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Synaptic localization of $GABA_A$ receptor subunits in the substantia nigra of the rat: effects of quinolinic acid lesions of the striatum

Fumino Fujiyama,[1] F. Anne Stephenson[2] and J. Paul Bolam[1]
[1]MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, Mansfield Road, Oxford, Oxford, OX1 3TH, UK
[2]School of Pharmacy, University of London, UK

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Abstract

The inhibitory amino acid, γ-aminobutyric acid (GABA), plays a critical role in the substantia nigra (SN) in health and disease. GABA transmission is controlled in part by the type(s) of GABA receptor expressed, their subunit composition and their location in relation to GABA release sites. In order to define the subcellular localization of $GABA_A$ receptors in the SN in normal and pathological conditions, sections of SN from control rats and rats that had received quinolinic acid lesions of the striatum were immunolabelled using the postembedding immunogold technique with antibodies against subunits of the $GABA_A$ receptor. Immunolabelling for α1, β2/3 and γ2 subunits was primarily located at symmetrical synapses. Double-labelling revealed that β2/3 subunit-positive synapses were formed by terminals that were enriched in GABA. Colocalization of α1, β2/3 and γ2 subunits occurred at individual symmetrical synapses, some of which were identified as degenerating terminals derived from the striatum. In the SN ipsilateral to the striatal lesion there was a significant elevation of immunolabelling for β2/3 subunits of the $GABA_A$ receptor at symmetrical synapses, but not of GluR2/3 subunits of the AMPA receptor at asymmetrical synapses. It was concluded that fast $GABA_A$-mediated transmission occurs primarily at symmetrical synapses within the SN, that different receptor subunits coexist at individual synapses and that the upregulation of $GABA_A$ receptors following striatal lesions is expressed as increased receptor density at synapses. The upregulation of $GABA_A$ receptors in Huntington's disease and its models is thus likely to lead to an increased efficiency of transmission at intact GABAergic synapses in the SN and may partly underlie the motor abnormalities of this disorder.

Introduction

The substantia nigra (SN) is a major division of the basal ganglia, a group of subcortical nuclei involved in a variety of functions including movement, memory and cognition (Alexander *et al*., 1990; DeLong, 1990; Graybiel, 1990, 1995; Chesselet & Delfs, 1996; Wise, 1996). The dorsal division (SN pars compacta; SNc) contains dopaminergic neurons that project to the forebrain, whereas the more ventral pars reticulata (SNr) contains one of the populations of GABAergic output neurons of basal ganglia. Within the SN there are at least three major populations of GABAergic synaptic terminals that are critical in sculpting the activity of both dopaminergic neurons and basal ganglia output neurons (Grace & Bunney, 1979, 1984a, b; Smith & Bolam, 1989; Bolam & Smith, 1990; Smith *et al*., 1998; Celada *et al*., 1999; Chan & Yung, 1999; Paladini *et al*., 1999). The first, and most numerous, are derived from the spiny projection neurons of the striatum. These terminals make synaptic contact with both SNr and SNc neurons (Somogyi *et al*., 1981; Wassef *et al*., 1981; Williams & Faull, 1985; Smith & Bolam, 1991; von Krosigk *et al*., 1992; Smith *et al*., 1998) and transmit the information carried by the so-called 'direct pathway' of information flow through the basal ganglia (Albin *et al*., 1989; Alexander & Crutcher, 1990; DeLong, 1990). Both populations of neurons also receive synaptic input from the globus pallidus (GP) and probably also from the local collaterals of GABAergic output neurons (Smith & Bolam, 1989, 1990; Tepper *et al*., 1995; Paladini *et al*., 1999).

The actions of γ-aminobutyric acid (GABA) in the SN are mediated by the ionotropic $GABA_A$ and the metabotropic $GABA_B$ receptors (Celada *et al*., 1999; Paladini & Tepper, 1999; Paladini *et al*., 1999). Most of the effects of GABA in the SN are mediated through postsynaptic $GABA_A$ receptors, which cause an increase in chloride conductance that underlies fast inhibitory postsynaptic potentials. Indeed, *in situ* hybridization and immunocytochemical studies have identified a variety of subunits of the $GABA_A$ receptor in neurons in the SN (Nicholson *et al*., 1992, 1995, 1996; Fritschy & Mohler, 1995, Pirker *et al*., 2000; Schwarzer *et al*., 2001).

Dysfunctions of GABAergic transmission in the SN have been implicated in a variety of diseases and their models. Excitotoxic lesions of the striatum leads to a loss of striatonigral GABAergic neurons and to changes in striatal targets that are similar to the changes that occur in Huntington's disease and, as such, have been used as models of this disease (for references see Brickell *et al*., 1999). One such change is an upregulation of $GABA_A$ receptors in the targets of the striatum, as demonstrated by increases in binding sites, mRNAs for subunits and in the proteins themselves in both

Correspondence: Dr J. P. Bolam, as above.
E-mail: paul.bolam@pharmacology.ox.ac.uk

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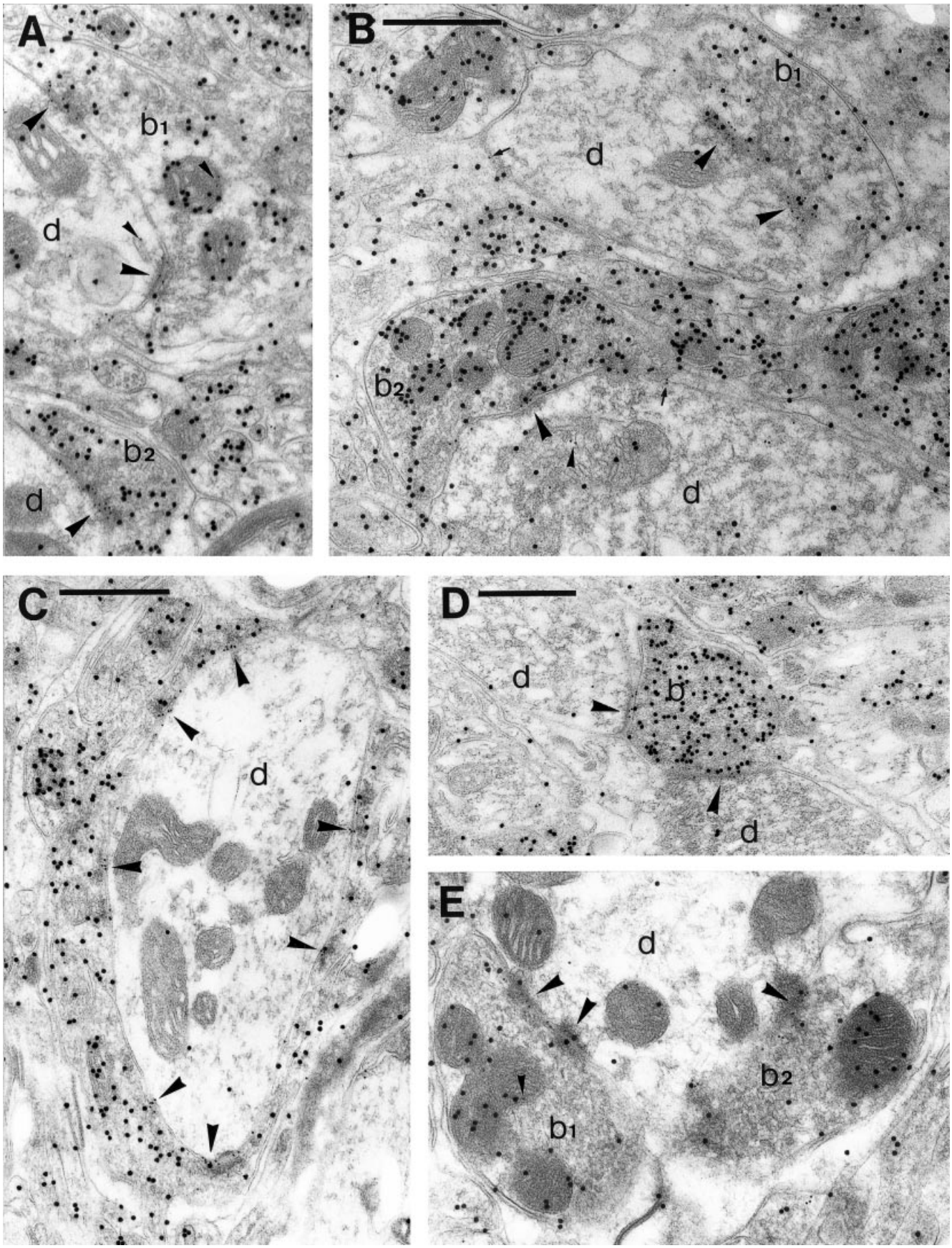
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models (Pan *et al*., 1983, 1984; Corda *et al*., 1986; Nicholson *et al*., 1995, 1996; Brickell *et al*., 1999) and Huntington's disease (Reisine *et al*., 1979, 1980; Penney & Young, 1982; Whitehouse *et al*., 1985; Faull *et al*., 1993; Glass *et al*., 2000). Furthermore, functional changes have been reported in nigral $GABA_A$ receptors in an *in vitro* expression system following excitotoxic lesions of the striatum in the rat (Sanna *et al*., 1998).

In view of the critical role of GABA and its receptors in SN function and their alteration in Huntington's disease and its models, it is important to characterize the localization and composition of GABA receptors in relation to the synaptic circuitry of the SN. Thus, the main objectives of this study were first to determine the subcellular localization of subunits of the $GABA_A$ receptor, in particular in relation to synaptic specializations; second to characterize the axon terminals presynaptic to the $GABA_A$ receptor-positive synapses by GABA-immunolabelling and/or anterograde degeneration; and third to test the hypothesis that the upregulation of $GABA_A$ receptors following excitotoxic lesions of the striatum is associated with an increased expression at synaptic specializations in the SN. These objectives were addressed using the postembedding immunogold technique on freeze-substituted tissue and antibodies against different subunits of the $GABA_A$ receptor.

Materials and methods

Intrastriatal injection of quinolinic acid

Female Wistar rats (200–250 g; Charles River, Margate, UK) were maintained on a 12-h light/12-h 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 UK, the European Communities Council Directive (80/609/EEC) and the policy on the use of animals in neuroscience research issued by the Society for Neuroscience. The animals (n = 4) were anaesthetized by intraperitoneal injection of neuroleptanalgesic consisting of fentanyl citrate/fluanison (0.135 mg/mL and 10 mg/mL, respectively; Hypnorm®, Janssen-Cilag, High Wycombe, UK) and midazolam (5 mg/mL; Hypnovel®; Roche Products, Welwyn Garden City, UK) diluted 1 : 1 : 2 with sterile water (2.7 mL/kg) and placed in a stereotaxic frame. Injections of the excitotoxin, quinolinic acid (QA; Sigma-Aldrich, Poole, UK), were stereotaxically placed in the dorsal region of the right striatum at two loci (coordinates: 8.0 mm anterior to bregma; 2.4 mm lateral to the midline; and 3.5 and 5.0 mm ventral to the pial surface) using a Hamilton syringe. Each rat received a total of 120 nmol of QA in 1 μL of phosphate-buffered saline (PBS; 0.01 M phosphate, pH 7.4) over a 10-min period (Chalon *et al*., 1996; Nakao *et al*., 1996; Nicholson *et al*., 1996). The cannula was then left in position for a further 5 min before being slowly removed.

Eleven or 14 days after the intrastriatal injection, the rats were anaesthetized with pentobarbitone (Sagatal, 200 mg/kg; Rhône Mérieux, Tallaght, Dublin, Ireland) and then perfused through the heart with ≈ 100 mL PBS over 1–2 min and then with 300 mL of 0.1% glutaraldehyde and 3% paraformaldehyde (both obtained from TAAB, Aldermaston, UK), made up in phosphate buffer (PB; 0.1 M, pH 7.4), over a period of 20 min. 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. Coronal sections of the SN (500 μm) were cut using a vibrating microtome and washed several times in PBS. Coronal sections of the forebrain (70 μm) of the QA-injected animals were cut, mounted on gelatine-coated slides and Nissl-stained to assess the extent of the lesions.

Freeze-substitution and Lowicryl embedding

A similar procedure was used to that described earlier (Baude *et al*., 1993). Small blocks of tissue of the SN ipsilateral and contralateral to the QA lesion were trimmed from the 500-μm sections. After washing in PB they were placed in 0.5 M sucrose in PB for 15 min followed by 1 M sucrose in PB for 2 h for cryoprotection. They were then slam-frozen on a polished copper block cooled with liquid nitrogen (Reichert MM80E; Leica, Milton Keynes, UK). The frozen blocks of tissue were transferred to a Leica CS Auto at –90 °C, where freeze-substitution and embedding in Lowicryl HM20 (Agar Scientific Ltd, Stansted, UK) was carried out as described before (Clarke & Bolam, 1998; Fujiyama *et al*., 2000). Once the resin was polymerized and the blocks restored to room temperature, they were removed for trimming and sectioning. Ultrathin sections (≈ 70 nm) were cut on a Reichert-Jung Ultracut-E ultramicrotome (Leica) and collected on adhesive-coated (coat-quick 'G' medium; TAAB) gold mesh grids. In addition to the material from the rats that had received striatal lesions, blocks of nigral tissue from rats prepared on previous occasions (Clarke & Bolam, 1998; Chatha *et al*., 2000) were also sectioned for postembedding immunolabelling.

Postembedding immunogold labelling

The sections were immunolabelled by the postembedding immunogold method essentially as described previously (Nusser *et al*., 1998). Briefly, the sections were treated with a saturated solution of sodium ethanolate for 3 s, washed in deionized water and then in 50 mM Tris

FIG. 1. Localization of β2/3 subunits of the $GABA_A$ receptor at synapses formed by GABA-immunolabelled boutons in control substantia nigra. In each micrograph, GABA-positive boutons are identified by the accumulation of 20-nm gold particles and immunolabelling for the β2/3 subunits identified by the 10-nm gold particles. (A) Two boutons (b_1 and b_2) in the substantia nigra pars compacta (SNc) that are GABA-positive and form symmetrical synapses (large arrowheads) with dendritic shafts (d). Both synapses are β2/3 subunit-positive as indicated by the 10-nm gold particles. Bouton b_1 is large, forms two active zones and is likely to be derived from the globus pallidus. Bouton b_2 has the features of a terminal derived from the striatum. This synapse is cut tangentially so the membranes are not visible, although the location of the synapses is indicated by the location of the receptor-immunolabelling. Note the receptor-immunolabelling associated with a membrane-bound organelle and a mitochondrion (small arrowheads). (B) Two GABA-positive boutons (b_1,b_2) that form β2/3 subunit-positive synapses (large arrowheads) with dendritic shafts (d) in the SNc. The synapse formed by b_1 is cut obliquely, but is strongly positive for the receptor subunits. This bouton has the features of a striatal terminal, whereas b_2 has the features of a terminal derived from the globus pallidus. Immunolabelling for the β2/3 subunits at extrasynaptic sites on the dendritic membrane is indicated by small arrows (a single gold particle in the upper dendrite and a pair in the lower dendrite). Note the receptor-immunolabelling associated with a mitochondrion (small arrowhead). (C) A dendritic shaft (d) in the substantia nigra pars reticulata (SNr) that is apposed by many GABA-positive terminals that have features of terminals derived from the striatum. They form symmetrical synapses (large arrowheads) that are positive for the β2/3 subunit, as indicated by the 10-nm gold particles. (D) A GABA-positive bouton (b) in the SNc that forms symmetrical synapses (large arrowheads) with two dendritic shafts (d). Both synapses are positive for the β2/3 subunits of the $GABA_A$ receptor. (E) Two GABA-positive boutons (b_1,b_2) in the SNr that form β2/3 subunit-positive synapses (large arrowheads) with the same dendritic shaft (d). The synapse formed by b_2 is cut tangentially. Note the receptor-immunolabelling associated with a mitochondrion (small arrowhead). Scale bars, 0.5 μm. The bar in B also applies to A and E.

(pH 7.6), containing 0.9% NaCl (TBS). They were then incubated for 30 min in TBS containing 2% human serum albumin and 0.01% Triton X-100 (TBST), followed by an overnight incubation at room temperature in various combinations of the primary antibody solutions (diluted in TBST) directed against subunits of the $GABA_A$ receptor, against GABA itself or against the GluR2/3 subunits of the α-amino-3-hydroxy-5-methyl-4-isoxaline propionic acid (AMPA) receptor (discussed later, and see Table 1). They were then washed in TBS and incubated in the appropriate secondary antibodies conjugated to colloidal gold (5–20 nm diameter; British BioCell International, Cardiff, UK) in TBST supplemented with 5 mg/mL polyethylene glycol (average molecular weight: 20 kDa) for about 2 h. They were washed in TBS, incubated in 2% glutaraldehyde in TBS for 2 min, contrasted in 1% uranyl acetate and lead citrate, and then examined in a Philips CM 10 transmission electron microscope.

Antibody preparations

Three antibodies against subunits of the $GABA_A$ receptor were used in this study. (i) A monoclonal antibody that recognizes extracellular domains of both the β2 and β3 subunits (gift from J.M. Fritschy and Chemicon International, Harrow, UK). This antibody has been extensively characterized (Harring *et al*., 1985; Schoch *et al*., 1985; Ewert *et al*., 1990) and used extensively in immunocytochemical studies (see for instance Harring *et al*., 1985; Somogyi *et al*., 1996; Waldvogel *et al*., 1998, 1999; Fujiyama *et al*., 2000). (ii) A polyclonal antibody raised in guinea pig against a synthetic peptide corresponding to the first 29 N-terminal amino acids of the rat γ2 subunit that was conjugated to keyhole limpet haemocyanin. Detailed characterization of the antibody has been described elsewhere (Benke *et al*., 1996; Somogyi *et al*., 1996). (iii) A rabbit antibody raised against the first 14 N-terminal amino acids of the rat α1 subunit (see Fujiyama *et al*., 2000). Two further antibodies were used. First, a rabbit antibody raised against the C-terminal peptide, EGYNVYGIE-SVKI, of the AMPA receptor that recognizes the GluR2 and GluR3 subunits but does not cross-react with GluR1 or GluR4 (Chemicon; Petralia & Wenthold, 1992; Wenthold *et al*., 1992; Chen *et al*., 1996). Second, a rabbit antibody against GABA (code 9; Hodgson *et al*., 1985; Somogyi & Hodgson, 1985; Somogyi *et al*., 1985). Details of the antibodies are summarized in Table 1.

Immunogold labelling

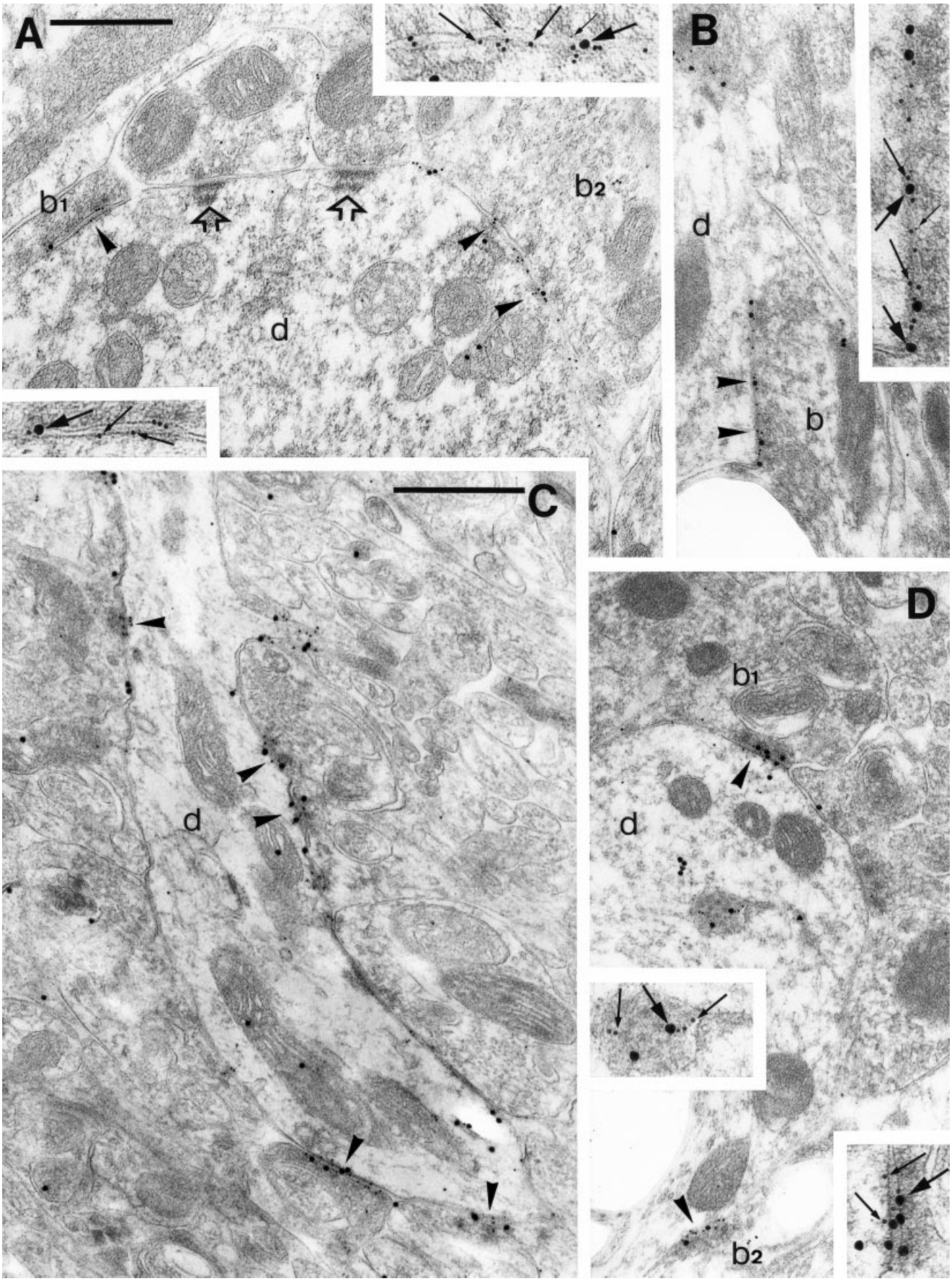
In addition to the single labelling of SN sections with the antibodies against subunits of the $GABA_A$ receptor, double-labelling and triple-labelling experiments were performed in which mixtures of primary antibodies of different species of origin were used. The following double-labelling and triple-labelling combinations of immunolabelling were performed.

(i) Mouse monoclonal antibodies against the β2/3 subunits of the $GABA_A$ receptor (10 μg/mL), together with rabbit antibodies against GABA (1 : 5000). After washes they were incubated in a mixture of goat antirabbit immunoglobulin G (IgG) coupled to 15-nm (1 : 60) or 20-nm (1 : 80) gold particles and goat antimouse IgG coupled to 10-nm gold particles (1 : 50).

TABLE 1. Details of antibody preparations

Antibody directed against	Species of origin	Dilution or concentration	Source and/or characterization
GABA	Rabbit	1 : 5000	Somogyi *et al*. (1985) Somogyi & Hodgson (1985) Hodgson *et al*. (1985)
α1 subunit of the $GABA_A$ receptor	Rabbit	11 μg/mL	Fujiyama *et al*. (2000)
β2/3 subunits of the $GABA_A$ receptor (bd-17)	Mouse (monoclonal)	10 μg/mL	Häring *et al*. (1985) Chemicon International Ltd
γ2 subunit of the $GABA_A$ receptor	Guinea pig	10 μg/mL	Benke *et al*. (1996)
GluR2/3 subunits of the AMPA receptor	Rabbit	10 μg/mL	Wenthold *et al*. (1992) Chemicon International Ltd
Rabbit IgG conjugated to 20-nm gold particles	Goat	1 : 60	British BioCell International
Rabbit IgG conjugated to 15-nm gold particles	Goat	1 : 80	British BioCell International
Mouse IgG conjugated to 10-nm gold particles	Goat	1 : 50	British BioCell International
Guinea pig IgG conjugated to 5-nm gold particles	Goat	1 : 40	British BioCell International

FIG. 2. Colocalization of subunits of the $GABA_A$ receptor at synapses in control substantia nigra (SN) revealed by triple-immunolabelling for α1 (20-nm gold; large arrows), β2/3 (10-nm gold; medium arrows) and γ2 (5-nm gold; small arrows) subunits. (A) A dendritic shaft (d) in the substantia nigra pars compacta (SNc) that receives synaptic input from four boutons. Boutons b_1 and b_2 form symmetrical synapses with the dendrite. The synapse formed by b_1 is immunopositive for the α1 and β2/3 subunits (see bottom left inset), whereas the synapse formed by bouton b_2 is positive for all three subunits, as indicated by the three sizes of gold particles (upper right inset). The two boutons in the centre form asymmetrical synapses (open arrows) and are not positive for the receptor subunits. (B) A bouton (b) forming symmetrical synaptic contact (arrowheads) with a dendritic shaft (d) in the substantia nigra pars reticulata (SNr). The synapse (inset) is associated with the three sizes of immunogold particles indicating the presence of α1, β2/3 and γ2 subunits. C. Longitudinal section of a dendrite (d) in the SNr. The dendrite is apposed by many boutons, some of which form symmetrical synapses. Many of the synapses (arrowheads) are associated with immunolabelling for α1 and β2/3 subunits, as indicated by the 20- and 10-nm immunogold particles, respectively. (D) A dendritic shaft (d) in the SNr apposed by several boutons, two of which (b_1,b_2) form synapses (arrowheads) that are positive for the α1 and β2/3 subunits, as indicated by the 20- and 10-nm gold particles (see lower right inset for synapse formed by b_1). Note the multivesicular body in the dendrite (inset on left) that is immunolabelled for α1 and β2/3 subunits, as indicated by the two sizes of gold particles. Scales bars, 0.5 μm for the main micrographs and 0.25 μm for the insets. The bar in A also applies to B and D.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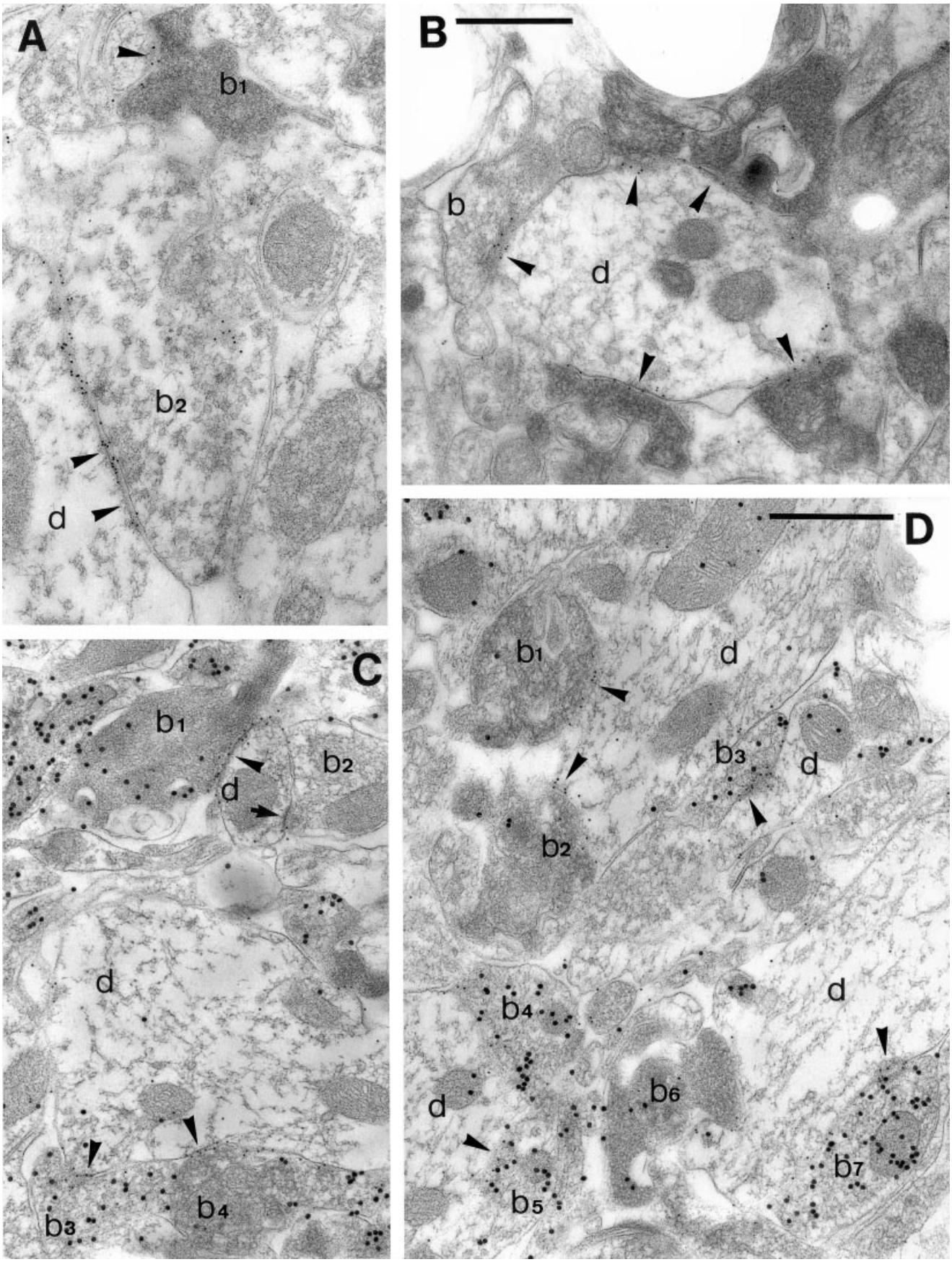
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(ii) Some of the sections of the ipsilateral and contralateral SN of the QA-injected animals were incubated in mixtures of mouse monoclonal antibodies against the β2/3 subunits of the $GABA_A$ receptor (10 μg/mL) and rabbit antibodies against the GluR2/3 subunits of the AMPA glutamate receptor (10 μg/mL). After washes they were incubated in a mixture of goat antirabbit IgG coupled to 15-nm (1 : 60) or 20-nm (1 : 80) gold particles and goat antimouse IgG coupled to 10-nm gold particles (1 : 50).

(iii) Rabbit antibodies against the α1 subunit of the $GABA_A$ receptor (11 μg/mL), mouse monoclonal antibodies against the β2/3 subunits of the $GABA_A$ receptor (10 μg/mL) and guinea pig antibodies against the γ2 subunit of the $GABA_A$ receptor (10 μg/mL) of the $GABA_A$ receptor. The secondary antibodies were goat antirabbit IgG coupled to 20-nm gold particles (1 : 60), goat antimouse IgG coupled to 10-nm gold particles (1 : 50) and goat antiguinea pig IgG coupled to 5-nm gold particles (1 : 40).

Analysis of material

The immunolabelled 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. Quantitative analyses of the distribution of immunogold particles were carried out on series of contiguous micrographs, the starting point of the series being selected at random or in systematic scans in the electron microscope.

The overall distribution in the tissue of immunogold particles immunolabelling the β2/3 subunits of the $GABA_A$ receptor was determined in sections of the ipsilateral and contralateral SN of two QA-lesioned rats. This was achieved by examination of the series of contiguous micrographs at a final magnification of ≈43 750 (total area examined: 473 μm^2 ipsilateral and 237 μm^2 contralateral to the lesion); the location of each gold particle was noted. In the same series of micrographs the density of immunogold particles at each immunopositive synapse was measured. The analysis was confined to synapses that were formed by boutons that possessed the ultrastructural characteristics of terminals derived from the striatum (see Smith *et al*., 1998). The width of each synaptic specialization was measured and the number of immunogold particles coding for the β2/3 subunits of the $GABA_A$ receptor was noted. Data were expressed as the number of gold particles per micron of synaptic specialization and compared using nonparametric statistics (Mann–Whitney *U*-test; $P = 0.05$ was considered significant).

In order to control for general differences in the quality of immunolabelling between the SN ipsilateral and contralateral to the lesion, the extent of synaptic-immunolabelling for the β2/3 subunits of the $GABA_A$ receptor and GluR2/3 subunits of the AMPA glutamate receptor was determined in the same sections (see Fig. 5). This was achieved by systematically scanning double-immunolabelled sections in the electron microscope. The number of immunogold particles at each synapse that possessed one or more immunogold particles was noted. The symmetrical synapses included those that were formed by boutons with the ultrastructural characteristics of striatal terminals, pallidal terminals, and terminals undergoing degeneration. The analysis was carried out on sections from two lesioned animals and was independently performed on two series of sections by two observers. The numbers of immunogold particles were compared by the Mann–Whitney *U*-test ($P = 0.05$ was considered significant).

Controls

In order to control for cross-reactivity of the secondary antibodies directed against the IgGs of the different species, sections were incubated with a mixture of two primary antibodies from different species, at the appropriate dilutions, and then with secondary, gold-conjugated antibodies directed against immunoglobulins of the third species. In the same immunolabelling experiment, 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 labelling of cytosolic and cellular elements in the case of the antibodies against GABA.

TABLE 2. Distribution of immunolabelling for β2/3 subunits of the $GABA_A$ receptor in the substantia nigra following quinolinic acid lesions of the striatum

	Membrane labelling (Percentage of total)	Synaptic labelling		
		(Percentage of total)	(Percentage of membrane)	*n*
Rat 1				
Lesion	55.5	32.8	59.2	1033
Control	41.4	17.6	42.4	319
Rat 3				
Lesion	63.1	32.0	50.7	753
Control	42.5	21.2	50.0	212

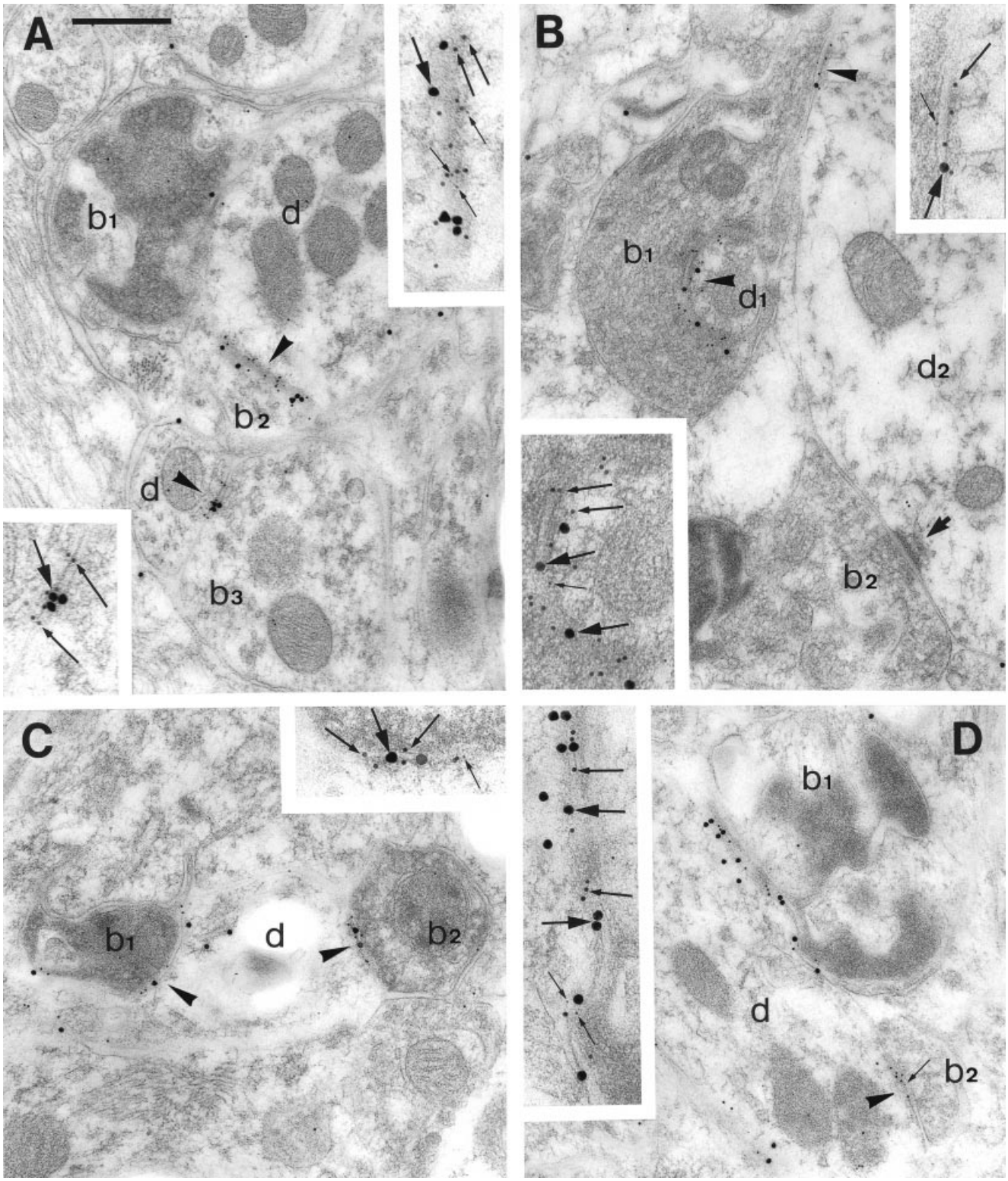
Figures represent the percentages of gold particles coding for the β2/3 subunits of the $GABA_A$ receptor observed in a series of random micrographs of the substantia nigra ipsilateral (lesion) and contralateral (control) to the quinolinic acid lesions of the striatum. Membrane labelling includes both synaptic and nonsynaptic labelling; *n* indicates number of gold particles analysed.

FIG. 3. Immunolabelling for the β2/3 subunits of the $GABA_A$ receptor (10-nm gold particles) alone (A and B) or together with immunolabelling for GABA itself (20-nm gold particles) in the substantia nigra (SN) following quinolinic acid lesions of the striatum. (A) Two boutons (b_1 and b_2) form symmetrical synapses (arrowheads) with small (b_1) or large (b_2) diameter dendrites (d) in the SN. Both synapses display immunolabelling for the β2/3 subunits of the GABA receptor, as indicated by the gold particles. Bouton b_1 shows the characteristic signs of degeneration and thus, is derived from the striatum. (B) A dendritic shaft (d) in the substantia nigra pars reticulata (SNr) that is surrounded by boutons forming β2/3 subunit-positive synapses (arrowheads), some of which are cut obliquely. Each bouton except one (b), shows the characteristic electron-dense appearance of degenerating boutons and are thus derived from the striatum. (C) Double-immunolabelling for β2/3 subunits (10-nm gold) and GABA (20-nm gold). Several boutons are shown forming β2/3 subunit-positive synapses (arrowheads) with dendritic shafts (d). Boutons b_1 and b_4 show characteristic electron-dense signs of degeneration and are thus derived from the striatum; they form receptor-positive synapses but have relatively low levels of GABA-immunolabelling. Bouton b_3, in contrast, is not degenerating, forms a receptor-positive synapse and is associated with a high level of GABA-immunolabelling. Bouton b_2 forms an asymmetrical synapse (arrow) and is negative for GABA. Two 10-nm gold particles (β2/3 subunits) are associated with the edge of this synapse. (D) Double-immunolabelling for β2/3 subunits (10-nm gold) and GABA (20-nm gold). Boutons b_1, b_2 and b_6 show characteristic electron-dense signs of degeneration and are thus derived from the striatum. Two of them (b_1 and b_2) are apposed to the same dendritic shaft (d) and are associated with immunolabelling for the β2/3 subunits (arrowheads). Note the low level of GABA labelling in these boutons. Boutons b_3–b_5 and b7, in contrast, are not degenerating and have higher levels of GABA-immunolabelling. Three of them contact dendritic shafts (d, arrowheads) and, although not clearly synaptic, are associated with immunolabelling for the β2/3 subunits. Scales bars, 0.5 μm. Bar in D also applies to A and C.

Results

Distribution of immunogold labelling for $GABA_A$ receptor subunits

Consistent with previous studies (Nicholson *et al*., 1992, 1995, 1996; Fritschy & Mohler, 1995; Pirker *et al*., 2000; Schwarzer *et al*., 2001), postembedding immunolabelling of sections of the SNc and SNr revealed the presence of sites that were immunoreactive for the α1, β2/3 and γ2 subunits of the $GABA_A$ receptor, identified by the presence of the immunogold particles. The antibody preparation 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 boutons were therefore carried out with the antibody directed against these subunits. Because there were no apparent qualitative differences in the labelling between the SN of noninjected rats and the SN contralateral to the QA lesions of the striatum, the distribution of labelling of the two groups shall be considered together. Furthermore, in describing the general principles of the labelling, the material from the SN ipsilateral to the QA lesions of the striatum will also be referred to.

Immunogold labelling for the β2/3 subunits was widely distributed in the SNc and SNr (Figs 1 and 2). Labelling was closely associated with membranes and was also localized at intracellular sites. The intracellular gold particles were associated with a variety of organelles, including membrane-bound organelles (Fig. 1A), mitochondria (Fig. 1A and E), multivesicular bodies (Fig. 2D) and the nuclear envelope (not shown). Although the intracellular labelling accounted for nearly 60% of the immunogold particles (Table 2), in contrast to the labelling observed at synapses (discussed later), it most commonly occurred as single gold particles and associations of two or more particles were only rarely observed. These gold particles probably include both specific labelling associated with the synthetic and transport machinery of the receptor, as well as nonspecific labelling. Over 41% of immunogold particles were associated with the plasma membrane, i.e. either touching the membrane or within about one gold particle's diameter of it. Of the membrane-associated gold particles, over 42% (Table 2) were localized at symmetrical synapses where they were often lined up along the synaptic specialization (Figs 1–5). In fact, labelling for the β2/3 subunits, as well as the other subunits, often indicated the presence of synaptic specializations even when the membranes were not clearly visible because the synapse was cut tangentially (Figs 1A, B, D and E, and 3B and 4A). The labelling associated with membranes at extrasynaptic sites usually consisted of isolated gold particles (Fig. 1B), although clusters of two or more particles were sometimes seen (Fig. 1B).

In the sections double-labelled for the β2/3 subunits of the $GABA_A$ receptor and for GABA itself, the receptor labelling was identified by the 10-nm gold particles and the GABA by 20-nm gold particles (Figs 1, and 3C and D). Consistent with other studies of GABA-immunolabelling in the SNc and SNr (for references see Smith *et al*., 1998), immunogold particles were widely distributed within the SN, but were mainly associated with axons and axon terminals forming symmetrical synapses (Figs 1, and 3C and D). The immunolabelling for GABA occurred as dense accumulations of gold particles over the terminals, often showing associations with the mitochondria (Figs 1, and 3C and D). Preterminal axons were also commonly labelled. The double-immunolabelling revealed that β2/3 subunit-positive synapses were mainly formed by GABA-positive boutons in both the SNc and SNr (Figs 1 and 3).

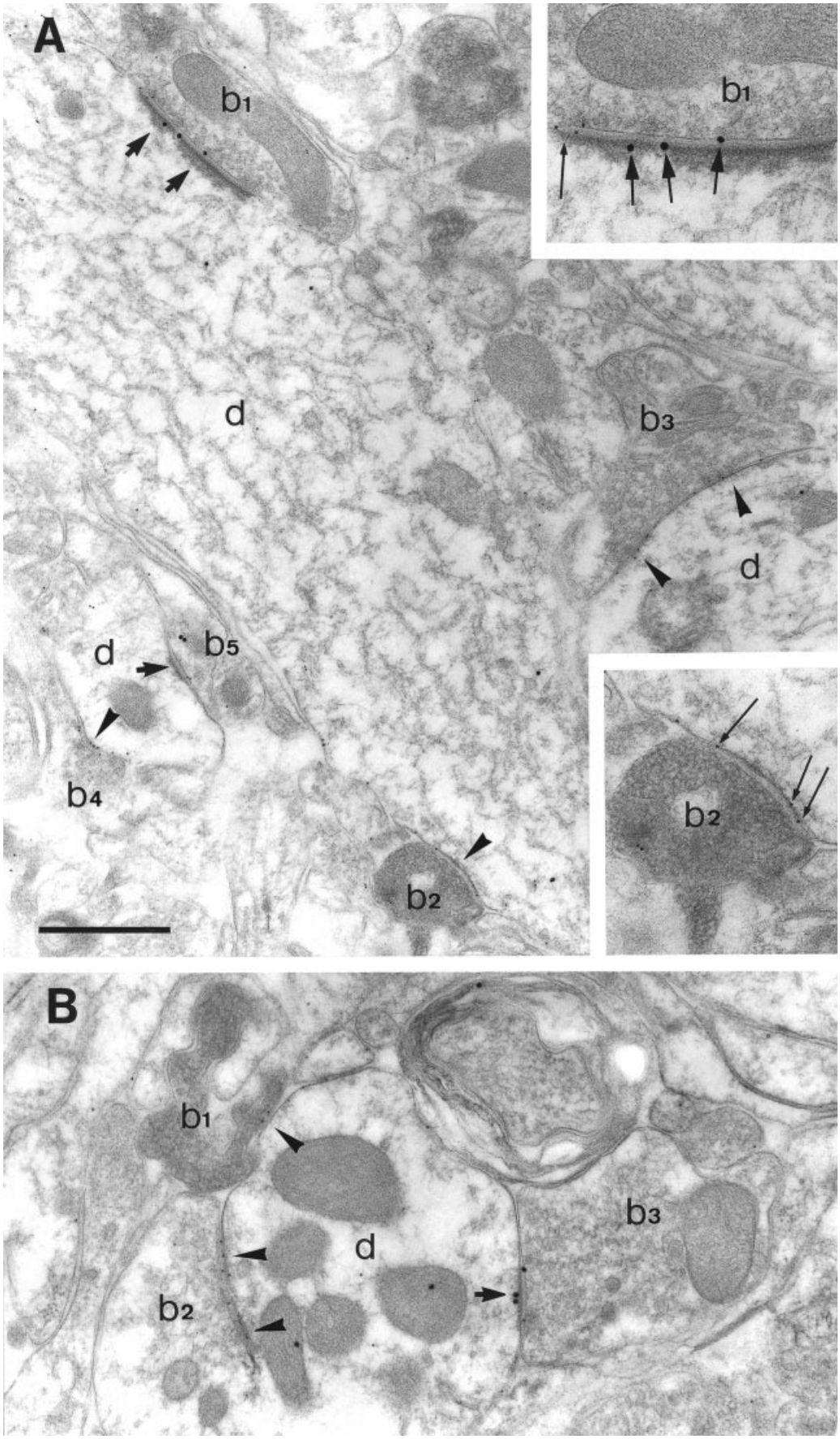
Morphological criteria and the GABA-immunolabelling revealed two classes of terminals forming β2/3 subunit-positive synapses. The first type was of medium size (≈ 0.5–1.0 μm diameter), possessed many vesicles, occasionally contained a single mitochondrion, was often interdigitated with other structures and usually possessed a single active zone (Figs 1A–D and 4B–D). In the SNr, this type of terminal commonly ensheathed dendritic shafts (Fig. 1C), but was more dispersed in the SNc. This same class of terminal was also identified in the triple-labelling studies (discussed later). The synaptology, and the morphological and chemical characteristics of these terminals are consistent with the features of terminals derived from the striatum (see Smith *et al*., 1998). The second class of GABA-positive terminal forming receptor-positive synapses was larger (generally greater than 1 μm diameter), usually contained several mitochondria, often formed more than one active zone and contained relatively small numbers of vesicles that congregated at the active zone(s) (Fig. 1A and B). This class of terminal was less commonly observed in both SNc and SNr. The features of these terminals are similar to those of terminals derived from the GP (see Smith *et al*., 1998) and the local collaterals of neurons of the SNr (Damlama *et al*., 1993). Terminals forming asymmetric synapses were also identified in both the SNc and SNr (Figs 2A and C, 3C, 4B and 5). These did not possess immunolabelling for GABA and their synapses were only rarely associated with immunogold particles coding for the $GABA_A$ receptor (Figs 3C and 5A).

TABLE 3. Synaptic labelling for β2/3 subunits of the $GABA_A$ receptor and GluR2/3 subunits of the AMPA receptor in the substantia nigra following quinolinic acid lesions of the striatum

	$GABA_A$ β2/3		GluR2/3	
	Particles per synapse	*n*	Particles per synapse	*n*
Rat 1				
Lesion	8.53 ± 6.69*	106	1.4 ± 0.56	30
Control	4.01 ± 2.12	86	1.77 ± 1.22	30
Rat 3				
Lesion	7.06 ± 3.81*	161	1.73 ± 0.94	45
Control	5.05 ± 3.2	162	1.79 ± 1.02	38

The figures represent the mean (± SD) of the number of gold particles coding for the β2/3 subunits of the $GABA_A$ receptor or the GluR2/3 subunits of the AMPA receptor at individual synapses in random analyses of the substantia nigra ipsilateral (lesion) and contralateral (control) to the quinolinic lesion of the striatum. *$P < 0.0001$, lesion side vs. control side (Mann–Whitney *U*-test). *n*, number of synapses analysed.

FIG. 4. Triple-immunolabelling for α1 (20-nm gold; large arrows), β2/3 (10-nm gold; medium arrows) and γ2 (5-nm gold; small arrows) subunits of the $GABA_A$ receptor in the substantia nigra (SN) following quinolinic acid lesions of the striatum. (A) Three boutons apposed to (b_1), or in synaptic contact (b_2 and b_3) with, dendritic shafts (d). Bouton b_1 is an electron-dense degenerating bouton derived from the striatum. It is almost engulfed by glial elements and no synapse is visible. Bouton b_2 does not show signs of degeneration. It forms a synapse (arrowhead) that is cut obliquely and is immunolabelled for the α1, β2/3 and γ2 subunits, as revealed by the three sizes of gold particles (indicated by the three sizes of arrow in the upper right inset). Bouton b_3 is also not degenerating and forms a synapse (arrowhead; lower left inset) that is positive for α1 and β2/3 subunits. (B) A degenerating bouton (b_1) derived from the striatum forms synaptic contacts (arrowheads) with two dendrites (d_1 and d_2). Both synapses are immunolabelled for α1, β2/3 and γ2 subunits, as indicated by the three sizes of immunogold particles (see insets). Bouton b_2 forms an asymmetric receptor-negative synapse (arrow) with dendrite d_2. (C) Two degenerating boutons (b_1 and b_2) that are derived from the striatum form receptor-positive synapses with a dendrite (d). The bouton on the right (b_2, inset) is immunolabelled for all three subunits, as indicated by the three sizes of gold particles. (D) Bouton b_1 is a partially engulfed, degenerating bouton derived from the striatum. It is apposed to a dendritic shaft (d), but the synaptic specialization is not clear in this section. The apposition is associated with the three sizes of gold particles (three arrow sizes) indicating the presence of α1, β2/3 and γ2 subunits (inset). Bouton b_2 forms a synapse (arrowhead) that is positive for the β2/3 subunits and a single 5-nm gold particle (small arrow) indicates the γ2 subunit. Scale bar, 0.5 μm for the main micrographs and 0.25 μm for the insets. The bar in A applies to all micrographs.


A
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Localization of α1 and γ2 subunits

Immunolabelling for the α1 and the γ2 subunits of the $GABA_A$ receptor was observed in both the SNc and SNr. The labelling was not as robust as that obtained with the antibodies against the β2/3 subunits and the labelling for the α1 was stronger and more consistent in the SNr than in the SNc. As with the labelling for the β2/3 subunits, labelling for the α1 and γ2 subunits occurred both on membranes and at intracellular sites, however, the most prominent labelling, in the form of groups of immunogold particles, occurred at symmetrical synapses mainly involving dendrites.

Triple-labelling experiments for the α1, β2/3 and γ2 subunits with three different sizes of gold particles revealed the colocalization of $GABA_A$ receptor subunits at individual symmetrical synapses (Fig. 2). All combinations of labelled synapses were observed. The most common forms of labelling, however, were the synapses that were double-labelled for the α1 and β2/3 subunits and synapses that were triple-labelled for the α1, β2/3 and γ2 subunits. Triple-labelling occurred at synapses formed by both the large- and medium-sized terminals in both the SNc (Fig. 2A) and SNr (Fig. 2B and D). Synapses labelled for β2/3 and γ2 subunits were less common and α1- and γ2-labelled synapses were not observed. Synapses singly labelled with each of the antibodies were also observed. Because of the variability in 'robustness' of immunolabelling between the different antibodies, no attempt was made to quantify the degree of colocalization of the different subunits.

Quinolinic acid lesions of striatum

The deposits of QA in the striatum resulted in a marked loss of neurons in the striatum as revealed by the Nissl-stained sections (data not shown). The SN from the four rats that received lesions was prepared for postembedding immunolabelling by the freeze-substitution, Lowicryl embedding method. The blocks of SN from two of these (R1 and R3) were sufficiently well-preserved, and antigenicity sufficiently well-maintained, to enable postembedding immunolabelling of receptors and ultrastructural analysis to be performed. In rat R1, the lesion occupied the dorsal half of the striatum rostral to the GP and caudal to the nucleus accumbens with damage to the overlying cortex due to the needle penetration. In rat R3, the lesion was larger, occupying the dorsal two-thirds of the striatum and extending to the rostral striatum overlying the nucleus accumbens; a larger volume of overlying cortex was also damaged. As far as we could tell the lesion did not encroach upon the GP but because the GP was prepared by the freeze-substitution, Lowicryl embedding method, we cannot be certain that the rostral pole was not affected by the injection. The blocks of SN included both SNc and SNr; only where a clear distinction could be made between the two divisions on the basis of morphology and synaptology was a structure ascribed to one region or the other. The quantitative data are thus derived from both SNc and SNr.

In the electron microscope, sections of SN ipsilateral to the lesions were similar in appearance to sections from the contralateral SN except that a proportion of axons and terminals forming symmetrical synapses exhibited characteristic signs of degeneration (Figs 3–5) and thus were likely to be derived from the striatum, although we cannot exclude the possibility of transneuronal degeneration (DeGiorgio *et al*., 1998). The axoplasm was darkened and amorphous. In the early stages of degeneration, vesicles and mitochondria showed an altered morphology and synaptic specializations were still visible (Figs 3, and 4B and C). At the later stages, vesicles and mitochondria disappeared, the structures became engulfed by glial cells and synaptic specializations were no longer visible (Fig. 4A and D). Degenerating axons and terminals were only rarely observed in the contralateral SN. Although not quantified, no evidence of increased tortuousity of synapses formed by degenerating or intact synapses was noted.

Postembedding labelling of the ipsilateral SN for the β2/3 subunits of the $GABA_A$ receptor revealed labelling at synapses formed by degenerating axon terminals (Figs 3 and 5), thus demonstrating that terminals in the SN that are derived from the striatum give rise to synapses that are positive for β2/3 subunits of the $GABA_A$ receptor. Furthermore, triple-labelling for the α1, β2/3 and γ2 subunits revealed the presence of degenerating striatal terminals that gave rise to synapses that were positive for all three subunits of the $GABA_A$ receptor, as well as double- and single-labelled synapses (Fig. 4B–D). Double-immunolabelling for β2/3 subunits and for GABA revealed that at least some of the degenerating striatal terminals were immunolabelled for GABA, albeit at lower levels than in nondegenerating terminals (Fig. 3C and D).

QA lesions of the striatum have been reported to lead to the upregulation of $GABA_A$ receptors in the SN (Nicholson *et al*., 1995,

TABLE 4. Density of synaptic labelling for β2/3 subunits of the $GABA_A$ receptor at striatal synapses in the substantia nigra following quinolinic acid lesions of the striatum.

	Width of synapse (μm)	Gold (particles per synapse)	Density of labelling (particles/μm)	*n*
Lesion	0.33 ± 0.16	7.98 ± 4.3	25.3 ± 9.8	65
Control	0.31 ± 0.09	5.2 ± 3.3*	17.2 ± 10.5*	29

Labelling for β2/3 subunits of the $GABA_A$ receptor at individual synapses in the substantia nigra ipsilateral (lesion) and contralateral (control) to the quinolinic acid lesions of the striatum (pooled data from rats 1 and 3). The figures represent the mean (± SD) of the width (μm), the level of labelling, as indicated by the number of gold particles and the density of labelling (particles/μm) at labelled synapses. The data were obtained from the same series of random micrographs used for the analysis in Table 2 and the analysis was confirmed to striatal-like boutons. *$P < 0.001$, lesion side vs. control side (Mann–Whitney *U*-test). *n*, number of synapses analysed.

FIG. 5. Double-immunolabelling for the β2/3 subunits of the $GABA_A$ receptor (10-nm gold) and GluR2/3 subunits of the AMPA receptor (20-nm gold) in the substantia nigra (SN) following quinolinic acid lesions of the striatum. (A) A dendritic shaft (d) receives synaptic input from two boutons. Bouton b_1 forms an asymmetric synapse (arrows) that is positive for the GluR2/3 subunits of the AMPA receptor (large arrows in upper right inset). Note the cluster of three 10-nm gold particles coding for the $GABA_A$ receptor (small arrow in the inset) associated with this synapse. Bouton b_2 is a degenerating bouton that is derived from the striatum. The symmetrical synapse it forms with the dendrite (arrowhead) is positive for the β2/3 subunits of the $GABA_A$ receptor (medium arrows, bottom right inset). Three other boutons make synapses on other dendritic profiles; b_3 and b_4 form symmetrical synapses (arrowheads) that are positive for the β2/3 subunits of the $GABA_A$ receptor and b_5 forms an asymmetric synapse (arrow). This asymmetric synapse has two immunogold particles coding for the β2/3 subunits at the periphery of the synaptic specialization. (B) A dendritic shaft (d) receives synaptic input from three boutons, b_1 is degenerating and forms a β2/3 subunit-positive symmetric synapse (arrowhead); b_2 is not degenerating but also forms a β2/3 subunit-positive symmetric synapse (arrowheads); and b_3 forms an asymmetric synapse (arrow) that is positive for the GluR2/3 subunits of the AMPA receptor. Note the single small gold particle coding for β2/3 subunits at the periphery of the synapse. Scales bar, 0.5 μm for the main micrographs and 0.375 μm for the insets. The bar in A also applies to B.

1996). Analysis of the distribution of the immunolabelling for β2/3 subunits of the $GABA_A$ receptor revealed that there was an altered distribution of labelling in the control vs. lesioned hemispheres. Fifty-five to 63% of immunoparticles were associated with plasma membranes in the SN ipsilateral to the QA lesions, as opposed to 41–42% in the SN on the nonlesioned side (Table 2). Synaptic labelling, as a proportion of total labelling, was also greater on the lesioned side as opposed to the control side, although the synaptic labelling as a proportion of membrane labelling was variable (Table 2).

In order to determine whether the altered distribution of labelling in favour of membranes and synapses was reflected in an increase in the level of immunolabelling at *individual synapses*, the number of immunogold particles at synapses in SN on the ipsilateral and contralateral side were counted in systematic scans in the electron microscope. To control for variability of immunolabelling between different blocks and between the two hemispheres, the sections were double-labelled for β2/3 subunits of the $GABA_A$ receptor and for the GluR2/3 subunits of the AMPA receptor (Fig. 5). In these sections, immunolabelling for the GluR2/3 subunits of the AMPA receptor occurred primarily at asymmetrical synapses, as described previously (Chatha *et al*., 2000). There was a significantly greater number of immunogold particles coding for the $GABA_A$ receptor at symmetrical synapses in the SN on the lesioned compared with the nonlesioned side. In contrast, there were no significant differences between the ipsilateral and contralateral SN in the number of gold particles coding for the AMPA receptor at asymmetrical synapses (Table 3).

In order to determine whether the increased labelling at symmetrical synapses was due to an alteration in size of the active zone, the density of immunolabelling at symmetrical synapses was estimated. This was carried out on the same micrographs that had been used for the analysis of the overall distribution of immunogold particles. The analysis was confined to terminals that had morphological features of striatal terminals and revealed that the mean width of immunopositive symmetrical synapses was not significantly different between the SN ipsilateral and contralateral to the QA lesion of the striatum. In contrast, the number of gold particles and density of immunolabelling was significantly greater in labelled synapses in the SN on the lesioned side compared with the control side (53.5 and 47.1% increase, respectively) (Table 4). There was a much lower density of large terminals that were probably derived from the GP or from interneurons, which meant there were insufficient to allow statistical analysis.

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 formed by specific populations of axon terminals in the SN of the rat. The findings demonstrate first, in agreement with previous studies (Nicholson *et al*., 1992, 1995, 1996; Fritschy & Mohler, 1995; Pirker *et al*., 2000; Schwarzer *et al*., 2001), that immunolabelling for the α1, β2/3 and γ2 subunits of the $GABA_A$ receptor is widely distributed in the SN. A high proportion of immunogold particles labelling the β2/3 subunits is located on the plasma membrane, about half of which is located at symmetrical synaptic specializations, and immunolabelling for all three subunits colocalizes at individual synapses. Second, they demonstrate that the terminals forming the $GABA_A$ receptor-positive synapses are enriched in GABA and that at least some of them are derived from the striatum. Third, they demonstrate that the previously reported upregulation of $GABA_A$ receptors that occurs following lesions of the striatum leads to increased labelling for the β2/3 subunits at individual synapses. The findings suggest, therefore, that (i) fast GABAergic transmission mediated by $GABA_A$ receptors containing α1, β2/3 and/or γ2 subunits occurs primarily at synapses within the SN, (ii) the GABAergic boutons giving rise to receptor-positive synapses are, at least in part, derived from the striatum and (iii) the upregulation of GABA receptors that occurs in the QA model of Huntington's disease is likely to lead to an increased efficiency of transmission at individual GABAergic synapses.

Subcellular distribution of immunolabelling for $GABA_A$ receptor subunits

The most prominent immunolabelling for $GABA_A$ receptor subunits occurred at symmetrical axodendritic synapses in both the SNc and SNr. The immunogold particles often lined up along the synaptic specialization, and although we cannot define the localization as pre- or postsynaptic using the current method, the results of previous studies imply that they are primarily associated with the postsynaptic membrane. The concentration of immunogold particles at these sites was higher than at other sites. Thus, in the SN contralateral to the QA lesion of the striatum, about 20% of total immunogold particles labelling β2/3 subunits were associated with synaptic specializations. This figure is consistent with findings of the distribution of immunolabelling for $GABA_A$ receptor subunits in the striatum (Fujiyama *et al*., 2000) and of other ionotropic receptor subunits in the SN (Chatha *et al*., 2000). The findings indicate a selective enrichment of receptors at synapses because the area in a micrograph, or the volume in the tissue, occupied by symmetrical synaptic specializations, as a proportion of total, is very low. We conclude therefore, that fast GABAergic transmission in the SN mediated by $GABA_A$ receptors occurs primarily at symmetrical synaptic specializations. The immunolabelling occurring at nonsynaptic sites on the membrane may also represent functional receptors. The labelling at intracellular sites is likely to represent receptors in the process of synthesis, degradation and/or trafficking. The significance of labelling at asymmetrical synapses remains to be established and the occasional immunogold particles associated with mitochondria is likely to be an artefact. The triple-immunolabelling for the α1, β2/3 and γ2 subunits confirms the presence of these subunits in the SN. Although we cannot determine the subunit composition of an individual receptor using the present method, colocalization of α1, β2/3 and γ2 subunits at *individual* symmetrical synapses in both the SNc and SNr suggests that GABAergic transmission at individual synapses occurs via receptors containing these subunits.

Characterization of the axon terminals presynaptic to $GABA_A$ receptor-positive synapses

Three methods were used to characterize the boutons forming the receptor-positive synapses. First, on the basis of morphological criteria, we identified at least two populations of terminals that formed the receptor-positive synapses, i.e. those with the morphological features of terminals derived from the striatum and those with the morphological features of terminals derived from the GP and/or local collaterals of SNr neurons (see Smith *et al*., 1998). Synapses formed by both of these classes of terminals were immunolabelled for the α1, β2/3 and/or γ2 subunits. Second, the combination of GABA-immunolabelling with immunolabelling for the β2/3 subunits revealed that that the majority of axon terminals forming receptor-positive synapses express relatively high levels of GABA. Third, by anterograde degeneration, we demonstrated that at least some of the GABA-positive boutons that form synapses positive for the β2/3

subunits of the $GABA_A$ receptor are derived from the striatum. Furthermore, we demonstrated that axon terminals derived from the striatum form synapses that express α1, β2/3 and/or γ2 subunits. Thus, GABAergic transmission at striatonigral terminals is likely to occur at synapses that express these subunits of the $GABA_A$ receptor. It should be noted that excitotoxic lesions of the striatum cause transneuronal degeneration of neurons in the SNr, thus at least some of the degenerating terminals that we observed may be derived from the collaterals of SNr neurons (DeGiorgio *et al*., 1998).

The effect of quinolinic acid lesions of the striatum on $GABA_A$ receptor subunit labelling in the SN

In Huntington's disease or the QA lesion model of the disease, there is an upregulation of mRNA for $GABA_A$ receptor subunits, an upregulation of the proteins and an upregulation of $GABA_A$ binding sites in the SN and internal segment of the GP (GPi, or entopeduncular nucleus), i.e. the major targets of the 'direct' striatofugal projections (see Introduction). The use of immunogold labelling enabled us to determine whether there were alterations in receptor labelling at specific subcellular sites in the SN after the striatal lesion and whether the upregulation of receptors is reflected in increased labelling at synapses that may underlie a functional change in GABAergic transmission in the SN. In qualitative terms, a similar pattern of labelling was observed at synapses in the SN on the ipsilateral and contralateral sides. Thus, degenerating terminals derived from the striatum, nondegenerating striatal-like terminals and terminals presumed to be derived from the GP or collaterals of SNr neurons expressed GABA-immunolabelling and their synapses were positive for the β2/3 subunits of the $GABA_A$ receptor. Similarly, at least some examples of each class of terminal were found to form synapses that were positive for all three of the antibodies recognizing α1, β2/3 and γ2 subunits. The quantitative analysis revealed that a higher proportion of immunolabelling was associated with the plasma membrane on the lesioned side. This finding is consistent with the reports of increased binding sites for GABA in the SN following striatal lesions (Pan *et al*., 1983, 1984; Corda *et al*., 1986; Nicholson *et al*., 1995, 1996) and the indirect data derived from an *in vitro* expression system (Sanna *et al*., 1998). The main finding of this part of the study was, however, that the level of labelling for the β2/3 subunits of the $GABA_A$ receptors was greater at symmetrical synapses in the SN ipsilateral than in the SN contralateral to the QA-lesioned striatum. The increased labelling was unlikely to be due to general differences in the preservation of antigenicity in the blocks of the SN ipsilateral and contralateral to the lesion, nor to a generalized increase in receptor labelling, as there was no difference in labelling for the GluR2/3 subunits of the AMPA receptor at asymmetrical synapses in the same sections. Furthermore, increased labelling at synapses is unlikely to be due to an increase in the size of the active zone of the synapse and consequent increase in number of receptors, as the mean width of striatal-like synapses was not significantly altered by the lesion. Thus, the changes that we observed are likely to be a reflection of an increased *density* of receptors at individual synapses.

The majority of synapses that were analysed were formed by apparently intact axonal boutons and part of the analysis was confined to terminals with a morphology typical of striatal terminals (see Smith *et al*., 1998). Thus, QA lesions of the striatum, which result in striatal cell death and degeneration of striatonigral axons, lead to an upregulation of $GABA_A$ receptors at synapses formed by intact GABAergic terminals. Thus, the plastic response of neurons of the SN to a reduction in GABAergic input from the striatum is an increase in the number of $GABA_A$ receptors at the remaining, intact, striatonigral (and possibly other) GABAergic synapses. Because differences in receptor numbers at GABAergic synapses on cerebellar stellate cells have been proposed to underlie variations in the amplitude of the response to GABA (Nusser *et al*., 1997), it is likely that the increased receptor labelling at synapses in the SN observed in the present study will lead to increased efficiency of GABA transmission at these synapses. Indeed, in other lesion models, receptor upregulation is associated with increased responsiveness. For instance, the unilateral 6-hydroxydopamine lesion model of Parkinson's disease is associated with an upregulation of mRNAs for subunits of the $GABA_A$ receptor in the SN (Chadha *et al*., 2000a, b) and an increased responsiveness of neurons in the SNr to striatal stimulation (MacLeod *et al*., 1990). Similarly, sectioning of the sciatic nerve leads to a selective upregulation of AMPA receptors at primary afferent synapses in the substantia gelatinosa and an increased excitability of neurons in this region (Popratiloff *et al*., 1998). We conclude therefore, that one of the compensatory responses of SN neurons to a reduction in GABAergic input is likely to be increased efficiency of transmission at remaining GABAergic synapses.

Conclusions

The present findings in the rat suggest that the upregulation of GABA receptors that occurs in Huntington's disease (see Introduction for references) is likely to be associated with increased expression of receptors at intact GABAergic synapses and hence increased efficiency of transmission at these synapses. Increased efficiency of GABAergic transmission at striatonigral and at pallidonigral synapses (and possibly also at GABAergic synapses in the GPi), together with the loss of striatal terminals, will lead to inappropriate inhibition of basal ganglia output neurons and hence inappropriate disinhibition of basal ganglia targets. Indeed, striatal lesions have been reported to alter the pattern and rate of firing in the GP and entopeduncular nucleus of the cat (Levine *et al*., 1982; Sachdev *et al*., 1989). Increased and/or inappropriate disinhibition of basal ganglia targets have been proposed to underlie the abnormal involuntary movements associated with Huntington's disease (Albin *et al*., 1989; DeLong, 1990). The altered levels of $GABA_A$ receptors at synapses in the output nuclei of the basal ganglia may, in part, underlie the motor abnormalities of Huntington's disease.

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Abbreviations

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxaline propionic acid; GABA, γ-aminobutyric acid; GP, globus pallidus; GPi, internal segment of the globus pallidus; IgG, immunoglobulin G; PB, phosphate buffer; PBS, phosphate-buffered saline; QA, quinolinic acid; SN, substantia nigra; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; TBS, Tris-buffered saline; TBST, TBS containing 2% human serum albumin and 0.01% Triton X-100.

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