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# Cortical input to parvalbumin-immunoreactive neurones in the putamen of the squirrel monkey

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The cortex projects heavily to the striatum and makes asymmetrical synaptic contact mainly with the spines of medium-sized densely spiny neurones. The possibility exists that corticostriatal terminals also make synaptic contact with classes of striatal interneurones. The primary objective of the present experiment was to determine whether parvalbumin-immunoreactive neurones, which represent a class of GABAergic interneurones in the striatum, also receive a direct synaptic input from corticostriatal fibres. The anterograde tracer biocytin was injected into the motor and premotor cortices of the squirrel monkey (*Saimiri sciureus*). Following perfuse-fixation, sections of the striatum were processed histochemically to reveal the transported biocytin using an avidin-biotin-peroxidase complex and diaminobenzidine as the chromogen. They were then immunostained to reveal parvalbumin using benzidine dihydrochloride as the chromogen. In both the light and electron microscopes, the morphological features and the afferent synaptic input of the parvalbumin-immunoreactive neurones were similar to those observed in other species. Similarly, the morphology and postsynaptic targets of the corticostriatal terminals were similar to those described in other species. Light microscopic examination revealed that the anterogradely labelled corticostriatal terminals were often in close apposition to the parvalbumin-positive neurones. At the electron microscopic level the biocytin-positive corticostriatal terminals were found to make asymmetrical synaptic contacts mainly with spines. The parvalbumin-positive neurones were seen to have an invaginated nucleus, extensive cytoplasm and relatively few spines. Parvalbumin-immunoreactive dendrites received a dense synaptic input consisting mainly of asymmetric synapses and only a few symmetric synapses. Biocytin-labelled corticostriatal terminals were often seen in asymmetrical synaptic contact with parvalbumin-immunoreactive dendrites. These results show that GABAergic interneurones identified on the basis of parvalbumin immunoreactivity, in addition to the projection neurones of the striatum, are under the direct influence of the cerebral cortex.

## INTRODUCTION

The mammalian striatum (caudate nucleus/putamen) contains many classes of neurones identified not only on the basis of morphological characteristics but also on their chemical characteristics, i.e., the nature of their chemical transmitter. One such chemical transmitter is the inhibitory amino acid, gamma-aminobutyric acid (GABA), which is in fact present in the majority of striatal neurones. Morphological analysis of these GABAergic elements identified by immunocytochemistry for GABA itself[44,53] or its synthetic enzyme, glutamate decarboxylase (GAD)[5,35,48] or by the local uptake of [$^3$H] GABA combined with Golgi-impregnation[4] have revealed the existence of at least two distinct populations of GABAergic neurones in the striatum. The most common type are medium-sized densely spiny neurones[21,22,35,56] which send their axons to the substantia nigra and/or the globus pallidus[14,21,55]. The second type of GABAergic neurones in the striatum are medium-sized aspiny neurones which have been identified as interneurones on the basis of their morphological features in Golgi-impregnated material[4], their ultrastructural features[5,44] and their failure to become retrogradely labelled following injections of tracers in the output regions[2,3,55].

The striatum also contains a small population of neurones which contain the calcium-binding protein, parvalbumin. This protein is distributed throughout the brain of both the rat[13] and monkey[17] and can be localised to various neuronal populations such as cerebellar Purkinje, basket and stellate cells, hippocampal basket cells[28,37], septohippocampal cells[24], cells of the dorsal geniculate nucleus[57] and neocortical interneurones[11]. In all of these neuronal systems parvalbumin is co-localised with GABA[13,24,37,57]. Parvalbumin is also present in fast twitch muscle fibres, where it is thought to participate in postcontraction relaxation[8,28,46] and occurs in fast spiking hippocampal neurones[30] where its calcium-binding capacities are thought to play a role in conferring rapid firing properties to these neurones. It has been suggested that parvalbumin may allow specific neurones to maintain a fast firing rate by reducing the calcium-dependent

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$K^+$ outflow[9,10]. In the striatum, parvalbumin-containing neurones are indistinguishable from GABAergic interneurones on the basis of morphological features and distribution, and indeed, co-localisation studies have revealed that all parvalbumin-immunoreactive neurones in the rat striatum display GABA-immunoreactivity[19,36].

Ultrastructural analysis of striatal GABAergic interneurones identified by Golgi impregnation combined with the uptake of [$^3$H]GABA[4] and by GAD or GABA immunocytochemistry[5,44] have shown that these neurones receive both symmetrical and asymmetrical synaptic inputs onto their perikarya as well as proximal and distal dendrites. A similar pattern of synaptic innervation was recently described for the parvalbumin-immunoreactive cells in the rat striatum[36]. Asymmetrical synapses in the striatum are usually considered excitatory in nature and to originate from extrinsic sources, including the cerebral cortex and the thalamus (see Smith and Bolam[54]). Both of these regions project extensively to the striatum[31,43,50,51,62] where they make asymmetrical synaptic contact mainly with the output neurones but also with cells that display morphological features of interneurones[20,32,33,52,56]. The possibility exists therefore that the GABAergic interneurones of the striatum receive a direct synaptic input from the cerebral cortex. Pharmacological evidence supports this possibility, since it has been shown that both application of excitatory amino acids and stimulation of the cerebral cortex directly affect the levels of GABA in the striatum[23,27,49,60]. However, these results do not give any indication as to whether these changes in GABA levels are due to stimulation of the GABAergic interneurones or GABAergic projection neurones. The primary objective of the present experiment was therefore to determine whether GABAergic interneurones in the putamen of the monkey, as revealed by immunocytochemistry for parvalbumin, receive a direct synaptic input from corticostriatal terminals. The second objective was to compare the morphology and afferent synaptic input of the parvalbumin-immunoreactive neurones in the putamen of the monkey with GABAergic interneurones in the striatum of the rat[4,5,35,36,48] and to compare the morphology and postsynaptic targets of corticostriatal terminals in the primate with those described in other species[20,25,26,32,56,61].

## MATERIALS AND METHODS

*Animals and preparation of tissue.* Two adult (800–1,000 g), male, squirrel monkeys (*Saimiri sciureus*) were used in this study. All procedures were carried out under deep anaesthesia using ketamine hydrochloride (40 mg/kg/i.m.). A 5% solution of biocytin (Sigma Chemical Co.) in 0.05 M Tris-HCl buffer (pH 7.4; Tris buffer) was stereotaxically injected through multiple penetrations ($n$ = 10) into the motor and pre-motor cortices (areas 4 and 6). Injections of 300 nl of tracer were made at each site using a 5 $\mu$l Hamilton syringe. Following a survival time of 48 h the monkeys were anaesthetised and transcardially perfused with fixative consisting of 2% paraformaldehyde and 1% glutaraldehyde (1.5 l) in 0.1 M phosphate buffer (pH 7.4; PB). After perfusion the brains were removed and regions including the caudate nucleus, putamen and globus pallidus were cut on a vibrating microtome at 50 $\mu$m in the coronal plane. The sections were collected in phosphate-buffered saline (PBS; 0.01 M, pH 7.4) and treated with 1% sodium borohydride in PBS for 5–10 min. They were then washed extensively, equilibrated with a cryoprotectant consisting of 25% (w/v) sucrose and 10% (v/v) glycerol in 50 mM phosphate buffer (pH 7.4) and frozen initially in isopentane cooled in liquid nitrogen and then in liquid nitrogen. The sections were then processed to reveal first, the injected and transported biocytin[29,34] and to reveal secondly neurones immunoreactive for parvalbumin, using a double peroxidase procedure[6,40].

*Biocytin histochemistry and parvalbumin immunohistochemistry.* To localise the injected and transported biocytin, sections were washed thoroughly in PBS before being incubated overnight in an avidin-biotin-peroxidase complex (ABC, Vector Labs; 1:100 in PBS) solution including 1% bovine serum albumin (BSA, Sigma Chemical Co.) at 4°C. Following incubation, the sections were washed in PBS followed by Tris buffer before being pre-incubated in a diaminobenzidine (DAB) solution (25 mg/100 ml Tris buffer) and then incubated in the same solution but including 0.006% hydrogen peroxide for 10–20 min. After several washes in Tris buffer followed by PBS, sections of the caudate nucleus, putamen and globus pallidus were preincubated in 5% normal horse serum (NHS) diluted in PBS, rinsed and then incubated for 48 h at 4°C with a monoclonal antibody directed against parvalbumin (1:5,000, diluted in PBS containing 1% NHS). The parvalbumin antibody (No. 235) was kindly provided by Dr. M.R. Celio. Detailed description of the method of production of this antibody and tests demonstrating its specificity have been published elsewhere[12]. After incubation with the primary antibody, the sections were washed in PBS and incubated for 1 h at room temperature in biotinylated horse anti-mouse IgG (1:200 diluted in PBS; Vector Labs., Burlingame, CA), washed in PBS and then incubated for 1 h at room temperature in ABC (1:100 in PBS containing 1% BSA). Sections were washed in PBS followed by 0.01 M phosphate buffer solution (pH 6.8) before being pre-incubated in a solution of benzidine dihydrochloride (BDHC) consisting of 5 mg BDHC and 25 mg sodium nitroprusside in 50 ml 0.01 M phosphate buffer (pH 6.8) for 10 min. They were then incubated in the same solution but including 0.0024% hydrogen peroxide for 5–10 min before being washed thoroughly in 0.01 M phosphate buffer (pH 6.8). The sections were then post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 6.8) for 25 min, dehydrated through a graded series of alcohols (including 1% uranyl acetate in 70% ethanol), infiltrated with Durcupan resin (ACM, Fluka), mounted on slides, coverslipped and cured at 60°C for 48 h.

*Analysis of material.* The sections were examined in the light microscope and areas of interest were drawn, photographed, cut out from the slides and glued with cyanoacrylic glue onto the top of resin blocks. Serial ultrathin sections were cut on a Reichert-Jung Ultracut E microtome and collected onto pioloform-coated single-slot copper grids. The sections were stained with lead citrate[47] before being examined on a Philips 410 electron microscope.

The morphology and synaptic input of 12 parvalbumin-immunoreactive neurones, first identified at the light microscopic level, were examined in serial sections in the electron microscope. In addition, all the sections were scanned for anterogradely labelled corticostriatal fibres and parvalbumin-immunoreactive structures. The targets of the corticostriatal terminals and inputs to the parvalbumin-immonoreactive neurones were noted.

*Control experiments.* As controls, a series of sections were processed to localise biocytin with DAB (see above) and then incubated in solutions in which the primary antibody against parvalbumin was omitted. Experiments were also carried out in which the parvalbumin was revealed alone using the peroxidase-anti-peroxi-

dase method (PAP). In such cases the sections were incubated overnight in PBS containing 1% BSA but omitting the ABC, followed by the DAB reaction (as described above). The sections were then incubated for 48 h at 4°C with a monoclonal antibody directed against parvalbumin. After incubation with the primary antibody, the sections were washed in PBS, incubated for 1 h at room temperature in horse anti-mouse IgG (1:50 diluted in PBS; Dakopatts), washed again in PBS and then incubated for 1 h in rat PAP (1:100, diluted in PB; Sternberger Meyer). They were then washed in phosphate buffer (0.01 M, pH 6.8) and incubated to reveal parvalbumin-immunoreactive structures with BDHC as described above.

## RESULTS

### *Anterograde labelling of corticostriatal fibres*

*Light microscopy*. In the light microscope, the DAB-labelled (biocytin-positive) structures were brown and displayed an amorphous appearance. The injection sites of biocytin were located mainly in the motor cortex (area 4) with some extension into the premotor cortex (area 6). An extensive terminal field of corticostriatal fibres was seen in the striatum, being mainly confined to the postcommissural lateral half of the putamen with almost no labelling in the caudate nucleus. The rostral pole of both structures appeared almost devoid of labelling. The corticostriatal terminals had a heterogeneous pattern of labelling with areas of dense labelling interspersed with areas almost devoid of labelled elements. At some levels this heterogeneous staining produced bands of labelling which possessed a dorsolateral orientation. Upon examination at higher magnification each band of labelling was seen to be composed of a dense plexus of labelled fibres and punctate structures distributed throughout the neuropil.

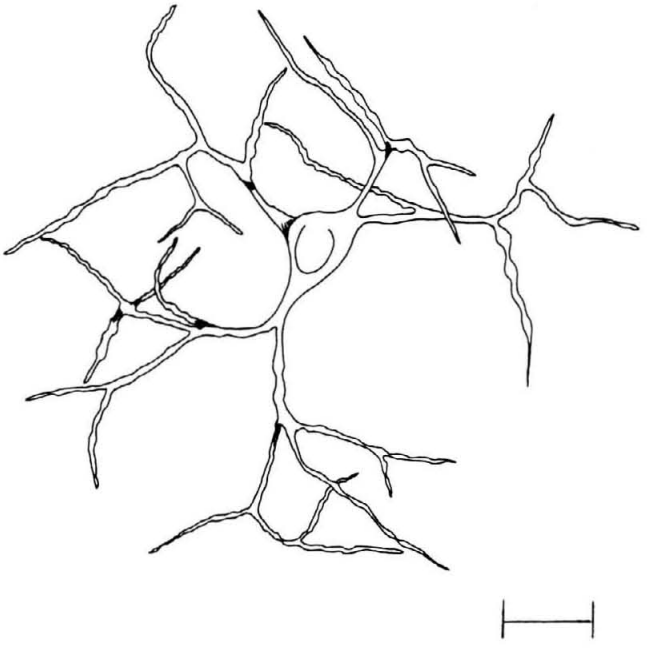

Fig. 1. Drawing of a parvalbumin-immunoreactive neurone in the putamen of the monkey. The cell possesses a medium-sized perikaryon and extensively branching, aspiny dendrites which are varicose in appearance. Scale: bar = 20 $\mu$m.

*Electron microscopy*. In the electron microscope, the DAB-labelled structures (corticostriatal axons and terminals) were recognised by the presence of an electron dense amorphous reaction product associated with the surface of all cell organelles and the inner surface of the plasma membrane. The peroxidase-labelled structures were easily distinguishable from unlabelled elements as their dark staining stood out clearly from the background. The biocytin-positive corticostriatal fibres and punctate structures seen in the light microscope were identified as vesicle-containing axons and boutons. The corticostriatal terminals were of variable sizes (diameter 0.2–0.6 $\mu$m) and contained densely packed, round vesicles and often a single mitochondrion (Fig. 4). All corticostriatal terminals that were seen to make synapses formed asymmetrical specialisations, mainly with unlabelled spines ($n$ = 220; 85%) and some unlabelled dendrites ($n$ = 40; 15%).

### *Parvalbumin-immunoreactive neurones*

*Light microscopy*. In the light microscope, the BDHC-labelled neurones displaying parvalbumin immunoreactivity appeared blue and had a granular appearance. The parvalbumin-immunoreactive neurones were distributed throughout the entire extent of the putamen and the caudate nucleus but were more intensely stained in the rostral regions of the striatum. The extent of the labelling of these cells was variable between different regions of the striatum and between the two animals. The labelling was usually limited to the cell body and the proximal dendrites. However, in a few regions the staining was more extensive and the distal dendrites were also labelled (Fig. 1). The parvalbumin-immunoreactive neurones had a medium sized, round or oval cell body (15 $\mu$m) from which several dendrites (4–5) were seen to emerge. The dendrites branched frequently, often at 90°, did not possess spines and often had a varicose appearance.

*Electron microscopy*. In the electron microscope the BDHC reaction product appeared as granules or aggregates of extremely electron dense crystalline material that was distributed throughout the perikaryal and dendritic cytoplasm of parvalbumin-immunoreactive neurones (Figs. 2–4). There was no consistent association of the reaction product with membranes or subcellular organelles. In distal dendrites the granular reaction product occasionally filled the whole of the cytoplasm (Fig. 3A). In confirmation of the light microscope findings, the parvalbumin-immunoreactive neurones had medium-

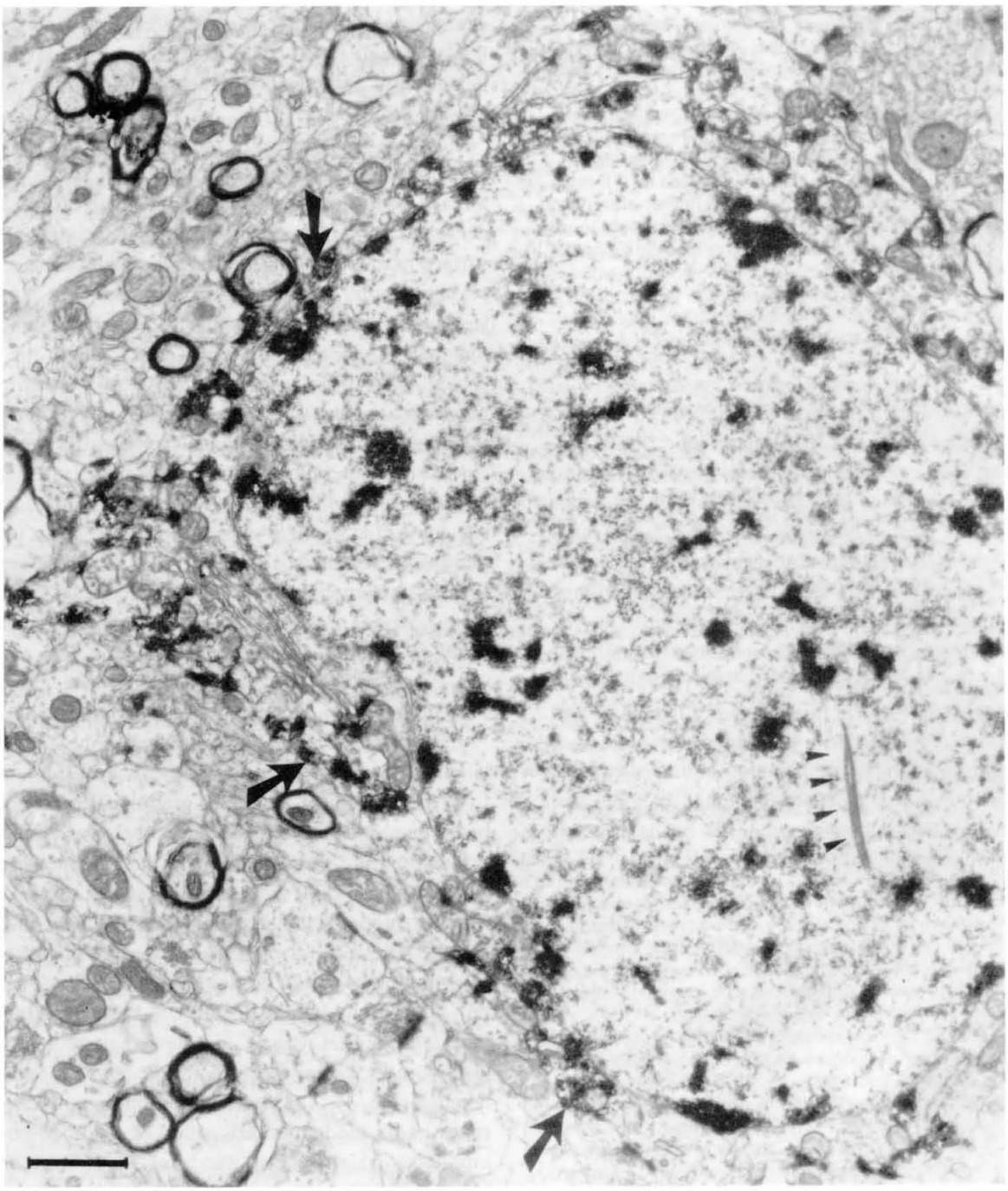

Fig. 2. Electron micrograph of the parvalbumin-immunoreactive neurone shown in Fig. 1. The cell body contains the BDHC reaction product, some of the granules of which are indicated by arrows. The nucleus contains an inclusion body (arrowheads) and the nuclear membrane in this micrograph shows an indentation. At other levels of the cell the nuclear membrane was more deeply invaginated. The cytoplasm contains numerous organelles. Scale: bar = 1.0 $\mu$m.

sized (15 $\mu$m) cell bodies and contained a greater volume of cytoplasm than that seen in neighbouring unlabelled cells. The cytoplasm was rich in various organelles including endoplasmic reticula, mitochondria and Golgi apparatus. The nuclear membrane was always indented (Fig. 2) and in some sections these indentations appeared as deep invaginations. The nucleus often contained an inclusion body (Fig. 2). The dendrites were aspiny and varicose. A few mitochondria were often seen in the varicose swellings along the dendrites. The parvalbumin-immunoreactive neurones received numerous synaptic inputs of both the symmetrical and asymmetrical varieties, especially onto the distal dendrites (Fig. 3 and Table I). The majority of inputs to the dendrites were of the asymmetrical type, whereas most of the inputs on the cell body were of the symmetrical va-

TABLE I

*Afferent synaptic input to parvalbumin-immunoreactive neurones in the putamen of squirrel monkey*

Figures represent the numbers of different categories of terminals seen in synaptic contact with the parvalbumin-immunoreactive neurones. Figures in parentheses represent percentages.

| | *Corticostriatal asymmetrical* | *Unlabelled asymmetrical* | *Unlabelled symmetrical* |
|---|---|---|---|
| Distal dendrites | 16 (57) | 54 (77) | 15 (60) |
| Proximal dendrites | 9 (32) | 14 (20) | 5 (20) |
| Perikarya | 3 (11) | 2 (3) | 5 (20) |

riety. It is worth noting that the density of synapses onto perikarya was far less than that seen on the dendrites. Distal dendrites were occasionally seen ensheathed by unlabelled terminals that formed asymmetrical synaptic contacts (Fig. 3).

*Double labelling of corticostriatal terminals and parvalbumin-immunoreactive neurones*

At the light microscopic level corticostriatal fibres and the extensively labelled parvalbumin-immunoreactive neurones were seen to overlap in the caudal lateral regions of the putamen. In this region biocytin-labelled punctate structures were often found in close apposition to parvalbumin-immunoreactive cell bodies and dendrites. In the electron microscope, sections from the lateral regions of the putamen were seen to contain structures positive for biocytin as revealed with DAB and structures immunoreactive for parvalbumin as revealed with BDHC. The different structures could be readily distinguished due to the different appearance of the two reaction products (Fig. 4). Corticostriatal terminals were often seen in close apposition to parvalbumin-immunoreactive cell bodies and dendrites as observed at the light microscopic level. Examination of these terminals in serial sections revealed that they often formed synaptic contact with parvalbumin-immunoreactive neurones (Fig. 4). All the synapses between the biocytin-labelled corticostriatal terminals and parvalbumin-immunoreactive neurones displayed asymmetrical membrane specialisations. The majority occurred on distal dendrites (Fig. 4C and D), but anterogradely labelled corticostriatal terminals were also found in contact with perikarya and proximal dendrites of parvalbumin-positive cells (Fig. 4A and B and Table I). The corticostriatal asymmetrical synapses were occasionally associated with postjunctional dense bodies (Fig. 4B).

*Control experiments*

Following omission of the primary antibody directed against parvalbumin, only DAB-labelled corticostriatal fibres and terminals were observed throughout the striatum. On the other hand, in cases where ABC was omitted only BDHC-labelled parvalbumin-immunoreactive cells were visualised.

## DISCUSSION

The main finding of the present experiment is that in the putamen of the monkey, parvalbumin-immunoreactive neurones which represent a class of GABAergic interneurones, receive direct synaptic input from neurones located in the motor and premotor cortices. The corticostriatal terminals made asymmetrical synaptic contact with all regions of parvalbumin-immunoreactive neurones, the majority of which were on distal, i.e., small diameter, dendrites. The results of the present study also demonstrate: (1) that parvalbumin-immunoreactive neurones have ultrastructural characteristics and synaptic inputs similar to those of GABAergic interneurones identified in the rat and (2) that the ultrastructural features and synaptology of the corticostriatal system in primate is similar to that in other species.

*Technical considerations*. The main objective of this experiment was to determine whether GABAergic interneurones, identified by immunocytochemistry for parvalbumin, receive direct synaptic input from the cerebral cortex in the squirrel monkey (*Saimiri sciureus*). The method chosen to address this problem was to label the corticostriatal terminals by the anterograde transport of biocytin[29,34] and to combine this with immunocytochemistry for parvalbumin. The GABAergic interneurones were labelled by parvalbumin immunocytochemistry rather than GABA or GAD immunocytochemistry for two reasons: first, because parvalbumin is selective for the GABAergic interneurones in the striatum and does not stain the GABAergic projection neurones and secondly, because the antiserum to parvalbumin gives much more extensive staining than do antibodies to GABA or GAD. Since synaptic connections can only be identified at the electron microscopic level, a double peroxidase method that is suitable for electron microscopy[40] was used to simultaneously localize the transported biocytin and the parvalbumin-immunoreactive structures. The essence of this approach is the use of diaminobenzidine (DAB) and benzidine dihydrochloride (BDHC) as chromogens for the peroxidase reactions. Each of these produce reaction products that are distinguishable in both the light and electron microscopes[6]. In the present experiments the biocytin-containing corticostriatal terminals were labelled with DAB and the parvalbumin-immunoreactive structures labelled with BDHC. The material stained in this way enabled us to identify corti-

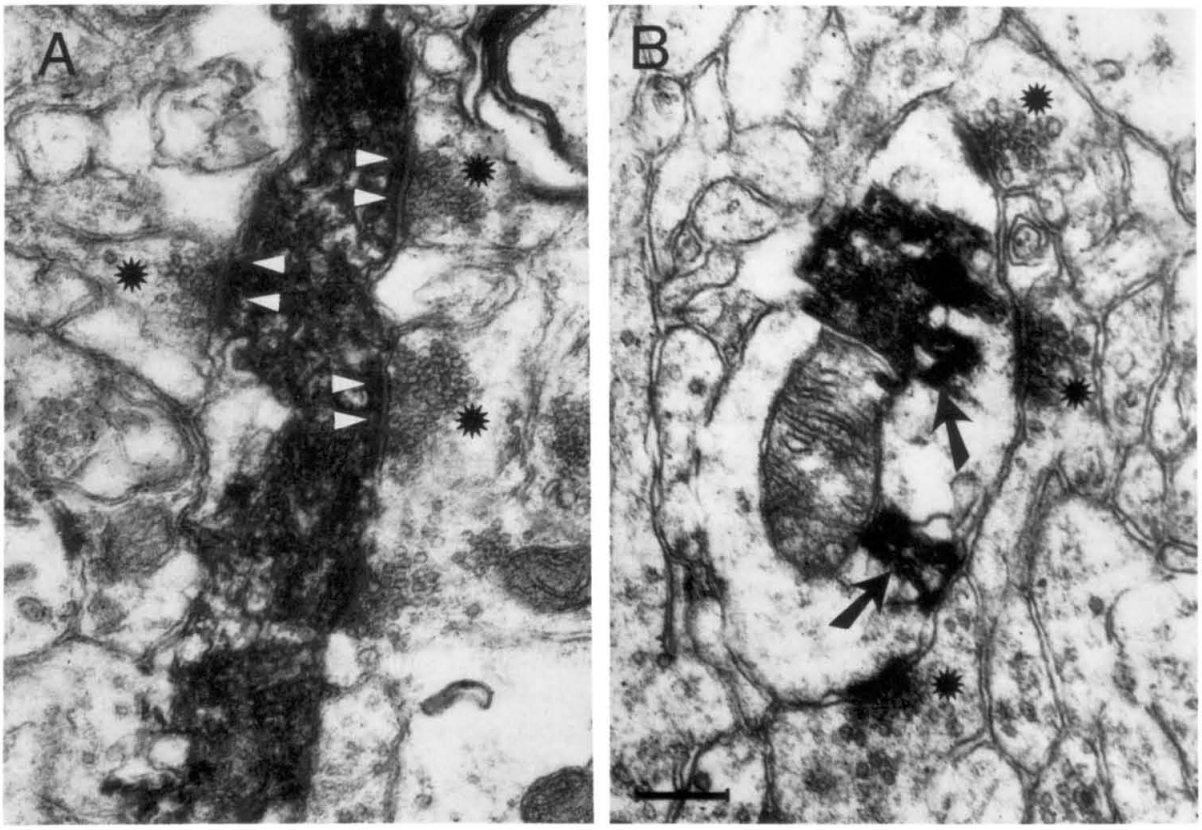


Fig. 3. Electron micrographs of parvalbumin-immunoreactive dendrites receiving synaptic inputs (arrowheads) from unlabelled terminals (stars). In these micrographs all of the synaptic specialisations are of the asymmetrical type. In (A) the BDHC reaction product appears to fill the entire dendrite whereas in (B) the reaction product is in the form of granules (arrow). Scales: both micrographs at the same magnification: bar = 0.25 $\mu$m.

costriatal terminals in contact with parvalbumin-immunoreactive neurones. The technical considerations concerning this type of double labelling study have been discussed extensively on previous occasions[6,7,40].

*Corticostriatal terminals*. The pattern of distribution of corticostriatal fibres from the motor and the premotor cortices reported in the present study is consistent with previous findings in primate[38,41,62]. This projection is mainly confined to the postcommissural putamen where it arborises in the form of bands that are interspersed with areas almost devoid of labelling. The band-like pattern displayed by corticostriatal afferents has been shown to correlate with the somatotopic representation of the different body parts in the monkey putamen[1].

The corticostriatal terminals labelled with biocytin displayed morphological features similar to those identified by anterograde degeneration and anterograde labelling in the cat[25,26,32] and rat[20,56,61]. Thus, the terminals were of variable size, containing densely packed round vesicles and occasional mitochondria. Similarly, as in other species, in all the cases where the terminals were seen forming synapses, the membrane specialisations were of the asymmetrical type. Most of the postsynaptic targets of the corticostriatal terminals were dendritic spines. This is consistent with the observations in other studies[20,25,26,32,56,61]. It has been demonstrated that the spines of medium-size densely spiny neurones are the major target of cortical input to the striatum[25,32,56]. Furthermore, the spiny neurones postsynaptic to the cortical terminals have been characterised as striatonigral neurones by the retrograde transport of horseradish peroxidase[56].

In addition to dendritic spines the corticostriatal terminals identified in the present study also made synaptic contacts with dendritic shafts. In fact, analysis of anterogradely labelled terminals identified in scans of electron microscope sections revealed that at least 15% of cortical terminals formed asymmetrical synaptic contact with dendritic shafts in the striatum of the monkey. Since spiny neurones rarely receive asymmetrical input on their dendritic shafts[54], then this observation implies that other classes of neurones also receive cortical input. Indeed, the present study demonstrates that GABA interneurones, identified on the basis of parvalbumin immunoreactivity, receive an input from the cerebral cortex. Furthermore, recent evidence suggests that the somatostatin/neuropeptide Y interneurones also receive cortical input

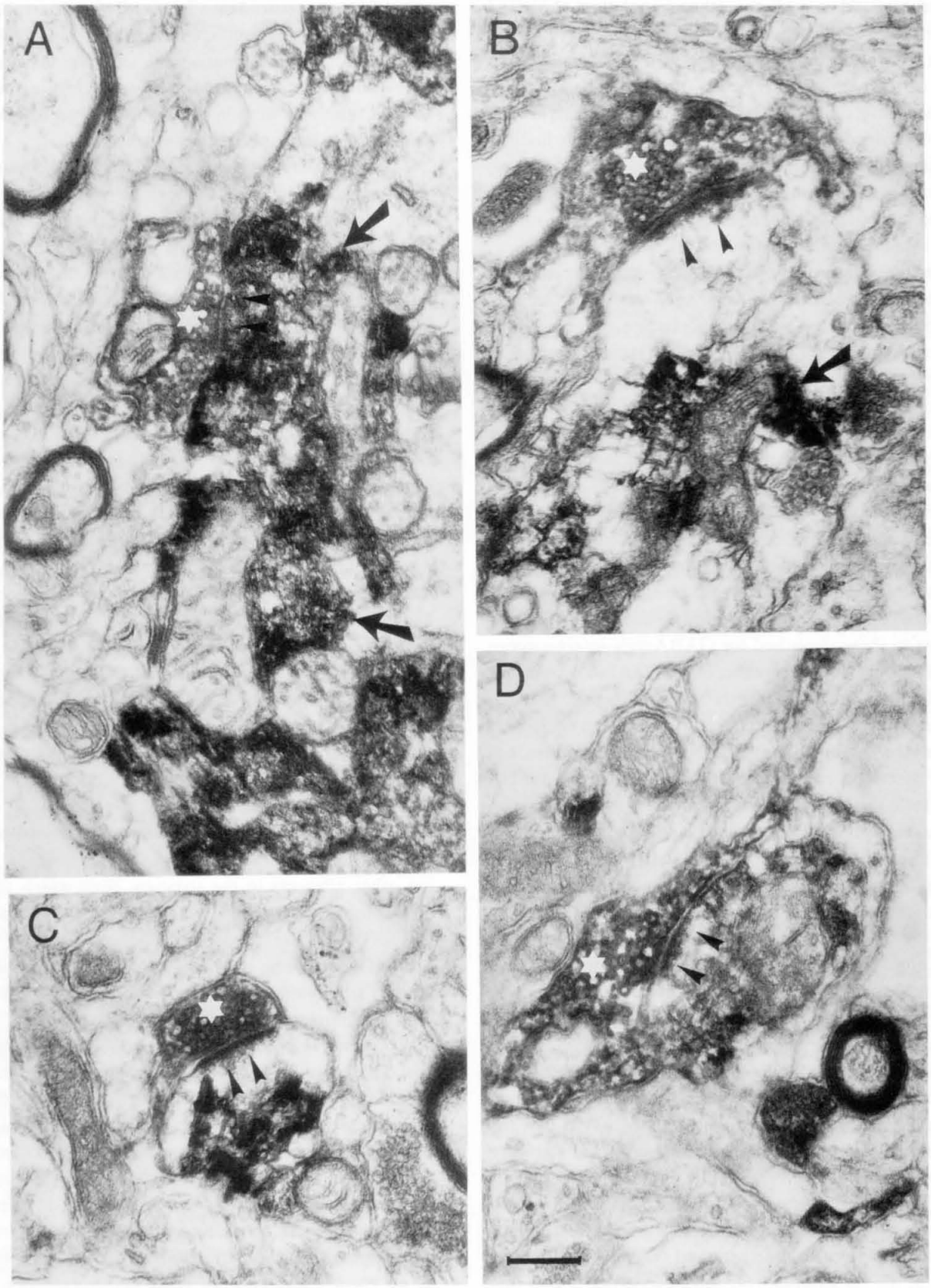


Fig. 4. Electron micrographs of corticostriatal terminals in synaptic contact with parvalbumin-immunoreactive neurones. Parvalbumin-immunoreactive cell body (A); proximal dendrite (B) and distal dendrites (C, D) contain the electron dense crystalline BDHC reaction product (arrows). Each parvalbumin-immunoreactive structure receives asymmetrical synaptic input (arrowheads) from boutons containing the DAB reaction product identifying them as corticostriatal terminals (stars). The synapse in (B) is associated with post-junctional dense bodies. Scales: all micrographs at the same magnification: bar in (D) = 0.25 $\mu$m.

in the rat[59]. However, the other population of striatal interneurones, those containing acetylcholine, do not seem to receive input from corticostriatal terminals, at least in their proximal regions[39,42].

The figure of 15% of corticostriatal terminals in contact with dendritic shafts is in agreement with findings obtained in the rat following injections of biocytin into the frontal cortex (unpublished observations), where 13% of the anterogradely labelled terminals made asymmetrical synaptic contact with dendritic shafts.

*Parvalbumin-immunoreactive neurones.* Parvalbumin-immunoreactive neurones were seen scattered throughout both the putamen and the caudate nucleus in the squirrel monkey[17]. The intensity of staining varied in different regions of the striatum. The parvalbumin-immunoreactive cells located in the dorsal and lateral regions of the putamen and the caudate nucleus were more intensely stained than neurones located more centrally. Areas that were devoid of staining were also present. This variability of staining is consistent with other studies in the monkey[17] and the rat[13,36] using the same antibodies.

The morphology of parvalbumin-immunoreactive cells is similar to that described previously in the rat[13,19,36] and monkey[17]. The morphological characteristics are also similar to those described for GABAergic interneurones labelled by the local uptake of [$^3$H]GABA[4], GAD or GABA immunocytochemistry[5,21,35,44,48], or in situ hybridization for GAD mRNA[15]. In each of these studies at least one population of GABA interneurones has been described as medium-sized neurones, the nucleus of which is indented and often contains an inclusion body. The numbers and types of afferent synaptic boutons in contact with the perikarya and dendrites of parvalbumin-positive neurones in primate striatum are similar to previous descriptions in other species[36]. In keeping with the general pattern of synaptic innervation of striatal cells[54] the proximal part of parvalbumin-containing neurones were found to be contacted by a few symmetrical synapses. However, with increasing distance from the soma the incidence of asymmetrical synapses and the density of synaptic inputs increase.

*Cortical input to parvalbumin-immunoreactive neurones.* The major finding of the present study is that the parvalbumin-immunoreactive neurones in the putamen of the monkey receive synaptic input from terminals identified as originating from the motor and premotor cortices. Since there is a 100% correspondence between parvalbumin immunoreactivity and GABA immunoreactivity in the rat striatum[19,36], this observation shows that corticostriatal neurones make contact with striatal GABAergic neurones. The labelled corticostriatal terminals formed asymmetrical synapses and were occasionally associated with postjunctional dense bodies. The cortical input occurred on all regions of the parvalbumin-immunoreactive neurones, but, consistent with the overall distribution of asymmetrical and symmetrical synapses onto striatal neurones[54], the majority of labelled cortical terminals were in contact with distal dendrites of parvalbumin-immunoreactive neurones.

Individual parvalbumin-immunoreactive neurones that were examined in serial sections were found to receive input from only a small number of labelled corticostriatal terminals. This, however, is unlikely to be a true representation of the input to a single neurone from an individual cortical field, since it is difficult to label *all* terminals from a particular region by anterograde tracing techniques, especially when the material is prepared for electron microscopy. Furthermore, differential penetration into the tissue of the two sets of reagents used to localize the biocytin and the parvalbumin may lead to false negative results, i.e., the failure to reveal parvalbumin immunoreactivity in a structure postsynaptic to a labelled corticostriatal terminal or the failure to reveal the biocytin in a terminal forming synaptic contact with a parvalbumin-immunoreactive structure. It is thus likely that, from a given cortical region, in this case the motor and premotor cortices, individual parvalbumin-containing neurones will receive a quantitatively greater input than that reported in the present study.

In addition to the labelled corticostriatal terminals, the parvalbumin-immunoreactive neurones also received input from many unlabelled terminals forming asymmetrical synaptic specializations. As mentioned above, some of these may be derived form the same region of cortex that either did not transport the biocytin or were not revealed by the histochemical reaction. However, there are other afferents to the striatum, from which the terminals form asymmetrical synapses. These include other regions of the cerebral cortex, the thalamus[16,20,39,52] and the 5-HT-containing terminals from the dorsal raphe nucleus[18,45]. Any or all of these may contact the parvalbumin-immunoreactive neurones.

*Functional implications.* Although the major targets of corticostriatal fibres are the output neurones of the striatum[56] the present study demonstrates that GABA interneurones also receive direct cortical input. Since these neurones have been suggested on the basis of Golgi-impregnated material[58] and more directly by the analysis of parvalbumin-immunostained terminals[36], to form synaptic contact with the output neurones of the striatum, they then represent a feed-forward inhibitory system. Thus, cortical activation of spiny neurones may be subjected to a feed-forward inhibition by concomitant activation of GABA interneurones. In effect, the GABA interneurones modulate the activity of the output neurones following cortical stimulation.

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## ABBREVIATIONS

| | |
|---|---|
| PB | phosphate buffer |
| PBS | phosphate-buffered saline |
| NHS | normal horse serum |
| ABC | avidin-biotin-peroxidase complex |
| PAP | peroxidase antiperoxidase |
| DAB | diaminobenzidine |
| BDHC | benzidine dihydrochloride |
| BSA | bovine serum albumin |
| GABA | gamma-aminobutyric acid |
| GAD | glutamate decarboxylase |
| 5-HT | 5-hydroxytryptamine |
| i.m. | intramuscularly |

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