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The Distribution of Thirteen $GABA_A$ Receptor Subunit mRNAs in the Rat Brain. III. Embryonic and Postnatal Development

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The embryonic and postnatal expression of 13 $GABA_A$ receptor subunit genes in the rat CNS was studied by *in situ* hybridization. Each transcript exhibited a unique regional and temporal developmental expression profile. For example, in both embryonic and early postnatal cortex and thalamus, expression of the α_2, α_3, α_5, and β_3 mRNAs was pronounced. In particular, the α_5 gene expression underwent a prominent peak in early brain. Subsequently, the thalamocortical expression of these four genes substantially diminished and was superseded in the adult by the α_1, α_4, β_2, and δ subunit mRNAs. Similarly, γ_1 and γ_3 gene expression also dropped markedly during development, their initial stronger expression being restricted to relatively few structures. In contrast, γ_2 gene expression was widespread and mostly remained constant with increasing age. The medial septum and globus pallidus were regions expressing few subunits in both early postnatal and adult stages, allowing clear developmental combinatorial changes to be inferred ($\alpha_2/\alpha_3\beta_2\gamma_2$ to $\alpha_1\beta_2\gamma_2$, $\alpha_2/\alpha_3\beta_2\gamma_1$ to $\alpha_1\beta_2\gamma_1/\gamma_2$, respectively). In contrast, cerebellar Purkinje cells exhibited no developmental switch, expressing only the α_1, β_2, β_3, and γ_2 mRNAs from birth to adult. Certain $GABA_A$ transcripts were also detected in germinal zones (e.g., β_1, β_3, γ_1) and in embryonic peripheral tissues such as dorsal root ganglia (e.g., α_2, α_3, β_3, γ_2) and intestine (γ_3). Some parallels in regional and temporal CNS expression were noted (e.g., $\alpha_1\beta_2$, $\alpha_2\beta_3$, $\alpha_4/\alpha_6\delta$), whereas the α_5 and β_1 regional mRNA expressions converged over time. The changes of $GABA_A$ receptor subunit gene expression suggest a molecular explanation for earlier observations on changing ligand binding affinities. Thus, the composition, and presumably properties, of embryonic/early postnatal rat $GABA_A$ receptors differs markedly from those expressed in the adult brain.

In the adult vertebrate brain, the inhibitory neurotransmitter GABA (γ-aminobutyric acid) mediates fast inhibitory neurotransmission by gating chloride channels intrinsic to $GABA_A$ receptors (Olsen and Tobin, 1990). Although both GABAergic neurons and high levels of GABA are found in the fetal and neonatal CNS (Coyle and Enna, 1976; Lauder et al., 1986; Seress and Ribak, 1988; Meinecke and Rakic, 1990; Cobas et al., 1991), the role of the perinatal $GABA_A$ system appears to differ substantially from that in adult CNS. For example, the immature brain is poorly protected against seizure disorders by the $GABA_A$ system (Aicardi and Chevrie, 1970; Mecarelli et al., 1988). In the fetal and neonatal hippocampus, GABA-activated chloride channels lead to marked membrane *de*polarization (Ben-Ari et al., 1989; reviewed by Cherubini et al., 1991). Furthermore, activation of neonatal $GABA_A$ receptors induces a rise in intracellular calcium concentration in both cerebellar and cortical neurons (Connor et al., 1987; Yuste and Katz, 1991), probably as a result of membrane depolarization and activation of voltage-sensitive calcium channels. Raised intracellular calcium is an important factor in neuronal growth and differentiation (Kater and Guthrie, 1990; Spitzer, 1991). Consistent with this observation, in primary culture of several embryonic and neonatal brain tissues, GABA, perhaps in concert with glutamate, exerts a variety of pronounced neurotrophic actions, including promotion of neurite extension, synaptogenesis, and the synthesis of its own receptors (Hansen et al., 1987; Meier et al., 1987; Wolff et al., 1987; Kater and Guthrie, 1990). Consistent with an important role of $GABA_A$ receptors in development, experimental administration of benzodiazepines during pregnancy causes biochemical and behavioral manifestations in the progeny that can persist into adulthood (Simmons et al., 1984a,b; Kellogg, 1988).

Perhaps commensurate with the different roles of GABA in the neonate and adult, the pharmacological properties of $GABA_A$ receptors change during rat and primate brain development. For example, the proportions of $GABA_A$ and benzodiazepine (BZ) receptor subtypes alter, with low-affinity $GABA_A$ receptors appearing later than those of high affinity, and type II and I BZ receptors predominating in the neonate and adult, respectively (Chisholm et al., 1983; Madtes, 1987; Meier et al., 1987; Reichelt et al., 1991).

The subunit composition of perinatal $GABA_A$ receptors remains undefined. $GABA_A$ receptors are believed to be pentameric and composed, in unknown ratios, of subunits from several related sequence classes (Unwin, 1989). In the rodent, 13 subunit genes have been identified and subdivided into α, β, γ, and δ classes (reviewed by Olsen and Tobin, 1990; Seeburg et al., 1990; Lüddens and Wisden, 1991; Wisden and Seeburg, 1992). The properties of recombinant $GABA_A$ receptors depend upon the subunits from which they are assembled (Lüddens and Wisden, 1991; Wisden and Seeburg, 1992). Regional differences

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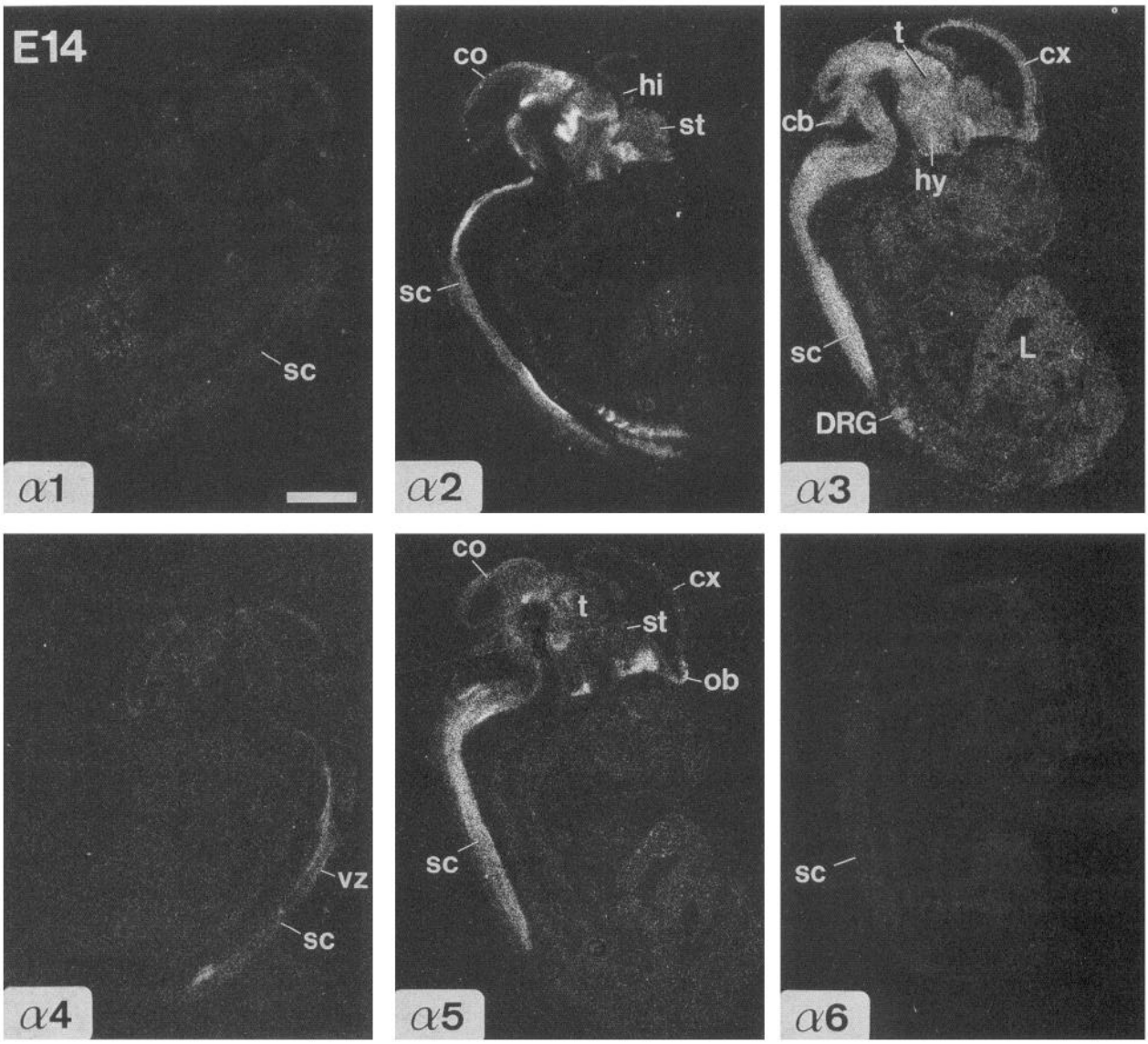


Figure 1. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor α_1–α_6 mRNAs in sagittal sections of E14 rat embryos. See Appendix for abbreviations. The apparent labeling of liver by some probes was nonspecific. Scale bar, 2 mm.

in subunit gene expression (Laurie et al., 1992; Wisden et al., 1992) could therefore account for the pharmacological diversity of $GABA_A$ receptors in the adult rat CNS (Unnerstall et al., 1981; Young et al., 1981; Niddam et al., 1987; Olsen et al., 1990), and probably also underlie the changing $GABA_A$ pharmacology during development. Northern and Western analyses have indicated age-related changes in α_1, α_2, α_3, α_5, and β_1 subunit expression in rat brain, but have not shown in detail which brain regions are affected (Montpied et al., 1989; Garrett et al., 1990; MacLennan et al., 1991; McKernan et al., 1991a). Regional changes in expression of a limited number of subunit genes (α_1, β_1, β_2, β_3, and γ_2) in postnatally developing rat brain have been reported using *in situ* hybridization (Gambarana et al., 1990, 1991; Zhang et al., 1991). We recently mapped the mRNA distribution of the 13 $GABA_A$ subunits (α_1–α_6, β_1–β_3, γ_1–γ_3, δ) in adult rat brain to deduce subunit combinations found *in vivo* (Laurie et al., 1992; Wisden et al., 1992). We have extended these studies with a comprehensive examination of $GABA_A$ receptor subunit gene expression during both embryonic and postnatal development. We now report that expression of each $GABA_A$ receptor subunit gene changes during early development. These changes appear to coincide with the alteration of GABA's role from putatively excitatory, neurotrophic factor to inhibitory neurotransmitter.

Materials and Methods

For each $GABA_A$ receptor subunit (α_1–α_6, β_1–β_3, γ_1–γ_3, δ), a unique 45-base antisense oligonucleotide was employed. Note that the subunit termed α_5 by us and others (Malherbe et al., 1990; Pritchett and Seeburg, 1990) has also been termed α_4 by Khrestchatisky et al. (1989). The α_4 subunit referred to in this article is as described by Ymer et al. (1989) and Wisden et al. (1991a). The oligonucleotide sequences and experimental procedures (labeling, hybridization, posthybridization washing) were as described by Wisden et al. (1991b, 1992). Probes were 3′ end-labeled using terminal deoxynucleotidyl transferase (Bethesda Research Labs) and a 30:1 molar ratio of (α-^{35}S)dATP (1200 Ci/mmol; Amersham). Nonperfused rat brains or whole rat embryos were removed and frozen on dry ice prior to sectioning on a cryotome. *In situ* hybridization was performed on sagittal sections (14 μm) of whole embryos of 14, 17, and 19 d of gestation (E14, E17, and E19), and on horizontal sections

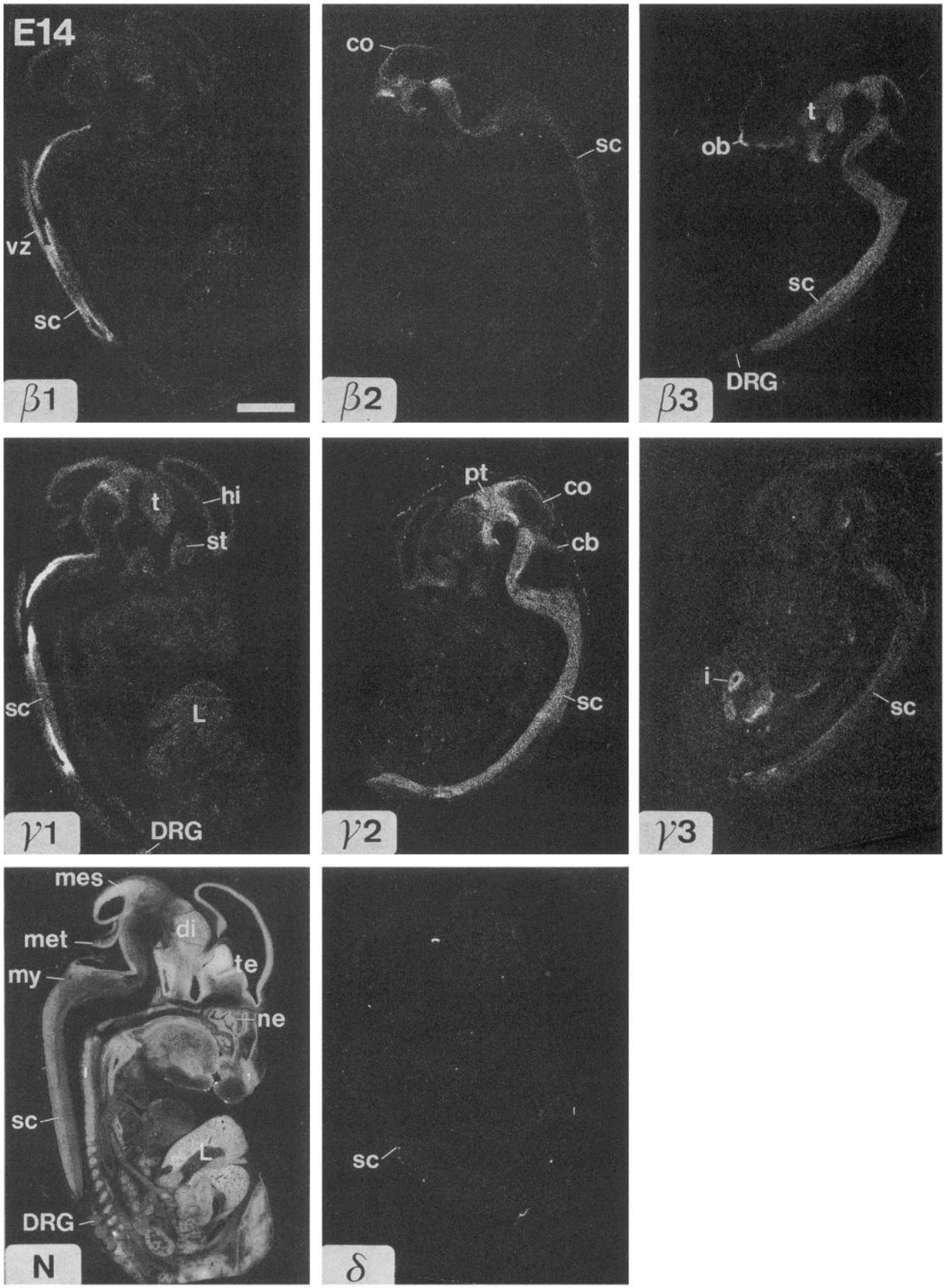


Figure 2. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor β_1–β_3, γ_1–γ_3, and δ mRNAs in sagittal sections of E14 rat embryos. *N*, Nissl (thionin) stain of whole embryo section. See Appendix for abbreviations. The apparent labeling of liver by some probes was nonspecific. Scale bar, 2 mm.

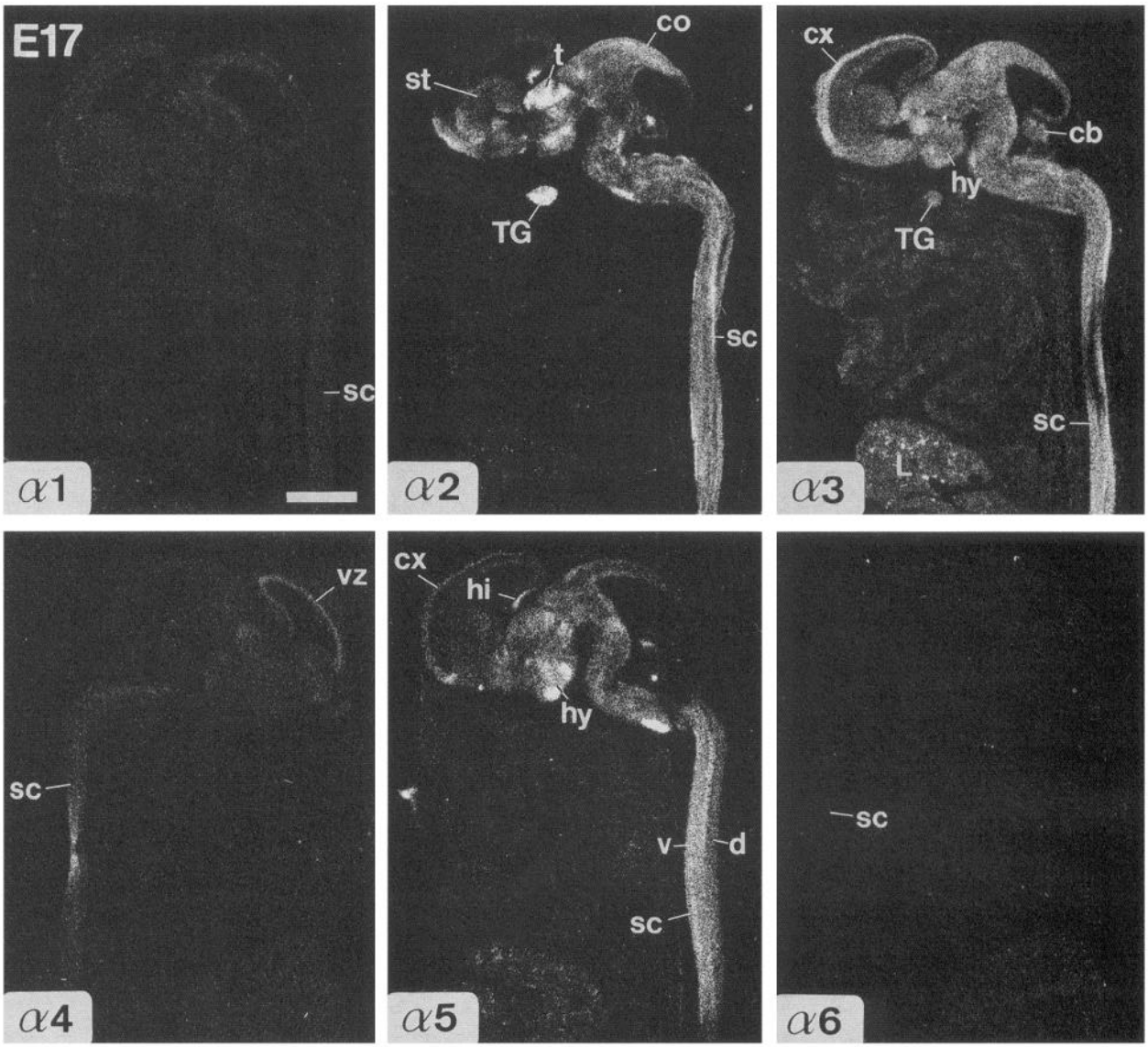


Figure 3. X-ray film autoradiographs illustrating distribution of GABA$_A$ receptor α_1–α_6 mRNAs in sagittal sections of E17 rat embryos. See Appendix for abbreviations. The apparent labeling of liver by some probes was nonspecific. Scale bar, 2 mm.

of brains taken from rats of postnatal ages 0, 6, and 12 d (P0, P6, P12) and from adult males. Embryonic ages were calculated from the end of a 4 hr mating period and confirmed by examination of paw development (Rugh, 1991). P0 indicates the day of birth. Sex determination was performed only on adult rats. In order to confirm developmental changes, two sections from each of three animals at each age were hybridized and examined for each probe. After hybridization [50% formamide, 4× saline–sodium citrate (SSC), 10% dextran sulfate; 42°C] and washing (1× SSC, 60°C), sections were exposed to Kodak SB-5 film or dipped in photographic emulsion (Ilford K5). Anatomy of autoradiographs and thionin-stained sections was determined using the atlases of Paxinos and Watson (1986) and Paxinos et al. (1991). Microscopic examination of emulsion-coated sections was performed for every described structure in order to determine the cellular locations reported. Signal specificity was assessed by competition experiments in which radiolabeled probes were hybridized to sections in the presence of excess (100-fold) unlabeled probe. This resulted in virtually blank autoradiographs, except for some nonspecific labeling of peripheral tissues. The assessment of the specificity of the probes has been described previously (Wisden et al., 1992). Photomicrographs were obtained using a Zeiss Axioplan microscope under bright- and dark-field optics.

Expression levels of representative mRNAs (α_1, α_2, α_5, β_3, γ_2) were quantified in selected brain regions by measurement of x-ray film optical densities using an Amersham RAS optical densitometric system. The weak signals due to nonspecific hybridization in each region were subtracted from the corresponding total hybridization value to yield a value for specific hybridization. The values from three subjects were averaged.

Results

In the developing brain, a specific regional and temporal expression pattern occurred for each of the 13 GABA$_A$ receptor subunit mRNAs (illustrated in Figs. 1–15, with quantified values presented in Fig. 16; summarized in Tables 1 and 2). Embryonic stage E14 was chosen as an initiation point as this age is prior to generation, migration, and differentiation of most telencephalic and mesencephalic neurons (Jacobson, 1978; Jones, 1985). The α_1, α_6, and δ mRNAs appeared postnatally and increased in expression markedly with age (see Table 2). The transcript for α_6 was confined to the postnatal cerebellum. (Note that in this context, the term "transcript" used throughout the text denotes mature mRNA and not necessarily the primary mRNA.) The β_2 and γ_2 transcripts were present in the embryo, and their

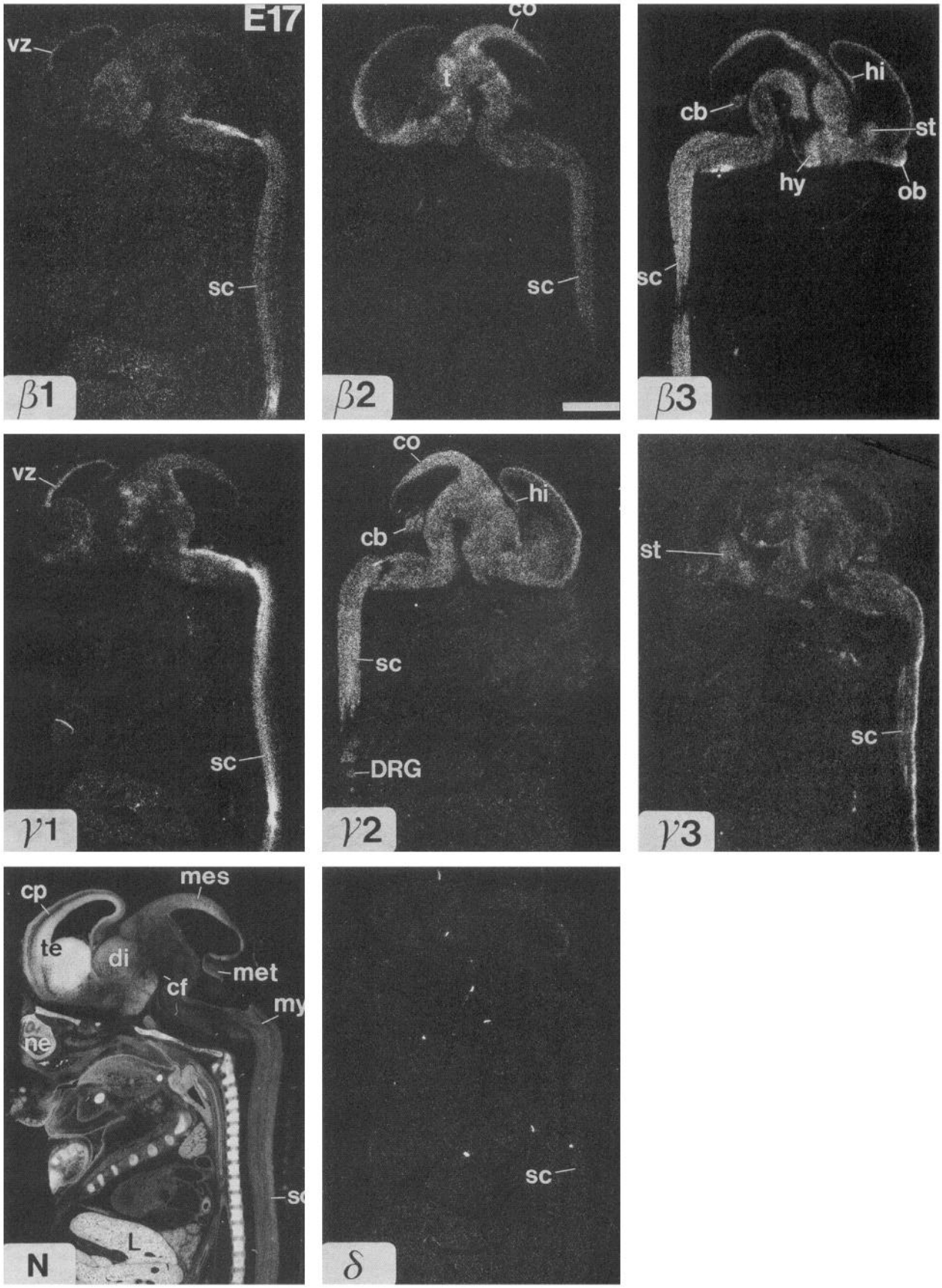


Figure 4. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor β_1–β_3, γ_1–γ_3, and δ mRNAs in sagittal sections of E17 rat embryos. *N*, Nissl (thionin) stain of whole embryo section. See Appendix for abbreviations. The apparent labeling of liver by some probes was nonspecific. Scale bar, 2 mm.

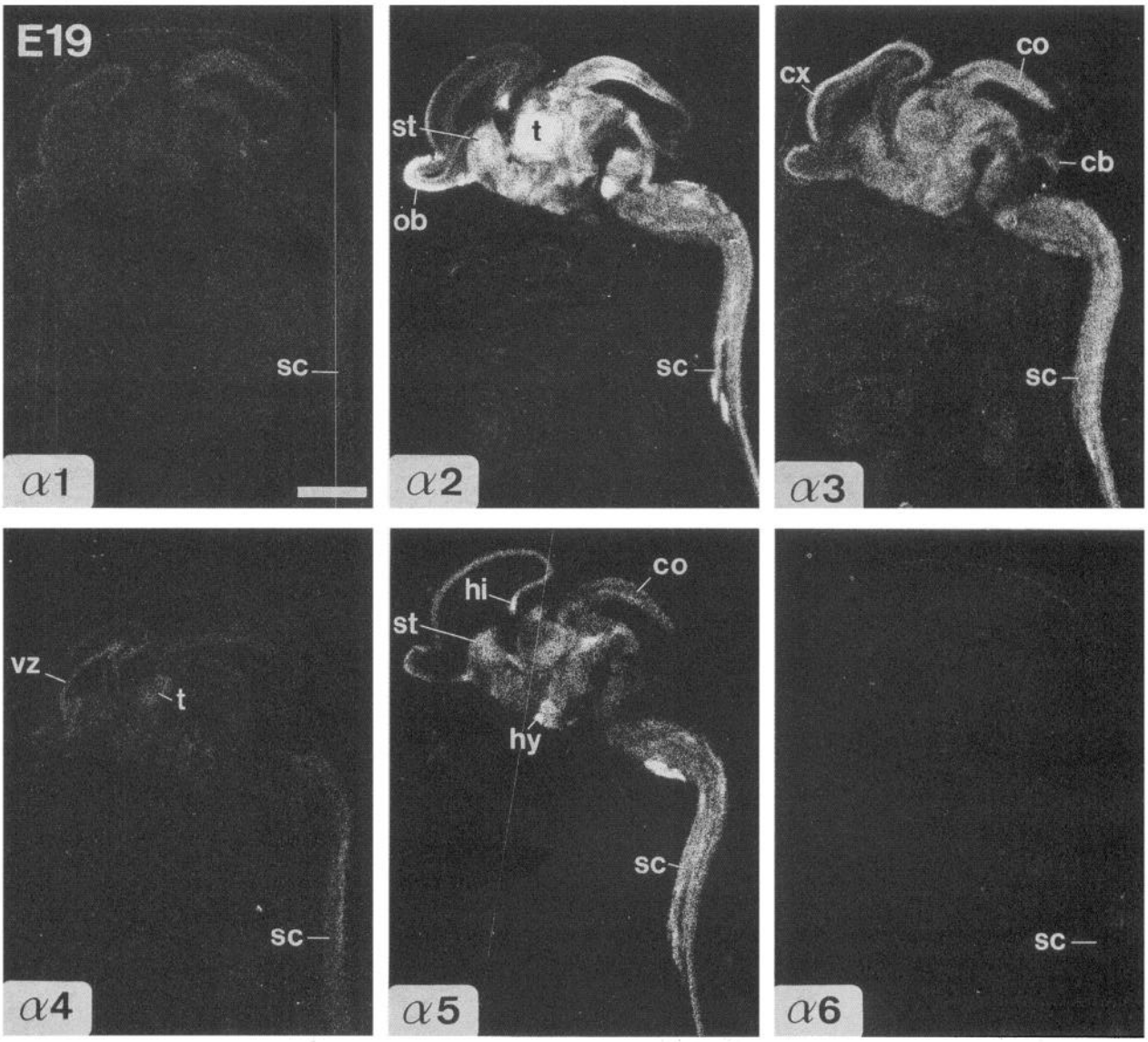


Figure 5. X-ray film autoradiographs illustrating distribution of GABA$_A$ receptor α_1–α_6 mRNAs in sagittal sections of E19 rat embryos. See Appendix for abbreviations. The apparent labeling of liver by some probes was nonspecific. Scale bar, 2 mm.

Table 1. Summary of primary GABA$_A$ receptor subunit mRNAs expressed in selected regions of perinatal and adult rat brain

Structure	Perinate (E19–P6)	Adult
Olfactory bulb		
Mitral cells	$\alpha_1\alpha_2\alpha_3(\alpha_4)\alpha_5$	$\alpha_1(\alpha_3\alpha_4)$
	$\beta_1\beta_2\beta_3\gamma_1\gamma_2$	$\beta_1\beta_2\beta_3\gamma_2$
Thalamus	$\alpha_2\alpha_3\alpha_4\alpha_5\beta_3\gamma_1$	$\alpha_1\alpha_4\beta_2\delta$
Medial septum	$\alpha_2\alpha_3\beta_2\gamma_2$	$\alpha_1\beta_2\gamma_2$
Striatum		
Caudate	$\alpha_2\alpha_3\beta_3(\gamma_3)$	$\alpha_2\alpha_4\beta_3\delta$
Globus pallidus	$\alpha_2(\alpha_3)\beta_2\gamma_1$	$\alpha_1\beta_2(\gamma_1\gamma_2)$
Cerebellum		
Purkinje cells	$\alpha_1\beta_2\beta_3\gamma_2$	$\alpha_1\beta_2\beta_3\gamma_2$
Granule cells	$(\alpha_1\alpha_2\alpha_3\alpha_4\alpha_6)$	$\alpha_1\alpha_6\beta_2\beta_3\gamma_2\delta$
	$(\beta_1\beta_2\beta_3\gamma_1\gamma_2\gamma_3)$	

Parentheses indicate lesser, but not insignificant, levels of expression. Adult expression data were constructed from Wisden et al. (1992), Laurie et al. (1992), and present results.

expression increased after birth. In contrast, a postnatal decline in expression was observed for α_3 mRNA, which was abundant in the embryo. Finally, a third pattern involving a peak of expression was apparent for α_2 (E19–P6), α_4 (P12), α_5 (P6), β_1 (P6–P12), β_3 (P12), γ_1 (P0–P6), and γ_3 (P6) transcripts (see Table 2). Regional expression changes are described below. Since expression was not examined between P12 and adult ages, descriptions of peak expression at P12 relate to comparison of P12 and adult levels and do not preclude the possibility of maximal expression at a later age.

Cortex

In neurons of the developing rat cortex, considerable changes occurred between the principal subunit mRNAs expressed perinatally and in maturity. The α_3 transcript was the most prominent throughout pre- and early postnatal development (Figs. 1–7), declining after P12 to relatively low adult levels (Figs. 9–13, Table 2). The α_2 and α_5 mRNAs appeared later in cortical neurons (E17/E19; Figs. 3, 5), quickly increased (four- and two-

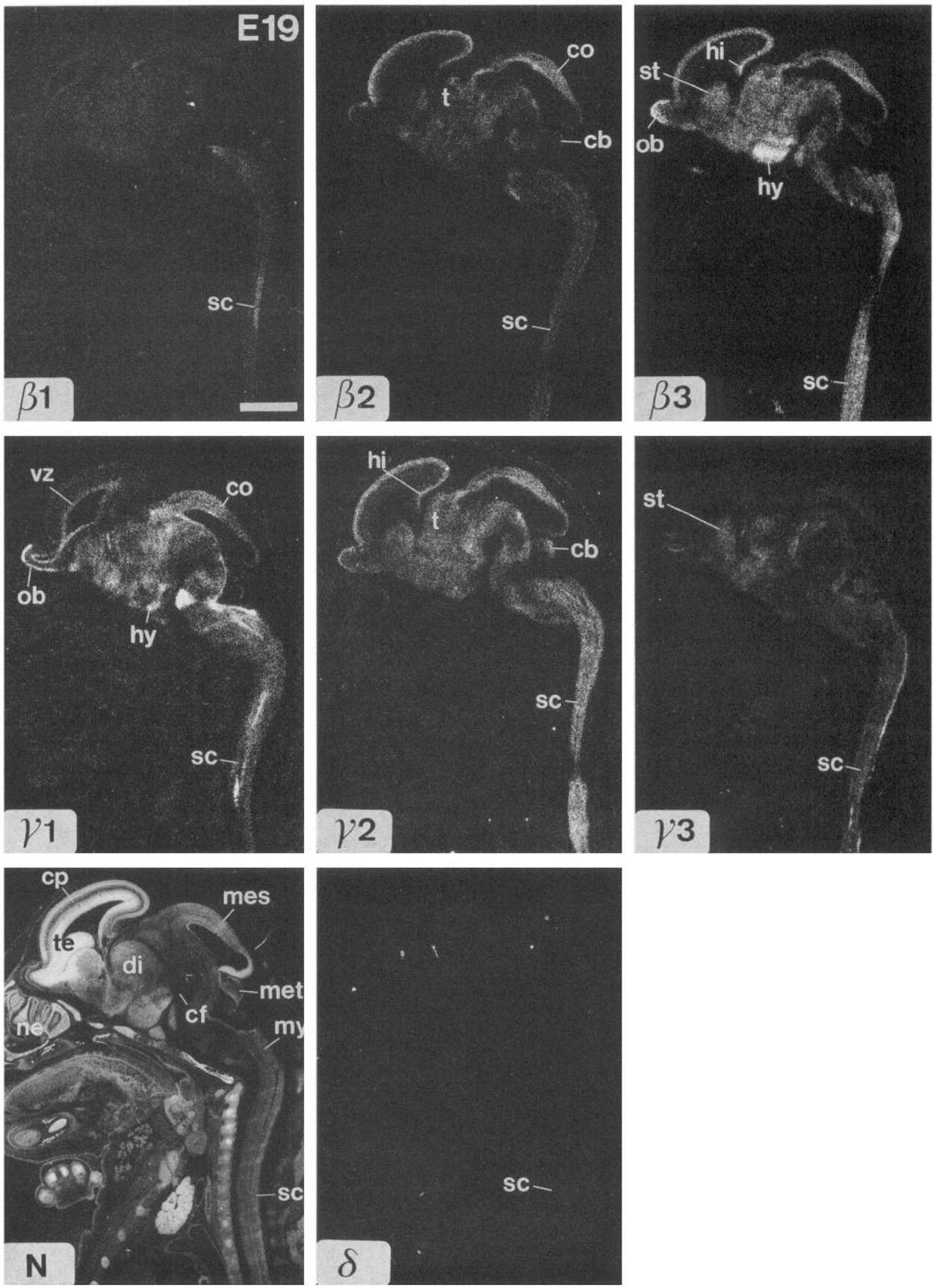


Figure 6. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor β_1–β_3, γ_1–γ_3, and δ mRNAs in sagittal sections of E19 rat embryos. *N*, Nissl (thionin) stain of whole embryo section. See Appendix for abbreviations. The apparent labeling of liver by some probes was nonspecific. Scale bar, 2 mm.

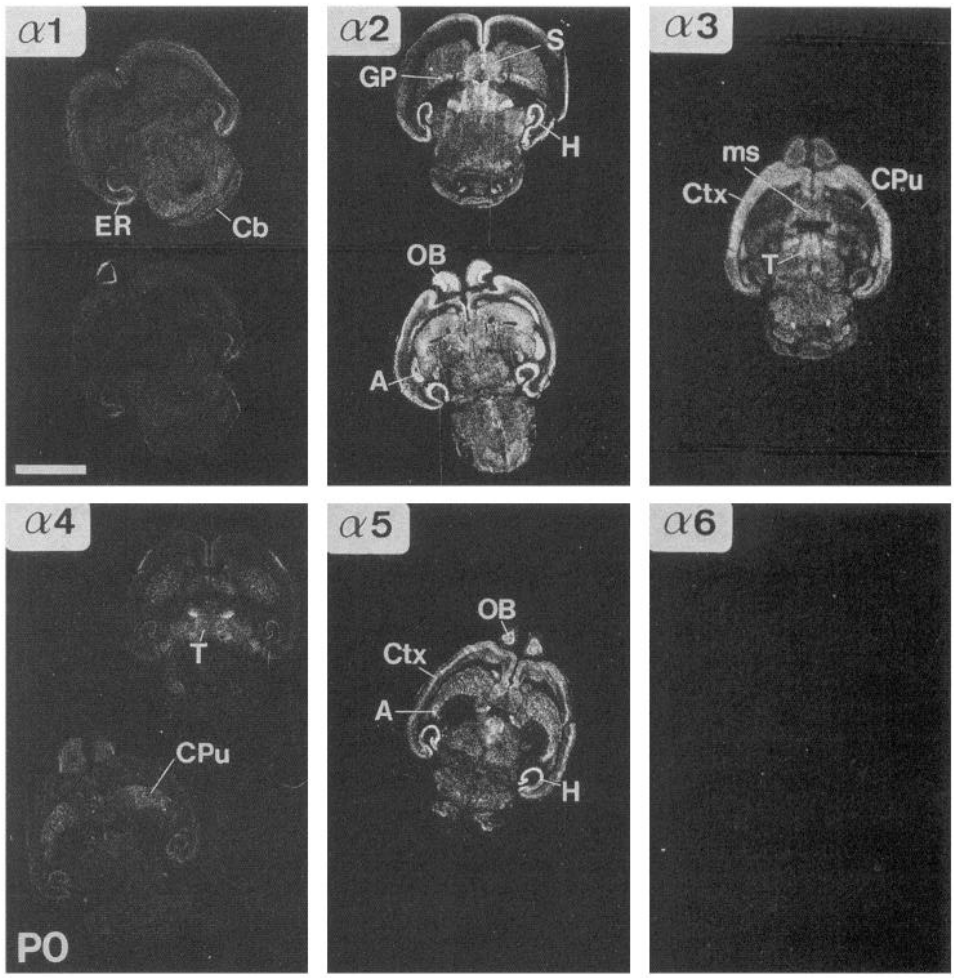


Figure 7. X-ray film autoradiographs illustrating distribution of GABA$_A$ receptor α_1–α_6 mRNAs in horizontal sections of P0 rat brain. See Appendix for abbreviations. Scale bar, 4 mm.

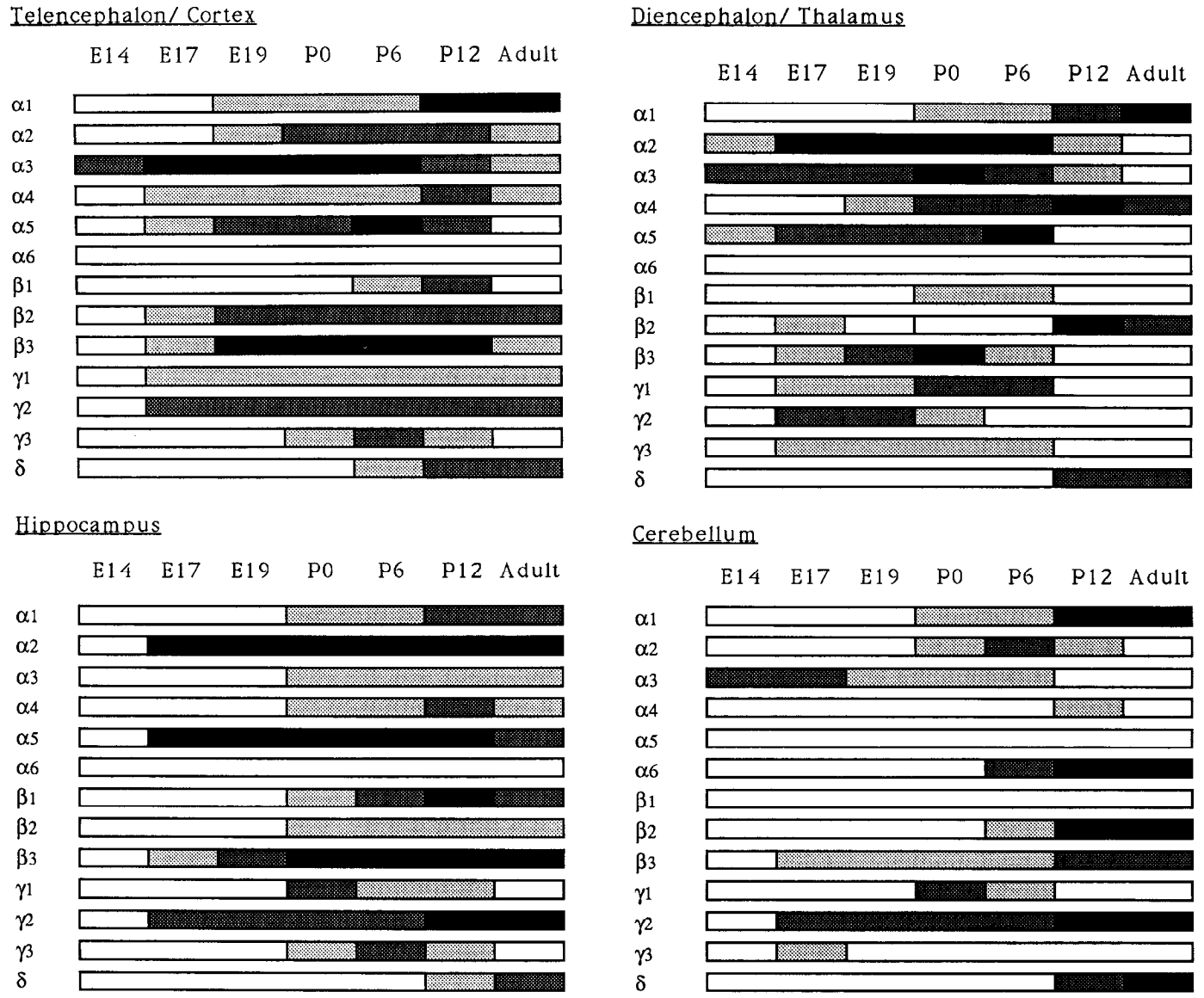
fold, respectively, in whole neocortex as measured by optical densities) to prominent peaks of expression around P6 (Figs. 7, 9, 16), and subsequently also declined to adult levels (one-half and one-sixth of the respective peak expression; Figs. 11, 13, 16; Table 2). In contrast, cortical expression of the α_1 and α_4 genes increased (Figs. 1–5), especially after birth (Figs. 7, 9, 16), reaching levels at or slightly above adult by P12 (fivefold increase for α_1 mRNA; Figs. 11, 13, 16; Table 2). Beginning at low degrees of expression at E17 (Fig. 4), the β subunit mRNAs increased at different rates ($\beta_3 > \beta_2 > \beta_1$), each reaching a peak in expression (β_1 and β_2 at P12, β_3 at E19–P12; Figs. 6–14, 16). Transcripts for γ_1 and γ_2 were detected only at a very low level at E14 (Fig. 2). Thereafter, whereas cortical γ_1 mRNA expression showed a slight peak at P0, γ_2 mRNA reached a moderate level by birth, which continued into maturity (Figs. 4–14, 16). Cortical expression of γ_3 and δ mRNAs appeared around P0 and peaked at P6 and P12, respectively (Figs. 8–14, Table 2).

Each subunit transcript, besides exhibiting its own temporal pattern, also showed regional specificities of expression within the cortex. At E14, the cortex consists of a primordial plexiform layer that is split before E17 by the cortical plate of postmitotic neuroblasts (Jacobson, 1978; Miller, 1988). At E17 and E19, the α_1, α_2, α_5, β_2, and β_3 mRNAs were most abundant in the outer cortical layer (layer I), the α_3 and γ_2 transcripts were found in both outer and inner (subplate) layers, and the α_2, α_4, β_1, and γ_1 mRNAs were mainly located in the lower intermediate or ventricular zones (Figs. 3–6). No subunit transcript appeared to be expressed in the cortical plate. Stratification of cortical neurons (layers I–VI) is established by P6 (Jacobson, 1978; Miller, 1988) and the laminated patterns of transcript expression at this age were mostly continued into adulthood (see also Wisden et al., 1992). The α_2, α_4, and δ mRNAs were found principally in outer layers, while α_3 mRNA expression became restricted to more deeper regions. The γ_1 and γ_3 mRNAs were expressed almost homogeneously, whereas the α_1, the three β, and the γ_2 transcripts were detected mainly in superficial and deep cortical layers (Figs. 9–12). In contrast, α_5 mRNA was predominantly found in middle layers until P12, but in deep cortical layers in the adult (Figs. 7–13). In the postnatal entorhinal cortex, expression of all subunit mRNAs, except that of α_3 and δ, was higher than in neighboring cortex (e.g., Figs. 9–12). These patterns were maintained into adulthood, despite changes in absolute abundance.

Hippocampus and septum

Gene expression in the hippocampal formation was examined from the beginning of its formation (after E14; Stanfield and Cowan, 1988). In contrast to the changing predominance of GABA$_A$ subunit mRNAs in the cortex, the main transcripts found in the mature hippocampal CA regions (α_2, α_5, β_3, γ_2) (Figs. 13, 14; Table 2) predominate from an early stage (E17; Figs. 3, 4), soon after the neurons have finished migrating (Stan-

Table 2. Schematic representation of the expression of the $GABA_A$ receptor α_1-α_6, β_1-β_3, γ_1-γ_3, and δ subunit mRNAs in selected entire regions of the embryonic and postnatal rat brain



Black, strong signal; dark gray, moderate signal; light gray, weak signal; white, very weak or undetectable signal.

field and Cowan, 1988). In the dentate gyrus, a structure formed mainly postnatally (Stanfield and Cowan, 1988), adult expression patterns are quickly established (Figs. 7–10). No subunit mRNA was detected in E14 hippocampus (Figs. 1, 2), but at E17 and thereafter, marked expression of α_2 and α_5 mRNAs was obvious throughout the hippocampus (Figs. 3–11, 16). The expression of both mRNAs declined slightly (by 25%) after P12 in CA1 and CA3, and α_5 mRNA was found at lower levels in adult dentate gyrus (Figs. 13, 16). Transcripts for β_3 and γ_2 were also detected at low levels in E17 hippocampus, and subsequently increased rapidly (Figs. 4, 6), β_3 mRNA reaching a strong perinatal expression (150% of adult; Figs. 6, 8, 16). Expression of both transcripts resolved to moderate expression (Figs. 10–14, 16; Table 2). All other subunit transcripts were detectable only in postnatal hippocampus. Temporal expression patterns included a continuous low expression (β_2), a gradual increase to adult levels (δ), a decrease after P6 (γ_1), or a peak around P6 (γ_3) or P12 (α_3, α_4, β_1, γ_3; Figs. 7–14, 16; Table 2). Expression of α_1 mRNA also peaked at P12, being 12 times that at E19 and a third more than adult, as assessed by optical density measurement (Fig. 16).

Mature spatial mRNA patterns in the hippocampal pyramidal cells and dentate gyrus granule cells were established soon after birth with homogeneous expression of α_1, α_2, β_1, β_2, β_3, γ_1, γ_2, and γ_3 transcripts, concentration of α_3 and α_5 mRNAs in CA3, and concentration of α_4 and δ mRNAs in dentate gyrus (e.g., Figs. 9–14; see also Wisden et al., 1992). Strong expression of α_2, α_5, β_2, β_3, and γ_1 transcripts was noted in the early postnatal subiculum (Figs. 7–10), which in adulthood was replaced by lower expression of α_1, α_2, β_2, β_3, and γ_2 mRNAs (Figs. 11–14).

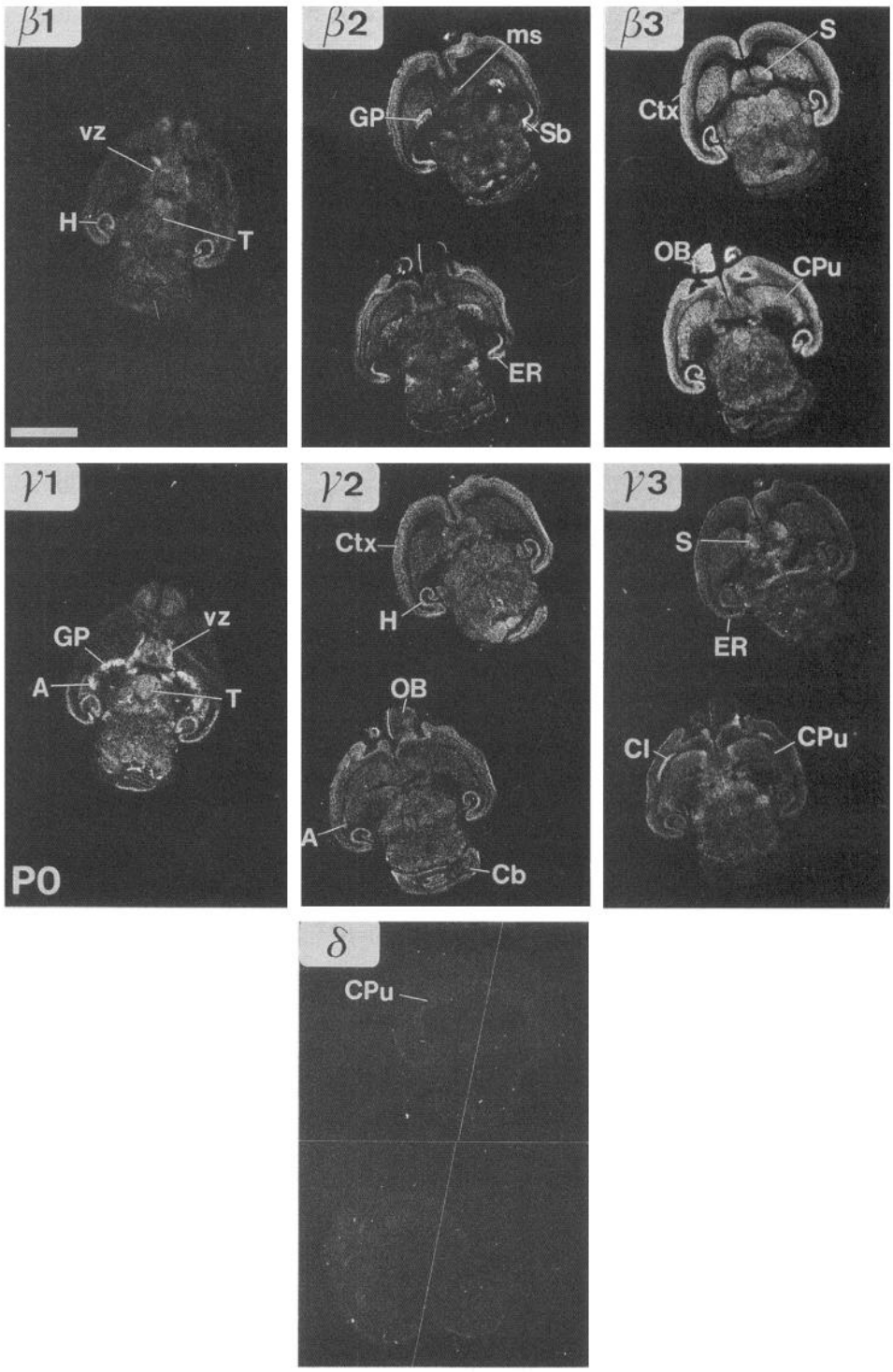


Figure 8. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor β_1–β_3, γ_1–γ_3, and δ mRNAs in horizontal sections of P0 rat brain. See Appendix for abbreviations. Scale bar, 4 mm.

In addition, α_1, α_2, α_4, β_2, β_3, and γ_2 mRNAs were detected postnatally in hippocampal interneurons, on examination of sections dipped in photographic emulsion (data not shown).

In the postnatal lateral septum, α_2 mRNA was expressed strongly at all ages, while expression of α_3 and γ_1 mRNAs declined from moderate neonatal levels to low adult levels (Figs. 7–14). Expression of other subunit transcripts declined gradually from moderate (α_5, β_3, γ_3) and low (β_1, γ_2) levels at P0 (Figs. 7, 8) to very low levels in the adult (Figs. 9–14). In contrast, in the postnatal medial septum the β_2 and γ_2 transcripts were moderately expressed, and α_1 mRNA increased as α_2 and α_3 mRNAs declined (Figs. 7–14).

Olfactory bulb

Subunit mRNAs expressed by each particular cell type of the olfactory bulb were similar at adult (see also Laurie et al., 1992) and perinatal stages, as assessed on emulsion-dipped sections. Thus, β_1 mRNA was found only in mitral cells; α_1 mRNA in mitral and tufted cells; β_2 mRNA in mitral, tufted, and periglomerular cells; α_2, α_5, γ_1, γ_3, and δ mRNAs in postmigratory

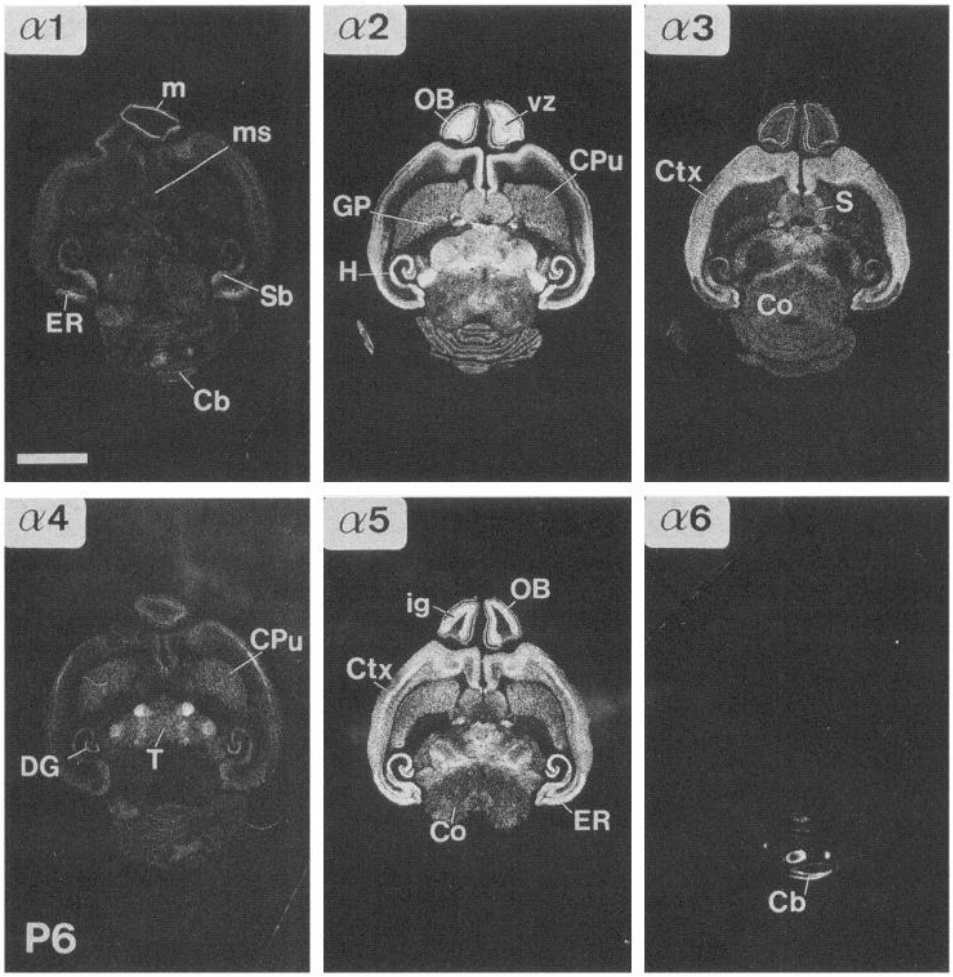


Figure 9. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor α_1–α_6 mRNAs in horizontal sections of P6 rat brain. See Appendix for abbreviations. Scale bar, 4 mm.

granule and periglomerular cells; and α_3, α_4, β_3, and γ_2 transcripts in all these cell types (e.g., Figs. 9, 10). At perinatal ages, α_2, α_5, and γ_1 mRNAs were also strongly expressed in mitral cells (Figs. 5–10, 15). In addition, the α_2 and γ_1 transcripts were present in immature, premigratory granule cells in the ventricular zone (e.g., Figs. 9, 10). The subunit mRNAs most prominently expressed throughout development were those of α_2, α_5, and β_3, which attained perinatal peaks of strong expression (Figs. 5–12). Some others, which were much more weakly expressed, also peaked around E19 (γ_1), E19–P6 (α_3), or P6–P12 (γ_3). In contrast, yet other mRNAs, detectable at E19 (Figs. 5, 6), rapidly reached persistently strong (α_1, β_2, γ_2), moderate (β_1), or low (α_4) levels (Figs. 7–14). The δ transcript was detectable at P6 and reached moderate expression by P12 (Figs. 10–14).

Thalamus

In the diencephalon of developing rats, marked changes occurred in transcript predominance (summarized in Table 2). Of the α subunits, α_2, α_3, and to a lesser extent α_5 were the major mRNAs in the embryonic diencephalon (Figs. 1, 3, 5; Table 2). Expression of α_3 mRNA peaked slightly at P0 (Fig. 7), while marked α_2 and α_5 mRNA expression continued until P6 (Figs. 9, 16), after which all three transcripts declined dramatically before P12 and were only faintly detectable in the midline adult thalamus (Figs. 11, 13; Table 2). Expression of the α_2 and α_5 mRNAs in whole adult thalamus was approximately 15% and 5%, respectively, of P6 levels (Fig. 16). Expression of β_3, γ_1, γ_2, and γ_3 mRNAs underwent similar temporal patterns, albeit at a generally lower intensity (Figs. 2–14, 16; Table 2). Expression of β_1 mRNA remained at a continuously low level (Figs. 4–14). Diencephalic α_4 mRNA gradually increased from low embryonic levels (Figs. 3, 5), reaching a strong peak at P12 (Figs. 7–13), while the slowly increasing production of α_1, β_2, and δ mRNAs (Figs. 1–10) accelerated after P6 to adult levels by P12 (Figs. 11–14, 16; Table 2). The variety of expression patterns in the various thalamic nuclei of neonates and adults was remarkable (see also Wisden et al., 1992). In brief, throughout development, α_3, β_1, γ_1, and γ_3 transcripts tended to be expressed in midline thalamic nuclei (although α_3 and γ_3 mRNAs were also found in reticular thalamus and medial geniculate, respectively), as did α_2, α_5, and β_3 mRNAs before and after their peaks of expression (Figs. 7–14). The α_2 and α_5 mRNAs at their maxima and the α_1, α_4, β_2, and γ_2 mRNAs were found throughout the thalamus, while δ mRNA was located in lateral thalamic nuclei (Figs. 7–14).

Striatum

Caudate putamen

Transcript expression in the embryonic striatum and postnatal caudate followed a temporal pattern similar to that described for the thalamus. Expression of α_2, α_3, α_5, and β_3 mRNAs gradually increased from low levels at E14 (Figs. 1–8); peaked in caudate prior to birth (α_3; Figs. 5–8), at P0 (α_2, β_3), or at P6 (α_5; Figs. 7–10); and subsequently decreased to moderate (α_2) or very

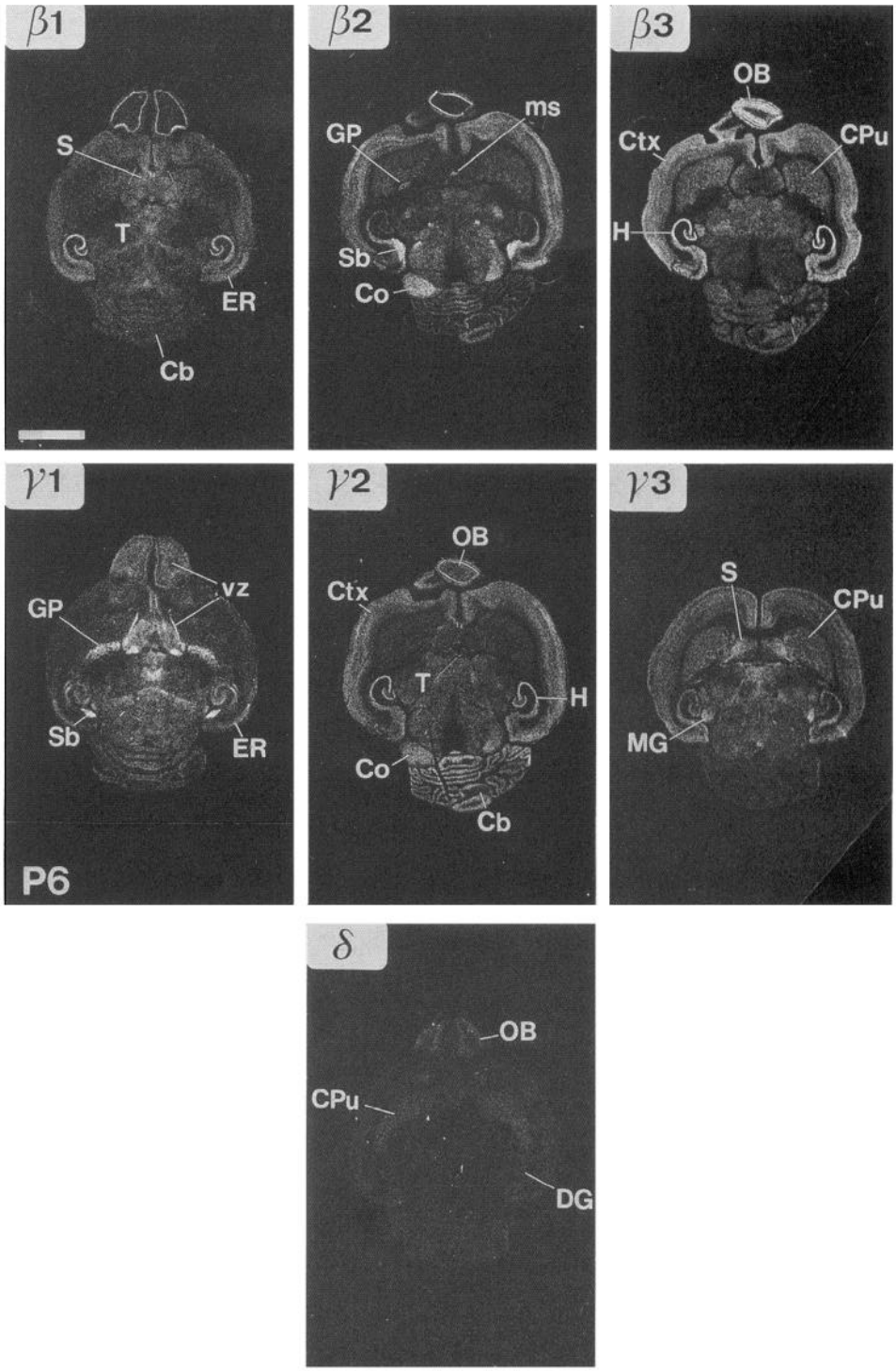


Figure 10. X-ray film autoradiographs illustrating distribution of GABA$_A$ receptor β_1–β_3, γ_1–γ_3, and δ mRNAs in horizontal sections of P6 rat brain. See Appendix for abbreviations. Scale bar, 4 mm.

low (α_3, α_5, β_3) expression in the adult caudate (Figs. 11–14). Expression of α_1 and β_2 mRNAs was detected at all stages of postnatal caudate at a low level (Figs. 7–14). Production of α_4 mRNA began in caudate around E19 (Fig. 5) and gradually increased until P12 (Figs. 7–11) before declining slightly to moderate adult levels (Fig. 13). The γ_1, γ_2, and γ_3 mRNAs were expressed only to a low degree in embryonic, postnatal, and adult caudate (Figs. 2–14). Expression of the δ gene was just detectable in neonatal caudate (Fig. 8) and gradually increased to a moderate adult level (Figs. 10, 12, 14).

Globus pallidus

The globus pallidus exhibited an expression profile that often contrasted strongly with that in caudate. No α_4, α_5, or δ transcripts were detected in the globus pallidus at any age. Expression levels in globus pallidus declined from pronounced (α_2, γ_1) or low (α_3, β_1, β_3, γ_2, γ_3) expression at P0 down to low (γ_1) or very low levels in P12 and adult (Figs. 7–14). In contrast, postnatal expression of the α_1 gene gradually increased to strong in P12 and adult globus pallidus (Figs. 7–13), while β_2 mRNA

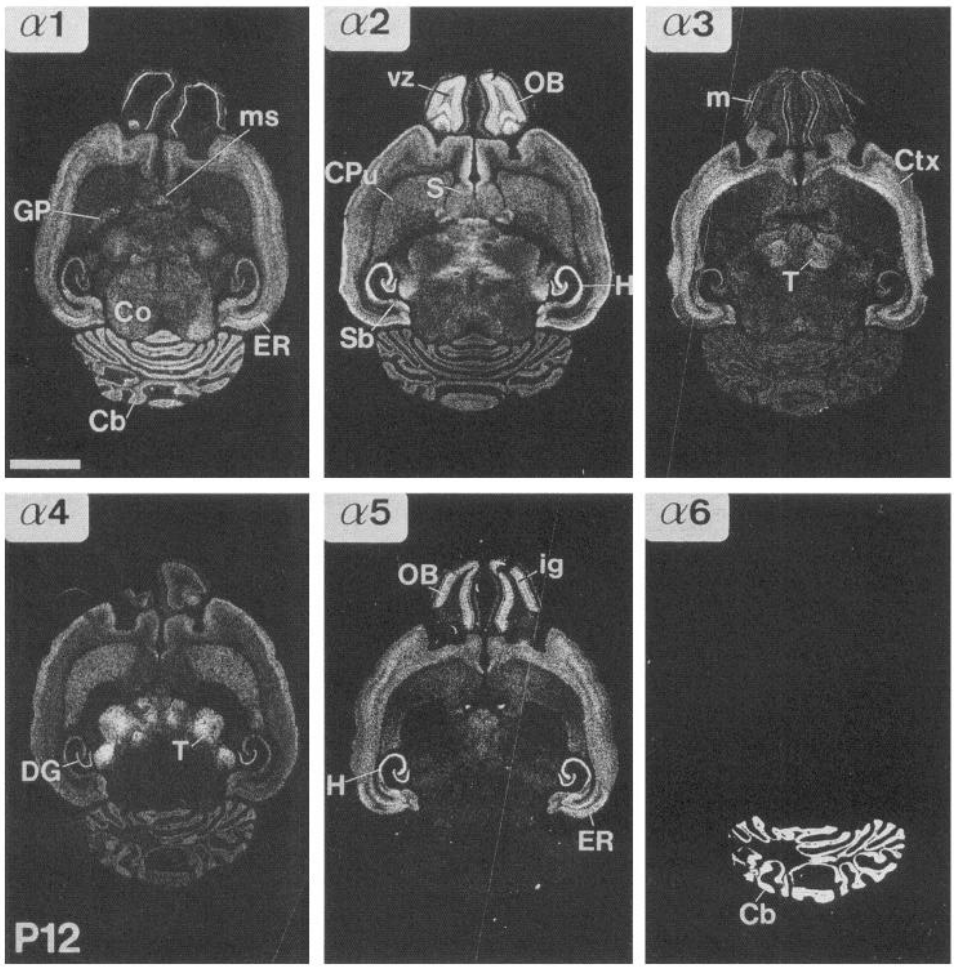


Figure 11. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor α_1–α_6 mRNAs in horizontal sections of P12 rat brain. See Appendix for abbreviations. Scale bar, 4 mm.

remained continuously strong (Figs. 8–14). In young postnatal brain (P0–P12), β_1 and γ_1 mRNAs were also observed in the caudate ventricular zone (Figs. 8–12).

Colliculi

The α_2, α_3, and α_5 mRNAs were detected at moderate or low levels in the E14 mesencephalon (Fig. 1) and remained at the same levels, or increased (α_2), until E19 (Figs. 3, 5). After birth, expression of these gradually declined to low (α_2, α_3) or undetectable (α_5) levels (Figs. 9–13). Expression of β_3 and γ_1 mRNAs followed a similar temporal profile (Figs. 2–14), while β_1 and γ_3 mRNAs remained very low throughout development (Figs. 2–14). In contrast, other mRNAs exhibited low (β_2, γ_2) or undetectable (α_1) levels at E14 (Figs. 1–6) and then gradually increased (Figs. 3–6), especially in the inferior colliculi, to adult levels by P12 (Figs. 9–14). Transcripts for α_4 and δ were not detectable in mesencephalon (Figs. 1–14).

Cerebellum

In the rat cerebellum, most $GABA_A$ receptor mRNA expression was restricted to the period of postnatal development (Table 2). Prior to birth, homogeneous, low cerebellar expression of α_3, β_3, and γ_2 transcripts was observed (Figs. 1–6). At P0, hybridization over the multilayered Purkinje cells (Jacobson, 1978) was observed with the α_1, β_2, β_3, and γ_2 probes (Figs. 7, 8, 15). By P6, the Purkinje cells had resolved into a monolayer, with strong signals over each cell. This picture continued into adulthood (Figs. 9–15; see also Laurie et al., 1992). Transcripts for α_2, α_3, β_3, γ_1, and γ_2 were observed in the external granule cell layer from the thick layer at P0 to the very thin layer at P12, and in granule cells migrating through the molecular layer to the internal granule cell layer. By P6, all subunit mRNAs, except those for α_5 and δ, could be detected at low levels in postmigratory granule cells (Figs. 9, 10). After P6, some transcripts showed small changes of expression in postmigratory granule cells: a decline (α_2, α_3, β_1, γ_1, γ_3) or a slight peak at P12 (α_4; Figs. 11–14). In contrast, others (α_1, α_6, β_2, β_3, γ_2, δ) exhibited a very pronounced increase between P6 and P12 to adult levels of expression (Figs. 11–14). Labeling of stellate/basket cells in the molecular layer by the probes for α_1, β_2, and γ_2 mRNAs was observed only in the adult cerebellum. A "halo" of hybridization for the α_2 and γ_1 mRNAs in the molecular layer at its border with the granule cell layer, consistent with labeling of putative Bergmann glia, was apparent at and after P12 (Figs. 11–14; see also Laurie et al., 1992).

Spinal cord

As sections were not taken through postnatal spinal cord, only embryonic $GABA_A$ receptor mRNA expression can be described here. Transcripts for α_1 and δ were not detected (Figs. 1–6). All other subunit mRNAs remained at continuously strong (α_2, α_3, α_5, β_3, γ_1, γ_2), low (β_1), or very low levels (α_4, β_2, γ_3; Figs. 1–6). At E14, expression of α_4, β_1, and γ_1 mRNAs appeared to be restricted to the ventricular germinal zone surrounding the cen-

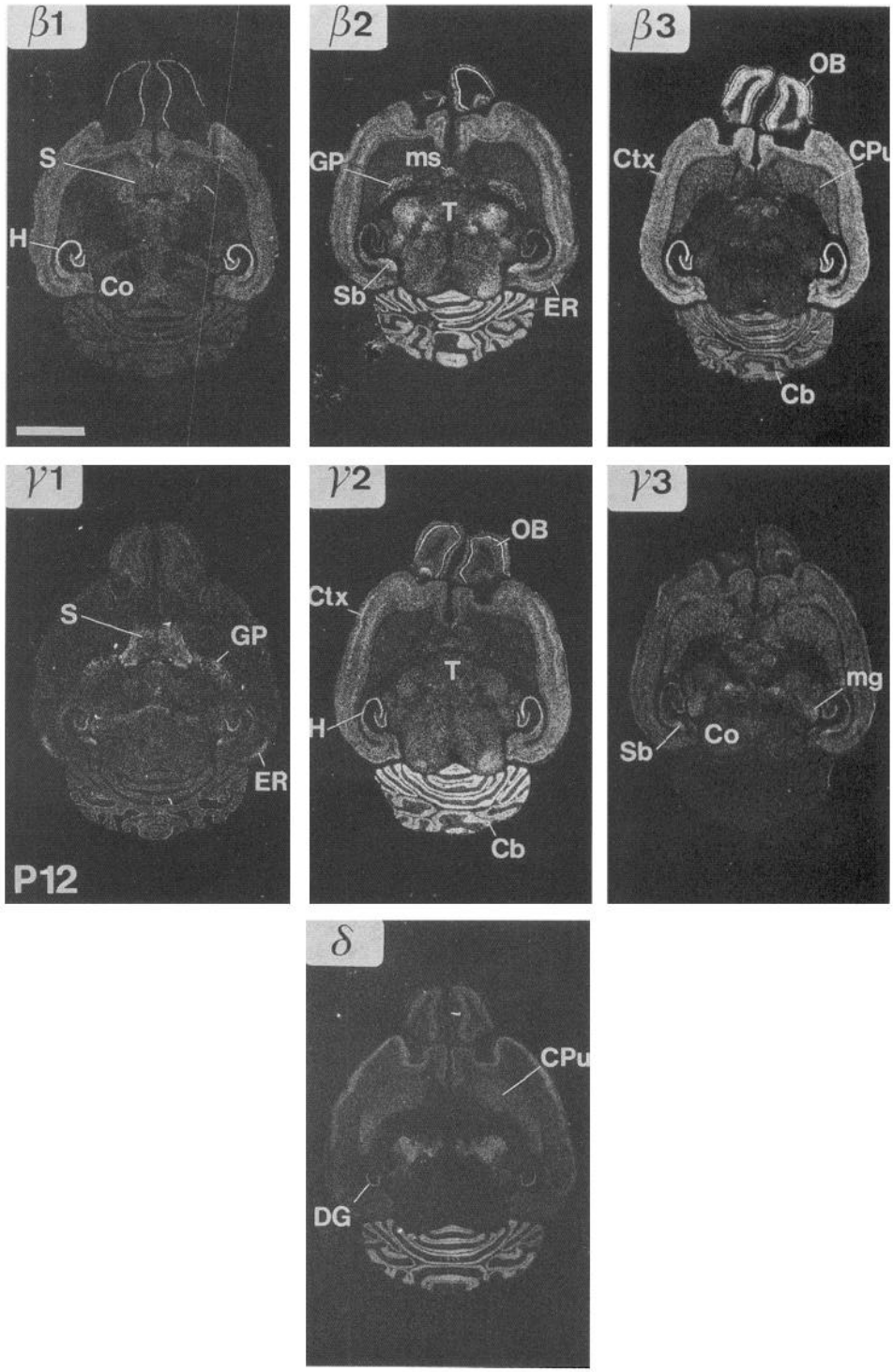


Figure 12. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor β_1–β_3, γ_1–γ_3, and δ mRNAs in horizontal sections of P12 rat brain. See Appendix for abbreviations. Scale bar, 4 mm.

tral canal (Figs. 1, 2). Some subunit transcripts were more concentrated in ventral than in dorsal spinal cord at stages of embryonic development (e.g., α_5, β_1, γ_1), while others showed a homogeneous distribution (α_3, β_3, γ_2; Figs. 1–6). The γ_3 mRNA expression in E17 and E19 spinal cord was concentrated primarily in the most dorsal region (Figs. 4, 6).

Peripheral localization

In the course of this study, specific signals for certain subunit mRNAs were also found in peripheral embryonic nervous tissues. The α_2, α_3, β_3, γ_1, γ_2, and γ_3 transcripts were moderately expressed in dorsal root ganglia at all embryonic ages (Figs. 1, 2), and moderate signals for α_2, α_3, β_3, and γ_2 transcripts were detected in the E17 trigeminal ganglion (Fig. 3). The γ_3 mRNA was strongly expressed in embryonic (E14–E19) intestine (Fig. 2), although the cellular resolution of this signal could not be determined.

Discussion

In this study, different ontogenic progressions in expression have been revealed for each of 13 $GABA_A$ receptor subunit genes (see Table 2 for summary). The results suggest that the adult subunit

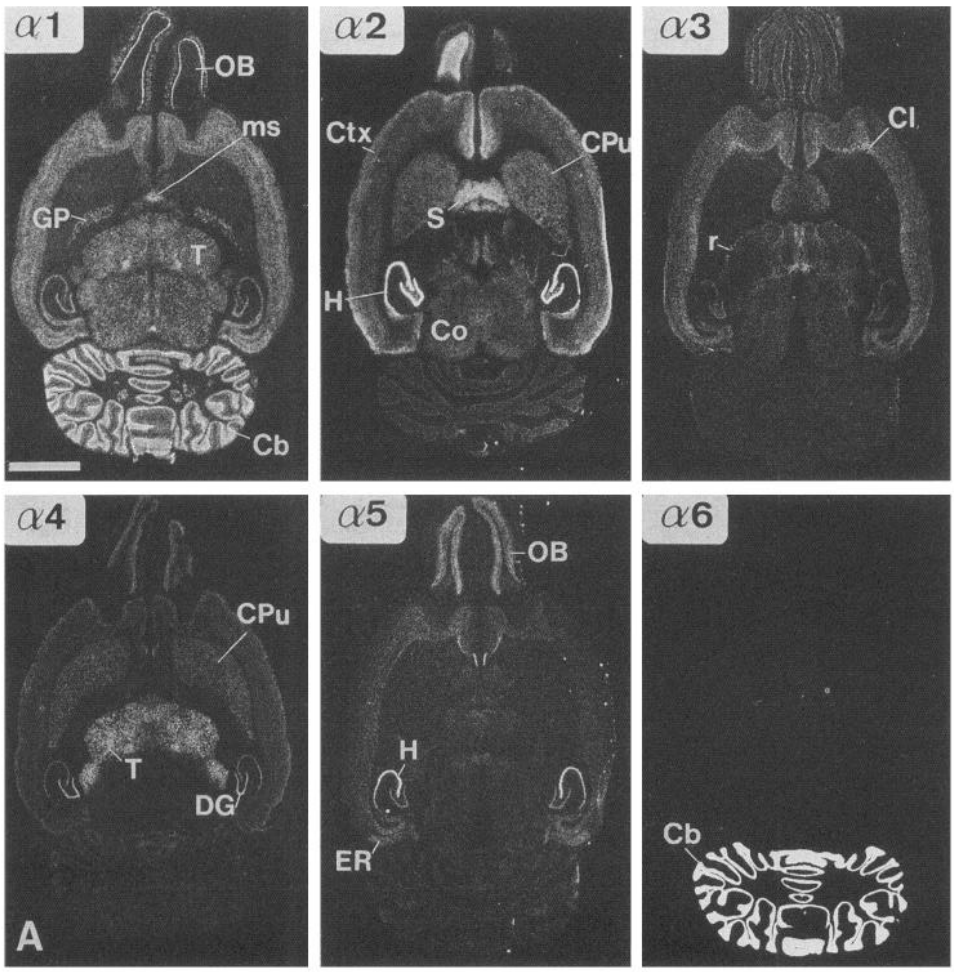


Figure 13. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor α_1–α_6 mRNAs in horizontal sections of adult rat brain. See Appendix for abbreviations. Scale bar, 4 mm.

composition of the $GABA_A$ receptor in certain brain regions (e.g., cortex) differs substantially from that in the embryo and neonate and that some receptor populations persist throughout development (e.g., in hippocampus) while others proceed postnatally almost directly to an adult form (e.g., in cerebellum; summarized in Tables 1, 2). One of the most striking features of $GABA_A$ subunit expression during brain development is the marked, widespread expression of the α_2 and α_5 mRNAs, which reaches an early peak and then declines (Figs. 9, 16), while in contrast, α_1 mRNA expression increases (Figs. 9, 11, 13, 16). Expression of β_1 mRNA mimics that of the α_2 and α_5 transcripts, albeit at a much lower intensity. Such developmental changes of α_1, α_2, α_5, and β_1 mRNA expression agree with Northern analysis of whole rat brain (Garrett et al., 1990; MacLennan et al., 1991). Note that the sequence termed α_5 by us is referred to as α_4 by MacLennan et al. (1991; see Materials and Methods). The expression patterns revealed in this study provide a possible molecular explanation for earlier observations (see introductory remarks) concerning global and regional changes in the pharmacological properties of $GABA_A$ receptors during development.

Regional changes in expression during development as detected by film autoradiography probably reflect both changes in the expression repertoire of neurons, and alterations in neuronal density. The developing vertebrate brain produces an excess of neurons, many of which are selectively eliminated in early development (Oppenheim, 1991). A point yet to be examined is whether the widespread loss of early-type gene expression (e.g., α_2 and α_5 in cortex and thalamus) is due to changes of gene expression within neurons or to death of a population of cells specifically expressing these genes. The main period of developmental neuronal death, however, happens after mature synaptogenesis (Oppenheim, 1991), which in the rat cortex occurs principally in the third and fourth postnatal weeks (Aghajanian and Bloom, 1967). This may therefore argue against marked, selective destruction between P6 and P12 of neurons expressing the early $GABA_A$ gene profile.

With regard to regional expression, $GABA_A$ receptors of the developing cortex, hippocampus, and cerebellum have been most extensively studied by previous binding and electrophysiological experiments and will be discussed in some detail.

Cortical receptors

The pronounced cortical expression of α, β, and γ subunit genes by birth (Figs. 7, 8; Table 2) predicts the early formation of functional $GABA_A$ receptors. Paradoxically, although cortical BZ binding is already substantial at birth (60% of adult; Candy and Martin, 1979; Lippa et al., 1981; Chisholm et al., 1983) and is coupled to $GABA_A$ receptors (Palacios et al., 1979; Eichinger and Sieghart, 1986; Kellogg and Pfleger, 1989), the number of rat cortical GABA and muscimol binding sites is low at birth (25% of adult) and increases only after P8 (Coyle and Enna, 1976; Vitorica et al., 1990). The appearance of most cortical $GABA_A$ binding coincides with the replacement of perinatal

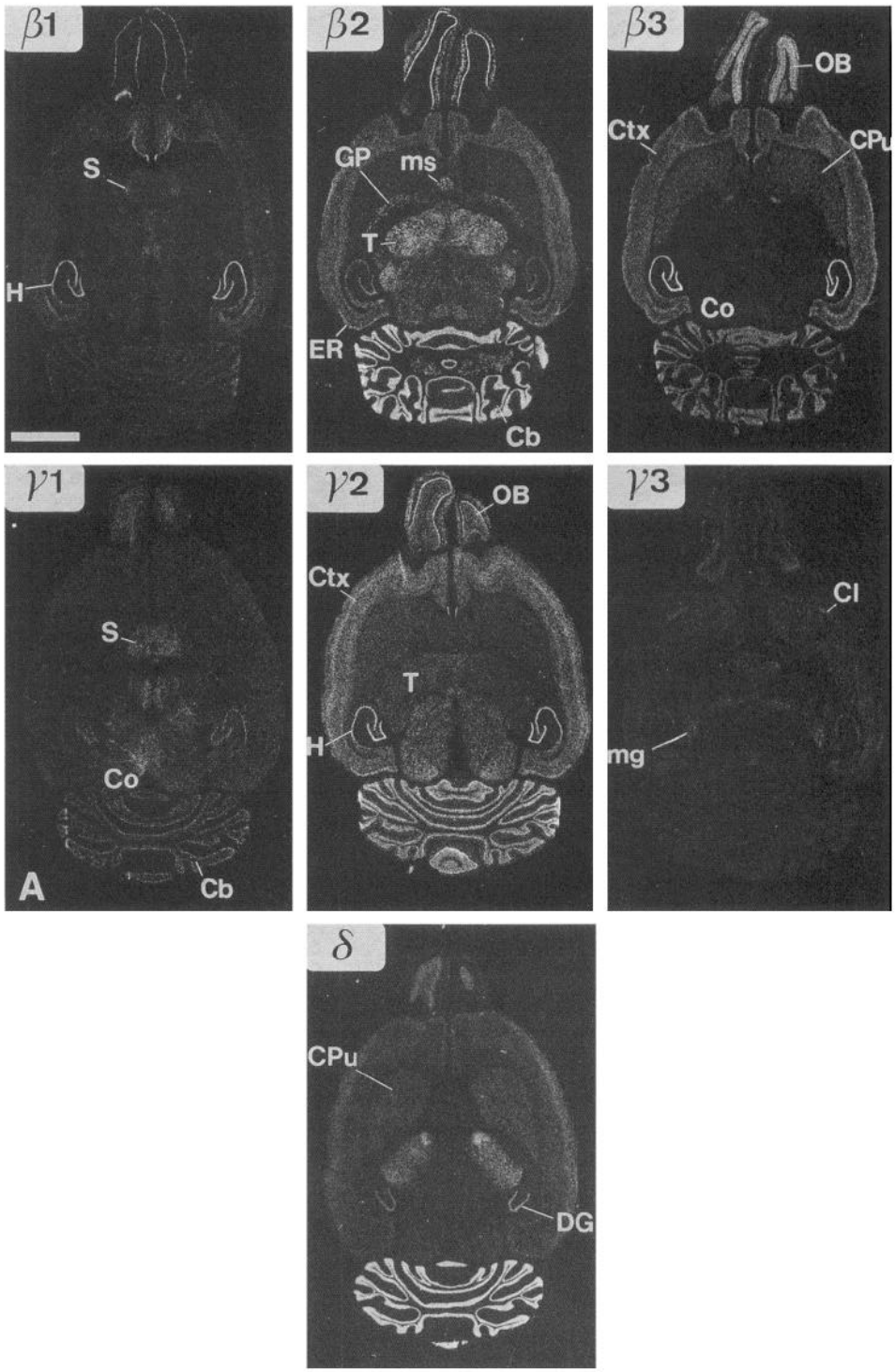


Figure 14. X-ray film autoradiographs illustrating distribution of $GABA_A$ receptor β_1–β_3, γ_1–γ_3, and δ mRNAs in horizontal sections of adult rat brain. See Appendix for abbreviations. Scale bar, 4 mm.

transcripts (e.g., α_2, α_3, α_5, β_3) with adult ones (e.g., α_1, α_4, β_2, δ; Figs. 9–12, Table 2). The early subunit composition or post-translational subunit modifications may impede marked $GABA_A$ binding. In adult rat brain, such mismatches in the densities of $GABA_A$ and BZ binding sites occur in regions such as the cerebellum, thalamus, and hippocampus (Unnerstall et al., 1981; Olsen et al., 1990).

Recombinant $GABA_A$ receptors of the combination $\alpha_1\beta_x\gamma_2$ (where x = 1–3) exhibit type I BZ binding, whereas $\alpha_2\beta_x\gamma_2$, $\alpha_3\beta_x\gamma_2$, and $\alpha_5\beta_x\gamma_2$ assemblies display type II BZ pharmacology (Pritchett et al., 1989a; Pritchett and Seeburg, 1990). Receptors of composition $\alpha_5\beta_x\gamma_2$ also exhibit very low affinity for the partial agonist zolpidem (Pritchett and Seeburg, 1990). $GABA_A$ receptors exhibiting such pharmacologies are precipitated from brain homogenates by the appropriate α subunit antibodies (McKernan et al., 1991b). From their mRNA expression levels, the α_2, α_3, α_5, β_1, β_2, β_3, γ_2, and γ_3 subunits are predicted to exist in perinatal cortical receptors, the composition depending on the

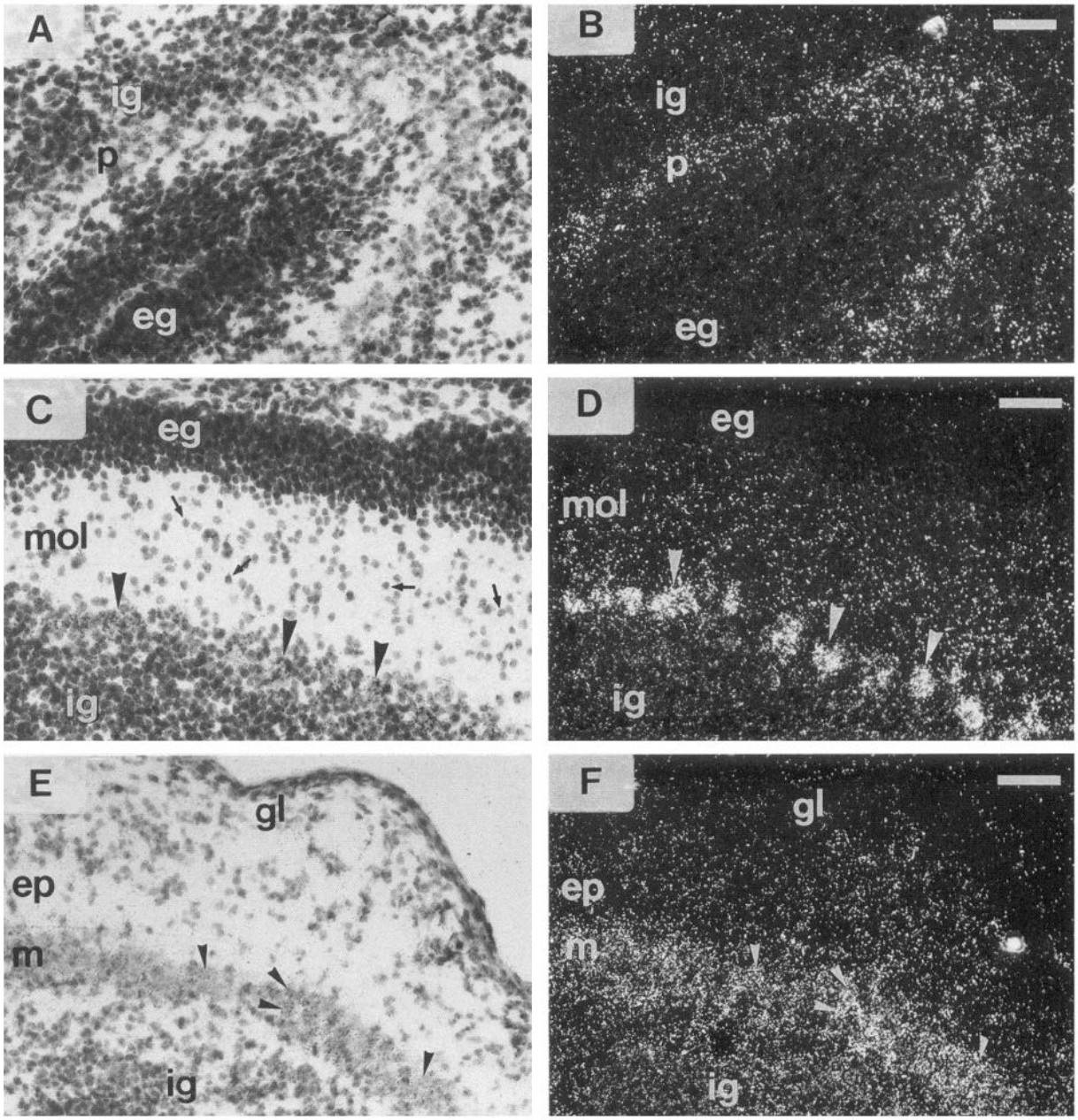


Figure 15. Microscopic resolution of hybridization in thionin-stained sections. *Left column,* bright-field optics; *right column,* corresponding dark-field optics. *A* and *B*, α_1 mRNA in P0 cerebellum; *C* and *D*, α_1 mRNA in P12 cerebellum; *E* and *F*, α_2 mRNA in P0 olfactory bulb. See Appendix for abbreviations. *Arrows* in *C* and *D*, migrating granule cells. *Arrowheads*, in *C* and *D*, purkinje cells; in *E* and *F*, mitral cells. Scale bar, 50 μm.

cortical layer (e.g., $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_2\gamma_3$, $\alpha_5\beta_1\gamma_2$; Figs. 3–10). Later expression patterns indicate a majority of $\alpha_1\beta_2\gamma_2$ assemblies (Figs. 13, 14; see also Benke et al., 1991; Gambarana et al., 1991; Wisden et al., 1992). These expression changes are therefore consistent with (1) type II and type I BZ receptors predominating in neonatal and adult cortex, respectively (Lippa et al., 1981; Chisholm et al., 1983); (2) zolpidem having very low affinity for P6 rat brain homogenates (Sieghart and Schlerka, 1991); and (3) ^{3}H-flunitrazepam photolabeling mainly three α subunit proteins (55, 59, 62 kDa) in neonatal cortex, and mainly one (51 kDa) in adult cortex (Eichinger and Sieghart, 1986; Sato and Neale, 1989; Fuchs et al., 1990; Vitorica et al., 1990).

Hippocampal receptors

Based on transcript levels, most perinatal hippocampal $GABA_A$ receptors would be formed from a selection of α_2, α_5, β_1, β_3, γ_1, and γ_2 subunits, with fewer receptors containing α_3, α_4, and γ_3 subunits (Figs. 7–10, Table 2). By P12, all subunits except α_6 could contribute to hippocampal $GABA_A$ receptors, although by this time γ_1 and γ_3 would have a minor contribution. The expression levels of each hippocampal transcript during development could explain both the dense BZ binding at birth and the continuous predominance of type II BZ receptors in the hippocampus (Chisholm et al., 1983; Sieghart and Schlerka,

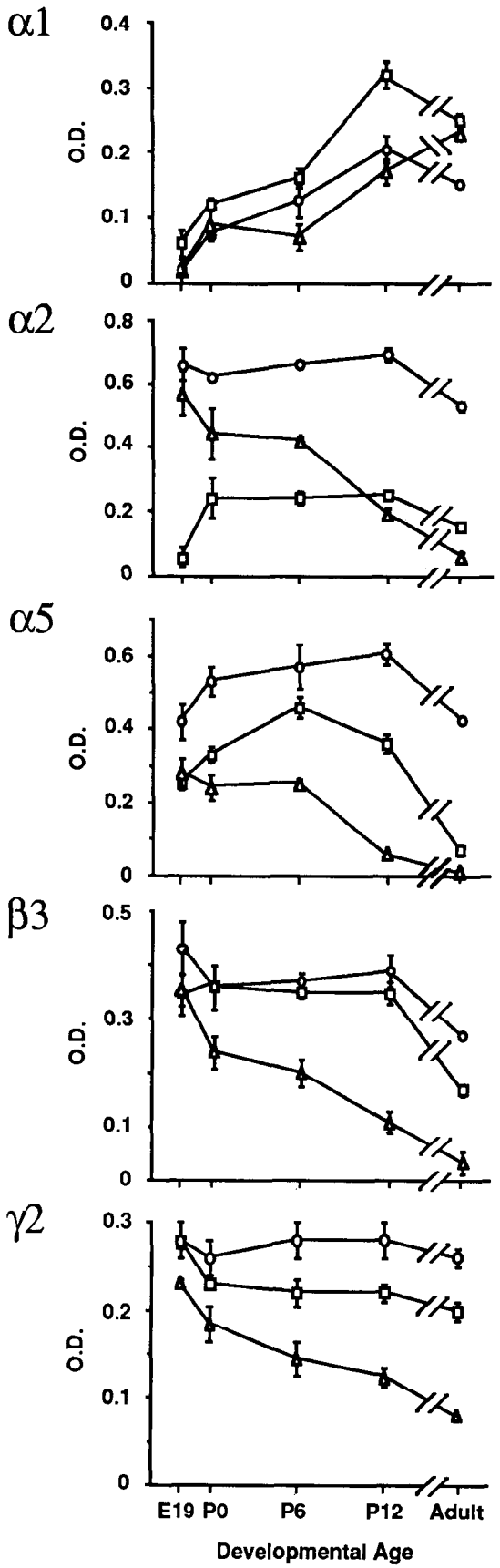


Figure 16. Developmental expression of α_1, α_2, α_5, β_3, and γ_2 mRNAs, as representative mRNAs, in regions of rat brain as measured by optical densities (*O.D.*) of x-ray film images. Data points represent means ± SEM of values from three animals: *squares*, neocortex (all layers); *circles*, hippocampus (CA1 + CA3); *triangles*, thalamus (all nuclei). Nonspecific hybridization consistently gave signals on the same autoradiograms of optical density <0.08.

1991). Diazepam-resistant Ro15-4513 binding and type I BZ binding (Turner et al., 1991; Wisden et al., 1991a) would be predicted to appear gradually with increasing age, while pharmacology contributed by the γ_1 subunit (Puia et al., 1991) should diminish.

Cerebellar receptors

The results described for α_1, β_1, β_2, β_3, and γ_2 mRNA expression agree with similar studies on the postnatal rodent cerebellum (Gambarana et al., 1990, 1991; Zdilar et al., 1991; Zhang et al., 1991). Consistent with our results, functional $GABA_A$ receptors are present on Purkinje cells at birth (Woodward et al., 1971), and cerebellar α_1-like immunoreactivity on Western blots increases postnatally (McKernan et al., 1991a).

The increasing postnatal expression of $GABA_A$ transcripts in both Purkinje and postmigratory granule cells (α_1, β_2, β_3, γ_2, δ) is paralleled by increases in $GABA_A$ and type I BZ binding after P8 (Coyle and Enna, 1976; Candy and Martin, 1979; Palacios and Kuhar, 1982; Chisholm et al., 1983; Zdilar et al., 1991). An acceleration of BZ binding mainly in the molecular layer between P14 and P28 is probably due to the coincident development of the Purkinje cell dendritic tree (Jacobson, 1978) and extensive production of receptors containing α_1, β_2/β_3, and γ_2 subunits. The transient production by postmigratory granule cells of almost all $GABA_A$ transcripts (e.g., $\alpha_2\beta\gamma_2$, $\alpha_3\beta\gamma_2$ assemblies) explains the initial heterogeneity of cerebellar BZ binding sites and BZ-photolabeled proteins (Chisholm et al., 1983; Sieghart, 1986). Worthy of note is the absolute restriction of the α_6 mRNA to postmigratory granule cells (Figs. 9–13), indicating a highly cell-specific control of gene expression. This mRNA could not be detected anywhere else in the developing brain or embryo. Thus, α_6 gene expression could be used as a unique indicator for cerebellar granule cells, although, in cultured hippocampal neurons, the suppression of α_6 expression can be overridden temporarily (Killisch et al., 1991). Diazepam-resistant ^{3}H-Ro15-4513 binding, a feature of $\alpha_6\beta_x\gamma_2$ receptors (Lüddens et al., 1990), appears in the cerebellar granule cell layer after P6 (Uusi-Oukari et al., 1991; D. J. Laurie, unpublished observations), coinciding with the production of α_6 mRNA. Whereas moderate BZ binding is present in neonatal cerebellum, ^{3}H-muscimol and ^{3}H-GABA binding only begins 1–2 weeks after birth (Coyle and Enna, 1976; Palacios and Kuhar, 1982). This temporal disparity is similar to that in the cortex and may have a similar basis.

Other brain structures: thalamus, globus pallidus, medial septum, and spinal cord

In the developing thalamus there is a dramatic and almost complete switch of $GABA_A$ subunit gene expression from α_2, α_3, α_4, α_5, β_1, β_3, γ_1, γ_2, and γ_3 mRNAs in specific nuclei, to a virtually homogeneous adult expression of α_1, α_4, β_2, and δ mRNAs (Table 2; see Wisden et al., 1992, for discussion of adult thalamic receptors). Thalamic receptors should therefore bind $GABA_A$ ligands at all ages but should lose affinity for BZ analogs (Pritchett et al., 1989b; Ymer et al., 1990; Puia et al., 1991; Herb et

al., 1992), due to the predicted decline of thalamic γ subunit production. The large number of transcripts in the early thalamus makes simple predictions of $GABA_A$ receptor subunit composition impossible. However, in the globus pallidus an obvious developmental switch in the few transcripts expressed allows the deduction of $\alpha_2\beta_2\gamma_1$ and $\alpha_1\beta_2\gamma_1/\gamma_2$ assemblies in the neonate and adult, respectively. Similarly, $GABA_A$ receptor composition in the medial septum should change from $\alpha_2/\alpha_3\beta_2\gamma_2$ to $\alpha_1\beta_2\gamma_2$. Exchange of α_1 for α_2 or α_3, and γ_1 for γ_2 in recombinant $\alpha\beta\gamma$ receptors drastically alters their responses to GABA and BZ agonists and inverse agonists (Ymer et al., 1990; Puia et al., 1991). Thus, these combinatorial switches in the globus pallidus and medial septum should significantly change $GABA_A$ receptor properties. In embryonic spinal cord, the strong expression of α, β, and γ genes is consistent with the very high densities (~400% of adult) of $GABA_A$ and BZ binding (type II), which then decline after birth (Saito et al., 1983).

Caudal to rostral expression of subunit genes

In cell culture, GABA stimulates autodevelopment of its own receptors through $GABA_A$ sites (Meier et al., 1987). GABA-immunoreactive neurons and fibers are detectable from an embryonic age (E13), appearing first in caudal structures and later in rostral structures and cerebellum (Lauder et al., 1986; Seress and Ribak, 1988; Meinecke and Rakic, 1990; Cobas et al., 1991). Consistent with GABA-promoted development of $GABA_A$ receptors, fetal subunit transcripts also appear in a caudal to rostral manner (Figs. 1–4), approximately 1 d after the GABA-immunoreactive fibers (Lauder et al., 1986). Autoradiographic patterns of ^{3}H-flunitrazepam binding in the brains of developing rat embryos (Schlumpf et al., 1983) very closely follow the caudal to rostral appearance of γ_2 mRNA (Figs. 1–6), supporting the proposal, based on *in vitro* expression studies, that the γ_2 subunit is necessary for high-affinity BZ agonist binding (Pritchett et al., 1989b). In contrast, α_3 mRNA is already ubiquitously expressed in the E14 brain (Fig. 1), suggesting that its expression is not dependent on GABA and indeed that GABA may stimulate the expression of the other subunits through receptors containing the α_3 subunit.

Receptors on neuroblasts and embryonic peripheral neurons

Although the majority of $GABA_A$ subunit transcripts were restricted to mature postmigratory neurons, expression of some (α_2, α_3, β_1, β_3, γ_1, γ_2) was detected in germinal zones and in migrating neurons. The β_3 transcript has also been noted by others in mitotic zones of the forebrain and cerebellum (Gambarana et al., 1991; Zhang et al., 1991). Mitotic cells in the germinal zones therefore express a variety of $GABA_A$ genes that is potentially sufficient to form fully functional receptors (Seeburg et al., 1990; Puia et al., 1991), through which GABA could exert neurotrophic or mitotic effects (Hansen et al., 1987).

GABA has a role in several peripheral organs (Erdo and Wolff, 1990), especially in neuronal signaling. Two embryonic structures strongly expressing $GABA_A$ transcripts (dorsal root ganglion, trigeminal ganglion) are derived from the neural crest (Jacobson, 1978), indicating a common origin and an association with the CNS. The peripheral localization of several $GABA_A$ subunit transcripts shows the involvement of some subunits in both central and peripheral $GABA_A$ receptors. The detection of only γ_3 mRNA in intestine suggests that other $GABA_A$ subunits may await discovery in the PNS. Whether these expression patterns are maintained into maturity remains to be examined, but α_1, α_2, β_2, and γ_2 mRNAs are also found in adult dorsal root ganglia (Persohn et al., 1991).

Changing subunit combinations in development

In the developing rat CNS, a wide range of regional expression schemes are apparent. Subunit mRNAs found in early brain are sometimes conserved (e.g., hypothalamus) or replaced (e.g., thalamus, globus pallidus), other times supplemented (e.g., hippocampus, spinal cord) or deleted (e.g., lateral septum, cerebellar granule cells; Tables 1, 2). Even within a structure, each subunit mRNA often follows a different program (e.g., caudate, globus pallidus, mitral cells; Table 1). The neonatally expressed transcripts may belong to a default expression profile that can be retained (e.g., in hippocampus) or lost (e.g., in thalamus) when other $GABA_A$ transcripts appear during neuronal maturation.

Based on overlapping mRNA expression profiles, a wide variety of receptor combinations could be formed during development. No two subunit transcripts exhibit identical temporal and spatial patterns. However, the α_2, α_3, and α_5 transcripts often colocalize in time and region with the β_3 transcript (e.g., Figs. 7, 8), while α_1 and β_2 mRNA often codistribute (Figs. 11–14). These are not absolute relationships, as, for example, in the young cortex and globus pallidus β_2 and β_3 mRNAs are found with α_2 and α_3 mRNAs (Figs. 9, 10), and the expression of the β_2 transcript in several regions precedes that of α_1. Although the α_5 and β_1 mRNA expressions are markedly different at perinatal stages (Figs. 3–10), there is a gradual convergence over time such that by P12 and beyond, the distributions are very similar (Figs. 11–14) (except in olfactory bulb), suggesting the $\alpha_5\beta_1$ combination as a mature pairing. Similarly, the sum of the α_4 and α_6 mRNA expression patterns shows a strong similarity to that of the δ transcript, although the expression of the latter lags behind that of the former two. The $\alpha_1\beta_2$, $\alpha_2\beta_3$, $\alpha_5\beta_1$, $\alpha_4\delta$, and $\alpha_6\delta$ pairings have already been suggested by us from their mRNA distributions in the adult rat brain (Laurie et al., 1992; Wisden et al., 1992), and the present study adds some weight to these combinatorial proposals.

A variety of other regionally specific combinations are also possible, and subunits may proceed through a series of partnerships before converging to final adult assemblies. The spatial and temporal expression patterns of the γ subunit mRNAs did not follow the pattern of any α, β, or δ subunit transcript. Based on mRNA levels, the γ_2 subunit should participate in many adult and neonatal $GABA_A$ receptors, while the majority of $GABA_A$ receptors containing the γ_1 and γ_3 subunits would be found perinatally. However, there are certain areas in the adult rat brain (e.g., medial amygdala, septum, Bergmann glia) where γ_1 gene expression remains substantially elevated (Laurie et al., 1992; Wisden et al., 1992). The γ subunits therefore appear more promiscuous than members of the α, β, and δ classes, and exhibit a variety of partnerships that change with region and alterations in α and β expression (e.g., $\alpha_3\beta_3\gamma_2$ and $\alpha_1\beta_2\gamma_2$ in neonatal and mature cortex).

The intensity of α_2, α_3, and α_5 subunit gene expression in P6 brain (Fig. 9) contrasts with the more limited expression of β and γ subunit genes (Fig. 10). If the corresponding protein levels are in proportion, there might be a surplus of these α subunits unless they combine with other, as yet unidentified, $GABA_A$ subunits such as a possible rodent homolog of the avian β_4 subunit (Bateson et al., 1991). Alternatively, an excess of α

subunits may promote the assembly of heteromeric $GABA_A$ receptor complexes.

Rationale for subunit gene expression changes

The changes of $GABA_A$ subunit mRNA expression imply a rationale for the receptor combinations at each age. The most marked change in expression occurs for the α subunit mRNAs (Tables 1, 2), which may occur as a result of the changing role of GABA during development. Until P8–P12, GABA apparently mediates neuronal depolarization through $GABA_A$ receptors rather than hyperpolarization as found in the adult brain (Ben-Ari et al., 1989; reviewed by Cherubini et al., 1991). This difference is thought to be due to opposite electrochemical chloride gradients. The perinatal neurotrophic action of GABA, probably released from growth cones (Gordon-Weeks et al., 1984), could be related to the GABA-induced depolarization and subsequent calcium ion entry via voltage-sensitive calcium channels (Connor et al., 1987; Hansen et al., 1987; Wolff et al., 1987; Spitzer, 1991; Yuste and Katz, 1991). This GABA-induced elevation of basal intracellular calcium levels can persist for several minutes and may result in development and/or modification of future inhibitory synapses (Yuste and Katz, 1991).

Mature synapse formation in rat brain principally occurs in the third and fourth postnatal weeks (Aghajanian and Bloom, 1967). Thus, most $GABA_A$ receptors present on neurons before this age would be expected to be extrasynaptic. For GABA to exert a neurotrophic effect, such receptors may have to exhibit greater sensitivity to GABA than those eventually located in synapses, because perinatal $GABA_A$ binding is unusually low, and because ambient concentrations of GABA would probably be lower than those later occurring in synaptic clefts. Thus, GABA should be more efficacious at fetal and neonatal $GABA_A$ receptors (mainly containing α_2, α_3, or α_5) than at those of the adult (mainly containing α_1). Such a hypothesis fits nicely with electrophysiological data indicating that recombinant $\alpha_3\beta_2\gamma_2$ and $\alpha_5\beta_2\gamma_2$ receptors exhibit greater sensitivity to GABA than do $\alpha_1\beta_2\gamma_2$ receptors, and similarly that GABA is more potent on $\alpha_2\beta_1$ and $\alpha_5\beta_1$ combinations than on $\alpha_1\beta_1$ assemblies (Levitan et al., 1988; Malherbe et al., 1990; Sigel et al., 1990).

Genesis of mature-type synapses accelerates dramatically after P12 (Aghajanian and Bloom, 1967), at which stage GABA is proposed to become an inhibitory transmitter (Kriegstein et al., 1987; Ben-Ari et al., 1989; Swann et al., 1989; reviewed by Cherubini et al., 1991). As a consequence of the increasing synaptic localization of $GABA_A$ receptors, such high efficacy of GABA would no longer be required and more assemblies could begin to contain α_1 subunits. The depolarizing and neurotrophic actions of GABA (protein synthesis, neurite/axon extension, synaptogenesis) may therefore be mediated through $GABA_A$ receptors constructed from a perinatal group of subunits (e.g., α_2, α_3, α_5, β_3, γ_2) until mature-type neuronal connections are established. It is of interest that in the adult hippocampus, which also contains predominantly α_2 and α_5 mRNAs, $GABA_A$ receptors operate both hyperpolarizing *and* depolarizing chloride currents (Wong and Watkins, 1982; Michelson and Wong, 1991).

Summary and conclusions

We have demonstrated by film and slide-emulsion autoradiography that all $GABA_A$ subunit genes exhibit different developmental expression patterns in rat brain. Based on our observations in the cortex, hippocampus, and thalamus, it seems likely that $GABA_A$ receptors are expressed on many telencephalic and mesencephalic neurons as soon as they cease migration. $GABA_A$ receptors in the perinatal brain are proposed to contain combinations of α_2, α_3, α_5, β_1, β_2, β_3, γ_1, γ_2, and γ_3 subunits. These receptors are often superseded in the adult by others containing α_1, α_4, α_6, β_2, γ_2, and δ subunits, except in some regions such as hippocampus that in maturity express both neonatal and adult receptor forms. The combinations of $\alpha_1\beta_2$, $\alpha_2\beta_3$, $\alpha_4\delta$, and $\alpha_6\delta$ are largely conserved during development, while the $\alpha_5\beta_1$ pairing converges over time. Based on mRNA levels, the γ subunits could combine with whatever α and β subunits are coexpressed in cells. The events controlling each spatial and temporal gene expression pattern are unknown, but the late-appearing transcripts (α_1, α_4, α_6, β_2, δ) coincide with the genesis of mature synapses. Receptors for many other transmitter systems (e.g., glycine, glutamate, 5-HT) also undergo marked changes in gene expression during rat brain development (Malosio et al., 1991; Monyer et al., 1991; Spitzer, 1991; Voigt et al., 1991). Any ontogenetic effects of the GABAergic system most likely involve interactions with these other systems as well. A better understanding of why these changes should occur during brain development, and what significance these may have in terms of the function and pharmacological manipulation of the fetal and neonatal GABAergic systems, is an intriguing prospect.

Appendix

List of anatomical abbreviations

A	Amygdala
Cb	Cerebellum
cb	Fetal cerebellum
cf	Cephalic flexure
Cl	Claustrum
Co	Colliculi
co	Fetal colliculi
cp	Cortical plate
CPu	Caudate putamen
Ctx	Neocortex
cx	Fetal cortex
d	Spinal cord, dorsal
DG	Dentate gyrus
di	Diencephalon
DRG	Dorsal root ganglia
eg	External granule cell layer
ER	Entorhinal cortex
gl	Glomerular layer
GP	Globus pallidus
H	Hippocampus
hi	Fetal hippocampus
hy	Fetal hypothalamus
i	Intestine
ig	Internal granule cell layer
L	Liver (nonspecific labeling)
m	Mitral cell layer
mes	Mesencephalon
met	Metencephalon
mg/MG	Medial geniculate nucleus
mol	Molecular layer
ms	Medial septum
my	Myelencephalon
ne	Nasal epithelium
OB	Olfactory bulb
ob	Fetal olfactory bulb
p	Purkinje cell layer
pt	Fetal pretectal area
r	reticular thalamic nucleus
S	Septum
sc	Fetal spinal cord
st	Fetal striatum
T	Thalamus

t	Fetal thalamus
te	Telencephalon
TG	Trigeminal ganglion
v	Spinal cord, ventral
vz	Ventricular zone

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