THE JOURNAL OF COMPARATIVE NEUROLOGY 416:158–172 (2000)

Synaptic Localization of $GABA_A$ Receptor Subunits in the Striatum of the Rat

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ABSTRACT

The inhibitory amino acid γ-aminobutyric acid (GABA) is widely distributed in the basal ganglia. It plays a critical role in the functioning of the striatum as it is the transmitter of projection neurons and sub-populations of interneurons, as well as afferents from the globus pallidus. Some of the factors controlling GABA transmission are the type(s) of GABA receptor expressed at the site of transmission, their subunit composition, and their location in relation to GABA release sites. To address these issues, we examined the sub-cellular localization of subunits of the $GABA_A$ receptor in the striatum of the rat. Sections of freeze-substituted, Lowicryl-embedded striatum were immunolabelled by the post-embedding immunogold technique with antibodies specific for subunits of the $GABA_A$ receptor. Immunolabelling for α1, β2/3, and γ2 $GABA_A$ receptor subunits was primarily located at symmetrical synapses on perikarya, dendrites, and spines. Quantitative analysis of the distribution of immunolabelling for the β2/3 subunits revealed that the majority of membrane associated immunogold particles were at synapses and that, on average for the whole population, they were evenly distributed across the synapse. Double labelling for the β2/3 subunits and for GABA itself revealed that receptor-positive synapses were formed by at least two populations of terminals. One population (59.3%) of terminals forming receptor-positive synapses was positive for GABA, whereas the other (40.7%) had low or undetectable levels of GABA. Furthermore, the post-synaptic neurons were characterised on neurochemical and morphological grounds as both medium spiny neurons and GABA interneurons. Triple immunolabelling revealed the co-localization of α1, β2/3, and γ2 subunits at some symmetrical axodendritic synapse. It is concluded that fast $GABA_A$-mediated transmission occurs primarily at symmetrical synapses within the striatum, that the populations of boutons giving rise to receptor-positive synapses are heterogeneous, and that previously reported co-existence of different subunits of the $GABA_A$ receptor at the cellular level also occurs at the level of individual synapses. J. Comp. Neurol. 416:158–172, 2000.

Indexing terms: basal ganglia; synapses; immunolabeling; GABA

The inhibitory amino acid γ-aminobutyric acid (GABA) plays a critical role in the neuronal networks of the basal ganglia. Most of the major classes of neurons in the basal ganglia utilize GABA as a neurotransmitter. Within the striatum, medium size densely spiny neurons, which are the major projection neurons of the striatum and give rise to extensive local axon collaterals, are GABAergic (Smith and Bolam, 1990). The striatum also contains populations of GABAergic interneurons (Bolam et al., 1983, 1985) that express different calcium binding proteins (Cowan et al., 1990; Kita et al., 1990; Kubota et al., 1993; Clarke and Bolam, 1997) or synthesize nitric oxide (Kubota et al., 1993). Furthermore, the striatum receives GABAergic afferents from the globus pallidus (Bevan et al., 1998; Smith et al., 1998) and possibly the substantia nigra (van der Kooy et al., 1981).

The effects of GABA are mediated by three sub-populations of GABA receptors, the ionotropic $GABA_A$ and

Grant sponsor: Medical Research Council; Grant sponsor: Japanese Ministry of Education; Grant sponsor: Saga Medical School.

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Received 11 May 1999; Revised 7 September 1999; Accepted 7 September 1999

$GABA_C$ receptors and the metabotropic $GABA_B$ receptors. Most of the effects in the striatum are mediated through $GABA_A$ receptors, which cause an increase in Cl^- conductance that underlies early inhibitory postsynaptic potentials. Molecular studies have identified that the $GABA_A$ receptor, in keeping with other ionotropic receptors, consists of a combination of receptor subunits in a pentameric structure (Backus et al., 1993; Smith and Olsen, 1995; Stephenson, 1995; McKernan and Whiting, 1996). At least 15 subunits have been identified and, on the basis of both in situ hybridization and immunocytochemistry, the most frequently occurring combination of subunits in the rat brain is α1, β2/3, and γ2 (Fritschy and Mohler, 1995; McKernan and Whiting, 1996). Recent reports suggest that different cell types in the same brain area may contain distinct complements of subunits (Persohn et al., 1992; Wisden et al., 1992; Gao et al., 1993; Fritschy and Mohler, 1995; Gao et al., 1995) that give rise to different pharmacological profiles (McKernan et al., 1991; Smith and Olsen, 1995; Stephenson, 1995) and may mediate different forms of inhibition (Brickley et al., 1996; Wall and Usowicz, 1997). Furthermore, differential localization of GABA receptor subunits in relation to synaptic specializations has been proposed to underlie phasic and tonic inhibition in the cerebellum (Nusser et al., 1998).

In situ hybridization studies have identified a variety of $GABA_A$ receptor subunits in the striatum (Zhang et al., 1991; Araki et al., 1992; Persohn et al., 1992; Wisden et al., 1992; Fritschy and Mohler, 1995). Immunocytochemical studies using antibodies that recognize different subunits of the $GABA_A$ receptor have identified α, β, and γ subunits that are differentially distributed within the striatum. For instance, several small sub-populations of morphologically distinct striatal neurons are strongly immunoreactive for α1 subunits, whereas antibodies against α2 or β2/3 subunits label larger populations of neurons that are widely distributed in the striatum (Fritschy and Mohler, 1995; Hartig et al., 1995; Caruncho et al., 1996, 1997; Waldvogel et al., 1997, 1998; Riedel et al., 1998). The α1 subunit-positive neurons also express β2/3 and γ2 subunits and glutamate decarboxylase and are thus GABAergic. Two of the populations have light and electron microscopic features of interneurons, whereas the third is similar in morphology and neurochemistry to a type of projection neuron (Waldvogel et al., 1997, 1998; Riedel et al., 1998) that probably represents ectopic pallidal neurons (Bolam et al., 1981, 1985; Penny et al., 1988; Bennett and Bolam, 1994a). Electron microscopic analysis of the α1 and β2/3 subunit-immunolabelled tissue in rat and baboon has revealed the presence of receptor-positive synapses of the symmetrical type associated with the immunoreactive neurons (Waldvogel et al., 1997, 1998). In addition, receptor immunolabelling was also detected at asymmetrical synapses, ie, the type usually associated with excitatory transmission, and it was also detected at non-synaptic sites (Waldvogel et al., 1997, 1998). These analyses were carried out using immunoperoxidase techniques; to define antigenic sites precisely at the subcellular or subsynaptic level, it is necessary to compliment this approach with immunolabelling techniques in which the antigenic sites are identified by non-diffusible markers (Baude et al., 1993, 1994; Nusser et al., 1994; Ottersen and Landsend, 1997).

In view of the widespread distribution of GABA and its receptors in the striatum and its critical role in the function in the striatum, and the basal ganglia in general, it is important to characterise the position and composition of GABA receptors in relation to the synaptic circuitry of the striatum. In this study we examine the localization of subunits of the $GABA_A$ receptor in the striatum of the rat. The primary objectives were threefold: first, to determine the sub-cellular localization of subunits of the $GABA_A$ receptor, in particular to determine their spatial relationship to synaptic specializations; second, to attempt to characterize the axon terminals presynaptic to the $GABA_A$ receptor-positive synapses by GABA immunolabelling; and third, to determine whether different subunits of the $GABA_A$ receptor that have been shown to be co-expressed at the cellular level are co-expressed at individual synapses. These issues were addressed by using the post-embedding immunogold technique on freeze-substituted tissue.

MATERIALS AND METHODS

Preparation of tissue

The tissue was obtained from four female Wistar rats (Charles River, Margate, Kent; 200–250 g) maintained on a 12 hour light/12 hour dark cycle with free access to food and water. Environmental conditions for housing of the rats, and all procedures that were performed on them, were in accordance with the Animals (Scientific Procedures) Act 1986 and also the European Communities Council Directive (80/609/EEC).

The animals were anesthetized with pentobarbitone (Sagatal, 200 mg/kg; Rhône Mérieux, Tallaght, Dublin, Ireland) and then perfused through the heart with 100 ml phosphate-buffered saline (PBS; 0.01 M phosphate, pH 7.4) or saline (0.9% NaCl) over 1–2 minutes and then with 300 ml of 0.025–0.5% glutaraldehyde and 3% paraformaldehyde (with or without 0.2% picric acid), made up in phosphate buffer (PB; 0.1 M, pH 7.4), over a period of 20 minutes. Following fixation, the brain was removed from the cranium, divided into 5-mm-thick coronal slices, and stored in PBS at 4°C prior to further processing. From each 5-mm slice, coronal sections (500 µm) through the striatum were taken by using a vibrating microtome. The sections were collected and washed several times in PBS.

Freeze-substitution and Lowicryl embedding

A similar procedure was used as described earlier (Baude et al., 1993). Small blocks of the striatum were trimmed from the 500-µm sections. After washing in PB, they were placed in 0.5 M sucrose in PB for 15 minutes followed by 1 M sucrose in PB for 2 hours for cryoprotection. They were slammed on a polished copper block cooled with liquid nitrogen (Reichert MM80E). The slammed blocks of tissue were transferred to a Leica CS Auto at −90°C where freeze-substitution and embedding in Lowicryl HM20 (Agar Scientific, Stansted, UK) was carried out as described before (Clarke and Bolam, 1998). Once the resin was polymerized and the blocks elevated to room temperature, they were removed for trimming and sectioning. Ultrathin sections (70 nm) were cut on a Reichert-Jung Ultracut-E ultramicrotome and collected on gold or nickel single-slot grids coated with pioloform (Agar Scientific) or on adhesive-coated (Coat-quick "G" medium; Daido Sangyo, Japan) gold or nickel mesh grids.

Post-embedding immunogold labelling

The sections were then immunolabelled by the post-embedding immunogold method essentially as described

TABLE 1. Details of Antibody Preparations

Antibody directed against	Species of origin	Dilution or concentration	Source and/or characterization
GABA	Rabbit	1:5,000	Somogyi et al. (1985); Somogyi and Hodgson (1985) Hodgson et al. (1985)
$GABA_A$ α1 subunit	Rabbit	11 μg/ml	Figure 1
$GABA_A$ β2/3 subunits, bd-17	Mouse (monoclonal)	10 μg/ml	Häring et al. (1985) Chemicon International Inc.
$GABA_A$ γ2 subunit	Guinea pig	10 μg/ml	Benke et al. (1991)
Rabbit IgG conjugated to 20-nm gold particles	Goat	1:60	British BioCell Int.
Rabbit IgG conjugated to 15-nm gold particles	Goat	1:80	British BioCell Int.
Mouse IgG conjugated to 10-nm gold particles	Goat	1:50	British BioCell Int.
Guinea pig IgG conjugated to 5-nm gold particles	Goat	1:40	British BioCell Int.

previously (Nusser et al., 1998). Briefly, the sections were treated with a saturated solution of sodium ethanolate for 3 seconds and washed in deionized water and then in 50 mM Tris-HCl, pH 7.6, containing 0.9% NaCl (TBS). They were then incubated for 30 minutes in 2% human serum albumin (HSA) in TBS containing 0.01% Triton X-100 (TBST) followed by an overnight incubation in the primary antibody solutions (diluted in TBST containing 2% HSA) directed against subunits of the $GABA_A$ receptor, or against GABA itself, or incubated in mixtures of antibodies (see below and Table 1). They were then washed in TBS and incubated in the appropriate secondary antibodies conjugated to colloidal gold (5–20 nm diameter; all obtained from British BioCell, Cardiff, UK) in 2% HSA in TBST supplemented with 5 mg/ml polyethylene glycol for about 2 hours. They were washed in TBS, incubated in 2% glutaraldehyde in TBS for 2 minutes, contrasted in 1% uranyl acetate and lead citrate, and then examined in a Philips CM 10 transmission electron microscope.

In double-labelling and triple-labelling experiments, mixtures of primary antibodies against GABA and the β2/3 subunits of the $GABA_A$ receptor or antibodies against different subunits of the $GABA_A$ receptor were applied to the sections. In these experiments the species of origin of the primary antibodies were different. The following double-labelling and triple-labelling combinations were performed:

1. Rabbit antibodies against GABA (1:5,000; Table 1) and mouse monoclonal antibodies against the β2/3 subunits of the $GABA_A$ receptor (10 μg/ml; Chemicon, Harrow, UK; Table 1). After washes they were incubated in a mixture of goat anti-rabbit IgG coupled to 15- (1:60) or 20-nm (1:80) gold particles and goat anti-mouse IgG coupled to 10-nm gold particles (1:50).

2. Rabbit antibodies against the α1 subunit (11 μg/ml; Table 1), mouse monoclonal antibodies against the β2/3 subunits of the $GABA_A$ receptor (10 μg/ml; Table 1), and guinea pig antibodies against the γ2 subunit (10 μg/ml; Table 1) overnight. The secondary antibodies were goat anti-rabbit IgG coupled to 20-nm gold particles (1:60), goat anti-mouse IgG coupled to 10-nm gold particles (1:50), and goat anti-guinea pig IgG coupled to 5-nm gold particles (1:40).

Antibody preparations

Three antibodies were used in this study:

1. A monoclonal antibody that recognizes extracellular domains on both the β2 and β3 subunits of the $GABA_A$ receptor. This antibody has been extensively characterized (Härtig et al., 1985; Schoch et al., 1985; Ewart et al., 1990) and used extensively in immunocytochemical studies (see for instance Fritschy and Mohler, 1995; Somogyi et al., 1996; Waldvogel et al., 1998).
2. A polyclonal antibody raised in guinea pigs against a synthetic peptide corresponding to the N-terminal 1–29 amino acids of the rat γ2 subunit that was conjugated to keyhole limpet hemocyanin. Detailed characterization of the antibody has been described elsewhere (Benke et al., 1996; Somogyi et al., 1996).
3. A rabbit antibody raised against the N-terminal 1–14 amino acids of the rat α1 subunit. Affinity-purified anti-α1 1–14 Cys peptide antibodies were prepared following the method of Stephenson and Duggan (1991). The peptide QPSQDELKDNTTVFC, which corresponds to the rat $GABA_A$ receptor α1 1–14 subunit sequence with a C-terminal cysteine was coupled to the carrier protein, thyroglobulin, via the *m*-maleimidobenzoic acid *N*-hydroxysuccinimide ester method. Rabbits were injected with the peptide-carrier conjugate emulsified with Freund's complete adjuvant at two sites intramuscularly (0.1 μmol peptide/site). Subsequent immunizations were in Freund's incomplete adjuvant again at two sites intramuscularly. Animals were ear bled at 7 days following the second and subsequent immunizations. The anti-α1 1–14 Cys peptide antibodies were affinity-purified by α1 1–14 Cys peptide affinity chromatography where the peptide was coupled to Activated Thiol Sepharose 4B via the terminal cysteine. Affinity-purified antibodies were stored at 4°C in the presence of 0.02% NaN_3 until use. The specificity of the affinity-purified anti-α1 1–14 Cys antibodies were assessed by immunoblots (Fig. 1).

Analysis of material

The immunolabeled sections were examined in a Philips CM 10 transmission electron microscope. Immunoreactive sites were identified by the presence of the colloidal gold particles that were attached to the secondary antibodies. The distribution of immunogold particles coding for the β2/3 subunits was determined by systematic examination of adjacent photomicrographs at a final magnification of 33,000 (366 gold particles; 138 μm^2) or by systematic (non-overlapping) scans of sections on mesh grids (2,760 gold particles; approximate area: 8,980 μm^2). The location of each gold particle was noted. To determine the association between GABAergic terminals and β2/3 subunits adjacent photomicrographs (area: 2,723 μm^2) were examined or systematic, non-overlapping, scans of sections on mesh grids (approximate area: 7,480 μm^2) were made. A synapse was considered positive by the presence of two or more immunogold particles along the synaptic membranes. Each receptor-positive synapse and the level of immunogold labelling for GABA in the presynaptic bouton was assessed. A bouton was considered to be GABA negative or to have low levels of GABA if the number of gold particles was two or less.

Quantitative analysis of the distribution of immunogold particles for the β2/3 subunits along the synaptic membrane specialization of striatal synapses was performed on electron micrographs. A total of 155 β2/3 subunit-positive synapses (746 gold particles) from two animals were analyzed. All synapses in series of adjacent photomicro-

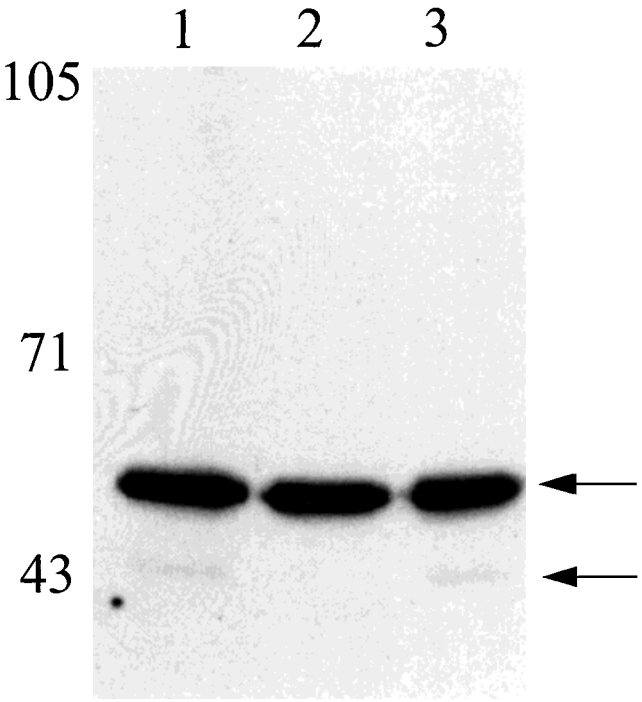

Fig. 1. Immunoblot demonstrating the specificity of the affinity-purified anti-α1 1–14 Cys antibodies. Immunoblotting was carried out as previously described using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions in 10% polyacrylamide slab gels and the ECL method for detection (e.g. Pollard et al., 1995) using either membranes (20 µg protein) prepared from adult rat cerebellum (lane 1) and cerebral cortex (lane 2) or GABAA receptors purified from adult rat cerebral cortex by benzodiazepine affinity chromatography (lane 3) as antigens and affinity-purifed anti-α1 1-14 Cys antibodies at a final concentration of 1 µg/ml. The positions of pre-stained protein standards (kDa x 10^{-3}) are shown on the left. The antibodies recognised a major band with M_r 53 000 daltons, the α1 subunit; immunoreactivity was also associated with a M_r 46 000 molecular weight species. This is a known proteolytic fragment of the α1 subunit termed α1′.

graphs of well-preserved strips of ultrathin sections were analysed. In addition, micrographs containing synapses that had been selected for other parts of the current study were also analysed. Since the data from the random and non-random analyses were essentially similar, they were pooled. The distance of each immunoparticle from the nearest edge of the synapse was measured and normalised to take into account different widths of synapses. The data were expressed as the proportion of immunoparticles in five bins along the half width of the synapse (see Bernard et al., 1997; Clarke and Bolam, 1998).

Quantification of the proportion of receptor-positive synapses that express immunoreactivity for α1, β2/3 and γ2 subunits was performed by photographing every immunopositive synapse in well-preserved strips of an ultrathin section. Micrographs were printed at a final magnification of 33,000. The gold particles for α1, β2/3, and γ2 subunits were counted on 50 synapses. The proportions of receptor-positive synapses expressing the different subunits were calculated.

Controls

In these experiments primary antibodies raised in three species (mouse, guinea pig, and rabbit) and goat secondary antibodies against immunoglobulins of the three species were used. Since double and triple labelling was performed, it is necessary to control for the specificity of the secondary antibodies. Sections were incubated with a mixture of two primary antibodies from different species, at the appropriate dilutions, and then with secondary, gold-conjugated antibody directed against immunoglobulins of the third species. In the same immunolabelling run, positive controls for each antibody were included. In each case the inappropriate gold-conjugated secondary antibody failed to produce specific labelling, although the positive controls revealed specific labelling of symmetrical synapses and membranes in the case of the antibodies against receptor subunits, or intracellular labelling of cellular elements in the case of the antibodies against GABA.

RESULTS

Distribution of immunogold labelling for GABA$_A$ receptor subunits

Consistent with previous in situ hybridization and pre-embedding immunocytochemical studies (see opening paragraphs), post-embedding immunolabelling of sections of the striatum revealed the presence of sites that were immunoreactive for the α1, β2/3, and γ2 subunits of the GABA$_A$ receptor. They were identified by the presence of accumulations of immunogold particles. The antibody preparations directed against the β2/3 subunits gave the most robust labelling; the analyses of the distribution of immunoparticles and the relationship of receptor-positive terminals to GABA-positive synapses were therefore carried out with antibodies against these subunits.

Immunogold particles coding for the β2/3 subunits were widely distributed in the sections of the striatum (Figs. 2–4). They were closely associated with membranes (Figs. 2–4) and were also localized at intracellular sites. The intracellular gold particles were associated with a variety of organelles including saccules of endoplasmic reticulum, mitochondria, and the nuclear envelope. Although the intracellular labelling accounted for 54.4% of the gold particles, they most commonly occurred as single gold particles (Figs. 2D,F, 3A, 4C) and only rarely were associations of two or more particles observed (Fig. 3A,E). This contrasts with the labelling observed at symmetrical synapses (see below) and probably includes both specific labelling associated with the synthetic and transport machinery of the receptor as well as non-specific labelling. Forty-four percent of gold particles were associated with membranes, i.e., either touching the membrane or within 1 diameter of it. Of the membrane-associated particles, 55.5% were localized at symmetrical synapses where gold particles lined up along the synaptic specialization (Figs. 2–4). Immunopositive asymmetrical synapses were not observed. A large number of the gold particles at synapses were located on the external surface of the plasma membrane (see insets in Figs. 2, 3D,E, 4A–D). The antibody used recognized the N-terminal sequence of the β2 and β3 subunits; thus extracellular labeling is consistent with the predicted topography of the receptor subunits. However, it is not possible to localize an antigen unequivocally to the internal or external surface of the membrane, nor to the pre- or post-synaptic structure using post-embedding techniques because of the distortion between the image of the membrane specialization formed from the whole thickness

of the section and the most superficial layer of the section available for the antibody. This is exemplified by the tangentially cut synapses where there is an accumulation of immunogold particles but no apparent synaptic membranes (Fig. 2D). The labelling associated with membranes at extrasynaptic sites consisted mainly of isolated gold particles (Fig. 3A), although clusters of two or more particles were sometimes seen (Fig. 2C).

Synaptology of synapses positive for the β2/3 subunits

The post-synaptic structures at synapses positive for the β2/3 subunits of the $GABA_A$ receptor included dendrites (Figs. 2B–E, 3A,B,D,E, 4A,C,D,E, 5), spines (Fig. 2D,F), and perikarya (Figs. 2A, 3C, 4B). In keeping with the known data concerning symmetrical synapses in the striatum (Ingham et al., 1998), the majority of receptor-positive synapses were axodendritic (Figs. 2B–E, 3A,B,D,E, 4A,C,D,E, 5). Synapses with both small (presumably distal) and large diameter dendrites, as well as proximal dendrites emerging from perikarya, were positive for the $GABA_A$ receptor subunits. At least two types of dendrites were identified: first, dendrites with the characteristics of medium size densely spiny neurons (Fig. 3E), i.e., they gave rise to dendritic spines and possessed morphological features of the dendrites of spiny neurons (Somogyi and Smith, 1979; Wilson and Groves, 1980); and second, in the double-labeled material, dendrites were identified that displayed immunolabelling for GABA (Fig. 3A,B,D; see below), which is indicative of the GABA interneurons of the striatum (Bolam et al., 1983; Kawaguchi et al., 1995; Kawaguchi, 1997).

Dendritic spines were the second most frequently observed post-synaptic structure at synapses positive for the β2/3 subunits (Fig. 2D,F). The synapses generally occurred on the neck of the spine (when visible), and the spine was often post-synaptic to another terminal that formed an asymmetric synapse (Fig. 2D,E).

Synapses positive for the β2/3 subunits of the $GABA_A$ receptor were observed on neuronal perikarya that had the ultrastructural characteristics of medium size densely spiny neurons, i.e., large non-indented nucleus and a relatively small volume of cytoplasm that was poor in organelles (Somogyi and Smith, 1979; Dimova et al., 1980; Wilson and Groves, 1980) (Figs. 2A, 4B). Multiple receptor-positive synapses were often detected on spiny neuron perikarya (Fig. 2A). Receptor-positive synapses were also formed on the perikarya of neurons characterised as GABA interneurons on the basis of both morphology and neurochemistry (Fig. 3C; see below).

GABA labelling of presynaptic boutons at synapses positive for the β2/3 subunits

To gain insight into the nature of the terminals that form synapses positive for the β2/3 subunits, sections of the striatum were double immunostained to reveal both GABA and the β2/3 subunits. The immunolabelling for GABA (20-nm gold particles) was similar to that described previously for the striatum and other regions of the basal ganglia (Clarke and Bolam, 1997; Smith et al., 1998). The GABA immunolabelling was widely distributed in the striatum with marked accumulations of immunogold particles over a subset of axons, axon terminals, dendrites, and perikarya (Figs. 2–4). Systematic scans of double-labelled sections revealed that most (59.3%) of the boutons forming synapses that were positive for the β2/3 subunits were also positive for GABA (Figs. 2, 3, 4A,C–E). The boutons were of variable size and made symmetrical synaptic contacts with dendrites, spines, and perikarya. They contained from 0 to 4 mitochondria and sometimes formed synapses with more than one structure in the same plane (Figs. 2C,E). In addition to the GABA-positive boutons, 40.7% of receptor-positive synapses were formed by boutons that possessed low or undetectable levels of GABA (Fig. 4). These synaptic boutons were identified in sections in which structures that were strongly labelled for GABA were identified in the close vicinity. Postsynaptic targets included spines (not shown), dendrites (Fig. 4A,C–E), and perikarya (Fig. 4B). A similar analysis of the boutons in the entopeduncular nucleus, the substantia nigra pars reticulata, and pars compacta revealed that only 9.3%, 4.1%, and 3.8% of boutons forming receptor-positive synapses, respectively, possessed low or undetectable levels of GABA (unpublished observations).

The double-labelled sections also enabled the chemical characterization of the post-synaptic structures involved in receptor-positive synapses. Thus, some of the dendrites and perikarya were themselves immunopositive for GABA (Fig. 3). The GABA-positive perikarya (Fig. 3C) possessed indentations in the nuclear membrane and a relatively large volume of cytoplasm (compared with spiny neuron perikarya) that was rich in organelles. These morphological features and the presence of GABA immunolabeling are characteristics of the GABA interneuron of the striatum that has been characterized on the basis of the uptake

Fig. 2. Localization of β2/3 subunits of the $GABA_A$ receptor at synapses formed by GABA-immunolabelled boutons. In each micrograph GABA-positive boutons, identified by the accumulation of 20 nm immunogold particles, form symmetrical synapses (large arrows) that are positive for the β2/3 subunits, identified by the 10 nm immunogold particles. The insets show the synapses at higher magnification. **A:** A GABA-positive axon gives rise to two boutons (b1 and b2) both of which form symmetrical synapses (large arrows) that are β2/3-positive (10 nm gold particles). The postsynaptic structure is a perikaryon (p) that has morphological features of a spiny neuron. The inset shows the synapse formed by bouton b2 at a higher magnification. **B:** GABA-positive bouton (b) that forms a β2/3 subunit-positive symmetrical synapse (large arrow) with a dendrite (d). **C:** A GABA-positive bouton (b) that forms receptor-positive symmetrical synapses (large arrows) with two dendrites (d). The upper of the two synapses (shown at higher magnification in the inset) has several immunogold particles associated with it whereas the lower synapse has only two immunogold particles (small arrow). The small arrowhead indicates immunogold particles close to the membrane but not at a synaptic specialization. **D:** Two GABA-positive boutons, b1 and b2, form β2/3 subunit-positive symmetrical synapses (large arrows) with a dendrite (d) and a spine (s) respectively. The synaptic specialization between b2 and the spine is not clear as it is cut tangentially. Both the dendrite and the spine are also post-synaptic to GABA-negative boutons forming receptor-negative asymmetrical synapses (arrowheads). **E:** A GABA-positive bouton (b) that forms receptor-positive symmetrical synapses (large arrows) with two dendrites (d). The inset shows the lower of the two synapses at higher magnification (rotated through 90° in a counter-clockwise direction). **F:** A GABA-positive bouton forms a receptor-positive symmetrical synapse with a spine (s) that also is postsynaptic to an immunonegative bouton possibly forming an asymmetrical immunonegative synapse (arrowhead). The bouton is also apposed to a perikaryon (p), one immunogold particle coding for the β2/3 subunits of the $GABA_A$ receptor is associated with the apposition (small arrow). Scale bars = 0.5 μm in A (also applies to D, E & F) for the main micrographs and represents 0.25 μm for the insets; 0.5 μm in B (also applies to C) for the main micrographs and represents 0.28 μm for the insets.

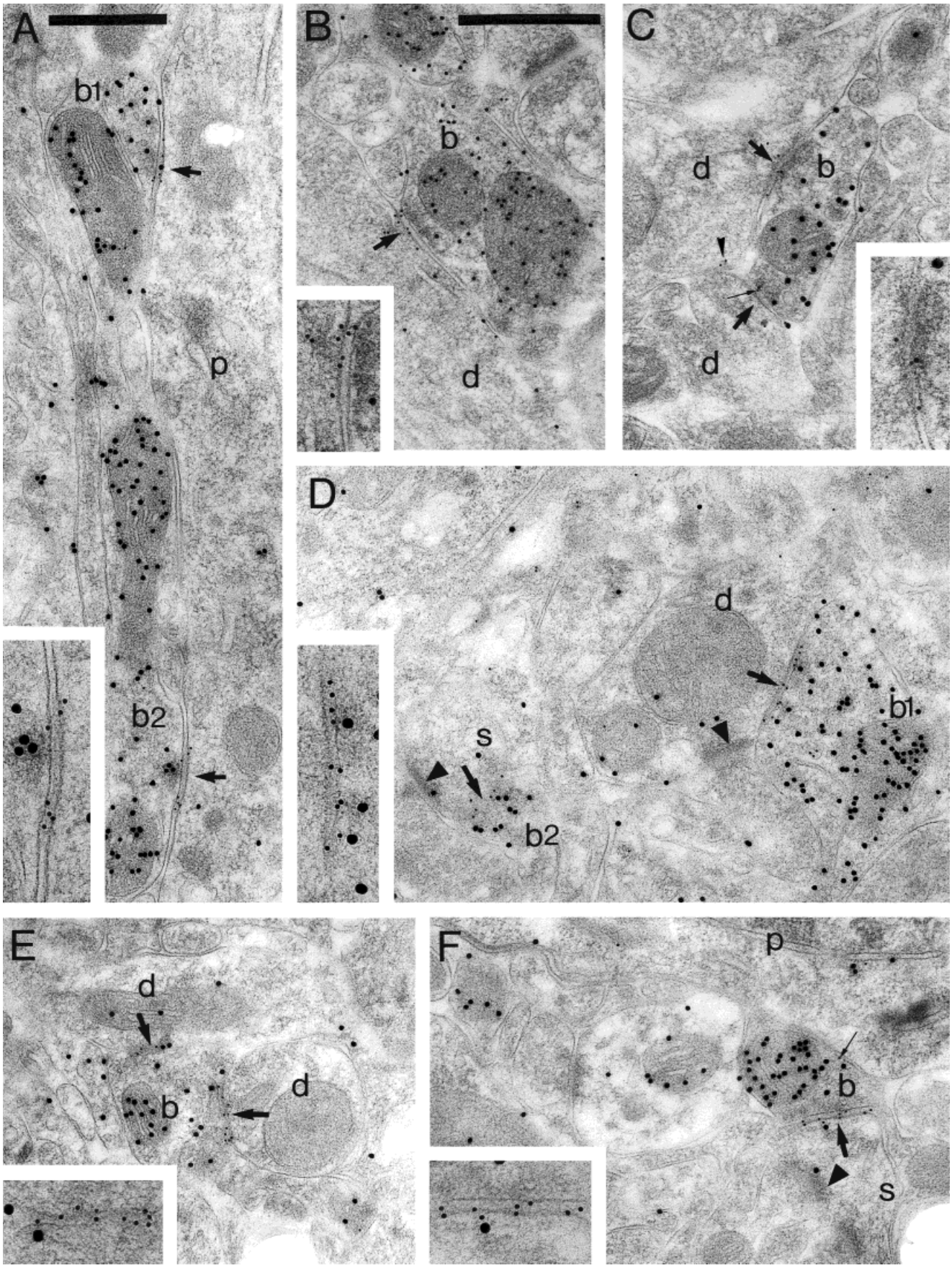

Figure 2

of exogenous GABA, GAD immunocytochemistry, and parvalbumin labelling (Bolam et al., 1983, 1985; Kita and Kitai, 1988; Cowan et al., 1990; Kita et al., 1990).

Localization of α1 and γ2 subunits

Sections immunolabelled to reveal α1 and γ2 subunits of the $GABA_A$ receptor revealed labelling for both subunits, although the labelling was not as robust as that obtained with the antibodies against the β2/3 subunits. Immunogold particles were observed both on membranes and at intracellular sites, but the most prominent labelling, in the form of groups of immunogold particles, occurred at symmetrical synapses (Fig. 5). Labelling for each of the subunits was observed at symmetrical synapses involving spines, perikarya, and, most frequently, dendritic shafts (Fig. 5).

Triple-labelling experiments for the α1, β2/3, and γ2 subunits with three different sizes of gold particles revealed the co-localization of the $GABA_A$ receptor subunits at individual symmetrical synapses (Fig. 5). In a systematic analysis of receptor-positive synapses, 42%, 92%, and 26% were positive for α1, β2/3, and γ2 subunits, respectively. Colocalization for all three subunits occurred at 12% of the synapses, colocalization of α1 and β2/3 subunits at 22%, and colocalization of β2/3 and γ2 subunits at 14%. Synapses were detected that exhibited labelling for only α1 (8%) or only β2/3 (44%) subunits. In this analysis, synapses with only single gold particles coding for a particular subunit were considered positive.

Distribution of immunolabelling for β2/3 subunits across synapses

The quantitative analysis in the striatum revealed that the immunoparticles labelling β2/3 subunits were, on average, evenly distributed across the width of the synaptic specialization, although there was a tendency for reduced levels toward the edge (Fig. 6). Very few particles fell apparently outside the synaptic specialization at perisynaptic sites. The exact location of immunoparticles at the edge of the synapse, however, is difficult to judge because of steric distortion between the image of the membrane specialization formed from the whole thickness of the section and the most superficial layer of the section available for the antibody and the difficulty of identifying the edge of symmetrical synapses in freeze-substituted tissue where the preservation is not optimal.

DISCUSSION

The results of the present study provide a detailed analysis of the distribution of subunits of the $GABA_A$ receptor in relation to synaptic specializations in the striatum of the rat. They demonstrate first that subunits of the $GABA_A$ receptor are widely distributed in the striatum, that most of the immunolabelling is associated with the plasma membrane, and that almost half of this is associated with symmetrical synaptic specializations. The labelling for the β2/3 subunits, when considered as an average of the whole population, is evenly distributed across the synaptic specialization. Second, the receptor-positive synapses are heterogeneous with respect to both the pre- and post-synaptic structures. The post-synaptic neurons included medium spiny neurons, identified on the basis of morphological characteristics, and GABA interneurons, identified on the basis of both morphological and neurochemical characteristics (Bolam and Bennett, 1995; Kawaguchi et al., 1995). About 60% of the pre-synaptic boutons forming β2/3 subunit-positive synapses are GABA-positive; the remainder are formed by boutons that have low or undetectable levels of GABA. Finally, the present results demonstrate the colocalization of α1, β2/3, and γ2 subunits of the $GABA_A$ receptor at individual symmetrical synapses. These findings suggest therefore that fast GABA transmission mediated by $GABA_A$ receptors containing α1, β2/3, and γ2 subunits occurs primarily at synapses within the striatum, that the boutons giving rise to receptor-positive synapses are neurochemically heterogeneous, and that previously reported co-existence of different subunits of the $GABA_A$ receptor at the cellular level also occurs at the level of individual synapses.

Subcellular distribution of $GABA_A$ receptor subunit immunolabelling

The present findings demonstrate that there is a selective association of immunogold particles coding for subunits of the $GABA_A$ receptor with symmetrical synapses in the striatum. Although data is not available for the striatum, it has been estimated that in the hippocampus only 1–2% of neuronal membrane is occupied by asymmetric synapses and that the area of membrane occupied by symmetrical synapses is negligible (Rusakov et al., 1998). In the striatum about one-fifth of synapses are of the symmetrical type (Ingham et al., 1998). If the overall proportion of membrane occupied by synapses in the striatum is similar to that in the hippocampus, then 0.2–0.4% of neuronal membrane is occupied by symmetrical synapses. The finding of about 25% of total gold particles associated with symmetrical synapses represents

Fig. 3. Localization of β2/3 subunits of the $GABA_A$ receptor at synapses formed by GABA-positive boutons (b) and GABA-positive structures. In each micrograph GABA-positive boutons, identified by the accumulation of 20 nm (A, B, D & E) or 15 nm (C) gold particles, form receptor-positive (10 nm gold particles), symmetrical synapses (large arrows) with GABA-positive structures. The insets show the synapses at higher magnification and allow comparison of the different size gold particles. **A:** A GABA-positive bouton (b) that forms a receptor-positive synapse (large arrow) with a GABA-positive dendrite (d). The dendrite also receives input from two immunonegative boutons forming asymmetrical, receptor-negative synapses (arrowheads). The small arrowheads indicate immunogold particles coding for the β2/3 subunits at non-synaptic sites. The left hand arrowhead indicates a single gold particle associated with the membrane, the right hand arrowhead indicates a cluster of gold particle but it is difficult to identify the underlying structures. **B, D, E:** Boutons (b) that display high levels of GABA immunolabelling form receptor-positive synapses (large arrows) with dendrites (d) that display GABA-immunolabelling, albeit at a lower level. The synapses and the immunolabelling for the β2/3 subunits are shown at higher magnification in the insets in D and E. Note in B, the immunonegative boutons forming receptor-immunonegative, asymmetrical synapses (arrowheads) with spines. Note also the two spines (asterisks) emerging from the postsynaptic dendrites in E suggesting that this is a dendrite of a spiny projection neuron. The small arrowhead in E indicates labelling at a non-synaptic site. **C:** A GABA-positive bouton (b) forms a receptor-positive synapse (large arrow) with a perikaryon that is also positive for GABA and possesses characteristics of a GABA interneuron (not shown). The GABA was revealed with 15 nm gold particles and the β2/3 subunits with 10 nm gold particles. Each of the 10 nm gold particles coding for the β2/3 subunits is indicated by a small arrow. Scale bars = 0.5 μm in A (also applies to B); 0.5 μm in C (also applies to E) for the main micrographs and represents 0.24 μm for the inset in E; 0.5 μm in D for the main micrographs and 0.24 μm for the inset.

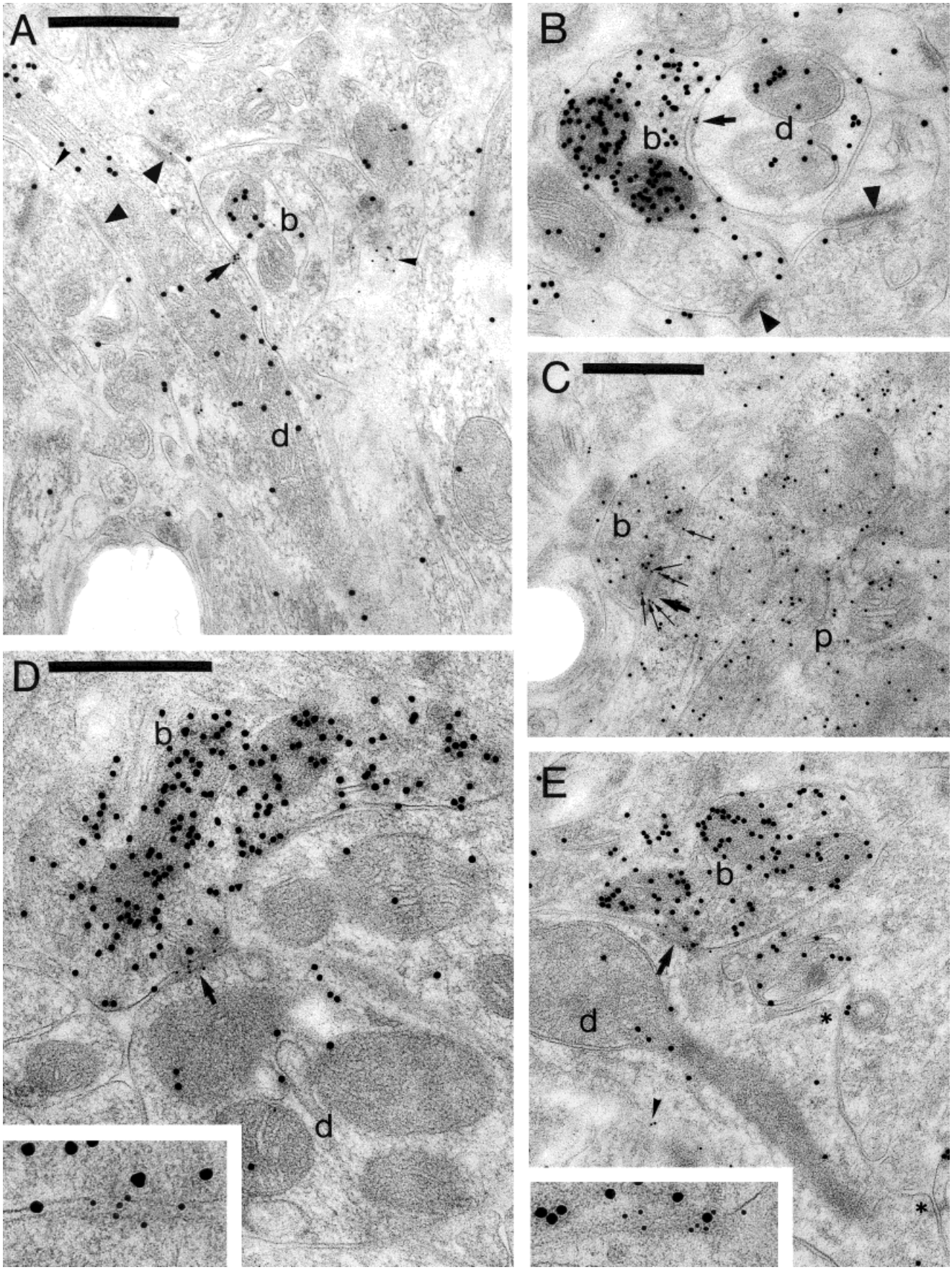

Figure 3

a 125–250-fold enrichment at synapses. When considering the number of gold particles at synapses as a proportion of membrane-associated particles, then our results reveal a 220–440-fold enrichment at synapses. These findings are consistent with those observed in other regions of the brain including the globus pallidus, cerebellum, and hippocampus, where at least some of the subunits of the $GABA_A$ receptor are preferentially localized at synapses (Nusser et al., 1995a,b, 1996a,b, 1997, 1998; Somogyi et al., 1996; Nusser and Somogyi, 1997; but see below). Furthermore, it seems to be a general principle that subunits of fast ionotropic receptors are preferentially localized at synapses, as ionotropic glutamate receptors (both AMPA and NMDA) in the basal ganglia (Bernard et al., 1997; Bernard and Bolam, 1998; Clarke and Bolam, 1998) as well as in other regions of the brain (Baude et al., 1994, 1995; Nusser et al., 1994; Kharazia et al., 1996; Kharazia and Weinberg, 1997; Ottersen and Landsend, 1997; Popratiloff et al., 1998) are selectively associated with synapses whereas at least some metabotropic receptors are preferentially located at perisynaptic sites (Baude et al., 1993; Lujan et al., 1996; Ottersen and Landsend, 1997). The nature of the immunolabelling outside of synapses or at intracellular sites remains to be established. The membrane-associated receptors may represent true "extrasynaptic receptors" that will only be exposed to GABA that has diffused from the release site in the synapse; alternatively, they may be non-functional or receptors that are in the process of being transported to the synapse. Similarly, intracellular label may represent receptors undergoing synthesis, transport, degradation, or recycling.

The detection of immunolabelling at symmetrical synapses is consistent with previous findings in the rat and baboon (Waldvogel et al., 1997, 1998). However, in these studies the postsynaptic density of some asymmetrical synapses were reported to be immunopositive for α1 and β2/3 subunits, an observation not made in the present study. The most likely explanation for this discrepancy is technical, relating to the techniques that were used. It is well recognised that peroxidase reaction products are diffusible and readily adhere to membranes and to postsynaptic densities and can thus give false-positive labelling. Colloidal gold when attached to the secondary antibody does not diffuse. Thus the detection of labelling only at symmetrical synapses is probably a reflection of the true distribution of immunolabelling. It should be noted, however, that post-embedding labelling on freeze-substituted tissue is less sensitive that other methods; it may be that the level of receptor subunits at asymmetric synapses as well as at other sites is below the detection limit of the technique (Baude et al., 1994; Nusser et al., 1994). Nevertheless, our findings indicate that the highest concentration or density of $GABA_A$ receptor subunits occurs at symmetrical synapses.

The quantitative analysis revealed that, when considered as a population, immunolabeling for the β2/3 subunits of the $GABA_A$ receptor was evenly distributed across the width of the synapse. Labelling became negligible within a few nanometers of the edge of the synaptic specialization. This indicates that GABA transmission mediated through $GABA_A$ receptors that possess β2 and/or β3 subunits is likely to occur almost exclusively within the synapse and that there is a homogeneous distribution of receptors in the post-synaptic membrane. These findings are consistent with previous findings of the distribution of $GABA_A$ receptors subunits in the hippocampus (Nusser et al., 1995a, 1996a) and subunits of ionotropic glutamate receptors in the striatum (Bernard et al., 1997; Bernard and Bolam, 1998), entopeduncular nucleus, and subthalamic nucleus (Clarke and Bolam, 1998). It must be noted, however, that GABAergic synapses in the striatum are heterogeneous with respect to their origin; the average data that we generated may obscure any variations in the distribution of immunolabelling at sub-populations of synapses.

It is not possible to determine whether immunogold labelling, using the freeze-substitution, post-embedding method, is associated with the pre- or post-synaptic membrane because of sterical distortion between the image of the membrane specialization formed from the whole thickness of the section and the most superficial layer available for the antibody. On the basis of in situ hybridization and immunocytochemical studies, the weight of evidence is that most, if not all, of the immunolabelling that we observed is associated with the post-synaptic element (Wisden et al., 1992; Fritschy and Mohler, 1995; Hartig et al., 1995; Caruncho et al., 1996, 1997; Liste et al., 1997; Waldvogel et al., 1997, 1998; Kultas-Ilinsky et al., 1998).

Identity of boutons forming receptor-positive synapses

At least two classes of axon terminals were identified that formed $GABA_A$ receptor-positive synapses in the striatum, those that were associated with a high density of GABA immunogold particles and those with low or undetectable levels. There are at least four possible origins of the terminals with high levels of GABA:

1. The medium spiny projection neuron, which accounts for the majority of striatal neurons and gives rise to

Fig. 4. Synapses positive for the β2/3 subunits of the $GABA_A$ receptor formed by boutons with low or undetectable levels of GABA. In each micrograph there are boutons or axons with a high density of GABA immunogold particles overlying them demonstrating that GABA immunoreactivity was adequately maintained in the tissue. **A:** Bouton, b1, has high levels of GABA immunogold particles and forms a $GABA_A$ receptor-positive, symmetrical synapse (large arrow) with a dendrite (d). In contrast, b2 which also forms a receptor-positive synapse (arrow) with a dendrite (d), possesses only one immunogold particle coding for GABA. Note the GABA-positive bouton to the right of b2. The insets show the synapses at high magnification. **B:** Two boutons (b1 and b2) form $GABA_A$ receptor-positive synapses (arrows) with a perikaryon (p) which has characteristics of a medium spiny neuron (not shown). Both boutons possess low numbers of GABA immunogold particles in contrast to the GABA-positive structures within the same field. It is possible that the boutons arise from the same axon but continuity is not evident in this micrograph. The inset shows the synapse formed by bouton b2 at higher magnification. **C:** Boutons, b1 and b2, both form receptor-positive symmetrical synapses with a dendrite (d). The number of GABA immunogold particles associated with b1 is higher than that associated with b2. The two synapses are shown at higher magnification in the inset. **D:** Three boutons form $GABA_A$ receptor-positive synapses (arrows). Two of them (b2, b3) possess low (b2) or undetectable (b3) levels of GABA immunolabelling. In contrast, b1 possesses high levels of GABA immunolabelling. The inset shows the synapse formed by b2 at higher magnification. Note that the synapse formed by b3 has been cut tangentially and so the membranes are not visible. **E:** Two boutons form receptor-positive synapses (arrows) with dendrites (d); b1 is GABA-positive whereas b2 has relatively low levels of GABA immunolabelling. Scales bar = 0.5 μm in A (applies to all main micrographs) and represents 0.24 μm for the insets in a A, C & D and 0.25 μm for the inset in B.

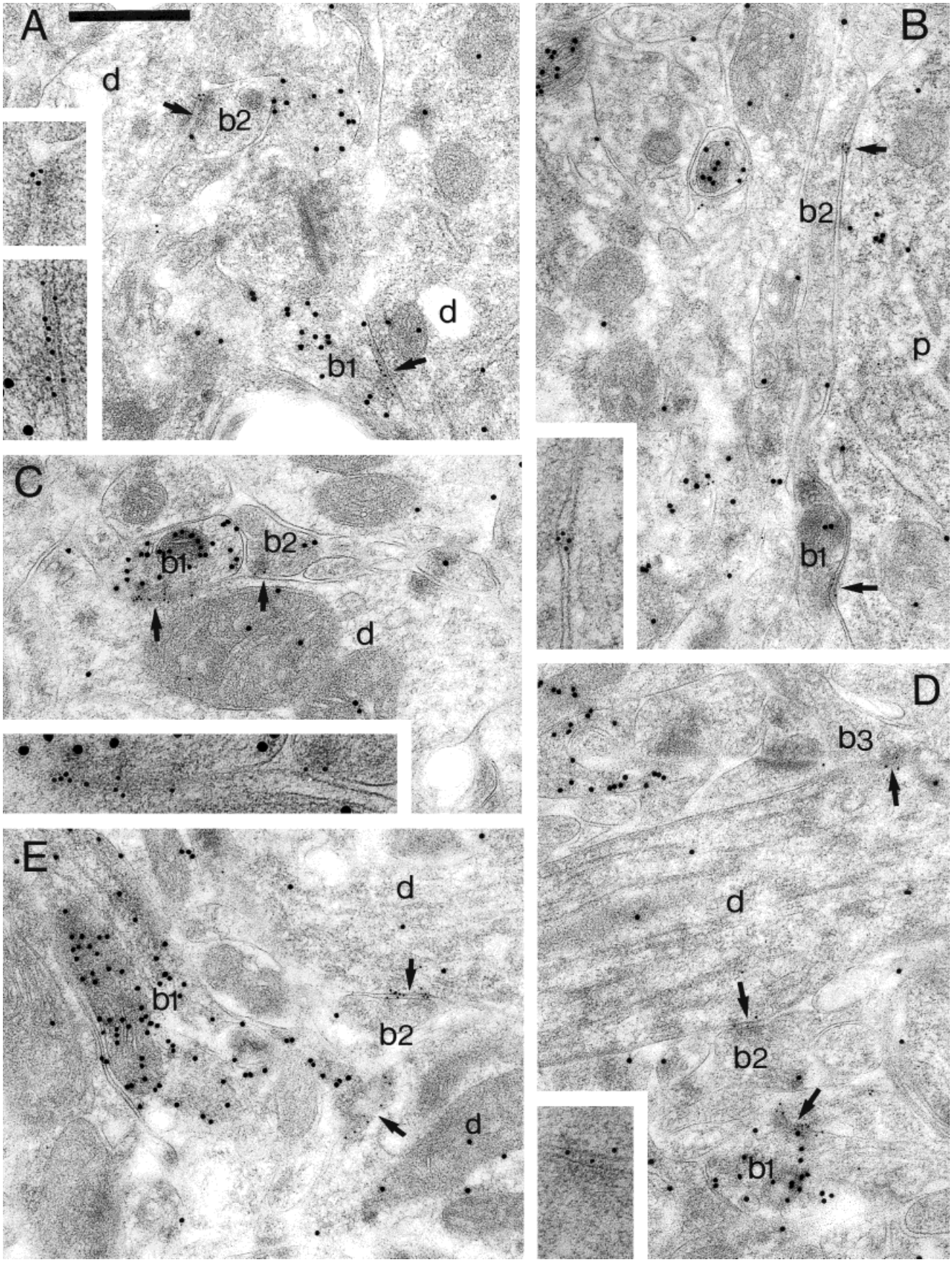

Figure 4

local axon collaterals that form symmetrical synapses (Wilson and Groves, 1980; Somogyi et al., 1981; Yung et al., 1996), are GABAergic neurons (see Smith and Bolam, 1990). Their axon terminals in the globus pallidus, entopeduncular nucleus, and substantia nigra have been shown to be enriched in GABA (see Smith et al., 1998).

2. The population of GABA interneurons identified on the basis of the uptake of [^{3}H]GABA (Bolam et al., 1983) and by GAD immunolabeling (Bolam et al., 1985; Kita and Kitai, 1988). These neurons stain more strongly for GAD than do the medium spiny neurons (Bolam et al., 1985; Kita and Kitai, 1988) and express immunoreactivity for parvalbumin (Cowan et al., 1990; Kita et al., 1990); their axon terminals within the striatum have been shown to form symmetrical synapses (Cowan et al., 1990; Kita et al., 1990; Bennett and Bolam, 1994a, 1994b) and are GABA-positive (Kubota et al., 1993; Bennett and Bolam, 1994b).
3. The terminals formed by striatal interneurons that express nitric oxide synthase and those expressing the calcium binding protein calretinin, form symmetrical synapses (Bennett and Bolam, 1993; Morello et al., 1997)) and have been reported to be GABA positive (Kubota et al., 1993; Clarke and Bolam, 1997).
4. A sub-population of neurons in the globus pallidus gives rise to a projection to the striatum (Staines et al., 1981; Beckstead, 1983; Jayaraman, 1983; Staines and Fibiger, 1984; Shu and Peterson, 1988; Walker et al., 1989; Kita and Kitai, 1994; Rajakumar et al., 1994; Spooren et al., 1996; Nambu and Llinas, 1997; Bevan et al., 1998). These neurons are GABAergic (Smith et al., 1998); they give rise to symmetrical synapses in the striatum and selectively innervate striatal interneurons (Bevan et al., 1998).

The postsynaptic neurons included both medium spiny and GABA interneurons identified on the basis of both morphological and neurochemical criteria. From the known synaptology of the striatum (Bolam and Bennett, 1995), we can conclude that the $GABA_A$ receptor-positive synapses on spiny neurons are formed by the collaterals of spiny neurons themselves (Wilson and Groves, 1980; Somogyi et al., 1981; Yung et al., 1996; but see below) and/or the terminals of GABA interneurons (Kita et al., 1990; Kita, 1993; Bennett and Bolam, 1994a,b) but not those of pallidostriatal neurons (Bevan et al., 1998). Those GABA-positive terminals forming receptor-positive synapses with GABA-positive neurons are likely to be derived from the globus pallidus (Bevan et al., 1998) and possibly other GABA interneurons (Bolam et al., 1985).

There are several possible explanations for the presence of the second population of axon terminals that formed $GABA_A$ receptor-positive synapses, i.e., those possessing low or undetectable levels of GABA. First, it is possible that the low or undetectable levels of GABA are a technical artifact caused by the failure to maintain the antigenicity for GABA within those boutons. This, however, is unlikely, as other boutons that were strongly positive for GABA (and formed receptor-positive synapses) were found in the vicinity of the boutons with the low levels of GABA. Furthermore, similar analyses in other regions of the basal ganglia (entopeduncular nucleus, substantia nigra pars reticulata and compacta) revealed the presence of much smaller proportions of boutons with low or undetectable levels of GABA-forming receptor-positive synapses (unpublished observations). This suggests that the higher proportion of GABA-poor boutons that formed receptor-positive synapses in the striatum is a characteristic of the striatum and not the technicalities of the procedures. A second possibility is that the terminals with low or undetectable levels of GABA truly represent a population of terminals that are GABAergic but are at the lower end of the spectrum in terms of their content of GABA and are simply below the level of detection in this tissue. There is evidence, albeit indirect, that different populations of GABAergic terminals in the striatum are derived from neurons that express different levels of GABA. Thus the parvalbumin-expressing GABA interneurons stain more strongly for GAD than striatal spiny neurons (Bolam et al., 1985; Kita and Kitai, 1988; Kawaguchi et al., 1995). Similarly, the terminals of globus pallidus neurons in the entopeduncular nucleus or the substantia nigra have higher levels of GABA immunoreactivity than do the terminals of spiny projection neurons in these regions (Smith et al., 1998). It is possible, therefore, that in the striatum the terminals of GABA interneurons and the terminals of globus pallidus neurons are those terminals with high levels of GABA and that the terminals with low or undetectable levels of GABA are the local axon terminals of the spiny projection neurons.

A third possible explanation is that the terminals are indeed non-GABAergic but form synapses that are positive for GABA receptor subunits. Mismatches between the putative transmitter of synaptic terminals and the receptor located within the synapse have been reported for GABA and for other receptors. In the subthalamic nucleus, a population of terminals that have low levels of glutamate immunoreactivity and high level of GABA forms synapses that are positive for the NR1 subunit of the NMDA receptor and the GluR2/3 subunits of the AMPA receptor (Clarke and Bolam, 1998). Furthermore, the γ2, β2/3, and α6 subunits of the $GABA_A$ receptor have been shown to be concentrated in some glutamatergic mossy fibre synapses in the cerebellum (Nusser et al., 1996b, 1998). In the striatum, non-GABAergic terminals that form symmetrical synapses include dopaminergic axon terminals derived from the substantia nigra pars compacta (Bouyer et al., 1984; Freund et al., 1984; Smith et al., 1994; Hanley and Bolam, 1997) and cholinergic terminals derived from cholinergic interneurons (Wainer et al., 1984; Izzo and Bolam, 1988; Pickel and Chan, 1990). The possibility of the presence of $GABA_A$ receptors at synapses formed by these classes of terminals cannot, as yet, be excluded.

Fig. 5. Co-localization of subunits of the $GABA_A$ receptor at synapses in the striatum (α1 subunits: 20 nm gold particles; β2/3 subunits: 10 nm gold particles; γ2 subunits: 5 nm gold particles). **A:** The synapse formed between the bouton (b) and a dendrite (d) is positive for α1 (long arrows), and β2/3 subunits (medium arrows). **B:** A bouton (b) forms two synapses with two dendrites (d). The synapse on the right is positive for α1 (long arrows), and β2/3 subunits (medium arrows) and possesses one gold particle coding for the γ2 subunit (small arrow). The synapse on the left (cut tangentially) is apparently positive for β2/3 subunits only (medium arrows). **C, D, E:** Boutons (b) forming receptor-positive synapses with dendrites (d). In each case immunolabelling for α1 (long arrows), β2/3 (medium arrows), and γ2 (small arrows) subunits occurs at the synapse. **F:** A synapse between a bouton (b) and dendrite (d) that is positive for β2/3 (medium arrows) and γ2 (small arrow) subunits. Scale bars = 0.2 µm. The bar in B also applies to C and the bar in D also applies to E & F.

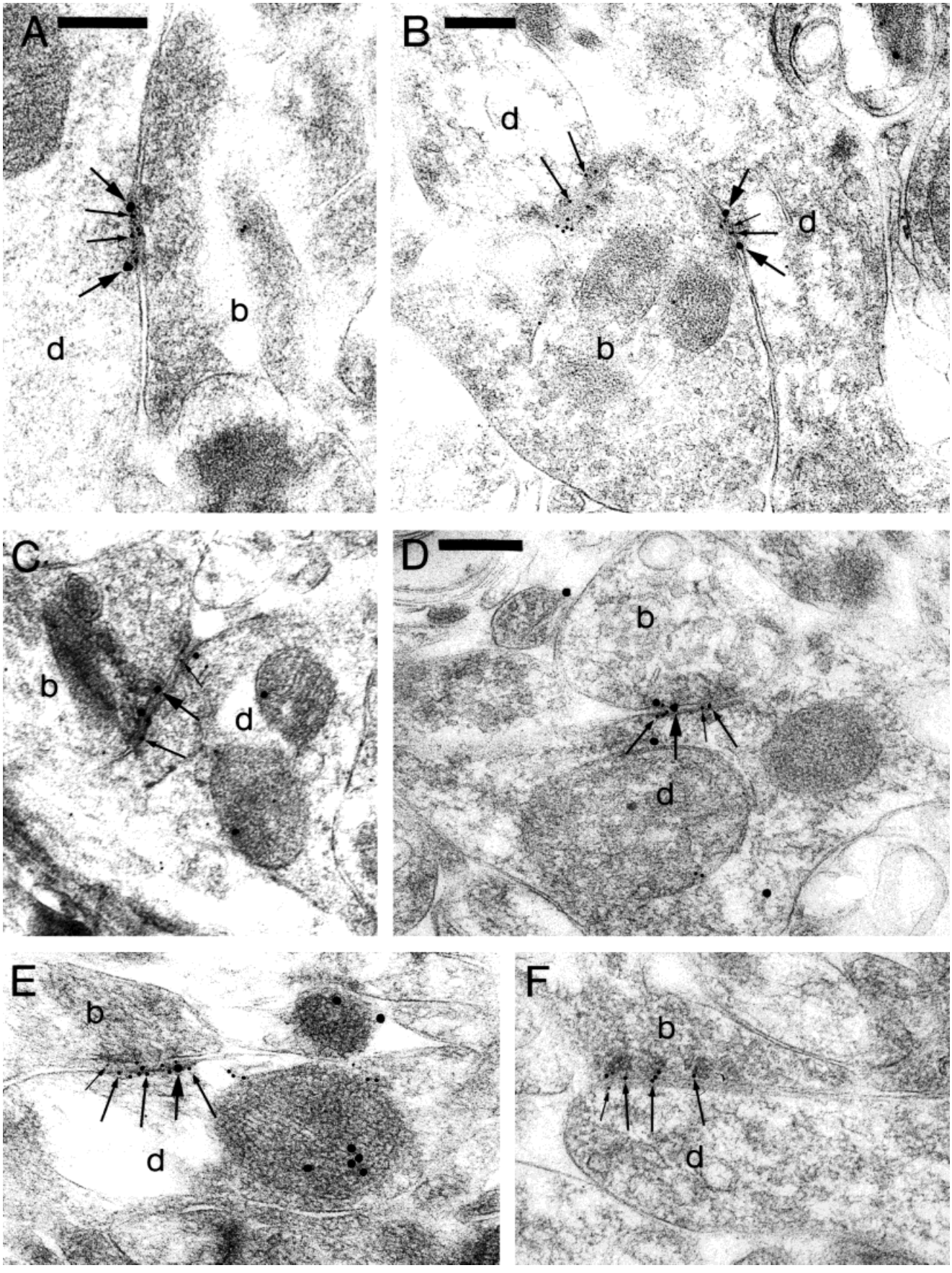

Figure 5

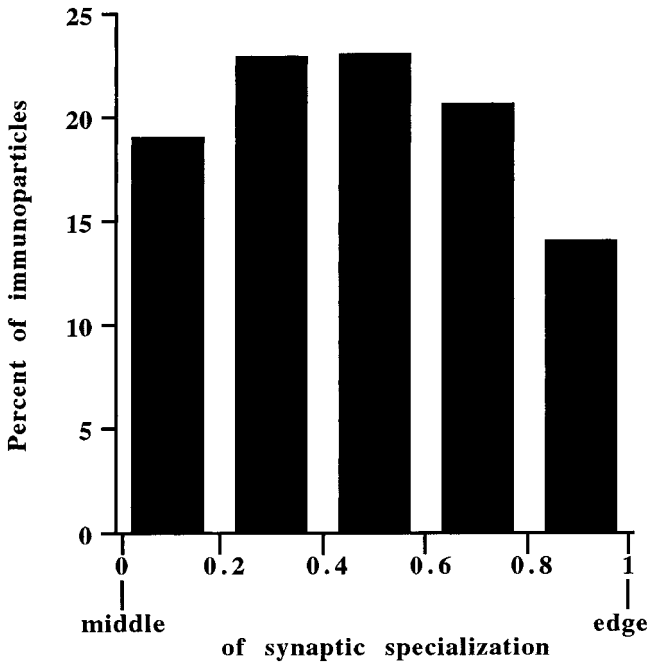

Fig. 6. The average distribution of immunoparticles coding for the β2/3 subunits of the GABAA receptor along symmetrical synaptic membranes in the striatum labelled by the post-embedding immunogold method. The distribution was even over the width of the synapses with a slight reduction towards the edge. A total of 155 synapses from two animals (746 gold particles) were analyzed. The gold particles were assigned to five bins over the half width of the synapses. Only synapses labelled with two or more immunoparticles were included in the analysis.

Co-localization of $GABA_A$ receptor subunits at synapses

The triple-labelling study revealed the presence of symmetrical synapses that were positive for various combinations of the α, β, and γ subunits. Thus synapses were observed that were labelled by one of the three antibodies, two out of the three antibodies and synapses that were positive for all three. Although negative findings are difficult to interpret, the present study indicates that α1, β2/3, and γ2 receptor subunits of the $GABA_A$ receptor co-localize at individual synapses. This finding corroborates previous radioligand-binding, in situ hybridization, and immunohistochemical studies indicating the co-localization of $GABA_A$ receptor subunits in the striatum at the regional and cellular levels (Fritschy and Mohler, 1995; Caruncho et al., 1996, 1997; McKernan and Whiting, 1996; Waldvogel et al., 1997, 1998; Riedel et al., 1998). In fact the α1, β2/3, and γ2 receptor subunit configuration has been proposed as the most common for $GABA_A$ receptors in the mammalian brain. From pre-embedding immunocytochemical and in situ hybridisation studies (Fritschy and Mohler, 1995; Caruncho et al., 1996, 1997; McKernan and Whiting, 1996; Liste et al., 1997; Waldvogel et al., 1997, 1998; Riedel et al., 1998), it is evident that the α1 subunit in not expressed, or expressed at low levels, by medium spiny neurons but is expressed by small populations of striatal neurons, which include GABA interneurons and a large type of projection neuron (Caruncho et al., 1996; Waldvogel et al., 1997, 1998). It is thus likely that the synapses that were positive for the α1 subunits with or without the β2/3 and γ2 subunits are formed by these latter populations of neurons. Those synapses that were negative for the α1 subunits may express some other α subunit, possibly α2, and may thus represent the synapses of spiny neurons. It is thus evident that there are differences in the $GABA_A$ receptor subunit profiles at synapses on medium-sized projection spiny neurons and those on interneurons in the striatum.

CONCLUSIONS

The present findings demonstrate the precise localization of subunits of the $GABA_A$ receptor in relation to symmetrical synaptic specializations in the rat striatum. The main conclusions that we can draw from this study are that $GABA_A$ receptor are primarily located at symmetrical synapses formed by boutons that are heterogeneous with respect to their morphology and neurochemistry and that different subunits colocalize at the level of individual synapses. The findings represent the first step in the elucidation of the chemical anatomy of the GABA-mediated synaptic circuits of the striatum in which the anatomical connections, transmitter neurochemistry, and transmitter receptors are localized. Experiments are in progress to identify the origin of the synaptic boutons involved in these circuits.

ACKNOWLEDGMENTS

The authors thank Caroline Francis, Paul Jays, Liz Norman, and David Roberts for technical assistance and Peter Somogyi for the antibodies against GABA. The α1 antibody was prepared in collaboration with Dr. S. Pollard. Thanks to Jason Hanley for comments on the manuscript. F.F. was supported by the Japanese Ministry of Education and Saga Medical School. F.A.S. is supported by a Medical Research Council ROPA award.

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