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AMPA Receptor Subunits Underlying Terminals of Fine-Caliber Primary Afferent Fibers

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Postembedding immunogold electron microscopy was used to determine the relation of primary afferent terminals in superficial laminae of the spinal dorsal horn with AMPA receptor subunits. Immunogold particles coding for GluR1 and GluR2/3 were concentrated at synaptic sites, between 30 nm outside and 40 nm inside the postsynaptic membrane. Immunopositive synapses displayed round vesicles and asymmetric specializations, characteristic of terminals releasing excitatory neurotransmitters; symmetric synapses, characteristic of terminals releasing inhibitory amino acids, were immunonegative.

In superficial laminae, large terminals of two main types at the center of a synaptic glomerulus originate from primary afferents: C1 terminals are mainly endings of unmyelinated afferent fibers; C2 terminals are mainly endings of thinly myelinated afferent fibers. Terminals of both types were presynaptic to AMPA subunits, but in different proportions: C1 terminals were related more to GluR1 than to GluR2/3, whereas the reverse was true for C2 terminals. These results suggest that functional properties of peripheral afferents to the spinal cord may be specified by the density and combination of receptor subunits in the postsynaptic membrane, and raise the possibility that calcium-permeable AMPA channels may play a special role in the mediation of sensory input by unmyelinated fibers.

Key words: glutamate receptors; dorsal horn; nociception; substantia gelatinosa; C fibers; postembedding immunogold

The superficial laminae of the spinal cord are of special interest for their role in pain, but they receive other types of primary afferent fibers besides those innervating peripheral nociceptors. Indeed, input from afferents that mediate different types of stimuli may impinge onto the same neuron (Willis and Coggeshall, 1991). How spinal neurons decode this convergent information is still a matter of speculation. One possibility is that different submodalities are mediated by different transmitters. However, all primary afferents terminating in superficial laminae appear to use glutamate (Rustioni and Weinberg, 1989; Salt and Herrling, 1995), although the amount of glutamate released may differ in different afferents, and other agents may be coreleased with glutamate (De Biasi and Rustioni, 1988; Merighi et al., 1991; Tracey et al., 1991; Levine et al., 1993; Valtschanoff et al., 1994).

Postsynaptic factors that may contribute to the diversity of signals in response to release of glutamate include the diversity of ionotropic glutamate receptors. Light microscopic (LM) studies have demonstrated high concentrations of AMPA receptor subunits in neurons of superficial laminae of the dorsal horn (Furuyama et al., 1993; Henley et al., 1993; Tölle et al., 1993; Tachibana et al., 1994; Kondo et al., 1995). Electron microscopy (EM) is required to verify the presence of receptor subunits at synaptic sites and to explore the relations between receptor subunits and primary afferent terminals. EM evidence for glutamate receptors in superficial laminae of the dorsal horn has been provided so far only with preembedding immunocytochemistry (Liu et al., 1994; Tachibana et al., 1994; Vidnyanszky et al., 1994). Preembedding was recently used in an effort to relate glutamate receptor subunits to primary afferent terminals (Alvarez et al., 1994). However, this method is not well suited for quantitative study, both because of variable antibody penetration and because of difficulty in quantifying the density of immunoreaction at EM. Moreover, the tendency of the peroxidase reaction product to migrate and to deposit on membranes limits the accuracy of antigen localization.

Postembedding immunocytochemistry with colloidal gold can in principle surmount the above technical limitations (Nusser et al., 1995a,b). However, osmic acid abolishes or seriously impairs the antigenicity of many large molecules, including glutamate receptor subunits. We have introduced a method that replaces osmic acid with tannic acid and uranyl salts in material originally fixed with glutaraldehyde, thus yielding good structural preservation together with precise localization of multiple receptor subunits (Phend et al., 1995). With this method, we here report that AMPA receptor subunits are highly concentrated at synapses. We also show that different types of primary afferent terminals in superficial laminae of the spinal cord are associated with different receptors, suggesting a postsynaptic mechanism by which the same transmitter may convey functionally distinct peripheral messages.

MATERIALS AND METHODS

Eight male Sprague–Dawley rats (200–350 gm) were anesthetized with pentobarbital (50 mg/kg) and perfused via the aorta with fixative, after brief flush with heparinized saline. After perfusion, the spinal cords were removed and post-fixed for 2 hr in the same fixative used for perfusion. Immunocytochemistry was performed using polyclonal affinity-purified antibodies raised in rabbit against peptide fragments homologous to the C terminus of GluR1, or to a sequence shared by GluR2 and GluR3 ("GluR2/3"), conjugated with glutaraldehyde to bovine serum albumin. These antisera have been shown by Western blot analysis to recognize the respective native proteins, and have been characterized and widely used for immunocytochemical studies (Wenthold et al., 1992; Petralia et al., 1994; Tachibana et al., 1994).

Light microscopy. Three rats were used for light microscopy. These were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4

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(PB). Transverse 25- to 50-μm-thick sections were cut on a Vibratome and placed into glass vials for immunocytochemistry. Sections were incubated on a shaker in 50% alcohol for 20 min, rinsed in PBS, and incubated 10 min in 3% H_2O_2 in PBS. They were then rinsed and incubated for 10 min in normal goat serum (NGS) in PBS and then overnight at room temperature in either anti-GluR1 or anti-GluR2/3 primary antibodies, diluted 1:1000–2000 in PBS. Sections were rinsed in PBS, incubated in 2% NGS in PBS for 10 min, and then treated with biotinylated goat anti-rabbit IgG (Sigma, St. Louis, MO), 1:200 in PBS for 2 hr. Sections were then rinsed, incubated in ExtrAvidin (Sigma), 1:5000 in PBS, rinsed in PBS and twice in PB, and were processed according to a nickel-intensified diaminobenzidine (DAB; Sigma) protocol for peroxidase. This consisted of incubation for 10 min in a freshly prepared solution comprising 4.7 ml of PB, 2.5 mg of DAB, 50 μl of a 0.4% solution of ammonium chloride, 50 μl of a 20% solution of D-glucose, and 200 μl of a 1% solution of nickel ammonium sulfate (Llewellyn-Smith et al., 1993). After adding 5 μl glucose oxidase solution (Sigma), sections were incubated for another 10–30 min and rinsed in PB.

For visual examination, sections were mounted on gelatin-coated slides, dehydrated through ascending alcohols, cleared in xylene, and coverslipped. Reconstructions of the staining in superficial laminae, including lamina I and two portions (outer, o; inner, i) of lamina II distinguishable by the apparent absence of myelinated fibers and sparse cellular density in IIi, were made from the dorsal horn at low and high magnification using a drawing tube. For photomicroscopy, sections were treated with 2% OsO_4 in PB, pH 6.0, for 2 hr, dehydrated in graded alcohols, and then embedded in Epon–Spurr resin as described in detail below. Chips from superficial laminae were glued to plastic blocks; 0.5- to 1.0-μm-thick sections were collected on glass slides, coverslipped, and photographed with a Leitz DMR microscope.

Electron microscopy. Five rats were used for EM; the material had good structural preservation and provided good immunocytochemical yield, consistent despite minor protocol variations. These rats were fixed by intra-aortic perfusion with a mixture of 2.5% glutaraldehyde and 0.5–4% paraformaldehyde, in some cases with 0.1% picric acid. Fifty-micrometer-thick transverse sections from two selected spinal cord segments (C5 and L4) were cut on a Vibratome and stored in PB. Sections were processed for osmium-free embedment according to Phend et al. (1995), with minor modifications. Briefly, sections on ice were treated for 45 min in 1% tannic acid in 0.1 M maleate buffer (MB), pH 6.0, rinsed in MB, subsequently immersed for 40 min in 1% uranyl acetate, 0.5% iridium tetrabromide (Pfaltz and Bauer, Waterbury, CT) in MB, 50 and 70% ethanol for 5 min, 1% phenylenediamine hydrochloride in 70% ethanol for 15 min, 1% uranyl acetate in 70% ethanol for 40 min, and then dehydrated in 80%, 95%, and 2×5 min in 100% ethanol. Sections were then immersed in propylene oxide and infiltrated with Epon or Epon–Spurr (4:6) resin. After overnight infiltration in 100% resin, sections were sandwiched between strips of plastic film, flattened between microscopic slides, and polymerized at 60°C for 24 hr. Chips from laminae I–II of the dorsal horn were glued onto plastic blocks. Thin sections were cut and collected on 300-mesh uncoated nickel grids that were treated with Quick-Coat (Kiyota Express, Elk Grove, IL) for improved section adhesion.

Postembedding immunocytochemistry was performed essentially as described previously (Phend et al., 1995). Briefly, grids were washed with Tris-buffered saline containing detergent (TBS/D; 0.1 M Tris, pH 7.6, with 0.1% Triton X-100 or 0.005% Tergitol NP-10 added) incubated overnight at 37°C in primary antibody (1:1000), rinsed in TBS/D, pH 7.6, transferred to TBS/D, pH 8.2, and incubated in goat anti-rabbit IgG conjugated to either 18 nm colloidal gold (Jackson ImmunoResearch, West Grove, PA) or to 10 nm colloidal gold (Amersham, Arlington Heights, IN) 1:15 in TBS/D, pH 8.2, for 1 hr, rinsed, and dried. Grids were examined with a JEOL 200CX transmission EM at 80 kV.

Data collection and analysis. To determine the spatial relationship between gold particles and synaptic membrane at immunopositive active zones, photographs were taken at 40,000×. All terminals within random grid squares that were identifiable as primary afferents on the basis of their large size and glomerular arrangement (as defined by the presence of synapses onto at least two postsynaptic profiles) were photographed, if at least one active zone was immunopositive (defined by the presence of at least one gold particle within 100 nm of the postsynaptic membrane). Synaptic contacts were further selected from photographs to include only those that displayed well defined active zones with distinct pre- and postsynaptic membranes. Negatives containing the selected terminals were digitized at 400 pixels/inch resolution with a Hewlett Packard Scan Jet II connected to a Macintosh Quadra 850. The distance between the center of each gold particle and the cytoplasmic leaflet of the postsynaptic membrane was measured. All sampled terminals were pooled together.

To determine the numerical relationship between primary afferent terminals and receptor subunits, two types of terminals identified in laminae I and II (see Results) were treated separately. For this part of the study, synaptic contacts associated with at least one gold particle within ~50 nm of the synaptic specialization were considered positive, counting directly from the microscope screen. All terminals from random grid squares that could be unequivocally classified as one of these two morphological types were included in the counts. χ^2 tests were performed to evaluate the null hypothesis that the proportion of synapses presynaptic to the different subunits was the same for each type of terminal.

RESULTS

Light microscopy

With the nickel-intensified DAB–peroxidase procedure, immunostaining produced a fine granular product in cells and neuropil. Cellular staining could be identified in somata and proximal dendrites; nuclei were unstained. Because of differences in staining intensity, the dendritic arbor was more visible in material stained with GluR2/3 than with GluR1. Staining with the GluR1 antibody was concentrated in the superficial dorsal horn (Fig. 1*A*). Stained neurons in other regions of the spinal cord were small and sparsely distributed, except in the region around the central canal. Neurons immunoreactive for GluR2/3 were also concentrated in superficial laminae (Fig. 1*B*); however, this antibody also stained a substantial number of larger neurons throughout the rest of the cord.

In lamina I, neurons stained with GluR1 were concentrated laterally; a larger population of neurons was intensely stained with GluR2/3 (Fig. 2). Fine punctate neuropil staining was organized in small bundles oriented mediolaterally, especially for GluR1. Neurons in lamina II were more intensely immunopositive than in lamina I. The density of neurons immunostained for GluR1 was highest near the IIo/IIi border; few stained cells were seen in deep IIi (Fig. 2, *top*). Neuropil staining with GluR1 overlapped the staining of somata, gradually disappearing at the ventral border of II. With the GluR2/3 antibody, the staining was markedly different: density of cellular and neuropil staining was relatively low at the IIo/IIi border, and highest deep in lamina IIi extending into III (Fig. 2, *bottom*).

Electron microscopy

Sections showed generally good structural preservation in the absence of osmium. Myelin was poorly preserved, but clear and dense core vesicles, as well as synaptic specializations, were well preserved and contrasted. With both antibodies, gold particles were too sparse over cell bodies and dendrites to warrant collection of quantitative data. Gold particles were instead clustered over the postsynaptic density, postsynaptic membrane, and cleft of a large number of asymmetric synapses. A significant fraction of terminals with positive synaptic zones could be recognized as originating from primary afferents (see below), but synaptic zones of many terminals lacking characteristic glomerular organization, likely to originate mainly from intrinsic neurons, were also immunopositive. Labeling was not observed over active zones of symmetric synapses. Ninety-four percent of gold particles tallied (410/437) from a sample of 215 glomerular terminals from lamina II were in a region between 30 nm outside and 40 nm inside the postsynaptic membrane. Most particles were associated with the postsynaptic membrane; although particles at a distance up to 200 nm from the synapse were considered, none was more than 90 nm from the synapse (Fig. 3). The distribution of gold particles was

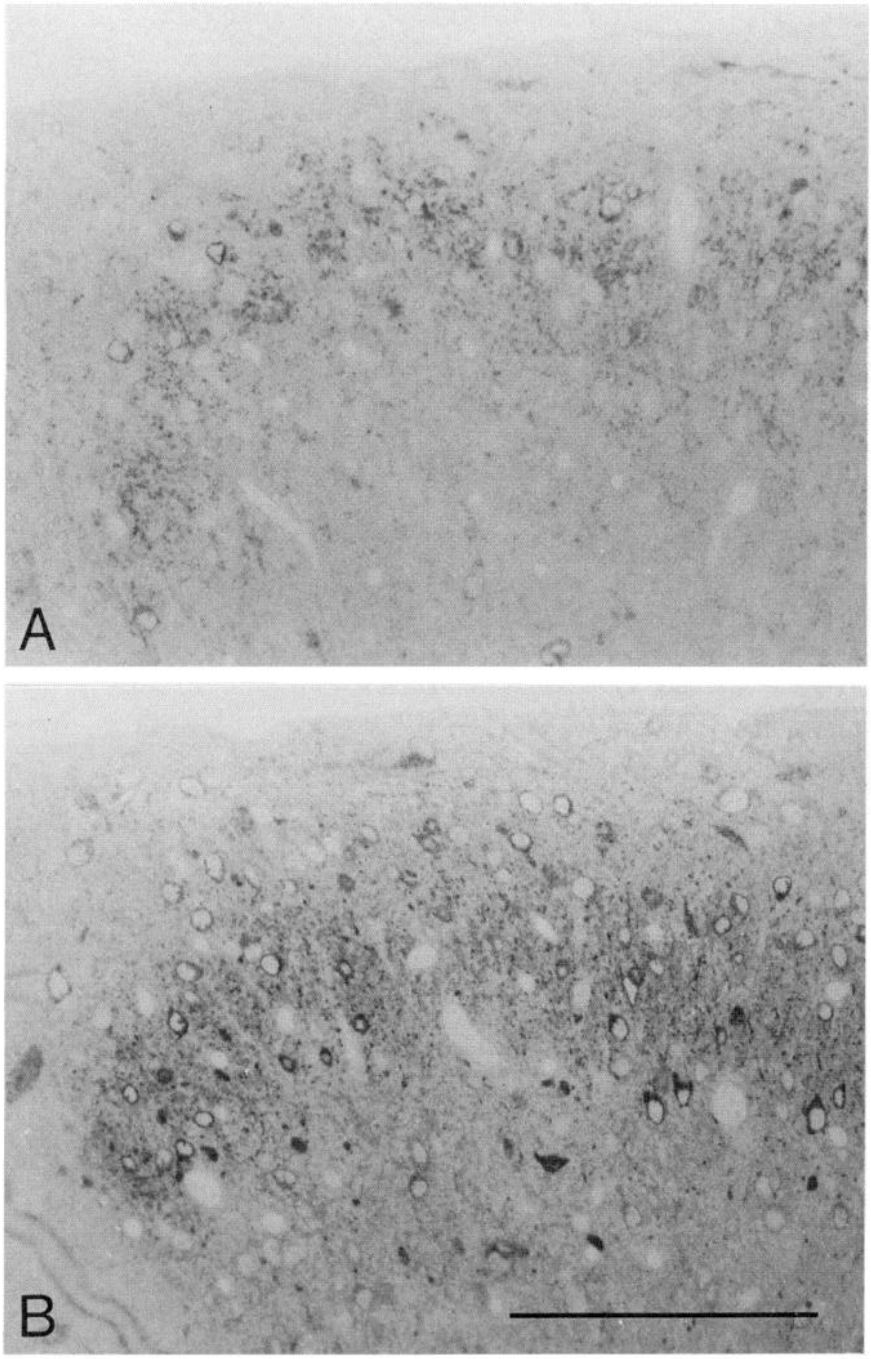

Figure 1. Photomicrographs of the dorsolateral portion of two 0.5-μm-thick sections from L4, showing immunostaining for GluR1 (*A*) and for GluR2/3 (*B*). Note staining in cells and punctate staining in neuropil, generally more superficial for GluR1 than for GluR2/3. Lateral is to the *left* of the photomicrographs. Scale bar, 200 μm.

similar for GluR1 and GluR2/3. The very low density of gold particles away from the active zone implies that even a single particle at the active zone is strong evidence for immunopositivity. Examination of serial thin sections confirmed this interpretation, because synapses first identified as labeled by the presence of one dot on one section displayed one or more dots also in contiguous sections (Fig. 4). The same did not hold true for gold particles at nonsynaptic sites (Fig. 4*A*,*B*).

Types of terminals

Two types of glomerular terminals could be identified in superficial laminae. One was scalloped, with densely packed clear vesicles of variable size, dark axoplasm, and few mitochondria (Figs. 4*A*,*B*, 5*A*,*C*). These terminals, which contacted several postsynaptic dendrites, correspond to the central terminals of type 1 glomeruli (C1) described by Ribeiro-da-Silva and Coimbra (1982). They are likely to be terminals of unmyelinated primary afferents (Ribeiro-da-Silva, 1994). Terminals of the second type were also scalloped, but with loosely packed clear vesicles of uniform size, light axoplasm, and many mitochondria (Figs. 4*C*,*D*, 5*B*,*D*). These terminals, contacting several postsynaptic profiles and involved in axo-axonic contacts with symmetric active zones, correspond to the central terminals of type 2 glomeruli (C2) described by Ribeiro-da-Silva and Coimbra (1982). These are likely to arise from thinly myelinated primary afferents. C1 terminals are concentrated in lamina IIo and dorsal IIi, whereas C2 terminals are concentrated in ventral lamina IIi (Bernardi et al., 1995).

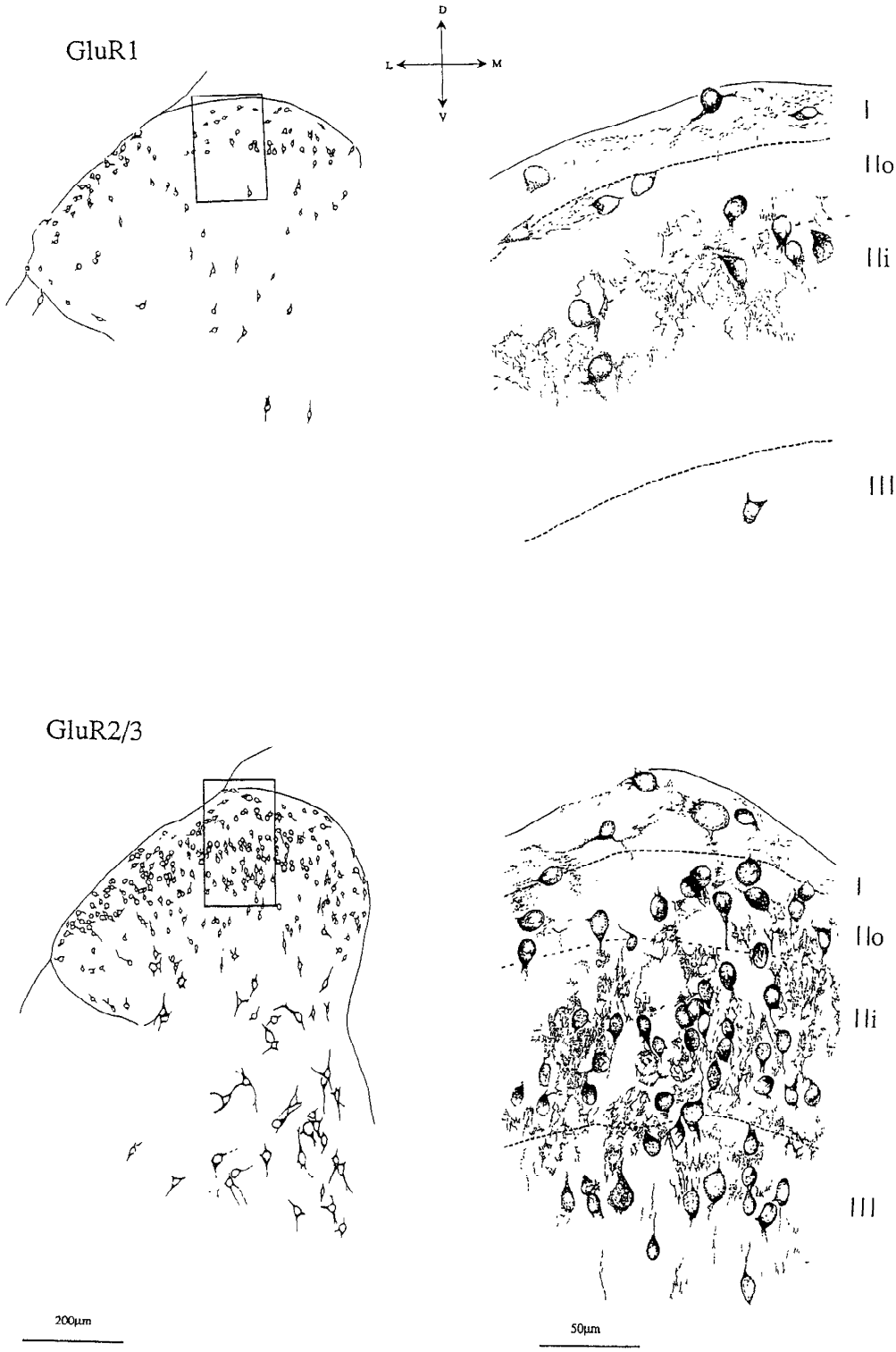

Figure 2. Reconstructions of immunostaining for GluR1 and GluR2/3 in the dorsal horn, from transverse, 25-μm-thick sections of L4. On the *left*, only immunopositive cell bodies are represented. On the *right* is a higher magnification of the area in the *inset* at *left* also showing neuropil stain. Staining for GluR1 is generally more dorsal than for GluR2/3. *D*, Dorsal; V, ventral; M, medial; L, lateral.

Relationship between types of terminals and different receptor subunits

Terminals of both types were presynaptic to both GluR1 and GluR2/3, but to a different extent. C1 synapses were predominantly GluR1-positive, and C2 synapses were predominantly positive for GluR2/3 (Fig. 6). These differences were highly significant by χ^2 tests; the null hypothesis (that both types of terminal were equivalently immunopositive for a receptor subunit) was rejected, for both cervical and lumbar levels, at $p < 0.0001$. To exclude that this might arise from a single nonrepresentative case, we performed χ^2 tests on the data grouped separately for the two levels, from each of three animals; the same pattern was seen in each case, with p-values ranging from <0.02 to <0.0001, despite the relatively small sample sizes.

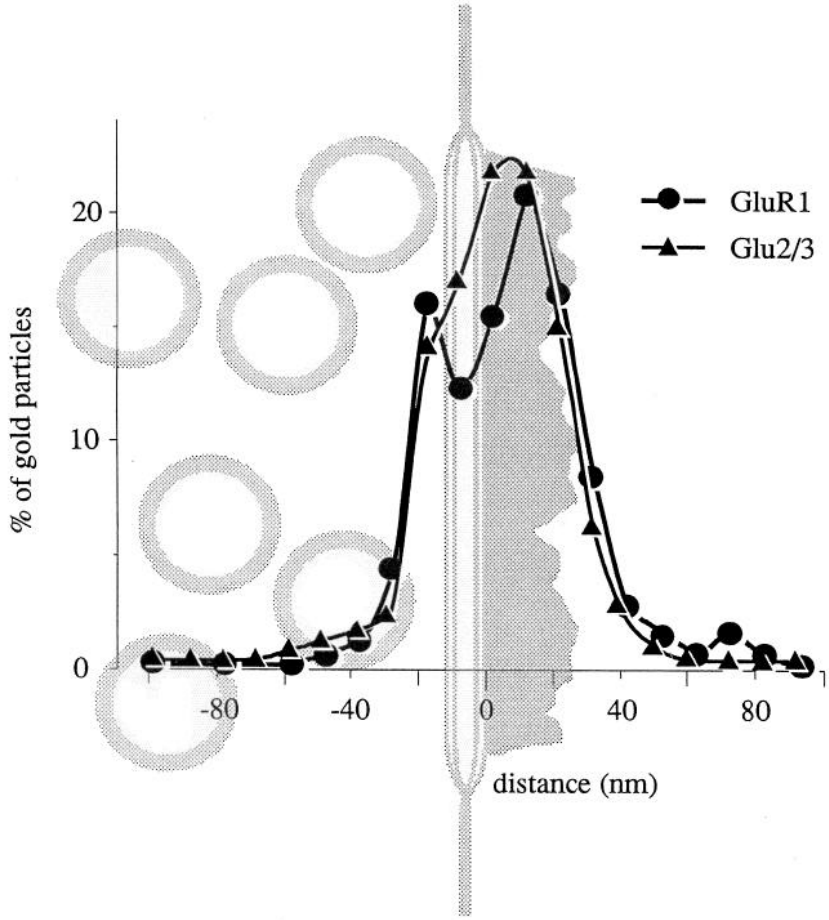

Figure 3. Distribution of gold particles coding for AMPA receptor subunits at the pre- and postsynaptic region of tallied active zones. These were at synaptic endings recognizable as terminals of primary afferents on the basis of their large size and glomerular arrangement. Stippled artwork shows synaptic vesicles, synaptic cleft, and postsynaptic specialization as an aid to relate the quantitative data to the microscopic structures. The data for each curve are pooled together from 131 active zones from 112 terminals for GluR1 (total number of gold particles: 209); 115 active zones from 103 terminals for Glu R2/3 (total number of gold particles: 229). The zero point corresponds to the cytoplasmic leaflet of the postsynaptic membrane.

Interpretation of the quantitative differences reported above is complicated by the possibility that unlabeled synaptic sites might nonetheless contain receptor subunits, or that the concentration of subunits may vary at different types of synapses. To explore this issue, we counted gold particles underlying each active zone of randomly photographed primary afferent terminals (Fig. 7). The counts were roughly Poisson-distributed, reflecting the random exposure of epitopes in a thin section. However, heterogeneity of synaptic contacts was also suggested, especially for C2 terminals immunopositive for GluR2/3. Immunopositive C1 synapses contained a similar number of gold particles coding for GluR1, on average, as did immunopositive synapses of C2 terminals (1.88 vs 2.10), confirming that a higher proportion of C2 than of C1 synapses expressed little or no GluR1. On the other hand, immunopositive synapses of C1 terminals contained a markedly lower mean number of gold particles coding for GluR2/3 than did synapses of C2 terminals (1.92 vs 2.79). This could not be explained by differences in dimensions of active zones, because C1 and C2 had active zones of similar lengths (322 ± 13 vs 341 ± 11 nm, respectively).

DISCUSSION

Light microscopy

The present data on LM distribution of AMPA subunits are generally consistent with previous studies (Furuyama et al., 1993; Henley et al., 1993; Tölle et al., 1993, 1995; Tachibana et al., 1994; Kondo et al., 1995). The high density of AMPA receptor expression in superficial laminae of the dorsal horn is consistent with the presence in these laminae of numerous glutamatergic synapses both from peripheral afferents (Broman et al., 1993; Valtschanoff et al., 1994) and from local interneurons (Rustioni and Cuénod, 1982). GluR1-positive neurons are concentrated at the IIo/IIi border and are generally superficial to the GluR2/3-positive neurons. Because previous studies with *in situ* hybridization suggest that the GluR3 subunit is only weakly expressed in the superficial dorsal horn (Furuyama et al., 1993; Henley et al., 1993; Tölle et al., 1993, 1995; Pellegrini-Giampietro et al., 1994), our staining for GluR2/3 is likely to reveal mainly the GluR2 subunit. By extrapolation from observations in the cortex (Kharazia et al., 1996) and in the dorsal column nuclei (our unpublished observations), at least a fraction of GluR1-positive neurons in superficial laminae may be GABAergic. Nitric oxide synthase (NOS) coexists with GABA in cells in these laminae (Valtschanoff et al., 1992), and NOS-positive neurons in forebrain lack GluR2 (Catania et al., 1995); however, because nitric oxide-synthesizing neurons in the spinal cord are concentrated at the border between laminae II and III (ventral to GluR1-positive neurons), only a modest fraction of GluR1-stained neurons may synthesize nitric oxide.

Relationship of LM and EM results

The laminar distribution of staining for the two antibodies was similar at LM and EM. However, staining of somata was prominent at LM, but sparse at EM. This apparent discrepancy is presumably explained by the characteristics of the techniques: immunoperoxidase exhibits high sensitivity (because of amplification of weak signals by the DAB reaction), but is less well

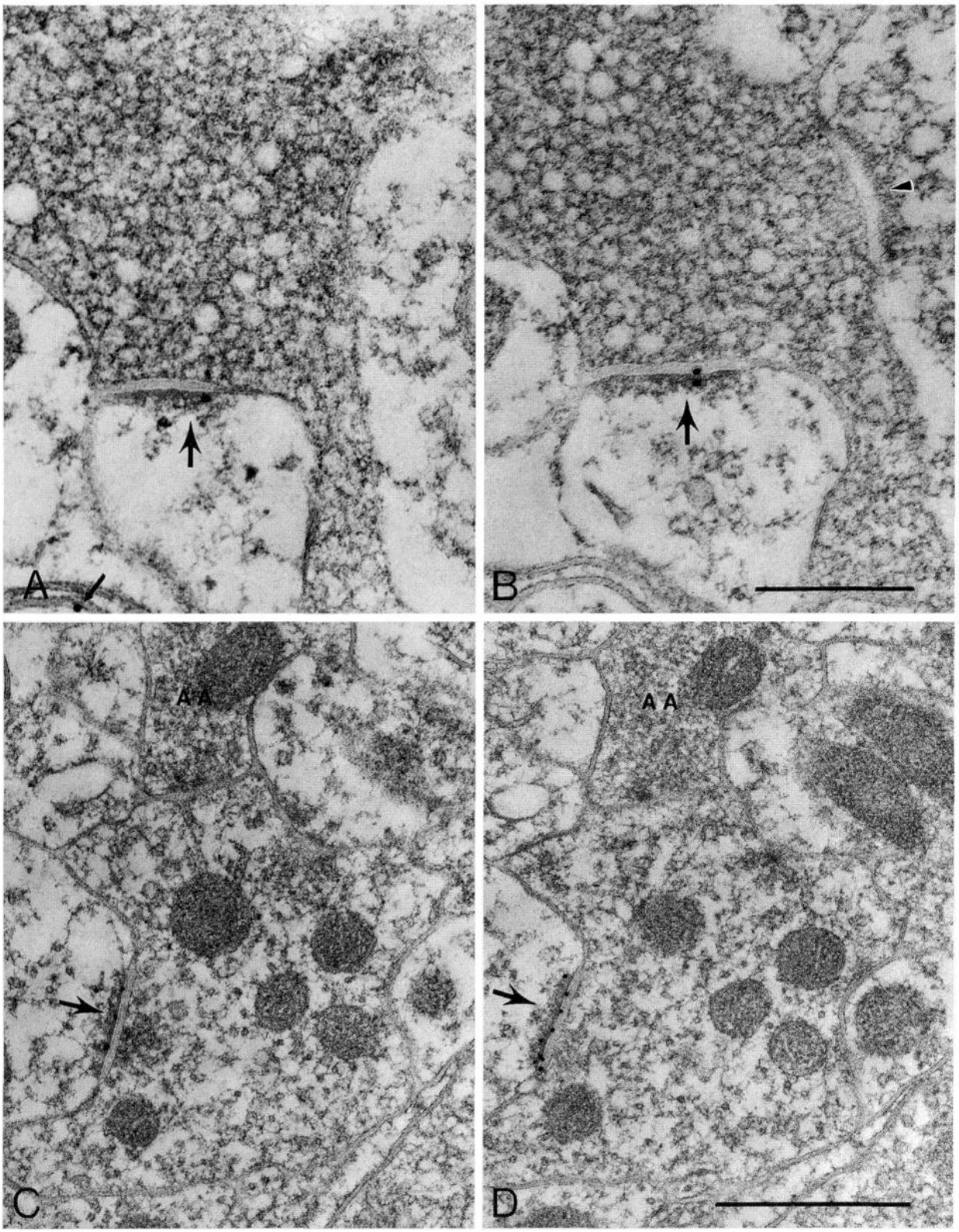

Figure 4. Photomicrographs of serial sections from L4. *A* and *B* are from a C1 terminal characterized (among other features) by vesicles of variable size and sparseness of mitochondria; *large arrows* point at gold particles coding for GluR1. *Small arrow* at bottom of *A* points at a gold particle at a nonsynaptic site; no gold particle is present at the same site in *B*. *C* and *D* are from a C2 terminal containing several mitochondria and vesicles of uniform size. *Large arrows* point at gold particles coding for GluR2/3. *Arrowhead* in *B* points at a negative synapse. Note immunonegative (presumably GABAergic) axo-axonic (AA) synapse. Scale bars: *A*, *B*, 0.25 μm; *C*, *D*, 0.5 μm.

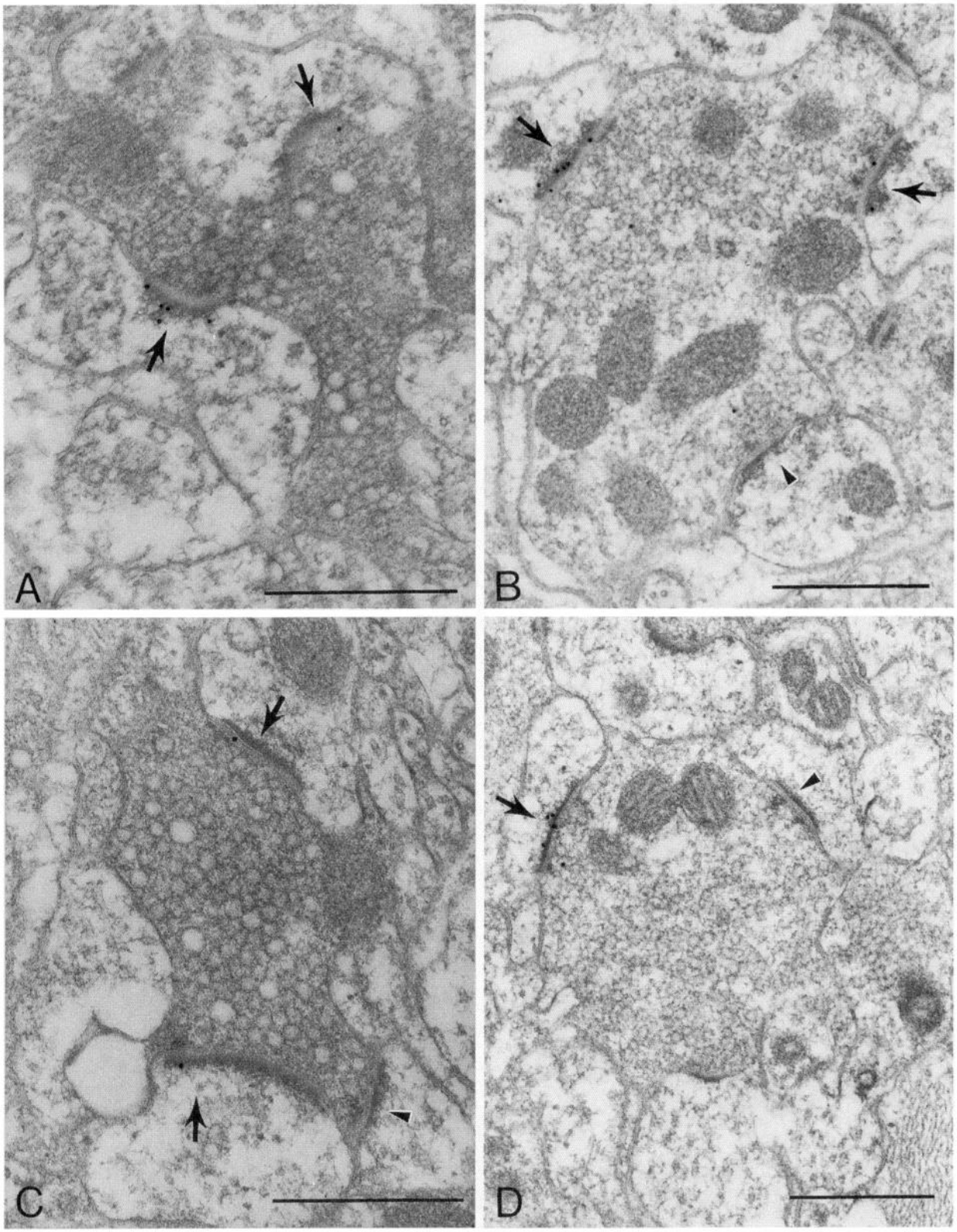

Figure 5. Photomicrographs of sections from cervical cord showing gold particles (*large arrows*) coding for GluR1 in C1 terminals (*A*, *C*) and in C2 terminals (*B*, *D*). *Arrowheads* point at examples of negative synapse. Scale bars, 0.5 μm.

localized than immunogold and does not accurately reflect quantitative differences (Griffiths, 1993). Alternatively, the immunogold labeling may require antigen concentration to exceed a threshold value. Finally, because cytoplasmic receptors in the cell body are presumably not in their final conformation, they may not be recognized by our EM method.

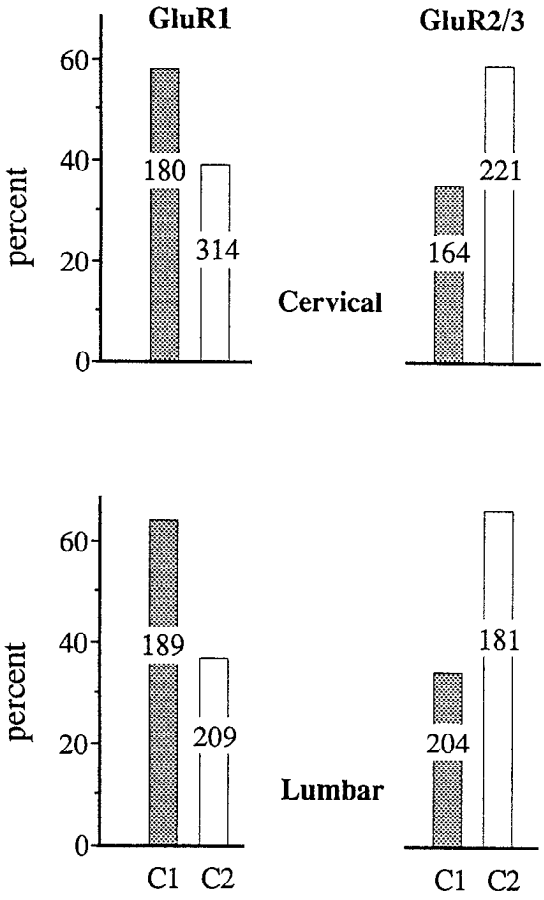

Figure 6. Bars show percentages of different types of terminals that have synaptic contacts with AMPA subunits. Total number of active zones from which percentages were computed is presented over each bar.

Synaptic clustering of AMPA receptors

Craig et al. (1993) provided LM evidence for clustering of AMPA/kainate subunits at synapses in cultured neurons; this was supported by EM immunogold performed on frozen or freeze-substituted sections (Nusser et al., 1994, 1995a,b) and by the present results. Localization in material fixed with high concentrations of glutaraldehyde (a highly effective protein cross-linker) is more reliable than in previous reports based on the use of very low concentrations of glutaraldehyde. The immunoglobulin bridge introduces a localization error of ~20 nm for the gold particles (Kellenberger and Hayat, 1991); because staining is confined to the surface, obliquity of synaptic membranes in the section may introduce an additional error of similar magnitude. These errors do not affect the present data concerning the modal location of particles but suggest that our results documenting a strong association of AMPA receptors with the postsynaptic membrane underestimate the precision of this association. The close match between glutamate-enriched terminals and sites immunopositive for glutamate receptors (Craig et al., 1994; Phend et al., 1995) shows that the labeling is selective for excitatory synapses, a conclusion supported by the absence of gold labeling at symmetric synapses.

Number of receptors at a synapse

The exact numerical relationship between gold particles and receptor molecules cannot yet be determined, but in other systems, one gold particle represents 20–200 molecules of antigen (Kellenberger et al., 1987; Kellenberger and Hayat, 1991; Griffiths, 1993). This ratio reflects various factors: (1) only antigen molecules presenting an epitope at the surface can be recognized and, even for thin (100 nm) sections, a majority of the epitopes are not exposed; (2) many of the epitopes may be denatured by the fixation and processing; and (3) steric constraints permit only a fraction of surface epitopes to bind immunoglobulin. Thus, although even a single gold particle over a synapse is likely to indicate presence of receptor, its absence cannot be taken as proof of the lack of receptor. Nevertheless, because there is an approximately linear relationship between gold particles and antigen density (Ottersen, 1989; Griffiths, 1993), it is possible to estimate the relative densities of subunits at different synapses. The mean number of particles associated with synapses made by primary afferent fibers is significantly higher than in cortical synapses from the same animals (our unpublished observations), consistent with the view that primary afferent fibers are strongly coupled to their postsynaptic neurons.

We studied subunits, not functional receptors. However, con-

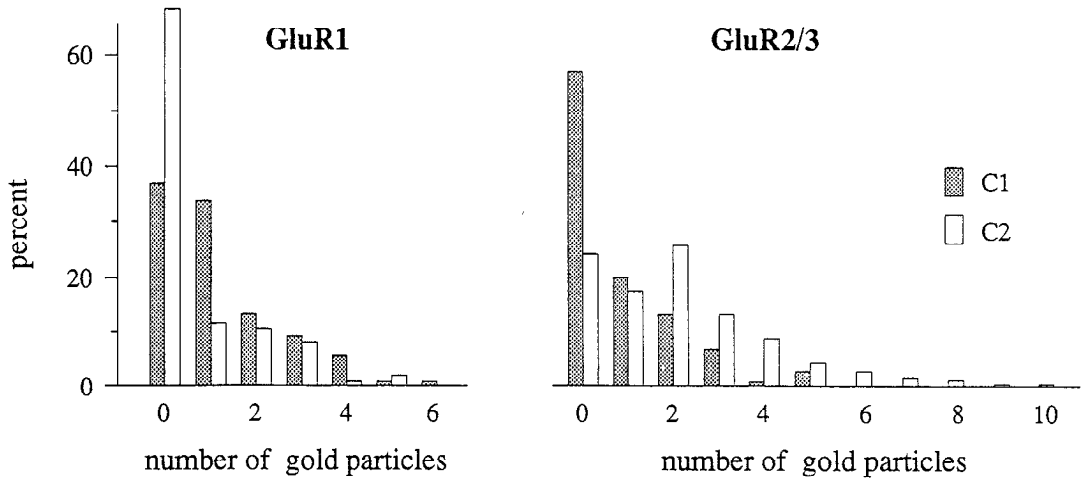

Figure 7. Histograms showing distribution of counts of gold particles over active zones associated with the two types of glomeruli, for both antibodies. Counts are from 130 active zones, associated with 52 C1 terminals, and 198 active zones, associated with 74 C2 terminals for GluR1; and from 116 active zones associated with 49 C1 terminals, and 183 active zones, associated with 74 C2 terminals, for GluR2/3.

sidering the high concentration of gold in the vicinity of the postsynaptic membrane, most of these subunits were already in a functionally appropriate position. In cortex and hippocampus, the labeling density seen using the present method corresponds well to biophysically derived estimates of functional receptors, assuming a labeling efficiency of 1–2% (Hestrin, 1992; Stern et al., 1992; Griffiths, 1993). It is thus plausible that most subunits inserted into the synaptic membrane have been assembled into functional pentameric receptors.

Relation of receptors to types of synapses

Central terminals of glomeruli were identified as endings of primary afferents. Two main types of central boutons (C1 and C2) in glomerular structures are likely to be mainly terminals of unmyelinated and small myelinated afferent fibers, respectively (Ribeiro-da-Silva, 1994). Although yielding a very limited sample, especially when dealing with unmyelinated afferents, the results from intracellular staining of physiologically identified peripheral afferents are generally compatible with an association of unmyelinated fibers with C1 and of myelinated mechanoreceptors with C2 terminals (Alvarez et al., 1992, 1993; Light, 1992). C1 terminals contain a low density of mitochondria and a high density of glutamate (Broman et al., 1993; Valtschanoff et al., 1994), both features perhaps related to their lower tonic activity and need for a larger pool of vesicular glutamate. C1 terminals are frequently presynaptic to GABAergic dendrites, whereas C2 terminals are more frequently postsynaptic to GABAergic profiles, possibly reflecting the generally lower spatiotemporal resolution of unmyelinated versus small myelinated fibers (Bernardi et al., 1995).

The present quantitative data show that both types of primary afferent terminals are associated with subtypes of AMPA receptors, but in different proportions. The preference of C1 for GluR1 contrasts with the preference of C2 terminals for GluR2/3 subunits. While the relative role of presynaptic and postsynaptic factors in establishing and maintaining these differences remains to be determined, the contrasting distribution of GluR1- and GluR2/3-immunopositivity raises the possibility that some neurons in the superficial dorsal horn may express only one of the two receptor subunits. Because AMPA receptors lacking GluR2 are calcium-permeable (Hollman and Heinemann, 1994), some neurons in dorsal substantia gelatinosa may experience AMPA-mediated calcium transients in response to glutamatergic synaptic input, particularly that originating from unmyelinated afferents, thus potentially activating second-messenger cascades. Recent work in primary culture demonstrates calcium-permeable AMPA channels in some neurons in the dorsal horn (Kyrozis et al., 1995). The apparent bias of terminals of unmyelinated fibers toward GluR2-poor AMPA receptors may bear on the issue of hyperalgesia. Sugimoto et al. (1990) proposed that central hyperalgesia secondary to peripheral neuropathy may involve NMDA-mediated excitotoxic damage to inhibitory interneurons. The present data raise the possibility that GABAergic interneurons in substantia gelatinosa may suffer excitotoxic damage from sustained abnormal activity in unmyelinated fibers synapsing onto calcium-permeable AMPA channels.

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