following its local administration; on the other hand, cerebellar local circuit neurons do accumulate GABA (Sotelo et al., '72; Hökfelt and Ljungdahl, '72; Iversen, '78) and are also immunoreactive for GAD (Fonnum and Storm-Mathisen, '78; Oertel et al., '81).

Possible functional implications

If we assume that the fact that certain neurons in the striatum selectively take up GABA indicates that they use GABA as a transmitter, then our findings are relevant to the functioning of the striatum. The ability to study the Golgi-impregnated GABA-accumulating cells and their dendritic processes in the electron microscope allows us to make some provisional suggestions about the role of the neurons in the neuronal circuits in the striatum. The GABA-accumulating neurons received synapses on their cell bodies (mainly symmetrical) and dendrites (mainly asymmetrical). Boutons forming asymmetrical synapses in the striatum are believed to originate mainly from extrinsic neurons in the cortex, thalamus, and substantia nigra, while boutons forming symmetrical synapses probably originate from intrinsic neurons (Kemp and Powell, '71b; Chung et al., '77; Hassler et al., '78; Wilson and Groves, '80; Chang et al., '81; Somogyi et al., '81a; Frotscher et al., '81). It is thus likely that the GABA-accumulating local circuit neurons receive input both from outside and from within the striatum. The nature and origin of these synaptic inputs can now be studied by combining the present procedure with anterograde degeneration (for extrinsic inputs) and with Golgi-staining of other neurons with local axons (for intrinsic inputs).

In conclusion, we suggest that the type of neuron we have described is a GABAergic local circuit neuron and that such neurons could well mediate many of the physiological and pharmacological effects ascribed to GABA in the striatum and may play an important role in integrating distant and local input.

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