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The Distribution of 13 $GABA_A$ Receptor Subunit mRNAs in the Rat Brain. I. Telencephalon, Diencephalon, Mesencephalon

W. Wisden, D. J. Laurie, H. Monyer, and P. H. Seeburg

Laboratory of Molecular Neuroendocrinology, Zentrum für Molekulare Biologie, University of Heidelberg, D-6900 Heidelberg, Germany

The expression patterns of 13 $GABA_A$ receptor subunit encoding genes (α_1–α_6, β_1–β_3, γ_1–γ_3, δ) were determined in adult rat brain by *in situ* hybridization. Each mRNA displayed a unique distribution, ranging from ubiquitous (α_1 mRNA) to narrowly confined (α_6 mRNA was present only in cerebellar granule cells). Some neuronal populations coexpressed large numbers of subunit mRNAs, whereas in others only a few $GABA_A$ receptor–specific mRNAs were found. Neocortex, hippocampus, and caudate-putamen displayed complex expression patterns, and these areas probably contain a large diversity of $GABA_A$ receptors. In many areas, a consistent coexpression was observed for α_1 and β_2 mRNAs, which often colocalized with γ_2 mRNA. The $\alpha_1\beta_2$ combination was abundant in olfactory bulb, globus pallidus, inferior colliculus, substantia nigra pars reticulata, globus pallidus, zona incerta, subthalamic nucleus, medial septum, and cerebellum. Colocalization was also apparent for the α_2 and β_3 mRNAs, and these predominated in areas such as amygdala and hypothalamus. The α_3 mRNA occurred in layers V and VI of neocortex and in the reticular thalamic nucleus. In much of the forebrain, with the exception of hippocampal pyramidal cells, the α_4 and δ transcripts appeared to codistribute. In thalamic nuclei, the only abundant $GABA_A$ receptor mRNAs were those of α_1, α_4, β_2, and δ. In the medial geniculate thalamic nucleus, α_1, α_4, β_2, δ, and γ_3 mRNAs were the principal $GABA_A$ receptor transcripts. The α_5 and β_1 mRNAs generally colocalized and may encode predominantly hippocampal forms of the $GABA_A$ receptor. These anatomical observations support the hypothesis that $\alpha_1\beta_2\gamma_2$ receptors are responsible for benzodiazepine I (BZ I) binding, whereas receptors containing α_2, α_3, and α_5 contribute to subtypes of the BZ II site. Based on significant mismatches between α_4/δ and γ mRNAs, we suggest that *in vivo*, the α_4 subunit contributes to $GABA_A$ receptors that lack BZ modulation.

GABA is the principal inhibitory transmitter in vertebrate brain. GABA produces its inhibitory effect by interacting with two classes of molecules on the target cell: (1) $GABA_A$ receptors, which are ligand-gated anion channels that exhibit a diverse and clinically important pharmacology, being the locus of action for barbiturates, benzodiazepines (BZs), and steroids, which all act alosterically to modify the efficacy of GABA (Haefely and Polc, 1986; Lambert et al., 1987; Puia et al., 1990); ethanol also appears to mediate some of its effects through this receptor (Wafford et al., 1990, 1991); and (2) $GABA_B$ receptors, which are coupled to G-protein–mediated cellular responses (Bowery, 1989). These two GABA receptor classes act on different time scales, often in the same synapse (Dutar and Nicoll, 1988).

Our knowledge regarding the molecular composition of the $GABA_A$ receptor has increased considerably over recent years. Once thought to be a single molecular species (Häring et al., 1985), this receptor now presents a staggering molecular diversity revealed by cDNA cloning of $GABA_A$ receptor subunits. In keeping with other members of the ligand-gated ion channel superfamily (Unwin, 1989; Cooper et al., 1991), the $GABA_A$ receptor is probably assembled as a pentameric structure from a number of possible subunit classes. The subunit stoichiometry in any given $GABA_A$ receptor complex is unknown. In the rodent there are currently six α-subunits (α_1–α_6), three β-subunits (β_1–β_3), three γ-subunits (γ_1–γ_3), and a δ-subunit (Olsen and Tobin, 1990; Seeburg et al., 1990; Herb et al., 1992; Lüddens and Wisden, 1991; Wilson-Shaw et al., 1991). Additionally a ρ-subunit cDNA has recently been isolated from human retina (Cutting et al., 1991). Molecular diversity rather than uniformity probably accounts for the pharmacological heterogeneity of $GABA_A$ receptors seen by numerous laboratories (Unnerstall et al., 1981; Young et al., 1981; Sieghart, 1989; Olsen et al., 1990).

Different subunit combinations confer disparate pharmacologies on $GABA_A$ receptors expressed from combinations of cDNAs. For example, the γ-subunit class is required to confer a generally robust BZ responsiveness on any α/β subunit combination (Pritchett et al., 1989b), such that a minimum requirement for conventional $GABA_A$ receptor pharmacology would be an $\alpha_x\beta_x\gamma_x$ combination (where x is any variant). However, studies on $\alpha_x\beta_x\gamma_2$ receptors reveal that it is the members of the α-subunit class that dictate which type of BZ ligand binds to, and allosterically modulates, the receptor complex (Pritchett et al., 1989a,b; Lüddens et al., 1990; Pritchett and Seeburg, 1990; Seeburg et al., 1990; Lüddens and Wisden, 1991). The α_1-containing complexes exhibit high affinity for CL 218-872 and β-carbolines, whereas the complexes containing α_2, α_3, and α_5 show lower affinity for these compounds (Pritchett et al., 1989a; Pritchett and Seeburg, 1990). However, all display high affinity for the BZ antagonist Ro 15-1788 and, particularly, for the

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Correspondence should be addressed to W. Wisden, Laboratory of Molecular Neuroendocrinology, Zentrum für Molekulare Biologie, University of Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg, Germany.

"alcohol antagonist" Ro 15-4513. The α_4 and α_6 subunits in combination with a $\beta_2\gamma_2$ subunit pair bind Ro 15-4513 at a site insensitive to diazepam (Lüddens et al., 1990; Wisden et al., 1991b), and hence $\alpha_4\beta\gamma_2$ and $\alpha_6\beta\gamma_2$ receptors do not bind BZ agonists. The β-subunits have been reported to modulate current amplitudes (Sigel et al., 1990) and appear to differ in binding of GABA analogs and pentobarbital (Bureau and Olsen, 1990). The role of the δ-subunit remains unclear, but this subunit forms homomeric channel complexes gated by GABA (Shivers et al., 1989). The ρ-subunit appears to be quantitatively unimportant in rodent CNS, with its mRNA restricted to retina (Cutting et al., 1991).

Clearly, in order to proceed with electrophysiological and pharmacological analyses, it is important to know which subunit combinations occur *in vivo.* Two approaches are available: mapping the site of protein expression using subunit-specific antibodies (immunocytochemistry), or tracing sites of gene expression using *in situ* hybridization. The immunocytochemical approach, although providing useful information about the cellular distribution of $GABA_A$ receptors, is severely hindered by the difficulty of raising unique antibodies. Previously used antibodies, for example, monoclonal antibody (mAb) bd-17 and mAb 62-3G1 (Richards et al., 1986, 1987; de Blas et al., 1988), react with both β_2 and β_3 subunits (Fuchs et al., 1988; Ewert et al., 1990, 1991). However, one recent report using synthetic peptide-derived antibodies has demonstrated differential localization of immunoreactivity specific for α_1, α_2, and α_3 subunits in rat brain (Zimprich et al., 1991). The γ_2 and δ-subunits have also been detected immunohistochemically using peptide-specific antisera (Benke et al., 1991a,b).

The sites of gene expression of some of the subunits have been partially mapped by *in situ* hybridization. In rodent brain these are the mRNAs for α_1 (Séquier et al., 1988; Khrestchatisky et al., 1989; Lolait et al., 1989; Hironaka et al., 1990; Malherbe et al., 1990b; Seeburg et al., 1990; MacLennan et al., 1991), α_2 (Seeburg et al., 1990; MacLennan et al., 1991; Persohn et al., 1991; Wisden et al., 1991a), α_3 (Seeburg et al., 1990; Persohn et al., 1991; Wisden et al., 1991a), α_4 (Wisden et al., 1991b), α_5 (Khrestchatisky et al., 1989; MacLennan et al., 1991, termed α_4 by these authors), α_6 (Kato, 1990; Lüddens et al., 1990), β_1 (Séquier et al., 1988; Malherbe et al., 1990b; Seeburg et al., 1990; Zhang et al., 1990), β_2 and β_3 (Lolait et al., 1989; Seeburg et al., 1990; Zhang et al., 1990), γ_1 (Ymer et al., 1990), γ_2 (Shivers et al., 1989; Malherbe et al., 1990b; Persohn et al., 1991; Wisden et al., 1991a), and δ (Shivers et al., 1989). In bovine brain, the α_1, α_2, and α_3 mRNAs (Wisden et al., 1988, 1989a,b) and β_1 mRNAs (Siegel, 1988), and in chicken brain the α_1 mRNA (Bateson et al., 1991a), have also been studied.

However, no systematic comparison is currently possible. First, studies have selectively focused on different brain regions and/or different species, and second, the mRNA abundance has been assessed by oligonucleotides, cRNA, or DNA restriction fragment probes. The combined results make a systematic comparison between data difficult. In addition, studies employing extended cRNA or DNA probes may produce misleading results, since such probes may cross-hybridize to closely related gene family members.

In this and the accompanying article (Laurie et al., 1992), we have undertaken a systematic comparison of the brain distribution of the 13 currently known rat $GABA_A$ receptor transcripts by *in situ* hybridization using unique oligonucleotide probes specific for each subunit mRNA.

Materials and Methods

For detection of $GABA_A$ receptor subunit transcripts, 45-base antisense oligonucleotides were synthesized, each of a unique sequence often taken from the region encoding the divergent intracellular area between putative transmembrane domains M3 and M4. The oligonucleotides were constructed complementary to rat cDNA encoding subunit residues as follows: α_1, 342–356 (Khrestchatisky et al., 1989); α_2, 340–344 (Khrestchatisky et al., 1991); α_3, 361–375 (Malherbe et al., 1990a); α_4, 15–30 of the signal peptide (Ymer et al., 1989a; Wisden et al., 1991b); α_5, 355–369 (Khrestchatisky et al., 1989; Malherbe et al., 1990a; the subunit termed α_5 by us is termed α_4 by Khrestchatisky et al., 1989); α_6, 342–356 (Lüddens et al., 1990); β_1, 382–396 (Ymer et al., 1989b); β_2, 382–396 (Ymer et al., 1989b); β_3, 380–394 (Ymer et al., 1989b); γ_1, 341–354 (Ymer et al., 1990); γ_2, 338–352 (Shivers et al., 1989); γ_3, 343–358 (Herb et al., 1992; see also Wilson-Shaw et al., 1991, for the mouse sequence); δ, 335–349 (Shivers et al., 1989).

The procedures used (Monyer et al., 1991; Wisden et al., 1991c) were a modification of those by Young et al. (1986). Probes were 3′ end labeled using a 30:1 molar ratio of α-^{35}S-dATP (1200 Ci/mmol; Amersham) to oligonucleotide, and terminal deoxynucleotidyl transferase (Boehringer Mannheim). Unincorporated nucleotides were removed by Bio-Span 6 chromatography columns (Bio-Rad). Nonperfused brains were removed and frozen on dry ice. Sections (14 μm) were cut on a cryostat, mounted onto poly-L-lysine-coated slides, and dried at room temperature. Sections were fixed in 4% paraformaldehyde, washed in phosphate-buffered saline, and dehydrated into 95% ethanol for storage at 4°C until required. Prior to hybridization, sections were removed from ethanol and allowed to air dry. Labeled probe dissolved in hybridization buffer (0.06 fmol, 1000 dpm/μl) was then applied to sections. Hybridization buffer contained 50% formamide/4× SSC (1× SSC:0.15 M NaCl, 0.015 M Na-citrate)/10% dextran sulfate. Hybridization was at 42°C overnight. Sections were washed to a final stringency of 1× SSC at 60°C, before alcohol dehydration and exposure to Kodak XAR5 film. Anatomy of sections and autoradiographs was determined using the atlas of Paxinos and Watson (1986), and for thalamus the monograph of Jones (1985) was consulted. Signal specificity was assessed by use of competition experiments in which radiolabeled probes were hybridized to sections in the presence of an excess (50-fold) unlabeled probe. This resulted in blank autoradiographs. Specificity was also confirmed by reference to previous reports of the distribution of $GABA_A$ transcripts in rat performed by other laboratories or other methods (e.g., Northern blot analysis): the distribution of α_1 (Northern blot and *in situ* hybridization-cRNA probes, Khrestchatisky et al., 1989), α_2 (Northern blot, Khrestchatisky et al., 1991; *in situ* hybridization-cRNA probe, MacLennan et al., 1991), α_5 (Northern blot and *in situ* hybridization-cRNA probes, Khrestchatisky et al., 1989; MacLennan et al., 1991), α_6 (Northern blot and *in situ* hybridization-cDNA probe, Kato, 1990), β_1 (Northern blot, Garrett et al., 1990), β_2 and β_3 (Northern blot and *in situ* hybridization-oligonucleotides, Lolait et al., 1989; Ymer et al., 1989b; Zhang et al., 1990), γ_2 (*in situ* hybridization-cRNA probes, Shivers et al., 1989; Malherbe et al., 1990b), and γ (*in situ* hybridization-cDNA probe, Shivers et al., 1989). Our results were in general agreement with these studies.

Results

Within the predicted cytoplasmic loop between transmembrane domains M3 and M4, all $GABA_A$ receptor subunits carry divergent amino acid sequences (Seeburg et al., 1990). Therefore, this segment is ideal for designing subunit-specific nucleic acid probes that will not cross-hybridize to transcripts of related genes. However, several subunit genes (γ_2 and avian β_4) have been recently shown to be differentially spliced in this region (Whiting et al., 1990; Bateson et al., 1991b; Kofuji et al., 1991; Wafford et al., 1991). These new findings mean that we have to be cautious in evaluating whether our probes would be truly selective (or nonselective) for any, as yet undiscovered, splice products. For example, our γ_2 probe detects both known versions of the γ_2 mRNA.

In situ hybridization was performed with subunit mRNA-specific ^{35}S-labeled oligonucleotide probes on various horizontal and coronal sections through the rat brain in order to cover a

Table 1. Distribution of α_1–α_6, β_1–β_3, γ_1–γ_3, and δ mRNAs of $GABA_A$ receptors in the CNS

	α1	α2	α3	α4	α5	α6	β1	β2	β3	γ1	γ2	γ3	δ
Olfactory bulb													
Periglomerular	0	+	(+)	(+)	0	0	0	++	+	(+)	+	(+)	(+)
Tufted cells	++	(+)	+	0	0	0	0	+++	++	0	++	0	0
Mitral cells	+++	0	+	0	0	0	++	+++	+++	0	+++	0	0
Granule cells	0	+++	(+)	++	++	0	0	0	+++	(+)	(+)	(+)	+
Neocortex													
layer II/III	++	++	+	++	(+)	0	(+)	++	++	(+)	++	+	+
layer IV	+	+	+	+	(+)	0	(+)	+	+	(+)	+	+	(+)
layer V/VI	++	+	++	+	+	0	+	++	++	(+)	++	+	(+)
Pyriform cortex	+++	+++	++	++	0	0	+	+++	+++	+	+++	+	+
Hippocampus													
CA1 str. pyramidalis	++	+++	(+)	++	+++	0	+++	+	+++	+	+++	(+)	(+)
CA3 str. pyramidalis	+	+++	(+)	++	+++	0	+++	+	+++	+	+++	(+)	(+)
DG granule cells	++	+++	+	+++	+	0	+++	++	+++	+	+++	(+)	++
Tenia tecta	+	+++	0	+++	++	0	+++	0	+++	0	0	0	0
Basal nuclei													
Caudate -putamen	(+)	++	(+)	++	0	0	(+)	(+)	++	(+)	+	+	+
Nucleus accumbens	(+)	++	(+)	++	0	0	(+)	(+)	++	(+)	+	+	+
Globus pallidus	+++	+	(+)	0	0	0	(+)	+++	(+)	++	+	0	0
Endopeduncular n.	+++	(+)	0	0	0	0	0	+++	0	0	+	0	0
Claustrum	++	++	+++	+	+	0	++	++	++	(+)	+	++	(+)
Subthalamic nucleus	++	0	0	0	0	0	0	++	(+)	0	++	(+)	0
Amygdala													
central amygdaloid n.	(+)	++	(+)	(+)	0	0	+	+	+	++	+	(+)	0
med. amygdaloid n.	(+)	+++	+	+	0	0	+	++	++	+++	+	0	0
lateral amygdaloid n.	++	+++	+	+	(+)	0	+	+	++	(+)	++	(+)	0
Septum													
bed nucleus s. t.	+	+++	++	+	(+)	0	++	+	++	+++	+	(+)	0
lateral septum	+	+++	++	+	0	0	+	(+)	+	++	+	(+)	0
medial septum	+++	0	(+)	0	0	0	(+)	+++	0	0	++	0	0
diagonal band	+++	+	+	(+)	0	0	(+)	+++	+	(+)	++	+	0
Medial Habenula	(+)	++	(+)	0	0	0	(+)	(+)	++	+	++	(+)	0
Thalamus													
Medio dorsal	++	0	0	+++	0	0	0	+++	0	(+)	(+)	(+)	+
Paraventricular n.	+	++	+	+++	(+)	0	(+)	+++	(+)	(+)	++	(+)	0
Rhomboid nucleus	++	++	++	+++	0	0	+	+++	(+)	+	+	(+)	0
Dorsolat. geniculate	++	0	0	+++	0	0	0	+++	0	(+)	(+)	(+)	+
Ventrolat. geniculate	++	0	0	+++	0	0	0	+++	0	(+)	(+)	(+)	++
Medial geniculate	++	0	0	+++	0	0	0	+++	0	0	+	++	++
Parafascicular n.	+++	+	(+)	+++	0	0	(+)	+++	(+)	(+)	+	(+)	0
Reticular nucl.	+	(+)	+	(+)	0	0	0	(+)	(+)	(+)	+	(+)	0
Ventr. posterior n.	+	0	0	+++	0	0	0	+++	0	0	(+)	(+)	++
Zona incerta	++	0	(+)	0	0	0	0	+++	0	0	+	(+)	0
Hypothalamus													
Medial preoptic area	+	+++	+	(+)	+	0	+	(+)	++	+++	+	(+)	0
Arcuate nucl.	(+)	++	0	0	+	0	0	0	++	+	+	0	0
Dorsomedial nucl.	(+)	+	(+)	(+)	0	0	0	0	+	(+)	+	0	0
Ventromedial nucl.	(+)	++	+	0	+	0	+	0	++	+	++	0	0
Midbrain													
Red nucleus	+++	(+)	0	0	0	0	0	++	(+)	(+)	++	(+)	0
Inferior colliculi													
Central nucleus	+++	+	(+)	0	0	0	0	++	0	(+)	++	(+)	0
Substantia nigra													
Pars reticulata	+++	(+)	(+)	0	0	0	0	++	(+)	+	+	(+)	0
Pars compacta	(+)	0	+	+	0	0	(+)	(+)	+	(+)	+	(+)	0
Cerebellum													
Stellate/Basket cells	+++	0	(+)	0	0	0	0	(+)	0	0	++	0	0
Purkinje	+++	0	0	0	0	0	0	+++	+++	0	+++	0	0
Bergmann glia	0	+	0	0	0	0	0	0	0	++	0	0	0
Granule cells	+++	0	0	(+)	0	+++	(+)	+++	+++	0	++	(+)	+++

In situ hybridization signals obtained with ^{35}S-labeled oligonucleotide probes on serial sections were assessed as intense, +++; strongly positive, ++; positive, +; weakly positive, (+); or not detectable, 0.

broad range of structures. The results compiled from the figures and also unpublished data are summarized in Table 1. Results for the olfactory bulb and cerebellum are described and discussed in detail in the accompanying article (Laurie et al., 1992).

Telencephalon (cortex, hippocampal formation, septum, striatum)

Neocortex

In the neocortex and hippocampus, 12 of the 13 subunits are present (Figs. 1–14). The α_6 mRNA is restricted completely to the cerebellum (Fig. 1), and its expression will not be further described here (see accompanying article, Laurie et al., 1992). Considering first the α-subunit class, α_1 mRNA is present in cortex in a laminated pattern, with layers II/III and V/VI expressing higher levels than layer IV (Figs. 1, 5, 11). In contrast, the α_2 mRNA is most predominant in layer II, although it is present in deeper layers (Figs. 1, 3, 5, 7, 9). The α_3 mRNA occurs in a gradient reciprocal to that of α_2, with layer VI expressing most of this transcript (Figs. 1, 5, 7, 9). The α_4 mRNA appears to be highest in layers II and III, although significant levels are present in deeper layers (Figs. 1, 3, 7, 9). The α_5 mRNA is rare in cortex, but the expression pattern appears weakly delineated in layer VI (Figs. 1, 3, 5, 7, 9).

For the β-subunit mRNAs in cortex (Figs. 2, 4, 6, 8, 10), that of β_1 resembles α_5 mRNA in that it is present at overall uniformly low levels, but layer VI has slightly higher levels. The β_2 and β_3 transcripts are present in similar amounts in the same pattern of lamination, with layers II/III, V, and VI having higher levels than layer IV (Figs. 2, 4, 6, 8, 10).

All three γ-subunit genes are expressed in cortex (Figs. 2, 4, 6, 8, 10), although only the pattern of γ_2 expression appears strongly laminated, similar to that of the α_1, β_2, and β_3 mRNAs. The γ_1 mRNA is present at uniformly low levels throughout all layers of the cortex. Interestingly, parts of the corpus callosum appear to contain targets hybridizing with the γ_1 probe, as witnessed by the lack of a signal boundary between cortex and caudate putamen for the γ_1 autoradiographs (e.g., Figs. 2, 4, 6). In addition, white matter tracts in the hippocampus also appear to be weakly labeled. This effect seems specific because (1) two independent γ_1 oligonucleotides recognizing different parts of the mRNA (Ymer et al., 1990) give the same result, (2) the signal can be competed by competition with unlabeled probe, and (3) γ_1 probes do not label white matter tracts in the cerebellum or olfactory bulb (Fig. 2; Laurie et al., 1992). No other subunit mRNAs could be detected in corpus callosum, and the demarcation between cortex and caudate-putamen was always pronounced for these subunit mRNAs.

The δ-subunit mRNA pattern resembles that of the α_2 and α_4 mRNAs, with layer II showing moderate levels (Figs. 3, 5, 7, 9, 11).

Piriform cortex

The piriform cortex expresses every subunit mRNA, to a greater or lesser extent, except α_6 (data not shown). The most abundant transcripts are α_1–α_4, β_2, β_3, γ_2, and δ (Figs. 3–6). The autoradiographic images obtained over this area are often extremely intense.

Hippocampus

Examining the hippocampal expression of the α-subunit genes, the α_2 mRNA is consistently the most abundant product and is expressed at high levels in the CA1, CA3, and dentate gyrus cell layers (Figs. 1, 7, 9, 12). The α_1 and α_4 mRNAs are also found in all sectors of the hippocampus (Figs. 1, 7, 9, 11, 12). The α_3 mRNA occurs mainly in the dentate granule cells, with some pyramidal cell expression also. The α_5 mRNA appears as abundant as the α_2 transcript in the CA1 and CA3 areas, but is less prominent in the dentate gyrus (Figs. 1, 7, 9, 12). In fact, the α_5 mRNA appears to encode a predominantly hippocampal subunit, since it is virtually absent from most other areas of the brain (see also Khrestchatisky et al., 1989).

Regarding β-subunit mRNAs, all three are present in CA1, CA3, and dentate gyrus cell layers (Figs. 2, 8, 10, 13), with β_1 and β_3 being more abundant than β_2 mRNA. Reminiscent of the α_5 mRNA, the β_1 mRNA is expressed at its highest levels in the hippocampus but is rare elsewhere. All three γ-subunit mRNAs are detectable in the hippocampus (Figs. 2, 8, 10, 13), with the γ_2 mRNA being most abundant. The γ_3 mRNA is rather rare, cortical levels being higher than those in the hippocampus. The δ mRNA is restricted to dentate granule cells at the resolution of x-ray film autoradiographs (Figs. 7, 9, 11, 12).

Tenia tecta

The tenia tecta, a structure embryologically related to hippocampus, expresses a number of subunit genes at very high levels (Figs. 3, 4). These abundant mRNAs are α_2, α_4, α_5, β_1, and β_3. No γ-subunit or δ-subunit mRNAs are detected in this structure.

Septum

The lateral septum contains a variety of subunit mRNAs, the most abundant of which are α_2, α_3, β_3, and γ_1 transcripts (Figs. 3, 4). Some subunit mRNAs such as α_5, γ_2, and γ_3 are completely absent from the lateral septum. The medial septal nucleus (data not shown; Wisden et al., 1991b) and the nucleus of the diagonal band contain very high levels of α_1, β_2, and γ_2 mRNAs (Table 1; Figs. 5, 6). This is consistent with these two nuclei being anatomically linked and containing the same cell types (Bleier and Byne, 1985). In the ventral septum, a different profile of subunit transcripts is found. The bed nucleus of the stria terminalis contains very high levels of α_2 and γ_1 transcripts (saturating autoradiographic signals), moderate levels of α_3, β_1, and β_3 mRNAs and low levels of α_1, α_4, β_2, and γ_2 mRNAs (Table 1; Figs. 5, 6).

Basal ganglia: caudate-putamen, nucleus accumbens, and globus pallidus

In the caudate nucleus the most prevalent α-subunit mRNAs are α_2 and α_4 (Figs. 1, 3, 5). However, longer exposure times also reveal the presence of α_1 and α_3 mRNAs. The α_5 mRNA was undetectable. The predominant β-subunit in the caudate is β_3 (Figs. 2, 4, 6), followed in order of abundance by β_2 and β_1 mRNAs. All three γ-subunit mRNAs are present at low levels in caudate, with the γ_3 mRNA slightly elevated relative to the others (Figs. 2, 4, 6). The δ-subunit mRNA is moderately expressed in the caudate nucleus (Figs. 3, 5, 11).

Levels of subunit transcripts in the nucleus accumbens parallel their respective levels in caudate. Subunit mRNAs abundant in the caudate are also abundant in the nucleus accumbens, while those mRNAs rare in nucleus accumbens are also rare in caudate. The most abundant accumbens transcripts are those of α_2, α_4, and β_3. However, the γ_3 mRNA appears to be elevated in the medial parts of the nucleus accumbens (Fig. 4).

In the globus pallidus, the major α-subunit mRNA is that of α_1 (Fig. 5). There are also smaller but significant amounts of α_2

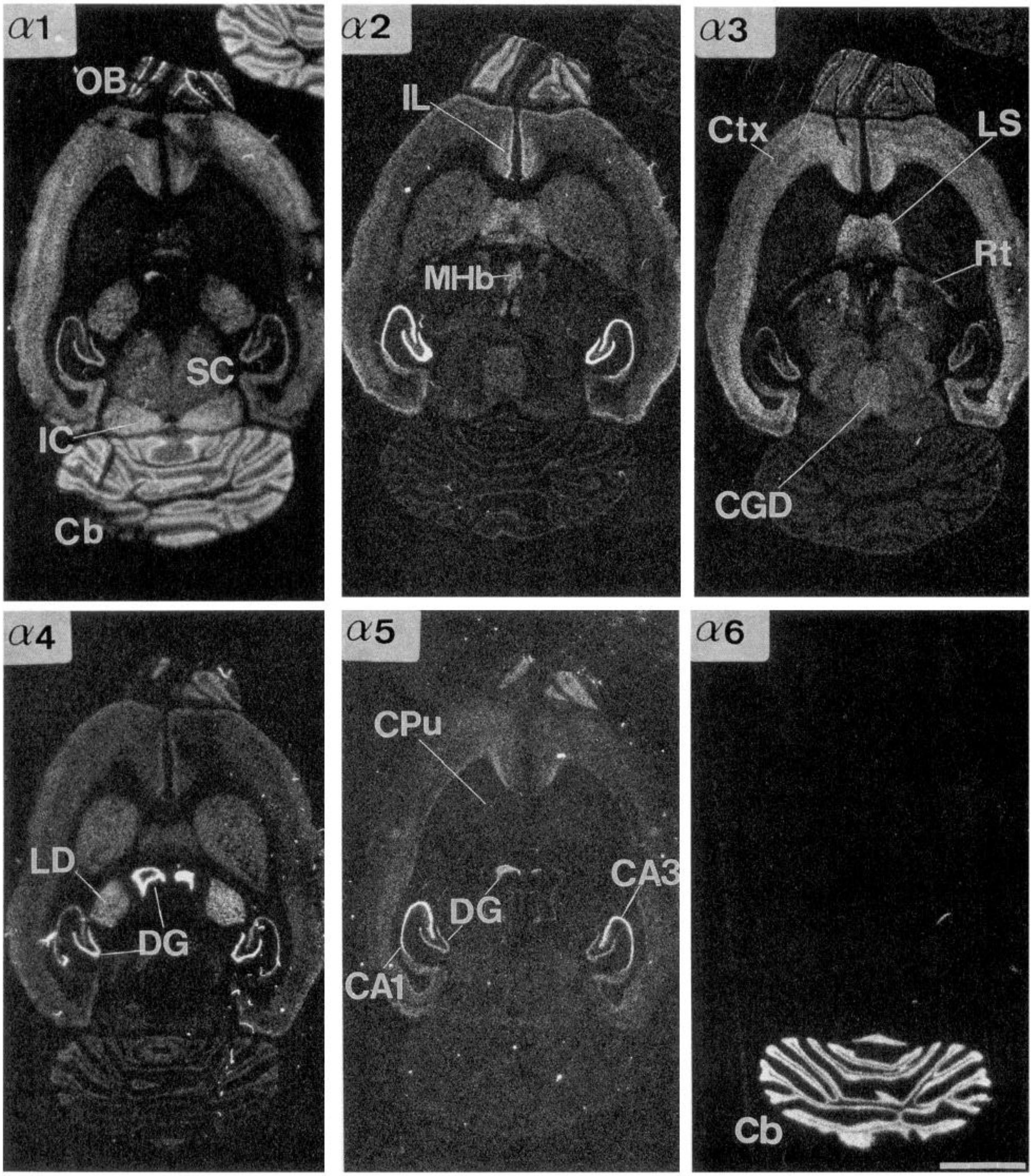

Figure 1. Distribution of GABA$_A$ receptor α-subunit mRNAs (α_1–α_6) in horizontal rat brain sections. See Appendix for abbreviations. Scale bar, 4.4 mm.

and α_3 mRNAs in this area (Fig. 5). The β_2 mRNA is the only β mRNA found in the globus pallidus (Fig. 6). Regarding the γ-subunit class, γ_1 mRNA is marginally the more abundant γ-transcript in this region (Fig. 6). The δ and γ_3 mRNAs are not expressed in the globus pallidus (Figs. 5, 6).

Subthalamic nucleus

In the subthalamic nucleus, the α_1 and β_2 mRNAs are the most significant GABA$_A$ receptor transcripts present (Figs. 9, 10). γ_2 mRNA is also in this area, but at lower levels (Fig. 10). Longer exposure times also indicate the presence of γ_3 mRNA (not shown).

Amygdala

In the amygdaloid complex, α_2 mRNA is found at very high levels and is the predominant α-subunit mRNA in the medial amygdaloid nucleus (Fig. 9), lateral amygdaloid nucleus (Fig. 7), and posterior medial cortical nucleus (not shown). All other

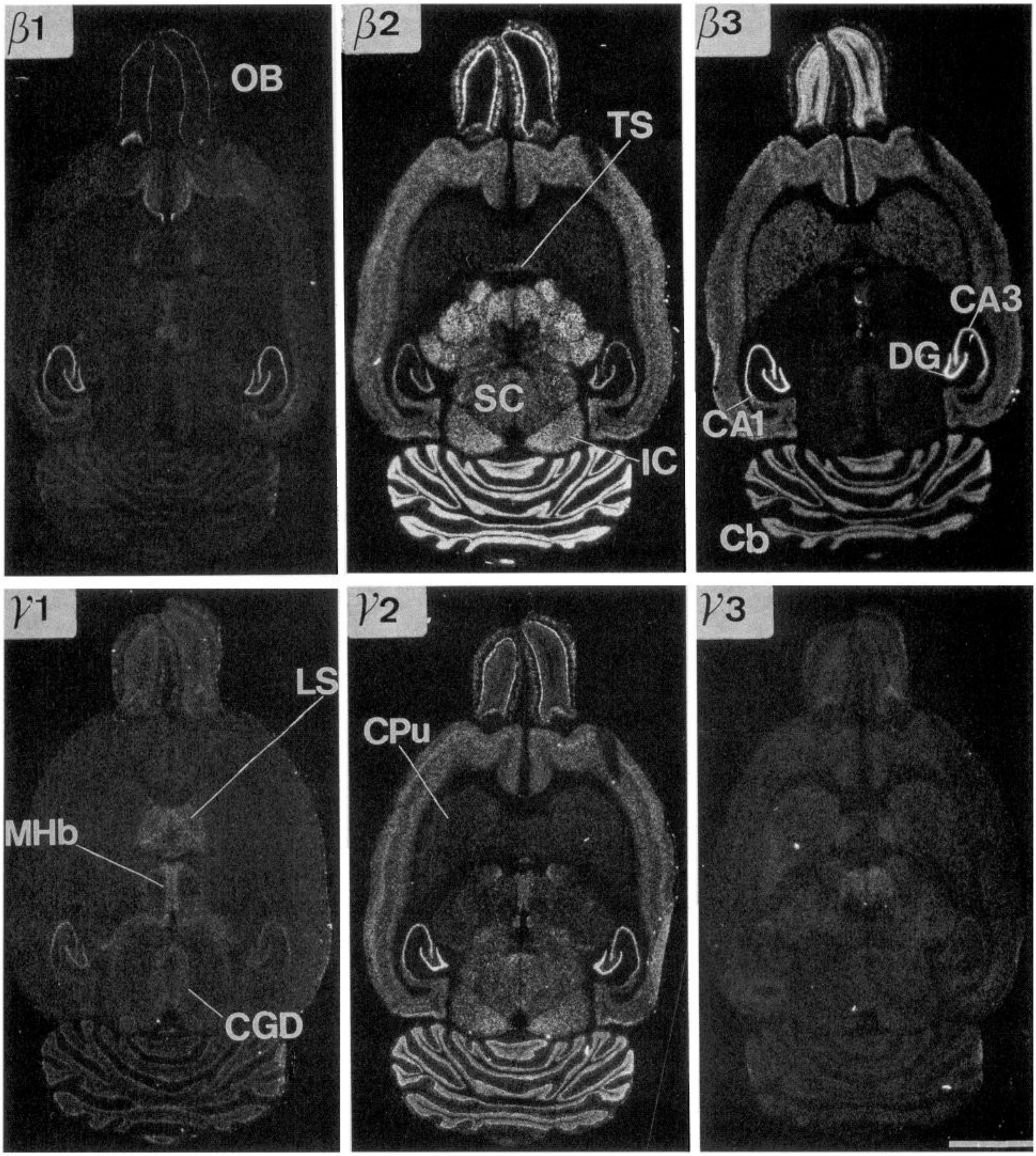

Figure 2. Distribution of $GABA_A$ receptor β-subunit mRNAs (β_1–β_3) and γ-subunit mRNAs (γ_1–γ_3) in horizontal rat brain sections. See Appendix for abbreviations. Scale bar, 4.4 mm.

α-subunit mRNAs, except α_6, are also present in these nuclei but at differing degrees. The rarest is α_5 mRNA. Similarly, all β-genes are expressed in these nuclei, with β_3 generally being the best expressed β-subunit mRNA (Figs. 8, 10).

The differential expression of the γ-subunit genes in the amygdaloid nuclei is noteworthy. For example, γ_1 mRNA is expressed at striking levels in the medial amygdaloid nuclei (Fig. 10), an area where there is relatively little of the γ_2 and γ_3 mRNAs. Indeed, this nucleus contains some of the highest amounts of γ_1 mRNA in the brain. However, γ_2 mRNA is the main representative of the γ-class in the general amygdaloid area (Figs. 8, 10). The γ_3 mRNA is present in diffuse, low levels throughout the complex. The δ-transcript is absent from the amygdala (Figs. 7, 9).

Diencephalon (epithalamus, thalamus, and hypothalamus)

The medial habenula expresses significant quantities of α_2 and β_3 mRNAs and some γ_1 and γ_2 mRNAs (Figs. 7, 8). All other subunit mRNAs are rare or undetectable.

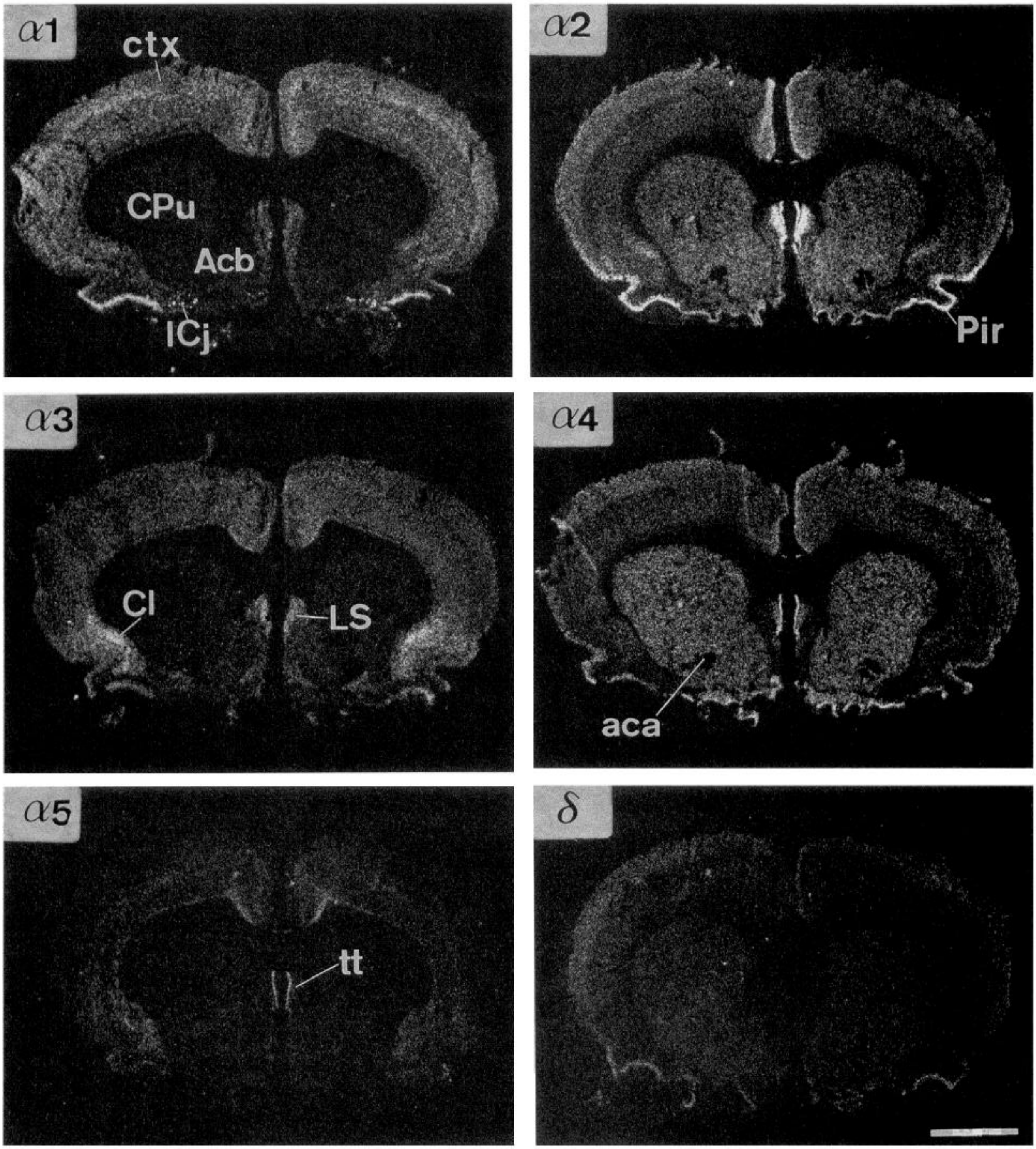

Figure 3. Distribution of α_1–α_5 and δ GABA$_A$ receptor subunit mRNAs in coronal sections at the level of caudate nucleus and nucleus accumbens. See Appendix for abbreviations. Scale bar, 2.8 mm.

Thalamus

The α_1 and α_4 transcripts are the most prominent α-subunit mRNAs throughout the whole thalamus. On sections through caudal portions of the thalamus, the dorsal lateral geniculate, ventral lateral geniculate, and the ventral posterior nuclei are clearly positive with the α_1 and α_4 probes (Figs. 7, 9, 11, 14), as is the lateral dorsal complex (Fig. 1). The α_2 and α_3 mRNAs are also present in the thalamus, but in more restricted subpopulations (Figs. 7, 9, 14). The α_5 mRNA was absent from all thalamic nuclei examined (Figs. 7, 9).

Examining the thalamic distribution of the α-subunit mRNAs in more detail, several features become apparent. The α_4 mRNA is very abundant in most nuclei of the thalamus, with some exceptions. For example in the zona incerta, a part of the ventral thalamus, α_1 mRNA is the only α-variant present (Fig. 9), while in the reticular thalamus α_4 mRNA is absent but α_3 mRNA is present (Figs. 7, 9). The α_2 and α_3 mRNAs are absent from the ventral posterior nucleus where the α_1 and α_4 mRNAs appear (Figs. 7, 9). In very caudal parts of the thalamus, such as the medial geniculate nucleus, the α_1 and α_4 mRNAs predominate, with α_4 mRNA being the most abundant (Fig. 12). The α_2 and

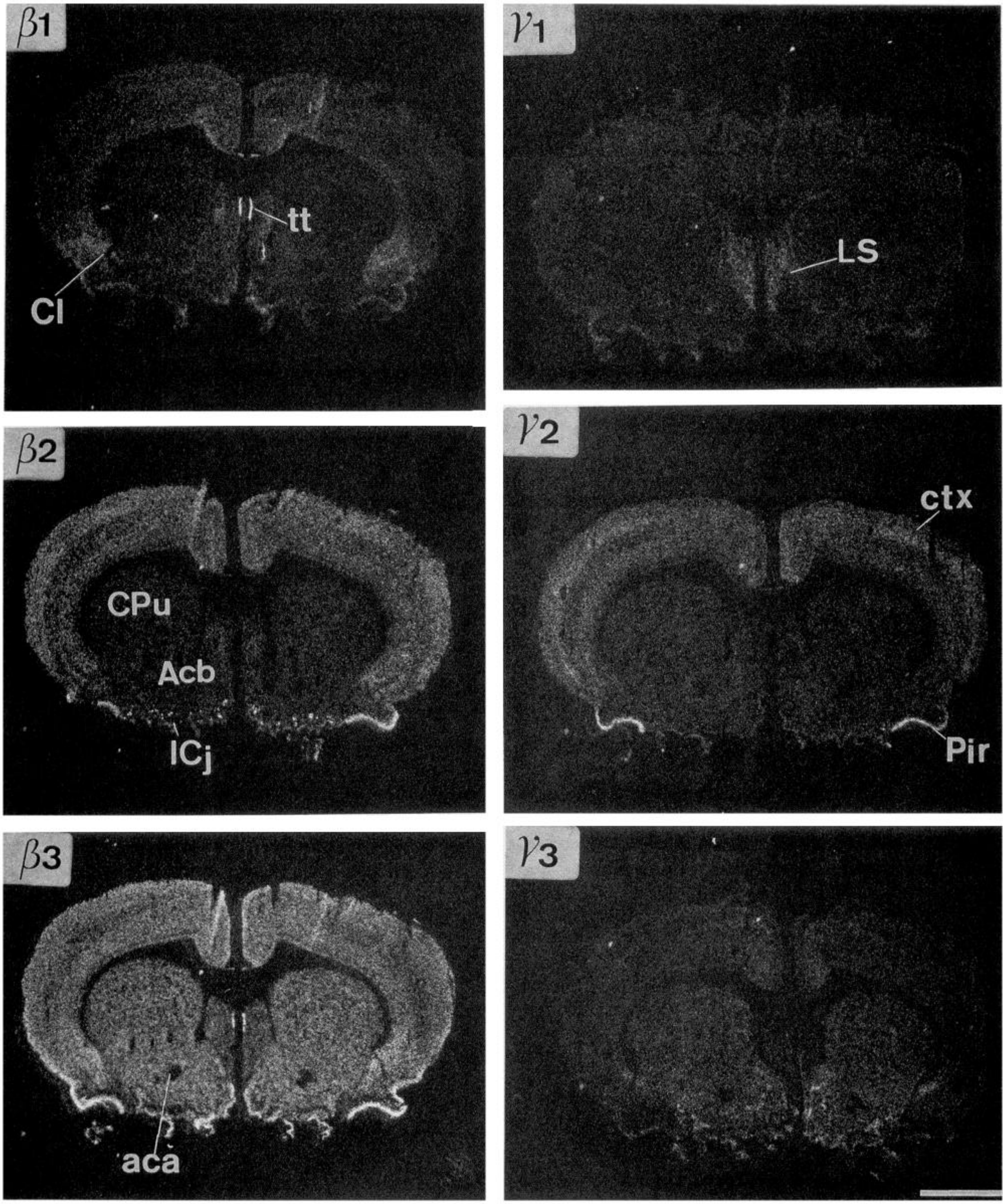

Figure 4. Distribution of β_1–β_3 and γ_1–γ_3 $GABA_A$ receptor subunit mRNAs in coronal sections at the level of caudate nucleus and nucleus accumbens. See Appendix for abbreviations. Scale bar, 2.8 mm.

α_3 mRNAs appear to be restricted to predominantly midline nuclei (e.g., paraventricular nuclei, rhomboid nuclei) and also the central lateral nucleus, and seem to colocalize in these structures. Both the α_2 and α_3 mRNAs are absent from the mediodorsal nucleus (Fig. 7).

Certain areas of the thalamus show a surprising degree of microheterogeneity with regard to α-subunit expression. This is illustrated by the parafascicular thalamic nucleus (Fig. 14, PF), which encircles the fasciculus retroflexus (fr) fiber tract (Jones, 1985; Paxinos and Watson, 1986). The α_4 transcript is the high-

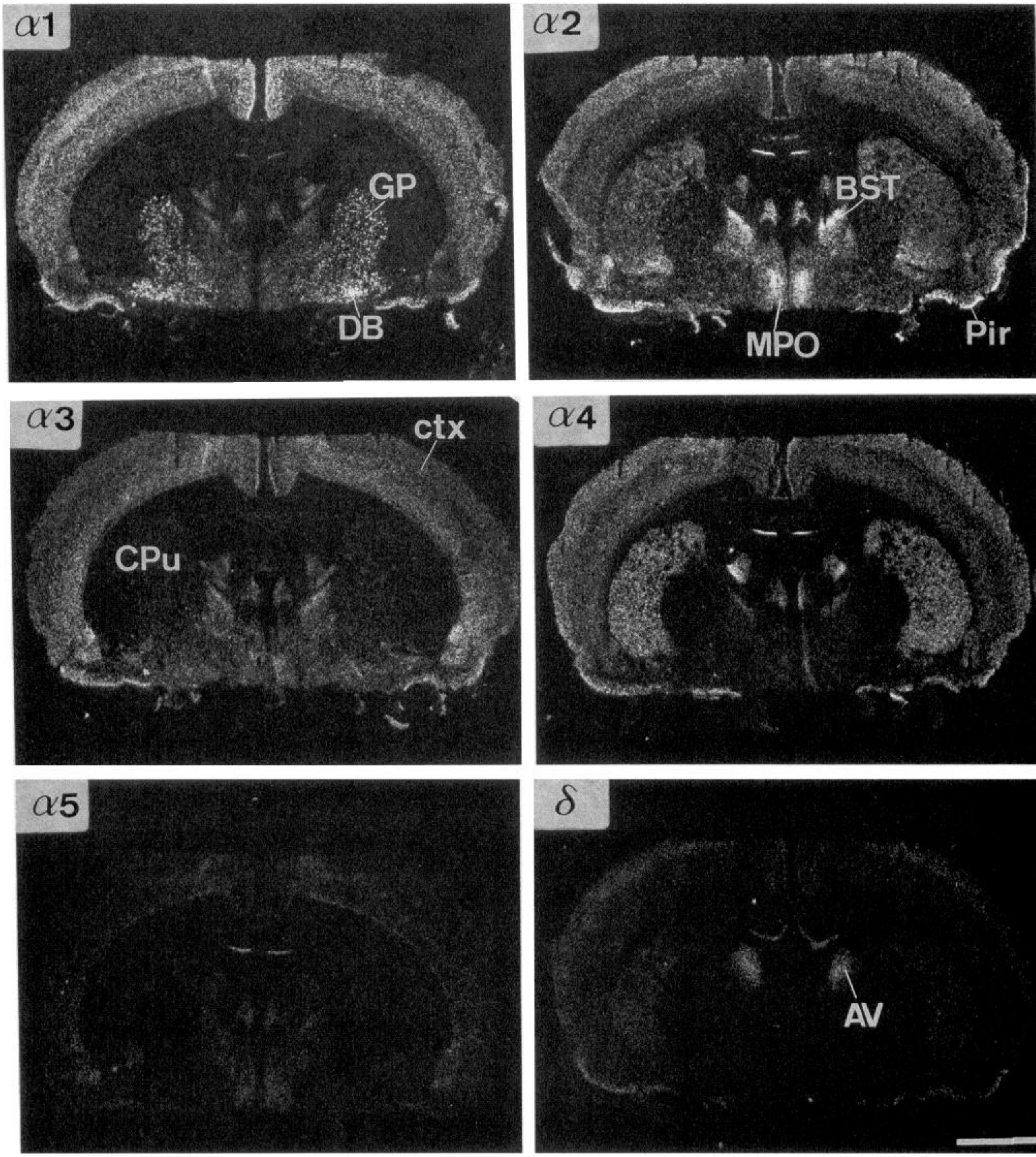

Figure 5. Distribution of α_1–α_5 and δ GABA$_A$ receptor subunit mRNAs in coronal sections at the level of globus pallidus and medial preoptic hypothalamic area. See Appendix for abbreviations. Scale bar, 2.8 mm.

est overall in this nucleus and is more abundant in the lateral and ventral parts than in the most medial part (Fig. 14). In contrast, α_1 mRNA is largely restricted to the lateral portion (Fig. 14 α_1, arrowheads), and the α_2 mRNA is present in the most medial midline portion (Fig. 14, α_2, arrowhead). The α_3 mRNA is present at diffuse low levels throughout the parafascicular nucleus (Fig. 9).

A pronounced feature of β-subunit mRNA expression in thalamus is the diffuse low-level expression of β_1 and β_3 mRNAs. Levels for these mRNAs increase in midline nuclei, for example, paraventricular, rhomboid, and central lateral nuclei. By contrast, β_2 mRNA is ubiquitous in thalamus (Figs. 2, 8, 10, 13, 14), and its pattern resembles in detail that of α_1 mRNA, both in spatial distribution and relative abundance.

All members of the γ-subunit mRNA class are poorly expressed in the thalamus (Figs. 2, 8, 10, 13). Of these, the γ_2 mRNA is the most abundant, with the γ_1 and γ_3 probes giving weaker, diffuse signals. The γ_3 mRNA is present at moderate

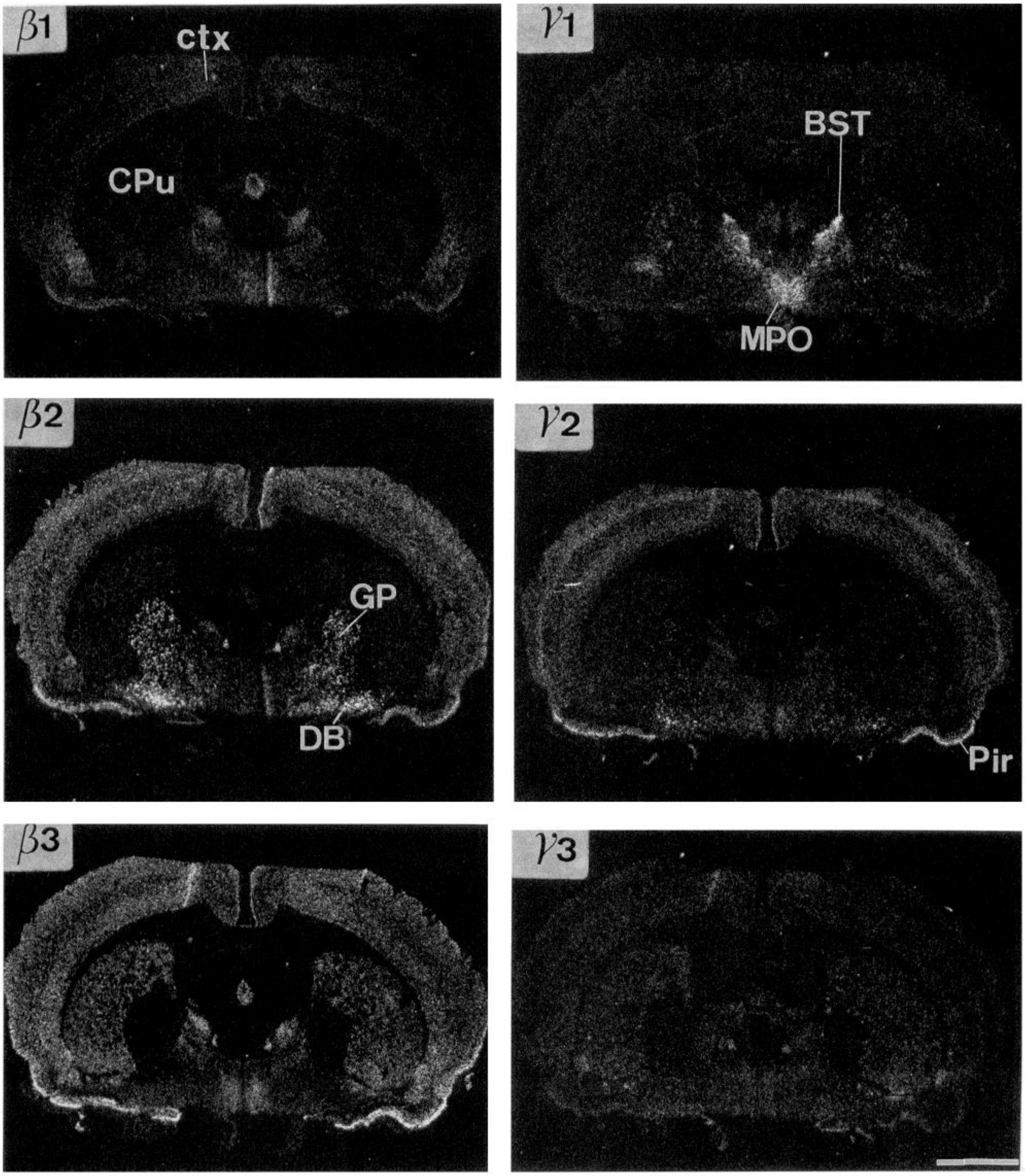

Figure 6. Distribution of β_1–β_3 and γ_1–γ_3 $GABA_A$ receptor subunit mRNAs in coronal sections at the level of globus pallidus and medial preoptic hypothalamic area. See Appendix for abbreviations. Scale bar, 2.8 mm.

levels in the medial geniculate nucleus (Fig. 13), being more abundant than γ_1 and γ_2 mRNAs in this nucleus.

The δ-subunit mRNA is observed in a broad range of thalamic nuclei and colocalizes with the α_4 mRNA in the medial geniculate, ventral posterior, ventral lateral geniculate, and dorsolateral geniculate nuclei (Figs. 5, 7, 9, 11, 12). However, unlike the α_4 mRNA, δ mRNA is absent from the parafascicular thalamic nucleus (Fig. 9). The δ mRNA also appears to be absent from midline nuclei such as the paraventricular nucleus and rhomboid nucleus, and is also absent from the reticular nucleus (Fig. 7). However, it is found in the mediodorsal nucleus (Fig. 7). In this respect, δ-gene expression is reciprocal to the thalamic nuclei expressing the α_2 and α_3 subunit genes. For example, the autoradiographic signals for α_2 and α_3 mRNAs result in the formation of a trident-like pattern (comprising the centrolateral, paraventricular, and rhomboid nuclei) in the middle of the thal-

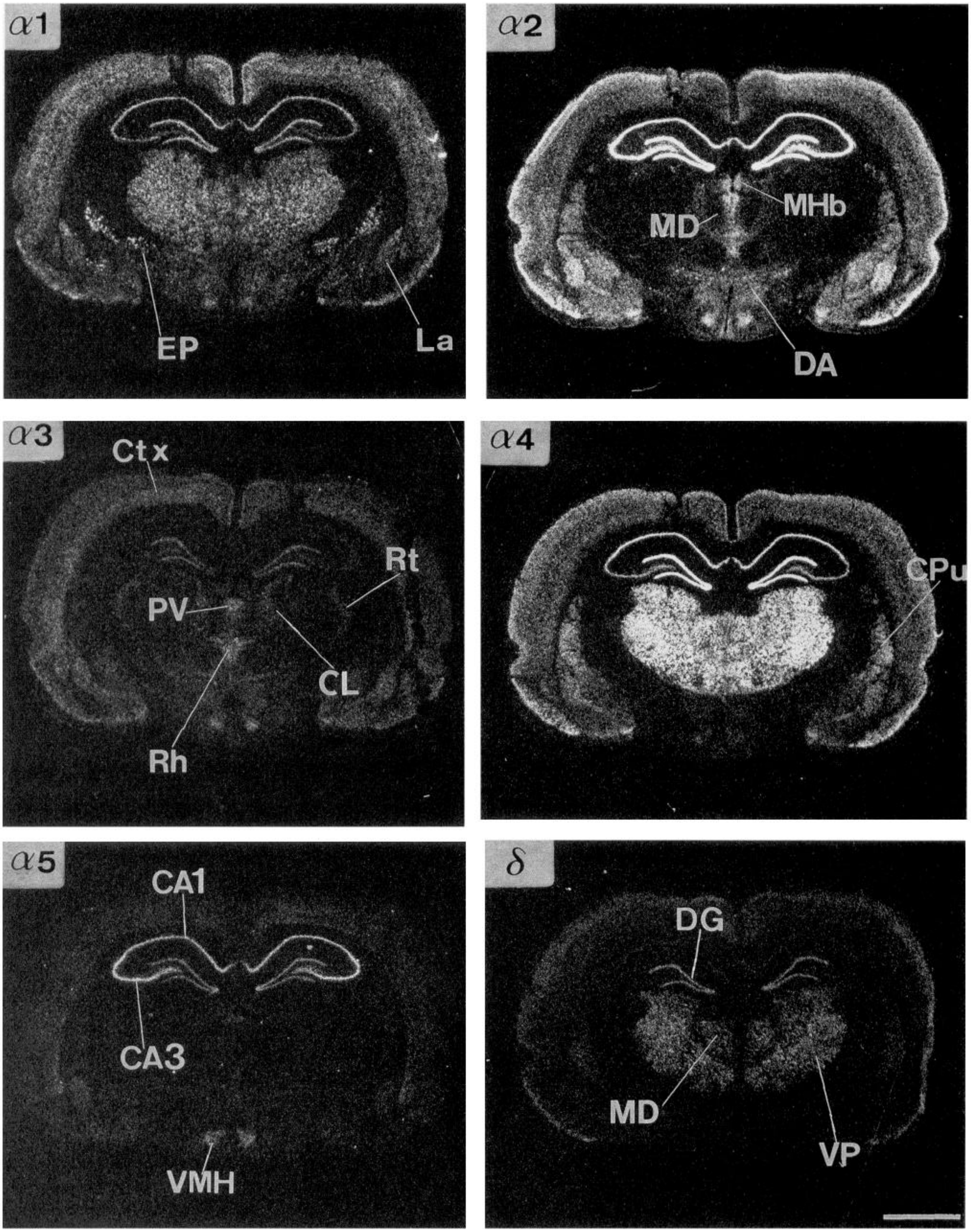

Figure 7. Distribution of GABA$_A$ receptor α-subunit mRNAs (α_1–α_5 and δ) in coronal sections of rat brain at the level of medial habenula. See Appendix for abbreviations. Scale bar, 3 mm.

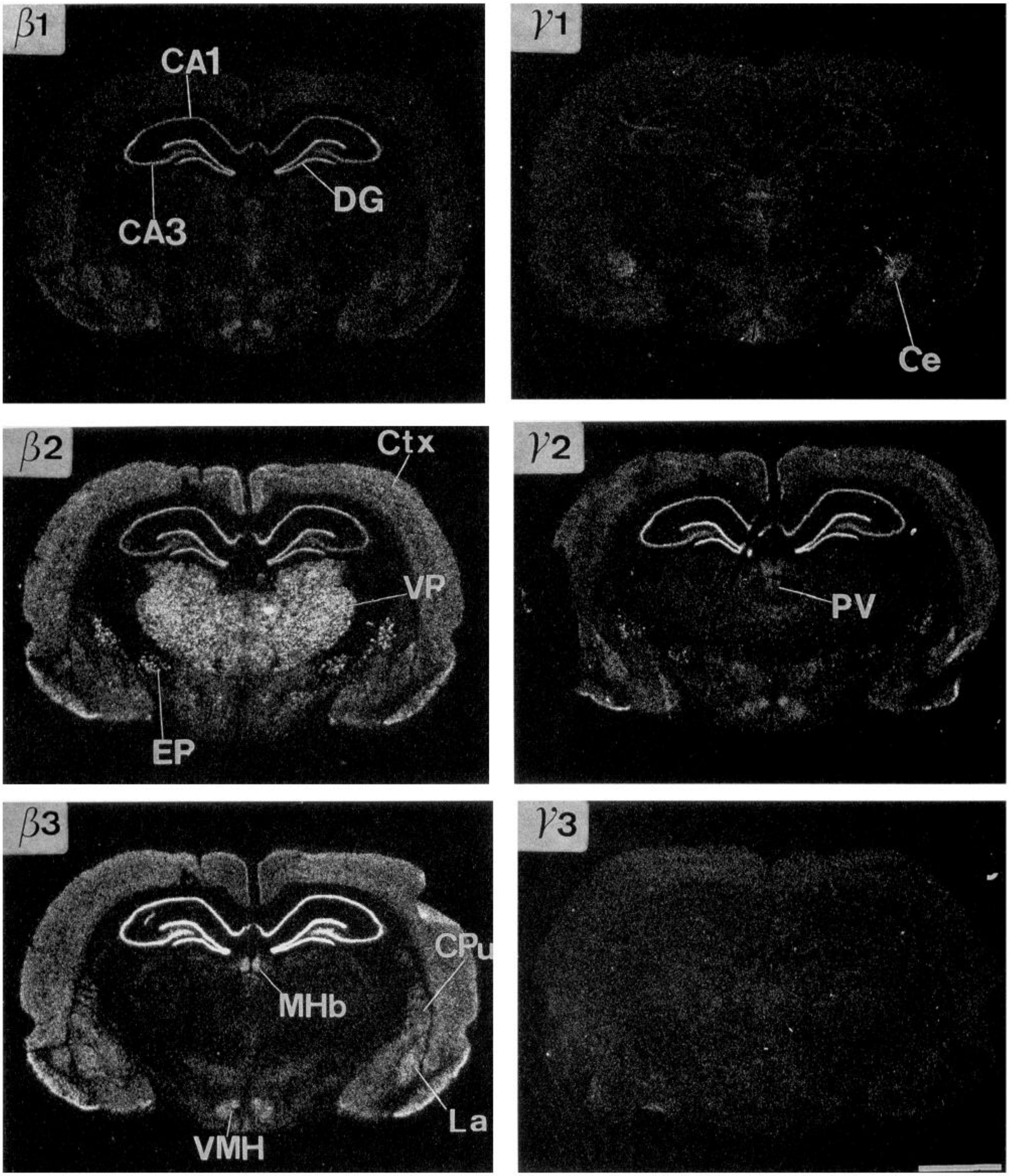

Figure 8. Distribution of $GABA_A$ receptor β-subunit mRNAs (β_1–β_3) and γ-subunit mRNAs (γ_1–γ_3) in coronal sections at the level of medial habenula. See Appendix for abbreviations. Scale bar, 3 mm.

amus (Fig. 7), and the δ mRNA distribution traces the negative image of this pattern (Fig. 7).

Hypothalamus

In the hypothalamus, the most prominent mRNA is that of α_2 (Figs. 5, 7, 9, 14). This mRNA is abundantly present in the medial preoptic, dorsomedial, ventromedial, and arcuate nuclei and in the dorsal hypothalamic area. The α_1, α_3, and α_5 subunit mRNAs are also found in these nuclei, but in lower amounts. The α_4 mRNA appears to be absent from the hypothalamus (Fig. 14).

Regarding β-subunits, the β_3 mRNA predominates in the me-

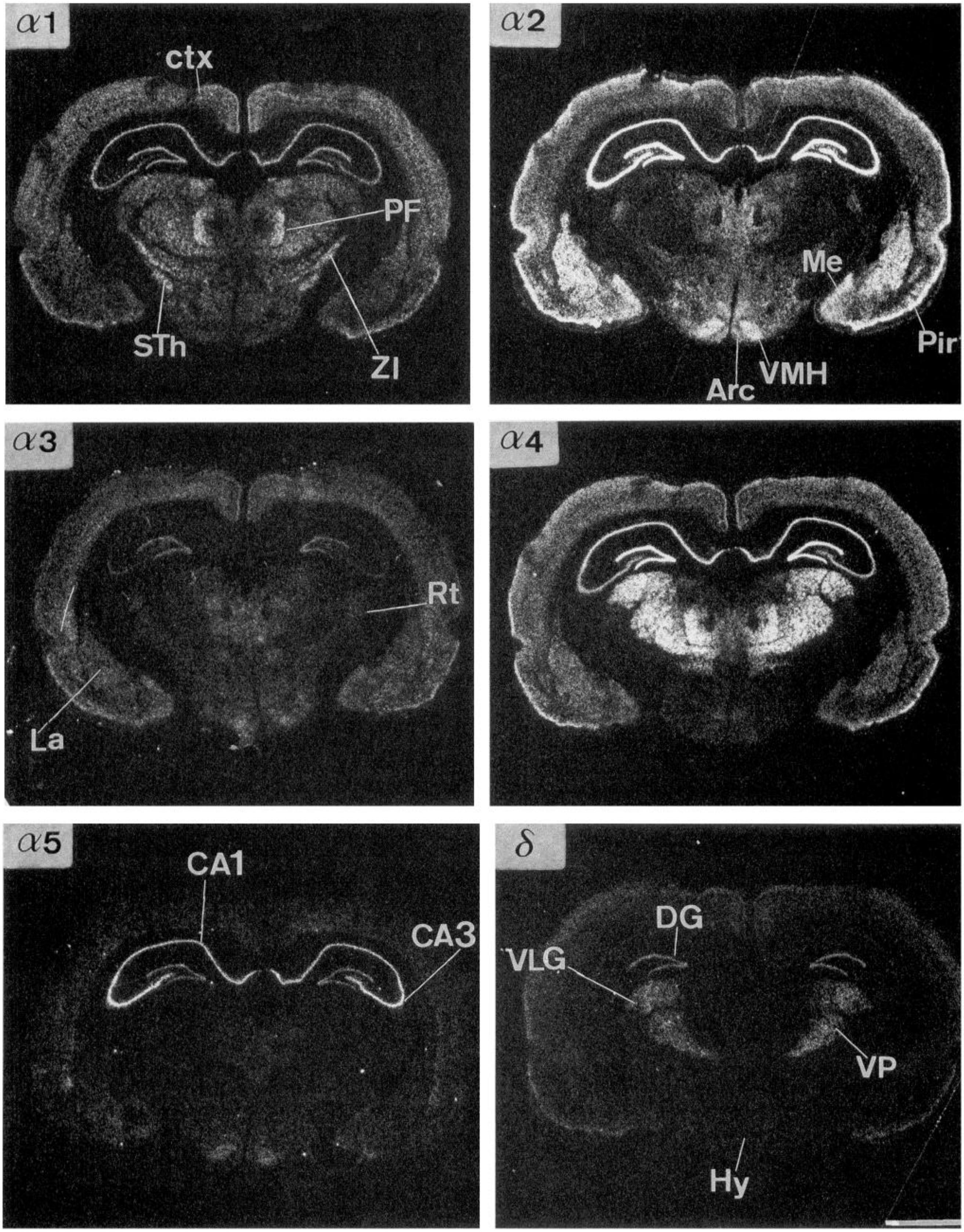

Figure 9. Distribution of α_1–α_5 and δ GABA$_A$ receptor subunit mRNAs in coronal sections at the level of the parafascicular nucleus. See Appendix for abbreviations. Scale bar, 3 mm.

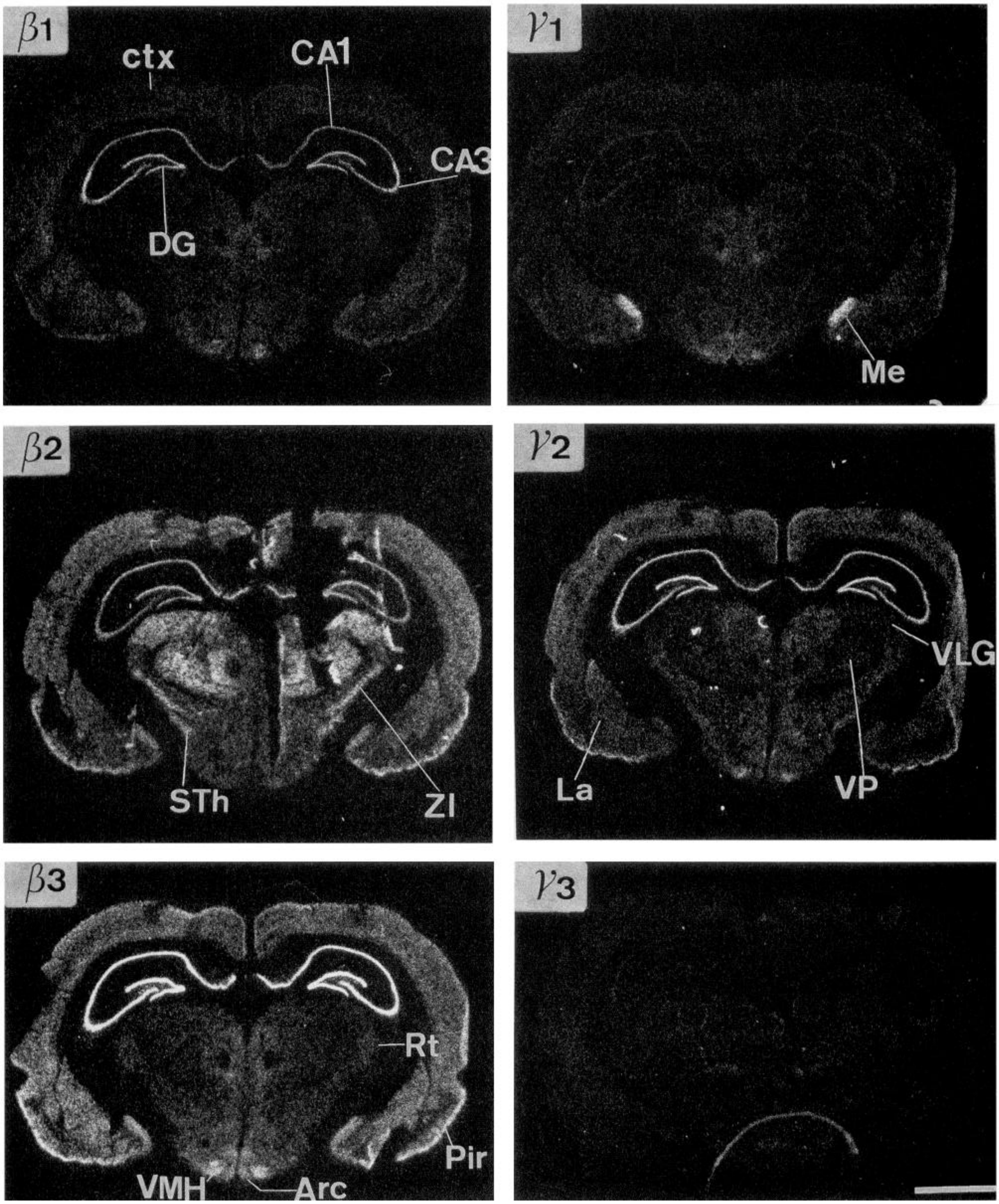

Figure 10. Distribution of β_1–β_3 and γ_1–γ_3 $GABA_A$ receptor subunit mRNAs in coronal sections at level of the parafascicular nucleus. See Appendix for abbreviations. Scale bar, 3 mm.

dial preoptic, dorsomedial, ventromedial, and arcuate nuclei (Figs. 6, 8, 10). A low amount of β_1 mRNA is seen in the arcuate and the ventromedial nuclei, whereas β_2 mRNA is rare in all hypothalamic nuclei examined. Considering the γ-subunit class, γ_1 and γ_2 mRNAs are found in dorsomedial, ventromedial, and arcuate nuclei (Figs. 8, 10). The γ_1 mRNA conspicuously predominates in the medial preoptic area (Fig. 6). The δ-subunit mRNA is undetectable in hypothalamus.

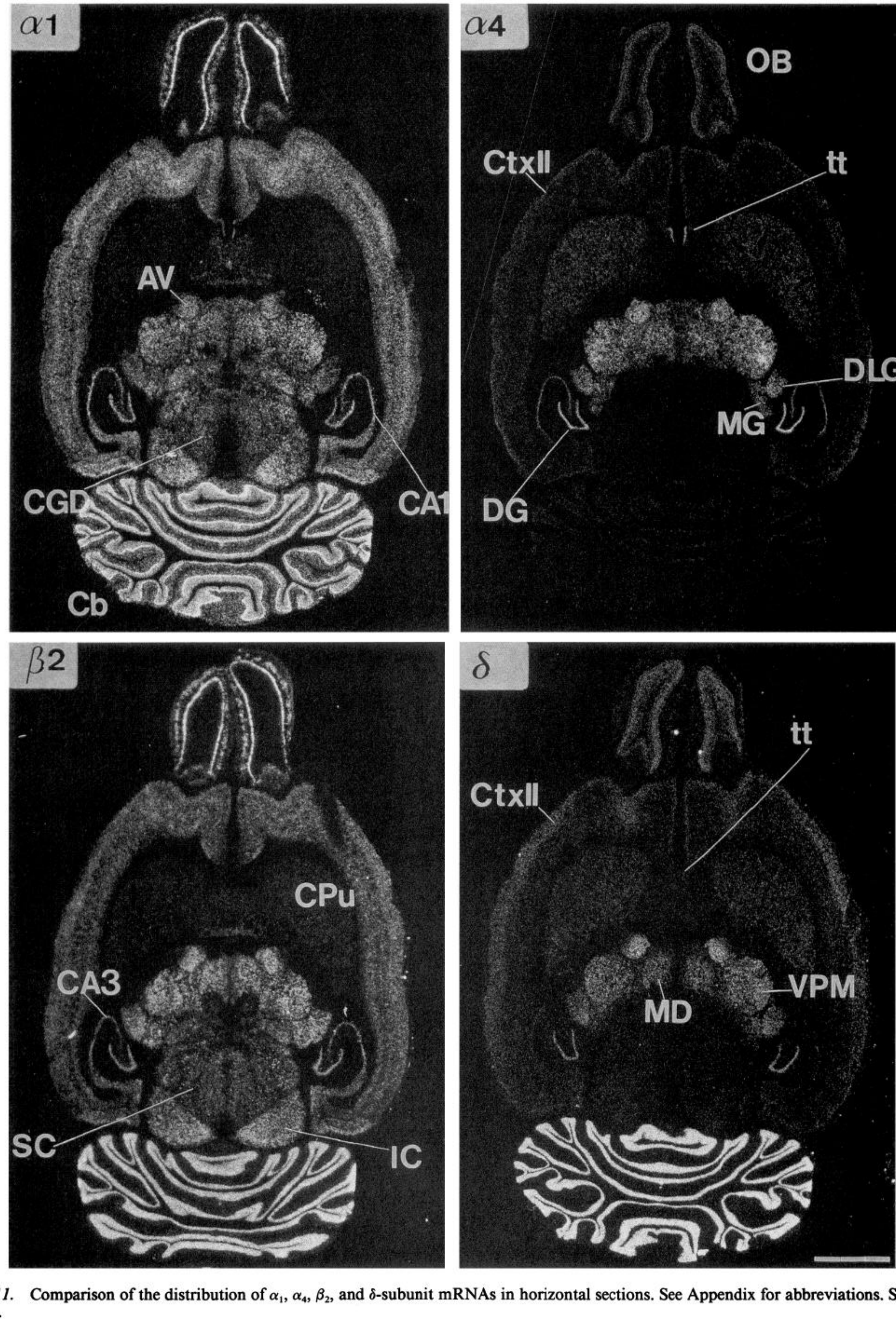

Figure 11. Comparison of the distribution of α_1, α_4, β_2, and δ-subunit mRNAs in horizontal sections. See Appendix for abbreviations. Scale bar, 3.1 mm.

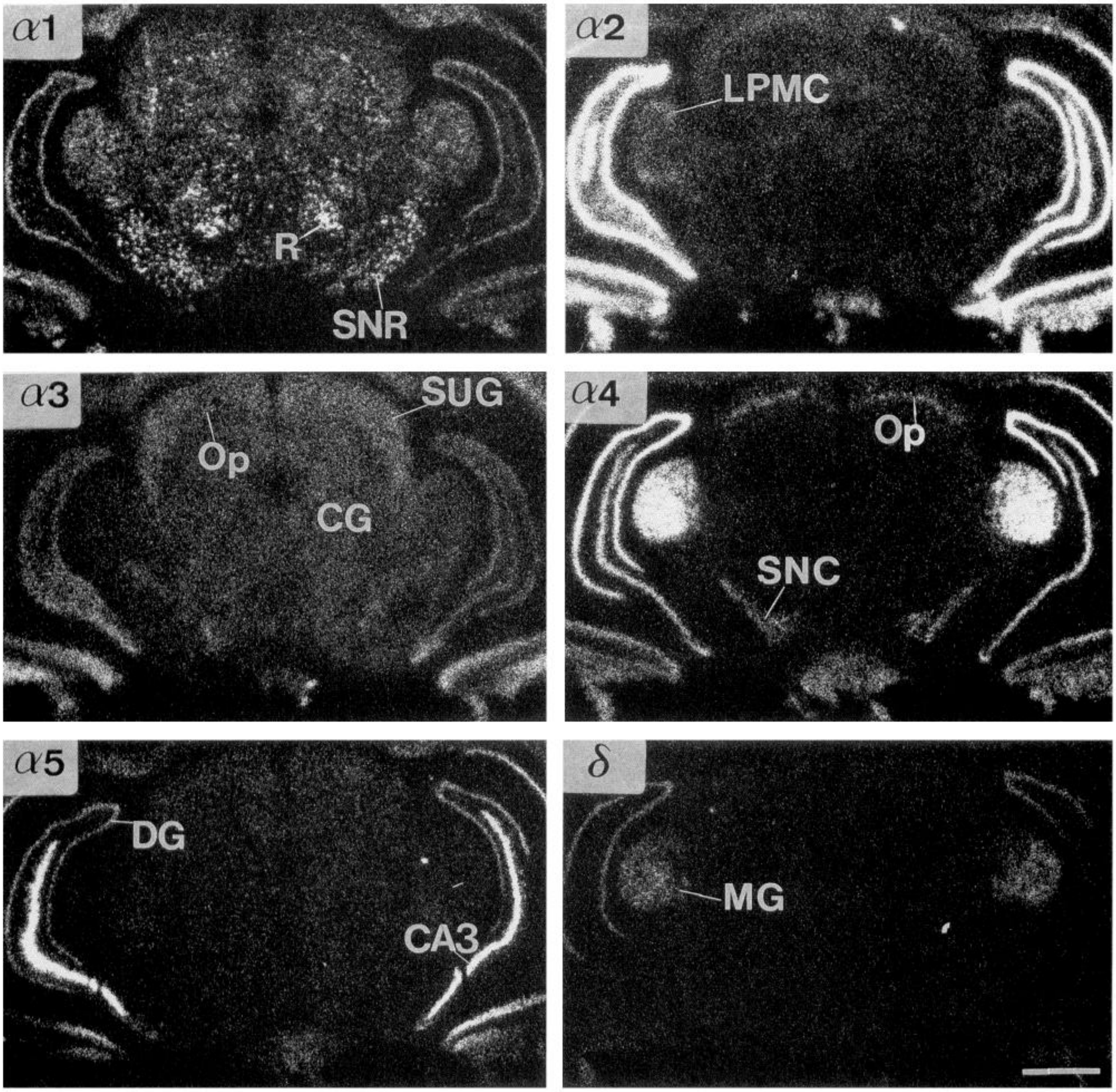

Figure 12. Coronal sections at the level of the medial geniculate nucleus and substantia nigra illustrating differential patterns of α_1–α_5 and δ mRNAs. See Appendix for abbreviations. Scale bar, 1.6 mm.

Midbrain (colliculi, substantia nigra, red nucleus)

Throughout the general midbrain area, the most noticeable mRNAs are α_1, α_3, β_2, β_3, and γ_2 (Figs. 12, 13). However, the autoradiographic signals obtained with the α_1, β_2, and to some extent the γ_2 probes are very punctate, suggesting expression in large cells. The patterns obtained with the α_3 and β_3 probes are more uniform and diffuse (Figs. 12, 13). Some subunit mRNAs (α_6, β_1, and δ) are entirely absent from any midbrain structures examined, whereas others are generally absent but very prominent in certain nuclei, for example, α_4 in substantia nigra compacta (Fig. 12).

Substantia nigra

The substantia nigra pars reticulata contains high levels of α_1 (see also Hironaka et al., 1990) and β_2 mRNAs with γ_1 and γ_2 transcripts also present (Figs. 12, 13). The substantia nigra pars compacta contains α_3, α_4, β_3, and γ_2 mRNAs (Figs. 12, 13).

Red nucleus

The red nucleus (see also Hironaka et al., 1990, for α_1) expresses the same $GABA_A$ receptor subunit genes as that of the substantia nigra reticulata, that is, α_1, β_2, and γ_2 mRNAs, with only borderline to zero degrees of expression for the others (Figs. 12, 13).

Superior colliculus

All levels of the superior colliculus contain α_1, β_2, and γ_2 mRNAs, although deeper layers contain larger amounts (Figs. 12, 13). In contrast, the α_2, α_3, and α_5 transcripts are largely found in the superficial layer. The α_4 mRNA occurs mainly in the optic nerve layer. The β_1 mRNA is restricted to the optic layer, and the β_3

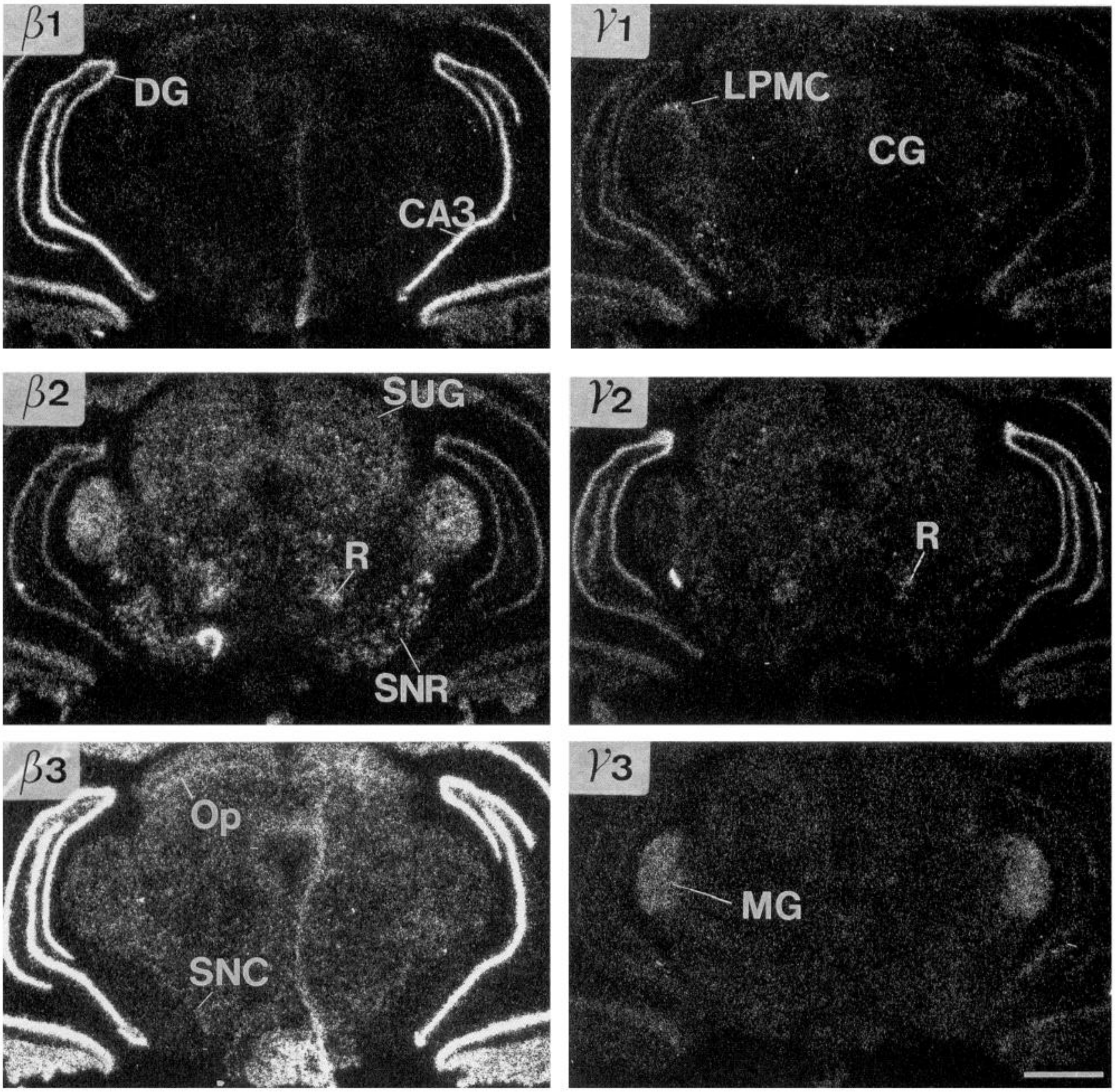

Figure 13. Coronal sections at the level of the medial geniculate nucleus/substantia nigra illustrating patterns of β_1–β_3 mRNAs and γ_1–γ_3 mRNAs. See Appendix for abbreviations. Scale bar, 1.6 mm.

mRNA is more highly expressed throughout the superior colliculi.

Inferior colliculus

In the inferior colliculus (central nucleus), the main GABA$_A$ receptor transcripts are α_1, β_2, and γ_2 (Figs. 1, 2, 11).

Discussion

In this study we have documented the regional brain distribution of 13 rat GABA$_A$ receptor subunit mRNAs. Their expression patterns can be analyzed to deduce plausible *in vivo* subunit combinations that may constitute molecularly and functionally distinct receptor subtypes. In the following discussion, we list such combinations and endeavor to match these suggested subtypes with previously published pharmacological characteristics in both brain membranes and engineered expression systems.

To derive plausible combinations, brain regions in which a limited subset of subunit genes was expressed were naturally more amenable than regions with highly complex expression patterns. For example, dentate granule cells pose an extreme problem, since they seem to contain every subunit mRNA with the exception of that encoding α_6. Thus, either a large complexity of GABA$_A$ receptors exists on one cell type or there are subpopulations of granule cells, each expressing particular subsets of receptors. Sophisticated multiple-labeling experiments with antibodies will be required to address this problem. However, there is evidence in favor of more than one GABA$_A$ receptor on different parts of hippocampal neurons. GABA$_A$ receptors on pyramidal cell soma differ from those on dendrites in terms

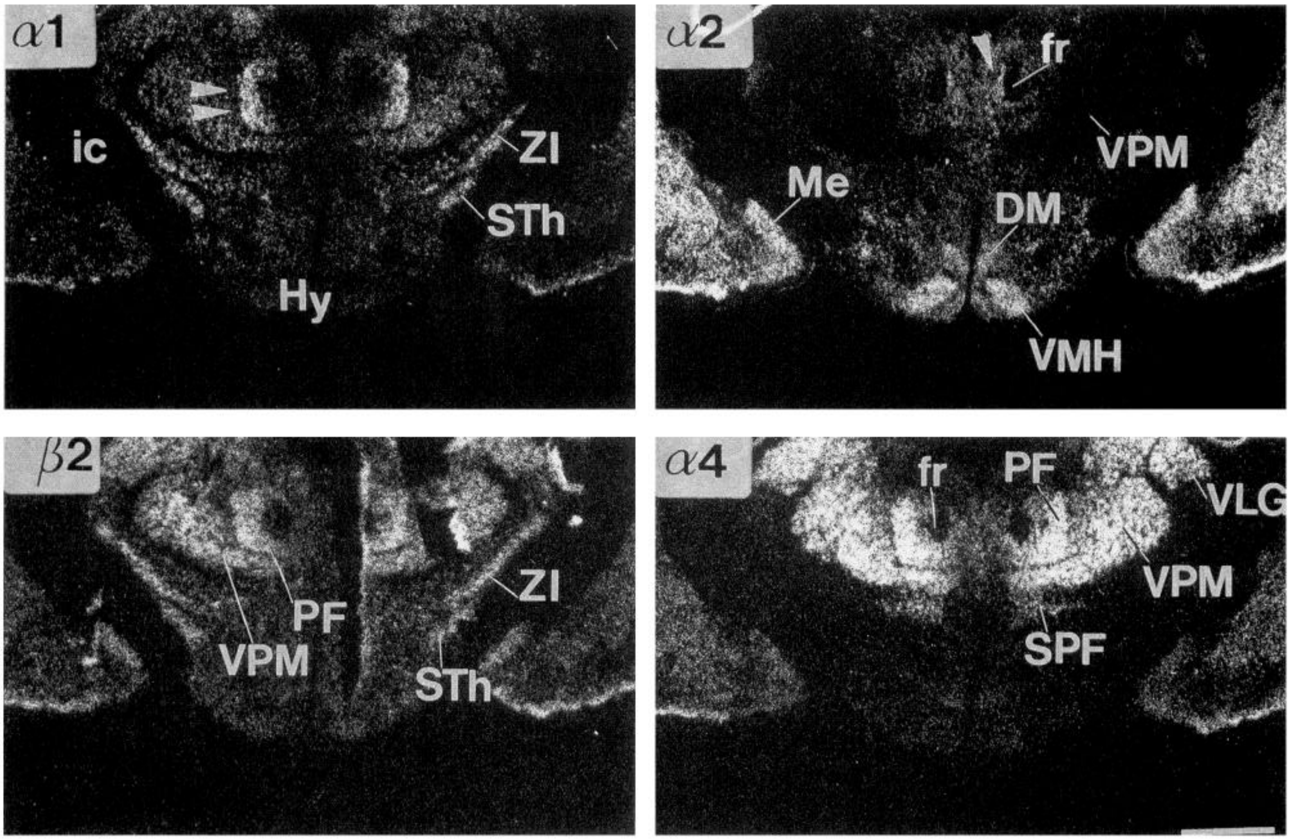

Figure 14. Differential distribution of α_1, α_2, α_4, and β_2 mRNAs in the thalamus (enlargements from Figs. 9, 10). *Arrowheads* defined in text. See Appendix for abbreviations. Scale bar, 1.8 mm.

of agonist preference (Alger and Nicoll, 1982; Nicoll and Dutar, 1989).

Similarly, the neocortex seems refractory to analysis owing to the large repertoire of different cell populations. However, there are certain shared regional patterns of subunit mRNAs with regard to different laminae. For example, the α_2, α_4, and δ mRNAs appear higher in layers II and III, relative to other laminae (Table 1). The α_3, α_5, and β_1 transcripts are highest in layer VI relative to their abundance in other layers. Although the α_1, β_2, β_3, and γ_2 mRNAs are in every layer, they appear highest in layers II/III and V/VI. These correlations may be significant. For the α_1, α_2, and α_3 subunits, the cortical polypeptide distribution correlates with the mRNA pattern (Zimprich et al., 1991), and the same mRNA pattern is observed in bovine cortex (Wisden et al., 1988). Moreover, these broad groupings of mRNAs are consistent with groupings in other brain regions (thalamus, colliculi, caudate nucleus). For example α_4 and δ mRNAs often codistribute, the α_5 and β_1 mRNA patterns look very similar, and the $\alpha_1\beta_2\gamma_2$ combination appears frequently (see below). This is suggestive of at least three cortical receptor subtypes containing $\alpha_4\delta$, $\alpha_5\beta_1$, and $\alpha_1\beta_2\gamma_2$. Other subunits, most obviously γ-variants, would presumably also be a part of these "cores."

Deduced GABA$_A$ receptor subunit combinations

$\alpha_1\beta_2(\gamma_2)$. The α_1 and β_2 mRNAs are most widely codistributed in the brain (Table 1). In addition, the γ_2 mRNA often colocalizes with this pair. In areas such as the central nucleus of the inferior colliculi and the red nucleus, only α_1, β_2, and γ_2 mRNAs are found. This core combination is also found in mitral cells of the olfactory bulb and in cerebellar Purkinje cells (accompanying article, Laurie et al., 1992). In coexpression experiments, the $\alpha_1\beta_2\gamma_2$ receptor embodies the complete profile of "classical" GABA$_A$ electrophysiological responses (Sigel et al., 1990; Verdoorn et al., 1990). This subunit combination is also supported by immunoprecipitation studies (Benke et al., 1991a). However, it appears that other γ-variants can be used. For example, in the globus pallidus, both $\alpha_1\beta_2\gamma_1$ and $\alpha_1\beta_2\gamma_2$ combinations may occur. Some regions, such as the zona incerta, islands of Calleja, and the subthalamic nucleus, express very high levels of α_1 and β_2 mRNAs, with no other GABA$_A$ receptor mRNAs present except for moderate levels of γ_2 mRNA.

$\alpha_2\beta_3(\gamma_x)$, $\alpha_5\beta_1(\gamma_x)$. Another frequently occurring combination is that of α_2 and β_3 mRNAs. For example, in the nucleus accumbens, caudate nucleus, medial habenula, numerous amygdaloid nuclei, and in many hypothalamic nuclei, the $\alpha_2\beta_3$ pair occurs in combination with various γ-variant mRNAs. In the spinal cord, the $\alpha_2\beta_3$-pair rule also seems to hold, with probable $\alpha_2\beta_3\gamma_2$ complexes occurring on motor neurons (Persohn et al., 1991; Wisden et al., 1991a). The α_2 and β_3 mRNAs also colocalize in the olfactory bulb granule cell layer (Laurie et al., 1992). It is interesting to note that the α_2 and β_3 together with α_5 and β_1 are the most abundant hippocampal mRNAs. The distribution and intensity of the α_5 and β_1 probe hybridization signals appear to be identical throughout most of the brain (with the exception of the olfactory bulb), suggesting that they could well

be partners. Such an $\alpha_5\beta_1$-containing receptor would occur mainly in the hippocampus. The properties of both $\alpha_5\beta_1\gamma_2$ and $\alpha_5\beta_2\gamma_2$ recombinant receptors have been studied in *Xenopus* oocytes and transfected kidney cells (Sigel et al., 1990; Puia et al., 1991), with receptors containing the β_1 subunit being less sensitive to diazepam potentiation of GABA responses (Sigel et al., 1990).

$\alpha_1\alpha_4\beta_2\delta$—thalamic receptors. This set of mRNAs is highly expressed in overlapping areas of the thalamus. With the exception of γ_3 mRNA in the medial geniculate nucleus, γ mRNAs are exiguous in thalamus. This data may be suggestive of an *in vivo* receptor complex containing α_2, α_4, β_2, and δ-subunits of unknown stoichiometry. Such a receptor in the absence of a γ-subunit might be expected to bind $GABA_A$ ligands but not BZs (Pritchett et al., 1989a,b; Shivers et al., 1989), and indeed, the diencephalic distribution of high-affinity ^{3}H-muscimol and ^{3}H$GABA_A$ sites (Palacios et al., 1981; Bowery et al., 1987; Olsen et al., 1990) strikingly resembles the distribution of the α_4 mRNA. For example, high-affinity ^{3}H-muscimol sites are prevalent in thalamus and rare in the hypothalamus. Thalamic BZ sites as assessed by ^{3}H-flunitrazepam binding are fivefold less abundant than ^{3}H-muscimol sites (Olsen et al., 1990), suggesting that the majority of thalamic $GABA_A$ receptors do not bind BZs (Unnerstall et al., 1981). The δ-subunit is probably present in a subset of the high-affinity muscimol binding receptors since it is present in a more limited number of thalamic nuclei than the α_1, α_4, and β_2 mRNAs. Thus, it is possible that many thalamic receptors may be $\alpha_1\alpha_4\beta_2$ receptors. In certain parts of the thalamus, such as the reticular nucleus, α_3 mRNA appears to "replace" that of α_4, a result consistent with immunocytochemical studies using α_3-specific antibodies (Zimprich et al., 1991).

With regard to $\alpha_1\alpha_4\beta_2\delta$ receptors, the evidence for the occurrence of two different α-variants in the same complex is uncertain. An antibody specific for the α_1 subunit coprecipitates under nondenaturing conditions other photolabeled subunits in addition to that of α_1 (tagged by either ^{3}H-flunitrazepam or ^{3}HRo 15-4513), whereas only the labeled α_1 subunit is precipitated under denaturing conditions (Lüddens et al., 1991). On the other hand, other immunoprecipitation studies on bovine brain suggest that the α_1 and α_2 subunits are in largely distinct complexes (Duggan and Stephenson, 1990), in agreement with our *in situ* hybridization data. In *Xenopus* oocytes, addition of α_1 or α_3 to $\alpha_5\beta_1\gamma_2$ combinations made little difference to the receptor properties (Sigel et al., 1990).

Correlation of mRNA levels with protein levels

Our inferences of subunit combinations from differential mRNA distributions is critically dependent on the hypothesis that mRNA levels reflect protein levels. Unfortunately, there is currently no proof that this assumption holds. Additionally, the protein might be located in processes (dendrites, axon terminals) far from the soma where the mRNA resides. Nevertheless, the relative subunit mRNA abundances correlate well with the immunocytochemical results obtained with the small number of specific antibodies tested so far (Benke et al., 1991a,b; Zimprich et al., 1991). For example, using α_1, α_2, and α_3 subunit-specific antibodies on globus pallidus, an anti-α_1-subunit antibody gives a very strong signal, α_2 is present in a small number of cells, and α_3 is absent (Zimprich et al., 1991). Of the three α-subunit antibodies, that of α_2 produces the most intense reaction in dentate gyrus (Zimprich et al., 1991), in line with our *in situ* hybridization results. The protein levels of the δ-subunit appear to follow mRNA levels and distribution faithfully (Shivers et al., 1989; Benke et al., 1991b). There are, however, some discrepancies with the γ_2 results. Some of the strongest γ_2-immunoreactive labeling appeared in the islands of Calleja and in the substantia nigra (Benke et al., 1991a), areas that, although positive for γ_2 mRNA, are not the most marked areas of γ_2 mRNA abundance (Shivers et al., 1989; Malherbe et al., 1990b; present results). The hippocampus, while containing high levels of γ_2 mRNA (considerably higher than substantia nigra), contains only moderate levels of γ_2 immunoreactivity. Although arguments could be made regarding the relative specificites of immune sera, it is possible that some degree of distortion may be present in our inferences resulting from differences in mRNA turnover rates in different cell types.

$GABA_A$ receptor mRNA distribution and pharmacology

The α-subunits. Expression studies on recombinant receptors show that it is the α-subunit class that confers major pharmacological differences with respect to BZs on the receptor complexes $\alpha_x\beta_y\gamma_z$. The α_1 $\beta_2\gamma_2$ complexes display BZ I-type binding, whereas $\alpha_2\beta_3\gamma_2$ and $\alpha_3\beta_1\gamma_2$ combinations display indistinguishable BZ II binding (Pritchett et al., 1989a). $GABA_A$ receptors containing the α_5 subunit also display a BZ II-like pharmacology (Pritchett and Seeburg, 1990), whereas those containing α_4 do not appear to bind BZ agonists (Wisden et al., 1991b). The brain areas expressing the highest level of α_1 mRNA, that is, olfactory bulb, medial septum (Wisden et al., 1991b), globus pallidus, zona incerta, central nucleus of the inferior colliculi, red nucleus, substantia nigra pars reticulata, and cerebellum are precisely those regions that are mainly of the BZ I type (Young et al., 1981; Niddam et al., 1987; Sieghart, 1989). These areas contain relatively low levels of mRNA for the other BZ agonist-binding α-subunit variants (α_2, α_3, α_5). One potential difficulty with the assignment of the $\alpha_1\beta_2\gamma_2$ combination as a BZ I subtype is that BZ I binding is enriched in cortical layer IV (Young et al., 1981; Niddam et al., 1987; Olsen et al., 1990), while the mRNAs are not. However, this could be due to spatial mismatches between receptor protein present in dendrites and mRNA in the soma.

Conversely, the spinal cord (Persohn et al., 1991; Wisden et al., 1991a), the nucleus accumbens, caudate nucleus, and parts of the amygdala have relatively little α_1 mRNA but express highly the α_2 and/or α_3 mRNAs. These areas contain predominantly BZ II sites (Young et al., 1981; Niddam et al., 1987). These data are concordant with *in vitro* expression binding data on the α-subunits. Additionally, areas that have mixed populations of BZ I and BZ II binding sites (e.g., cortex, hippocampus) have mixed populations of mRNAs encoding BZ agonist-binding subunits.

A puzzling observation is that, in receptor autoradiography, the GABA agonists ^{3}H-muscimol and ^{3}H-GABA fail to decorate hypothalamic and amygdaloid areas (Bowery et al., 1987; Olsen et al., 1990), even though in both of these regions α_2 and β_3 mRNAs are well expressed. It is possible that α_2/β_3 subunit-containing receptors have a high affinity for certain GABA antagonists, since the distribution of the $GABA_A$ antagonist ^{3}H-SR-95531 matches the distribution of α_2 mRNA (Bristow and Martin, 1988; Olsen et al., 1990); its binding in cortex decreases from superficial to deep layers, and the highest density of binding is in the hippocampus and nucleus accumbens, with intermediate levels present in caudate nucleus. Low densities of sites are observed in thalamic nuclei, substantia nigra, and both layers of the cerebellum.

It appears that forebrain also contains an unusual type of $GABA_A$ receptor, constructed in part from the α_4 subunit. The recombinant ($\alpha_4\beta_x\gamma_2$) is characterized by ^{3}H-Ro 15-4513 binding not displaceable by diazepam (Wisden et al., 1991b). The properties of this $\alpha_4\beta_x\gamma_2$ subtype are reminiscent of cerebellar α_6 subunit–containing $GABA_A$ receptors (Lüddens et al., 1990). Although α_4 mRNA is abundant in many forebrain areas, Ro 15-4513 binding that is resistant to BZ agonist displacement does not seem very common except in the cerebellum (Sieghart et al., 1987; Turner et al., 1991). However, a low amount of diazepam-insensitive Ro 15-4513 binding has been detected in cortex, hippocampus, and striatum, although not in the thalamus (Turner et al., 1991). This may suggest that a fraction of the α_4 subunits combines with γ-subunits in some brain regions (cortex, hippocampus, striatum), but not in other areas. For example, the thalamus is the principal region for α_4 gene expression, but because of the relative scarcity of γ-subunit mRNAs, thalamic α_4 containing receptors may exhibit binding of muscimol but not BZs (see below).

The β-subunits. In recombinant receptors assembled from α-, β-, and γ-subunits, the three β-subunits appear to be functionally interchangeable isoforms (Pritchett et al., 1989a; Ymer et al., 1989b), yet clearly their marked differential distributions of mRNAs (see also Lolait et al., 1989; Zhang et al., 1990; Laurie et al., 1992) would suggest some functional significance for β-variants. Recently, a fourth β cDNA variant has been isolated from avian brain cDNA libraries (Bateson et al., 1991b), but it is not at present clear if a rat β_4 homolog exists or whether it represents an avian idiosyncrasy. So far, the only differences reported for recombinant β-subunits (β_1 vs. β_2) are those of current amplitudes in receptors expressed in *Xenopus* oocytes (Sigel et al., 1990). This could simply be due to different efficiencies of protein expression in the oocyte system. However, other subtle differences may emerge if the cloned β-subunits are tested with their most likely *in vivo* partners. For example, it has been suggested that natural receptors containing β_2 and β_3 subunits differ in their affinity to GABA analogs and pentobarbital (Bureau and Olsen, 1990).

The γ-subunits. The γ-subunits are required for allosteric potentiation by BZs of α- and β-subunit–containing complexes (Pritchett et al., 1989b; Ymer et al., 1990; Herb et al., 1992). Of the three known members, the γ_2 mRNA is the most universal and abundant. It is also the most studied in terms of function (Pritchett et al., 1989a,b; Sigel et al., 1990; Verdoorn et al., 1990). Additional complexity has recently arisen in that the γ_2 mRNA exists in two splice versions, resulting from an exonic insertion into the cytoplasmic loop between transmembrane segments M3 and M4 (Whiting et al., 1990; Kofuji et al., 1991; Wafford et al., 1991). This splicing event is predicted to generate a γ_2 polypeptide with the addition of a target sequence for protein kinase C. As assessed by PCR analysis, the relative abundances of the γ_2 splice variants depend on the brain region (Whiting et al., 1990). However, our γ_2 probe would hybridize to both of these mRNA versions.

In certain limbic areas of the brain such as amygdala, hypothalamus, and septum, γ_2 mRNA appears to be replaced by considerable amounts of γ_1 mRNA. Thus, future compounds selective for γ_1 subunit–containing $GABA_A$ receptors might be expected selectively to modulate neurons of affective circuits. The γ_3 subunit would appear to contribute to only a minority of CNS $GABA_A$ receptors, as assessed by its low mRNA abundance. Yet other regions, for example, the tenia tecta and the thalamus, contain none or very little, respectively, of any of the known γ-subunit mRNAs, even though high levels of non-γ-subunit transcripts are detected in these regions. How the different γ-subunits affect the functional properties of the α- and β-subunits is a largely unexplored area, and many of the combinations we have suggested here have not yet been subjected to binding or electrophysiological analysis. However, different γ-subunits can differentially modulate responsiveness of α-subunits to BZs and related compounds (Ymer et al., 1990; Herb et al., 1992). Specifically, the γ_1 subunit confers positive modulation by β-carbolines and DMCM (6,7-dimethoxy-4-ethyl-β-carboline-3-carbolic acid methyl ester) on $\alpha_x\beta_1$ complexes (Puia et al., 1991), and the replacement of γ_2 with either γ_1 or γ_3 leads to a general lowering of the response to BZs. Consequently, the γ_2 subunit may not always be the appropriate γ-subunit to use when testing α-subunit pharmacology *in vitro*.

The δ-subunit. This subunit's role in $GABA_A$ receptor function has remained an enigma. In a large number of regions (principally thalamic nuclei) it colocalizes with the α_1, α_4, and β_2 mRNAs, although its distribution is more restricted (see Fig. 11). These results suggest that *in vitro* experiments should be designed with recombinant receptors containing both α_4 and δ-subunits. As originally suggested for the δ-subunit alone (Shivers et al., 1989), $\alpha_1\alpha_4\beta\delta$-containing receptors may have high affinity to muscimol but lack BZ binding sites. In the cerebellum, $\alpha_1\alpha_6\beta\delta$-containing receptors would correspond to high-affinity muscimol sites in the granule cell layer (Laurie et al., 1992). Thus, δ-subunits may preferentially associate with α-subunits that do not bind BZ agonists.

Conclusions

A number of plausible $GABA_A$ receptor combinations have been inferred based on mRNA distribution. Immunoprecipitation and immunocytochemical studies in addition to modern patch-clamping methodology on brain slices (Edwards et al., 1989) could be used to test the validity of these predictions, using recombinant receptors as a reference. Although the original classification of BZ I and BZ II receptors could be criticized for being too reductionistic, it seems that a systematic comparison of $GABA_A$ receptor subunit distributions in the brain still enables this distinction to be maintained with qualifications. Thus, based on their patterns of gene expression and recombinant expression studies, we would propose that the $\alpha_1\beta_2\gamma_2$ receptors correspond to the BZ I subtype, and receptors containing $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_x\gamma_2$, and $\alpha_5\beta_1\gamma_2$ are three subtypes of BZ II receptor. Additional as yet unclassified subtypes of receptor would emerge if these subunits occur with the γ_1 or γ_3 subunits. The receptors containing α_4 and α_6 would diverge from the main family of BZ receptors because of their very restricted BZ ligand binding profile *in vitro*. Indeed, in some brain regions the α_4 subunit may contribute to BZ-insensitive $GABA_A$ receptors because its mRNA fails to colocalize with that of any known γ-subunit.

The physiological significance of such $GABA_A$ receptor complexity remains to be determined. Low-affinity receptors on presynaptic terminals could serve as autoreceptors mediating negative feedback of transmitter release. Additionally, different $GABA_A$ receptor complexes could differ in mean channel open time or desensitization rate, with constraints of neuronal geometry dictating the type of desensitization rate or channel conductance that is required to produce a given hyperpolarization per unit time. Alternatively, neurons receiving a strong excitatory input may require $GABA_A$ receptors of greater conductance and slower densensitization rates than neurons in which such input is less. Analogous situations for receptor diversity also extend to other ligand-gated ion channels in brain, most notably the neuronal nicotinic receptors (Wada et al., 1989; Morris et al., 1990), glycine receptors (Betz, 1991; Malosio et al., 1991), and glutamate receptors (Bettler et al., 1990; Sommer et al., 1990; Monyer et al., 1991; Werner et al., 1991). Future experimental directions could involve homologous recombination experiments to see if it is possible to dissect out the function of the different receptor subtypes.

Appendix

List of anatomical abbreviations

aca	anterior commissure, anterior
Acb	accumbens nucleus
Arc	arcuate hypothalamic nucleus
AV	anteroventral thalamic nucleus
BST	bed nucleus, stria terminalis
CA1–4	fields 1–4 of Ammon's horn
Cb	cerebellum
Ce	central amygdaloid nucleus
CG	central gray
CGD	central gray, dorsal
Cl	claustrum
CL	centrolateral thalamic nucleus
CPu	caudate putamen
Ctx	neocortex
CtxII	neocortex, layer 2
DA	dorsal hypothalamic area
DB	diagonal band
DG	dentate gyrus
DLG	dorsal lateral geniculate thalamic nucleus
DM	dorsomedial hypothalamus
EP	endopeduncular nucleus
fr	fasciculus retroflexus
GP	globus pallidus
Hy	hypothalamus
ic	internal capsule
IC	inferior colliculus
ICj	islands of Calleja
IL	infralimbic cortex
La	lateral amygdaloid nucleus
LD	laterodorsal thalamic nucleus
LPMC	lateral posterior thalamic nucleus, mediocaudal
LS	lateral septum
MD	mediodorsal thalamic nucleus
Me	medial amygdaloid nucleus
MG	medial geniculate nucleus
MHb	medial habenular nucleus
MPO	medial preoptic nucleus
OB	olfactory bulb
Op	optic nerve layer, superior colliculus
PF	parafascicular thalamic nucleus
Pir	piriform cortex
PV	paraventricular thalamic nucleus
R	red nucleus
Rh	rhomboid thalamic nucleus
Rt	reticular thalamic nucleus
SC	superior colliculus
SNC	substantia nigra pars compacta
SNR	substantia nigra pars reticulata
SPF	subparafascicular thalamic nucleus
STh	subthalamic nucleus
SUG	superficial gray layer, superior colliculus
TS	triangular septal nucleus
tt	tenia tecta
VLG	ventral lateral geniculate nucleus
VMH	ventromedial hypothalamic nucleus
VP	ventral posterior thalamic nucleus
VPM	ventral posteromedial thalamic nucleus
ZI	zona incerta

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