Brain Research, 504 (1989) 173–179
Elsevier

BRES 23841

Immunocytochemical localization of the $GABA_A$/benzodiazepine receptor in the guinea pig cochlear nucleus: evidence for receptor localization heterogeneity

José M. Juiz[1], Robert H. Helfert[1], Robert J. Wenthold[2], Angel L. De Blas[3] and Richard A. Altschuler[1]

[1]*Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, MI 48109 (U.S.A.),* [2]*Laboratory of Neuro-Otolaryngology, NINCDS, NIH, Bethesda, MD 2025 (U.S.A.) and* [3]*Department of Neurobiology and Behaviour, SUNY, Stony Brook, NY 11794 (U.S.A.)*

(Accepted 15 August 1989)

Key words: Cochlear nucleus; Neurotransmitter receptor; γ-Aminobutyric acid A-receptor; Benzodiazepine receptor; Receptor heterogeneity; Immunocytochemistry

Immunocytochemistry with a monoclonal antibody against the $GABA_A$/benzodiazepine receptor showed labeled axo-dendritic synapses in the anteroventral cochlear nucleus. In the dorsal cochlear nucleus, label was seen apposing both axo-somatic and axo-dendritic terminals. The results suggest a heterogeneous distribution of GABA receptors, together with a possible segregation of receptor subtypes between somata and dendrites in certain neurons. The presence of cytoplasmic labeling in some neurons might reflect a higher receptor turnover rate in these neurons.

Many studies indicate that γ-aminobutyric acid (GABA) is an important neurotransmitter mediating inhibition in the cochlear nucleus (CN) (for review see refs. 7, 58). GABA and glutamic acid decarboxylase (GAD) levels are high in the cochlear nucleus[12,15,16,62], particularly in the dorsal CN[15]. GABA and GAD have been localized immunocytochemically in cells, fibers and terminals in the CN[1,27,29,31,32,38,44,52,61]. GABA is released in a calcium-dependent manner from CN slices[6,56] and uptake of tritiated GABA has been demonstrated in the CN[34,42]. Iontophoretic application of GABA inhibits neuronal firing in the CN[8,24,25,62] and this effect is abolished with the GABA antagonist bicuculline[25].

Receptor binding sites for GABA are present in the CN, as binding of the GABA agonist muscimol has been demonstrated autoradiographically in the CN at the light microscopic level[13,14]. However, a comparison of the distribution of the muscimol binding with that of the distribution of GABA and GAD-like immunoreactivity in the CN shows some degree of mismatch. In the central portions of the ventral CN, where there is a high density of GABA-immunoreactive terminals, a low density of [^{3}H]muscimol binding sites was observed. Furthermore, recent findings indicate that not only is GABA co-localized with glycine in CN cells, fibers and terminals[30,57] (personal observations), but glycine receptor immunoreactivity is seen apposing GABA-immunoreactive terminals in the CN[30]. These results raise questions as to the correlation between GABA receptors and GABA-ergic terminals. With the recent development of monoclonal antibodies to the $GABA_A$/benzodiazepine (BDZ) receptor complex[10,37,40,55], it is now possible to study the distribution of this receptor complex with great sensitivity and resolution. For this study, we have used a monoclonal antibody against the $GABA_A$/BDZ receptor[55] to investigate, at both the light and electron microscopic level, the distribution of $GABA_A$/BDZ receptor-like immunoreactivity (LI) in the CN.

Twelve adult guinea pigs were heavily anesthetized with chloral hydrate and perfused intravascularly with saline followed by a fixative containing 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer. The brainstem was removed, placed in the same fixative for 4 h at 4 °C and washed overnight in cold buffer. Sections through the CN of 50 μm thickness, obtained using an oscillating tissue slicer (Vibratome), were incubated for 72 h in the monoclonal antibody *62-3G1*[10,55] diluted 1:100 in phosphate-buffered saline (PBS). This antibody has been shown to react with a 57,000 M_r (β) subunit of the $GABA_A$/BDZ receptor complex in immunoblots, and its characteristics and specificity have been described in detail[55]. The avidin–biotin–peroxidase complex (ABC) technique of Hsu et al.[19] (Vectastain kit, Vector Labs) was used to visualize

Correspondence: J.M. Juiz, Kresge Hearing Research Institute, The University of Michigan, 1301 East Ann St., Ann Arbor, MI 48109, U.S.A.

the immunoreaction product, with diaminobenzidine as the chromogen. As a control for the specificity of this method, alternate sections were incubated either in non-immune mouse serum or in PBS substituted for the primary antibody. Sections for light microscopy (LM) were mounted onto slides, allowed to dry overnight, coverslipped and observed with a Leitz Dialux photomicroscope equipped with differential interference contrast optics. Sections for electron microscopy (EM) were postfixed in a mixture of 1% osmium tetroxide and 1.5% potassium ferricyanide, dehydrated in graded ethanols and embedded in EMbed 812 (EMSciences). Sections of 75–80 nm thickness of the anteroventral (AVCN) and dorsal (DCN) cochlear nucleus were observed without uranyl acetate or lead citrate counterstaining, using a JEOL EM-1200 electron microscope.

Immunostaining was not observed, either at the LM or EM levels, in the control sections of which the incubation in primary antibody was omitted. As uranyl acetate and lead citrate were not used to counterstain ultrathin sections, the synaptic densities were undetectable electron microscopically. Thus, it was assumed that the labeling observed in the experimental tissue was related to the presence of specific immunolabeling.

Apart from some scattered granule cells in the granule cell layer, no immunostaining was evident in the VCN (AVCN and PVCN) at the LM level after incubation with the *62-3G1* antibody (Fig. 1a). In contrast, the DCN showed intense $GABA_A$/BDZ receptor-LI, particularly in the superficial (molecular and fusiform) layers. Me-

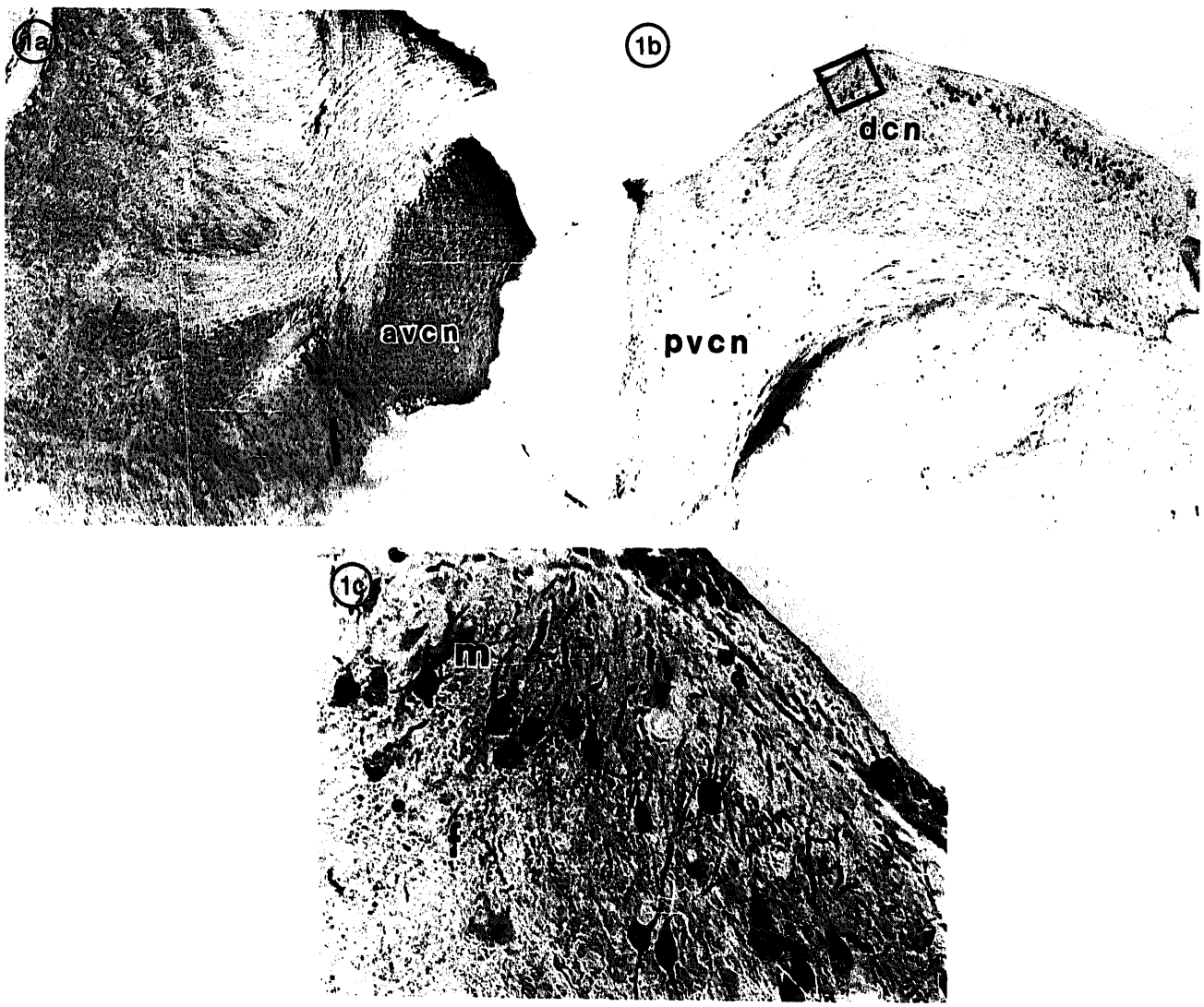


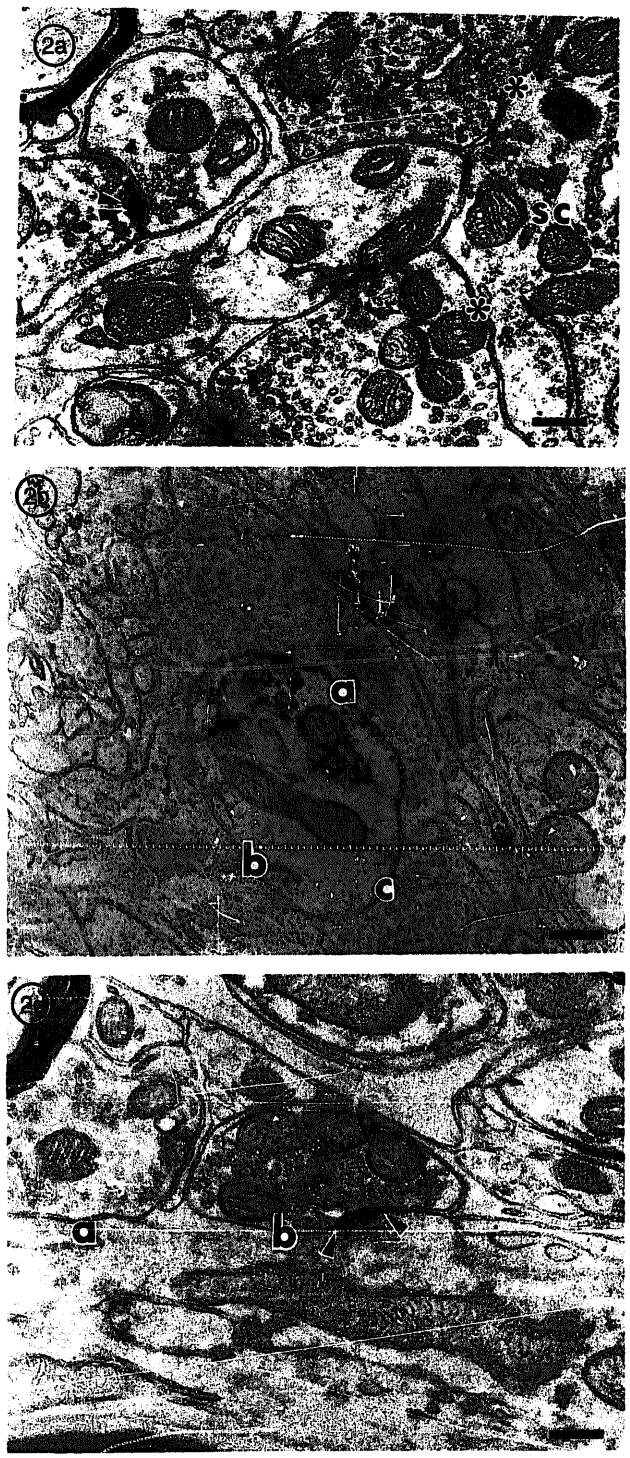
Fig. 1. $GABA_A$/benzodiazepine receptor-like immunoreactivity in the cochlear nucleus. The anteroventral (avcn, 1a) and posteroventral (pvcn, 1b) cochlear nucleus show only background staining and some red blood cells. No immunolabeled neurons are recognizable. In contrast, heavily stained neuronal profiles are evident in the dorsal cochlear nucleus (dcn, 1b), specifically in the molecular and fusiform layers (m and f in 1c). The area boxed in 1b is shown enlarged in 1c. 1a: 21×. 1b: 21×. 1c: 340×.

dium- and small-sized cell bodies in these layers were labeled (Fig. 1b,c), as were fusiform cell bodies (Fig. 1c). Immunoreactive dendritic profiles were also abundant (Fig. 1c).

Immunostaining was evident at the EM level in the rostral AVCN, despite its lack of visibility at the LM level. $GABA_A$/BDZ receptor-LI was observed in medium and small caliber dendrites, postsynaptic to terminals containing oval/pleomorphic vesicles (Fig. 2a,b) and, less frequently, in primary dendritic trunks (Fig. 2c). No immunoreactivity was seen apposing terminals on spherical/bushy cell bodies (Fig. 2a), despite the fact that these somata have been shown to receive a significant number of GABA-like immunoreactive terminals[30,31,38,44,50,52,61]. In the DCN, however, $GABA_A$/BDZ receptor-LI apposed both axo-somatic (Fig. 3a) and axo-dendritic (Fig. 3b,c) terminals on fusiform cells. Some of the medium and small cells in the molecular and fusiform cell layers also had $GABA_A$/BDZ receptor-LI apposing terminals on both somata and dendrites. While most terminals apposing $GABA_A$/BDZ receptor immunolabel contained oval/pleomorphic vesicles, some possessed flattened vesicles (Fig. 2c). Occasionally, presynaptic localization of the $GABA_A$/BDZ receptor was observed both in DCN (Fig. 3a) and AVCN. In both subdivisions, the immunolabeling was always associated with the cytoplasmic side of the junctional membrane (Figs. 2a,b,c and 3a,b,c). However, the labeling was not restricted to synaptic membranes. Intense cytoplasmic labeling was also seen deep within fusiform and small cell bodies in the DCN (Figs. 1b,c and 3a), as well as in dendritic profiles in both AVCN (Fig. 2b) and DCN (Fig. 3c). The immunolabel was usually associated with cytoskeletal structures and mitochondrial membranes (Figs. 2b and 3a,c). Some granule cell bodies and dendrites also contained abundant cytoplasmic staining. No label was ever found in glial cells.

The present study demonstrates the existence of $GABA_A$/BDZ receptor-LI in the guinea pig CN and adds further evidence to support the role of GABA as an inhibitory neurotransmitter in the CN.

The distribution of the immunoreactivity for the *62-3G1* antibody is heterogeneous. In the superficial layers of the DCN, many terminals on both neuronal somata and dendrites apposed immunolabeled postsyn-



→

Fig. 2. Subcellular distribution of the $GABA_A$/benzodiazepine receptor-like immunoreactivity in the anteroventral cochlear nucleus. The immunoreactivity is found postsynaptic to terminals contacting small and medium caliber dendrites (2a, arrowheads and 2b, synapse 'a') and some primary dendritic trunks (2c, synapse 'b'). No immunoreactivity is seen postsynaptic to terminals contacting spherical cell bodies (2a, asterisks. sc = spherical cell). Note the localization of the immunoreaction product in the cytoplasmic side of the postsynaptic membrane and the presence of cytoplasmic labeling (2b). Unlabeled synapses in 2a (asterisks) are at the same depth of the labeled synapse (arrowheads), relative to the cutting surface. In 2b, unlabeled synapses 'b' and 'c' are actually closer to the cutting surface than 'a'. Thus, artifacts related to the penetration of the antibodies can be safely ruled out. As uranyl acetate and lead citrate were not used, there are no postsynaptic densities that could be mistakenly interpreted as immunoreactivity (2a, 2b, 2c). Scale bars — 2a:200 nm. 2b:400 nm. 2c:250 nm.

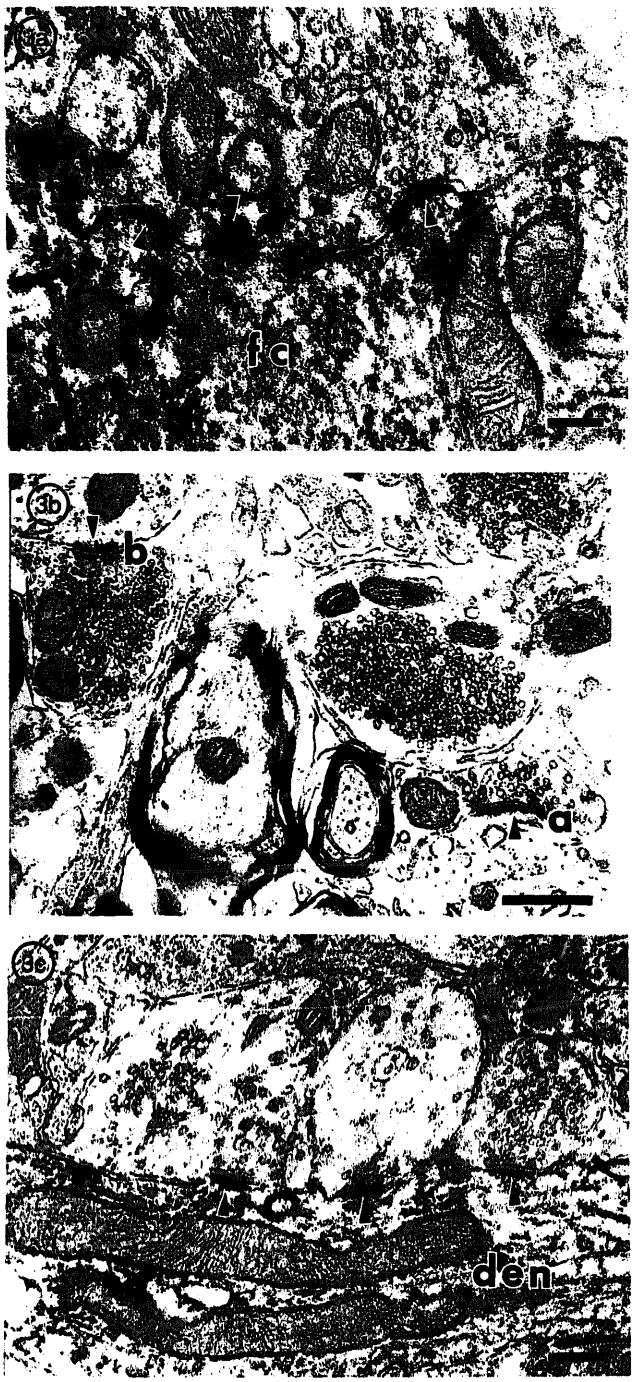


Fig. 3. Subcellular distribution of the $GABA_A$/benzodiazepine receptor-like immunoreactivity in the dorsal cochlear nucleus. A synapse on a fusiform cell body (3a,fc) shows post- and presynaptic immunoreaction product (3a, arrowheads). In the fusiform (3b) and molecular (3c) layers, terminals contacting dendrites appose postsynaptic immunoreactivity (3b, 'a' and 'b'. 3c, arrowheads). Cytoplasmic labeling is also obvious (3a, 3c). In 3b, synapse 'a', which is closer to the cutting surface, is more intensely stained than synapse 'b' (arrowheads). Non counterstained material. Scale bars — 3a:125 nm. 3b:500 nm. 3c:200 nm.

aptic membranes. These findings are similar to those obtained with other receptor-related antibodies in different areas of the central nervous system[2,3,37,53,54,60]. In the AVCN, however, $GABA_A$/BDZ receptor-LI was only seen apposing some axo-dendritic terminals. None was observed apposing axo-somatic terminals on spherical/bushy cells, despite observations that many of the terminals contacting these cells exhibit GABA-LI[30,31,38,44,50,52,61].

Several possibilities exist to explain the finding of a heterogeneous localization of the $GABA_A$/BDZ receptor-LI in the CN and the mismatch with the distribution of GABA-LI terminals. It is possible that the structure of $GABA_A$/BDZ receptors on spherical/bushy cell bodies is different than those on their dendrites, or in DCN cell bodies and dendrites. As such they would not be recognized by the monoclonal antibody used in this study. *62-3G1* recognizes an epitope in the 57,000 M_r (β) subunit (GABA/muscimol binding site) of the receptor complex[10,55]. In this regard, our results correlate well with the distribution of [^{3}H]muscimol binding sites in the CN observed in other studies[13,14]. However, the $GABA_A$/BDZ receptor complex has been shown in other areas to exhibit considerable structural heterogeneity as demonstrated by differences in ligand binding, photoaffinity and immunoaffinity labeling properties[21,33,35,36]. Molecular heterogeneity has also been described using recombinant DNA techniques, where the different polypeptide subunits of the receptor complex seem to exhibit important variations in their respective primary structures[4,22,36,41]. Furthermore, in situ hybridization studies indicate a differential distribution in the CNS of the mRNAs coding for different subunits of the $GABA_A$/BDZ receptor complex[22,43,46]. These results suggest that a particular antibody to the $GABA_A$/BDZ receptor might not recognize all its forms. How these possible structural differences may affect the binding properties of the receptor (muscimol binding, for example) must be addressed in the future.

The subcellular localization of $GABA_A$/BDZ receptor-LI (see Figs. 2 and 3) suggests that the antigenic determinant recognized by the antibody resides either in the cytoplasmic or in the intramembrane domain of the β subunit. This means that if there are structural variations in the $GABA_A$ receptors of the CN (see above), this variation resides, at least, in this region(s) of the β subunit. The use of antibodies against other epitopes of the $GABA_A$/BDZ receptor[37,48] would be helpful to address the extension and location of these structural heterogeneities, whose functional role, at present, is not clear.

Another possible explanation for our observations of receptor heterogeneity could be that there are GABA-

ergic terminals not apposed by postsynaptic $GABA_A$/BDZ receptors, but rather by $GABA_B$ receptors. $GABA_B$ receptors are believed to be mainly presynaptic[5]. However their involvement in postsynaptic inhibition has also been proposed[5,11,28]. In the CN, Baclofen, an agonist of GABA at $GABA_B$ receptors, reduces tone-evoked activity[7]. Although this effect has been linked to inhibition of the release of glutamate and aspartate through a presynaptic $GABA_B$ receptor[7], the presence, distribution and function of postsynaptic $GABA_B$ receptors on CN neurons needs to be determined. The availability of GABA antagonists at $GABA_B$ receptors[11] and the recent development of monoclonal antibodies directed against Baclofen[17] will help to address the question of $GABA_B$ receptor localization in the CN.

Regardless of the type ($GABA_A$ or $GABA_B$), our results suggest that different subpopulations of GABA receptors could be segregated to different receptive regions (somata and dendrites) in some neurons. This would raise questions regarding the possible functions of receptor segregation in terms of auditory signal processing. Also, a differential receptor distribution between somatic and dendritic synapses might be important in phenomena involving specific recognition between cells. Our observation of $GABA_A$/BDZ label presynaptically and its significance also adds to questions deserving future study.

$GABA_A$/BDZ receptor-LI was observed in the cytoplasm of fusiform and small neurons in the DCN. Intracellular labeling has been previously described using this antibody and other anti $GABA_A$ receptor monoclonal antibodies in different regions of the brain of various species[10,18,23,37,48,63]. At the EM level, the cytoplasmic label appeared within the perikarya and dendrites of DCN fusiform cells as well as in some granule cells both in DCN and VCN. This labeling is only seen in cells that also have $GABA_A$/BDZ receptor-LI apposing terminals. A likely explanation for this finding, also observed in other receptors and neuronal surface proteins[20,39,47,49,51], is that it might represent the receptor undergoing synthesis or degradation. The observation that some neuronal types display more intracellular immunoreactivity than others might reflect different receptor turnover rates. It should be noted that an internal pool for $GABA_A$/BDZ receptors has been described in cultured neurons, using biochemical techniques[9].

The results of the present study provide further evidence of GABA receptor heterogeneity in the central nervous system, with the additional intriguing suggestion of differential localization and segregation of GABA receptor types between the soma and dendrites within individual neurons. The functional implications of this receptor heterogeneity for the processing of auditory information remains to be determined.

The authors are most grateful to Ms. J.M. Bonneau for her expert technical assistance. This work was supported by a Generalitat Valenciana (Spain) Fellowship to José M. Juiz and NIH-NIDCD Grant NS 24369.

1 Adams, J.C. and Mugnaini, E., Patterns of GAD immunostaining in the feline cochlear nuclear complex studied with silver enhancement and electron microscopy, *J. Comp. Neurol.*, 262 (1987) 375–401.
2 Altschuler, R.A., Betz, H., Parakkal, M.H., Reeks, K.A. and Wenthold, R.J., Identification of glycinergic synapses in the cochlear nucleus through immunocytochemical localization of the postsynaptic receptor, *Brain Research*, 369 (1986) 316–320.
3 Aoki, C., Zemcik, B.A., Strader, C.D. and Pickel, V.M., Cytoplasmic loop of beta-adrenergic receptors: synaptic and intracellular localizations in the neocortex, striatum and brainstem of rat brains using a monoclonal antibody, *Soc. Neurosci. Abstr.*, 1988, p. 924.
4 Blair, L.A., Levitan, E.S., Marshall, J., Dionne, V.E. and Barnard, E.A., Single subunits of the $GABA_A$ receptor form ion channels with properties of the native receptor, *Science*, 242 (1988) 577–579.
5 Bormann, J., Electrophysiology of $GABA_A$ and $GABA_B$ receptor subtypes, *Trends Neurosci.*, 11, 3 (1988) 112–116.
6 Canzek, V. and Reubi, J.C., The effect of cochlear nerve lesion on the release of glutamate, aspartate and GABA from cat cochlear nucleus in vitro, *Exp. Brain Res.*, 38 (1980) 437–441.
7 Caspary, D.M., Cochlear nuclei: functional neuropharmacology of the principal cell types. In R.A. Altschuler, R.P. Bobbin and D.W. Hoffman (Eds.), *Neurobiology of Hearing: the Cochlea*, Raven, New York, 1986, pp. 303–332.
8 Caspary, D.M., Rybak, L.P. and Faingold, C.L., The effects of inhibitory and excitatory amino-acid neurotransmitters on the response properties of brainstem auditory neurons. In D.G. Drescher (Ed.), *Auditory Biochemistry*, Charles C. Thomas, Springfield, 1985, pp. 198–226.
9 Cjazowski, C. and Farb, D.H., Transmembrane topology and subcellular distribution of the benzodiazepine receptor, *J. Neurosci.*, 6 (1986) 2857–2863.
10 De Blas, A.L., Vitorica, J. and Friedrich, P., Localization of the $GABA_A$ receptor in the rat brain with a monoclonal antibody to the 57000 M_r peptide of the $GABA_A$ receptor/benzodiazepine receptor/Cl^- channel complex, *J. Neurosci.*, 8, 2 (1988) 602–614.
11 Dutar, P. and Nicoll, R.A., A physiological role for $GABA_B$ receptors in the central nervous system, *Nature (Lond.)*, 332 (1988) 156–158.
12 Fisher, S.K. and Davies, W.E., GABA and its related enzymes in the lower auditory system of the guinea pig, *J. Neurochem.*, 27 (1976) 1145–1155.
13 Frostholm, A. and Rotter, A., Autoradiographic localization of receptors in the cochlear nucleus of the mouse, *Brain Res. Bull.*, 16 (1986) 189–203.
14 Glendenning, K.K. and Baker, B.N., Neuroanatomical distribution of receptors for three potential inhibitory neurotransmitters in the brainstem auditory nuclei of the cat, *J. Comp. Neurol.*, 275, 2 (1988) 288–308.
15 Godfrey, D.A., Carter, J.A., Berger, S.J., Lowry, O.H. and Matchinsky, F.M., Quantitative histochemical mapping of candidate transmitter aminoacids in cat cochlear nucleus, *J. Histochem. Cytochem.*, 25 (1977) 417–431.
16 Godfrey, D.A., Carter, J.A., Lowry, O.H. and Matchinsky, F.M., Distribution of gamma-aminobutyric acid, glycine, glutamate and aspartate in the cochlear nucleus of the rat, *J.*

Histochem. Cytochem., 26 (1978) 118–126.
17 Holstein, G., Martinelli, G., Zamir, I. and Pasik, P., Visualization of probable $GABA_B$ receptor sites by monoclonal anti-baclofen antibodies, *Soc. Neurosci. Abstr.*, 1988, p. 780.
18 Hughes, T.E., Carey, R.G., Vitorica, J., De Blas, A.L. and Karten, H.J., Immunohistochemical localization of $GABA_B$ receptors in the retina of the primate *Samiri sciureus, Visual Neurosc.*, in press.
19 Hsu, S.M., Raine, L. and Fanger, H., Use of avidin–biotin–peroxidase complex (ABC) and unlabeled antibody (PAP) procedures, *J. Histochem. Cytochem.*, 29 (1981) 577–580.
20 Jacob, M.H., Lindstrom, J.M. and Berg, D.W., Surface and intracellular distribution of a putative neuronal nicotinic acetylcholine receptor, *J. Cell Biol.*, 103 (1986) 205–214.
21 Klepner, C.A., Lippa, A.S., Benson, D.I., Sano, M.C. and Beer, B., Resolution of two biochemically and pharmacologically distinct benzodiazepine receptors, *Pharmacol. Biochem. Behav.*, 11 (1979) 456–462.
22 Levitan, E.S., Schofield, P.R., Burt, D.R., Rhee, L.M., Wisden, W., Kohler, M., Fujita, N., Rodriguez, H.F., Stephenson, A. and Darlison, M.G., Structural and functional basis for $GABA_A$ receptor heterogeneity, *Nature (Lond.)*, 335 (1988) 76–79.
23 Mariani, A.P., Cosenza-Murphy, D. and Barker, J.L., GABAergic synapses and benzodiazepine receptors are not identically distributed in the primate retina, *Brain Research*, 415 (1987) 153–157.
24 Martin, M.R., The pharmacology of amino acid receptors and synaptic transmission in the cochlear nucleus. In D. Drescher (Ed.), *Auditory Biochemistry*, Charles C. Thompson, Springfield, 1985, pp. 184–197.
25 Martin, M.R., Dickson, J.W. and Fex, J., Bicuculline, strychnine and depressant aminoacid responses in the anteroventral cochlear nucleus of the cat, *Neuropharmacology*, 21 (1982) 201–207.
26 Montpied, P., Martin, B.M., Cottingham, L., Stubblefield, B.K., Ginns, E.I. and Paul, S.M., Regional distribution of the $GABA_A$/benzodiazepine receptor (alpha subunit) mRNA in rat brain, *J. Neurochem.*, 15 (1988) 1651–1654.
27 Moore, J.K. and Moore, R.Y., Glutamic acid decarboxylase-like immunoreactivity in brainstem auditory nuclei of the rat, *J. Comp. Neurol.*, 260 (1987) 157–174.
28 Mott, D.D., Lewis, D.V., Bragdon, A.C. and Wilson, W.A., Evidence that depression of recurrent inhibition following tetanic stimulation in the rat dentate gyrus is mediated by $GABA_B$ receptors, *Soc. Neurosci. Abstr.*, 1988, p. 809.
29 Mugnaini, E., GABA neurons in the superficial layers of the rat dorsal cochlear nucleus: light and electron microscopy immunocytochemistry, *J. Comp. Neurol.*, 235 (1985) 61–81.
30 Oberdorfer, M.D., Parakkal, M.H., Altschuler, R.A. and Wenthold, R.J., Co-localization of glycine and GABA in the cochlear nucleus, *Soc. Neurosci. Abstr.*, 1987, p. 544.
31 Oberdorfer, M.D., Parakkal, M.H., Altschuler, R.A. and Wenthold, R.J., Ultrastructural localization of GABA immunoreactive terminals in the anteroventral cochlear nucleus of the guinea pig, *Hearing Res.*, 33, 3 (1988) 229–238.
32 Ottersen, O.P. and Storm-Mathissen, J., Neurons containing or accumulating transmitter aminoacids. In: *Handbook of Chemical Neuroanatomy, 3: Classical Transmitters and Transmitter Receptors in the CNS, Part I*, Elsevier, Amsterdam, 1984, pp. 141–246.
33 Park, D., Vitorica, J. and De Blas, A.L., Molecular heterogeneity of the $GABA_A$/benzodiazepine receptor complex, *Soc. Neurosci. Abstr.*, 1988, p. 977.
34 Potashner, S.J., Lindberg, N. and Morest, D.K., Uptake and release of GABA in the guinea pig cochlear nucleus after axotomy of cochlear and centrifugal fibers, *J. Neurochem.*, 44 (1985) 1558–1566.
35 Pritchett, D.B., Stonheimer, H., Gorman, C.M., Kettenman, H., Seeburg, P.H. and Schofield, P.R., Transient expression shows ligand gating and allosteric potentiation of $GABA_A$ receptor subunits, *Science*, 242 (1988) 1306–1308.
36 Pritchett, D.B., Stonheimer, H., Shivers, B.D., Ymer, S., Kettenmann, H., Schofield, P.R. and Seeburg, P.H., Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology, *Nature (Lond.)*, 338 (1989) 582–585.
37 Richards, J.G., Schoch, P., Haring, P., Takacs, B. and Mohler, H., Resolving $GABA_A$/benzodiazepine receptors: cellular and subcellular localization in the CNS with monoclonal antibodies, *J. Neurosci.*, 7, 6 (1987) 1866–1886.
38 Roberts, R.C. and Ribak, C.E., GABAergic neurons and axon terminals in the brainstem auditory nuclei of the gerbil, *J. Comp. Neurol.*, 258 (1987) 267–280.
39 Schmidt, J.W. and Catteral, W.A., Biosynthesis and processing of the alpha subunit of the voltage-sensitive sodium channel in rat brain neurons, *Cell*, 46 (1986) 437–444.
40 Schoch, P., Richards, J.G., Haring, P., Takacs, B., Stahli, C., Staehelin, T., Haefely, W. and Mohler, H., Co-localization of $GABA_A$ receptors and benzodiazepine receptors in the brain shown by monoclonal antibodies, *Nature (Lond.)*, 314 (1985) 168–171.
41 Schofield, P.R., Darlison, M.G., Fujita, N., Burt, D.R., Stephenson, F.A., Rodriguez, H., Rhee, L.M., Ramachandran, J., Reale, V. and Glencorse, T.A., Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super family, *Nature (Lond.)*, 328 (1987) 221–227.
42 Schwartz, I.R., The differential distribution of label following uptake of H^3-labeled aminoacids in the DCN of the cat, *Exp. Neurol.*, 73 (1981) 601–607.
43 Sequier, J.M., Richards, J.G., Malherbe, P., Price, G.W., Mathews, S. and Mohler, H., Mapping of brain areas containing RNA homologous to the cDNAS encoding the alpha and beta subunits of the rat $GABA_A$ receptor, *Proc. Natl. Acad. Sci. U.S.A.*, 85 (1988) 7815–7819.
44 Shiraisi, T., Senba, E., Tohyama, M., Wu, J., Kubo, T. and Matsunaga, T., Distribution and fine structure of neuronal elements containing glutamic acid decarboxylase in the rat cochlear nucleus, *Brain Research*, 347 (1985) 183–187.
45 Sieghart, W. and Karobath, M., Molecular heterogeneity of benzodiazepine receptors, *Nature (Lond.)*, 286 (1980) 285–287.
46 Siegel, R.E., Differential distribution of $GABA_A$/benzodiazepine receptor subunit mRNAs in the bovine cerebellum, *Soc. Neurosci. Abstr.*, 1988, p. 977.
47 Smith, M.M., Lindstrom, J. and Merlie, J.P., Formation of the alpha-bungarotoxin binding site and assembly of the nicotinic acetylcholine receptor subunits occur in the endoplasmic reticulum, *J. Biol. Chem.*, 262 (1987) 4367–4376.
48 Somogyi, P., Takagi, H., Richards, J.G. and Mohler, H., Subcellular localization of benzodiazepine/$GABA_A$ receptors in the cerebellum of rat, cat and monkey, using monoclonal antibodies, *J. Neurosci.*, 9 (1989) 2197–2209.
49 Swanson, L.W., Simmons, D.M., Whiting, P.J. and Linstrom, J., Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system, *J. Neurosci.*, 7 (1987) 3334–3342.
50 Tachibana, M. and Kuriyama, K., Gamma-aminobutyric acid in the lower auditory pathway of the guinea pig, *Brain Research*, 69 (1974) 370–374.
51 Tamkum, M.M. and Fambrough, D.M., The Na^+-K^+ ATP-ase of chick sensory neurons. Studies on biosynthesis and intracellular transport, *J. Biol. Chem.*, 261 (1986) 1009–1119.
52 Thompson, G.C., Cortex, A.M. and Lam, D.K.M., Localization of GABA immunoreactivity in the auditory brainstem of guinea pigs, *Brain Research*, 339 (1985) 119–122.
53 Triller, A., Cluzeaud, F., Pfeiffer, F. and Korn, H., Distribution of glycine receptors at central synapses: an immunoelectron microscopy study, *J. Cell Biol.*, 101 (1985) 683–688.
54 Van den Pol, A.N. and Gores, T., Glycine and glycine receptor immunoreactivity in brain and spinal cord, *J. Neurosci.*, 8, 2 (1988) 472–492.
55 Vitorica, J., Park, D., Chin, G. and De Blas, A.L., Monoclonal

antibodies and conventional antisera to the $GABA_A$ receptor/benzodiazepine receptor/Cl channel complex, *J. Neurosci.*, 8, 2 (1988) 615–622.

56 Wenthold, R.J., Release of endogenous glutamic acid, aspartic acid and GABA from cochlear nucleus slices, *Brain Research*, 162 (1979) 338–343.

57 Wenthold, R.J., Huie, D., Altschuler, R.A. and Reeks, K.A., Glycine immunoreactivity localized in the cochlear nucleus and superior olivary complex, *Neuroscience*, 22 (1987) 897–912.

58 Wenthold, R.J. and Martin, M.R., Neurotransmitters of the auditory nerve and central auditory system. In C. Berlin (Ed.), *Hearing Science*, College-Hill Press, San Diego, 1984, pp. 341–370.

59 Wenthold, R.J. and Morest, D.K., Transmitter related enzymes in the guinea pig cochlear nucleus, *Soc. Neurosci. Abstr.*, 1976, p. 28.

60 Wenthold, R.J., Parakkal, M.H., Oberdorfer, M.D. and Altschuler, R.A., Glycine receptor immunoreactivity in the ventral cochlear nucleus of the guinea pig, *J. Comp. Neurol.*, 276 (1988) 423–435.

61 Wenthold, R.J., Zempel, J.M., Parakkal, M.H., Reeks, K.A. and Altschuler, R.A., Immunocytochemical localization of GABA in the cochlear nucleus of the guinea pig, *Brain Research*, 380 (1986) 7–18.

62 Whitfield, I.C. and Comis, S.D., The role of inhibition in information transfer: the interaction of centrifugal and centripetal stimulation on neurones of the cochlear nucleus, Final Report, European Office of Aerospace Research, U.S.A.F., 1966, Grant 63–115.

63 Yazulla, S., Studholme, K.M., Vitorica, J. and De Blas, A.L., Immunocytochemical localization of $GABA_A$ receptors in goldfish and chicken retinas, *J. Comp. Neurol.*, 280 (1989) 15–26.