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Subcellular and subsynaptic distribution of the NR1 subunit of the NMDA receptor in the neostriatum and globus pallidus of the rat: co-localization at synapses with the GluR2/3 subunit of the AMPA receptor

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Abstract

Glutamatergic neurotransmission in the neostriatum and the globus pallidus is mediated through NMDA-type as well as other glutamate receptors and is critical in the expression of basal ganglia function. In order to characterize the cellular, subcellular and subsynaptic localization of NMDA receptors in the neostriatum and globus pallidus, multiple immunocytochemical techniques were applied using antibodies that recognize the NR1 subunit of the NMDA receptor. In order to determine the spatial relationship between NMDA receptors and AMPA receptors, double labelling was performed with the NR1 antibodies and an antibody that recognizes the GluR2 and 3 subunits of the AMPA receptor.

In the neostriatum all neurons with characteristics of spiny projection neurons, some interneurons and many dendrites and spines were immunoreactive for NR1. In the globus pallidus most perikarya and many dendritic processes were immunopositive. Immunogold methods revealed that most NR1 labelling is associated with asymmetrical synapses and, like the labelling for GluR2/3, is evenly spread across the synapse. Double immunolabelling revealed that in neostriatum, over 80% of NR1-positive axospinous synapses are also positive for GluR2/3. In the globus pallidus most NR1-positive synapses are positive for GluR2/3. In both regions many synapses labelled only for GluR2/3 were also detected.

These results, together with previous data, suggest that NMDA and AMPA receptor subunits are expressed by the same neurons in the neostriatum and globus pallidus and that NMDA and AMPA receptors are, at least in part, colocalized at individual asymmetrical synapses. The synaptic responses to glutamate in these regions are thus likely be mediated by both AMPA and NMDA receptors at the level of individual synapses.

Introduction

Glutamate is the major neurotransmitter of cortical and thalamic afferents of the basal ganglia and of the intrinsic basal ganglia neurons in the subthalamic nucleus (see reviews Smith & Bolam, 1990; Gerfen & Wilson, 1996; Smith *et al.*, 1998). In addition to its physiological role in neurotransmission, glutamate may also play pathological roles in the cell death observed in neurodegenerative diseases like Huntington's disease and Parkinson's disease (Beal *et al.*, 1991; Albin & Greenamyre, 1992; Young, 1993). Indeed, overactivity of the glutamatergic projection from the subthalamic nucleus to the output nuclei of the basal ganglia, and possibly of the glutamatergic corticostriatal projection, are associated with the symptoms of Parkinson's disease (Bergman *et al.*, 1990; Lindefors & Ungerstedt, 1990; Pollak *et al.*, 1993).

The actions of glutamate are mediated by two types of receptors, the ionotropic receptors and the metabotropic receptors. Ionotropic receptors are further subdivided, on the basis of selective agonists, into α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, *N*-methyl-D-aspartate (NMDA) receptors and kainate receptors. The genes coding the four subunits (GluR1–4) of the AMPA receptor have been cloned. These subunits combine in an hetero-oligomeric complex to form a functional receptor, although each subunit has been reported to be functional in the homomeric form in transfected cells (Boulter *et al.*, 1990; Keinänen *et al.*, 1990; Nakanishi *et al.*, 1990; Sommer *et al.*, 1990; Hollmann *et al.*, 1991; Hume *et al.*, 1991; Gasic & Hollmann, 1992; Keller *et al.*, 1992; Brose *et al.*, 1994; Hollmann & Heinemann, 1994; Huntley *et al.*, 1994; Puchalski *et al.*, 1994; Wenthold *et al.*, 1996). Five subunits of the NMDA receptor have been identified: NR1 and NR2A–NR2D and alternative

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splicing generates eight receptor isoforms (Nakanishi *et al.*, 1992; Durand *et al.*, 1993; Hollmann *et al.*, 1993). Functional NMDA receptors comprise both NR1 and NR2 subunits, although NR1 subunits may form a functional homomeric receptor channel when expressed *in vitro* (Moriyoshi *et al.*, 1991; Nakanishi *et al.*, 1992; Yamazaki *et al.*, 1992; Sucher *et al.*, 1996).

Anatomical, physiological and pharmacological studies have demonstrated that AMPA and NMDA receptors are widely expressed in the neostriatum and globus pallidus (Galli *et al.*, 1992; Soltis *et al.*, 1994; Maione *et al.*, 1995; Chen *et al.*, 1996; Kita, 1996; Bernard *et al.*, 1997; Götz *et al.*, 1997). Indeed, responses in the basal ganglia to stimulation of excitatory afferents or the administration glutamate receptor agonists, involve both AMPA and NMDA receptors. Stimulation of AMPA receptors in striatal and pallidal neurons results in fast EPSPs, whereas the stimulation of NMDA receptors is considered as a mechanism to modulate the fast component of the glutamate-induced excitatory postsynaptic potential (EPSP) (Soltis *et al.*, 1994; Götz *et al.*, 1997). The possible cellular and subcellular sites of interaction between these two classes of receptors in the neostriatum and globus pallidus are unknown.

We have recently demonstrated that all medium spiny neurons and most interneurons in the neostriatum express immunoreactivity and possess mRNAs for at least one subunit of the AMPA receptor and the NR1 subunit of the NMDA receptor (Lannes *et al.*, 1995; Bernard *et al.*, 1996, 1997). Moreover, all neurons in the globus pallidus express the NR1 subunit (mRNAs and immunoreactivity) (Petralia *et al.*, 1994; Standaert *et al.*, 1994) and a high proportion expresses AMPA receptor subunit mRNAs and immunoreactivity (Martin *et al.*, 1993; Sato *et al.*, 1993; Bernard *et al.*, 1996; Paquet *et al.*, 1997). These observations suggest that the two classes of ionotropic receptors colocalize in the majority of neurons in the neostriatum and in at least a subpopulation of neurons in the globus pallidus. We have recently demonstrated that immunoreactivity for subunits of the AMPA receptor is mainly located in the postsynaptic membrane of asymmetric synapses in the neostriatum (Bernard *et al.*, 1997). Little is known about the NMDA receptors in this respect. We have also shown that subunits of AMPA and NMDA receptors colocalize at individual synapses in the subthalamic nucleus and entopeduncular nucleus (Clarke & Bolam, 1998). The first objective of the present study was to determine the cellular, subcellular and subsynaptic localization of immunoreactivity for the NR1 subunit of the NMDA receptor in the neostriatum and globus pallidus. The second objective was to examine the anatomical basis of the interaction between NMDA and AMPA receptors in the neostriatum and globus pallidus by determining whether NMDA receptor and AMPA receptor subunits are colocalized at individual synapses.

Materials and methods

Animals and tissue preparation

Wistar rats (Charles River, Margate, Kent; 200–250 g) were used in this study. 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 in accordance with the European Communities Council Directive (80/609/EEC). They were deeply anaesthetized with sodium pentobarbitone (Sagatal, Rhône Mérieux, Tallaght, Dublin, Ireland; 60 mg/kg, i.p.) and then perfused transcardially with 50–100 mL of 0.9% NaCl followed by 250 mL of fixative consisting of 3% paraformaldehyde with 0.2% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4), and then with 100 mL of 3% paraformaldehyde alone, at a rate of about 15 mL/min. The brain was quickly removed and sections from neostriatum were cut on a vibrating microtome at about 70 μm and collected in phosphate-buffered saline (PBS, 0.01 M phosphate, pH 7.4). In order to enhance the penetration of the immunoreagents in the pre-embedding procedures, the sections were equilibrated in a cryoprotectant solution (PB 0.05 M, pH 7.4, containing 25% sucrose and 10% glycerol) and freeze-thawed by freezing in isopentane (BDH Chemicals, Letterworth, Leicestershire, UK) that had been cooled in liquid nitrogen and then in liquid nitrogen and thawing in PBS (von Krosigk & Smith, 1991). The sections were then preincubated in 4% normal goat serum (NGS) in PBS for 30 min at room temperature.

Immunohistochemistry

Immunoreactivity for the NR1 subunit of the NMDA receptor was detected using two antibodies: a monoclonal antibody raised in mouse [mNR1 (MAB363); Pharmingen, Becton and Dickenson, Oxford, UK] and a polyclonal antibody raised in rabbit [rNR1 (AB1516); Chemicon International Ltd, Harrow, UK]. The mNR1 antibody was raised against a recombinant fusion protein containing the amino acids 660–811 of the NR1 subunit, and thus recognizes all eight splice variants of NR1 (Siegel *et al.*, 1994). The rNR1 antibody was selective for splice variants NR1-1a, NR1-1b, NR1-2a, NR1-2b (Petralia *et al.*, 1994). The GluR2/3 AMPA receptor subunits were detected using a polyclonal antibody raised in rabbit [GluR2/3 (AB1506); Chemicon]. These antibodies were obtained against synthetic peptides derived either from an intracellular loop between putative transmembrane regions III and IV of NR1 (mNR1: Siegel *et al.*, 1994), or the C-terminus part of the NR1 subunit (rNR1: Petralia *et al.*, 1994) or from intracellular sequences of the GluR2 subunit (GluR2/3: Wenthold *et al.*, 1992). The anti-GluR2/3 antibody recognizes GluR2 and GluR3 subunits of the AMPA receptor. The antibodies have been characterized and widely used for immunohistochemical studies [mNR1 (Huntley *et al.*, 1994; Siegel *et al.*, 1994; Farb *et al.*, 1995); rNR1 (Petralia *et al.*, 1994; Farb *et al.*, 1995; Gracy & Pickel, 1995); GluR2/3 (Tachibana *et al.*, 1994; Matsubara *et al.*, 1996; Popratiloff *et al.*, 1996; Wenthold *et al.*, 1996; Bernard *et al.*, 1996, 1997)] and the specificity has been described in detail (Wenthold *et al.*, 1992).

Pre-embedding immunoperoxidase method

The sections were incubated for 15 h at room temperature with constant gentle shaking in primary antibody solutions (mNR1, rNR1 or GluR2/3 at a concentration of 0.4 μg/mL, 1 μg/mL and 1 μg/mL, respectively) diluted in PBS that was supplemented with 1% NGS. They were then washed (3 × PBS) and incubated in biotinylated goat antirabbit or goat antimouse IgGs (1:100, Vector, Peterborough, UK) for 1.5 h at RT. The sections were then washed (3 × PBS) and incubated in an avidin–biotin–peroxidase complex (ABC), (1:100, Vector) for 1.5 h at RT. After washing with 2 × PBS and 1 × Tris buffer (TB) 0.05 M, pH 7.6, the immunoreactive sites were revealed by incubation in H_2O_2 (0.0048%) in the presence of 3,3′-diaminobenzidine (DAB; Sigma, Poole, Dorset, UK; 0.05% in TB). The reaction was stopped by several washes in TB.

Pre-embedding immunogold method

The pre-embedding immunogold method was carried out as previously described (Baude *et al.*, 1993; Yung *et al.*, 1995; Bernard *et al.*, 1997). Briefly, the sections were incubated in primary antibody solutions as described above. After washing [2 × PBS, 2 × PBS supplemented with 0.5% bovine serum albumin and 0.1% gelatin (PBS-BSA)], they

were incubated in goat antirabbit or goat antimouse IgGs conjugated to colloidal gold (1.4 nm diameter; Nanoprobes, Stony Brook, NY, USA; 1:100 in PBS-BSA) for 2 h at RT. The sections were then washed (2 × PBS-BSA, 2 × PBS) and postfixed in 1% glutaraldehyde in PBS for 10 min. After washing (2 × PBS; 2 × sodium acetate buffer, 0.1 M, pH 7.0), the colloidal gold labelling was intensified using a silver enhancement kit (HQ silver, Nanoprobes) for 3–5 min at RT in the dark. The sections were finally washed in acetate buffer and then in PB.

Preparation for electron microscopy

Immunoperoxidase- and immunogold-treated sections were postfixed in osmium tetroxide (1% in PB 0.1 M, pH 7.4) for 25 min for the DAB-reacted sections or 10 min for the immunogold-reacted sections at RT. After washing (3 × PB), they were dehydrated in an ascending series of dilutions of ethanol. Uranyl acetate (1%) was included in the 70% ethanol. They were then treated with propylene oxide (2 × 10 min) and equilibrated in resin overnight (Durcupan ACM, Fluka, Gillingham, Dorset, UK), mounted on glass slides and cured at 60 °C for 48 h. The sections were first examined in the light microscope. Areas of interest were sometimes photographed and were cut out from the slide and glued to blank cylinders of resin. Serial ultrathin sections were cut on a Reichert Ultracut E and collected on pioloform-coated single slot, copper or gold grids. The sections were stained with lead citrate and examined in a Philips CM10 electron microscope.

Post-embedding immunogold method

After perfusion as described above, 500-μm-thick slices of neostriatum and globus pallidus were cut on a vibrating microtome and were embedded in Lowicryl resin using the freeze substitution method as described by Baude *et al.* (1993) and Nusser *et al.* (1995a). Immunohistochemistry was carried out on ultrathin sections of the neostriatum collected on pioloform-coated single slot gold grids. The sections were incubated on drops of Tris-buffered saline (TBS) supplemented with 20% NGS for 45 min at RT, and then incubated for 15 h at RT on drops of primary antibody solutions (mNR1, rNR1, GluR2/3 at a concentration of 6.7 μg/mL, 2.5 μg/mL and 5 μg/mL, respectively, in TBS including 5% NGS). After several washes in TBS, the sections were incubated in goat antimouse IgG (GAM; for mNR1 incubated sections) or goat antirabbit IgG (GAR, for rNR1 and GluR2/3 incubated sections) conjugated to colloidal gold (1.4 nm, 1:100, Nanoprobes). They were then washed in PBS and postfixed in 2% glutaraldehyde in TBS for 2 min. They were washed at least three times in water and the labelling was silver-intensified using a silver enhancement kit (HQ Silver, Nanoprobes) for 5 min. After washing in water, they were incubated in 1% uranyl acetate in water for 25 min, washed in water, dried and stained with lead citrate.

Double detection of the NR1 and GluR2/3 subunits using the postembedding procedure

Two methods were used to determine whether the NR1 subunit of the NMDA receptor and the GluR2/3 subunit of the AMPA receptor are colocalized at synapses in the neostriatum and globus pallidus.

1 Double immunolabelling was carried out on the same ultrathin section using a mixture of anti-NR1 (mNR1) and anti-GluR2/3 antibodies. The sections were incubated on a drop of TBS supplemented with 20% NGS, then on drops of a cocktail of primary antibody solutions [mNR1 (6.7 μg/mL) + GluR2/3 (5 μg/mL)] in TBS with 5% NGS. After washing in TBS, the sections were incubated in a cocktail of GAM IgG conjugated to 10 nm colloidal gold (mNR1) and GAR IgG conjugated to 1.4 nm colloidal gold (GluR2/3) each at a dilution of 1:100 (Nanoprobes). The sections were then postfixed in glutaraldehyde, silver intensified and contrasted with uranyl acetate and lead citrate as described above. In order to control for the possibility that variability in the silver intensification procedure could give rise to false-positives in the double labelling, the diameter of the silver intensified gold particles labelling synapses were measured in the double-labelled sections and in sections labelled singly with the 1.4 nm or the 10 nm gold particles and plotted as a frequency distribution.

2 Pairs of adjacent sections collected on separate grids were treated for the single detection of the NR1 or the GluR2/3 subunits, secondary antibodies coupled to 1.4 nm gold immunoparticles and silver intensified as described above.

Controls of specificity of the immunolabelling

The specificity of the pre-embedding and postembedding labelling techniques was demonstrated by the absence of immunolabelling for the respective antigens when the primary antibody (single detection) or when one or both primary antibodies (double detection) were omitted.

Quantitative analysis

In order to estimate the proportion of asymmetrical axospinous synapses immunopositive for mNR1 or rNR1 (immunogold technique), we analysed two continuous strips of tissue (319 μm^2 each) in Lowicryl-embedded sections at a magnification of ×13 500, taken from two animals (one section per animal). A synapse was considered immunopositive when it was associated with two or more immunoparticles (Baude *et al.*, 1995; Popratiloff *et al.*, 1996); all positive synapses in the strip were evaluated in the analysis.

In order to quantify the proportion of NR1-immunopositive axospinous and axodendritic synapses that are also positive for GluR2/3 in the neostriatum, we analysed pairs of adjacent sections from two animals processed for the detection of NR1 or GluR2/3 using mNR1 or rNR1 and GluR2/3 antibodies. The NR1-immunopositive synapses (mNR1, $n = 49$; rNR1, $n = 67$) were identified and the same synapses were found on the adjacent GluR2/3-labelled section. The percentage of NR1-immunopositive synapses that were also immunopositive for GluR/3 was calculated. Quantification in the double immunolabelled sections (mNR1 and GluR2/3 antibodies) was performed by systematically scanning the sections and categorizing each labelled synapse (two or more immunoparticles) as GluR2/3-positive, NR1-positive or double labelled.

Quantitative analysis of the distribution of immunogold particles for the NR1 subunit and the GluR2/3 subunit along the synaptic membrane specialization of axospinous synapses was performed on electron micrographs of Lowicryl-embedded neostriatum. A total of 335 NR1 subunit-positive (1189 gold particles) and 314 GluR2/3 subunit-positive synapses (2080 gold particles) were analysed. The analysis was performed on synapses in the double immunolabelled adjacent sections. In systematic scans of the NR1-immunolabelled sections positive synapses were identified and photographed and then the same synapses were identified in the GluR2/3-immunolabelled sections; these synapses together with any other positive synapses in the same micrographs were included in the analysis. The distance of each immunoparticle from the nearest edge of the synapse was measured and normalized, to take into account different sizes of synapses. The data were expressed as the proportion of immunoparticles in five bins along the half width of the synapse.

Results

Light microscopic observations

Localization of the NR1 subunit of the NMDA receptor and the GluR2/3 subunit of the AMPA receptor in the neostriatum and globus pallidus

The neostriatum and the globus pallidus displayed immunoreactivity with the mNR1, rNR1 and GluR2/3 antibodies. The immunoperoxidase staining was homogeneous without obvious differences between the neostriatum and the nucleus accumbens and along the rostrocaudal and dorsoventral axes of the neostriatum and globus pallidus. In the neostriatum, the neuropil labelling was stronger for mNR1 and GluR2/3 than for rNR1. In both regions numerous immunolabelled perikarya and dendrites were detected with each of the antibodies (Fig. 1A–F). In the neostriatum, most of the perikarya that were immunopositive for mNR1, rNR1 and GluR2/3 were medium-sized with an unindented nucleus surrounded by a thin rim of cytoplasm (Fig. 1A,C,E). These characteristics are typical of medium-spiny neurons and indeed, almost all neurons with these characteristics displayed immunoreactivity for mNR1, rNR1 and GluR2/3. Immunoreactivity for mNR1 and rNR1 was also detected in some large-sized and medium-sized neurons with characteristics of aspiny interneurons in the neostriatum. Consistent with previous findings, GluR2/3 immunolabelling in neostriatum was also detected in some medium-sized, but not large, interneurons (Bernard *et al.*, 1997). In the globus pallidus immunostaining for rNR1, mNR1 and GluR2/3 was detected in perikarya and dendrites of most large-sized neurons in the globus pallidus (Fig. 1A–F). Similar patterns of labelling were detected by the pre-embedding immunogold method for each of the antibodies and in both regions although the intensity of labelling was lower. No labelling was seen in glial cells in either region in sections labelled by the peroxidase or immunogold methods.

Electron microscopic observations

Localization of the NR1 subunit of the NMDA receptor in the neostriatum and globus pallidus

The subcellular distribution of NR1 was determined using the pre-embedding immunoperoxidase, immunogold and the postembedding immunogold procedures.

In immunoperoxidase-labelled sections, the most commonly labelled profiles were spines and dendritic shafts in the neostriatum (Fig. 2B,D), and dendritic shafts in the globus pallidus (Fig. 3A,B). The peroxidase reaction product often filled these profiles (Fig. 2B,D) but also occurred as clumps (Fig. 3). In both regions most of the immunoreactive dendrites and all immunoreactive spines were postsynaptic to boutons forming asymmetrical synapses (Fig. 2B,D; 3 A,B). Some axons, but no boutons, were immunopositive with the NR1 antibodies in the neostriatum.

The analysis of the pre-embedding immunogold-labelled sections confirmed the presence of immunoreactivity for mNR1 and rNR1 in dendrites and spines in the neostriatum (Fig. 2C) and in dendrites in the globus pallidus. Immunoparticles were mainly associated with the internal surface of the plasma membrane at synaptic (Fig. 2C) and extrasynaptic sites (not illustrated).

Immunolabelling for mNR1 and rNR1 by both of the pre-embedding techniques was also detected in numerous perikarya that possessed a smooth unindented nucleus and a thin rim of cytoplasm (Fig. 2A). Immunolabelling was also detected in perikarya of neurons that possessed an indented nucleus and a large volume of cytoplasm, features that are characteristics of neostriatal interneurons (Fig. 2E). In the globus pallidus, immunolabelling was also detected in perikarya. In both regions, the immunoperoxidase product was seen as clumps in the cytoplasm (Fig. 2A). In both regions, in the pre-embedding immunogold-labelled sections, immunoparticles were mainly associated with the cytoplasmic face of endoplasmic reticulum, the cytoplasmic side of the nuclear membrane, and, albeit to a lesser extent, the Golgi apparatus (see Fig. 2E for neostriatum).

Localization of the GluR2/3 subunit of the AMPA receptor in the globus pallidus

In the immunoperoxidase and immunogold-treated sections, labelling for GluR2/3 was detected in perikarya and dendrites in the globus pallidus (not shown). As for the NR1 antibody, immunoparticles in perikarya were found in association with the endoplasmic reticulum, the cytoplasmic side of the nuclear membrane and cell membrane and the Golgi apparatus. Immunoperoxidase reaction product was detected in dendrites that had many axonal boutons associated with them forming primarily symmetrical synapses.

Localization of the NR1 subunit of the NMDA receptor in relation to synaptic junctions

In pre-embedding immunogold-labelled sections of the neostriatum and globus pallidus, immunoparticles were detected in association with axospinous and axodendritic asymmetrical synapses with both the antibodies (Fig. 2C). The immunogold labelling was generally found at the edges of the postsynaptic specialization, and only rarely within the body of the postsynaptic membrane. Immunoparticles were sometimes seen at sites on the internal membrane of spines and dendrites that were apparently not associated with synaptic specializations (not shown).

The postembedding immunogold method with both antibodies, revealed that most of the immunoparticles labelling NR1-immunoreactive sites were associated with asymmetrical synapses. In the neostriatum, axospinous and axodendritic synapses displayed immunolabelling for NR1 (Fig. 4A–C; 5A–D). In the globus pallidus immunolabelled axodendritic synapses were observed (Fig. 6A–E) some of which possessed clear subjunctional dense bodies (Fig. 6B). Most of the immunolabelling was found within the body of the synaptic specializations (Fig. 4A–C; 5A–D; 6A–E), only little immunolabelling was seen at extrasynaptic sites. The immunoparticles were distributed fairly evenly along the synaptic specialization (Figs 4–6). The quantitative analysis in the neostriatum revealed that the density of immunoparticles labelling NR1 subunits (with either antibody) was relatively even over the central 80% of the half width of the synapse but decreased in density in the outer 20% of the half width (Fig. 7). Very few particles fell outside of the synaptic specialization. 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.

In the neostriatum, 22% ($n = 60$) and 32% ($n = 83$) of asymmetric axospinous synapses were immunolabelled (two or more immunoparticles) with mNR1 and rNR1 antibodies, respectively. These values are likely to be underestimates because (i) synapses with one immunoparticle (7% for mNR1; 6% for rNR1) may, in fact, represent positive synapses, and (ii) it cannot be excluded that some immunonegative synapses do in fact contain receptors in a neighbouring section but was not available in the tested sections.

Localization of GluR2/3 subunit of the AMPA receptor in relation to synaptic junctions

The location of immunoparticles for GluR2/3 in relation to synapses was similar to that previously described in the neostriatum (Bernard

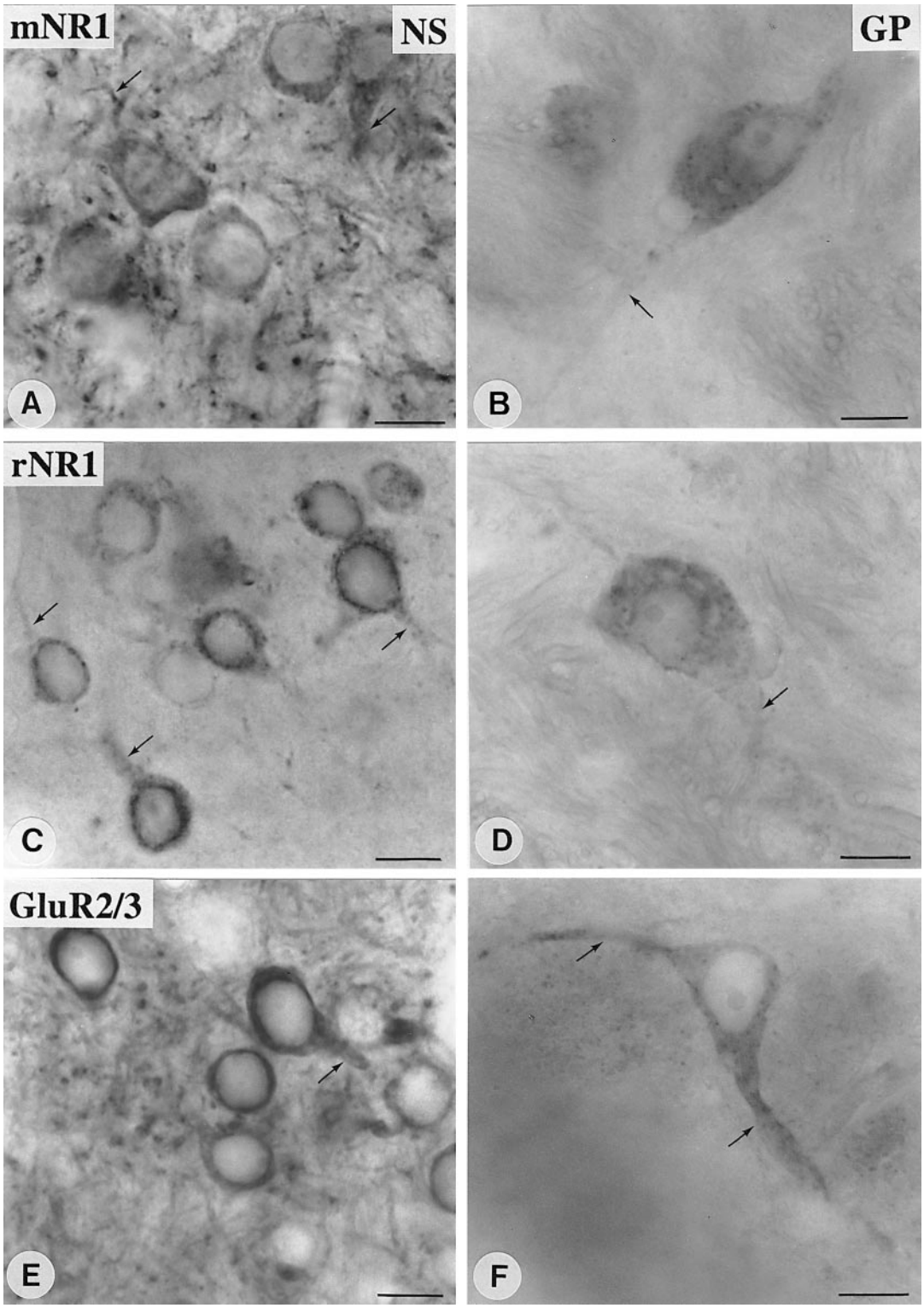

FIG. 1. Immunohistochemical detection of the NR1 subunits of the NMDA receptor and the GluR2/3 subunits of the AMPA receptor in the neostriatum (NS; A,C,E) and globus pallidus (GP; B,D,F) revealed by the peroxidase method at the light microscopic level. The NR1 subunit was detected using two antibodies, one raised in mouse (mNR1) the other in rabbit (rNR1). With both antibodies the majority of striatal (A,C,E) and pallidal (B,D,F) perikarya display immunoreactivity. Dense dendritic immunolabelling for mNR1 and GluR2/3 is present in the neostriatum, whereas the dendritic staining for rNR1 is weaker (arrows). The majority of striatal neurons that display immunoreactivity for the NR1 and GluR2/3 subunits have the characteristic features of medium spiny neurons. Dendritic labelling for both the NR1 and GluR2/3 subunits of glutamate receptors is also present in the globus pallidus (arrows). Scale bars: A–F, 10 μm.

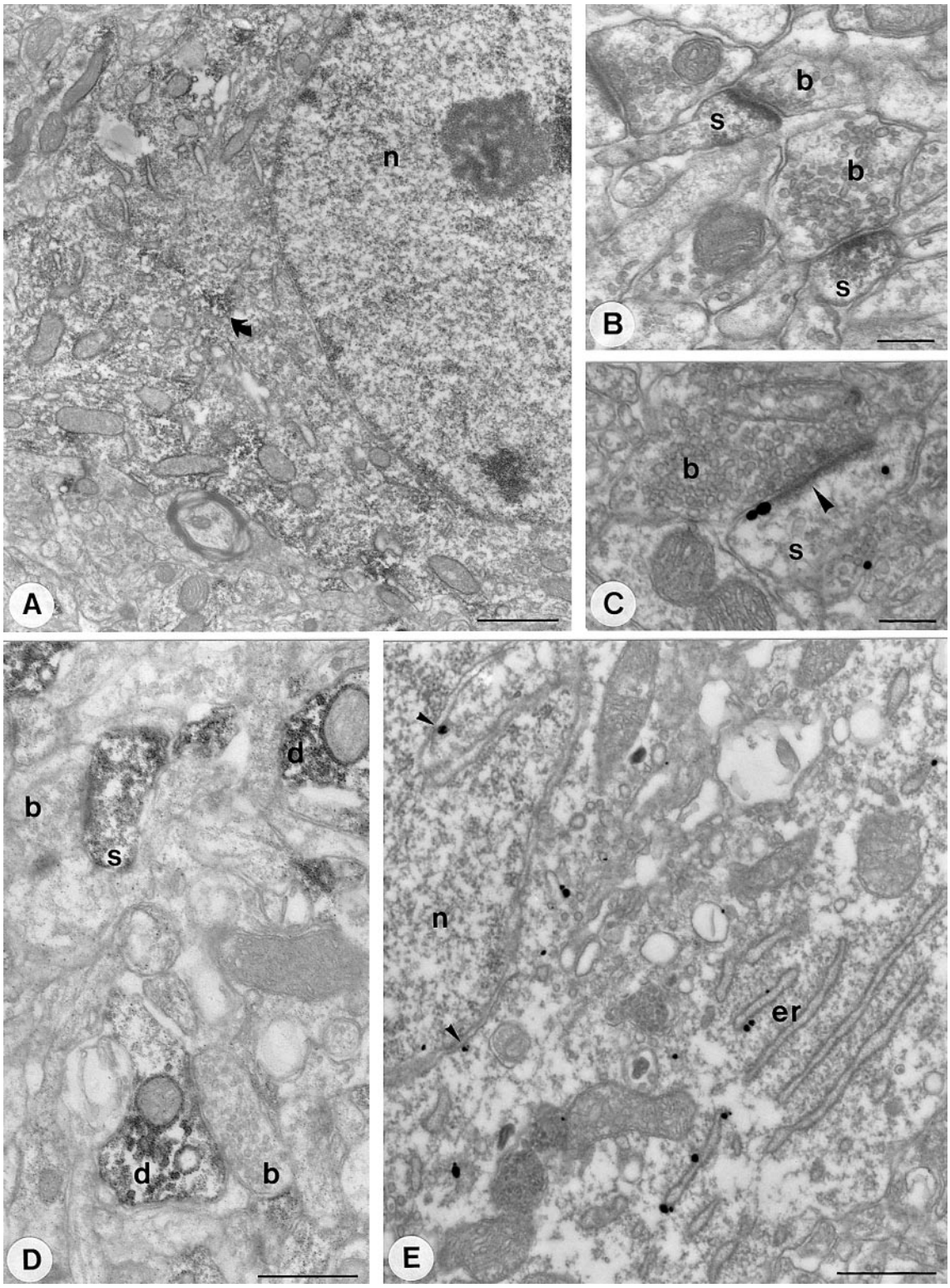

FIG. 2. Subcellular localization of NR1 in the neostriatum. NR1 was detected using the mNR1 (A,D) and rNR1 (B,C,E) antibodies by pre-embedding immunoperoxidase (A,B,D) and immunogold (C,E) methods. (A) and (E) Electron micrographs of NR1-immunoreactive perikarya revealed using the peroxidase (A) or pre-embedding immunogold (E) methods. The immunoreactive perikaryon in A has the characteristics of medium spiny neuron, i.e. an unindented nucleus (n) and a relatively small volume of cytoplasm. The immunoperoxidase reaction product occurs as aggregates within the cytoplasm (curved arrow). The labelled perikaryon in E possesses an indented nucleus (n) and a large volume of cytoplasm, and is thus identified as an aspiny interneuron. The immunoparticles are associated with the cytoplasmic surface of the endoplasmic reticulum (er) and the external surface of the nuclear membrane (arrowheads). (B–D) Electron micrographs of NR1-immunoreactive spines (s) and an immunoreactive dendrite (d) revealed using the peroxidase (B and D) or pre-embedding immunogold (C) methods. Many of the immunoreactive dendrites and spines are postsynaptic to boutons (b) forming asymmetrical synapses. In (C), the immunoparticles are associated with the edge of the postsynaptic membrane specialization. Scale bars: A, 1 μm; B and C, 0.5 μm; D and E, 0.2 μm.

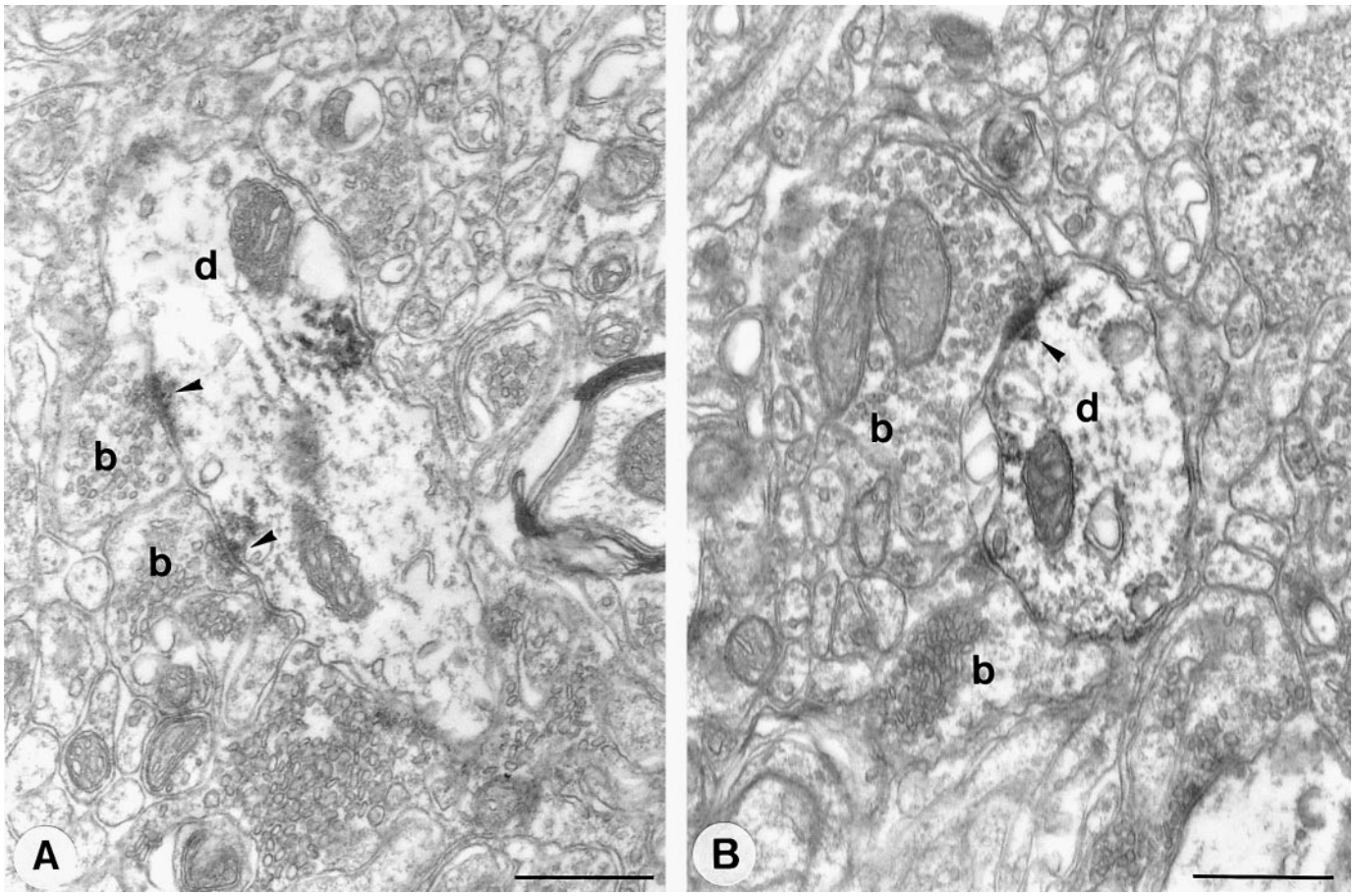

FIG. 3. Subcellular localization of NR1 immunoreactivity in the globus pallidus using the immunoperoxidase method. NR1 immunoreactivity was detected with the mNR1 (A) or the rNR1 (B) antibodies. The immunoreaction product is mainly present in dendrites (d) that have many axonal boutons associated with them (some indicated by b). Several of the boutons form synaptic contacts (arrowheads) with the dendrites. The upper synapse in (A) is asymmetric as indicated by the presence of subjunctional dense bodies. However, due the presence of the immunoperoxidase reaction product, it is often difficult to determine whether they form symmetrical or asymmetrical synapses. Scale bars: A and B, 0.5 μm.

et al., 1997). Briefly, immunoparticles were detected at axospinous (Fig. 4A′,B′; 5A–D) and axodendritic (Fig. 4C′) synapses in the neostriatum. The distribution of immunolabelling across the synapse was very similar to that of the NR1 immunolabelling, i.e. it was relatively even over the central 80% of the half width of the synapse but decreased in density in the outer 20% of the half width (Fig. 7).

In the globus pallidus most of the immunolabelling was detected at axodendritic, asymmetrical synapses (Fig. 6C–E) very little immunolabelling was detected at extrasynaptic sites. The presynaptic boutons were fairly large and contained one or more mitochondria. The postsynaptic specialization was often associated with subjunctional dense bodies (Fig. 6D,E).

Double immunolabelling for the NR1 subunit of the NMDA receptor and the GluR2/3 subunit of the AMPA receptor

The relative localization of the NR1 and GluR2/3 subunits at synapses was analysed by the postembedding immunogold technique using two approaches: (i) double detection of NR1 and GluR2/3 subunits on the same ultrathin sections of the neostriatum (Fig. 5) and globus pallidus (Fig. 6); (ii) double labelling for NR1 and GluR2/3 on serial ultrathin sections of neostriatum (Fig. 4). On double-labelled sections (Figs 5 and 6), the labelling for NR1 and GluR2/3 was distinguishable by the different size of silver-intensified immunogold particles coupled to the secondary antibodies. The diameters of the silver intensified immunogold particles labelling synapses in the double-labelled sections revealed a distinct bimodal distribution with peaks in the 10–15 nm and 40–45 nm bins (Fig. 8). The diameters of the immunoparticles in the single-labelled sections revealed distinct distributions that coincided with the two peaks in the double-labelled sections. The two distributions had only a small degree of overlap indicating the validity of the method. In the serial sections analysis, the same synapses were identified in the adjacent immunolabelled sections. Using both techniques, three subpopulations of labelled synapses were detected in neostriatum and globus pallidus: synapses immunopositive for only NR1, synapses immunopositive for only GluR2/3 and synapses immunopositive for both NR1 and GluR2/3. Quantitative analysis was performed in the neostriatum on the basis of the observations of the labelling for NR1 and GluR2/3 on adjacent sections and on the double-labelled single sections. In the adjacent section analysis all NR1-positive synapses in the neostriatum were identified on one section of the pair, the same synapses were then identified on the adjacent section to determine whether it was GluR2/3-positive. Of 49 mNR1-and 67 rNR1-immunopositive axospinous synapses, 43 (88%) and 50 (75%) were also positive for GluR2/3. Of six mNR1 and five rNR1 immunopositive axodendritic synapses, two of each were also positive for GluR2/3. In the double-labelled sections, a total of 100 NR1-positive axospinous synapses were identified, 82% of which were also GluR2/3-immunopositive.

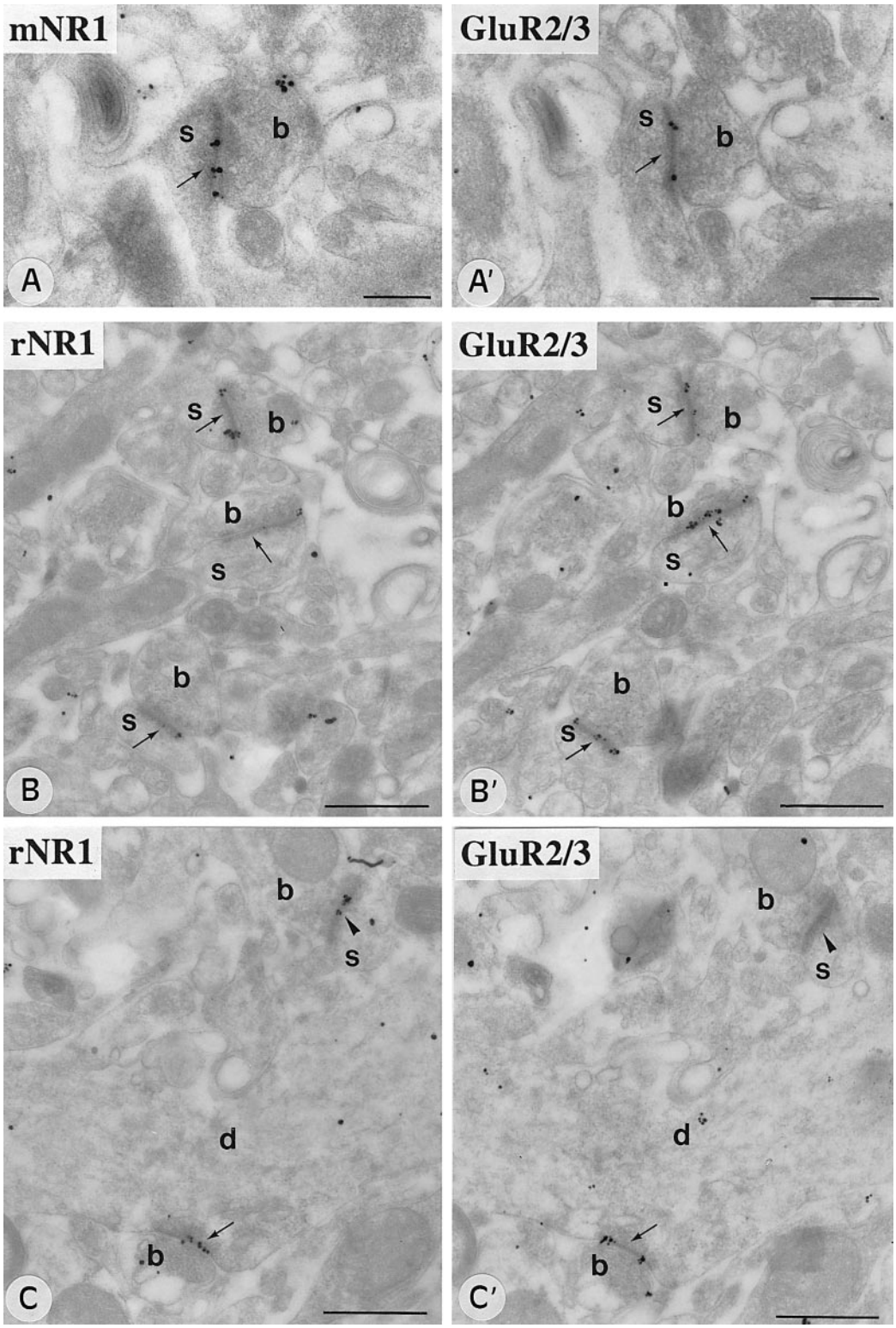

FIG. 4. Co-localization of the NR1 subunit of the NMDA receptor and the GluR2/3 subunit of the AMPA receptor at synapses in the neostriatum. Immunoreactive sites were revealed by the postembedding immunogold method on serial ultrathin sections. The sections in micrographs (A), (B) and (C) were immunolabelled to reveal NR1 and the serial sections in (A′), (B′) and (C′) were immunolabelled to reveal GluR2/3. The immunoparticles for NR1 (A, B, C) and GluR2/3 (A′, B′, C′) are mainly located within the body of the synaptic specialization. Synapses (arrows) between axonal boutons (b) and dendritic spines (s) (A, B) and between a bouton and dendritic shaft (d) (C) that are positive for the NR1 subunit are also positive for the GluR2/3 subunit (A′, B′, C′). Note in (C), an axospinous synapse (arrowhead) that is positive for rNR1 but negative for GluR2/3. Scale bars: A and A′, 0.5 μm; B and B′, C and C′, 0.2 μm.

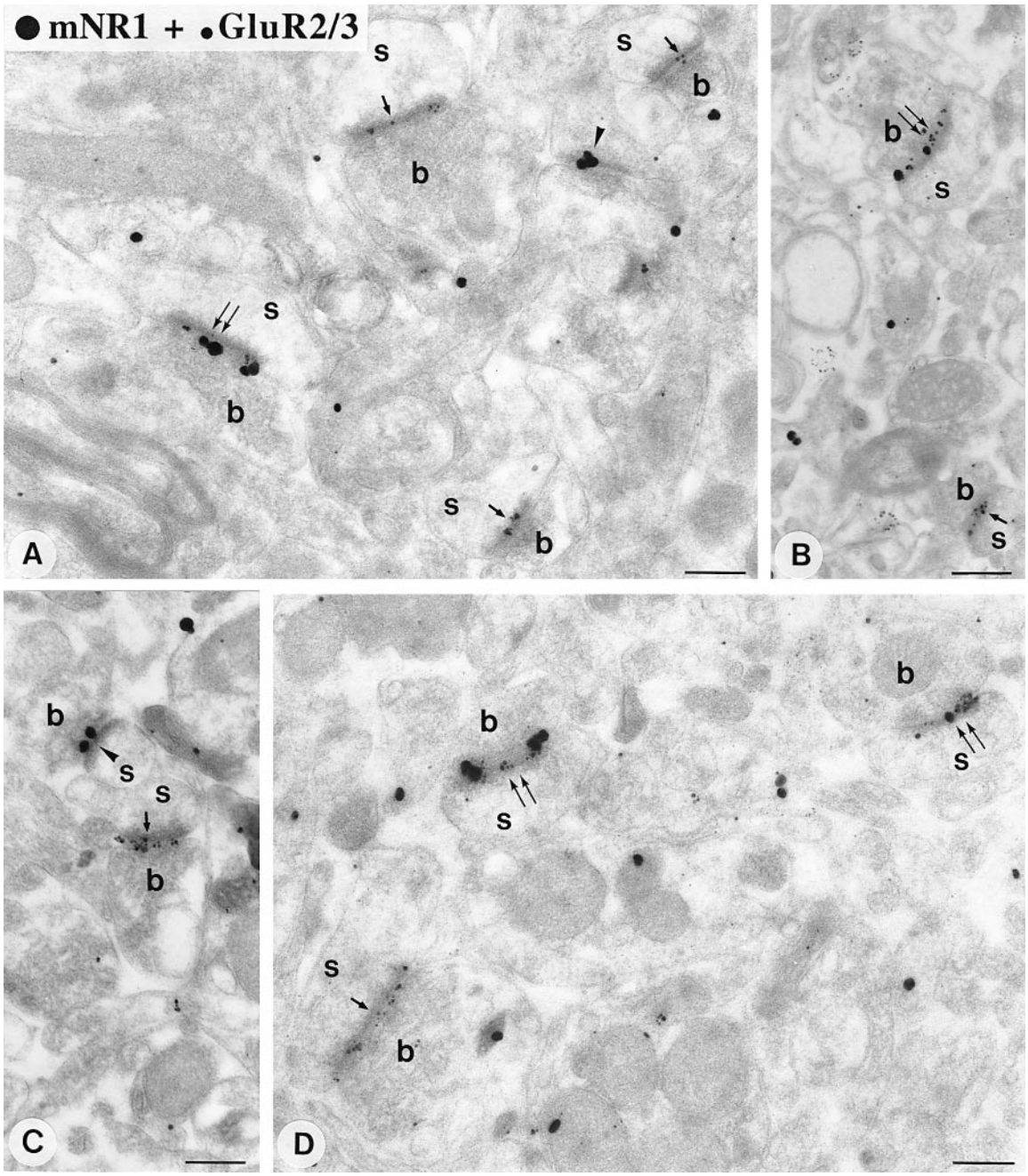

FIG. 5. Co-localization of the NR1 subunit of the NMDA receptor and the GluR2/3 subunit of the AMPA receptor at synapses in the neostriatum. Immunoreactive sites were revealed by a double postembedding immunogold method with silver intensification on the same sections. The NR1 subunit was detected using the mNR1 antibody. The secondary antibodies used were coupled either to 10 nm (mNR1; large immunoparticles) or 1.4 nm (GluR2/3; small immunoparticles) gold particles. Synapses between axonal boutons (b) and dendritic spines (s) are labelled for NR1 (A,C: arrowheads), for GluR2/3 (A–D: small arrows) or are double-labelled for both NR1 and GluR2/3 (A,B,D; double arrows). Scale bars: 0.5 μm.

Discussion

The results of the present study define the cellular and subcellular localization of the NR1 subunit of the NMDA receptor in both the neostriatum and the globus pallidus and demonstrate the colocalization of NMDA and AMPA receptor subunits at individual asymmetric synapses in both regions. This range of findings, from the cellular to the subsynaptic level, was made possible by the application of multiple immunohistochemical techniques at both the light and the electron microscopic levels. The immunogold methods revealed that NR1 is associated with the internal surface of the plasma membrane and that the highest density of immunoparticles is located at asymmetrical axospinous and axodendritic synapses, although extrasynaptic labelling is also present. It is not possible to localize the labelling to the pre- or postsynaptic membrane with the postembedding immunogold method 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 (Baude *et al.*, 1993). However, the results of the pre-embedding labelling studies lead to the conclusion that the labelling

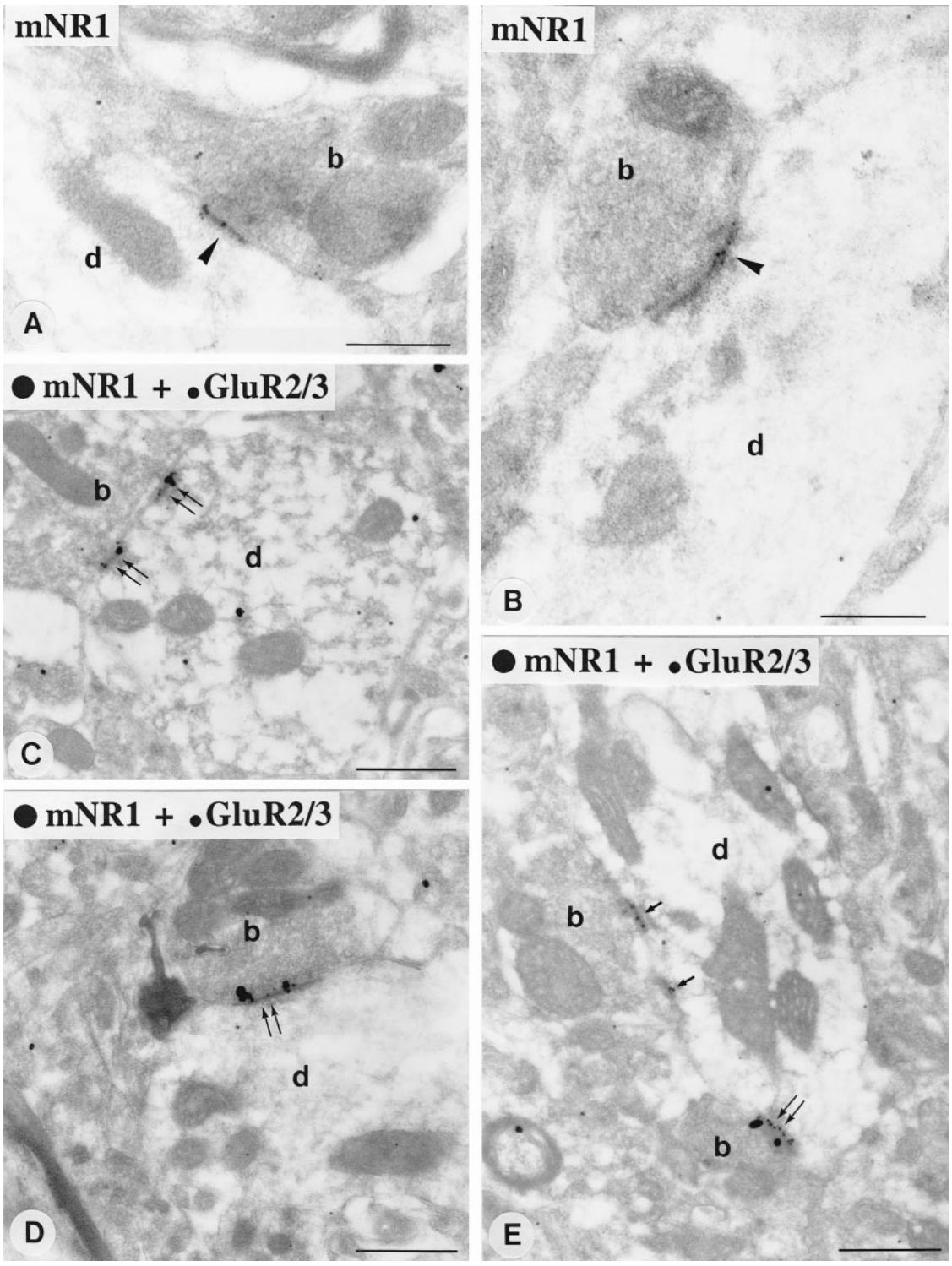

FIG. 6. Subcellular localization of the NR1 subunit of the NMDA receptor (A,B) in the globus pallidus and the colocalization with the GluR2/3 subunit of the AMPA receptor at individual synapses. Immunoreactive sites were revealed by a double postembedding immunogold method with silver intensification on the same sections (B–E). (A,B) Single immunolabelling for NR1 using the mNR1 antibody. Two dendrites (d) are postsynaptic to boutons (b) that form asymmetrical synapses (arrowheads). Immunolabelling for NR1 is located within the body of the specialization. In (B) the synapse is associated with subjunctional dense bodies. (C–E) Double immunolabelling for NR1 (large immunoparticles) and GluR2/3 (small immunoparticles) on the same section. Three dendrites (d) are postsynaptic to boutons (b) that form asymmetrical synapses (arrows). The synapses in (C) and (D) and the lower synapse in (E) display immunolabelling for both NR1 and GluR2/3 (C–E; double arrows). Note that the synaptic bouton in (C) possesses two active zones that both display immunolabelling for both NR1 and GluR2/3 (double arrows). In contrast, the upper of the two synaptic boutons in E, also possesses two active zones, both of which display immunolabelling for only GluR2/3 (small arrows). Scale bars: A,B, 0.3 μm; C–E, 0.5 μm.

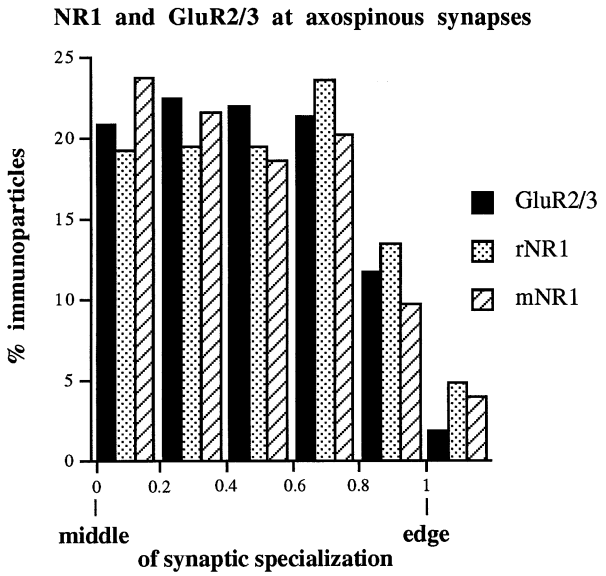

FIG. 7. Distribution of immunoparticles for NR1 and GluR2/3 subunits along the postsynaptic membrane specialization of axospinous synapses labelled by the postembedding immunogold method. The NR1 subunit was labelled using two antibodies. A similar distribution of immunolabelling at axospinous synapses occurred for both of the NR1 antibodies and this was similar to the distribution of immunolabelling for GluR2/3 subunits (rNR1, 194 synapses, 754 immunoparticles; mNR1, 141 synapses, 435 immunoparticles; GluR2/3, 314 synapses, 2080 immunoparticles). The mean length of synaptic specialization was 374.9 ± 18.7 nm (mean ± SEM) for all NR1 positive synapses and 387.1 ± 19.2 nm for GluR2/3-positive synapses. The immunoparticles are essentially evenly distributed across the synaptic membrane with a slight reduction at the periphery. Very few immunoparticle were observed outside of the synaptic specialization. Only synapses labelled by two or more particles are included in the analysis (mean number of particles per synapse NR1: 3.5; GluR2/3: 6.6).

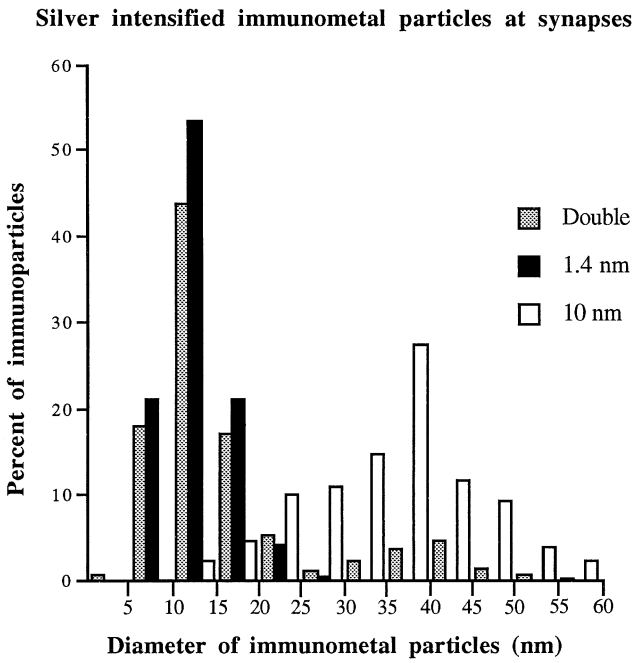

FIG. 8. Frequency distribution of the diameters of silver intensified immunogold particles labelling synapses in sections immunolabelled with 1.4 nm immunogold particles ($n = 186$), 10-nm immunogold particles ($n = 128$) or double-labelled with both ($n = 1365$). Measurements were made on projected images of electron micrograph negatives at final magnifications of ×180 800 or × 240 600. The distribution of particles in the double-labelled sections is bimodal, the peaks of which, coincide with the sizes observed in the single-labelled sections. Only a small degree of overlap exists between the two distributions from the single-labelled sections.

is associated with the postsynaptic membrane. The double post-embedding immunogold labelling revealed that in the neostriatum and globus pallidus a high proportion of synapses that are immuno-positive for NR1 are also immunopositive for GluR2/3 subunits of the AMPA receptor, although synapses positive for only one of the receptors were also present.

The present results thus provide an anatomical basis for a role of NMDA receptors in the regulation of the activity of neurons in the neostriatum and the globus pallidus. Indeed, physiological studies have demonstrated the involvement of NMDA receptors in excitatory synaptic transmission in the neostriatum and in the globus pallidus (see for instance Calabresi *et al.*, 1992a; Soltis *et al.*, 1994; Calabresi *et al.*, 1996; Kita, 1996; Götz *et al.*, 1997). Moreover, glutamate, through NMDA-dependent mechanisms, has been shown to mediate neurotransmitter release in the neostriatum (Scatton & Lehmann, 1982; Lannes & Micheletti, 1993 Galli *et al.*, 1994; Kendrick *et al.*, 1996). Receptors of the NMDA type have also been shown to be involved in the regulation of neuropeptide and receptor gene expression in the neostriatum (Lannes & Micheletti, 1993; Angulo *et al.*, 1995; Beckstead, 1995; Laprade & Soghomonian, 1995) and immediate-early gene expression following activation of the cortex (Liste *et al.*, 1995; Berretta *et al.*, 1997). Furthermore, NMDA receptors mediate long-term potentiation but not long-term depression of corticostriatal transmission (Calabresi *et al.*, 1992a;b, 1996).

Cellular and subcellular distribution of the NR1 subunit of the NMDA receptor in neostriatum and globus pallidus

The present findings relating to the cellular distribution of NR1 immunolabelling are in agreement with previous anatomical studies showing the presence of the NMDA receptor subunit and its mRNA associated with projection neurons and interneurons in the neostriatum and binding sites on striatonigral neurons (Tallakasen-Greene *et al.*, 1992; Petralia *et al.*, 1994; Landwehrmeyer *et al.*, 1995; Standaert *et al.*, 1994, 1996; Lannes *et al.*, 1995; Ghasemzadeh *et al.*, 1996; Ariano *et al.*, 1997). At the level of the perikaryon, both antibodies resulted in immunolabelling mainly associated with the endoplasmic reticulum but also with the internal side of the plasma membrane, the external side of the nuclear membrane and the Golgi apparatus. This pattern of localization is similar to that described for subunits of the AMPA receptor in the neostriatum (Bernard *et al.*, 1997). This labelling probably reflects newly synthesized receptors and thus are unlikely to be functional.

The most frequently labelled structures in the neostriatum were dendrites and spines. Dendritic labelling was stronger with the mNR1 than with the rNR1 which may be due to the differences in the spliced variants detected by the two antibodies. Thus, the mNR1 antibody recognizes all isoforms, whereas the rNR1 antibody recognizes only four of the eight variants (Petralia *et al.*, 1994). If indeed this is the case, these findings may reflect a differential addressing of the NR1 isoforms to the different subcellular compartments as has been demonstrated *in vitro* (Ehlers *et al.*, 1995).

Dendritic spines and shafts are the main synaptic targets of excitatory cortical and thalamic afferents and other excitatory inputs to the neostriatum (Kemp & Powell, 1970; Hattori *et al.*, 1978;

Frotscher *et al.*, 1981; Somogyi *et al.*, 1981; Kita & Kitai, 1987; Dubé *et al.*, 1988; Smith & Bolam, 1990; Xu *et al.*, 1991; Lapper & Bolam, 1992; Lapper *et al.*, 1992; Sadikot *et al.*, 1992; Bennett & Bolam, 1994). The demonstration that most immunolabelling is associated with the synaptic specializations of asymmetric axospinous and axodendritic synapses suggests that excitatory transmission at cortical and thalamic synapses in the neostriatum is mediated, at least in part, by NMDA receptors. This conclusion is thus in support of data derived from functional studies of the corticostriatal and thalamostriatal pathways (Kilpatrick & Phillipson, 1986; Kilpatrick *et al.*, 1986; Baldi *et al.*, 1995; Jolkkonen *et al.*, 1995; Calabresi *et al.*, 1996; Consolo *et al.*, 1996; Kita, 1996).

The findings relating to the cellular location of both the NR1 subunit and the GluR2/3 subunits in the globus pallidus are also consistent with previous observations using immunocytochemical or *in situ* hybridization techniques (see references above and Petralia & Wenthold, 1992; Martin *et al.*, 1993; Sato *et al.*, 1993). Thus the majority of neurons and many dendritic processes were labelled. The immunogold labelling revealed, as in other regions of the basal ganglia (Bernard *et al.*, 1997; Clarke & Bolam, 1998), that the highest density of immunolabelling for the subunits of both of the NMDA and AMPA receptors are localized at asymmetric synapses. Most terminals that form asymmetric synaptic specializations in the globus pallidus, are derived from the subthalamic nucleus (see review Smith *et al.*, 1998). Our findings suggest therefore, that glutamatergic transmission at subthalamopallidal synapses is also mediated by both NMDA and AMPA receptors.

In the pre-embedding immunogold labelling and occasionally in the postembedding labelling, immunoparticles were detected at extrasynaptic sites located both on the plasma membrane and at intracellular sites in both the neostriatum and the globus pallidus. The intracellular labelling was usually associated with saccules of endoplasmic reticulum (see also Gracy & Pickel, 1996, 1997; Gracy *et al.*, 1997) and thus presumably in the process of synthesis. Their detection at extrasynaptic sites on the plasma membrane is consistent with data obtained in pre-embedding studies of the ventral aspect of the neostriatum, the nucleus accumbens (Gracy & Pickel, 1996, 1997; Gracy *et al.*, 1997). The role of these receptors is still unclear; they may represent a pool of non-functional, stored receptor. However, the possibility cannot be excluded that they are functional and mediate non-synaptic transmission as has been suggested for the NMDA receptors in the cerebellum (Clark *et al.*, 1997). It has been suggested that the use of postembedding immunogold methods on sections prepared by freeze-substitution more accurately reflects the true distribution of the receptors as there is restricted access of the antibodies into the active zone in tissue prepared by conventional means (Baude *et al.*, 1995; Nusser *et al.*, 1995a; Bernard *et al.*, 1997). Because most of the receptor immunometal labelling occurred at synapses, we conclude that glutamatergic transmission mediated by NMDA receptors in the neostriatum and globus pallidus is likely to occur principally at synaptic specializations.

We very rarely detected immunoreactivity for NR1 in axons and or axon terminals in the neostriatum or globus pallidus which implies that presynaptic NMDA receptors are absent or that the density is far lower than at other sites. This finding does not favour a direct presynaptic modulation of neurotransmitter release by glutamate through NMDA receptors in the neostriatum and the globus pallidus. Interactions between glutamatergic and dopaminergic systems in the neostriatum have been extensively described; glutamate is reported to stimulate the release of dopamine through NMDA receptors (for review see (Lannes & Micheletti, 1993) however, the exact mechanisms remain to be resolved.

Comparison of the distribution of NR1 and GluR2/3

A key issue relating to the neurobiology of receptors is whether the selective placement of receptors in relation to specific synapses occurs and the mechanisms that underlie trafficking (Nusser *et al.*, 1994, 1995a,b, 1997; Nusser *et al.*, 1995b; Ottersen & Landsend, 1997; Rubio & Wenthold, 1997; Somogyi *et al.*, 1998). It is well established in the basal ganglia that excitatory responses are mediated by both AMPA and NMDA receptors (for references see above). Co-localization of the two classes of receptors is likely to occur at the cellular level and has been demonstrated in acutely dissociated neurons from adult rat striata (Ghasemzadeh *et al.*, 1996). Furthermore, the findings of the present study, that the NR1 subunit is present in virtually all spiny neurons in the neostriatum, taken together with our previous data and that of others demonstrating that these neurons also express AMPA receptor subunits, leads us to conclude that excitatory responses of individual spiny neurons are mediated by both NMDA and AMPA receptors. Our findings indicate that this is also the case for neurons in the globus pallidus. However, these findings and physiological analyses, do not tell us whether the response at an individual synapse is mediated by both types of receptors. To this end we performed the double-immunolabelling study which demonstrated that the NR1 subunit of the NMDA receptor and subunits of the AMPA receptor colocalize at individual axospinous and axodendritic synapses in the neostriatum and axodendritic synapses in the globus pallidus. These findings are similar to those found in other regions of the basal ganglia (Clarke & Bolam, 1998) and in other regions of the brain (Kharazia *et al.*, 1996). These anatomical data thus indicate that electrophysiological responses of excitatory inputs to neostriatal spiny neurons involving AMPA and NMDA receptors occurs at the same synapses (Cherubini *et al.*, 1988; Bekkers & Stevens, 1989; Calabresi *et al.*, 1990; Jiang & North, 1991; Kita, 1996). Furthermore, it is likely that the LTD that occurs following tetanic stimulation of the corticostriatal pathway, which is NMDA-independent but requires activation of AMPA receptors and the LTP that occurs following relief of the magnesium block of NMDA receptors, may well occur at the same corticostriatal synapses (Calabresi *et al.*, 1996).

In addition to those NR1-positive synapses that also displayed immunolabelling with the GluR2/3 antibody, we found that a proportion in both the neostriatum and globus pallidus that did not. There are several possible interpretations of this observation. One interpretation is that the failure to detect immunolabelling for GluR2/3 is because the level of the antigen is below the sensitivity of the postembedding technique applied to freeze-substituted tissue. Alternatively, if the synapses truly do not possess the GluR2 or 3 subunits of the AMPA receptor it raises interesting issues concerning the subunit composition of the AMPA receptor and glutamatergic transmission. It is unlikely that the GluR2/3-negative synapses possess AMPA receptors composed of GluR1 and/or GluR4 subunits since (i) GluR4 is not expressed by spiny neurons, and (ii) GluR1 is probably colocalized with GluR2/3 (Bernard *et al.*, 1997). This suggests that glutamate transmission via ionotropic receptors at some synapses in the neostriatum and globus pallidus occurs through NMDA receptors independent of AMPA receptors. Presumably the membrane depolarization required before NMDA receptors can participate in a glutamate response at these synapses is a function of the action of glutamate at kainate or metabotropic receptors, the activity of other excitatory afferents and/or intrinsic membrane properties (Testa *et al.*, 1994; Landwehrmeyer *et al.*, 1995; Bischoff *et al.*, 1997; Standaert *et al.*, 1994, 1996; Bernard *et al.*, 1996, 1997). Even if neurotransmission at most excitatory synapses is thought to involve

both AMPA and NMDA receptors, electrophysiological evidence suggest that activation of NMDA receptors alone can lead to LTP in the neostriatum (Calabresi *et al.*, 1996) and hippocampus (Bashir *et al.*, 1991). Synapses with only NMDA receptors may be silent at resting potentials, but they may acquire AMPA responses in case of stimulation, as it has been demonstrated in hippocampus during LTP (Isaac *et al.*, 1995; Liao *et al.*, 1995; Voronin *et al.*, 1996; Kiyosue *et al.*, 1997). The conversion from a silent to a functional synapse could thus be explained by the appearance of AMPA receptors in the synaptic specialization.

A further observation of the present findings was a population of synapses that were immunolabelled with the GluR2/3 antibody but were negative for NR1. As indicated above this finding may simply relate to the sensitivity of the technique and indeed, this is more likely in this case as the immunolabelling for the AMPA subunits was much more robust than for the NMDA receptor subunit. At this stage however, we cannot make firm conclusions; it may be the case that glutamatergic transmission is mediated at some axospinous and axodendritic synapses in the neostriatum and globus pallidus, only through non-NMDA glutamate receptors. Indeed functional synapses without NMDA receptors have been proposed to occur in Purkinje cells (Davies *et al.*, 1986; Dupont *et al.*, 1987; Honoré *et al.*, 1988). An additional possibility that can account for synapses labelled for only the NMDA subunit antibodies relates to the diversity of the isoforms of the NR1 subunits composing the NMDA receptor. It is possible that there are subpopulations of AMPA receptor-positive synapses that possess different isoforms of the NR1 subunit that are not recognized by the antibodies used in the present study.

Subsynaptic distribution of immunolabelling

The analysis of the distribution of immunogold particles revealed that the immunolabelling for both the NMDA subunit (with both of the antibodies) and the AMPA subunit is evenly distributed across the synaptic specialization with a slight fall-off at the periphery. The distribution of the GluR2/3 labelling is essentially the same as that found previously in the neostriatum (Bernard *et al.*, 1997) and in the entopeduncular nucleus and subthalamic nucleus (Clarke & Bolam, 1998). This distribution is also similar to that observed in the hippocampus (Somogyi *et al.*, 1998). The distribution of the NMDA receptor subunit is also similar to that observed in the entopeduncular nucleus and subthalamic nucleus (Clarke & Bolam, 1998). These findings suggest that both the NMDA receptor and AMPA receptor possessing the GluR2 or 3 subunit will have equal access to released glutamate. This situation however, is markedly different to that observed in the hippocampus (Somogyi *et al.*, 1998). In this study antibodies against two subunits of the NMDA receptor gave immunolabelling that, when averaged across a large population of spines, was concentrated at the centre of the synaptic specialization whereas AMPA receptor subunits were evenly dispersed across the synapse. The implication of these findings is that the mechanisms controlling the selective trafficking of receptors to synapses and within the synaptic specialization vary from region to region and synapse to synapse (Ottersen & Landsend, 1997; Rubio & Wenthold, 1997; Somogyi *et al.*, 1998).

Concluding remarks

In conclusion, the implication of the present findings is that corticostriatal and thalamostriatal transmission, and possibly other excitatory inputs to the neostriatum, occurs predominantly at synapses and is mediated, at least in part, by both AMPA and NMDA receptors. Similarly, transmission at subthalamic synapses in the globus pallidus is likely to mediated by similar mechanisms. The exact receptor profile of glutamate receptor subtypes and their subunit composition at characterized excitatory synapses remains to be established.

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Abbreviations

ABC	avidin-biotin-peroxidase complex
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionate
BSA	bovine serum albumin
DAB	3,3′-diaminobenzidine
EPSP	excitatory postsynaptic potential
GAM	goat antimouse
GAR	goat antirabbit
NGS	normal goat serum
NMDA	*N*-methyl-D-aspartate
PB	phosphate buffer
PBS	phosphate buffered saline
TB	Tris buffer
TBS	Tris buffered saline

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