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The Distribution of 13 $GABA_A$ Receptor Subunit mRNAs in the Rat Brain. II. Olfactory Bulb and Cerebellum

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In an effort to determine subunit compositions of *in vivo* $GABA_A$ receptors, the cellular localization of 13 subunit encoding mRNAs (α_1–α_6, β_1–β_3, γ_2–γ_3, δ) was determined in the rat olfactory bulb and cerebellum. Cerebellar granule cells expressed significant quantities of α_1, α_6, β_2, β_3, γ_2, and δ mRNAs. They contained very much lower levels of α_4, β_1, and γ_3 mRNAs, and the α_2, α_3, α_5, and γ_1 genes appeared to be silent. Purkinje cells contained only the α_1, β_2, β_3, and γ_2 mRNAs. Putative Bergmann glial cells were found to contain the γ_1 mRNA and possibly the α_2 mRNA. In the molecular layer, only the α_1, β_2 and γ_2 mRNAs were expressed in stellate/basket cells. The α_3 probe hybridized weakly to targets in the molecular layer. The inferior olivary nucleus contained significant quantities of α_2, α_4, and γ_1 transcripts, with the α_1, α_3, β_2, β_3, and γ_2 mRNAs also present. In the olfactory bulb, mitral cells were found to express the α_1, β_1, β_2, β_3, and γ_2 mRNAs strongly and the α_3 mRNA weakly. Tufted cells contained α_1, α_3, β_2, β_3, and γ_2 mRNAs and, occasionally, the α_2 mRNA. In the internal granule cells the α_2, α_4, α_5, β_3, and δ mRNAs were all present. Low levels of α_3, γ_1, γ_2, and γ_3 mRNAs were also noted in these cells. Periglomerular cells expressed low levels of α_2, α_3, α_4, β_2, β_3, γ_1, γ_2, and γ_3 mRNAs. No α_6 mRNA was present in the olfactory bulb. Correlations that are general ones from other brain regions are the colocalizations of $\alpha_1\beta_2$, $\alpha_2\beta_3$, and $\alpha_4\delta$ mRNAs. In both the olfactory bulb and cerebellum, $\alpha_1\beta_2\gamma_2$ receptor cores are probably employed. The δ-subunit mRNA appears to codistribute with α-subunit mRNAs (α_4 and α_6) associated with $GABA_A$ subunits that fail to bind benzodiazepine agonists.

The cerebellum and olfactory bulb are anatomically well-defined structures, consisting of stereotypic architectures and a small number of neuronal cell types identifiable by their position and size (Eccles et al., 1967; Palay and Chan-Palay, 1974; Switzer et al., 1985; Shepherd, 1988). The synaptic circuitry and neurochemistry of these structures are also relatively well known (Shepherd, 1988). In both regions, fast GABAergic inhibition via the $GABA_A$ receptor is very prominent (Halasz and Shepherd, 1983; Haefely and Polc, 1986; Richards et al., 1986; Ross et al., 1990). The detailed properties of individual $GABA_A$ receptors on neurons in these areas can now in principle be studied by novel patch-clamping methodology on tissue slices (Edwards et al., 1989). All of these features make these two areas attractive model systems to study the phenomenon of $GABA_A$ receptor diversity.

The $GABA_A$ receptor is a ligand-gated anion channel for which subunits of four subunit classes have been characterized in the rodent (α_1–α_6, β_1–β_3, γ_1–γ_3, and δ), and which probably exists as a pentameric complex of unknown stoichiometry (Seeburg et al., 1990). The mRNAs encoding these subunits in the brain exhibit enormously varied regional distributions, suggesting that a large repertoire of $GABA_A$ receptor isoforms are employed [Seeburg et al., 1990; Lüddens and Wisden, 1991; Wisden et al., 1992 (accompanying paper)]. However, for detailed pharmacological characterization of relevant recombinant receptors, and ultimately for the development of receptor-selective agents and drugs, it is important to have a precise notion of subunit compositions of $GABA_A$ receptors *in vivo*. The properties of recombinant receptors can then be compared to those *in vivo* by patch clamping in tissue slices (Edwards et al., 1989; Konnerth et al., 1990). To this end, we have studied the cellular localization of $GABA_A$ receptor subunit mRNAs in the olfactory bulb and the cerebellum.

Materials and Methods

Oligonucleotides for *in situ* hybridization and the procedure for the autoradiographic detection of $GABA_A$ receptor subunit mRNAs were exactly as described in the accompanying article (Wisden et al., 1992). In brief, 14-μm-thick rat brain sections were incubated overnight at 42°C with ^{35}S-labeled 45-base antisense oligonucleotides dissolved in hybridization buffer (50% formamide/4× saline–Na-citrate/10% dextran sulphate). Sections were then washed in 1 × SSC at 60°C, dehydrated in alcohol, and either exposed to Kodak X-Omat AR5 film for 3–4 weeks or dipped in photographic emulsion (Kodak NTB2, diluted 1:1 in water), allowed to dry, and then exposed at 4°C for 8–12 weeks. Dipped sections were developed in D19 (Kodak) at 17°C for 2 min, fixed, and subsequently stained with thionin. Identification of anatomical structures was performed according to the atlases of Paxinos and Watson (1986), Switzer et al. (1985), and Palay and Chan-Palay (1974). Photomicrographs were obtained with a Zeiss Axioplan microscope under bright- and dark-field optics.

The specificity of hybridization was determined by incubation of parallel sections with a mixture of both radiolabeled and excess (50-fold) unlabeled probe. Such procedures resulted in blank autoradiographs or a homogeneous, sparse distribution of silver grains in the developed emulsion.

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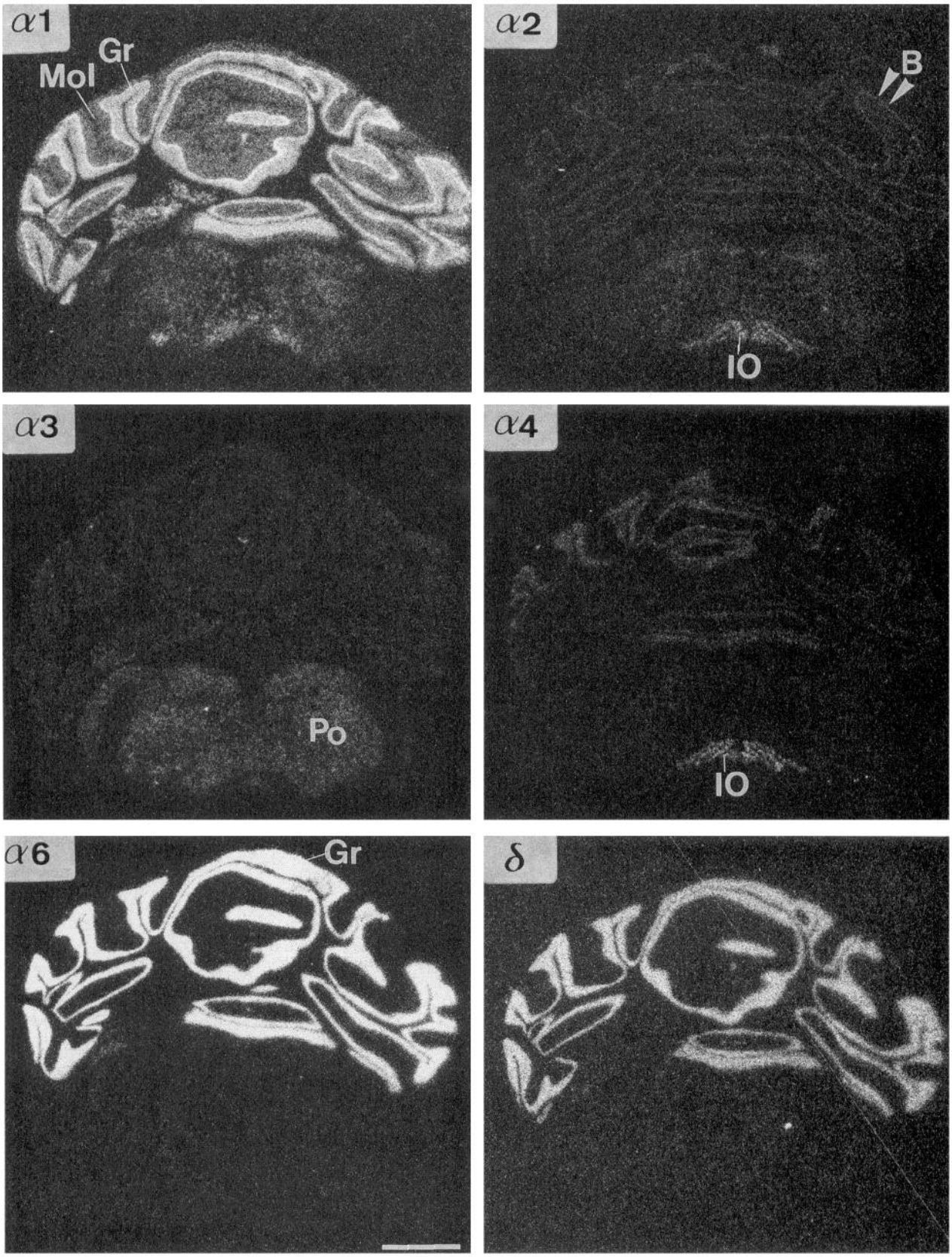

Figure 1. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor α-subunit and δ-subunit mRNAs in the cerebellum (coronal sections at the level of inferior olive). *Arrowheads* in *α2* indicate hybridizing Purkinje/Bergmann glia cell line. *B,* Bergmann glia/Purkinje cell line; *Gr,* granule cells; *IO,* inferior olivary nucleus; *Mol,* molecular layer; *Po,* pons. Scale bar, 1.7 mm.

Results

Cerebellum

The distribution of the $GABA_A$ receptor subunit transcripts was examined in coronal sections of the cerebellum at the level of the inferior olive. The results are summarized in Table 1.

In terms of mRNA abundance, the most significant transcripts in the cerebellum are those of α_1, α_6, β_2, β_3, γ_2, and δ (Figs. 1, 2). The autoradiographs indicate that all of these transcripts are expressed in granule cells (Figs. 1, 2). Examination of sections exposed to photographic emulsion confirms this (Fig. 3). Thus, the α_1 mRNA is present in granule cells, Purkinje cells, and stellate cells. The α_6 mRNA appears restricted to granule cells, with Purkinje and stellate/basket cells unlabeled by the α_6 probe. In fact, the cerebellar granule cells appear to be the sole site of expression of the α_6 gene throughout the entire CNS, suggesting a specific promoter control. Silver grains resulting from the β_2 probe hybridization are heavily localized over granule cells and Purkinje cells, with stellate/basket cells being weakly labeled. Similar results are observed with the β_3 probe, although the Purkinje cells are less heavily labeled. The γ_2 probe hybridizes to granule cells, Purkinje cells, and stellate/basket cells, with Purkinje cells being most strongly labeled (Fig. 3). The δ-probe hybridizes to targets restricted to granule cells (Fig. 3).

Other probes that give weak, but specific signals over the granule cell layer are α_4 (Fig. 1), β_1 (Fig. 2), and γ_3 (Fig. 2). In our hands, these autoradiographic signals are too weak to be detected with photographic emulsion. The α_3 probe hybridizes specifically but weakly to the molecular layer (Fig. 1). The α_5 mRNA is undetectable in cerebellum (data not shown; see also Wisden et al., 1992).

Putative Bergmann glia

The α_2 and γ_1 probes hybridize only to the Purkinje cell layer as assessed from the x-ray film autoradiographic images (Figs. 1, 2). The α_2 signal is very weak. Examination of the origins of this signal with photographic emulsion confirm that the signal results from hybridization to the Purkinje cell layer (Fig. 4*A,B*). The α_2 signal results in a "halo" of silver grains under dark-field optics, along the border between the granule and molecular cell layers, the granule cells themselves being unlabeled. Consistent with the x-ray film images, the γ_1 silver grain density of the photographic emulsion is much stronger (Fig. 4*B*). A dense cluster of silver grains originates at the granule cell layer/molecular layer border and extends out into the molecular layer (Fig. 4*B*). High-power bright-field optics reveal that the Purkinje cells themselves are unlabeled by the γ_1 probe (Fig. 4*C*). Silver grains are clustered over small cells surrounding the Purkinje cells, with the granule cells being unlabeled. The position of these labeled cells suggests they are Bergmann glia. High-power bright-field examination of the α_2 signal also reveals that the Purkinje cells are unlabeled (data not shown).

Brain stem/inferior olive

As assessed from the autoradiographs, the inferior olive contains a diversity of $GABA_A$ receptor subunit mRNAs. At a minimum, this nucleus expresses the α_1, α_2, α_4, β_3, and γ_1 genes (Figs. 1, 2). The abundance of the α_2, α_4, and γ_1 mRNAs in the inferior olive relative to their levels in surrounding brainstem or cerebellum is striking. The α_3, β_2, and γ_2 transcripts also appear to be in the inferior olive, but at levels comparable to those in the surrounding gray matter.

Table 1. Summary of detectable $GABA_A$ receptor subunit mRNAs in cerebellar and olfactory bulb cells

Cell type	Detectable mRNAs
Cerebellum	
Stellate/basket cells	α_1 (α_3) β_2 γ_2
Purkinje cells	α_1 β_2 β_3 γ_2
Granule cells	α_1 (α_4) α_6 (β_1) β_2 β_3 γ_2 (γ_3) δ
Putative Bergmann glia	α_2 γ_1
Olfactory bulb	
Mitral cells	α_1 (α_3) β_1 β_2 β_3 γ_2
Tufted cells	α_1 (α_2 α_3) β_2 β_3 γ_2
Putative short axon cells	α_1 β_2
Internal granule cells	α_2 (α_4) α_4 α_5 β_3 δ (γ_1 γ_2 γ_3)
Periglomerular cells	(α_2 α_3 α_4) β_2 β_3 (γ_1 γ_2 γ_3 δ)

Parentheses indicate minor mRNA species.

At this level of the brainstem, the predominant transcripts in the rest of the gray matter are α_1, α_3, β_3, and γ_2, with some β_2 mRNA as well. Some mRNAs (α_5, α_6, β_1, γ_3, and δ) are completely absent from any brainstem region examined (Figs. 1, 2).

Olfactory bulb

Hybridization signals for all of the examined $GABA_A$ receptor transcripts except for α_6 are detected in horizontal sections of the rat olfactory bulb, with each subunit mRNA exhibiting idiosyncracies in both signal intensity and distribution. The results are summarized in Table 1.

Main olfactory bulb. In granule cells of the internal granule cell and mitral cell layers, the most abundant $GABA_A$ receptor mRNAs encode the α_2 and β_3 subunits, with the α_5 mRNA also exhibiting a high degree of expression (Figs. 5–7). Moderate signals are detected for mRNAs of α_4 and δ-subunits in the granule cells, while those for α_3, γ_1, γ_2, and γ_3 are low (Figs. 5, 6). A gradient in signal intensity from outer to inner regions of the granule cell layer observed on autoradiographs (Figs. 5, 6) is due to a gradient in the density of granule cells. No signal is detectable for either α_1, β_1, or β_2 mRNAs in granule cells, although the α_1 and β_2 probes label occasional large cells in this layer (Figs. 5, 6; Table 1). These appear less stained than the surrounding granule cells when examined under high-power bright-field microscopy, and thus probably correspond to short axon cells.

Mitral neurons are intensely labeled by the probes complementary to the α_1, β_2, and β_3 mRNAs (Figs. 5–8). Moderately strong signals for γ_2 and β_1 mRNA are also detected over mitral cells (Figs. 6–8), while a weaker but definite signal is observed for α_3 mRNA (Figs. 5, 8). Transcripts for the α_2, α_4, α_5, γ_1, γ_3, and δ-subunits are absent from mitral cells (Figs. 5, 6, 8). In the tufted cells of the external plexiform layer the most abundant $GABA_A$ receptor subunit transcripts are those for α_1 and β_2 (Figs. 5–8). Strong hybridization signals are also observed for β_3 and γ_2 mRNAs, and signals of moderate strength for α_3 mRNA (Figs. 5–8). The transcript for α_2 is detected weakly in some tufted cells (Fig. 8), but many remain unlabeled. The mRNAs for α_4, α_5, γ_1, γ_3, and δ are undetectable in tufted cells (Figs. 5, 6). No $GABA_A$ receptor subunit transcript is present in large amounts in periglomerular cells, such cells being only moderately labeled by the β_2 and β_3 probes (Figs. 6, 7) and lightly labeled by probes for the α_2, α_3, α_4, γ_1, γ_2, γ_3, and δ-subunits (Figs. 5, 6). The α_1,

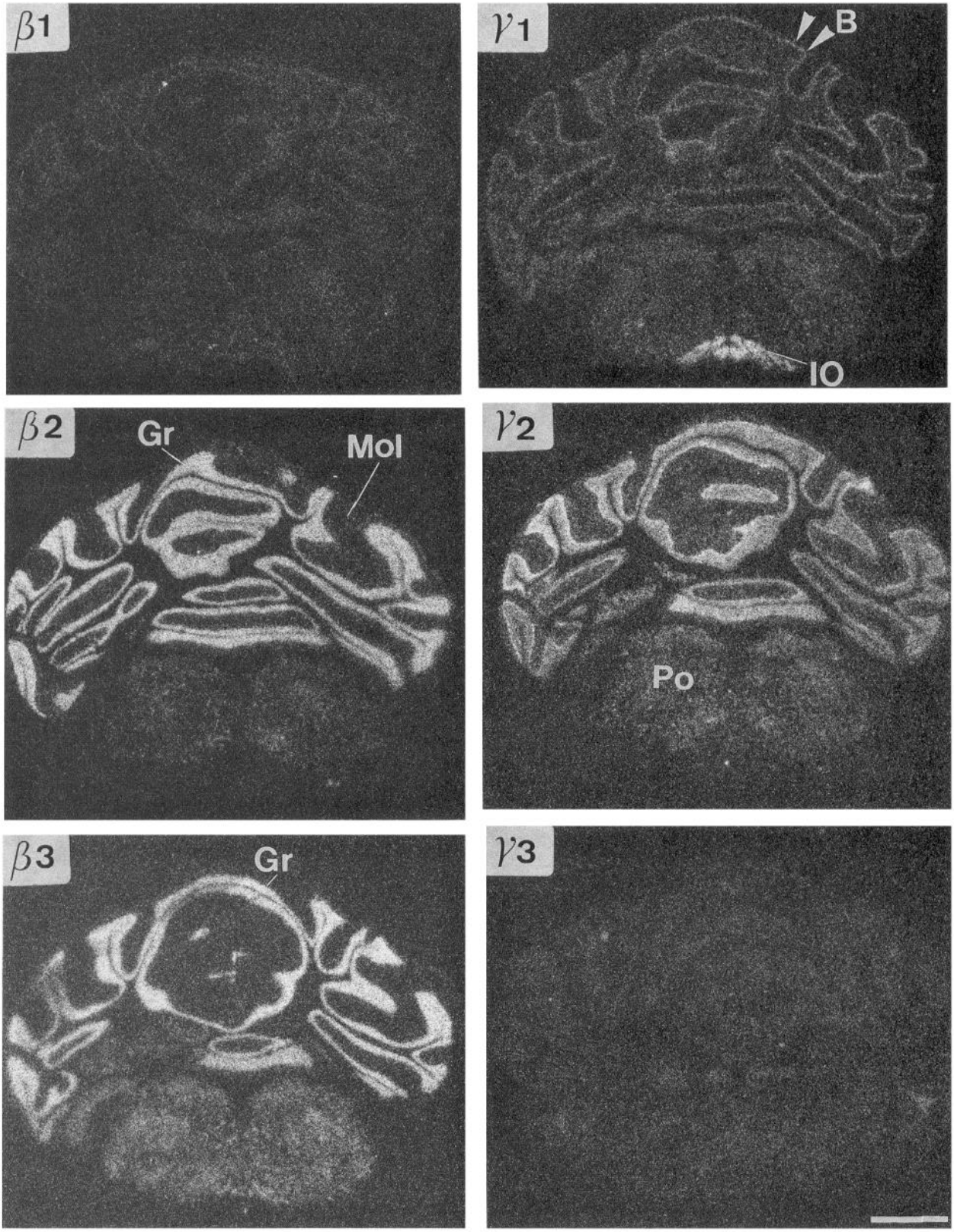

Figure 2. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor β and γ mRNA in cerebellum (coronal sections at level of inferior olive). Abbreviations and symbols are as for Figure 1. Scale bar, 1.7 mm.

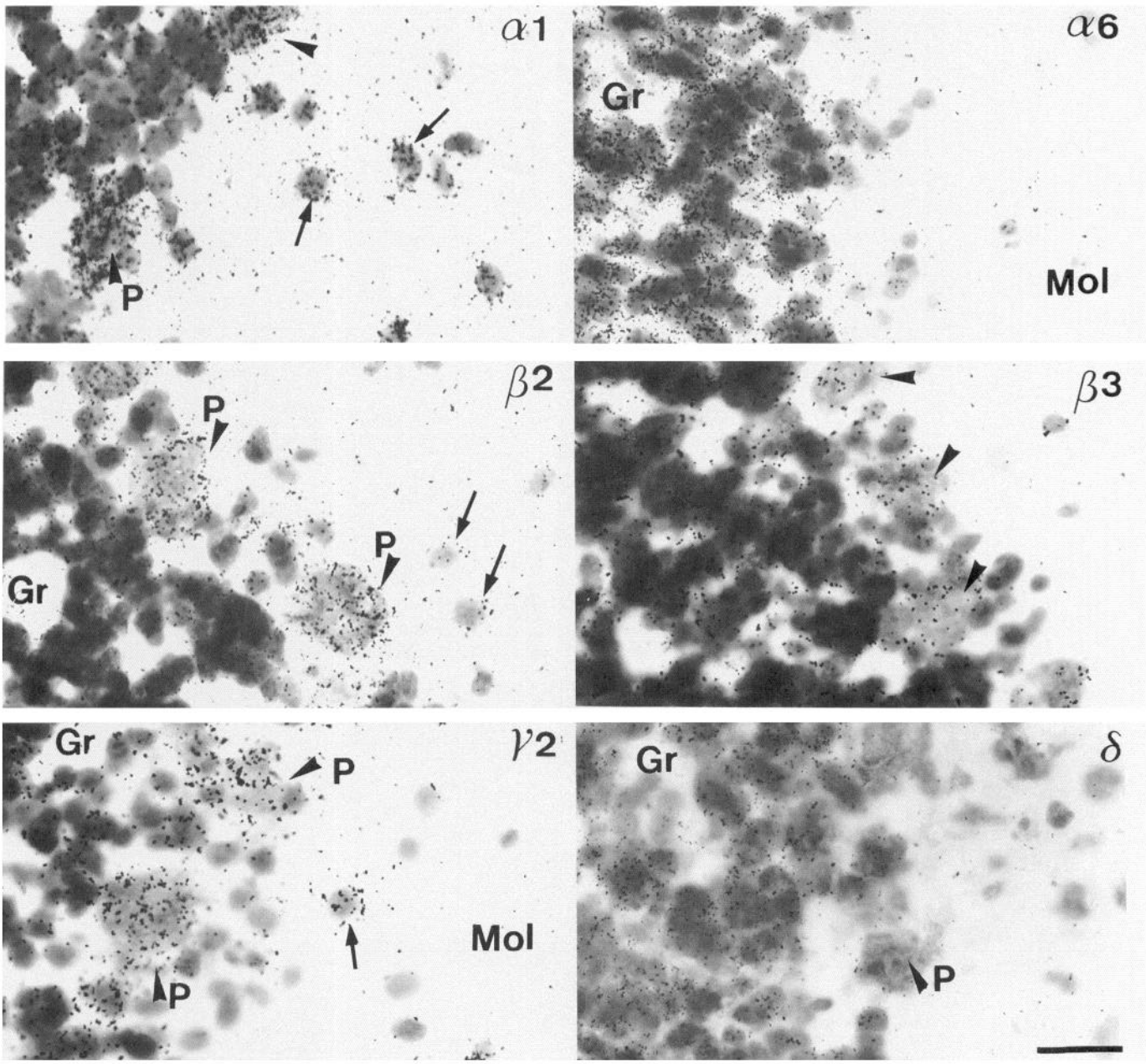

Figure 3. Bright-field photomicrographs showing cellular distribution of α_1, α_6, β_2, β_3, γ_2, and δ mRNAs in the cerebellum. *Arrows* indicate examples of labeled stellate/basket cells; *arrowheads* delineate Purkinje cells. *Gr,* granule cells; *Mol,* molecular layer; *P,* Purkinje cells. Scale bar, 50 μm.

α_5, and β_1 subunits are absent from periglomerular cells (Figs. 5, 6).

No $GABA_A$ receptor subunit mRNA is detectable in cells of the olfactory nerve layer or in ependymal cells lining the olfactory ventricle.

Accessory olfactory bulb. The patterns of expression of $GABA_A$ receptor subunit mRNA in the accessory olfactory bulb largely correspond to that in the main olfactory bulb with some differences in the degree of expression. In the granular cell layer, as in the main bulb, mRNA for α_2 and β_3 is highly expressed, while α_5 mRNA is present to a much smaller degree (Figs. 5, 6). Low to moderate levels of α_4, γ_1, γ_2, γ_3, and δ mRNAs are found, while α_1, α_3, β_1, and β_2 mRNAs are absent from accessory bulb granule cells (Figs. 5, 6). A high degree of expression is observed in cells of the external plexiform layer/mitral cell layer complex of transcripts for α_1, β_1, β_2, β_3, and γ_2, with mild expression of α_2 and α_3 mRNAs, other subunit mRNAs being undetectable (Figs. 5, 6). The weak expression of α_2 mRNA in the mitral cells of the accessory bulb matches the occasional expression of this subunit in tufted cells of the main bulb (Figs. 5, 8), since in the accessory bulb the external plexiform and mitral cell layers are merged (Switzer et al., 1985). Finally, a few cells of the glomerular layer contain moderate amounts of β_2 mRNA and low amounts of mRNAs for α_1, α_3, β_3, and γ_1 (Figs. 5, 6).

Discussion

In our accompanying article (Wisden et al., 1992) we have shown that $GABA_A$ receptor subunit gene expression in the brain is

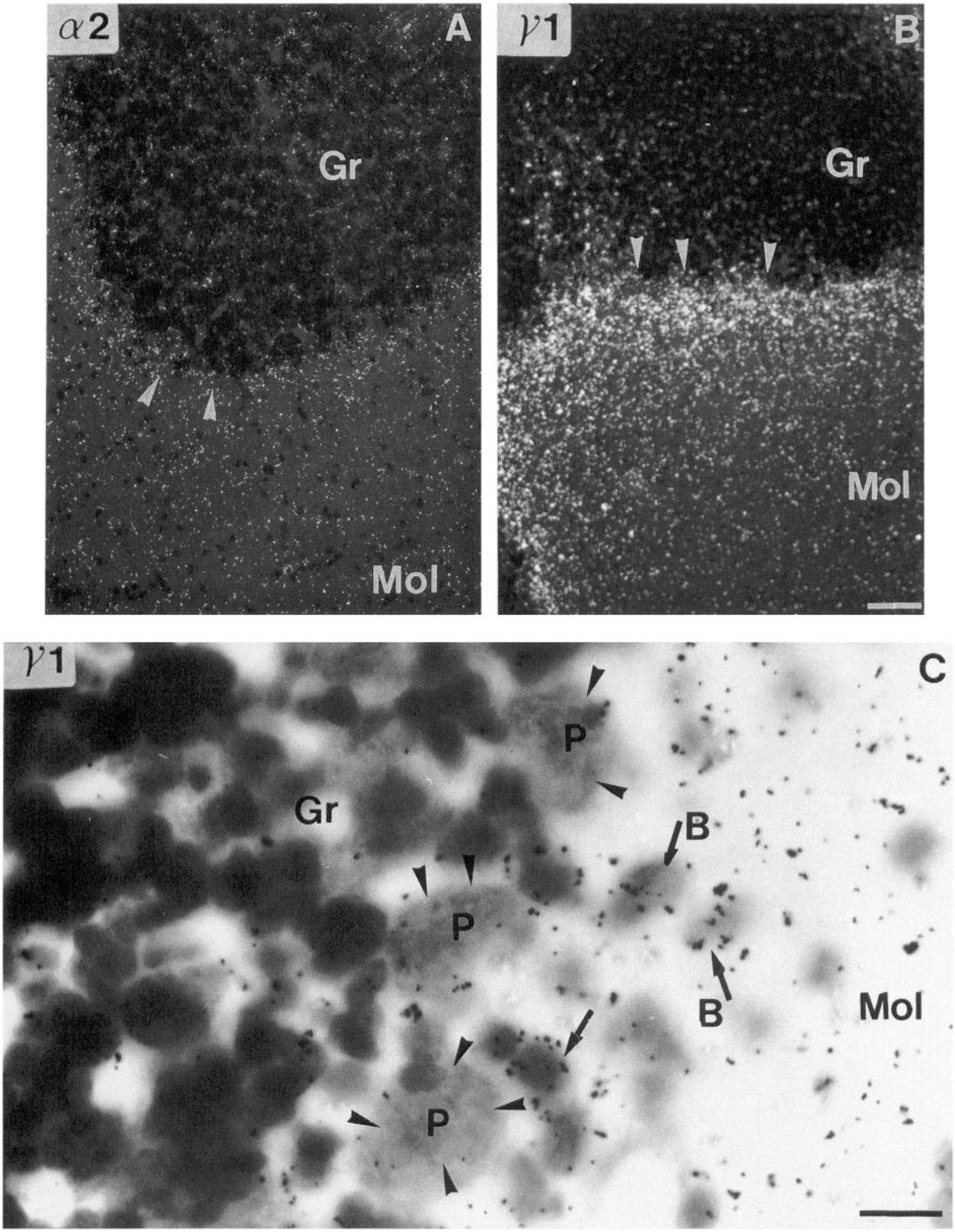

Figure 4. Cellular distribution of α_2 (*A*) and γ_1 (*B* and *C*) mRNAs in cerebellum. *A* and *B* are low-power dark field; *C* is high-power bright field of the image in *B*. *B*, putative Bergmann glia; *Gr*, granule cells; *Mol*, molecular layer; *P*, Purkinje cells. *Arrowheads* in *A* and *B* indicate "halo" of silver grains along the boundary of the granule cell/molecular layers. In a high-power bright-field view of the γ_1 probe autoradiographic signal (*C*), Purkinje cells (*arrowheads*) and granule cells appear to be unlabeled, whereas other small cells (*arrows*) in the Purkinje layer have clusters of silver grains over them. There is also a density of grains higher than background over the molecular layer in areas having no cell bodies. This could indicate possible labeling of glial cell process. Scale bars: *A* and *B*, 100 μm; *C*, 35 μm.

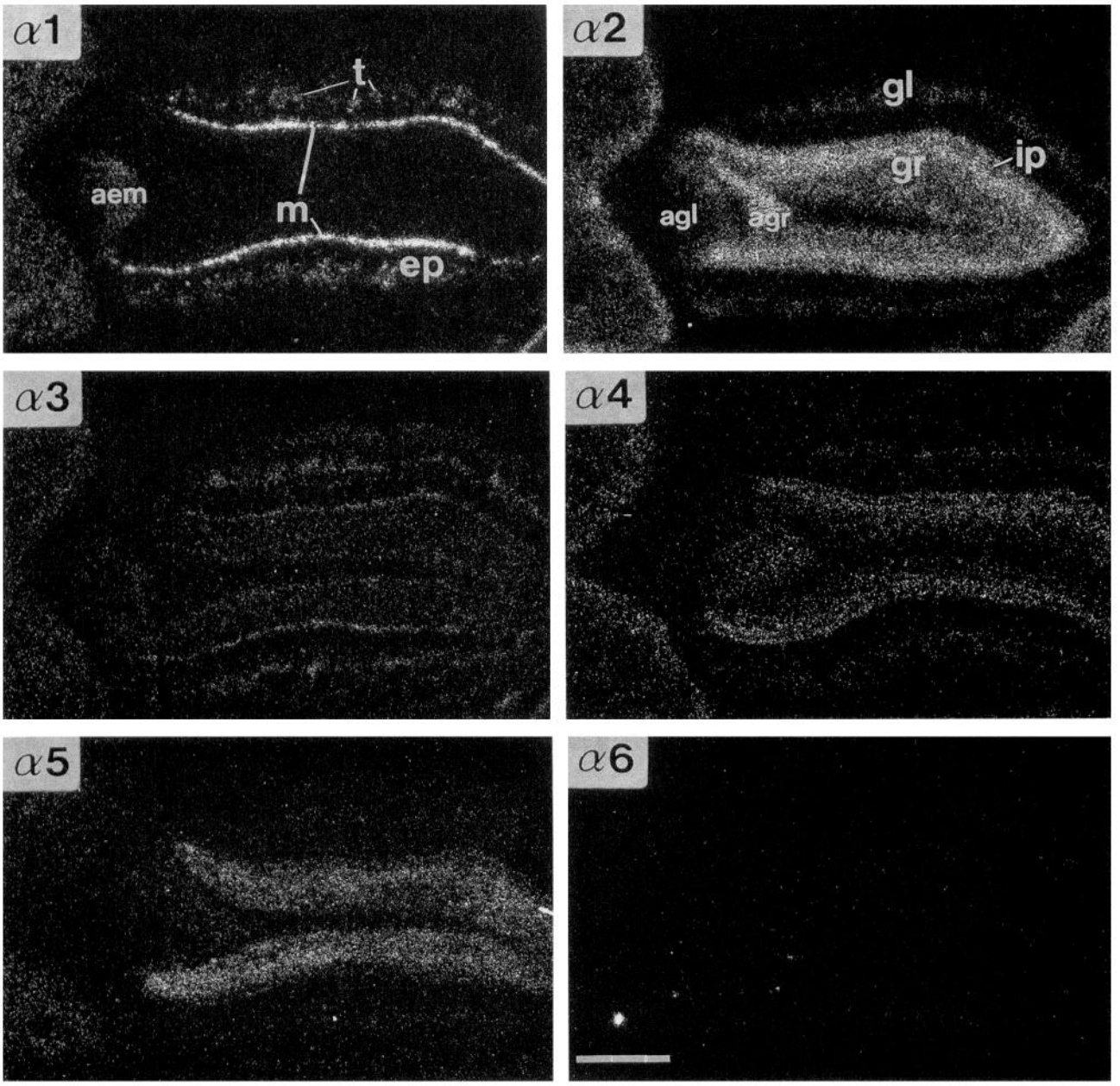

Figure 5. X-ray film autoradiographs illustrating regional distribution of $GABA_A$ receptor α-subunit mRNAs (α_1–α_6) in horizontal sections of rat olfactory bulb. Accessory olfactory bulb: *aem,* external plexiform–mitral cell layer; *agl,* glomerular layer; *agr,* granular cell layer. Main olfactory bulb: *ep,* external plexiform layer; *gl,* glomerular layer; *gr,* granule cell layer; *ip,* internal plexiform layer; *m,* mitral cell layer; *t,* tufted cells. Scale bar, 1 mm.

spatially very heterogeneous, varying between regions and even within regions in a complex manner. In this article the rat cerebellum and olfactory bulb were chosen for detailed study because in both these structures the cell populations are readily identifiable and well characterized. Proposals for naturally occurring combinations of $GABA_A$ receptor subunits can therefore be made more easily. However, there are problems even in these simplified systems if, for example, a defined cell population (e.g., granule cells) contains a large number of subunit mRNAs (see Table 1). Such cells may express $GABA_A$ receptor subtypes on different subcellular domains (e.g., soma vs. dendrites). Immunocytochemical studies at the resolution of the electron microscope (Somogyi et al., 1989), using subunit-specific antibodies will be required to resolve this problem.

$GABA_A$ receptors in the cerebellum

The GABAergic system in the cerebellum has been well documented (Mugnaini and Oertel, 1985; Haefely and Polc, 1986; Richards et al., 1986; Wuenschell et al., 1986; Shepherd, 1988; Ross et al., 1990). All neuronal cell types with the possible exception of the Golgi cells receive an inhibitory GABAergic input. The GABAergic Golgi cells synapse onto granule cells. GABAergic stellate/basket interneurons in the molecular layer synapse onto Purkinje cells. These send reciprocal inhibitory processes back onto the stellate/basket neurons as well as projecting to the deep cerebellar nuclei.

Since recombinant $GABA_A$ receptors are currently most easily distinguishable by their ligand binding profiles (Pritchett et al.,

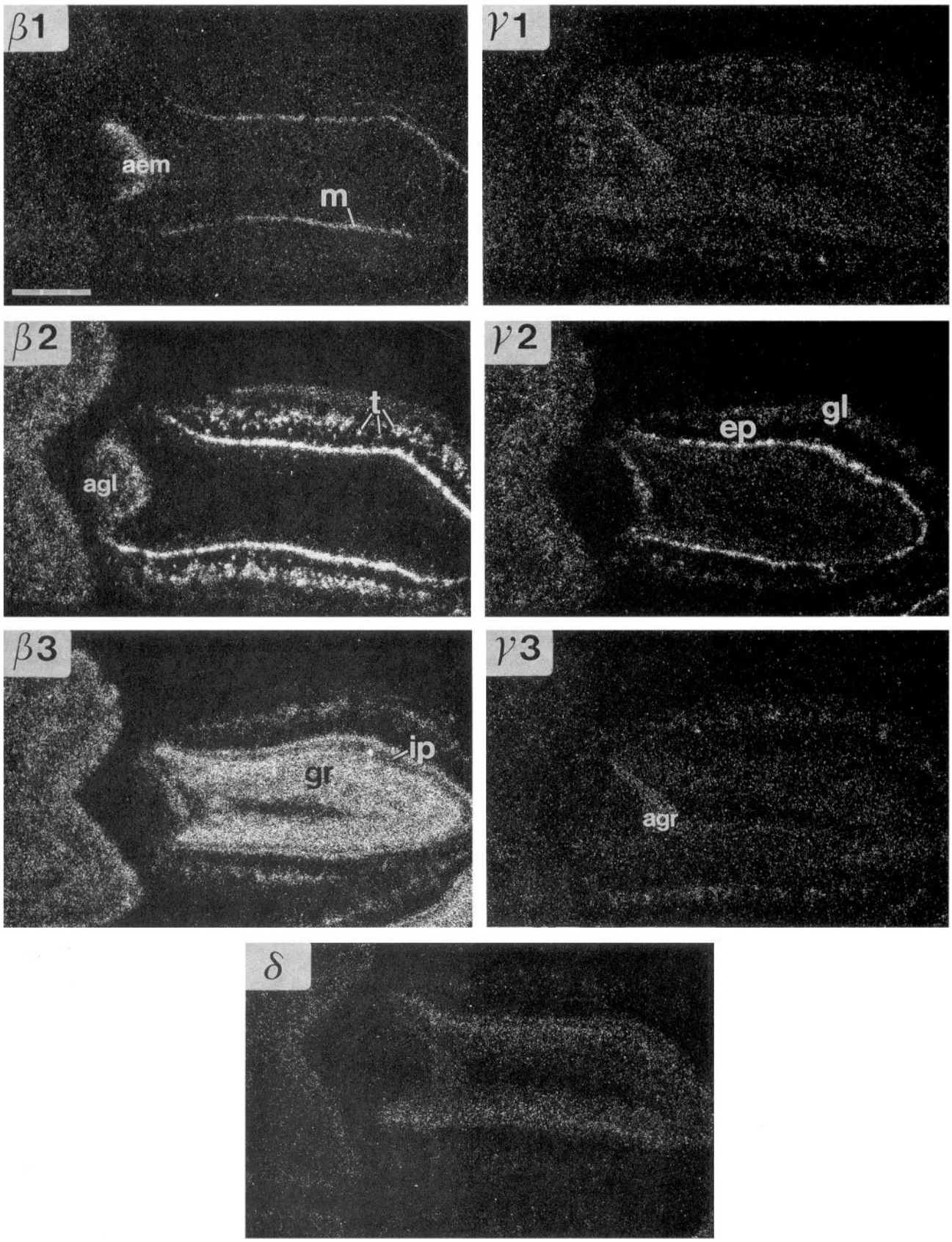

Figure 6. X-ray film autoradiographs illustrating regional distribution of $GABA_A$ receptor β (β_1–β_3), γ (γ_1–γ_3), and δ-subunit mRNAs in horizontal sections of rat olfactory bulb. Abbreviations are as for Figure 5. Scale bar, 1 mm.

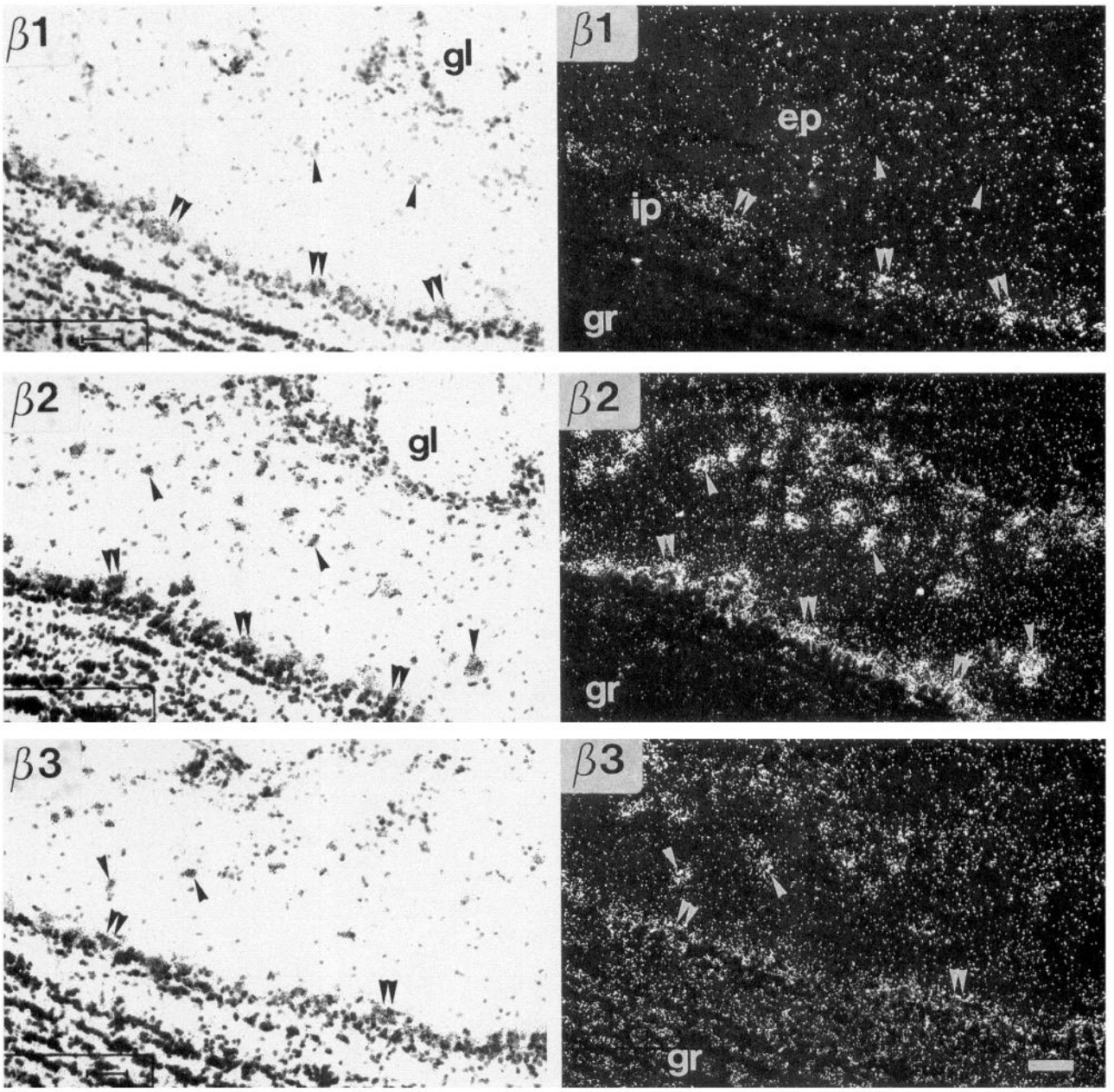

Figure 7. Cellular distribution of $GABA_A$ receptor β (β_1–β_3) mRNAs in rat olfactory bulb. *Left column,* bright-field optics; *right column,* dark-field optics. *Single arrowheads,* tufted cells; *double arrowheads,* mitral cells. Abbreviations are as for Figure 5. Scale bar, 50 μm.

1989a; Lüddens et al., 1990; Pritchett and Seeburg, 1990), it is appropriate to review autoradiographic studies in order to compare these results to the *in situ* hybridization data. For $GABA_A$ receptors in the rodent cerebellum, autoradiography has demonstrated interesting spatial mismatches between the binding patterns of different classes of ligands (reviewed in Olsen et al., 1990). Collectively, these data suggest the existence of $GABA_A$ receptor heterogeneity. Benzodiazepine (BZ) agonists bind predominantly to the molecular layer, whereas $GABA_A$ agonists (GABA, muscimol) bind mainly to sites in the granule cell layer (Palacios et al., 1980, 1981; Young and Kuhar, 1980; Unnerstall et al., 1981; Richards et al., 1986; Bowery et al., 1987; Niddam et al., 1987; Olsen et al., 1990). Nevertheless, even though high-affinity $GABA_A$ agonist sites are relatively scarce in the molecular layer, BZ binding is still enhanced by GABA in this sector, suggesting that these BZ sites are coupled to $GABA_A$ receptors (Unnerstall et al., 1981). $GABA_A$ sites are more prominent in the molecular layer when assessed with ^{3}H-bicuculline methochloride, which highlights low-affinity sites (Olsen et al., 1984). Subsequently, other ligands such as the $GABA_A$ antagonist SR 95531 have been shown to target a markedly reduced number of sites in cerebellum, with approximately equal binding densities in both granule cell and molecular layers (Bristow and Martin, 1988; Olsen et al., 1990). The majority of BZ binding sites in the molecular layer (greater than 90%) have a selectively high affinity for β-carbolines and CL 218, 872 (Young et al., 1981; Niddam et al., 1987; Sieghart and Schlerka, 1991) and have been termed BZ I. Tritiated flunitrazepam photolabels only

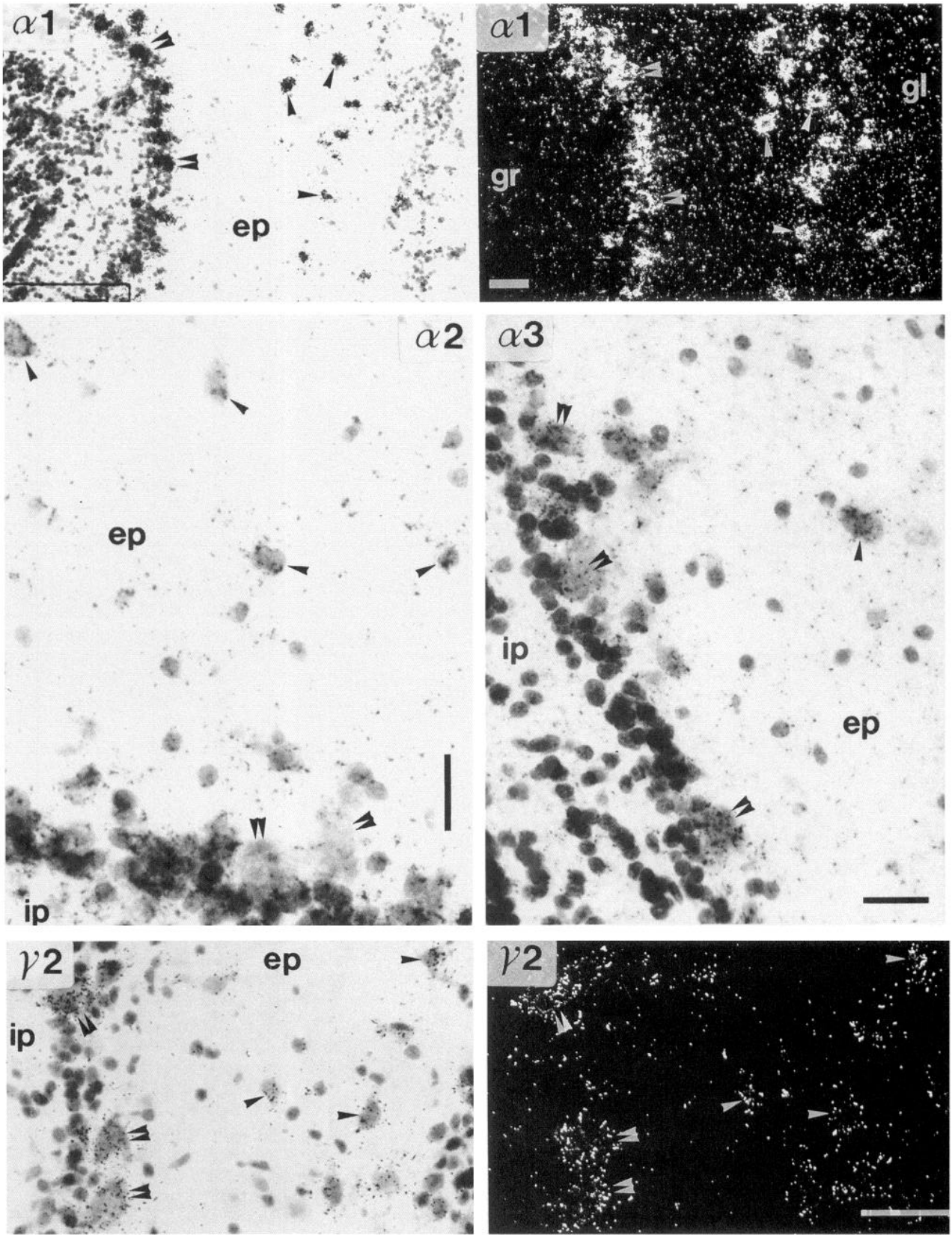

Figure 8. Cellular distribution of $GABA_A$ receptor α (α_1–α_3) and γ_2 mRNAs in rat main olfactory bulb. *Left column,* bright-field optics; *right column,* dark-field optics (except α_3, bright field). *Single arrowheads,* tufted cells; *double arrowheads,* mitral cells. Abbreviations are as for Figure 5. Scale bars: α_1 and γ_2, 50 μm; α_2 and α_3, 30 μm.

one polypeptide in cerebellum (P51), which exhibits a BZ I binding profile (Sieghart, 1989).

Purkinje cell $GABA_A$ receptors. The above binding data on the cerebellar molecular layer would concur with our *in situ* hybridization data, which suggest that Purkinje cells express α_1, β_2, β_3, and γ_2 subunits in a $GABA_A$ receptor complex. Immunocytochemical studies with common epitope β_2/β_3 antibodies (Richards et al., 1986, 1987; de Blas et al., 1988; Somogyi et al., 1989; Ewert et al., 1990, 1991), and a γ_2 antibody (Benke et al., 1991b) suggest that such a receptor would be located mainly on the Purkinje cell dendritic tree, since somatic labeling is weak. The photolabeled cerebellar P51 protein presumably corresponds to the α_1 subunit. Both recombinant $\alpha_1\beta_2\gamma_2$ and $\alpha_1\beta_3\gamma_2$ receptors exhibit indistinguishable BZ I type binding (Pritchett et al., 1989a), consistent with the binding characteristics of the molecular layer. This subunit combination is also supported by immunoprecipitation studies (Benke et al., 1991b). A recombinant receptor with the inclusion of two different β-subunits has not yet been studied. It is possible that there may be two similar $GABA_A$ receptor subtypes ($\alpha_1\beta_2\gamma_2$, $\alpha_1\beta_3\gamma_2$) on the Purkinje cell processes, each displaying identical binding profiles, but differing in channel kinetics or desensitization rate. Careful matching of recombinant receptor electrophysiology to patch clamping on Purkinje cells in slices (Konnerth et al., 1990) will be required to distinguish between two such receptors or an $\alpha_1\beta_2\beta_3\gamma_2$ complex.

Granule cell $GABA_A$ receptors. In the granule cells of rodent cerebellum the most abundant mRNAs are α_1, α_6, β_2, β_3, γ_2, and δ, in agreement with the collective findings of previous studies (Khrestchatisky et al., 1989; Lolait et al., 1989; Shivers et al., 1989; Kato, 1990; Lüddens et al., 1990; Malherbe et al., 1990b; MacLennan et al., 1991). Granule cells also contain large quantities of α_1, β_2/β_3, and δ-subunits as assessed by immunocytochemistry (Richards et al., 1986; 1987; de Blas et al., 1988; Somogyi et al., 1989; Benke et al., 1991c; Zimprich et al., 1991), and moderate amounts of γ_2 immunoreactivity (Benke et al., 1991b). Other granule cell mRNAs that may contribute to a minority of receptors are α_4, β_1, and γ_3. In bovine granule cells, the β_1 mRNA also seems to be present at very low amounts (Siegel, 1988). The presence of six subunit mRNAs suggests the existence of at least two subtypes of pentameric $GABA_A$ receptors on the granule cells. Alternatively, there may be subtypes of granule cells expressing different $GABA_A$ receptors.

A major problem to address is why high-affinity muscimol/GABA binding occurs over the granule cells accompanied by reduced levels of BZ agonist binding. The BZ receptor partial inverse agonist Ro 15-4513 is unique in that it is the only BZ ligand to exhibit higher binding to the granule cell layer than to the molecular layer (Sieghart et al., 1987). This Ro 15-4513 binding site in granule cells was interpreted to be a nonspecific binding site (Sieghart et al., 1987). However, this finding was subsequently rationalized by the demonstration that an $\alpha_6\beta_2\gamma_2$ complex exhibited diazepam-insensitive Ro 15-4513 binding, as well as high-affinity muscimol binding (Lüddens et al., 1990). Such an $\alpha_6\beta_x\gamma_2$ core structure would be compatible with the granule cell mRNA distributions and meets the minimum requirement to reconstitute the *in vivo* pharmacology. The low degree of BZ agonist binding in the granule cell layer suggests that, despite containing large amounts of α_1, β_2, and β_3 transcripts, $\alpha_1\beta_x\gamma_2$ receptor complexes are not extensively constructed.

Role of the δ-subunit. The confinement of both δ mRNA and immunoreactivity to the granule cell layer makes their distribution complementary to that of BZ agonist binding sites (Shivers et al., 1989; Benke et al., 1991c). Thus, these results appear contradictory to the observation that anti-δ-subunit antibodies can precipitate receptors that bind BZs (Benke et al., 1991c), but agree with the failure of the δ-subunit to confer BZ-responsive properties on recombinant receptors (Shivers et al., 1989; H. Lüddens, personal communication). Additionally, immunoprecipitation of δ-containing receptors performed in this laboratory has precipitated receptors with high affinity for muscimol but that do not bind BZs (D. J. Laurie, I. Killisch, and H. Lüddens, unpublished observations). It has been noted previously that the δ-subunit mRNA appears to codistribute with α_1, α_4, and β_2 mRNAs (Wisden et al., 1992). This occurs particularly in the thalamus, which, like the cerebellar granule cells, exhibits elevated muscimol binding relative to BZ agonist binding (Unnerstall et al., 1981; Olsen et al., 1990). The α_4 and α_6 subunits are functionally related in that they seem to be associated with $GABA_A$ receptors that fail to bind BZ agonists (Lüddens et al., 1990; Wisden et al., 1991). Given the colocalization of α_4 and δ in the forebrain, it may be significant that δ mRNA colocalizes with the α_6 subunit mRNA in the cerebellar granule cells. Thus, by analogy with a forebrain mRNA colocalization ($\alpha_1\alpha_4\beta_2\delta$), it is possible that an $\alpha_1\alpha_6\beta_x\delta$ complex exists in granule cells. Such a complex might be predicted to exhibit high-affinity muscimol binding but lack agonist BZ binding.

Stellate/basket cell receptors. Using emulsion autoradiography, the only mRNAs readily detectable in the stellate/basket cells are α_1 and γ_2. There is also a weak signal obtained with the β_2 probe. Immunocytochemistry detects a moderate α_3 signal in the molecular layer (Zimprich et al., 1991), although this was not resolved at the cellular level. There clearly is a faint signal with the α_3 probe over the molecular layer (but not the granule cell layer), but the cellular origin of this was unresolvable.

Bergmann glia receptors. Earlier observations noted the presence of α_2 mRNA in putative Bergmann glia of bovine cerebellum (Wisden et al., 1989a). In rodent cerebellum, the α_2 mRNA is similarly expressed in the Purkinje cell layer as assessed by the x-ray film autoradiographic image (Fig. 1), but at apparently lower levels than in the cow. Examination of emulsions revealed that Purkinje cells clearly to be unlabelled by the rat α_2 oligonucleotide probe, suggesting that the origin of the signal observed on x-ray film was likely to result from Bergmann glia. Consistent with our results, a slight increase in labeling over cells at the border of the Purkinje cell and granule cell layers has also been observed in rat using an α_2 cRNA probe (MacLennan et al., 1991). In contrast, the γ_1 mRNA is abundant in these probable glial cells. However, no other $GABA_A$ receptor subunit mRNAs could be detected in these putative glia. Consistent with this, the common epitope β_2/β_3 antibody bd-17 fails to label Bergmann glia (Somogyi et al., 1989). However, a monoclonal antibody (E9) raised against affinity-purified $GABA_A$ receptors, but of unconfirmed specificity, does detect occasional Bergmann glial cells in immunocytochemical studies of cerebellum (Meinecke et al., 1989). If a β-subunit is needed for this $\alpha_2\gamma_1$ complex, it is possible that an as yet unidentified rat homolog of the avian β_4 subunit (Bateson et al., 1991) contributes to this glial receptor. Although Bergmann glial cultures have not been examined electrophysiologically for GABA receptors, other types of astrocytes in culture express GABA-gated chloride channels (Kettenmann et al., 1987; Bormann and Kettenmann, 1988). Interestingly, recombinant $\alpha_2\beta_x\gamma_1$ receptors mimic the

properties of native astrocytic $GABA_A$ receptors (Puia et al., 1991). It has been proposed that these glial $GABA_A$ receptors play a role in maintaining the local extracellular chloride concentrations in GABAergic synaptic clefts (Bormann and Kettenmann, 1988).

Bergmann glial cells also express a number of other ligand-gated channels or related proteins. For example, in avian brain they contain mRNA for the α_4 subunit of the neuronal nicotinic receptor (Morris et al., 1990) and a putative kainate binding protein (Somogyi et al., 1990; Ortega et al., 1991). In the rat, Bergmann glial cells contain the GluR-A Flip and GluR-D Flip mRNA forms of the AMPA/kainate receptor (Monyer et al., 1991). Thus, these glial cells are clearly involved in a range of cell–cell signaling processes.

$GABA_A$ receptors in the olfactory bulb

The structural organization of the olfactory bulb is superficially similar to that of the cerebellum (Switzer et al., 1985; Shepherd, 1988). There is a cell-dense granule cell layer, above which is a single layer of large mitral cells whose dendrites extend into and through a cell-sparse region, the external plexiform layer. The external plexiform layer contains tufted cells that are morphologically similar to mitral cells. Above the external plexiform layer are the spherical cell-free glomeruli, around which lie small interneurons.

As in the cerebellum, GABAergic inhibition plays an important role in the rodent olfactory bulb (Nicoll, 1971; Jaffe and Cuello, 1981; Halasz and Shepherd, 1983; Mugnaini and Oertel, 1985; Haefely and Polc, 1986; Richards et al., 1986). Around the glomeruli the primary dendrites of mitral and tufted cells are innervated by both excitatory olfactory neurons and by inhibitory GABAergic periglomerular cells. The secondary dendrites of these mitral and tufted neurons form reciprocal dendrodendritic synapses in the external plexiform layer with peripheral dendrites of the GABAergic internal granule cells. The internal granule and periglomerular cells in turn receive inhibitory input from glutamic acid decarboxylase–containing short-axon neurons. Thus, almost every type of olfactory bulb neuron receives some GABAergic input.

Autoradiographic studies examining the distribution of total $GABA_A$ and BZ binding sites in the rat olfactory bulb reveal a very high density of both these binding sites in the external plexiform layer (Young and Kuhar, 1980; Palacios et al., 1981; Marcel et al., 1986; Bowery et al., 1987; Niddam et al., 1987). A moderate density of both $GABA_A$ and BZ sites also occurs in the glomerular layer. However, the granule cell layer, although containing a marked density of $GABA_A$ sites labeled by [^{3}H]GABA (Bowery et al., 1987), exhibits only a low density of BZ sites and contains few high-affinity ^{3}H-muscimol binding sites (Palacios et al., 1981).

Mitral/tufted cell $GABA_A$ receptors. Our results confirm and extend previous *in situ* hybridization studies (Shivers et al., 1989; Wisden et al., 1989b; Malherbe et al., 1990a; Seeburg et al., 1990; Zhang et al., 1990; MacLennan et al., 1991). Mitral cells express large amounts of α_1, β_1, β_2, β_3, and γ_2 mRNAs and small amounts of α_3 mRNA. Tufted cells, which appear to be morphologically and functionally related to mitral cells, exhibit a very similar pattern of α-subunit mRNA expression except that some also express small amounts of α_2 mRNA. This indicates that there may be a small subpopulation of tufted cells expressing $GABA_A$ receptors of a different composition. The presence of β_2/β_3 and γ_2 subunits in the external plexiform layer and glomerular layer, presumably on dendrites of mitral and tufted cells, has been confirmed by immunohistochemistry (Richards et al., 1986, 1987; Benke et al., 1991b).

The presence in mitral cells of α_1, α_3, multiple β, and γ_2 mRNAs is reminiscent of the situation in Purkinje cells ($\alpha_1\beta_2\beta_3\gamma_2$). Clearly, there must be more than one pentameric $GABA_A$ receptor subtype present on mitral cells. A large number of combinations are possible. For example, $\alpha_1\beta_2\gamma_2$, $\alpha_1\beta_2\beta_3\gamma_2$, $\alpha_1\beta_1\beta_2\beta_3\gamma_2$, $\alpha_3\beta_1\gamma_2$, and $\alpha_1\alpha_3\beta_x\gamma_2$ could all be plausible combinations. At present, there are contradictory results as to whether two different α-subunits do (Lüddens et al., 1991) or do not (Duggan and Stephenson, 1990; Benke et al., 1991a) coexist in the same complex.

Consistent with the subunit mRNA distributions in the rest of the CNS (Wisden et al., 1992) it seems likely that at least one of the mitral cell receptors will consist of an $\alpha_1\beta_2\gamma_2$ core. This suggests that the majority of $GABA_A$/BZ receptors in the rat olfactory bulb are type I (i.e., high affinities for CL 218,872 and zolpidem; see Pritchett et al., 1989a). Photolabeling of olfactory bulb $GABA_A$ receptors with ^{3}H-flunitrazepam principally labels a protein of MW 51,000, consistent with that of the α_1 subunit, and to a much lesser extent, proteins of MW 53,000 and 59,000, consistent with those of the α_2 and α_3 subunits (Sieghart and Karobath, 1980; Sieghart and Drexler, 1983). In a homogenate binding study (Sieghart and Schlerka, 1991), the IC_{50} values of CL 218,872 and zolpidem for displacement of ^{3}H-flunitrazepam in rat olfactory bulb suggest a mixture of types I and II BZ receptors, with a predominance of the former. The source of low amounts of BZ II binding is probably the limited expression of α_2 and α_3 transcripts in tufted, periglomerular, and mitral cells, since recombinant $GABA_A$ receptors of composition $\alpha_2\beta_x\gamma_2$ and $\alpha_3\beta_x\gamma_2$ exhibit type II BZ binding (Pritchett et al., 1989a).

Olfactory bulb granule cell $GABA_A$ receptors. The most prominent mRNAs in the granule cell layer are α_2, α_4, α_5, β_3, and δ. Other transcripts present in rather low amounts are α_3, γ_1, γ_2, and γ_3. The subunit genes for α_1, α_6, β_1, and β_2 seem to be completely silent. As for the granule cell layer of the cerebellum, this cocktail of mRNA combinations complicates the assignment of subunits to receptors. However, two observations can be made: (1) the regional colocalization of α_2 and β_3 mRNAs and (2) the colocalization of α_4 and δ mRNAs. These two pairs of colocalizations seem to be a general rule and can be extended to many other regions of the CNS [Wisden et al., 1992 (accompanying paper)]. Nevertheless, it is not readily apparent what composition the receptors on granule cells are likely to have. It seems that the majority of granule cell $GABA_A$ receptors should not be subject to modulation by BZs for the following reasons. Based on mRNA levels, the three known γ-subunits seem to be minor participants in $GABA_A$ receptors in the rat olfactory bulb granule cells. Although recombinant receptors consisting only of α- and β-subunits are activated by GABA (Schofield et al., 1987; Sigel et al., 1990; Verdoorn et al., 1990), coexpression of a γ-subunit is necessary for robust BZ sensitivity (Pritchett et al., 1989b; Ymer et al., 1990; Herb et al., 1992). The borderline level of γ_2 mRNA in the granule cells, the paucity of detectable immune reaction for γ_2 (Benke et al., 1991b), and the very reduced levels of high-affinity BZ binding over this area (Young and Kuhar, 1980; Marcel et al., 1986; Niddam et al., 1987), together suggest that internal granule cells generally do not construct γ_2-containing $GABA_A$ receptors. Any contribution of γ_1 and γ_3 subunits to granule cell receptors would have gone undetected by previous autoradiographic studies, since replacement of these two subunits for γ_2 lowers the affinity of $\alpha\beta\gamma$

complexes for BZs by up to two orders of magnitude (Ymer et al., 1990; Herb et al., 1992).

Given the plethora of $GABA_A$ receptor subunit transcripts in the granule cells, it is somewhat surprising to note a very low density of ^{3}H-muscimol binding sites (Palacios et al., 1981; Richards et al., 1986) despite dense ^{3}H-$GABA_A$ binding over this sector (Bowery et al., 1987). This may indicate that granule cell receptor subunits form $GABA_A$ sites with unusual ligand affinities.

Conclusions

In summary, the cerebellum and olfactory bulb contain complex populations of $GABA_A$ receptors. For example, cerebellar granule cells probably contain at least two types of receptor, possibly $\alpha_6\beta_x\gamma_2$ and $\alpha_1\alpha_6\beta_x\delta$. These two combinations are the most economical way to explain the ligand binding data obtained for this region. Correlations that appear to be general ones from other brain regions (see Wisden et al., 1992) are the colocalizations of $\alpha_1\beta_2$, $\alpha_2\beta_3$, and $\alpha_4\delta$ mRNAs. The δ-subunit mRNA codistributes with α-subunit mRNAs (α_4 and α_6) associated with $GABA_A$ subunits that fail to bind BZ agonists. In both the olfactory bulb and cerebellum, $\alpha_1\beta_2\gamma_2$ receptor cores (BZ I type pharmacology) are probably used to construct prominent receptor isoforms.

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