Research Report

Distribution of GABA$_A$ and GABA$_B$ binding sites in the cochlear nucleus of the guinea pig

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Abstract

We compared the distribution of GABA$_A$ and GABA$_B$ binding sites in the cochlear nucleus using quantitative receptor autoradiography with [^{3}H]GABA. To visualize GABA$_A$ binding sites, GABA$_B$ binding sites were blocked with ±baclofen. To visualize GABA$_B$ binding sites, isoguvacine was used to block GABA$_A$ binding sites. GABA$_A$ binding sites predominated over GABA$_B$, although there were marked regional differences in the distribution of binding. In the ventral cochlear nucleus, GABA$_A$ and GABA$_B$ binding sites were concentrated in the peripheral granule cell cap, with low binding levels in the central region. In the dorsal cochlear nucleus, binding was concentrated in the superficial (fusiform and molecular) layers, with a distinct laminar pattern. GABA$_A$ binding sites predominated in the fusiform cell layer. The molecular layer contained the highest level of GABA$_B$ binding sites in the entire cochlear nucleus. These results suggest that GABAergic inhibition in the cochlear nucleus is mediated both by GABA$_A$ and GABA$_B$ receptors, particularly in the dorsal cochlear nucleus. However, low levels of binding in areas such as the magnocellular regions of the ventral cochlear nucleus, known to contain abundant GABAergic synapses, suggest heterogeneity of GABA receptors in this auditory nucleus.

Key words: GABA; GABA receptor; Auditory pathway; Cochlear nucleus; Neurotransmitter

1. Introduction

There is much evidence supporting gamma-aminobutyric acid (GABA) as a major inhibitory neurotransmitter in the cochlear nucleus (CN) (extensively reviewed in [3,19,82]). GABAergic inhibition of CN neurons arises from multiple 'non-primary' intrinsic and extrinsic (descending) inputs from a variety of sources [4,62,67,74]. Such extensive GABA-mediated inhibition would be crucial for CN processing of the primary acoustic input carried by the auditory nerve. The way in which GABA-mediated inhibition could modify or modulate the response of CN neurons to auditory stimuli would depend in good part on the type of GABA receptors and their distribution on CN neurons.

Two major classes of GABA receptors have been identified to date [8,24,27,72,75], termed respectively GABA$_A$ and GABA$_B$. They have different biochemical, physiological and pharmacological characteristics as well as distinct patterns of anatomical distribution [11,20,34,60]. At the cellular level, GABA$_A$ receptors are located preferentially in postsynaptic regions [2,21,35,64,73] contain an integral chloride channel [22,49,70,75,76] and mediate 'fast' inhibitory responses which can be blocked with the specific antagonist bicuculline. GABA$_B$ receptors may be pre- or postsynaptic and regulate K$^+$ or Ca^{2+} channels through a guanyl nucleotide-binding protein mechanism which is linked to some second messenger systems [1,24,27,33,53,71,83]. GABA$_B$ receptors produce slow and sustained inhibition insensitive to bicuculline blockade [7,9,23,53].

It is very likely that GABA$_A$ receptors are involved in inhibition in the CN, as GABA-mediated inhibition of spontaneous and tone-evoked activity of CN neurons is selectively blocked by bicuculline [15,16,18,19, 25], which also alters auditory evoked potentials [47].

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Also, GABA$_A$ receptors have been localized in the CN by receptor autoradiography with the agonist [^{3}H]muscimol [26,28] and by immunocytochemistry with an antibody against the β subunit of the GABA$_A$ receptor [35].

In contrast, much less is known about the function and distribution of GABA$_B$ receptors in the CN. The finding that the prototype GABA$_B$ agonist baclofen [9,10], alters auditory evoked potentials [46] and reduces tone-evoked activity of CN neurons [17,19,25] suggests involvement of GABA$_B$ receptors in CN inhibition. However, if GABA$_B$ receptors are present in the CN, their precise distribution in comparison to GABA$_A$ receptors is unknown.

This study addresses the question of the localization and comparative distribution of GABA$_A$ and GABA$_B$ receptors in the CN. For this purpose, we have used receptor autoradiography with [^{3}H]GABA under binding conditions which allow visualization of either GABA$_B$ or high affinity-GABA$_A$ binding sites [20].

2. Materials and methods

Six adult guinea pigs with normal pinna reflex were used in this study. The animals were deeply anesthetized with chloral hydrate (300 mg/kg b.w.). Their brain stems were dissected and immediately frozen in dry ice. Serial sections (20 μm thick) in the coronal plane were obtained in a Lipshaw cryostat. Sections containing the CN complex were identified and thaw-mounted onto gelatin coated slides. Slides containing the sections of interest were dried on a warming plate and stored at $-80°C$ until use.

The autoradiography technique followed the procedure of Chu et al. [20]. Sections were washed for 30 min in 50 mM Tris-2.5 mM CaCl$_2$ buffer (pH 7.4) at 4°C followed by drying under a stream of cool air. Serial sections were assayed for either GABA$_A$ or GABA$_B$ binding sites. In both cases [^{3}H]GABA (Amersham, specific activity 99.6 Ci/mmol) was used as radioligand at a concentration of 20 nM, well under the K$_d$ for both GABA$_A$ and GABA$_B$ binding sites [20]. All sections were incubated for 45 min. To examine GABA$_A$ receptors in the CN, sections were incubated with [^{3}H]GABA in Tris-CaCl$_2$ buffer (pH 7.4, 4°C) in the presence of 100 mM ($\pm$)baclofen (Ciba-Geigy) to block GABA$_B$ binding sites. To study the distribution of GABA$_B$ receptors, [^{3}H]GABA was used in the presence of isoguvacine (10 μM) to block GABA$_A$ binding sites. Non-specific [^{3}H]GABA binding was determined in sections incubated with a mixture of the radioligand, isoguvacine (100 μM) and baclofen (100 μM). After incubation, sections received 3 quick 3-ml rinses with buffer, followed by one 3-ml rinse with 2.5% glutaraldehyde in acetone and immediately dried under a stream of warm air. The slides were placed in X-ray cassettes and apposed to tritium-sensitive film (Ultrofilm, LKB) for 3 weeks at 4°C along with known radioactive ^{14}C standards (American Radiochemical Co., St. Louis, Mo., USA), after which the films were developed and dried. To facilitate identification of CN subdivisions and regions, selected sections were stained with Neutral red after film exposure.

Optical densities of specific binding in the different subdivisions of the CN were quantified using computer-assisted densitometry with an MCID system (Imaging Research Inc., St. Catherines, Ont., Canada). The optical density of the co-exposed ^{14}C standards was determined, and a standard curve was generated by fitting standard optical density values to standard radioactivity with a computer-gen-

Table 1

Comparative distribution of GABA$_A$ and GABA$_B$ binding sites in the cochlear nucleus of the guinea pig

CN area	GABA$_A$ binding	GABA$_B$ binding
	(fmol of GABA bound / mg of protein)	
AVCN-Central region	71 $\pm$ 10	10 $\pm$ 2
AVCN-Gran. cell cap	931 $\pm$ 12	63 $\pm$ 12
PVCN-Central region	54 $\pm$ 1	10 $\pm$ 3
PVCN-Gran. cell cap	807 $\pm$ 100	79 $\pm$ 3
DCN-Deep region	77 $\pm$ 15	18 $\pm$ 7
DCN-Fus. cell layer	662 $\pm$ 115	39 $\pm$ 4.8
DCN-Mol. layer	220 $\pm$ 3.2	154 $\pm$ 13.8
Cerebellum-Granule cell layer	1794 $\pm$ 7.5	114 $\pm$ 3
Cerebellum-Molecular layer	449 $\pm$ 3	237 $\pm$ 4.5

Values represent mean $\pm$ S.E.M. for three animals. Binding levels in the cerebellar cortex are given as reference.

erated fourth-degree polynomial regresion equation, as described in detail elsewhere [59]. The ^{14}C standards were calibrated against brain paste standards containing known amounts of tritium and protein [59,79]. Optical densities of regions of interest were expressed in fmoles of [^{3}H]GABA bound per mg of protein using the standard curve derived from the standards [59].

3. Results

To describe the density of GABA$_A$ and GABA$_B$ binding sites in the different regions of the CN, binding levels in the cerebellar cortex were used as reference values (see Table 1). Using an identical binding assay, Chu et al. [20] reported levels of GABA$_A$ and GABA$_B$ binding sites in the rat cerebellar cortex which were among the highest found in the rat central nervous sytem. As the values that we obtained in guinea pig were comparable to those reported by these authors, it was considered convenient to use the cerebellar cortex as a general reference to describe findings in the CN (Table 1).

For GABA$_A$ binding sites, values over 600 fmol of GABA bound per mg of protein were considered 'very high', values between 600 and 300 fmol/mg were considered 'high', 'moderate' between 300 and 100 fmol/mg, 'low' between 100 and 50 fmol/mg and 'very low' below 50 fmol/mg of protein. For GABA$_B$ binding sites, which usually occur at lower levels than GABA$_A$ sites, values above 300 fmol/mg were considered 'very high', values between 300 and 150 fmol/mg were referred to as 'high', 'moderate' between 150 and 75 fmol/mg, 'low' between 25 and 75 fmol/mg and 'very low' below 25 fmol/mg of protein.

Ventral cochlear nucleus (VCN). The distribution and levels of GABA$_A$ and GABA$_B$ binding sites was very similar in the anteroventral and posteroventral CN (AVCN and PVCN respectively). In both subdivisions of the VCN, the central region which contains

the main projection neurons (spherical, globular, multi-polar and octopus cells), had low levels of $GABA_A$ binding sites and very low, but detectable levels of

binding to $GABA_B$ sites (Figs. 1 and 4, Table 1). In contrast, the peripheral granule cell cap of the VCN, contained higher levels of both $GABA_A$ and $GABA_B$
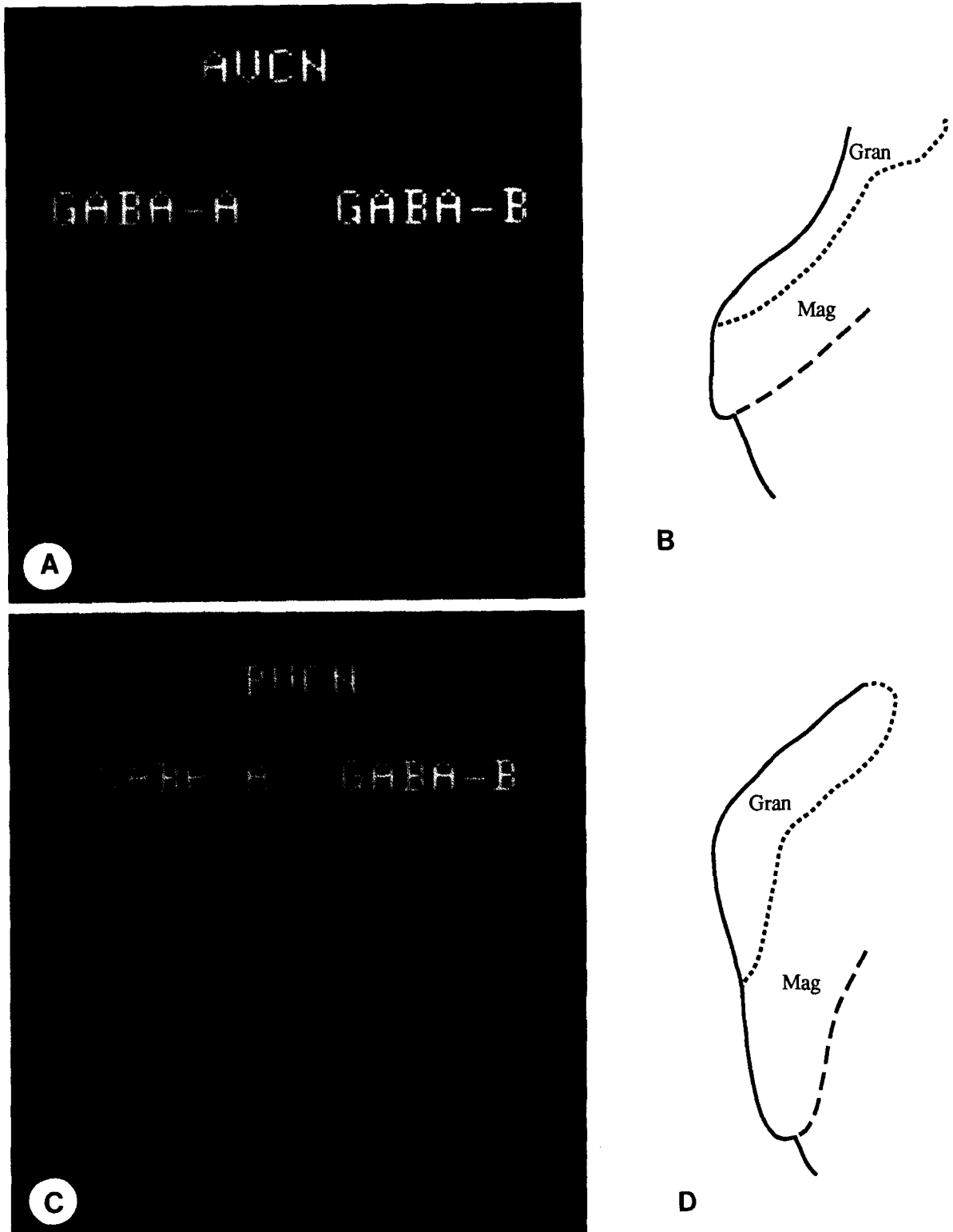

Fig. 1. False color images of autoradiograhy films showing the distribution of $GABA_A$ and $GABA_B$ binding sites in adjacent sections of the (A) anteroventral (AVCN) and (C) posteroventral (PVCN) cochlear nucleus. Red and yellow indicate high levels of binding, while different tones of blue code for lower binding levels. Drawings with the outlines of the magnocellular (Mag) and granule/small (Gran) cell regions are shown next to each photograph for orientation purposes (B, D).

binding sites. In this region, the level of $GABA_A$ binding sites was very high, and the level of $GABA_B$ binding sites low to moderate (Figs. 1 and 4, Table 1). Overall, the level of $GABA_A$ binding sites in the VCN was 5 to 15 times higher than that of $GABA_B$ binding sites (Table 1).

Dorsal cochlear nucleus (DCN). In the superficial layers of the DCN, $GABA_A$ and $GABA_B$ binding sites were abundant, and their distribution showed a clear laminar pattern. In the fusiform/granule cell layer, the level of $GABA_A$ binding sites was very high, while that of $GABA_B$ sites was low (Figs. 2 and 4, Table 1). In this layer, the level of $GABA_A$ sites was 17 times higher than that of $GABA_B$ sites. In the molecular layer, however, the density of $GABA_A$ binding sites was moderate and the density of $GABA_B$ binding sites high, the former being only 1.4 times more abundant than the latter (Figs. 2 and 4, Table 1).

In contrast, in the deep DCN the level of $GABA_A$ binding sites was low and the level of $GABA_B$ sites
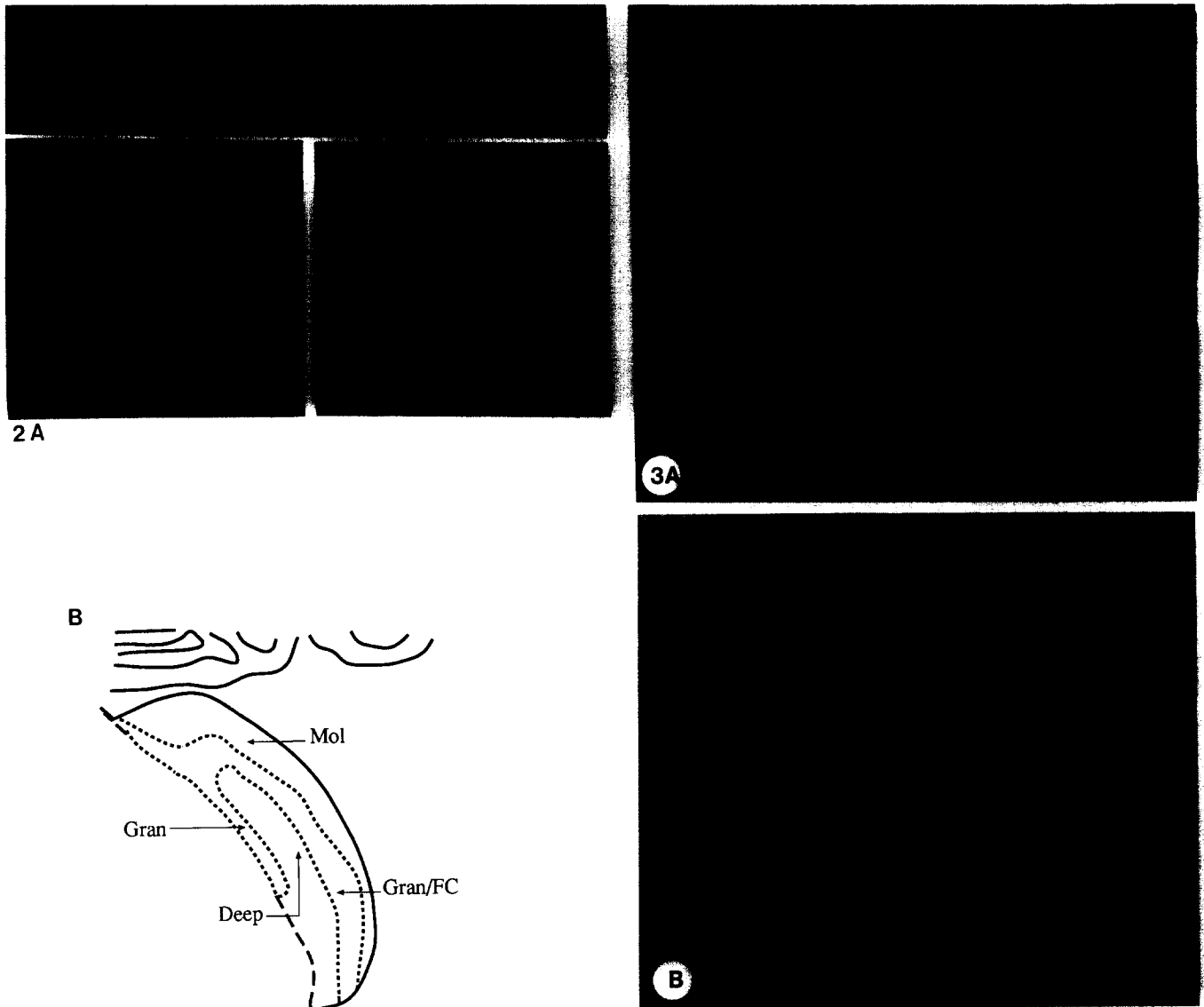

Fig. 2. (A) False color images of two autoradiography films from adjacent sections of the dorsal cochlear nucleus showing the comparative distribution of $GABA_A$ and $GABA_B$ binding sites. Color coding is described in Fig. 1. The drawing in B outlines the layers of the dorsal cochlear nucleus identified in sections stained with Neutral red. Mol: molecular layer; Gran, Gran/FC: granule cell regions of the DCN, including the fusiform/granule cell layer; Deep: deep region of the DCN.

Fig. 3. This figure illustrates the similarities in the distribution of $GABA_A$ and $GABA_B$ binding sites in the DCN and cerebellar cortex. In this figure the cerebellum was taken as reference for color coding. (A) $GABA_A$ binding sites predominate in the fusiform cell layer and granule cell regions of the DCN. In the cerebellar cortex, $GABA_A$ binding sites predominate in the Purkinje cell layer. (B) The density of $GABA_B$ binding sites is higher in the molecular layer of the DCN and the molecular layer of the cerebellar cortex, although the latter shows comparatively higher binding levels.
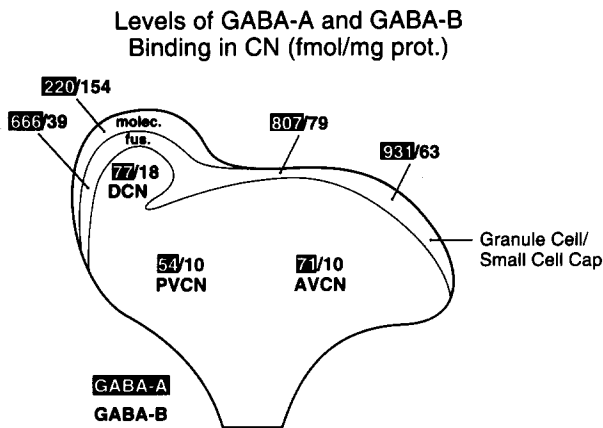
Levels of GABA-A and GABA-B
Binding in CN (fmol/mg prot.)

Fig. 4. Summary sketch of the distribution of $GABA_A$ and $GABA_B$ binding sites in the cochlear nucleus. The levels of binding shown in Table 1 have been collapsed into a single, idealized sagittal section through the cochlear nucleus to give a better idea of the distribution of binding.

very low, similar to those found in the central or magnocellular regions of the VCN (Figs. 2 and 4, Table 1).

Summarizing, under the conditions of this assay $GABA_A$ binding sites were concentrated in granule cell regions of the CN, including the fusiform/granule cell layer of the DCN (Figs. 1–3, 4). $GABA_B$ binding sites were found at moderate levels in the granule cell cap of the VCN (Figs. 1, 4) while the level was high in the molecular layer of the DCN (Figs. 2–4). $GABA_A$ binding sites clearly predominated over $GABA_B$ sites across the entire CN (Fig. 4), except in the molecular layer of the DCN, which contained the highest density of $GABA_B$ binding sites in the CN, approaching the density of $GABA_A$ sites. It is interesting to note that the distribution of binding in the superficial layers of the DCN was strikingly similar to that in the cerebellar cortex, where $GABA_A$ binding sites predominated in the granule and Purkinje cell layers, and the molecular layer was characterized by a high level of $GABA_B$ binding sites. However, the density of both $GABA_A$ and $GABA_B$ binding sites was higher in the cerebellar cortex than in the DCN (Table 1, Fig. 3).

4. Discussion

The general distribution of $GABA_A$ binding sites in the CN using [^{3}H]GABA as radioligand does not differ much from the pattern of [^{3}H]muscimol binding reported by Frostholm and Rotter [26] and Glendenning and Baker [28]. With either ligand, $GABA_A$ binding sites are highly concentrated in the granule/small cell cap of the VCN and are also abundant in the superficial layers (fusiform/granule cell layer and molecular layer) of the DCN, while binding levels are much lower

in the magnocellular regions of the VCN and deep DCN. There are, however, interesting new data derived from the use of quantitative densitometry of [^{3}H]GABA binding, which help to define better the pattern of distribution of $GABA_A$ binding sites in the CN. The great concentration of $GABA_A$ binding sites in the granule/small cell cap of the VCN compared with the rest of the nucleus is illustrated by the fact that levels of [^{3}H]GABA binding to $GABA_A$ binding sites in this VCN region probably are among the highest in the brain, extrapolating from the results obtained by Chu et al. [20]. Autoradiography with [^{3}H]GABA also reveals an abundance of $GABA_A$ binding sites in the superficial layers of the DCN, but with important differences between the fusiform/granule cell layer and molecular layer that have not been considered in previous studies [26,28]. With our binding assay, levels of $GABA_A$ binding sites are higher in the fusiform/granule cell layer than in the molecular layer. This finding suggests that there are potentially important differences in the distribution of $GABA_A$ receptors between the two layers that make up the 'cortex' of the DCN. The relevance of these differences in relation to auditory processing mechanisms in this nucleus needs to be evaluated.

Besides receptor autoradiography, immunocytochemistry also has been used to localize $GABA_A$ receptors in the CN [35]. The distribution of $GABA_A$ binding sites reported here and in previous receptor autoradiography studies [26,28] is in fact comparable to immunocytochemical findings in the CN [35] using an antibody against the β_2 subunit of the $GABA_A$/benzodiazepine receptor complex [21,35,81]. With this antibody, immunolabeling was intense in neurons in the superficial layers of the DCN, and very light in the central regions of the VCN [35]. Granule cells in granule cell domains also were intensely immunolabeled (unpublished observations).

In agreement with the high levels of $GABA_A$ receptors found in the granule/small cell cap of the VCN and the superficial layers of the DCN, GABA is abundant in both regions as determined by microneurochemical methods [29–31]. Accordingly, these same regions contain numerous GAD or GABA immunoreactive endings and cell bodies (reviewed in refs. 38 and 82). Pharmacological data also are consistent with these findings, as neurons in the superficial DCN are inhibited by GABA, and this inhibition is antagonized by bicuculline [15,19,25]. In contrast, the paucity of $GABA_A$ binding sites and $GABA_A$ receptor immunolabeling in the magnocellular regions of the VCN and the deep DCN does not match with the high levels of GABA found in these regions [29,30,31], or with the abundance of GABA or GAD immunoreactive endings and cell bodies, particularly in the magnocellular regions of the VCN (see refs. 4, 38 and 82 for reviews).

The presence of strong inhibitory responses to GABA, especially in magnocellular regions of the VCN [15,19,46] also is in contrast with this apparently low concentration of $GABA_A$ receptors. It should be kept in mind that conventional receptor autoradiography methods do not provide resolution at the synaptic level. Therefore, we cannot disregard the possibility that the low levels of $GABA_A$ binding sites found in the magnocellular regions of the VCN relative to other CN regions could be due in part to potential differences in the concentration of GABAergic synapses. However, in regions like the rostral part of the AVCN, where $GABA_A$ binding levels are very low, GABA immunopositive endings seem to be at least as abundant as in the superficial layers of the DCN [38], where $GABA_A$ binding levels are dramatically high. It is therefore unlikely that differences in synaptic concentration could account for all the observed mismatch between the distribution of GABA and $GABA_A$ binding sites in the CN. Another possible explanation for this mismatch is that $GABA_B$ rather than $GABA_A$ receptors could be associated with many GABAergic synapses in the central region of the VCN and in the deep DCN. The levels of $GABA_B$ binding sites in these regions, however, are too low to support this hypothesis (see discussion on $GABA_B$ receptors below). Furthermore, considering that GABA-mediated inhibition is essentially bicuculline-sensitive [15,16,19], one would expect to find indications of a greater concentration of $GABA_A$ receptors in these regions than actually found by autoradiography or immunocytochemistry. Therefore, another possibility to explain this mismatch is that there may be $GABA_A$ receptors in magnocellular regions of the VCN and deep DCN different from those visualized in granule cell domains and superficial layers of the DCN. Several lines of evidence support this possibility. First, [^{3}H]muscimol and [^{3}H]GABA under the conditions of our assay bind preferentially to the so called 'high-affinity' $GABA_A$ sites [8,12,55,56,58]. Reflecting their complex pharmacology, $GABA_A$ receptors can also be identified with assays for 'low affinity' binding sites, for the benzodiazepine binding sites, and for the picrotoxin binding sites [39,55,57,58,64,65]. In several instances, the regional distribution of these binding sites differs from that of 'high affinity' $GABA_A$ binding sites [50,58,64, 66,68,78,85]. Application of these autoradiographic assays to the CN might reveal the distribution of $GABA_A$ binding sites not visualized with our assay. Second, the possibility of heterogenous populations of $GABA_A$ receptors among different anatomical regions of the CN is further strengthened if one considers the structural diversity of $GABA_A$ receptors at the molecular level [41,70]. There are multiple isoforms of each of the subunits which constitute the $GABA_A$ receptor and they probably combine in many different ways to give

receptors with different structural and functional characteristics [22,43,56,63,70,80]. Mapping by in situ hybridization histochemistry the $GABA_A$ receptor subunits expressed in different CN regions and neurons [32] is another promising approach to address the possibility of $GABA_A$ receptor diversity in this nucleus.

Several pharmacological studies have addressed the inhibitory effects of the $GABA_B$ agonist baclofen in the CN [17,19,25,48]. However, the distribution of $GABA_B$ receptors in this nucleus was so far unknown. The heterogeneous distribution of $GABA_B$ binding sites in the CN and the high binding levels in the molecular layer of the DCN found in the present study, raise questions about the function of $GABA_B$ receptors in this auditory nucleus. Based on iontophoretic application of the prototype $GABA_B$ agonist baclofen [9,10] Caspary and coworkers have hypothesized that this substance may inhibit units in the VCN by blocking the release of glutamate or aspartate from auditory nerve endings [17,19], as suggested in other systems [9,14,37,54,61]. According to our results, the main territory of distribution of auditory nerve endings, including the central region of the VCN, deep DCN and fusiform/granule cell layer of the DCN contained low to very low levels of $GABA_B$ binding sites. The low level of $GABA_B$ binding sites found in these areas, does not necessarily argue against a role for $GABA_B$ receptors in synaptic events in the central region of the VCN. In most hippocampal regions, the level of $GABA_B$ binding sites is not particularly high [11,20], but physiological studies have demonstrated that presynaptic $GABA_B$ receptors have significant effects on the control of excitatory transmission in this region [40,53,54].

In contrast with findings in magnocellular regions of the VCN, levels of $GABA_B$ binding sites were remarkably higher in other areas of the CN, where they may be involved in inhibitory activity together with $GABA_A$ receptors. $GABA_B$ binding was moderate in the granule cell cap of the ventral CN and high in the molecular layer of the DCN. A feature common to both areas is the endings of granule cells [51,52]. Therefore, one possibility is that $GABA_B$ receptors might be presynaptic at the level of granule cell synapses, where they could modulate the release of glutamate, apparently used by CN granule cells as neurotransmitter [36,44,45]. However, there are also abundant GABA immunoreactive axo-dendritic endings in the granule cell cap of the VCN and the molecular layer of the DCN [38], and $GABA_B$ receptors might be postsynaptic to many of them. $GABA_B$ binding sites in the molecular layer of the cerebellum, whose organization shares analogies with the molecular layer of the DCN [5,6,51,52,84] are predominantly postsynaptic and located on Purkinje cells [69,77]. It is obvious that the ascertainment of pre-

or postsynaptic localization of $GABA_B$ receptors in the CN requires further experiments. In any case, the high level of $GABA_B$ binding sites in the molecular layer of the DCN, suggests that they have an important role in the integrative activity of this CN division. The apical dendrites of fusiform cells [6,13,42], the main projection neurons of the DCN, are located in the molecular as well as in the fusiform cell layers, which makes it likely that both $GABA_A$ and $GABA_B$ receptors contribute to the control of fusiform cell activity.

5. References

[1] Alford, S. and Grillner, S., The involvement of $GABA_B$ receptors and coupled G-protein in spinal GABAergic presynaptic inhibition, *J. Neurosci.,* 11 (1991) 3718–3726.

[2] Alger, B.E., GABA and glycine: postsynaptic actions. In M.A. Rogawski and J.L. Baker (Eds.). *Neurotransmitter Actions in the Vertebrate Nervous System,* Plenum Press, New York, 1985, pp. 33–69.

[3] Altschuler, R.A., Hoffman, D.W. and Wenthold, R.J., Neurotransmitters of the cochlea and cochlear nucleus: immunocytochemical evidence, *Am. J. Otolaryngol.,* 7 (1986) 100–106.

[4] Altschuler, R.A., Juiz, J.M., Shore, S.E., Bledsoe, S.C., Helfert, R.H. and Wenthold, R.J., Inhibitory amino acid synapses and pathways in the ventral cochlear nucleus. In M.A. Merchan, J.M. Juiz, D.A. Godfrey and E. Mugnaini (Eds.). *The Mammalian Cochlear Nuclei: Organization and Function,* Plenum Press, New York, 1993, pp. 211–224.

[5] Berrebi, A.S. and Mugnaini, E., Effects of the murine mutation 'nervous' on neurons in cerebellum and dorsal cochlear nucleus, *J. Neurocytol.,* 17 (1988) 465–484.

[6] Blackstad, T.W., Osen, K.K. and Mugnaini, E., Pyramidal neurones of the dorsal cochlear nucleus: a Golgi and computer reconstruction study in cat, *Neuroscience,* 13 (1984) 827–854.

[7] Bowery, N.G., $GABA_B$ receptors and their significance in mammalian pharmacology, *Trends Pharmacol. Sci.,* 10 (1989) 401–407.

[8] Bowery, N.G., Classification of GABA receptors. In S.J. Enna (Ed.). *The GABA receptors,* Humana Press, Clifton, NJ, 1984, pp. 177–213.

[9] Bowery, N.G., Hill, D.R., Hudson, A.L., Doble, A., Middlemiss D.N., Shaw, J. and Turnbull, M.J., ($-$)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor, *Nature,* 283 (1980) 92–94.

[10] Bowery, N.G., Hill, D.R. and Hudson, A.L., [^{3}H]($-$)Baclofen: an improved ligand for $GABA_B$ sites, *Neuropharmacology,* 24 (1985) 207–210.

[11] Bowery, N.G., Hudson, A.L. and Price, G.W., $GABA_A$ and $GABA_B$ receptor site distribution in rat central nervous system, *Neuroscience,* 20 (1987) 365–383.

[12] Bowery, N.G., Price, G.W., Hudson, A.L., Hill, D.R., Wilkin, G.P. and Turnbull, M.J., GABA receptor multiplicity, *Neuropharmacology,* 23 (1984) 219–231.

[13] Brawer, J.R., Morest, D.K. and Kane, E.C., The neuronal architecture of the cochlear nucleus of the cat, *J. Comp. Neurol.,* 155 (1974) 251–300.

[14] Cain, C.R. and Simmonds, M.A., Effects of baclofen on the olfactory cortex slice preparation, *Neuropharmacology,* 21 (1982) 371–373.

[15] Caspary, D.M., Havey, D.C. and Faingold, C.L., Effects of microiontophoretically applied glycine and GABA on neuronal response patterns in the cochlear nuclei, *Brain Res,* 172 (1979) 179–185.

[16] Caspary, D.M., Palombi, P.S., Backoff, P.M., Helfert, R.H. and Finlayson, P.G., GABA and glycine inputs control discharge rate within the excitatory response area of primary-like and phase-locked AVCN neurons. In M.A. Merchan, J.M. Juiz, D.A. Godfrey and E. Mugnaini (Eds.), *The Mammalian Cochlear Nuclei: Organization and Function,* Plenum Press, New York, 1993, pp. 239–252.

[17] Caspary, D.M., Rybak, L.P. and Faingold, C.L., Baclofen reduces tone-evoked activity of cochlear nucleus neurons, *Hear. Res,* 13 (1984) 113–122.

[18] 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 Thomas, 1985, pp. 198–226.

[19] Caspary, D.M., Cochlear nuclei: functional neuropharmacology of the different cell types. In R.A. Altschuler, R.P. Bobbin and D.W. Hoffman (Eds.), *Neurobiology of Hearing: The Cochlea,* Raven Press, New York, 1986, pp. 303–332.

[20] Chu, D.C.M., Albin, R.L., Penney J.B. and Young, A.B., Distribution and kinetics of $GABA_B$ bindig sites in rat central nervous system, *Neuroscience,* 34 (1990) 341–357.

[21] De Blas, A.L., Vitorica, J. and Friedrich, P., Localization of the $GABA_A$ receptor in rat brain with a monoclonal antibody to the 57,00 M_r peptide of the $GABA_A$ receptor/benzodiazepine receptor/Cl$^-$ channel complex, *J. Neurosci.,* 8 (1988) 602–614.

[22] DeFeudis F.W., Overview-$GABA_A$ receptors, *Ann. NY Acad. Sci.,* 585 (1990) 231–240.

[23] Dutar, P. and Nicoll, R.A., A physiological role for $GABA_B$ receptors in the central nervous system, *Nature,* 332 (1988) 156–158.

[24] Enna, S.J. and Gallagher, J.P., Biochemical and electrophysiological characteristics of mammalian GABA receptors, *Int. Rev. Neurobiol.,* 24 (1983) 181–212.

[25] Evans, E.F. and Zhao, W., Neuropharmacological and neurophysiological dissection of inhibition in the mammalian dorsal cochlear nucleus. In M.A. Merchan, J.M. Juiz, D.A. Godfrey and E. Mugnaini (Eds.). *The Mammalian Cochlear Nuclei: Organization and Function,* Plenum Press, New York, 1993, pp. 253–266.

[26] Frostholm, A. and Rotter, A., Autoradiographic localization of receptors in the cochlear nucleus of the mouse, *Brain Res. Bull.,* 16 (1986) 189–203.

[27] Gage, P.W., Activation and modulation of neuronal K+ channels by GABA, *Trends Neurosci.,* 15 (1992) 46–51.

[28] 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 (1988) 288–308.

[29] Godfrey, D., Parli, J., Dunn, J. and Ross, C., Microchemistry of the cochlear nucleus and superior olivary complex. In J. Syka and R. Masterton (Eds.), *Auditory Pathway: Structure and Function,* Plenum Press, New York, 1988, pp. 107–121.

[30] 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.

[31] Godfrey, D.A., Carter, J.A., Berger, S.J., Lowry, O.H. and Matschinsky, F.M., Quantitative histochemical mapping of candidate transmitter amino acids in the cochlear nucleus of the rat, *J. Histochem. Cytochem.,* 25 (1977) 417–431.

[32] Greene, D., Hunter, C., Wada, K., Lolait, S.J., O'Carroll A.M., Mahan, L.C. and Wenthold R.J., Pattern of gene expression of

GABA$_A$ receptor subunit mRNAS in the rat central auditory system, Assoc. Res. Otolaryngol. Abstr., 14 (1991) 11.

[33] Hill, D.R. and Bowery, N.G., ^{3}H Baclofen and ^{3}H GABA bind to bicuculline insensitive GABA$_B$ sites in rat brain, Nature, 290 (1981) 149–152.

[34] Hosli, E., Mohler, H., Richards, J.G. and Hosli, L., Autoradiographic localization of binding sites for [^{3}H]gamma-aminobutyrate, [^{3}H]muscimol, [^{3}H]bicuculline methiodide and [^{3}H]flunitrazepan in cultures of rat cerebellum and spinal cord, Neuroscience, 5 (1985) 1657–1665.

[35] Juiz, J.M., Helfert, R.H., Wenthold, R.A., De Blas, A.L. and Altschuler, R.A., Immunocytochemical localization of the GABA$_A$/benzodiazepine receptor in the guinea pig cochlear nucleus: evidence for receptor localization heterogeneity, Brain Res., 504 (1989) 173–179.

[36] Juiz, J.M., Rubio, M.E., Helfert, R.H. and Altschuler, R.A., Localizing putative excitatory endings in the cochlear nucleus by quantitative immunocytochemistry. In M.A. Merchan, J.M. Juiz, D.A. Godfrey and E. Mugnaini (Eds.), The Mammalian Cochlear Nuclei: Organization and Function, Plenum Press, New York, 1993, pp. 167–177.

[37] Kato, K., Goto, M. and Fukuda, H., Baclofen: inhibition of release of L-[3H]glutamate and L-[3H] aspartate from rat whole brain synaptosomes, Gen. Pharmac., 13 (1982) 445–447.

[38] Kolston, J., Osen, K.K., Hackney, C.M., Ottersen, O.P. and Storm-Mathissen, J., An atlas of glycine- and GABA-like immunoreactivity and colocalization in the cochlear nuclear complex of the guinea pig, Anat. Embryol., 186 (1993) 443–465.

[39] Kuhar, M.J., De Souza, E.B. and Unnerstall, J.R., Neurotransmitter receptor mapping by autoradiography and other methods, Annu. Rev. Neurosci., 9 (1986) 27–59.

[40] Lanthorn, T.H. and Cotman, C.W., Baclofen selectively inhibits excitatory synaptic transmission in the hippocampus, Brain Res., 225 (1981) 171–178.

[41] Levitan, E.S., Schofield, P.R., Burt, D.R., Rhee, L.M., Wisden, W., Kohler, M., Fujita, M., Rodriguez, H.F., Stephenson, A. and Darlison, M.G., Structural and functional basis for GABA$_A$ receptor heterogeneity, Nature, 335 (1988) 76–79.

[42] Lorente de No, R., In R. Lorente de No (Ed.), The Primary Acoustic Nuclei, Raven Press, New York, 1981.

[43] Luddens, W. and Wisden, W., Function and pharmacology of multiple GABA$_A$ receptor subunits, Trends Pharmacol. Sci., 12 (1991) 49–51.

[44] Manis, P.B., Responses to parallel fiber stimulation in the guinea pig dorsal cochlear nucleus, J. Neurophysiol., 61 (1989) 149–161.

[45] Manis, P.B., Pharmacology of synaptic transmission at parallel fiber synapses in the dorsal cochlear nucleus, Assoc. Res. Otolaryngol. Abstr., 13 (1990) 95.

[46] Martin, M.R., Dickson, J.W. and Fex, J., Bicuculline, strychnine and depressant amino acid responses in the anteroventral cochlear nucleus of the cat, Neuropharmacology, 21 (1982) 201–207.

[47] Martin, M.R. and Penix, L.P., Comparison of the effects of bicuculline and strychnine on brain stem auditory evoked potentials in the cat, Br. J. Pharmacol., 78 (1983) 75–77.

[48] Martin M.R., Baclofen and the brainstem auditory evoked potentials, Exp. Neurol., 76 (1982) 675–680.

[49] Mathers, D.A., The GABA$_A$ receptor: new insights from single-channel recording, Synapse, 1 (1987) 96–101.

[50] McCabe, R.T. and Wamsley, J.K., Autoradiographic localization of subcomponents of the macromolecular GABA receptor complex, Life Sci., 39 (1986) 1937–1946.

[51] Mugnaini, E., Warr, B.W. and Osen, K.K., Distribution and light microscopic features of granule cells in the cochlear nuclei of cat, rat and mouse, J. Comp. Neurol., 191 (1980) 581–606.

[52] Mugnaini, E., Osen, K.K., Dahl, A.L., Friedrich, V.L. and

Korte, G., Fine structure of granule cells and related interneurons (termed Golgi cells) in the cochlear nuclear complex of cat, rat and mouse, J. Neurocytol., 9 (1980) 537–570.

[53] Nicoll, R.A., The coupling of neurotransmitter receptors to ion channels in the brain, Science, 241 (1988) 545–551.

[54] Olpe, H.R., Baudry, M., Fagni, L. and Lynch, G., The blocking action of baclofen on excitatory transmission in the rat hippocampal slice, J. Neurosci., 2 (1982) 698–703.

[55] Olsen, R.W., Bergman, M.O., Van Ness, P.C., Lummis, S.C., Watkins, A.E., Napias, C. and Greenlee, D.W., Gamma-aminobutyric acid receptor binding in mammalian brain: Heterogeneity of binding sites, Mol. Pharmacol., 19 (1981) 217–227.

[56] Olsen, R.W., Bureau, M.H., Endo, S. and Smith, G., The GABA$_A$ receptor family in the mammalian brain, Neurochem. Res., 16 (1991) 309–315.

[57] Olsen, R.W. and Snowman, A.M., ^{3}H-bicuculline methochloride binding to low affinity GABA receptor sites, J. Neurochem., 41 (1983) 1653–1663.

[58] Olsen, R.W., Wong, E.H.F., Stauber, G.B. and King, R.G., Biochemical pharmacology of the gammaaminobutyric acid receptor/ionophore protein, Fed. Proc., 43 (1984) 2773–2778.

[59] Pan, H.S., Frey, K.A., Young A.B. and Penney J.B., Changes in ^{3}H muscimol binding in substantia nigra, entopeduncular nucleus, globus pallidus and thalamus after striatal lesions as demonstrated by quantitative receptor autoradiography, J. Neurosci., 3 (1983) 1189–1198.

[60] Penney, J.B., Pan, H.S., and Young, A.B., Quantitative autoradiography of ^{3}H-muscimol binding in rat brain, Science, 214 (1981) 1036–1038.

[61] Potashner, S.J., Baclofen: Effects on amino acid release and metabolism in slices of guinea pig cerebral cortex, J. Neurochem., 32 (1979) 103–109.

[62] Potashner, S.J., Benson, C.G., Ostapoff, E.M., Lindberg, N. and Morest, D.K., Glycine and GABA: transmitter candidates of projections descending to the cochlear nucleus. In M. Merchan, J.M. Juiz, D.A. Godfrey and E. Mugnaini (Eds.), The Mammalian Cochlear Nuclei: Organization and Function, Plenum Press, New York, 1993, pp. 196–210.

[63] Pritchett, D.B., Sontheimer, H., Shivers, B.D., Ymer, S., Kettenman H. Schofield, P.R. and Seeburg, P.H., Importance of a novel GABA$_A$ receptor subunit for benzodiazepine pharmacology, Nature, 338 (1987) 582–585.

[64] Richards, G., Mohler, H. and Haefely, W., Mapping benzodiazepine receptors in the CNS by radiohistochemistry and immunohistochemistry. In P. Panula, H. Paivarinta and S. Soinila (Eds.), Neurohistochemistry: Modern Methods and Applications, Alan Liss, New York, 1987, pp. 629–677.

[65] Richards, J.G., Glinz, R., Schoch, P. and Mohler, H., New trends in mapping benzodiazepine receptors, Adv. Biochem. Psycopharmacol., 45 (1988) 27–46.

[66] Richards, J.G., Schoch, P., Haring, P., Takacs, B. and Mohler, H., Resolving GABA$_A$/benzodiazepine receptors: cellular and subcellular localization with monoclonal antibodies, J. Neurosci., 7 (1987) 1866–1886.

[67] Saint Marie, R.L., Ostapoff, M.L., Benson, C.G., Morest, D.K. and Potashner, S.J., Non-cochlear projections to the ventral cochlear nucleus: are they mainly inhibitory?. In M.A. Merchan, J.M. Juiz, D.A. Godfrey and E. Mugnaini (Eds.), The Mammalian Cochlear Nuclei: Organization and Function, Plenum Press, New York, 1993, pp. 121–131.

[68] 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, 344 (1985) 168–171.

[69] Schreurs, B.G., Sanchez-Andres, J.V. and Alkon, D.L., GABA-induced responses in Purkinje cell dendrites of the rabbit cerebellar slice, Brain. Res., 597 (1992) 99–107.

[70] Seeburg, P.H., Wisden, W., Verdoorn, T.A., Pritchett, D.B., Werner, P., Herb, A., Luddens, H., Sprengel, R. and Sakmann, B., The GABA$_A$ receptor family: molecular and functional diversity, *Cold Spring Harb. Symp. Quant. Biol.*, 55 (1990) 29–40.

[71] Sekiguchi, M., Sakuta, H., Okamoto, K. and Sakai, Y., GABA$_B$ receptors expressed in Xenopus oocytes by guinea pig cerebral mRNA are functionally coupled with Ca^{2+}-dependent Cl^- channels and with K^+ channel through GTP-binding proteins, *Brain Res.-Mol.Brain Res.*, 8 (1992) 301–309.

[72] Sharif, N.A., Multiple synaptic receptors for neuroactive amino acid transmitters-New vistas, *Int. Rev. Neurobiol.*, 26 (1985) 85–125.

[73] 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 (1987) 2197–2209.

[74] Spangler, K.M., and Warr, B.W., The descending auditory system. In R.A. Altschuler, R.P. Bobbin, B.M. Clopton and D.W. Hoffman (Eds.). *Neurobiology of Hearing: The Central Auditory System*, Raven Press, New York, 1991, pp. 27–45.

[75] Stephenson, F.A., Understanding the GABA$_A$ receptor: a chemically gated ion channel, *Biochem. J.*, 249 (1988) 21–32.

[76] Stephenson, F.A. and Olson, R.W., Biochemical pharmacology of the GABA receptor-inophore protein complex. In P. Mandel and F.V. DeFeudis (Eds.), *CNS Receptors — From Molecular Pharmacology to Behaviour*, Raven Press, New York, 1983, pp. 71–80.

[77] Turgeon S.M., Albin R.L. and Gilman S., Development, pharmacology, distribution and cellular localization of GABA$_B$ binding in rat cerebellum, *Soc. Neurosci. Abstr.*, 17 (1991) 1572.

[78] Unnerstall, J.R., Kuhar, M.J., Niehoff, D.L. and Palacios, J.M., Benzodiazepine receptors are coupled to a subpopulation of GABA receptors: evidence from a quantitative autoradiographic study, *J. Pharmacol. Exp. Ther.*, 218 (1981) 797–804.

[79] Unnerstall, J.R., Niehoff, D.L., Kuhar, M.J. and Palacios, J.M., Quantitative receptor autoradiography using ^3H Ultrofilm: application to multiple benzodiazepine receptors, *J. Neurosci. Methods*, 6 (1982) 59–73.

[80] Verdoor, T.A., Draguhn, A., Ymer, S., Seeburg, P.H. and Sakmann, B., Functional properties of recombinant GABA$_A$ receptors depend upon subunit composition, *Neuron*, 4 (1990) 919–928.

[81] 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 (1988) 615–622.

[82] Wenthold, R.J., Neurotransmitters of brain stem auditory nuclei. In R.A. Altschuler, R.J. Bobbin, B.M. Clopton and B.W. Hoffman (Eds.). *Neurobiology of Hearing: The Central Auditory System*, Raven Press, NY, 1991, pp. 121–139.

[83] Wojcik, N.J. and Neff, N.H., Gamma-aminobutyric acid receptors are negatively coupled to adenylate cyclase in brain, and in the cerebellum these receptors may be associated with granule cells, *Mol. Pharmacol.*, 25 (1984) 24–28.

[84] Wouterlood, F.G. and Mugnaini, E., Cartwheel neurons of the dorsal cochlear nucleus: a Golgi-electron microscopic study in rat, *J. Comp. Neurol.*, 227 (1984) 136–157.

[85] Young, W.S. and Kuhar, M.J., Autoradiographic localization of benzodiazepine receptors in the brains of humans and animals, *Nature*, 280 (1979) 393–395.