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The Subcellular Localization of the G-Protein G$_i\alpha$ in the Basal Ganglia Reveals Its Potential Role in Both Signal Transduction and Vesicle Trafficking

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The G-protein G$_i$ is known to mediate signal transduction in cells by coupling its 41 kDa α-subunit to plasma membrane–bound receptors and inhibiting adenylyl cyclase or affecting ion channel function. Although this G-protein has been functionally associated with D$_2$/dopamine and μ-opioid receptors in striatal membranes, its localization to neurons of the neostriatum, a brain region rich in adenylyl cyclase activity, has not been established. Light and electron microscopic study of the basal ganglia was conducted using the immunoperoxidase method and an antiserum directed against the α-subunit of G$_i$. In the neostriatum, immunoreactivity was localized to medium-sized spiny and aspiny neurons and axon terminals that formed symmetric synapses. Some astrocytes and glial processes that encapsulated axospinous complexes were also labeled. Immunoreactive axon terminals were numerous in the globus pallidus and substantia nigra, where they exhibited a dense pattern of distribution characteristic of neostriatal spiny projection neurons. G$_i\alpha$ immunoreactivity was distributed to multiple subcellular compartments. In neostriatal somata and dendrites, labeling was present intermittently along plasma membranes, and on rough and smooth endoplasmic reticulum and microtubules. In axon terminals, reaction product appeared on plasma membranes and heavily labeled the membranes of synaptic vesicles.

The presence of G$_i\alpha$ in axon terminals was confirmed in purified synaptosome preparations. G-proteins consistent with the masses of G$_o\alpha$ and G$_i\alpha$, respectively, were ADP-ribosylated in the presence of pertussis toxin in striatal synaptosomes. Western blot analysis in purified synaptosome preparations of the neostriatum, globus pallidus, and substantia nigra with the same antiserum used in the immunohistochemistry demonstrated a predominant 41 kDa protein corresponding to the molecular mass of G$_i\alpha$. Immunohistochemical localization of G$_i\alpha$ with the immunogold method in a crude striatal synaptosome preparation showed gold particles associated with synaptic vesicles and plasma membranes.

Results provide the first direct evidence that G$_i\alpha$ is localized to medium-sized neostriatal projection neurons and interneurons, where it is likely to function in membrane-bound signal transduction at the postsynaptic and presynaptic level. The presence of G$_i\alpha$ in synaptic vesicle membranes points to another potentially important role for this G-protein in vesicle trafficking, such as that recently shown for smaller-molecular-mass G-proteins.

Binding of a ligand to its membrane-bound receptor leads to a change in the concentration of a second messenger (such as a cyclic nucleotide or Ca^{2+}), which then initiates other intracellular processes. A class of GTP-binding proteins, known as G-proteins, are heterotrimers (α-, β-, and γ-subunits), which couple receptors to their effectors (see reviews by Gilman, 1987; Neer and Clapham, 1988; Birnbaumer et al., 1990; Brown and Birnbaumer, 1990; Simon et al., 1991). Following activation of a receptor, the α-subunit of a G-protein binds to an intracellular domain of the receptor, replaces GDP for GTP, and dissociates from the $\beta\gamma$-subunits. In its activated GTP-bound state, the α-subunit of a G-protein (e.g., G$_s\alpha$) can interact with numerous other molecules in the plasma membrane (e.g., adenylyl cyclase) to produce amplification of the receptor-mediated signal and a variety of subsequent second messenger activities.

Neurons and neurally derived tissues utilize G-proteins found in non-neural organs (G$_s$ and G$_i$), but also appear to have developed specialized G-proteins that are either unique or particularly abundant in the nervous system (G$_t$, G$_o$, and G$_{olf}$). For example, in the retina, the G-protein transducin mediates rhodopsin activity in rod cells, and in the olfactory bulb adenylyl cyclase activity is mediated in part by G$_{olf}$ (Jones and Reed, 1989). The selective distribution of G-protein expression in the nervous system therefore appears to be an important component of information processing by neurons.

The G-protein G$_i\alpha$ is localized to brain, where it is heterogeneously distributed (Brann et al., 1987; Largent et al., 1988) and occurs in three isoforms, G$_i\alpha$-1, G$_i\alpha$-2, and G$_i\alpha$-3 (Itoh et al., 1986; Jones and Reed, 1987; Yatani et al., 1988; Carty et al., 1990; Senogles et al., 1990). G$_i\alpha$-1 is believed to be the most prevalent isoform in the brain (Neer and Clapham, 1988). G$_i\alpha$ transduces intracellular signals in diverse ways that include a direct influence on ion flux through K$^+$ or Ca^{2+} channels and the coupling of receptor stimulation to changes in the activity of membrane-bound enzymes (phospholipases, adenylate cyclase) (Birnbuamer et al., 1990). The best-described function of

$G_i\alpha$, and the one for which it was operationally named, is the inhibition of adenylyl cyclase activity (Gilman, 1987; Birnbaumer et al., 1990).

Receptor coupling through $G_i\alpha$ may mediate the effects of a number of neurotransmitters in the basal ganglia. For example, in the neostriatum, dopamine acts to stimulate or inhibit adenylyl cyclase activity (Kebabian et al., 1972) through D_1 and D_2 receptors, respectively (see review by Andersen et al., 1990). $G_i\alpha$ and another G-protein, $G_o\alpha$, have been functionally associated with the D_2 receptor in bovine striatal membranes (Elazar et al., 1989). Both $G_i\alpha$ and $G_o\alpha$ have also been linked to the coupling of μ-opioid receptors in guinea pig striatal membranes (Ueda et al., 1990). In addition, studies of target cells in other regions show that neurotransmitters and peptides such as ACh, norepinephrine, 5-HT, and somatostatin, which are also present in the neostriatum, activate receptor subtypes that are coupled to $G_i\alpha$ (Birnbaumer et al., 1990). Curiously, $G_i\alpha$ mRNA is notably lacking in neostriatal neurons (Brann et al., 1987), but has been found in dopaminergic cells of the substantia nigra (Vincent et al., 1990). In order to clarify the neuronal localization of $G_i\alpha$ in the basal ganglia, we used a combination of immunohistochemistry, Western blot analysis, and ADP-ribosylation of G-proteins in synaptosomal preparations to examine the neostriatum, globus pallidus, and substantia nigra. Our results demonstrate the presence of $G_i\alpha$ in neostriatal cells and their projections and raise the possibility that this G-protein has multiple functions in basal ganglia neurons.

Materials and Methods

Immunohistochemistry in brain sections. Rats ($n = 7$, Sprague–Dawley) were perfused transcardially under deep anesthesia (sodium pentobarbital, 35 mg/kg). One rat was anesthetized (sodium pentobarbital, 35 mg/kg) and injected intraventricularly with colchicine (70 μg in 1 μl) 24 hr prior to the perfusion. The fixative consisted of 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer at pH 7.4. Immunohistochemistry was performed as previously detailed from this laboratory (DiFiglia et al., 1989). Sections 40 μm thick were cut on a Vibratome and were serially incubated in 3% normal goat serum, anti-$G_i\alpha$ rabbit antiserum (1:800) for 48 hr at 4°C, and biotinylated goat anti-rabbit antiserum, followed by avidin-biotin-HRP complex (Vectastain, Vector Labs, Burlingame, CA). Detergents were not used to permeabilize membranes. Diaminobenzidine was used as the chromogen. Sections were washed in Tris-saline (TS) between each step. Tissue sections were either mounted on slides subbed with gelatin or prepared for electron microscopy as previously described (DiFiglia et al., 1989). Ultrathin sections were cut and mounted on Formvar-coated slot grids and examined in a JEOL-100CX electron microscope. Counterstaining with lead acetate was not employed to ensure optimal visualization of the peroxidase reaction product. Controls consisted of omission of the first antibody (anti-$G_i\alpha$) and preabsorption of $G_i\alpha$ with a fusion protein (generously provided by Eva Neer). Immunoreactivity was absent or greatly diminished under these conditions. Although the antiserum recognizes a 41 kDa protein in, by far, the greatest abundance (see Fig. 6), it does not detect $G_i\alpha$ exclusively; therefore, $G_i\alpha$-like immunoreactivity is implicitly understood if it is not overtly stated.

Immunohistochemistry in synaptosomes. Crude synaptosome pellets were fixed for 15 min in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.4). Pellets were rinsed with TS, freeze-thawed, and treated with 3% normal goat serum in TS for 1 hr. Pellets were rinsed in TS and incubated with gentle agitation overnight at 4°C in anti-$G_i\alpha$ antibody (1:800) containing 2% normal goat serum. After rinsing in TS, the pellets were incubated for 2 hr with a goat anti-rabbit IgG (1:10, Janssen Pharmaceuticals, Inc) to which 10 nm gold particles were attached. Pellets were rinsed in TS, postfixed in 2% glutaraldehyde followed by 1% osmium tetroxide, block stained with 1% uranyl acetate, and embedded in Epon.

Preparation and validation of purified synaptosomes. Synaptosomes were prepared by the method of Hajos (1975) and Krueger et al. (1979). Verification of the synaptosomal preparation was performed by electron

microscopic examination; the predominant neuronal element was an axon terminal without a postsynaptic membrane attached. Fewer than 5% of the elements were myelinated fibers or dendrites.

ADP-ribosylation of G-proteins by pertussis toxin in purified synaptosomes. Pertussis toxin catalyzes the transfer of an NADP-ribose group from nicotinamide adenine dinucleotide (NAD) to the α-subunit of a number of G-proteins including $G_i\alpha$. Pertussis toxin was activated by incubation in 50 mM Tris, pH 7.4, 5 mM $MgCl_2$, 50 mM dithiothreitol, for 10 min at 37°C, and then added (18 μg/ml) to the synaptosome preparation in buffer containing 25 mM HEPES, 1 mg/ml BSA, 2.5 mM $MgCl_2$, 0.3 mM EDTA, 10 mM thymidine, 1 mM ATP, 0.1 mM GTP, 5 μM ^{32}P-NAD, and 500 μM NADP. The ribosylation was carried out over 30 min at 30°C and terminated by the addition of EDTA and NAD. The tissues were centrifuged in the cold, washed in Tris buffer, heated at 95°C for 3 min, and submitted to SDS-polyacrylamide gel electrophoresis. The gel contained 8% acrylamide:bisacrylamide (29:1), and samples were run for 6 hr at 150 V constant. ^{14}C-labeled protein markers (Amersham) were included in each electrophoresis. Kodak XAR-5 film was used for the autoradiography. Omission of pertussis toxin eliminated the radiolabeling of proteins.

Western blot analysis. Purified synaptosome preparations from the neostriatum, globus pallidus, and substantia nigra, or whole cell extracts from these same regions, were applied to 8% SDS-polyacrylamide gel electrophoresis. The separated proteins were transferred to Immobilon-P using a semidry blotting method (Milliblot-SDE, Millipore, Bedford, MA). Immunoblotting was done as previously described from this laboratory (Aronin et al., 1991). ^{14}C protein molecular mass markers (Amersham) were used for size estimation. The anti-$G_i\alpha$ antiserum was generated against $G_i\alpha$-1 (generously provided by Dr. Eva Neer) and was *used at a 1:800 dilution. This antiserum is not known to distinguish* among the three isoforms of $G_i\alpha$ (Eva Neer, personal communication).

Results

Immunohistochemistry: light microscopy

Immunoreactive $G_i\alpha$ was localized to cell bodies in the neostriatum (Fig. 1*a,b*) and to axon terminals in all three basal ganglia regions (Fig. 1*a–c*, respectively). Immunoreactive somata were medium sized, and the majority of them exhibited only a light staining. Fewer but more intensely labeled cells of medium size were also seen; large striatal neurons were not immunoreactive. In the animal treated with colchicine, more of the lightly labeled medium-sized cells were revealed, and they often appeared in clusters throughout the caudate–putamen (results not shown). Reaction product in somata was confined to the cytoplasm, where it had an uneven, patchy appearance at the light microscopic level (Fig. 1*a,b*). Primary dendrites were occasionally labeled (Fig. 1*a*).

In the neostriatum, immunoreactive axon terminals were moderately distributed dorsally and increased significantly in density in the ventromedial regions, especially at the level of the anterior commissure and within the boundaries of the nucleus accumbens. Compared to the neostriatum, the globus pallidus and substantia nigra had a more dense labeling of axon terminals, which ensheathed the unlabeled cell bodies and dendrites of pallidal (Fig. 1*c*) and nigral (Fig. 1*d*) neurons.

Immunohistochemistry: electron microscopy

Neostriatum. At the ultrastructural level, the sparse, more intensely labeled medium-sized cells had an indented nucleus (Fig. 2*a*), a characteristic of the aspiny neuron (DiFiglia et al., 1980; Dimova et al., 1980); the more abundant, moderately immunoreactive neurons had an unindented nucleus (Fig. 2*b*), which identified them as medium spiny cells. Reaction product in both cell types was preferentially distributed to the rough and smooth endoplasmic reticulum, to free ribosomes and polyribosomes, as well as to cytoskeletal components in the cytoplasm. Immunoreactivity was easily detected in large-diameter (proximal)
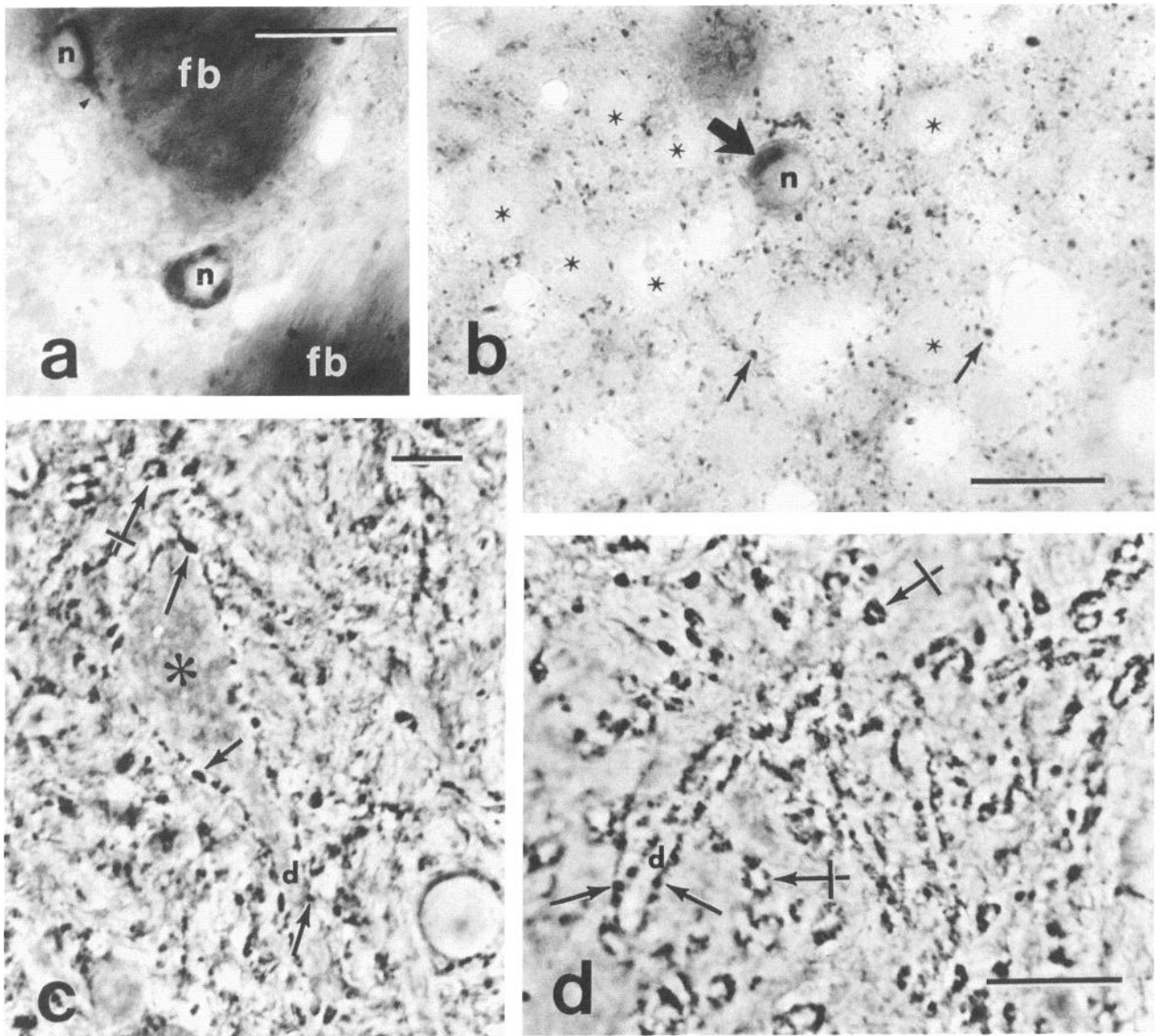
Figure 1. Immunoreactive $G_i\alpha$ in the basal ganglia. *a*, Two medium-sized immunoreactive neurons in the neostriatum exhibit reaction product in the cytoplasm and none in the nucleus (*n*). The neuron at upper left also has some immunoreactivity in a primary dendrite (*arrowhead*). This section was treated with osmium tetroxide, rendering the fiber bundles (*fb*) black. *b*, A moderate labeling of immunoreactive axon terminals (*small arrows*) is seen in the neostriatum. A labeled medium-sized neuron (*n* over the unlabeled nucleus) exhibits a patch of reaction product in the cytoplasm at the *large arrow*. Asterisks mark some of the unlabeled cells. *c*, Phase-contrast photomicrograph of immunoreactive axon terminals in the globus pallidus. Note the unlabeled pallidal neuron (*asterisk*), which is surrounded by labeled terminals (*arrows*). The primary dendrite (*d*) is also contacted by numerous terminals (*example at arrow*). The *crossed arrow* points to the cross section of an unlabeled dendrite that is surrounded by immunoreactive terminals. *d*, Phase-contrast photomicrograph of immunoreactive $G_i\alpha$ axon terminals in the substantia nigra. Note the unlabeled dendrite (*d*) that has been longitudinally sectioned and is surrounded by immunoreactive terminals (*arrows*). The rings of immunoreactivity (*crossed arrows*) represent cross-sectioned unlabeled dendrites that are surrounded by $G_i\alpha$-positive terminals. Scale bars: *a* and *b*, 30 μm; *c* and *d*, 10 μm.

dendrites (Fig. 2*c*), which had many synaptic contacts on their surface (Fig. 2*c*). This pattern of connectivity is a feature characteristic of medium aspiny neurons (DiFiglia et al., 1980). Dendrites with spines were occasionally found to be immunoreactive. Reaction product in dendrites was located intermittently along plasma membranes, and also appeared on microtubules, transport vesicles, and cisternae; it was generally absent from the inner mitochondrial membrane and cristae. $G_i\alpha$-positive axon terminals in the neostriatum varied in size (Figs. 2*a*, 3*a*) (range, 0.5–1.5 μm), and all formed symmetric synapses mostly with dendritic shafts. Dendritic spines in contact with

immunoreactive $G_i\alpha$ terminals were also usually postsynaptic to unlabeled terminals that made asymmetric synapses (Fig. 3*a*).

The somata and processes of some astroglia also contained immunoreactive $G_i\alpha$ (Fig. 3*b–d*). Similar to neurons, reaction product in astrocytes was attached to rough endoplasmic reticulum (Fig. 3*b*), ribosomes, and cytoskeletal components, and was absent from mitochondria (Fig. 3*d*). Reaction product was heavily deposited on the membranes of some small glial processes (Fig. 3*c*). $G_i\alpha$-immunoreactive glial processes invested some areas of striatal neuropil and abutted unlabeled spines (Fig. 3*c*) and axospinous complexes (Fig. 3*d*).
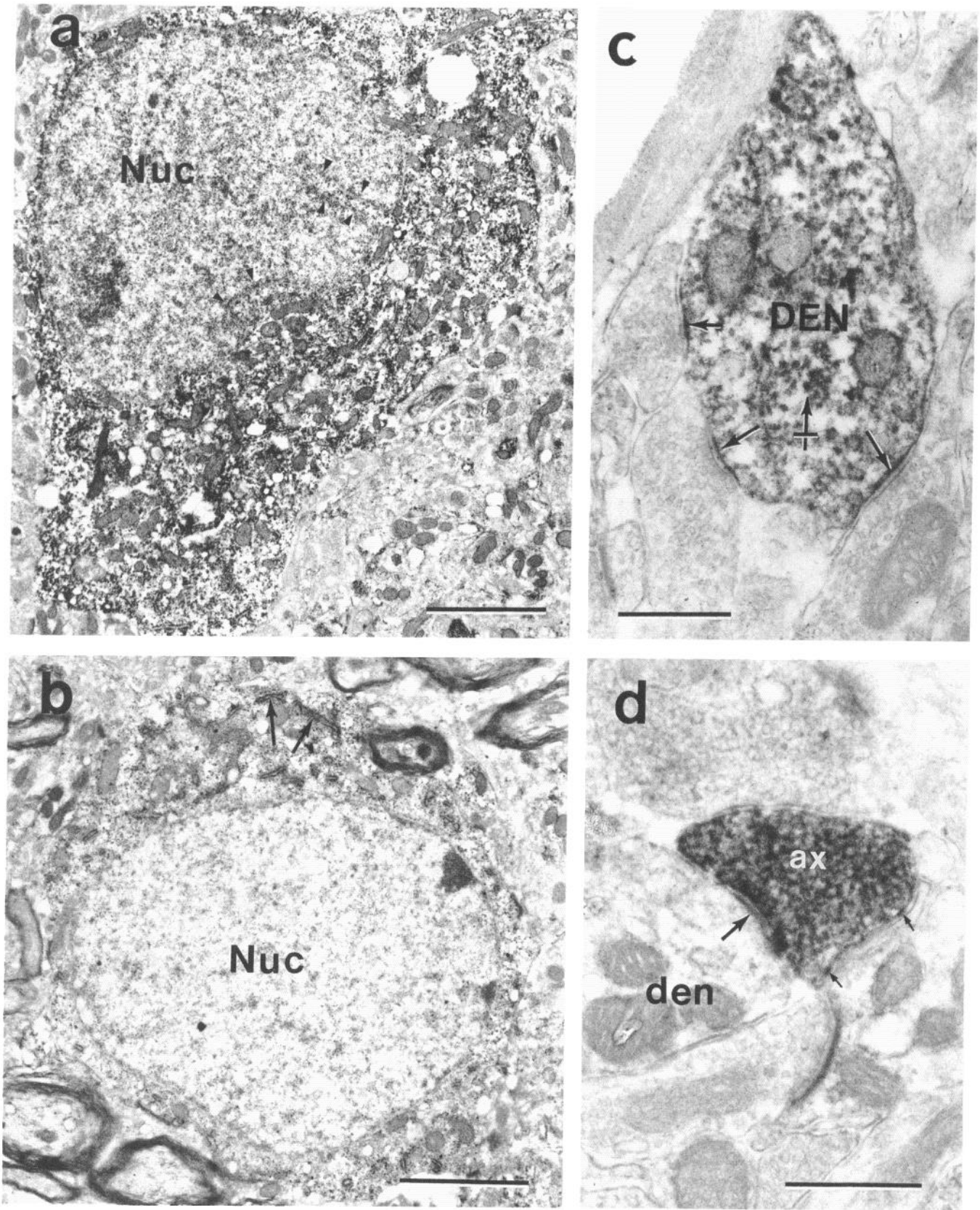
Figure 2. Ultrastructural localization of immunoreactive G$_i$α in the rat neostriatum. *a,* Immunoreactive medium-sized somata with indented (*arrowheads*) nucleus (*Nuc*), and abundant organelles, which are characteristics of the medium aspiny neuron. Reaction product appears only in the cytoplasm. *b,* A medium-sized neuron with unindented nucleus (*Nuc*) and few organelles, which are features of medium spiny cells. Compared to the aspiny neuron shown in *a,* the cytoplasm exhibits less immunoreactivity. Most of the reaction product is localized to ribosomes (not visible at this magnification) and the rough endoplasmic reticulum (*arrows*). *c,* G$_i$α-positive dendrite (*Den*) in the neostriatum is postsynaptic at *small*
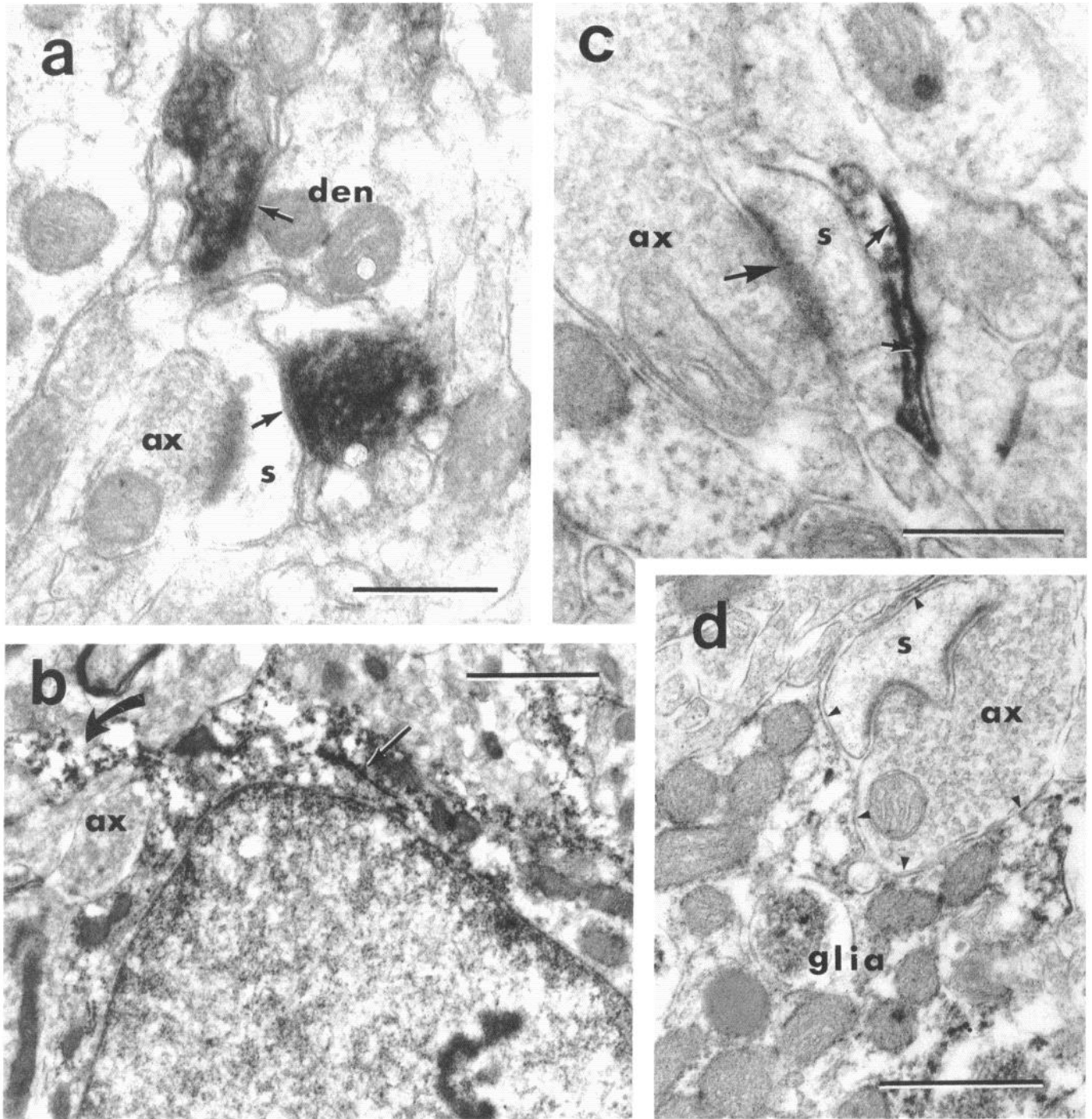
Figure 3. Ultrastructural localization of immunoreactive $G_i\alpha$ in the neostriatum. *a*, Two small $G_i\alpha$-positive terminals form symmetric synapses at *arrows* with a dendrite (*Den*) and a spine (*s*). The unlabeled terminal (*ax*) also makes an asymmetric contact with the spine. *b*, Immunoreactive astroglia with emerging process (*curved arrow*) shows some reaction product deposited on rough endoplasmic reticulum (*straight arrow*). *c*, A $G_i\alpha$-immunoreactive process, probably of glial origin, exhibits heavy deposits of reaction product on the plasma membrane (*small arrows*). The labeled process borders a dendritic spine (*s*), which receives an asymmetric contact at the *large arrow* from an unlabeled axon terminal (*ax*). *d*, Immunoreactive glia partly envelops (*arrowheads*) an axon terminal (*ax*) and dendritic spine (*s*), which form an asymmetric synapse. Scale bars: *a, c,* and *d,* 0.5 μm; *b,* 1 μm.

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arrows to three unlabeled axon terminals that form symmetric synapses. Reaction product is deposited heavily on microtubules (*crossed arrow*). *d,* Immunoreactive axon terminal (*ax*) exhibits a dense accumulation of reaction product over synaptic vesicles and forms a symmetric synapse at the *large arrow* with a dendrite (*Den*). More immunoreactivity in the terminal is seen at the presynaptic site. Patches of immunoreactivity (*small arrows*) are also visible along the plasma membrane. Scale bars: *a* and *b,* 2 μm; *c* and *d,* 0.5 μm.
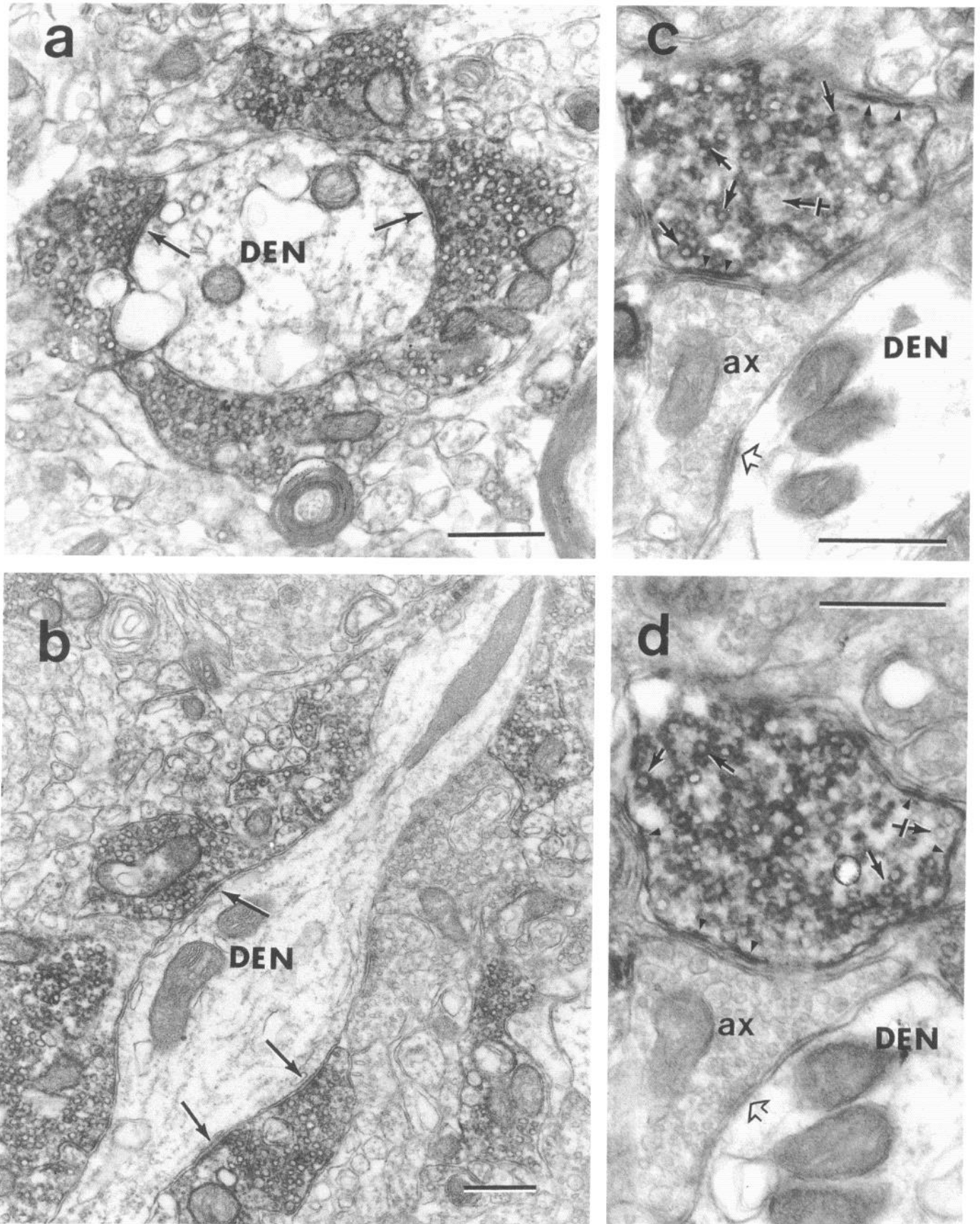
Figure 4. Ultrastructural localization of immunoreactive $G_i\alpha$ in the rat globus pallidus. *a*, $G_i\alpha$-positive axon terminals surround an unlabeled pallidal dendrite (*Den*), which is cut in cross section. Two of the terminals are making symmetric synapses (*arrows*). *b*, Longitudinal section through a pallidal dendrite (*Den*), which is ensheathed by $G_i\alpha$-positive and unlabeled axon terminals. Labeled terminals contain a loose mixture of round

Figure 5. Immunogold labeling of $G_i\alpha$ in neostriatal synaptosomes. *a* and *b* show examples from a crude synaptosome pellet that was incubated with anti-$G_i\alpha$ and then a gold-labeled second antib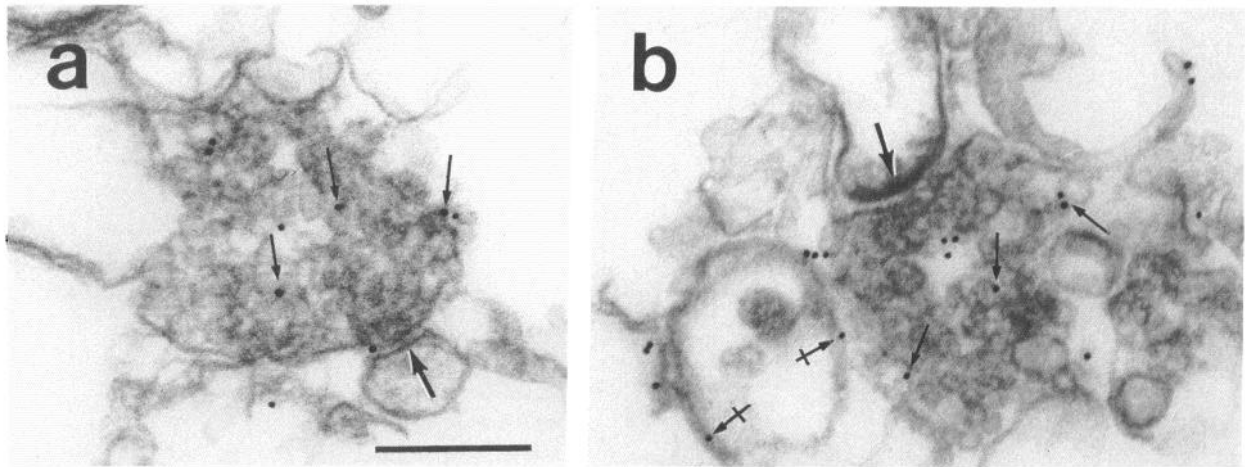ody. Ten nanometer gold particles are attached to vesicle membranes (*small arrows*) and to plasma membranes (*crossed arrows*). The postsynaptic membrane (at *large arrows*) is still attached in these examples but was not usually found. Control synaptosome pellets treated with no $G_i\alpha$ antibody followed by a gold labeled-second antibody were devoid of gold particles. Scale bar, 0.5 μm for *a* and *b*.

Globus pallidus. Immunoreactive terminals were abundant and consisted of a single population that contained round and ovoid vesicles (Fig. 4). These terminals formed symmetric synapses with cell bodies and small- and large-diameter dendrites (Fig. 4*a,b*). The high density and morphology of the $G_i\alpha$-positive terminals in the globus pallidus clearly identified them as belonging to neostriatal neurons (see review by DiFiglia and Rafols, 1988). Immunoreactive axon terminals in the substantia nigra were morphologically identical to those in the globus pallidus (data not shown), suggesting that they too originated from the neostriatum.

Axon terminals and synaptosomes. In all three regions examined, $G_i\alpha$ immunoreactivity in axon terminals was localized irregularly along the plasma membrane (Figs. 2*d*, 4*c,d*) and was very heavily disposed in synaptic vesicle membranes (Figs. 2*d*, 4*a–d*). The inner mitochondrial membranes and cristae were devoid of reaction product (Fig. 4*a*), as was the axoplasm (Fig. 4*c,d*). The heaviest accumulation of reaction product, particularly in neostriatal terminals, was seen in vesicles at presynaptic locations (Figs. 2*a*, 3*a*). In some boutons, only a subpopulation of synaptic vesicles was immunoreactive, suggesting that not all vesicle membranes contained $G_i\alpha$ (Fig. 4*c,d*). The heterogeneous labeling of synaptic vesicles was not related to the depth of penetration of the antibody, based on examination of individual boutons in serial sections (Fig. 4*c,d*). Immunogold labeling of a crude striatal synaptosome preparation showed gold particles distributed on both synaptic vesicle membranes and plasma membranes (Fig. 5*a,b*).

Biochemical characterization of $G_i\alpha$

ADP-ribosylation by pertussis toxin in purified synaptosomal extracts of the neostriatum labeled a broad band of proteins in the range of 39–41 kDa, corresponding to $G_o\alpha$ and $G_i\alpha$, respectively (Fig. 6*a*). To confirm the specificity of the antiserum, its reactivity was tested by Western blot analysis against a fusion protein containing $G_i\alpha$-1 (gift of Dr. E. Neer) (Fig. 6*b*). A major band consistent with the size of the fusion protein was identified. Western blot analysis of synaptosomal preparations made from the neostriatum, globus pallidus, and substantia nigra, using the same concentration of antiserum employed in the immunohistochemistry, revealed a predominant protein at 41 kDa (Fig. 6*c*). Proteins of other molecular weights were detected only in trace amounts. No distinct bands were observed in the range of 20–25 kDa, corresponding to small GTP-binding proteins, or at 43 kDa, corresponding to G_{olf}, or at 45 and 52 kDa, corresponding to G_s. The same pattern was revealed in whole extracts of the neostriatum, globus pallidus, and substantia nigra (data not shown).

Discussion

$G_i\alpha$ and other G-proteins coexist in neurons of the basal ganglia.

Data obtained from Northern blot analysis and *in situ* hybridization (Brann et al., 1987) have shown little or no detectable levels of $G_i\alpha$ in the caudate–putamen. Nevertheless, two pertussis toxin–sensitive G-proteins with molecular masses corresponding to $G_i\alpha$ and $G_o\alpha$ have been functionally associated with the D_2 receptor in bovine striatal membranes (Elazar et al., 1989) and with the μ-opioid receptor in guinea pig striatal membranes (Ueda et al., 1990). Medium-sized striatal spiny and aspiny neurons are known to be postsynaptic to dopaminergic inputs from the substantia nigra (Pickel et al., 1981; Kubota et al., 1987; Vuillet et al., 1989) and to the axon collaterals of intrinsic enkephalin-containing cells (DiFiglia et al., 1982). The demonstration by immunohistochemistry in this study, that

and pleomorphic vesicles and form symmetric synapses (*arrows*). *c* and *d*, Serial sections through a vesicle-filled bouton that contains immunoreactive (*arrows*) and unlabeled (*crossed arrows*) synaptic vesicles. Although the section in *c* is more superficial than in *d*, there is no difference in the proportion of vesicles labeled, suggesting that antibody penetration does not account for the heterogeneity in vesicle labeling. Reaction product is also localized at sites intermittently along the plasma membrane (*arrowheads*). An unlabeled axon terminal (*ax*) forms a symmetric synapse (*open arrow*) with the unlabeled dendrite (*Den*). Scale bars, 0.5 μm.
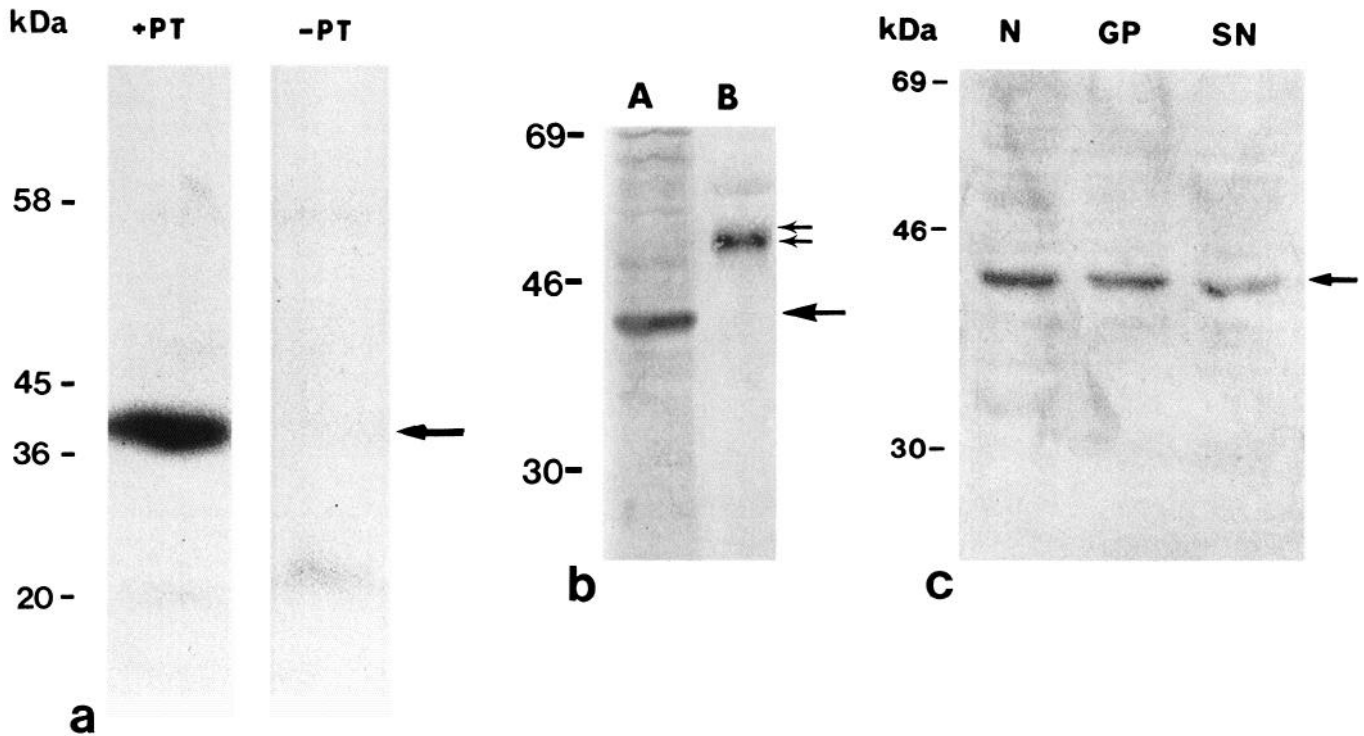
Figure 6. Pertussis toxin–induced ADP-ribosylation of GTPase and Western blot analysis of G$_i\alpha$ in synaptosomes from the basal ganglia. *a,* Synaptosomes from the neostriatum were incubated with pertussis toxin (*+PT*) or without pertussis toxin (*−PT*) in the presence of ^{32}P-NAD. ADP-ribosylated proteins were cholate extracted and electrophoresed by 8% SDS-PAGE (see Materials and Methods for details). The *arrow* shows a broad band of ADP-ribosylated proteins in the size range of 39–41 kDa on the autoradiogram, corresponding to the molecular masses of G$_o\alpha$ and G$_i\alpha$. *b,* Western blot analysis of whole neostriatal extract (*lane A*) and G$_i\alpha$-1 fusion protein (*lane B*) after 8% SDS-PAGE. The same antiserum used for immunohistochemistry was applied to the immunoblot. The *double arrow* designates the fusion protein, and the *single arrow,* the major protein band at 41 kDa, corresponding to the molecular mass of G$_i\alpha$. *c,* Western blot analysis of synaptosomal proteins prepared from the neostriatum (*N*), globus pallidus (*GP*), and substantia nigra (*SN*). The proteins were separated by 8% SDS-PAGE; the same concentration of antiserum was used as in the immunohistochemistry. The *arrow* identifies the major 41 kDa band corresponding to the molecular mass of G$_i\alpha$.

G$_i\alpha$ exists in medium-sized neurons, provides additional support that this G-protein is involved in coupling postsynaptically D$_2$ and μ-opioid receptor–induced activity in the striatum. G$_i\alpha$-immunoreactive axons were abundant in the globus pallidus and substantia nigra, which are the major targets of medium-sized spiny projection neurons. The characteristics of G$_i\alpha$-immunoreactive axons in the globus pallidus and substantia nigra, including their high density, ensheathment of dendrites, and ultrastructural morphology, are the same as those axons known to originate from the striatum (DiFiglia and Rafols, 1988).

In addition to G$_i\alpha$, other G-proteins are present in the neostriatum. For example, immunoreactive G$_z\alpha$ has been found in numerous neurons of the neostriatum (Hinton et al., 1990), where the abundance of immunoreactive cells is likely to include spiny neurons. Also, with ADP-ribosylation and Western blot analysis, G$_s$ and G$_{olf}$ have been identified in neostriatal synaptosomes, and are reduced by intrastriatal excitotoxic lesions that destroy medium-sized spiny neurons (N. Aronin and K. Chase, unpublished observations). Finally, G$_o$ immunoreactivity has been reported in the neostriatum and in the substantia nigra, where it is markedly diminished after lesions of the caudate–putamen (Worley et al., 1986), suggesting that striatonigral projection cells contain G$_o$. Thus, it appears that the principal input and projection neuron of the neostriatum, the medium spiny cell, contains an assortment of G-proteins including G$_s$/G$_{olf}$, G$_i$, G$_o$, and G$_z$, which are positioned to transduce receptor-initiated signals and possibly other functions (see below). The combi-

nation of G-proteins and the neurotransmitters they serve within individual striatal neurons remains largely unknown at this time.

Multiple sources may contribute to G$_i\alpha$-immunoreactive axons in the neostriatum. Probably a significant proportion of the G$_i\alpha$-immunoreactive terminals observed in the neostriatum belong to the intrinsic axons of medium-sized spiny and aspiny cells. The "symmetric" synaptic contact formed by G$_i\alpha$-immunoreactive boutons is also made by the axons of spiny and aspiny neurons (see review by Gerfen, 1988). However, it should be noted that "symmetric"-type synapses are also characteristic of the axon terminals of dopamine-containing afferents from the substantia nigra (Pickel et al., 1981), and the large aspiny "cholinergic" cells in the neostriatum (Phelps et al., 1985; DiFiglia, 1987). In fact, G$_i\alpha$ mRNA has been found in neurons of the substantia nigra pars compacta (Vincent et al., 1990), suggesting that at least some of the G$_i\alpha$-positive terminals in the caudate–putamen could arise from the midbrain. We cannot exclude the possibility that the cholinergic neuron also contains G$_i\alpha$ in its axon endings; it may simply express levels of the protein in its cell body that are too low to be detected by immunohistochemistry.

G$_i\alpha$ in astrocytes. The presence of immunoreactive G$_i\alpha$ in some astrocytes indicates that the signal transduction properties of this G-protein may not be limited to neurons of the striatum. Although the receptors to which G$_i\alpha$ may be functionally linked in astroglia are unknown, it is noteworthy that one class of

G-protein–coupled receptor, the β-adrenergic receptor, has been identified in cultured astroglia from the cortex and cerebellum (Trimmer et al., 1984; Voisin et al., 1987) and has been coupled to $G_s\alpha$ in a variety of non-neural tissues (Birnbaumer et al., 1990).

Different subcellular sites of immunoreactive $G_i\alpha$ localization suggest multiple functional roles. Consistent with their role in receptor-activated signal transduction, biochemical studies have shown that G-proteins, including $G_i\alpha$ in the striatum (Elazar et al., 1989; Ueda et al., 1990), are predominantly associated with plasma membranes (Gilman, 1987; Elazar et al., 1989; Ngsee et al., 1990; Ueda et al., 1990). The presence of immunoreactive $G_i\alpha$ in the membranes of striatal axon terminals as well as dendrites points to the potential importance of this G-protein in presynaptic signal transduction in the striatum and its target areas, the globus pallidus and substantia nigra. A noteworthy feature of $G_i\alpha$ distribution in the plasma membranes of both dendrites and axon terminals was its desultory pattern. A similar discontinuity has been noted in the localization of $G_i\alpha$ in the inner plasma membrane of murine Purkinje cells (Hinton et al., 1990).

The location of immunoreactive $G_i\alpha$ in plasma membranes was not necessarily correlated with synaptic contacts. Such a finding may reflect the capacity for transmembrane receptor-mediated signal transduction at nonsynaptic sites. Interestingly, although there are few studies on the immunohistochemical localization of receptors in the plasma membranes of neurons, the G-protein–linked β-adrenergic receptor has been localized to nonsynaptic locations along plasma membranes (Aoki et al., 1989).

$G_i\alpha$ immunoreactivity in the striatum also exhibited a cytoplasmic distribution, particularly in the endoplasmic reticulum and in microtubules. A similar cytoplasmic distribution of $G_s\alpha$ immunoreactivity was recently noted in cerebellar Purkinje cells (Hinton et al., 1990). Although their intracellular functions are unclear, there is evidence that under appropriate conditions the α-subunits of G-proteins can separate from the $\beta\gamma$-subunits at the plasma membrane and enter a cytoplasmic compartment (Sternweis, 1986; Haraguchi and Rodbell, 1990). Results of several studies also suggest that various G-proteins may be associated with the cytoskeleton. In the vagus nerve, GTPase activity regulates the anterograde movement of muscarinic cholinergic receptors by fast axonal transport (Zarbin et al., 1982). In synaptic membranes of cerebral cortex (Wang et al., 1989), $G_s\alpha$ and $G_i\alpha$-1 bind to tubulin. Also, G-proteins appear to have a role in actin polymerization in human neutrophils (Bengtsson et al., 1990). Whether the G-proteins associated with tubulin and actin are functioning at the plasma membrane in conjunction with signal transduction or play a separate role in regulating cytoskeletal function remains to be clarified.

Morphological substrate for $G_i\alpha$ in vesicle trafficking. The localization of immunoreactive $G_i\alpha$ by immunoperoxidase labeling was appreciably more abundant in axon terminals than in somata and dendrites. Western blot analysis of protein extracts, produced from synaptosomes of the three basal ganglia regions examined, also showed an abundance of protein corresponding to the molecular weight (41 kDa) of $G_i\alpha$. Immunogold labeling of synaptosomes confirmed that $G_i\alpha$ immunoreactivity was associated with the membranes of synaptic vesicles. Although the presence of $G_i\alpha$ in synaptic vesicle membranes was unexpected, this finding is consistent with the results of a number of recent studies implicating members of the G-protein family in the

formation, transport, and exocytosis of secretory vesicles (Bourne, 1988; Tooze et al., 1990). In a variety of cell types, a class of smaller-molecular-weight G-proteins (from 20 to 25 kDa), not recognized by our antibody, have been associated with vesicle membranes, including synaptic vesicles (Mizoguchi et al., 1990; Fischer von Mollard et al., 1991; Trimble et al., 1991) and shown to function in part in directing vesicles to release sites (Bourne, 1988; Balch, 1989; Fischer von Mollard et al., 1990, 1991; Mizoguchi et al., 1990).

There is some evidence for the association of G-proteins of larger molecular mass with secretory vesicles (Toutant et al., 1987; Rotrosen et al., 1988). For example, in chromaffin cells of the bovine adrenal medulla, several G-proteins including a 41 kDa species comparable to $G_i\alpha$ were detected in both plasma and granule membranes (Toutant et al., 1987). In the electric organ of the marine ray *Discopyge ommata*, two classes of G-proteins, differentiated by size, were discovered to be associated with cholinergic synaptic vesicles (Ngsee et al., 1990). One class was in the 37–41 kDa size range; the other was smaller, with several proteins between 20 and 29 kDa. Conceivably, these findings combined with our own observations raise the possibility that $G_i\alpha$ may serve a critical role(s) at synaptic endings directing the transport and fusion of synaptic vesicles to active zones and possibly other functions, such as neurotransmitter release and vesicle recycling.

Conclusions. The findings in this study demonstrated that $G_i\alpha$, a G-protein known to be involved in receptor-induced signal transduction, is prevalent in neostriatal neurons and in axon terminals within the neostriatum, globus pallidus, and substantia nigra. There is still relatively little known about the extent to which $G_i\alpha$ is coupled to different receptors in the basal ganglia, how its action is coordinated with that of other G-proteins, such as G_s and G_{olf}, and which ionic channels and second messengers it may regulate. The surprising abundance of $G_i\alpha$ in axon terminals of the basal ganglia may signify a highly important role for this G-protein in presynaptic signal transduction and in synaptic vesicle trafficking.

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