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Immunocytochemical Localization of the mGluR1α Metabotropic Glutamate Receptor in the Dorsal Cochlear Nucleus

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ABSTRACT

We demonstrate that the metabotropic glutamate receptor mGluR1α is enriched in two interneuron cell populations in the dorsal division of the cochlear nucleus. Electron microscopic analysis confirms that mGluR1α immunoreactivity is concentrated in the dendritic spines of cartwheel cells and in dendrites of the recently described unipolar brush cells. The cartwheel cells, which have many similarities to the Purkinje cells of the cerebellum, participate in a local neuronal circuit that modulates the output of the dorsal cochlear nucleus. Immunostained unipolar brush cells were observed in granule cell regions of the cochlear nucleus and the vestibulocerebellum. The presence of analogous cell types with similar patterns of immunolabeling in the cerebellum and in the dorsal cochlear nucleus suggests that a shared but as yet unknown mode of processing may occur in both structures.

Indexing terms: auditory system, cartwheel cell, cerebellum, ultrastructure unipolar brush cell

The dorsal cochlear nucleus (DCN) is a key structure in the auditory pathway. It receives direct connections from the cochlea by way of the auditory nerve and sends output directly to the inferior colliculus. How DCN cells process acoustic information depends on circuitry and intrinsic neuronal properties such as receptor types, ion channels, and/or second-messenger systems. Glutamate is the major excitatory neurotransmitter in the brain, and it is the proposed transmitter of the auditory nerve and the granule cell system of the cochlear nucleus (Schwartz, 1981; Greenamyre et al., 1984; Wenthold, 1985). Glutamate acts through ionotropic receptors, which are ligand-gated ion channels, and through G protein-linked metabotropic receptors (Nakanishi, 1992). Traditionally, excitatory responses of neurons in the cochlear nucleus have been considered in terms of stimulation of the ionotropic receptors. The recent localization of the mRNA of the metabotropic receptor subtype mGluR1 in the DCN (Shigemoto et al., 1992) has broadened our views on possible mechanisms for signal processing in this nucleus. Consequently, we have investigated the localization of a metabotropic glutamate receptor in the DCN in both rat and guinea pig.

Eight subtypes (mGluR1–8) of metabotropic glutamate receptors have been cloned to date (Houamed et al., 1991; Masu et al., 1991; Tanabe et al., 1992; Nakanishi, 1992; Nakajima et al., 1993; Okamoto et al., 1994; Pin and Duvoisin, 1995). Some subtypes stimulate the phosphoinositide (PI) second-messenger system, whereas others are coupled to adenylyl cyclase (Abe et al., 1992; Aramori and Nakanishi, 1992; Tanabe et al., 1992). mGluR1 exists as three distinct splice variants, mGluR1α, mGluR1β, and mGluR1c, and the coupling of the mGluR1α receptor subtype to the PI second-messenger cascade (Aramori and Nakanishi, 1992) is potentially important in auditory processing, because the inositol 1,4,5-trisphosphate receptor (IP_3r) is abundant in the DCN (Mignery et al., 1989; Rodrigo et al., 1994; Ryugo et al., 1995).

The DCN is a layered structure with a complex neuronal circuitry that is organized around the pyramidal cells of the nucleus (Blackstad et al., 1984). Pyramidal cells receive auditory nerve input and project to the inferior colliculus. They are analogous to neurons of the deep cerebellar nuclei, in that both are the main projection neurons of each respective structure. The activity of pyramidal cells is modulated by several types of interneurons. One interneuronal circuit in the superficial layers of the nucleus involves a granule cell system that shares anatomical, physiological, and biochemical similarities with that of the cerebellum (see, e.g., Mugnaini and Morgan, 1987; Berrebi and Mug-

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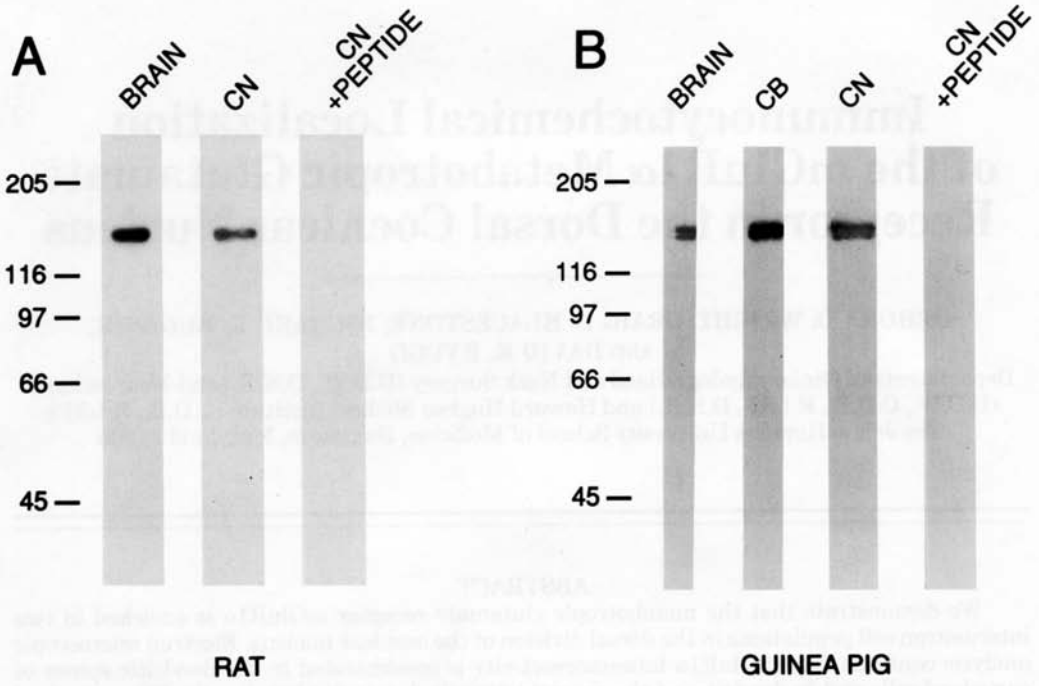

Fig. 1. Anti-mGluR1α antibodies. **A:** Total homogenates prepared from rat whole brain (50 μg/lane) or dissected rat cochlear nuclei (CN; 150 μg/lane) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with antipeptide antibodies against mGluR1α. A single band of protein having a relative molecular mass of 142,000 in both brain and cochlear nucleus was labeled with the mGluR1α antibody. **B:** Total homogenates from guinea pig whole brain (50 μg/lane), cerebellum (CB; 25 μg/lane) or cochlear nucleus (150 μg/lane) were dissected, homogenized, and analyzed as above. The mGluR1α antibody recognized a protein doublet in guinea pig whole brain, cerebellum, and cochlear nucleus that has a similar molecular mass to the labeled band in rat. When antibodies were preadsorbed with an excess of synthetic peptide (corresponding to the C-terminus of the mGluR1α receptor) prior to immunoblotting, immunoreactivity in the rat and guinea pig cochlear nucleus homogenates was completely abolished (A,B, far right lanes). Sizes of molecular mass standards (in kDa) are indicated on the left in A and B.

naini, 1991). Like the cerebellar cortex, the superficial DCN has granule cells, Golgi cells, stellate cells, unipolar brush cells (UBCs), and cartwheel cells, which are analogous in many ways to Purkinje cells. Dense immunoreactivity for the mGluR1α receptor has been demonstrated in Purkinje cells of the cerebellum (Martin et al., 1992).

In the present study, we demonstrate that the mGluR1α receptor subtype is concentrated in cartwheel cells, which are Purkinje cell analogs of the DCN. We also found that UBCs of the cochlear nucleus and the cerebellum are immunoreactive for the mGluR1α receptor antibody. The presence of the mGluR1α receptor subtype in these analogous cell classes is consistent with the notion that the function of the cartwheel cells and UBCs in the DCN is similar to their morphologic counterparts in the cerebellum.

MATERIALS AND METHODS

Antibody production and immunoblotting

Antipeptide antibodies were generated against a synthetic peptide corresponding to the C-terminal 20-amino-acid residues of the rat mGluR1α protein, as described previously (Blackstone et al., 1992a; Martin et al., 1992; Van den Pol et al., 1994). Antibodies were affinity purified prior to their use for immunoblotting of total tissue homogenates prepared from rat brain and from dissected rat cochlear nuclei as well as from guinea pig brain, dissected cerebella, and cochlear nuclei (Blackstone et al., 1992b).

Fig. 2. The dorsal cochlear nucleus (DCN) exhibits intense immunoreactivity to the mGluR1α antibody. A–E are rat tissue, and F and G are taken from guinea pig. Tissue in A–D was permeabilized with the detergent Triton X-100, whereas sections shown in E–G were treated with Photo-Flo or were frozen to allow access of the antibody into the tissue. **A:** Transverse section of the rat DCN demonstrates dense mGluR1α labeling in layer 1 and in superficial layer 2. DAS, dorsal acoustic stria; L, lateral; D, dorsal. **B:** A section of rat DCN processed using mGluR1α antibody preadsorbed with synthetic peptide shows an absence of nonspecific staining. **C:** mGluR1α staining in the neuropil of layers 1 and 2 is granular in appearance. Deeper regions of the DCN have scattered, intense mGluR1α label (arrowhead). **D:** Rat cerebellar cortex immunostained with mGluR1α has dark staining of the Purkinje cell processes found in the molecular layer, as previously reported by Martin et al. (1992). Similar to the DCN, the cerebellum has concentrated regions of label in the granule cell layer (arrowhead). ml, Molecular layer; pl, Purkinje cell layer; gl, granule cell layer. **E:** When rat tissue sections were permeabilized by freezing instead of by using Triton X-100 detergent, light staining was visible in medium-sized cell bodies (one is indicated by an arrowhead) that gave rise to labeled dendrites in layers 1 and 2 of the DCN. A small cell, presumably a unipolar brush cell (UBC), is also labeled (arrow). **F:** mGluR1α label in a section of guinea pig DCN that was permeabilized using Photo-Flo, a mild detergent. **G:**. Higher magnification view of layers 1 and 2 of guinea pig DCN shows labeling in a medium-sized cell body with labeled proximal dendrites (arrowhead) that have the granular staining that was seen throughout the superficial layers. A relatively unlabeled pyramidal cell is indicated by an arrow. Tissue in E–G was stained with osmium and embedded in plastic for electron microscopy; therefore, background staining appears darker than the light microscopic material in A–F. Scale bars = 250 μm in A,B, 50 μm in C–E,G, 100 μm in F.

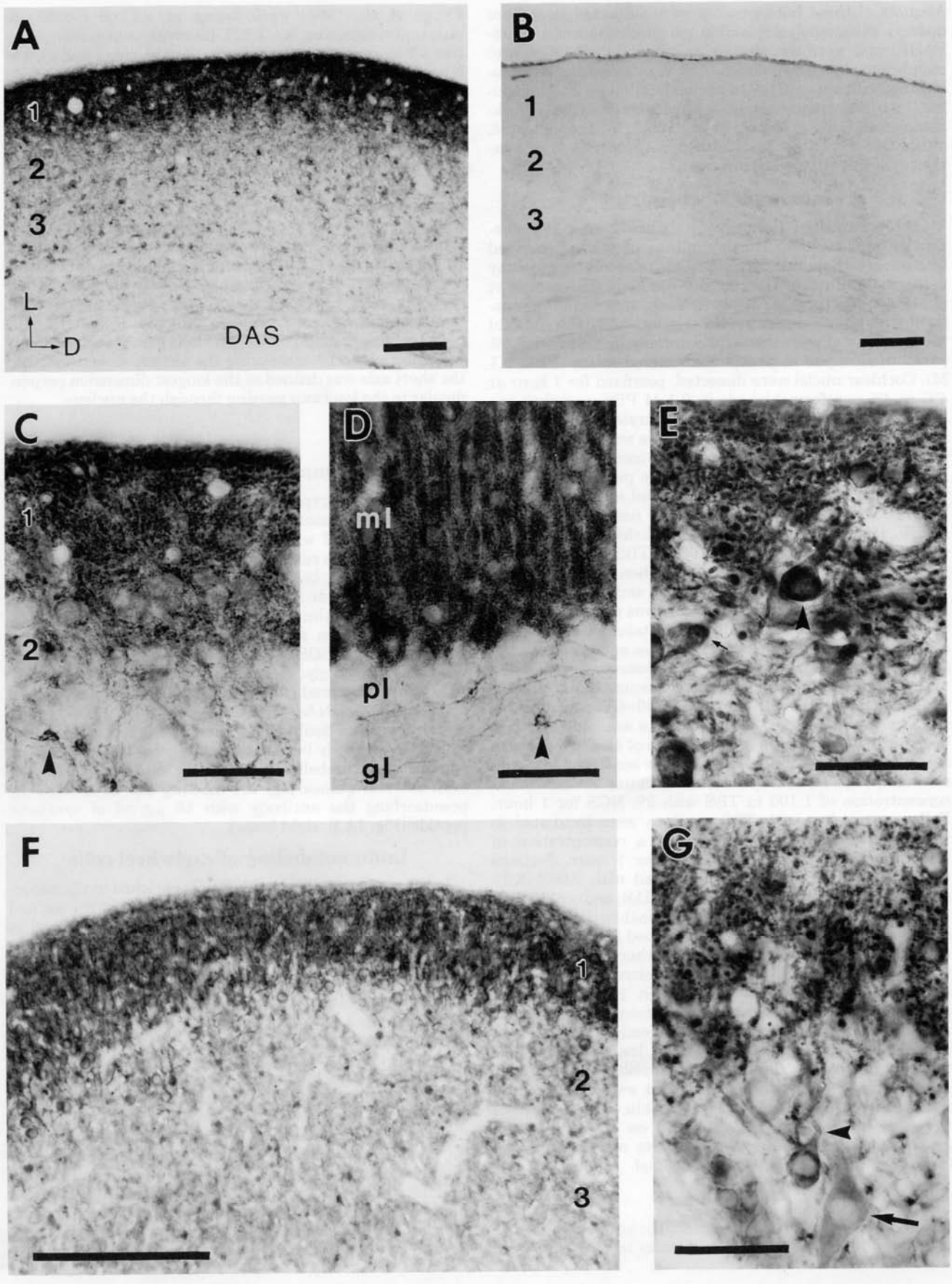

Figure 2

Aliquots of these homogenates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were transferred to Immobilon P (Millipore) membranes by electroblotting (30 V, overnight). Immunoblotting with anti-mGluR1α antibodies [0.5 μg immunoglobin (IgG)/ml] was performed as previously described (Blackstone et al., 1992a; Martin et al., 1992). Where indicated, antibodies were first preadsorbed with an excess of synthetic peptide (10 μg/ml) prior to use.

Immunohistochemistry

Cochlear nucleus tissue from six adult male Sprague-Dawley rats and two Hartley guinea pigs was processed immunocytochemically for light and electron microscopy using the anti-mGluR1α receptor antibody (Blackstone et al., 1992a; Martin et al., 1992). Animals were deeply anesthetized with chloral hydrate (400 mg/kg) or with Nembutal (50 mg/kg) and were then transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS; 0.1 M). Cochlear nuclei were dissected, postfixed for 1 hour at 4°C in 4% paraformaldehyde in 0.1 M PBS, and then cut into sections (40–50 μm thick) on a Vibratome.

All immunocytochemical incubations were performed at 4°C, unless otherwise stated. Prior to incubation with the primary antibody, tissue sections were permeabilized and then blocked for 1 hour in Tris-buffered saline (TBS; 0.05 M, pH 7.2; 1.5% NaCl) containing 4% normal goat serum (NGS). Permeabilization was accomplished by incubating sections with 0.08% Triton X-100 in TBS for 10 minutes prior to blocking or by using 0.1% Photo-Flo (Kodak) in TBS during incubations with primary and secondary antibodies. In one experiment, the tissue was permeabilized by first placing sections in a solution containing 25% sucrose and 10% glycerin in PBS for 20 minutes and then freezing them at −80°C for 20 minutes. Sections were incubated with the mGluR1α antibody at a concentration of 0.5–1.5 μg of IgG/ml in TBS with 2% NGS for 40–48 hours. Control sections were incubated with mGluR1α antibody that had been preadsorbed with a tenfold excess of blocking peptide. After washing with TBS, sections were incubated in a goat anti-rabbit IgG secondary (Jackson Immuno Research) at a concentration of 1:100 in TBS with 2% NGS for 1 hour. Following washes with TBS, sections were incubated in rabbit peroxidase antiperoxidase at a concentration of 1:200 (Jackson Immuno Research) for 1 hour. Sections were then washed in TBS and treated with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB) and 0.01% H_2O_2 in TBS for 5–10 minutes. Following washes in TBS, some sections were mounted on gelatin-subbed slides and coverslipped for light microscopy, whereas others were processed further for both light and electron microscopy. Electron microscopic material was postfixed in 1% OsO_4 for 20 minutes, stained en bloc with 1% uranyl acetate (overnight), dehydrated, infiltrated, and embedded in Epon between two sheets of Aclar (Ted Pella, Inc.). Sections were taped to microscope slides for examination with the light microscope. Selected regions of interest were dissected and reembedded in BEEM capsules. Ultrathin (75 nm) sections were cut on a microtome, collected on Formvar-coated grids, and stained with uranyl acetate and lead citrate. Some ultrathin sections were not stained.

Morphometry

Cells immunoreactive to the mGluR1α antibodies (guinea pigs 1 and 2) and to the IP_3r antibodies (guinea pigs from Ryugo et al., 1995) were drawn at ×2,500 (×100 oil-immersion objective, NA 1.25). Likewise, nonimmunoreactive silhouettes of pyramidal cells (guinea pigs 1 and 2), and Nissl-stained pyramidal cells (guinea pigs from Ryugo et al., 1995) were drawn at the same magnification. Unstained cells in immunoprocessed tissue were visualized using differential interference contrast microscopy, and pyramidal cells were identified by their distribution in layer 2, their elongated and relatively large cell bodies, and their centrally placed, round nuclei. In Nissl-stained, IP_3r-immunoprocessed tissue, pyramidal cells were identified by their elongated somatic shape, their prominent Nissl bodies, and their centrally placed, round, pale nuclei. Cartwheel cells were characterized by their medium-sized, round-to-oval cell bodies (roughly 15 μm in diameter), their centrally placed, deeply invaginated nucleus, and their immunoreactivity to antibodies directed against the IP_3 receptor (Ryugo et al., 1995). The long axis of each cell body was determined by measuring the longest dimension, and the short axis was defined as the longest dimension perpendicular to the long axis passing through the nucleus.

RESULTS

Immunoblot analysis

Immunoblots prepared from tissue homogenates of rat cochlear nuclei demonstrate that a single band of protein at 142 kD is labeled with the mGluR1α antibody (Fig. 1A, middle lane). This result is in agreement with the labeling seen in whole rat brain homogenates (Fig. 1A, left lane). Analysis of the amino acid sequence of the mGluR1α cDNA clone gives an estimated molecular mass of 133 kD. The difference between this estimated molecular weight and that seen using SDS-PAGE could be due to the in vivo posttranslational glycosylation at multiple N-glycosylation sites, which are predicted by consensus sequences seen in the mGluR1α cDNAs (Houamed et al., 1991; Masu et al., 1991). A protein doublet of similar size is labeled with the mGluR1α antibody in tissue homogenates from guinea pig whole brain, cerebella, and cochlear nuclei (Fig. 1B). In both rat and guinea pig, the labeling can be blocked by preadsorbing the antibody with 10 μg/ml of synthetic peptide (Fig. 1A,B, right lanes).

Immunolabeling of cartwheel cells

Immunoreactivity for mGluR1α is enriched in the superficial layers of the DCN. Transverse sections of the rat and guinea pig DCN exhibit intense labeling in layer 1 and in upper layer 2 (Fig. 2A,C,F). Control sections treated with mGluR1α antibody preadsorbed with the synthetic peptide to which it was raised show little nonspecific binding (Fig. 2B). At higher magnification, the dense label in the superficial layers has a granular appearance (Figs. 2C,E,G, 3A). A section of the rat cerebellum treated with the same protocol is shown in Figure 2D, demonstrating that our preparations have a level of immunoreactivity in the molecular layer similar to that shown previously using the same antibody and methodology (Martin et al., 1992).

Several different tissue processing techniques produced the same overall pattern of staining. Permeabilization of tissue sections by freezing, treatment with the mild detergent Photo-Flo, or treatment with Triton X-100 revealed cell bodies more clearly than when detergent was omitted. Otherwise, all procedures labeled dendrites of layers 1 and 2 in both rat and guinea pig DCN (Fig. 2E–G). In addition,

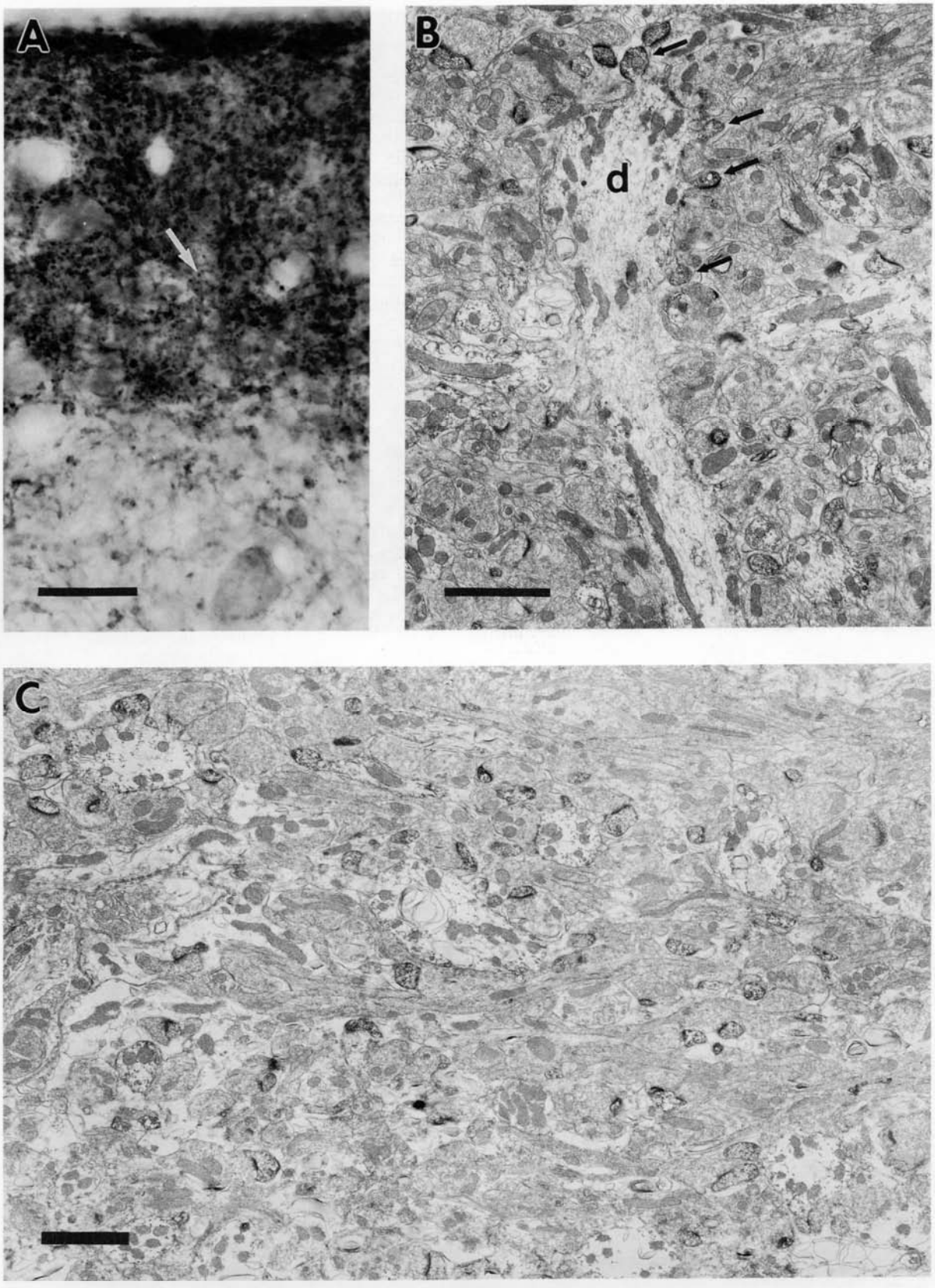

Fig. 3. Granular mGluR1α staining in the superficial layers of the rat DCN seen in the light microscope corresponds to labeled dendritic spines visualized with electron microscopy. **A:** At the light microscopic level, individual grains of mGluR1α label are concentrated in the neuropil (arrow). **B:** Labeled spines (arrows) arise from a relatively unlabeled dendritic shaft (d) in layer 1. **C:** Numerous labeled dendritic spines are seen throughout layers 1 and 2. Tissue in this figure was permeabilized with Triton X-100. Scale bars = 25 μm in A, 2 μm in B,C.

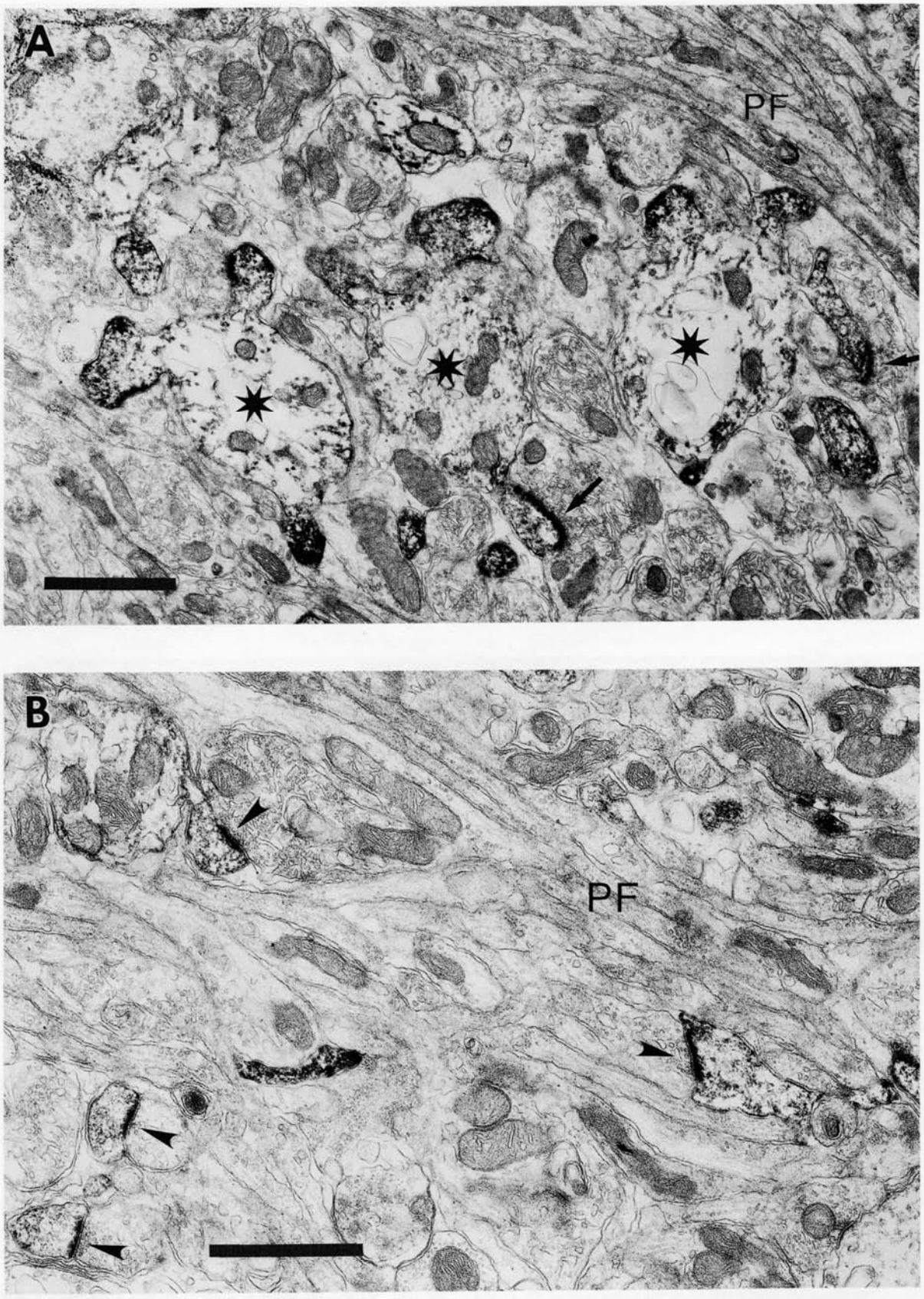

Fig. 4. Labeled spines in layers 1 and 2 of the rat DCN. **A:** Dendritic shafts (stars) have a high density of labeled spines. Axon terminals containing roundish vesicles make synapses with labeled spines (arrows) in layer 2. **B:** Unlabeled parallel fiber (PF) terminals synapse (arrowheads) with labeled spines in layer 1. Tissue was permeabilized with Triton X-100. Scale bars = 1 μm.

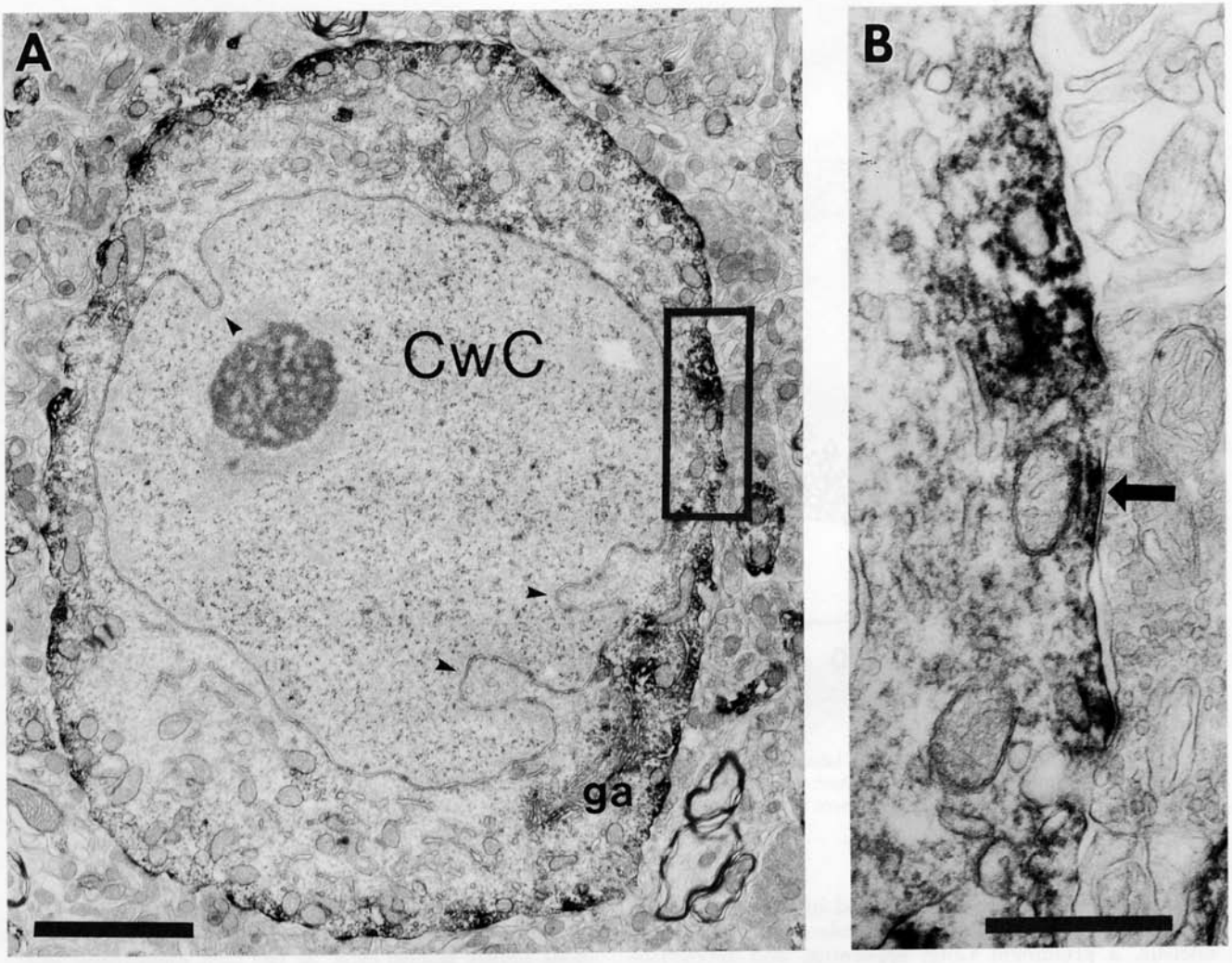

Fig. 5. The labeled cells in the rat DCN have the ultrastructural features of cartwheel cells. **A:** Medium-sized cell is labeled with the mGluR1α receptor antibody mainly along the plasma membrane of the soma. The labeled cell has characteristics of the cartwheel cells (CwC), including a conspicuous Golgi apparatus (ga), an irregular nuclear envelope (arrowheads), and subsurface cisternal-mitochondrial complexes (box). **B:** Subsurface cistern-mitochondrial complex (arrow) from the boxed area in A. Tissue in this figure was permeabilized by freezing. Scale bars = 2 μm in A, 0.5 μm in B.

tissue that was osmicated and embedded in plastic yielded more prominent cell body staining at the light microscopic level (Fig. 2E–G).

In order to determine the cellular localization of the mGluR1α receptor in the superficial DCN, we studied immunoreacted sections with electron microscopy. The granular appearance of immunolabeling in the superficial regions at the light microscopic level (Figs. 2E,G, 3A) corresponds to that seen in the electron micrographs of heavily labeled dendritic spines (Figs. 3, 4, 7). Cross sections of dendritic shafts with labeled dendritic spines are seen throughout layers 1 and 2 of the DCN. Frequently, these thin (75 nm) sections through the dendrites reveal a large number of labeled spines (Fig. 4A), indicating that the neurons labeled for the mGluR1α receptor have a very high density of dendritic spines. The high spine density of these labeled dendrites strongly suggests that they arise from cartwheel cells. Pyramidal and stellate cells have dendritic processes in the superficial layers, but do not exhibit numerous spines on their dendrites (Wouterlood et al., 1984; Hackney et al., 1990; Manis et al., 1994). No presynaptic mGluR1α label was observed.

In layer 1 of the DCN, groups of thin unmyelinated axons run parallel to the surface of the nucleus and produce unlabeled en passant terminals that contact labeled spines (Fig. 4B, arrowheads). The unlabeled terminals are filled with roundish synaptic vesicles, and they form asymmetric synapses with the labeled spines. These morphological characteristics indicate that the swellings arise from the excitatory parallel fibers of granule cells that are known to contact the dendritic spines of cartwheel cells in rat (Wouterlood and Mugnaini, 1984) and in guinea pig (Berrebi and Mugnaini, 1991).

In rat tissue processed with a higher concentration of antibody (1.5 μg/ml) and with no detergent, some cell bodies in layer 2 are lightly labeled along their plasma membranes, but they exhibit heavily labeled dendrites in layer 1. The labeled cells have the ultrastructural character-

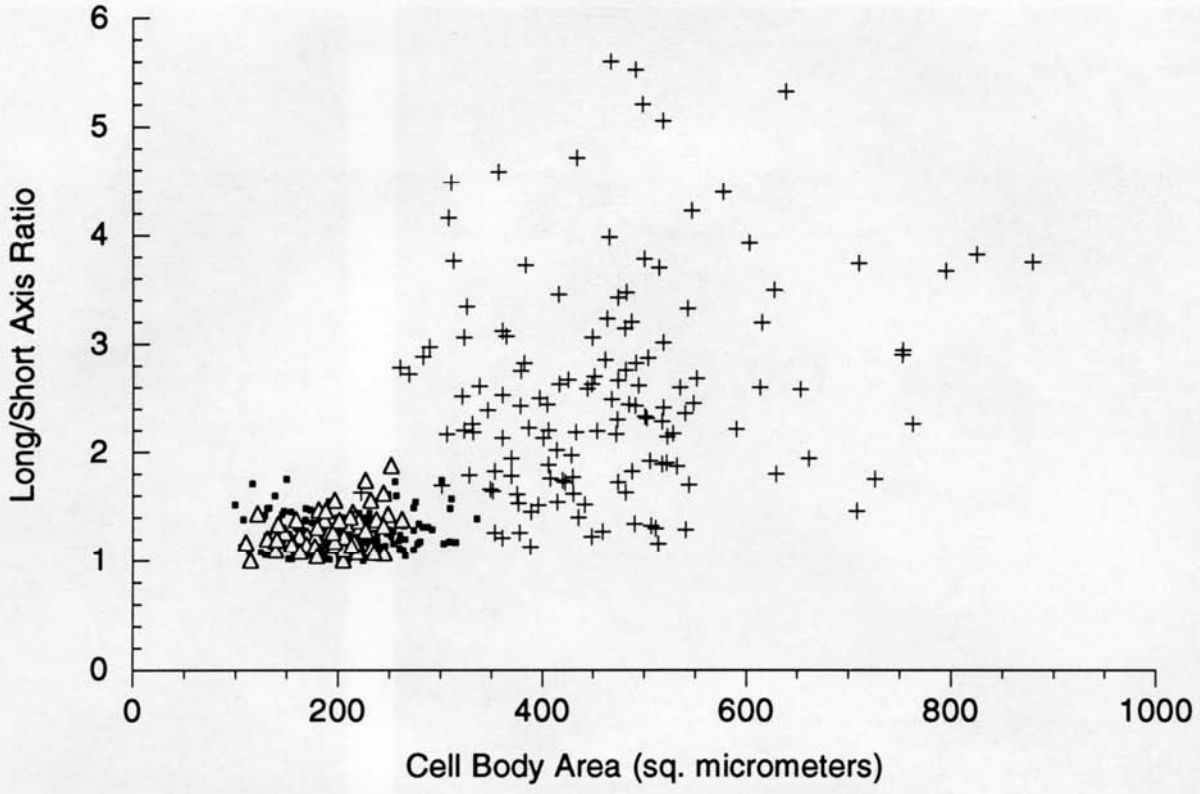

Fig. 6. The mGluR1α-labeled cells in layers 1 and 2 of guinea pig DCN have the dimensions of cartwheel cells. A plot of the long/short axis ratio vs. cell body area in square micrometers for mGluR1α-labeled cells (triangles; n = 56), compared to pyramidal cells (pluses; n = 137) and IP_3r-stained cartwheel cells (squares; n = 188).

istics of cartwheel neurons described by Wouterlood and Mugnaini (1984), including an indented euchromatic nucleus, a prominent Golgi apparatus, and subsurface cistern-mitochondrial complexes, which are the signature markers of this cell type in rat (Fig. 5). Although guinea pig cartwheel cells do not uniquely exhibit this subsurface organelle, the corresponding labeled cells in the superficial layers of the guinea pig DCN have the appearance of cartwheel cells, in that they are round medium-sized neurons containing large nuclei and have dendrites that project towards the ependymal surface of the cochlear nucleus (Ryugo et al., 1995). The size, shape, and distribution of the mGluR1α-labeled neurons in the guinea pig (Fig. 6) are identical to those of cartwheel cells described in previous studies (Manis et al., 1994; Ryugo et al., 1995). At the electron microscopic level, immunoreaction product in labeled cartwheel cells of the guinea pig is found along the inside of the plasma membrane (Fig. 7C). These observations are consistent with the conclusion that the mGluR1α receptor is enriched in cartwheel cells of the DCN of rat and guinea pig.

Immunolabeling of UBCs

Isolated clumps of label are also dispersed throughout the granule cell regions of the cochlear nucleus (Fig. 2C, arrowhead), in layers 2–4 of the DCN (Fig. 2A,F), and in the granule cell layer of vestibulocerebellum (exemplified in a section of the paraflocculus; Fig. 2D, arrowhead). In sections processed with higher concentrations of antibody and with no detergent, these dense regions of label appear to be concentrated in thin dendritic branchlets that arise from small, lightly labeled cells. At the light microscopic level, these small cells have the features of the recently characterized UBCs (Floris et al., 1994): The cell bodies are small (approximately 8–12 μm diameter in the cerebellum; 5–10 μm diameter in the DCN) and typically emit a single dendrite, which terminates in a brush-like spray of dendritic branchlets (Fig. 8). These labeled cells are most abundant in the granule cell regions of the cochlear nucleus and the vestibulocerebellum, including the nodulus (lobule X), ventral uvula (lobule IXd), lingula (lobule I), flocculus, and paraflocculus. This distribution corresponds to that of the previously described UBCs (Floris et al., 1994). Although UBCs were observed in granule cell regions throughout the cochlear nucleus, those described in this paper were taken exclusively from the DCN.

At the ultrastructural level, the labeled dendritic tufts of UBCs have a similar appearance in both rat and guinea pig cochlear nucleus and cerebellum (Figs. 9, 10A, 11A). The mGluR1α labeling is most dense in the dendritic appendages of these small cells. The long appendages are darkly labeled with the mGluR1α antibody, whereas the stalk of the dendrite contains less label (Figs. 9A,C, 11A). The UBC dendrites surround and synapse extensively with the mossy fiber terminals (Figs. 9, 10A, 11A). Mossy fiber terminals in the cochlear nucleus (named for their morphological similarities to those of the cerebellum) are defined as large central terminals filled with round synaptic vesicles and

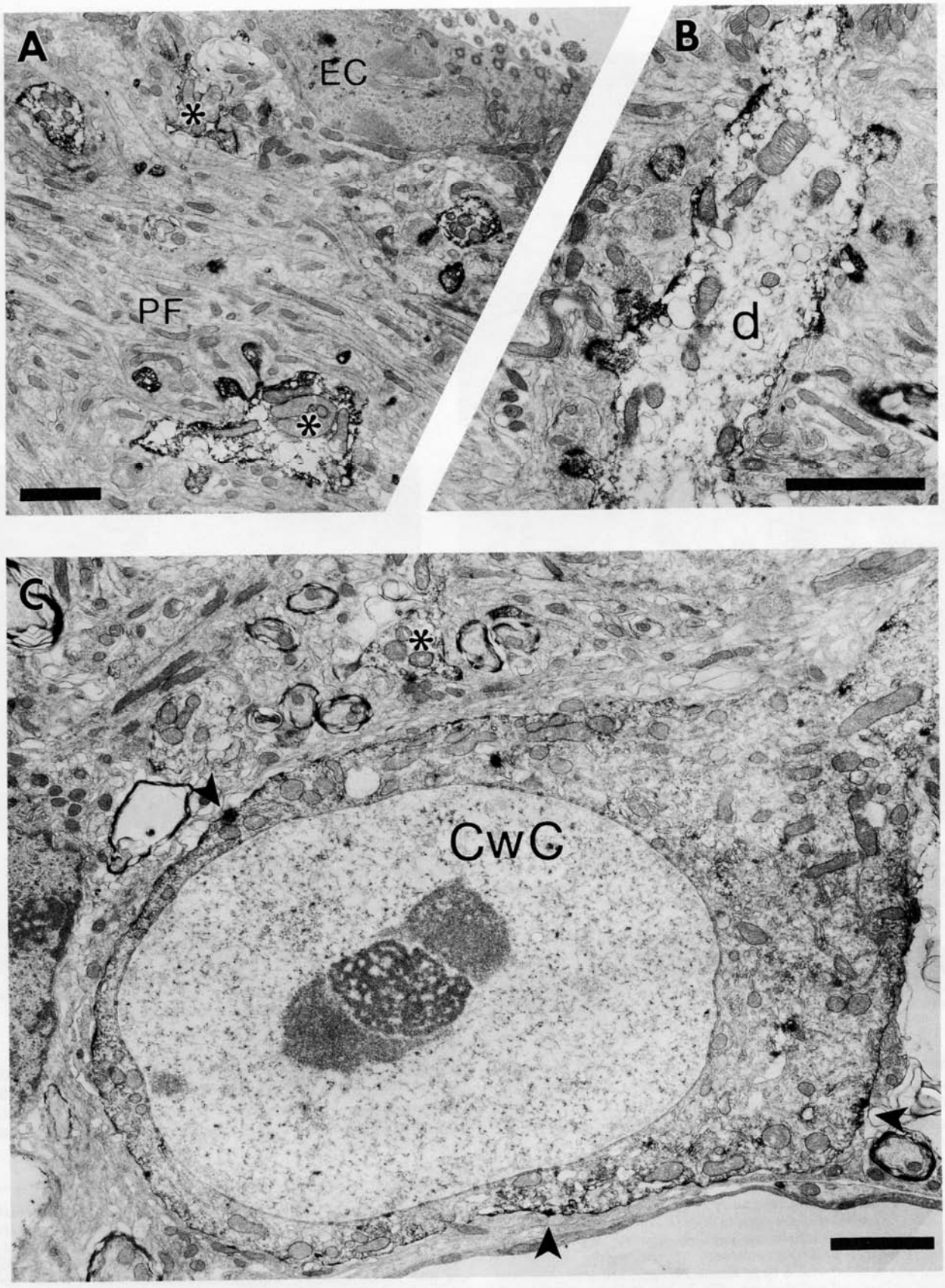

Fig. 7. At the ultrastructural level, mGluR1α labeling in the guinea pig DCN appears concentrated along the plasma membrane of cartwheel cell bodies and in their spiny dendrites. **A:** Cross sections of several labeled dendrites (asterisks) that give rise to darkly labeled spines in layer 1. PF, parallel fibers; EC, ependymal cell. **B:** This dendrite (d) in layer 2 exhibits several mGluR1α-labeled spines. **C:** Cartwheel cells (CwC) have light punctate mGluR1α immunoreactivity concentrated along the plasma membrane of the cell body. The most prominent regions of label are indicated by the arrowheads. A cross section of a labeled dendrite near the cell is indicated by the asterisk. Tissue was permeabilized with Photo-Flo. Scale bars = 2 μm.

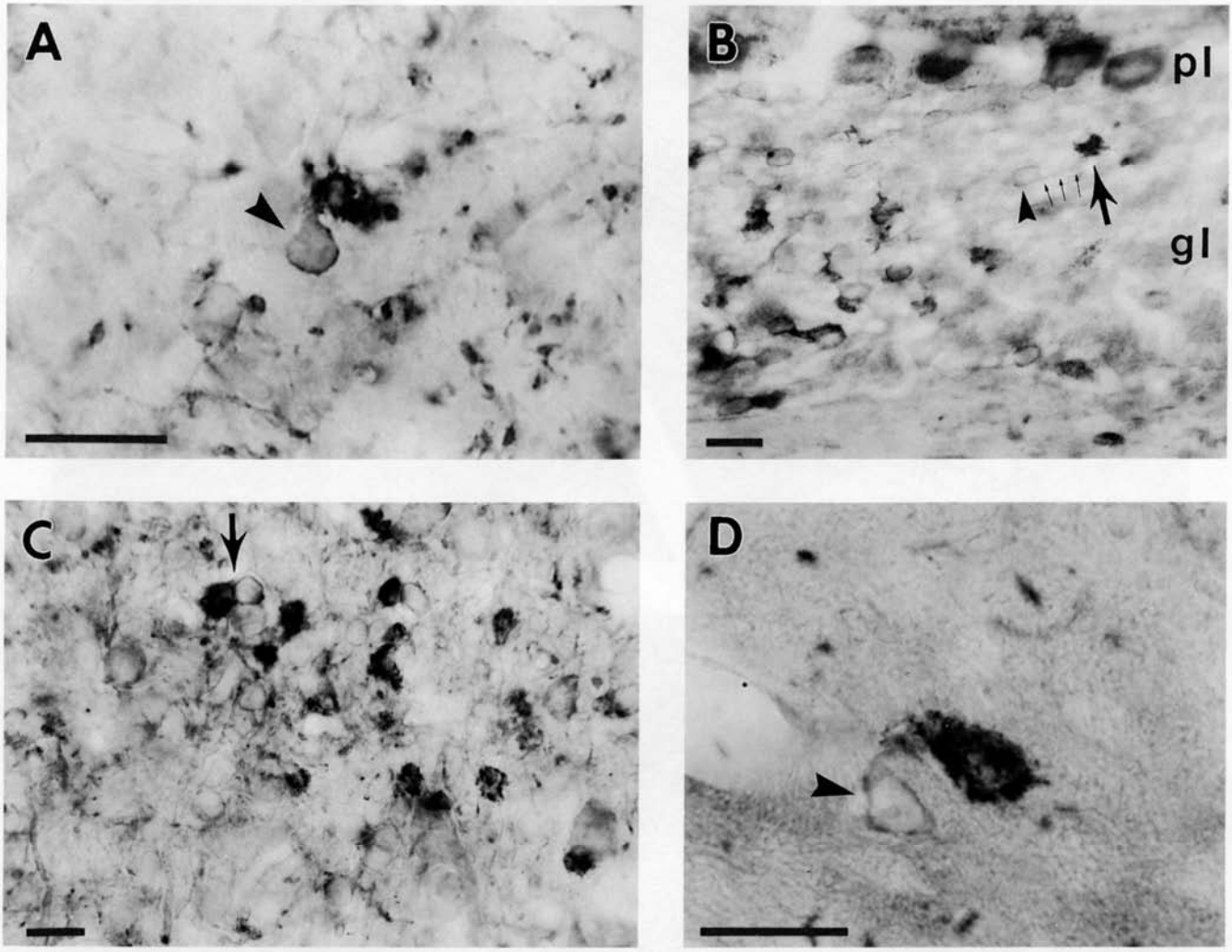

Fig. 8. UBCs in the granule cell regions of the cochlear nucleus and cerebellum are immunoreactive for the mGluR1α receptor antibody. Tissue in A and B were permeabilized by freezing tissue, whereas tissue in C and D were treated with Photo-Flo. **A:** In rat DCN, a labeled small cell (arrowhead) in layer 2 has a single thick dendritic stalk that gives rise to a tuft of dendritic branchlets, characteristic of UBCs. The periphery of the cell body and the dendritic brush are labeled. The brush appears to surround an unlabeled structure. **B:** In rat cerebellum, several labeled small cell bodies and dendritic tufts are seen in the granule cell layer (gl) of the nodulus. A heavily labeled dendritic tuft (large arrow) arises from a lightly labeled cell body (arrowhead) and a primary dendrite (small arrows). Purkinje cells, which are also known to have dense immunoreactivity for the mGluR1α receptor, are visible at the top. pl, Purkinje cell layer. **C:** In guinea pig DCN, a cluster of labeled cells in layer 3 has the appearance of UBCs previously described in rat. A labeled cell with a short dendritic stalk and labeled brush is indicated by the arrow. **D:** In guinea pig cerebellum, a small cell (arrowhead) that is located in the granule cell layer of the nodulus is labeled with the mGluR1α antibody. The extensive brush is darkly labeled and surrounds an unlabeled structure. Scale bars = 20 μm.

numerous mitochondria (Mugnaini et al., 1980). The small dendritic appendages of cerebellar UBCs have been described as nonsynaptic (Mugnaini et al., 1994), but we have observed clear examples of synapses between mossy fiber terminals and labeled appendages of the cochlear nucleus UBCs (Fig. 9A). In both the cochlear nucleus and the cerebellum, however, synapses between the UBC dendritic stalk and mossy fiber terminals are often distinctly separate

Fig. 9. Electron microscopic analysis demonstrates that dendrites with mGluR1α-labeled appendages in deep DCN surround and synapse with mossy fiber terminals. **A:** Labeled dendritic appendage that extends from the relatively unlabeled dendritic stalk (d) forms a synapse (arrowhead) with a large axonal ending containing numerous synaptic vesicles and mitochondria, called a mossy fiber terminal (mf). A synapse with another mossy fiber terminal (arrow) is unlabeled and is distant from the dense mGluR1α labeling in the dendrite. **B:** A labeled dendritic branch (d) of a cell that was identified as a UBC based on light microscopic appearance prior to thin sectioning surrounds and forms a synapse (arrowhead) with a mossy fiber terminal (mf). Small densely labeled appendages similar to the nonsynaptic appendages described for cerebellar UBCs are indicated by the arrows. **C:** The dendrite (d) of a UBC in guinea pig DCN is similar in appearance to those in rat UBCs and also makes synaptic contacts (arrowheads) with a mossy fiber terminal (mf). Many nonsynapsing appendages located to the right of the mossy fiber and along the outside of the main dendritic stalk are darkly labeled with the mGluR1α receptor antibody. Another section of dendrite is situated above the mossy fiber. Tissue was permeabilized by freezing. Scale bars = 1 μm.

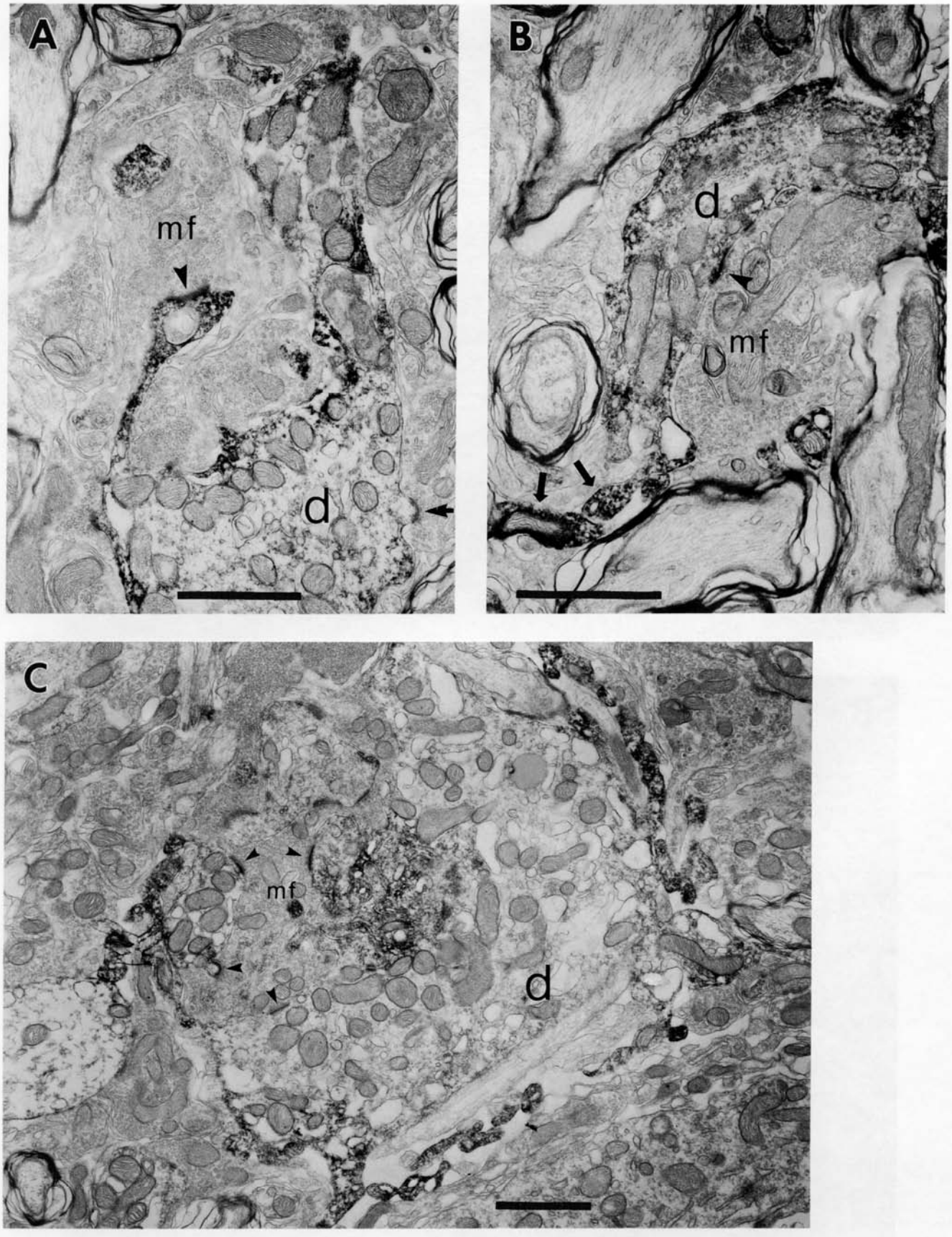

Figure 9

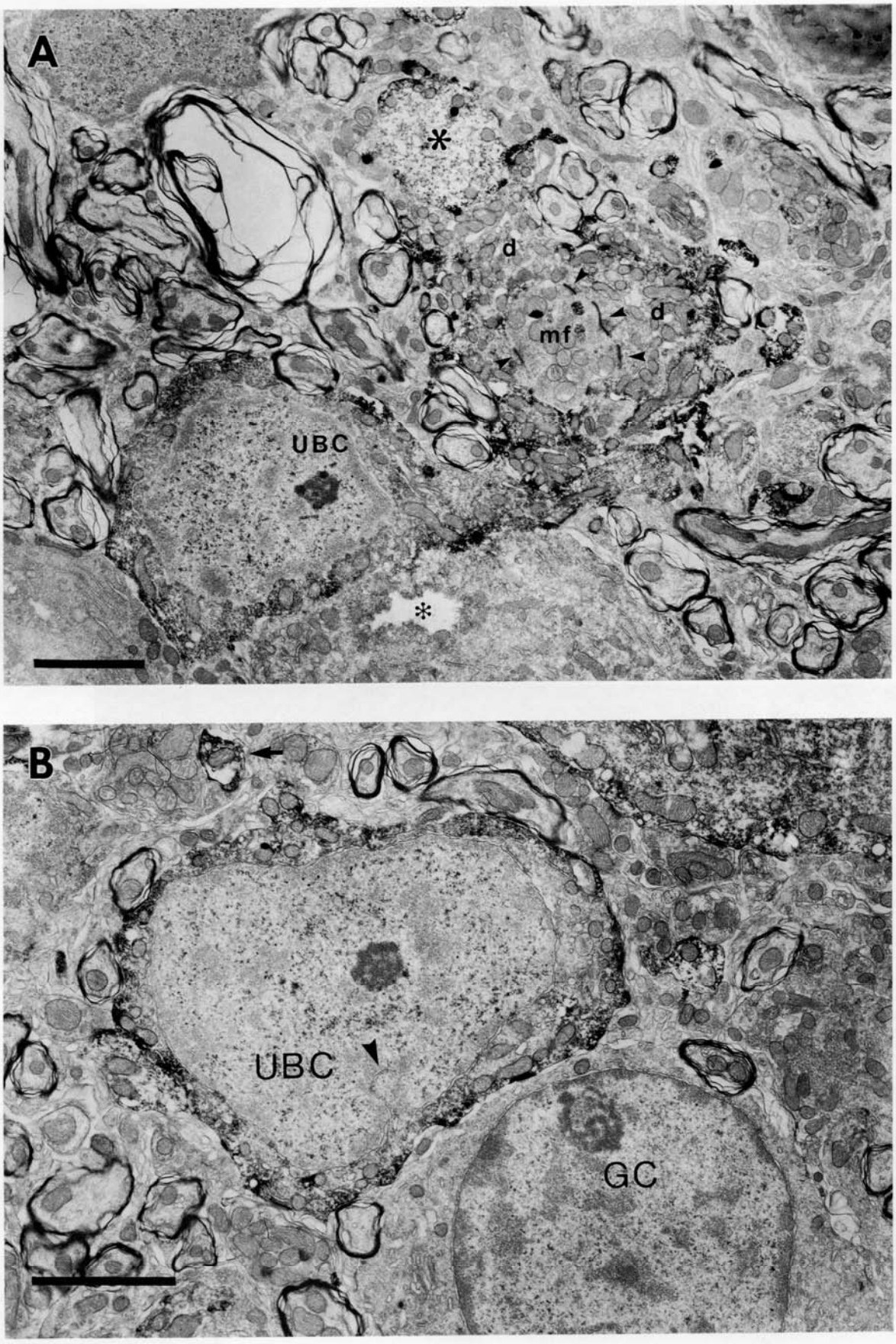

Figure 10

from the mGluR1α label in the dendrites (Fig. 9A, arrow; Figs. 9C, 10A, 11A, arrowheads). Many of the fine appendages of cochlear nucleus and cerebellar UBCs, which are darkly labeled for the mGluR1α receptor, are nonsynaptic (Figs. 9B,C, 11A).

Some UBC cell bodies are labeled along their periphery. One UBC in the cochlear nucleus was examined by both light and electron microscopy (Figs. 8A, 10A). At the light microscopic level, the labeled dendritic tuft envelopes an unlabeled structure. Ultrastructural analysis reveals that the unlabeled structure is a mossy fiber terminal that forms several synapses with the UBC dendrite. Cerebellar UBC cell bodies are also lightly labeled by the mGluR1α antibody with particularly dense labeling in somatic spines (Fig. 11B).

The labeled UBC cell bodies of the cochlear nucleus are similar in size to granule cells, but they can be distinguished from granule cells by the diffuse chromatin pattern of their nuclei and by the large number of mitochondria in their cytoplasm (Fig. 10B). Cerebellar UBCs are distinctly larger than the granule cells and frequently contain a ringlet body organelle (Fig. 11B, rb), which is unique to the UBC cell type (Mugnaini et al., 1994).

DISCUSSION

We used an antibody generated against the rat mGluR1α protein to study the localization of this metabotropic glutamate receptor in cochlear nucleus and cerebellum tissue from rat and guinea pig. Our Western blot analysis confirms the abundance of the mGluR1α receptor in the cochlear nucleus. A single band of protein with an approximate weight of 142 kD was labeled in homogenates prepared from rat cochlear nucleus. Previous studies with this antibody have demonstrated a band of similar size in rat cerebellum (Martin et al., 1992). In Western blots of guinea pig cochlear nucleus, cerebellum, and whole brain, a protein doublet of approximately the same size was labeled with the mGluR1α antibody. The presence of a protein doublet in the guinea pig may be the result of some receptor degradation or of differences in posttranslational modifications, such as glycosylation or phosphorylation.

Immunoreactivity in cartwheel cells

Dendritic spines in the molecular layer of the DCN are heavily labeled with the mGluR1α receptor antibody. The cell bodies that give rise to labeled spiny dendrites also display the size, shape, distribution, and ultrastructural features of cartwheel cells. In the rat, ultrastructural analysis confirmed that mGluR1α-immunoreactive cells contain subsurface cistern-mitochondrial complexes, which are organelle arrangements that are characteristically restricted to rat cartwheel cells (Wouterlood and Mugnaini, 1984). In guinea pig, the medium-sized, round shape and the laminar distribution of labeled cells within layers 1 and 2 of DCN are essentially identical to those of guinea pig cartwheel cells described previously (Manis et al., 1994; Ryugo et al., 1995).

We have found that the labeled spines in both rat and guinea pig DCN receive numerous synapses from axons that have the appearance of parallel fibers, in that they are thin, unmyelinated, and run parallel to the pial surface of the DCN. Our data are consistent with reports that parallel fibers provide excitatory (Manis, 1989) glutamatergic (Greenamyre et al., 1984; Schwartz, 1981) inputs to the molecular layer. In addition to mGluR1α glutamate receptors, other immunocytochemical studies have shown preliminary evidence for the presence of the GluR1 and GluR2-3 glutamate receptor subunits (Petralia and Wenthold, 1992) as well as the δ1 and δ2 glutamate receptor subunits (Petralia et al., 1995) in the cartwheel cells, which indicates that a complex glutamatergic signaling system exists in these interneurons.

In the cerebellum, the dendrites (particularly the spines) of Purkinje cells are known to contain high levels of the mGluR1α receptor immunoreaction product (Martin et al., 1992). This cerebellar localization of mGluR1α is of particular interest because of the distinguishing features shared by cochlear nucleus cartwheel cells and cerebellar Purkinje cells. Both exhibit high spine density on their dendrites (Palay and Chan-Palay, 1974; Hackney et al., 1990; Manis et al., 1994), and both respond to depolarizing stimuli with complex action potentials (Llinás and Sugimori, 1980; Manis et al., 1994). The Purkinje and cartwheel cells are highly immunoreactive for similar proteins: IP_3 receptors (Rodrigo et al., 1994; Ryugo et al., 1995), AMPA-type glutamate receptors GluR2/GluR3 (Martin et al., 1992; Petralia and Wenthold, 1992), the calcium-binding protein cerebellin (Mugnaini and Morgan, 1987), and the small peptides PEP-19 (Mugnaini et al., 1987) and L7 (Berrebi and Mugnaini, 1992). In several murine mutants, (e.g., staggerer," "lurcher," and "Purkinje cell degeneration;" Berrebi and Mugnaini, 1987), both Purkinje and cartwheel neurons are similarly affected, which indicates that the two cell types may have a common developmental origin. We have found that these two cell types also share a similar subcellular localization of the mGluR1α receptor. The growing list of specific similarities between these two cell types indicates the likelihood that they have analogous functions in their respective circuits.

Immunoreactivity of UBCs

In the granule cell areas of the cochlear nucleus and the vestibulocerebellum, dense regions of mGluR1α staining are found in dendrites of small cells. This pattern of cerebellar staining had been attributed to Golgi cells (Martin et al., 1992). Cells with the ultrastructural characteristics of UBCs in the cerebellum were previously lumped together with the Golgi cell class, but they now appear to be a separate class on the basis of their distinctive immunocytochemical staining patterns and their morphological characteristics (Harris et al., 1993; Floris et al., 1994; Mugnaini et al., 1994). A subset of small cells of the cochlear nucleus granule cell domain has been shown to have the morphologi-

Fig. 10. UBC cell bodies on the surface of the rat DCN tissue sections were darkly labeled with the mGluR1α antibody. **A:** A thin section of the labeled UBC that is also shown in the light micrograph in Figure 8A. The labeled cell body contains an irregularly shaped nucleus and emits a dendrite (d) that swirls around and forms multiple synapses (arrowheads) with a mossy fiber terminal (mf). This UBC was on the surface of the tissue section; therefore, some areas of the section are not complete (small asterisk). The dendritic stalk of a second presumed UBC (large asterisk) is adjacent to the dendritic tuft. **B:** A labeled UBC from rat DCN is adjacent to a granule cell (GC). The nucleus of the granule cell is round and has prominent patches of condensed chromatin, whereas the nucleus of the UBC is invaginated (arrowhead), and the chromatin is less pronounced. The UBCs also have numerous mitochondria in their cell bodies, whereas granule cells do not. A labeled UBC somatic spine is indicated with an arrow. Scale bars = 2 μm.

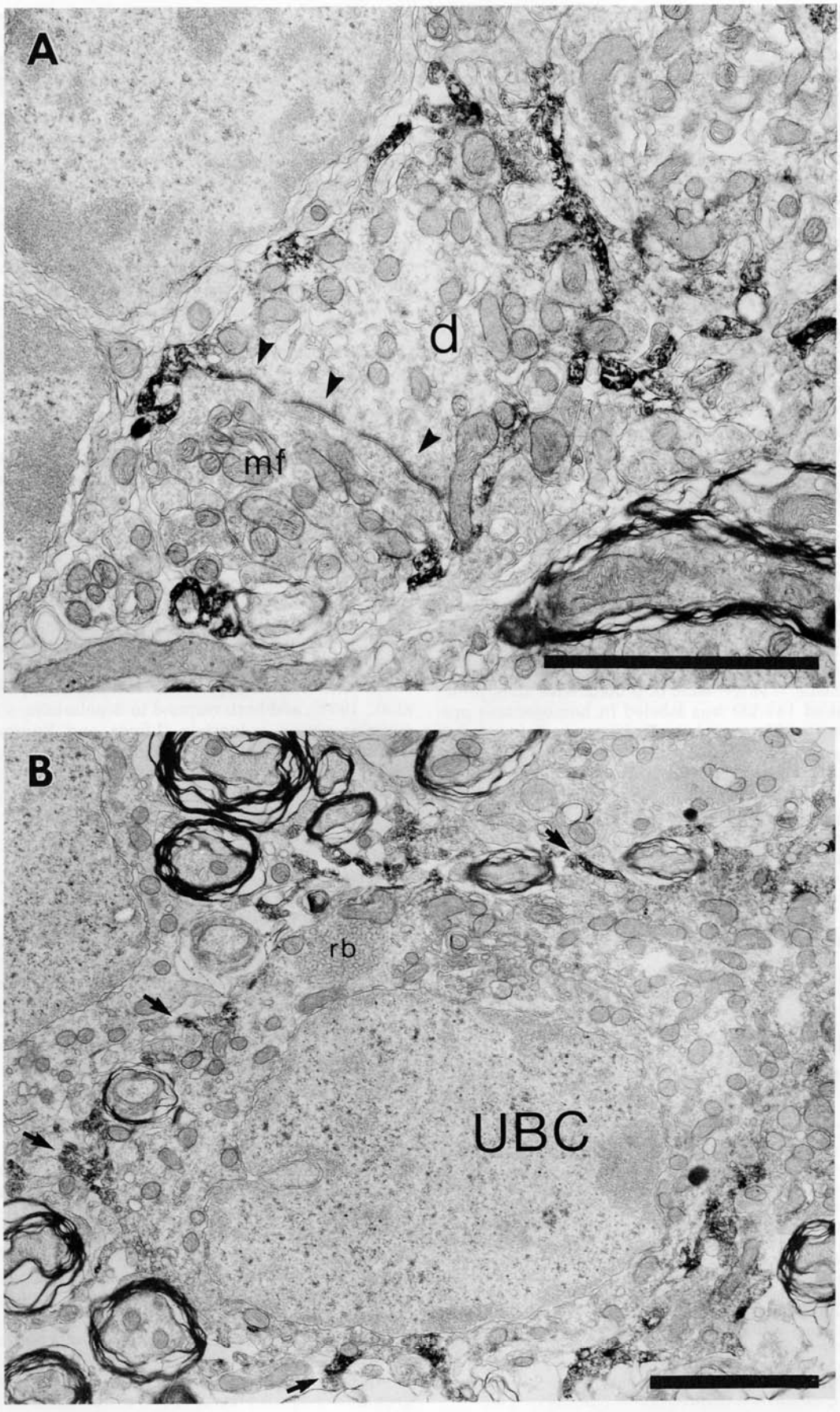

Figure 11

cal characteristics of the cerebellar UBCs (Floris et al., 1994). Although the electron microscopic character of cochlear nucleus UBCs was not described in previous studies, light microscopic evidence has shown that both cerebellar and cochlear nucleus UBCs have the unmistakable "paint-brush" spray of dendritic appendages arising from a single thick dendritic stalk and that both were strongly immunoreactive for the calcium-binding protein calretinin (Floris et al., 1994). We have determined that the mGluR1α-labeled cells in the granule cell regions of the cochlear nucleus and the cerebellum have the same size, distribution, and appearance of the recently characterized UBCs.

Ultrastructural analysis of the mGluR1α-labeled UBCs indicates that their brush-like dendrites surround and synapse with mossy fiber terminals. In the DCN, some long, labeled appendages that were spine-like in appearance made synapses with the mossy fibers. The concentrated areas of mGluR1α labeling within the UBC dendrites, however, were typically distant from the mossy fiber synapses. In fact, many of the small, thin dendritic appendages of cochlear nucleus and cerebellar UBCs are darkly labeled with the mGluR1α antibody, but they appear to be nonsynaptic. Cerebellar UBCs reportedly have many such nonsynaptic branchlets (Mugnaini et al., 1994).

The location of metabotropic glutamate receptors at sites distant from the main synapse is not a novel concept. Using immunogold electron microscopic techniques, Baude et al. (1993) found that mGluR1α receptor immunoreactivity was concentrated at the periphery of the anatomically defined synapses between granule cell axons and dendritic profiles in both the cerebellum and the hippocampus. It is conceivable that the mGluR1α receptors are sequestered in the dendritic appendages of UBCs and are thereby activated only after a build-up of glutamate in the extracellular space that might occur with repetitive firing of glutamatergic mossy fiber inputs (Rossi et al., 1994). Another indication that the mGluR1α receptors in UBCs have a unique mode of activation is that they are not immunoreactive for IP_3 receptors (isoform 1) like the Purkinje and cartwheel neurons are (Rodrigo et al., 1994; Ryugo et al., 1995). Although mGluR1α receptors may be coupled to a different isoform of the IP_3 receptor, it is plausible that the UBC mGluR1α receptors have a completely distinct mode of action. mGluR1 receptors have been linked to the cAMP and arachadonic acid second-messenger systems in vitro (reviewed by Pin and Duvoisin, 1995).

In any case, the immunocytochemical localization of a number of different glutamate receptors at the mossy fiber-UBC synapse indicates that these cells have a complex response to glutamatergic stimulation. Preliminary studies have demonstrated that cerebellar UBCs are immunoreactive for both AMPA and kainate glutamate receptors (Mugnaini and Jaarsma, 1995), and both cerebellar and cochlear nucleus UBCs are immunoreactive for the mGluR2-3 receptor subtype (Mugnaini and Jaarsma, 1995; Petralia et al., 1995).

The source of the glutamatergic inputs that stimulate the glutamate receptors on UBCs has not been demonstrated. Because cerebellar UBCs are concentrated mainly in regions that receive vestibular input, the vestibular ganglia and nuclei are strong candidate sources (Floris et al., 1994). The origin of analogous mossy fiber inputs to UBCs of the cochlear nucleus is unknown. One possible source is the mossy fiber projections from the cuneate region, which are immunoreactive for glutamate (Wright and Ryugo, 1995). Given the similarities between cerebellar and cochlear nucleus anatomy, however, the presence of this common cell type in the vestibulocerebellum and cochlear nucleus raises the possibility that mossy fibers of vestibular origins innervate the cochlear nucleus UBCs. In that regard, preliminary evidence exists for a direct projection of primary vestibular afferents to the granule cell regions of the cochlear nucleus in rodent (Burian and Gstoettner, 1988; Kevetter and Perachio, 1989), but ultrastructural studies are needed to determine whether they form mossy fiber-like terminals.

Our finding that both populations of UBCs are heavily labeled with the mGluR1α receptor indicates that these cells may share a similar function in the DCN and cerebellum. The exact role of the UBCs in the granule cell circuit of the cerebellum and the DCN, however, is still unclear. Based on the extensive contacts between mossy fibers and UBCs and on evidence that stimulation of mossy fibers leads to prolonged excitatory postsynaptic potentials in UBCs, it has been proposed that the extensive and enclosed synapses between mossy fibers and UBCs may be designed to increase the time that glutamate resides in the synaptic cleft (Rossi et al., 1994). The output of cerebellar UBCs also is most likely excitatory, because the cells are immunopositive for glutamate (Rossi et al., 1994) and are immunonegative for GABA and glycine (Mugnaini et al., 1994). Therefore, UBCs would propagate excitatory influences in response to mossy fiber activation. The targets of UBCs are not known for certain, but the axons of cerebellar UBCs ramify within the granule cell domains of the cerebellar cortex, forming large axon terminals (Rossi et al., 1994). In the cerebellum, at least, UBCs might function to augment the excitation within the granule cell domains.

Metabotropic glutamate receptors distant from the main synapses on UBCs in the cerebellum and the cochlear nucleus may aid in creating a strong and prolonged response to mossy fiber stimulation in the granule cell regions. Strong excitation by cochlear nucleus UBCs onto granule cells, in turn, would provide strong inhibition by cartwheel cells onto DCN projection neurons (Mugnaini, 1985; Osen et al., 1990). These synapses between the granule cell parallel fibers and cartwheel cells in the DCN resemble the granule-to-Purkinje cell synapse, where long-term synaptic changes are thought to occur. Metabotropic glutamate receptors are required for long-term depression observed at the granule-to-Purkinje cell synapse (Kano and Kato, 1987; Linden et al., 1991), which may be important for motor learning. Given the similarities between the DCN and the cerebellum, the mGluR1α receptors at the granule-to-cartwheel cell synapse may have a role in plasticity in this primary auditory nucleus.

Fig. 11. Nonsynaptic dendritic appendages and somatic appendages of UBCs in the rat cerebellum are darkly labeled with the mGluR1α antibody. **A:** The relatively unlabeled dendritic stalk (d) of a cerebellar UBC makes several synaptic contacts (arrowheads) with a mossy fiber terminal (mf). Many labeled nonsynaptic appendages emerge from the outer regions of the dendrite. **B:** Labeled cell containing a ringlet body (rb), the signature marker of the cerebellar UBCs. mGluR1α labeling of the cell body is heaviest typically in the somatic appendages (arrows). Scale bars = 2 μm.

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