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Lamina-Specific Expression and Activity-Dependent Regulation of Seven $GABA_A$ Receptor Subunit mRNAs in Monkey Visual Cortex

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Seven monkey-specific $GABA_A$ receptor subunit cDNAs were isolated and cloned; radioactive cDNA and cRNA probes derived from them were used for Northern blot analysis and *in situ* hybridization histochemistry of the primary visual cortex (area 17), with comparative observations on other cortical areas. cDNAs corresponding to $\alpha 1$, $\alpha 2$, $\alpha 4$, $\alpha 5$, $\beta 1$, $\beta 2$, and $\gamma 2$ $GABA_A$ receptor subunits were isolated and had sequences unique to the monkey but recognized mRNAs of distinct molecular weights consistent with those reported in other species.

mRNAs for the $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits were expressed at much higher levels in area 17 than in motor, somatosensory, or temporal association cortex, possibly reflecting the greater density of GABA cells and synapses in area 17. In areas 17 and 18, each of the seven subunit mRNAs showed individually distinct patterns of laminar distribution. $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit mRNAs, which are thought to form the basis of receptors with the full range of classical $GABA_A$ receptor properties in the adult, were particularly enriched in layers II–III, IVC, and VI of area 17, following patterns of receptor distribution previously demonstrated by radioligand binding and immunocytochemistry. $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\beta 1$ transcripts had quite different localization patterns that did not match the antoradiographic or immunocytochemical receptor localization patterns. $\alpha 2$ and $\alpha 5$ subunit mRNAs, which are thought to be the subunits mainly expressed in development, were enriched in layer VI and the underlying white matter, possibly reflecting the involvement of receptors formed from $\alpha 2$ and $\alpha 5$ polypeptides in trophic interactions in the cortical subplate zone during development of the cerebral cortex.

Following 8–21 d periods of monocular deprivation induced by intravitreal injection of TTX, levels of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit mRNAs were substantially reduced in deprived ocular dominance columns of layer IVC in area 17. The effect was greatest for the $\alpha 1$ subunit; for both $\alpha 1$ and $\gamma 2$ subunit mRNAs, it extended into deprived rows of cytochrome oxidase–identified periodicities in other layers. Apart from the $\alpha 5$ subunit mRNA, which showed reduced levels in layer VI, the other subunit mRNAs were unaffected by monocular deprivation.

These results demonstrate the heterogeneity of $GABA_A$ receptor subunit expression in a complex, multilaminar cortical area. They suggest that receptors with different functional properties may be assembled from different combinations of subunit polypeptides in different layers and show that subunit expression is differentially regulated under activity-dependent conditions.

[Key words: Northern blots, in situ hybridization, areas 17 and 18, monocular deprivation, cortical development]

GABA is the principal inhibitory neurotransmitter in the CNS (reviewed in Krnjević, 1987; Olsen, 1991). It exerts its fast inhibitory effect by opening anion channels intrinsic to $GABA_A$ receptors, increasing chloride conductance and leading to the generation of IPSPs (reviewed in Olsen and Tobin, 1990). The $GABA_A$ receptor is a member of a superfamily of ligand-gated receptors made up of membrane-spanning, hetero-oligomeric complexes of polypeptide subunits that form an ion channel (Barnard et al., 1987; Schofield et al., 1987; Schofield, 1990). It is one of two known forms of GABA receptor. The other form, or $GABA_B$ receptor, is linked by GTP-binding proteins to intracellular second messenger systems and thence to calcium and potassium channels (Bowery et al., 1980, 1984, 1987, 1990; Bowery, 1983, 1989; Matsumoto, 1989). The responses of $GABA_A$ receptors to GABA are modulated by allosteric binding of barbiturates, benzodiazepines, and steroids at different sites on the receptor. These tend to increase GABA affinity and enhance the frequency of channel opening (Stephenson, 1988; Schofield, 1989; Sieghart, 1989; Olsen and Tobin, 1990; Seeburg et al., 1990; Burt and Kamachi, 1991; Richards et al., 1991; Vicini, 1991). The $GABA_A$ receptor is assembled from combinations of polypeptide subunits grouped by amino acid sequence homology into at least five classes: α, β, γ, δ, and ρ. Molecular cloning of subunit-specific cDNAs has identified one or more members of each subunit class in rat, bovine, mouse, or human brain, and at least 15 $GABA_A$ receptor subunit genes are now recognized (Schofield et al., 1987; Garrett et al., 1988; Hirouchi et al., 1989; Khrestchatisky et al., 1989, 1991; Lolait et al., 1989a; Pritchett et al., 1989b; Shivers et al., 1989; Ymer et al., 1989a,b, 1990; Lüddens et al., 1990; Malherbe et al., 1990c; Olsen and Tobin, 1990; Pritchett and Seeburg, 1990; Whiting et al., 1990; Bateson et al., 1991a; Cutting et al., 1991; Lasham et al., 1991; Lüddens and Wisden, 1991; Olsen et al., 1991a,b; Wisden et al., 1991b; Herb et al., 1992). The exact subunit composition of native $GABA_A$ receptors is not known. However, immunoprecipitation studies show that probably all contain an α subunit, and most β and γ subunits as well (Duggan and Stephenson, 1989, 1990; Stephenson et al., 1990; Benke et

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al., 1991a,b; Lüddens et al., 1991; Endo and Olsen, 1992, 1993; Mertens et al., 1993). In recombinant receptors expressed *in vitro*, different combinations of subunits confer different kinetic and pharmacological properties (e.g., Levitan et al., 1988; Pritchett et al., 1988, 1989a,b; Puia et al., 1990, 1991; Sigel et al., 1990; Verdoorn et al., 1990; Von Blankenfeld et al., 1990; Knoflach et al., 1991, 1992; Smart et al., 1991; Angelotti and MacDonald, 1993; Angelotti et al., 1993). This may explain the heterogeneity of functional properties observed in native receptors studied *in vivo* (Stephenson, 1988; Sieghart, 1989, 1991; Olsen and Tobin, 1990; Burt and Kamatchi, 1991; DeLorey and Olsen, 1992; Angelotti and MacDonald, 1993).

Western blotting and immunoprecipitation studies have revealed region-specific variations in the abundance of different $GABA_A$ receptor subunit polypeptides in rat brain (de Blas et al., 1988; Fuchs and Sieghart, 1989; Benke et al., 1990, 1991a–d; Fuchs et al., 1990; Olsen et al., 1990a, 1991a; Burchstaller et al., 1991a,b; McKernan et al., 1991; Möhler et al., 1991; Park and de Blas, 1991; Park et al., 1991; Sieghart and Schlerka, 1991; Zezula and Sieghart, 1991; Zezula et al., 1991; Zimprich et al., 1991; Duggan et al., 1992; Endo and Olsen, 1992, 1993). Similar variations in localizations of related mRNAs have also been demonstrated in Northern blotting and *in situ* hybridization studies (Levitan et al., 1988; Lolait et al., 1989; Khrestchatisky et al., 1989; Ymer et al., 1989a,b; Malherbe et al., 1990b,c; MacLennan et al., 1991; Zhang et al., 1991a–c; Wisden et al., 1992). In rats, *in situ* hybridization histochemistry has revealed the localization of at least 13 subunit-specific mRNAs in the CNS, including the cerebral cortex, in which transcripts coding different subunit polypeptides show lamina-specific and developmentally regulated patterns of localization (Montpied et al., 1988; Séquier et al., 1988; Sigel, 1988; Wisden et al., 1988, 1989a,b, 1991a, 1992; Khrestchatisky et al., 1989, 1991; Lolait et al., 1989b; Pritchett et al., 1989b; Shivers et al., 1989; Hironaka et al., 1990; Kato, 1990; Lüddens et al., 1990; Malherbe et al., 1990c; Ymer et al., 1990a,b; Zhang et al., 1990, 1991a–c; Bateson et al., 1991; Churchill et al., 1991; Gambarana et al., 1991; MacLennan et al., 1991; Möhler et al., 1991; Persohn et al., 1991, 1992; Laurie et al., 1992a,b; Poulter et al., 1992). There have been no comparable studies on the primate cerebral cortex.

The importance of GABA-mediated inhibition in cortical function has been documented at many different levels. It is involved in shaping the receptive fields of neurons in sensory areas (Sillito, 1975; Hicks and Dykes, 1983; Alloway and Burton, 1986; Crook and Eysel, 1992), in representational plasticity (Jacobs and Donoghue, 1991), in fundamental mechanisms of cellular behavior (Scharfman and Sarvey, 1985, 1987; Avoli, 1986; Connors et al., 1988; McCormick, 1989; Luhmann and Prince, 1990; Kawaguchi, 1993), including mechanisms underlying the collective actions of cortical neurons which, when decompensated, can lead to convulsive activity (Kriegstein et al., 1987; Chagnac-Amitai and Connors, 1989a,b; Aram et al., 1991; Prince and Tseng, 1993). In the visual cortex, the capacity of many neurons to respond to stimuli of a particular orientation (Sillito, 1977; Tsumoto et al., 1979; Hatta et al., 1988) and to stimuli moving in a particular direction (Sillito, 1975, 1977) and certain other receptive field properties (Sillito et al., 1980; Sillito, 1984; Bolz and Gilbert, 1986) are dependent on GABA-mediated inhibition. GABA-based inhibition also appears to be a key element in the early postnatal maturation of visual cortical function, and in the phenomenon of critical period–dependent ocular dominance plasticity in cats (Wolff et al., 1986; Ramoa et al., 1988; Reiter and Stryker, 1988).

$GABA_A$ receptor localization has previously been studied in the monkey cerebral cortex by radioligand binding and autoradiography (Shaw and Cynader, 1986; Rakic et al., 1988; Hendry et al., 1990; Shaw et al., 1991) and by receptor immunocytochemistry (Hendry et al., 1990; Huntley et al., 1990; Meinecke and Rakic, 1992). In the monkey visual cortex, these investigations have demonstrated a lamina-specific pattern of localization that tends to follow that of the major concentrations of GABA-producing neurons (Fitzpatrick et al., 1987; Hendry et al., 1990). Immunocytochemistry with $\alpha 1$, $\beta 2/\beta 3$, or $\gamma 2$ subunit–specific antibodies suggests that these subunits may have even more specific sublamina-specific patterns of localization (Huntsman et al., 1991; Hendry et al., 1993). The localization of the neurons expressing the genes for these and other $GABA_A$ receptor subunits remains unknown. Differences in the cellular expression of $GABA_A$ receptor subunits could imply the assembly of lamina-specific combinations of receptor subunits to form receptors with different functional properties. Finally, immunodetectable levels of the $\alpha 1$, $\beta 2/\beta 3$, and $\gamma 2$ polypeptides can be reduced in deprived ocular dominance columns by blocking impulse activity in one optic nerve for brief periods in adult monkeys (Hendry et al., 1990, 1993; Huntsman et al., 1991). However, it is not known if this effect extends to other subunits or if it depends upon an activity-dependent regulation of mRNA levels, as revealed for glutamic acid decarboxylase (GAD), β-preprotachykinin, and a number of other neuroactive molecules (Benson et al., 1991a, 1993).

In the present study, seven monkey-specific $GABA_A$ receptor subunit cDNAs, including representatives of the α, β, and γ classes, were cloned and used for Northern blot analysis and *in situ* hybridization histochemistry. Although human $GABA_A$ receptors have been cloned and translated in expression systems (Schofield et al., 1987), the pattern of expression in the primate brain in general, and in the primate visual cortex in particular, has not previously been mapped, and the extent to which different subunit mRNAs are subjected to activity-dependent regulation is unknown.

A preliminary report has appeared (Huntsman et al., 1992).

Materials and Methods

Oligonucleotide preparation. Synthetic oligonucleotides were prepared to enable amplification of cDNAs for selected subunits of the α, β, and γ classes of the $GABA_A$ receptor by the polymerase chain reaction (PCR). The synthetic oligonucleotide primers (Operon) were constructed to correspond to regions that are conserved across species in the targeted subunit class variants (e.g., rat $\alpha 1$, bovine $\alpha 1$, and human $\alpha 1$), but which differ in their subunit classes (e.g., $\alpha 1$, $\alpha 2$, $\alpha 3$, etc.). $\alpha 1$, $\beta 2$, and $\gamma 2$ were selected because these subunits are widely expressed in the adult rat brain (Wisden et al., 1992) and because they are thought to be essential for forming a receptor with "classical" $GABA_A$ properties (Sigel et al., 1990). For comparison, $\alpha 4$ and $\beta 1$ were selected as subunits not highly expressed in rat brain (Wisden et al., 1992), and $\alpha 2$ and $\alpha 5$ as subunits expressed primarily in immature rat brain (Laurie et al., 1992b; Poulter et al., 1992).

The two oligonucleotides synthesized for PCR amplification of the monkey $\alpha 1$ subunit cDNA were 24 and 21 nucleotides long, and identical to published human (Schofield et al., 1989) and bovine (Schofield et al., 1987) cDNA sequences. The 5′ sense-strand oligonucleotide (5′-ATG GAA TAT ACA ATA GAT GTA TTT-3′) contained 24 bases corresponding to bases 468–491 of the human $\alpha 1$ cDNA (Schofield et al., 1989), and the 3′ antisense-strand oligonucleotide (5′-TTT GTT ATT CAA ACA TAC CTG-3′) contained 21 bases corresponding to bases 971–991. The resultant amplified cDNA was 524 bases.

The two oligonucleotides synthesized for PCR amplification of the

monkey $\alpha 2$ subunit cDNA were 21 nucleotides long, and identical to published rat (Khrestchatisky et al., 1991) cDNA sequences. The 5′ sense-strand oligonucleotide (5′-ATG GAA TAT ACA ATA GAT GTT-3′) contained 21 bases corresponding to bases 597–618 of the rat $\alpha 2$ cDNA (Khrestchatisky et al., 1991), and the 3′ antisense-strand oligonucleotide (5′-TGG AAA GTC CTC CAA GTG CAT-3′) contained 21 bases corresponding to bases 846–866 of the rat $\alpha 2$ cDNA (Khrestchatisky et al., 1991). The resultant amplified cDNA was 269 bases.

The two oligonucleotides synthesized for PCR amplification of the monkey $\alpha 4$ subunit cDNA were 21 nucleotides long, and identical to published rat (Wisden et al., 1991) cDNA sequences. The 5′ sense-strand oligonucleotide (5′-ATG GAA TAC ACA ATG GAT GTG-3′) contained 21 bases corresponding to bases 271–291 of the rat $\alpha 4$ cDNA (Wisden et al., 1991), and the 3′ antisense-strand oligonucleotide (5′-CGC ACT TAT GGT GAG TCT CAT-3′) contained 21 bases corresponding to bases 490–510 of the rat $\alpha 4$ cDNA (Wisden et al., 1991). The resultant amplified cDNA was 239 bases.

The two oligonucleotides synthesized for PCR amplification of the monkey $\alpha 5$ subunit cDNA were 21 nucleotides long, and identical to published rat cDNA sequences (referred to as $\alpha 5$ by Wisden et al., 1991a, but as $\alpha 4$ by Khrestchatisky et al., 1989). The 5′ sense-strand oligonucleotide (5′-GAG ACC AAT GAC AAC ATC ACA-3′) contained 21 bases corresponding to bases 250–270 of the rat cDNA (Khrestchatisky et al., 1989), and the 3′ antisense-strand oligonucleotide (5′-TCC ATT GCG CAC AAC ATG ACG-3′) contained 21 bases corresponding to bases 550–570 of the rat cDNA (Khrestchatisky et al., 1991). The resultant amplified cDNA was 300 bases.

The two oligonucleotides prepared for PCR amplification of the monkey $\beta 1$ subunit cDNA were 21 bases long and identical to human $\beta 1$ (Schofield et al., 1989), rat $\beta 1$ (Ymer et al., 1989a), and bovine $\beta 1$ (Schofield et al., 1987) cDNA sequences. The 5′ sense-strand oligonucleotide (5′-ATG GTT TGT TGT GCA CAC AGC-3′) contained 21 bases corresponding to bases 113–133 of the bovine $\beta 1$ cDNA (Schofield et al., 1987), and the 3′ antisense-strand oligonucleotide (5′-CTG GAG ATC GAA AGT TAT GGC-3′) contained 21 bases and corresponded to bases 580–600. The resultant amplified cDNA was 487 bases.

The two oligonucleotides prepared for PCR amplification of the monkey $\beta 2$ subunit cDNA were 21 bases long and identical to a published rat $\beta 2$ cDNA sequence (Ymer et al., 1989a). The 5′ sense-strand oligo nucleotide (5′-CCC CCT GTG GCA GTA GGA ATG-3′) contained 21 bases corresponding to bases 247–267 of the rat $\beta 2$ cDNA sequence (Ymer et al., 1989), and the 3′ antisense-strand oligonucleotide (5′-GGC AGC TGT AGT TGT GAT TCT-3′) contained 21 bases corresponding to bases 533–553 of the rat $\beta 2$ cDNA. The resultant amplified cDNA was 307 bases.

The two oligonucleotides prepared for PCR amplification of the monkey $\gamma 2$ subunit cDNA were 24 and 21 bases long and identical to published human (Pritchett et al., 1989b) and rat (Malherbe et al., 1990c) $\gamma 2$ cDNA sequences. The 5′ sense-strand oligonucleotide (5′-TAT GAC AGA CGT TTG AAA TTT AAC-3′) contained 24 bases and corresponding to bases 702–725 of the rat $\gamma 2$ cDNA (Malherbe et al., 1990c), and the 3′ antisense-strand oligonucleotide (5′-TAC TTT ACC ATC CAG ACC TAT-3′) contained 21 bases and corresponded to bases 1158–1179 of the rat $\gamma 2$ cDNA. The resultant amplified cDNA was 477 bases.

Subcloning of cDNAs by PCR. Two normal monkeys (*Macaca mulatta*) were given an overdose of Nembutal and the brains and liver were removed. Total RNA for cDNA synthesis and Northern blot analysis was extracted from the cerebral cortex and liver according to the methods of Chirgwin et al. (1979), and Chomczynski and Sacchi (1987). The amount and purity of RNA were measured by reading the absorbance at a wavelength of 260 nm, and a ratio of 2.0 at 260:280 nm. Five micrograms of total RNA was primed with the pd(N)6 random primer and transcribed with Moloney Murine Leukemia virus reverse transcriptase (Pharmacia). First-strand cDNA (1 μg/100 μl) was subjected to 36 cycles of PCR amplification (Perkin-Elmer Cetus, model 480), using 2.5 U/100 μl of AmpliTaq DNA polymerase (Perkin-Elmer Cetus), 2 mM $MgCl_2$, 800 μM dNTPs, and the appropriate oligonucleotide primers, described above.

EcoRI linkers were phosphorylated with polynucleotide kinase (Boehringer-Mannheim) and ligated with T4 DNA ligase (Boehringer-Mannheim) to the $\alpha 1$, $\beta 1$, $\beta 2$, and $\gamma 2$ amplified monkey subunit cDNAs, which were then digested with EcoRI, purified by electroelution from a 5% polyacrylamide gel, ligated to the pBS transcription vector (Stratagene), and transformed into 71.18 competent cells. Subcloning of the $\alpha 2$, $\alpha 4$, and $\alpha 5$ monkey cDNAs was obtained by insertion into the pBS vector and by using pCR-Script (Stratagene) in which amplified cDNAs were simultaneously digested with SrfI, ligated with T4 DNA ligase, and then transformed into XI1 Blue competent cells (Stratagene). The plasmids containing inserts were purified (QIAGEN) and sequenced by the dideoxy chain termination method (Sanger et al., 1977) using the Sequenase version 1.0 sequencing system (U.S. Biochemicals).

Preparation of cDNA and cRNA probes. cDNA probes to be used for Northern blot analysis were excised from the pBS transcription vector with EcoRI for the $\alpha 1$, $\beta 1$, $\beta 2$, and $\gamma 2$ receptor subunits, and with BamHI/PstI for cyclophilin (to be used as a control). The $\alpha 2$, $\alpha 4$, and $\alpha 5$ subunit cDNAs were excised from the PCR-Script vector (Stratagene) with BssHII. Digested cDNA was then electrophoresed on a 5% polyacrylamide gel and electroeluted. Template DNA (500 ng/ml) was labeled with α-^{32}P-dCTP (DuPont–New England Nuclear) with Klenow fragment DNA polymerase and random hexanucleotides to prime the DNA synthesis. Unincorporated label was removed by running the newly synthesized reaction mixture through a Sephadex G-50 column (Pharmacia).

For *in vitro* transcription, the $\alpha 1$, $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\gamma 2$ -clones were linearized with PvuII and the $\beta 1$ and $\beta 2$ clones with PvuI. Antisense-strand cRNA riboprobes to be used for *in situ* hybridization histochemistry were generated by *in vitro* transcription using T3 RNA polymerase in the presence of α-^{35}S- or α-^{33}P-dUTP (DuPont–New England Nuclear). Sense-strand riboprobes were generated with T7 RNA polymerase. Unincorporated label was removed by selective precipitation with 5 M ammonium acetate (pH 5.0), and double volumes of ethanol.

Northern blot analysis. Two normal monkeys (*Macaca mulatta*) were given an overdose of Nembutal, the brains and liver removed, and blocks taken from the occipital pole, pre- and postcentral gyri, and superior temporal gyri of the brains. Exactly 5 μg and, in certain cases (see Results), 10 μg of total RNA extracted from primary visual, motor, somatosensory, and superior temporal areas and liver were run through a denaturing mixture of 50% deionized formamide and 5% formaldehyde at 60°C for 15 min, and loaded onto a size-fractionating 1% agarose/formaldehyde gel (Sambrook et al., 1989). The RNA was transferred to a nylon membrane (Bio-Rad) overnight, and baked at 80°C for 1 hr. The nylon membrane was then pretreated with 10 ml of 7% SDS, 1 mM EDTA, and 40 mM sodium phosphate for 30 min. The membrane was then hybridized in the same solution containing the ^{32}P-labeled probe overnight, washed in decreasing concentrations of SDS for a total of 2 hr, and exposed to Kodak XR film for either 12 or 36 hr. Each cDNA probe used for Northern blot analysis was excised from the pBS plasmid by digestion with EcoRI, run though a 5% polyacrylamide gel, and electroeluted. To determine the quality of transfer of total RNA to the nylon membrane, all blots were stripped, and rehybridized with a BamHI/PstI restricted cDNA fragment for the ubiquitously expressed protein cyclophilin (Danielson et al., 1988). Molecular weights were determined by running 5 μg of RNA markers (Promega) along with the sample RNA.

In situ *hybridization histochemistry.* This portion of the study was carried out on the visual cortex from six additional macaque monkeys (three *Macaca mulatta,* three *Macaca fuscata*) ranging in age from 2 to 7 years. One monkey served as a control, and five were deprived of vision in one eye for periods ranging from 8 to 21 d by injecting 15 μg of the sodium channel blocker tetrodotoxin (TTX) in 10 μl of normal saline into the vitreous cavity at 4 d intervals. For the injections, the animals were anesthetized with ketamine. After the survival period, all animals were given an overdose of Nembutal and perfused transcardially with normal saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were postfixed overnight in 4% paraformaldehyde and cryoprotected in 30% sucrose/4% paraformaldehyde.

Blocks were frozen on dry ice, and 25-μm-thick serial sections were cut on a sliding microtome perpendicular or tangential to the pial surface of the lateral occipital lobe, and collected in groups of four. Sections for *in situ* hybridization histochemistry were kept in 4% paraformaldehyde at 4°C for at least 48 hr. One section of each four was labeled with the antisense probe for a particular $GABA_A$ receptor subunit, a second section was stained with 0.25% thionin, a third was stained for cytochrome oxidase (CO; Wong-Riley, 1979), and the fourth was used for controls and for repeat series if necessary.

Free-floating sections were pretreated for *in situ* hybridization by successive incubations in 0.1 M glycine in 0.1 M phosphate buffer (pH 7.4); 1 mg/ml of proteinase K (pH 8.0) for 30 min at 37°C; 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0); and 2× saline–sodium

citrate (SSC). Sections were then incubated in the hybridization solution containing 50% formamide, 10% dextran sulfate, 0.7% Ficoll, 0.7% polyvinyl pyrolidone, 0.5 mg/ml yeast tRNA, 0.33 mg/ml denatured herring sperm DNA, and 20 mM dithiothreitol (DTT) for 1 hr at 60°C, and then transferred to fresh hybridization solution containing an additional 20 mM DTT and 1×10^6 cpm/ml of the ^{35}S or ^{33}P antisense riboprobe for at least 20 hr at 60°C.

Following hybridization, sections were washed in 4× SSC at 60°C, digested with 20 mg/ml ribonuclease A (pH 8.0) for 30 min at 45°C, and washed through descending concentrations of SSC with 5 mM DTT to a final stringency of 0.1 × SSC at 60°C for 1 hr. Sections were mounted onto gelatin-coated slides, dried, and exposed to Amersham βMax film for 4 or 7 d. Following development of the film, the sections were lipid extracted in chloroform, dipped in Kodak NTB2 emulsion diluted 1:1 with water, exposed for 20–40 d at 4°C, developed in Kodak D-19, fixed, and stained through the emulsion with cresyl violet. Selected film autoradiographs from normal and deprived animals were quantified by densitometry using a microcomputer imaging device (MCID/M4; Imaging Research, Inc., St. Catherine's, Ontario). Optical density readings were taken in stripes of defined width through the full thickness of area 17 or horizontally across six or more deprived and normal eye columns in layer IVC, matching the readings to digitized images of adjacent CO- or Nissl-stained sections or to columns showing reduced and normal staining in adjacent CO-stained sections. Multiple readings were also taken from single columns. Background readings were taken over the subcortical white matter and subtracted from the others. Absolute values of radioactivity were determined from ^{14}C plastic standards (Amersham) exposed on the same sheet of film. Significance of differences was assessed using the two-tailed Student's t test. Sections hybridized with sense-strand subunit riboprobes showed no labeling above background levels (Fig. 1).

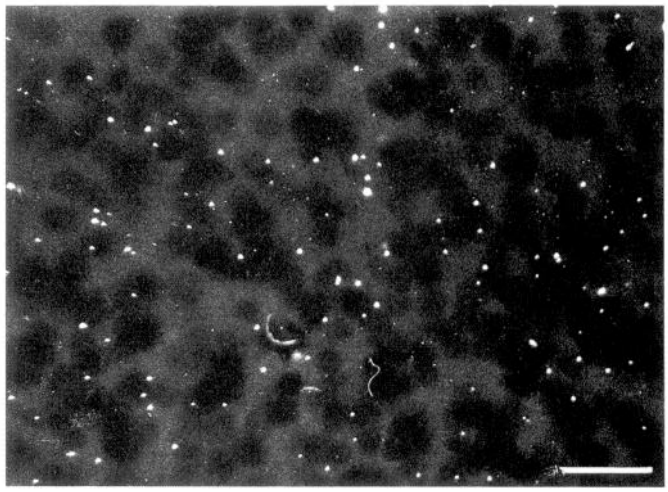


Figure 1. Dark-field photomicrograph from layer IVC of a control section hybridized with a sense cRNA probe for the γ2 receptor subunit, showing typical pattern of background labeling found with sense probes for all subunit mRNAs. Darker profiles in background are outlines of cell somata. Scale bar, 25 μm.

Results

Cloning and sequence analysis of monkey-specific cDNAs

PCR-generated cDNA clones for each of the seven $GABA_A$ receptor subunits were amplified from monkey cerebral cortex cDNA and sequenced. Multiple subclones for each of the receptor subunits were obtained and designated as follows: pBS $MGABA_A$ α1-39, -40, -42, and -45; pBS $MGABA_A$ α2-5; pCR-Script $MGABA_A$ α2-1, -2, -4; pBS $MGABA_A$ α4-25, -26; pCR-Script $MGABA_A$ α4-27, -28; pBS $MGABA_A$ α5-14; pCR-Script $MGABA_A$ α5-9, -10, -12; pBS $MGABA_A$ β1-16, -17, -18, -21; pBS $MGABA_A$ β2-24, -37, -47, -48; and pBS $MGABA_A$ γ2-47, -48, -49, -50. All monkey $GABA_A$ receptor cDNA sequences were colinear with the human and rat sequences. The monkey α1, β1, and γ2 cDNAs were nearly identical to their human counterparts. An occasional third base substitution occurred in codons which resulted in no net change in the amino acid encoded. Comparison of the rat α1, α2, α4, α5, β1, β2, and γ2 subunit cDNAs showed less similarity than between human and monkey, yet there was still a high degree of conservation between the sequences.

A comparison of portions of sequences that overlapped between the monkey cDNAs showed that the β1 and β2 subunits showed the highest percentage of sequence identity. This was expected because both of these subunits share unique restriction sites, which are different from those in the α1, α2, α4, α5 and γ2 subunit cDNAs.

Northern blot analysis

The α1 cDNA probe recognized a distinct mRNA transcript at 4.3 kilobase (kb) in all lanes containing RNA from the different areas of cerebral cortex (Fig. 2, α1). In underexposed autoradiograms, a second transcript was present at 3.9 kb, but was commonly obscured by the heavily expressed 4.3 kb transcript. When equal amounts (5 μg) of total RNA were loaded onto each gel, the α1 subunit transcript, as measured by densitometry, showed approximately 5–10 times higher levels in the visual cortex than in the temporal, motor, or somatosensory cortex. There was no detection of signal in the lane containing liver RNA for the α1, or any of the other subunit probes.

Due to expected low levels of expression of α2, α4, α5, and β1 mRNAs (Wisden et al., 1992), in these cases, 10 μg of total RNA was loaded onto the gel in order to obtain a detectable signal and in lanes approximately half the width of those loaded for α1, β2, and γ2 mRNAs (Fig. 2, bottom row). The monkey α2 cDNA recognized two distinct mRNA transcripts at 8.5 and 3.6 kb. The α2 probe hybridized to the two transcripts at equal intensity in each lane, but the highest levels of expression were seen in lanes containing RNA from the visual cortex.

The α4 cDNA recognized two distinct bands of 4 and 11 kb. The 11 kb transcript was the most abundant of the two transcripts. The monkey α4 mRNA was most abundant in visual and temporal areas and less abundant in the motor and somatosensory areas.

The α5 cDNA recognized a single distinct transcript at 2.8 kb. The α5 subunit mRNA was distinguished from the other α subfamily members in that hybridization was approximately equal in all four areas of the cerebral cortex examined.

The β1 cDNA recognized a single transcript at 13 kb. The 13 kb transcript showed the highest level of hybridization signal in the visual and temporal areas, with less in the motor and somatosensory areas.

The β2 cDNA recognized one distinct transcript at 8 kb in Northern blots containing 5 μg of total RNA (Fig. 2, β2). The β2 transcript was more abundant than the β1 transcript and with higher expression in the visual cortex than in the other three areas. Of the seven monkey cDNAs tested, the β2 and α1 transcripts were expressed in the visual cortex at levels many times higher than in the other three cortical areas.

The γ2 cDNA recognized one distinct transcript at 3.9 kb, in Northern blots containing 5 μg of total RNA (Fig. 2, γ2). The γ2 subunit mRNA was also highest in the lane containing visual cortex total RNA. Although there was a higher signal in the visual cortex, the overall density of hybridization for the γ2

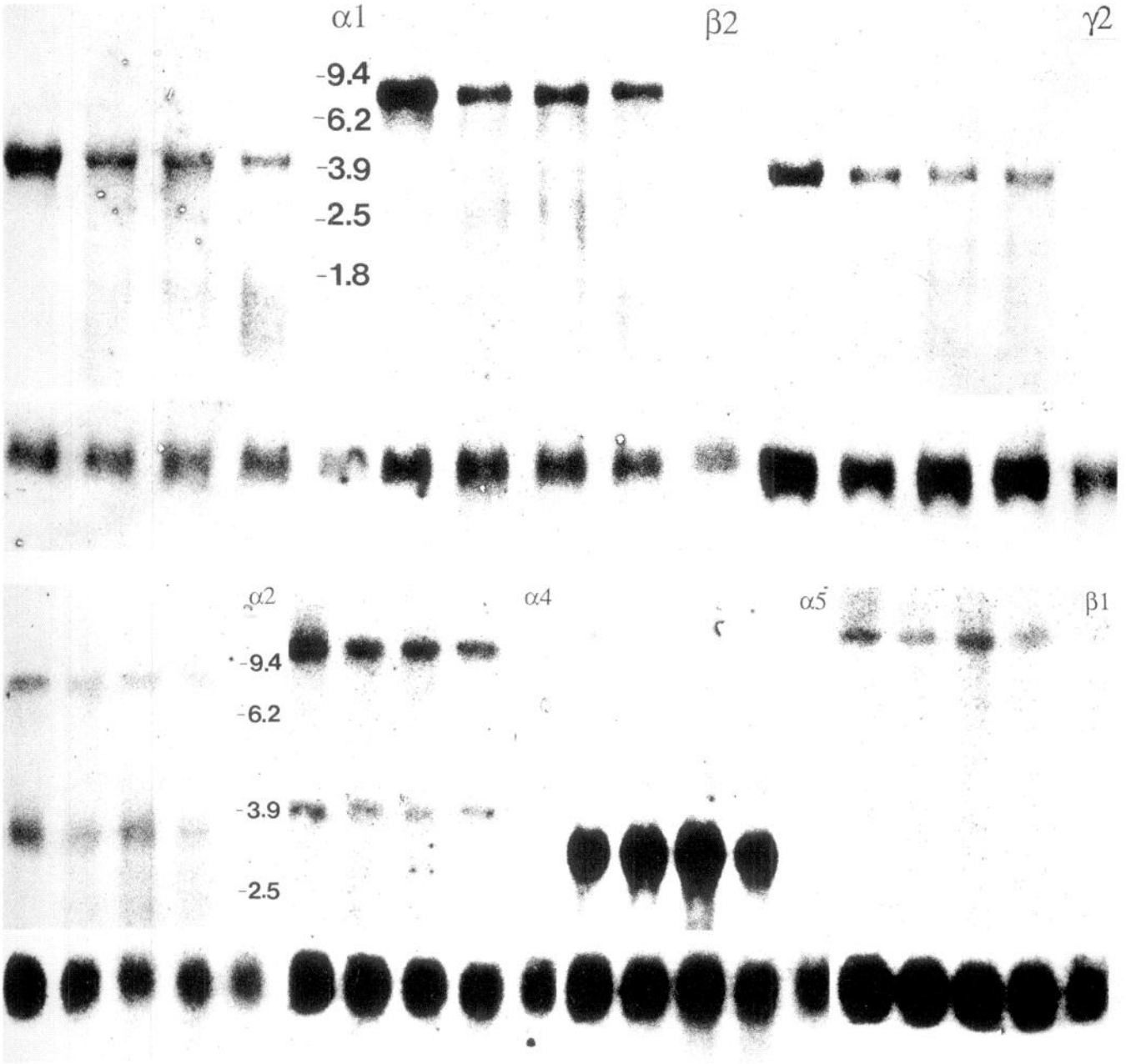


Figure 2. Photographs of Northern blots containing total mRNA hybridized with ^{32}P-labeled, subunit–specific cDNAs (*top member of each pair*) and, after stripping and rehybridization with a ^{32}P-labeled, cyclophilin cDNA (*bottom member of each pair*). In each blot, lanes *read from left to right* were loaded with equal amounts of total RNA from visual cortex, motor cortex, somatosensory cortex, temporal cortex, and liver. Size markers are indicated in kilobases. Note generally higher expression of $\alpha 1$, $\beta 2$, and $\gamma 2$ transcripts in visual cortex in comparison with other areas. Lanes showing $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit transcripts were loaded with 5 μg of total RNA; lanes showing $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\beta 1$ subunit transcripts were narrower and loaded with 10 μg of total RNA.

subunit did not differ as greatly between lanes in comparison with the $\alpha 1$ and $\beta 2$ subunits.

To determine the quality of transfer of total RNA to the nylon membrane, all blots were stripped and rehybridized with a BamHI/PstI restricted cDNA fragment coding the ubiquitously expressed protein cyclophilin (Fig. 2). In every case, the cyclophilin probe recognized a 1.0 kb transcript in all lanes containing monkey cerebral cortex RNA, and in the lane containing monkey liver RNA. Equal levels of expression were detectable in the four cortical areas, but there was a lower signal with liver RNA, which is consistent with the pattern of expression found in the rat (Danielson et al., 1988).

Normal distribution of $GABA_A$ receptor subunit mRNAs in monkey visual cortex

The distribution of $GABA_A$ receptor subunit mRNAs was examined in the visual cortex using *in situ* hybridization histochemistry with ^{35}S- or ^{33}P-labeled cRNA probes. In the emulsion autoradiographs, in regions containing significant labeling, silver grains were present in clusters over cresyl violet–counterstained

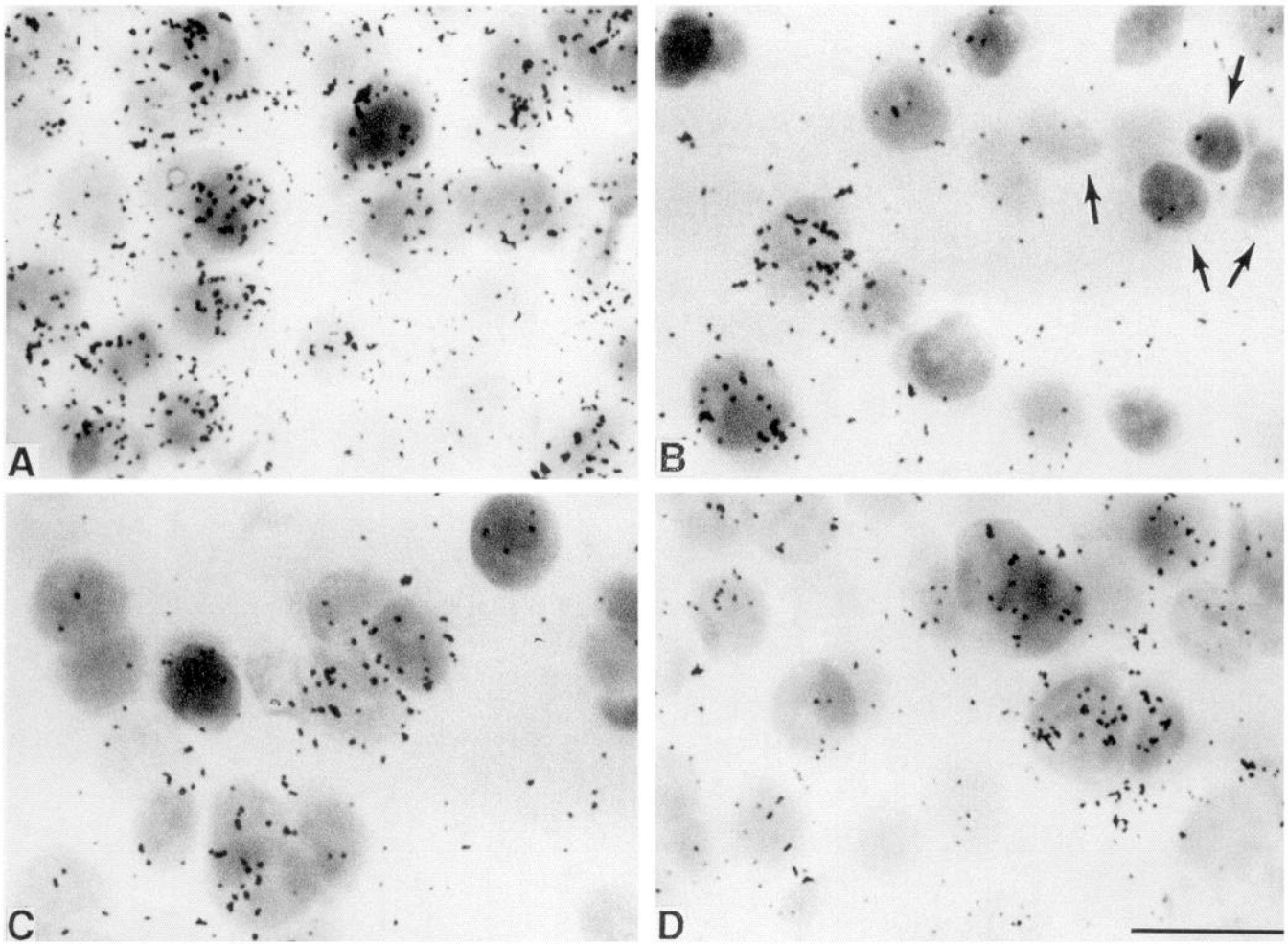


Figure 3. Bright-field photomicrographs from emulsion-dipped autoradiographs, showing localization of silver grains representing hybridization of radioactive cRNA probes complementary to $GABA_A$ receptor subunit–specific mRNAs. *A* shows $\alpha 1$ subunit–specific probe hybridization to large numbers of small neuronal somata in layer IVC of area 17. *B* shows $\gamma 2$ subunit–specific probe hybridization to neuronal somata in layer VI of area 17. Note lack of hybridization over neuroglial cells (*arrows*). *C* and *D* show $\alpha 1$ subunit–specific hybridization over selected cells in layers II (*C*) and V (*D*). Scale bar, 20 μm.

cell somata in areas 17 and 18 (Fig. 3). Cells were identified mainly by the size of the nucleus since RNase treatment after the hybridization step prevents most cytoplasmic staining with Nissl stains. Estimation of cell size is thus based on the sizes of grain clusters over a particular cell profile which bears a relationship to the size of the underlying cell (Benson et al., 1991b). Nuclei identified as those of astrocytes and oligodendrocytes on the basis of size and staining density were not labeled (Fig. 3B). T3 RNA polymerase-generated sense control riboprobes for all the subunit cDNAs were hybridized to sections but showed no labeling above background (Fig. 1).

Laminar distribution of receptor subunit mRNAs

With the exception of the $\beta 1$ subunit, which is expressed at relatively low levels in the cerebral cortex (see above), all subunits were expressed at high levels and *in situ* hybridization for their mRNAs led to relatively dense labeling of areas 17 and 18. There were, however, different densities overall, and patterns of labeling in relation to cortical layers and sublayers were unique to each of the probes. These differences reflect lamina-specific patterns of gene expression for each of the subunits.

Area 17

Intense autoradiographic labeling was seen on film autoradiograms after exposure times of 4 d for $\beta 2$ and $\gamma 2$ cRNA probes. Exposure times of 7 d were required to enhance labeling with $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\beta 1$ cRNA probes to a level suitable for assessing laminar distribution patterns.

$\alpha 1$ subunit. Autoradiographic labeling for $\alpha 1$ mRNA was denser overall than with all other subunit-specific probes (Figs. 4*A*, 5). The densest labeling by far was coextensive with layer IVC. Superficial to layer IVC, layer I showed only light labeling while layers II–III and IVA were merged as a single zone of homogeneous labeling of somewhat lower density than layer IVC. In emulsion autoradiographs, the majority of labeled cells in layers II–IVA were associated with grain clusters 12–15 μm in diameter, but in deeper parts of this zone a number of larger grain clusters approximately 25 μm in diameter were also seen (Fig. 6*A*,*B*). Layer IVB showed a low density of labeling, approximately the same as that of layer I but higher than levels over the white matter. Labeling in layer IVC was dense throughout, with little clear distinction between labeling of layers IV$C\alpha$ and IV$C\beta$. There was a slight tendency for an increase in density in the deepest part of layer IV$C\beta$. In emulsion autoradiographs,

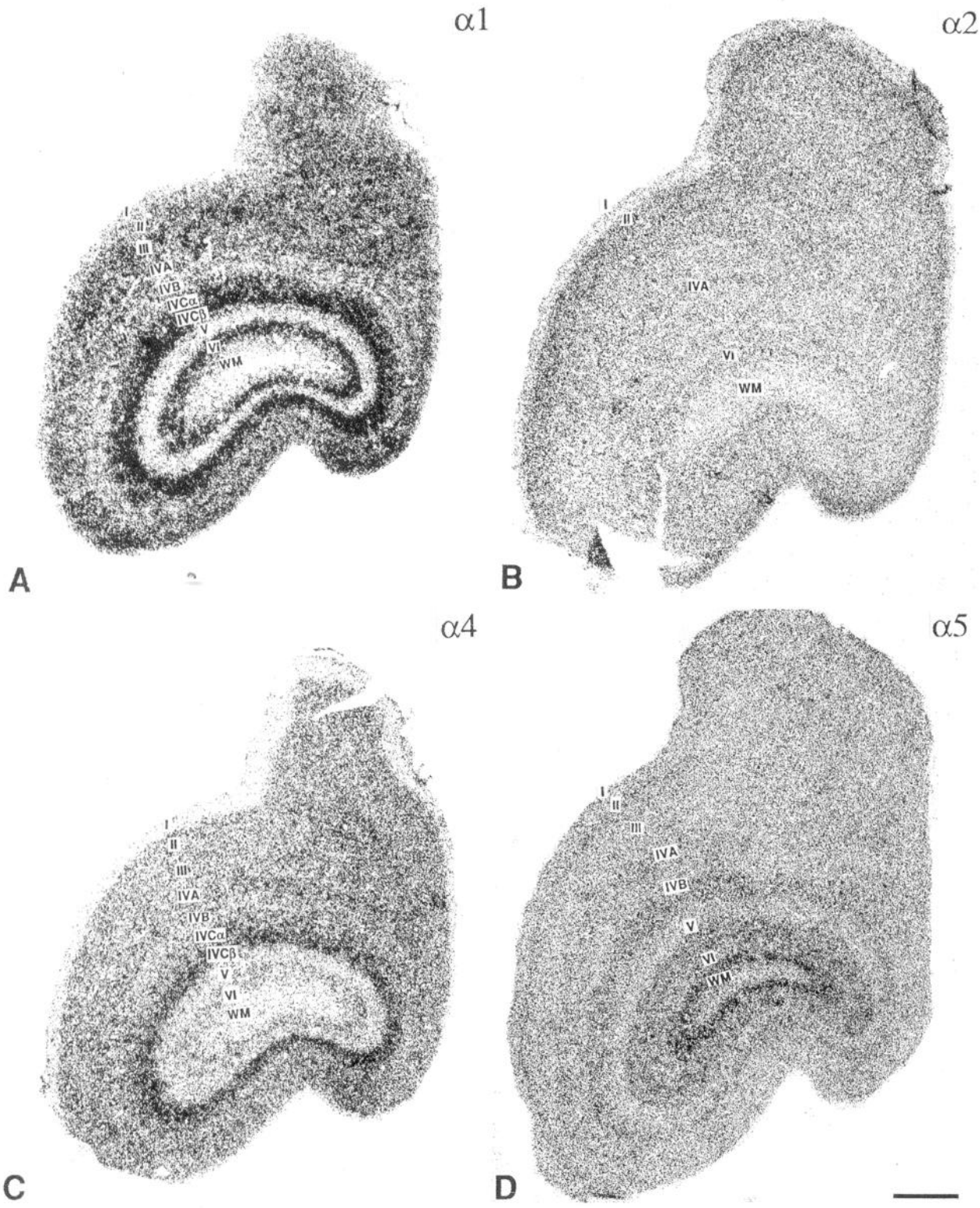


Figure 4. Photomicrographs from film autoradiograms (*A–G*) of adjacent sections through area 17, hybridized with $GABA_A$ receptor subunit–specific cRNA probes as indicated, and from a further adjacent section (*H*) stained for cytochrome oxidase (CO). These show the patterns of distribution of the seven subunit-specific mRNAs in relation to the cortical laminae. All autoradiograms were exposed for the same time (from *Macaca mulatta*). Scale bar, 1 mm.

the layer IVC labeling was almost universally in the form of small grain clusters 12–15 μm in size (Figs. 3*A*, 6*A*). In the deepest aspect of layer IVCβ where cell density falls off in most macaque monkeys (see Fig. 1 of Lund, 1973), there was a thin line of enhanced label. It was best seen in the Japanese monkeys (*Macaca fuscata*, Fig. 7*A,G*) and proved to be coextensive with a thin line of darkly stained cells consistently found at that level in this species (Fig. 7*G*).

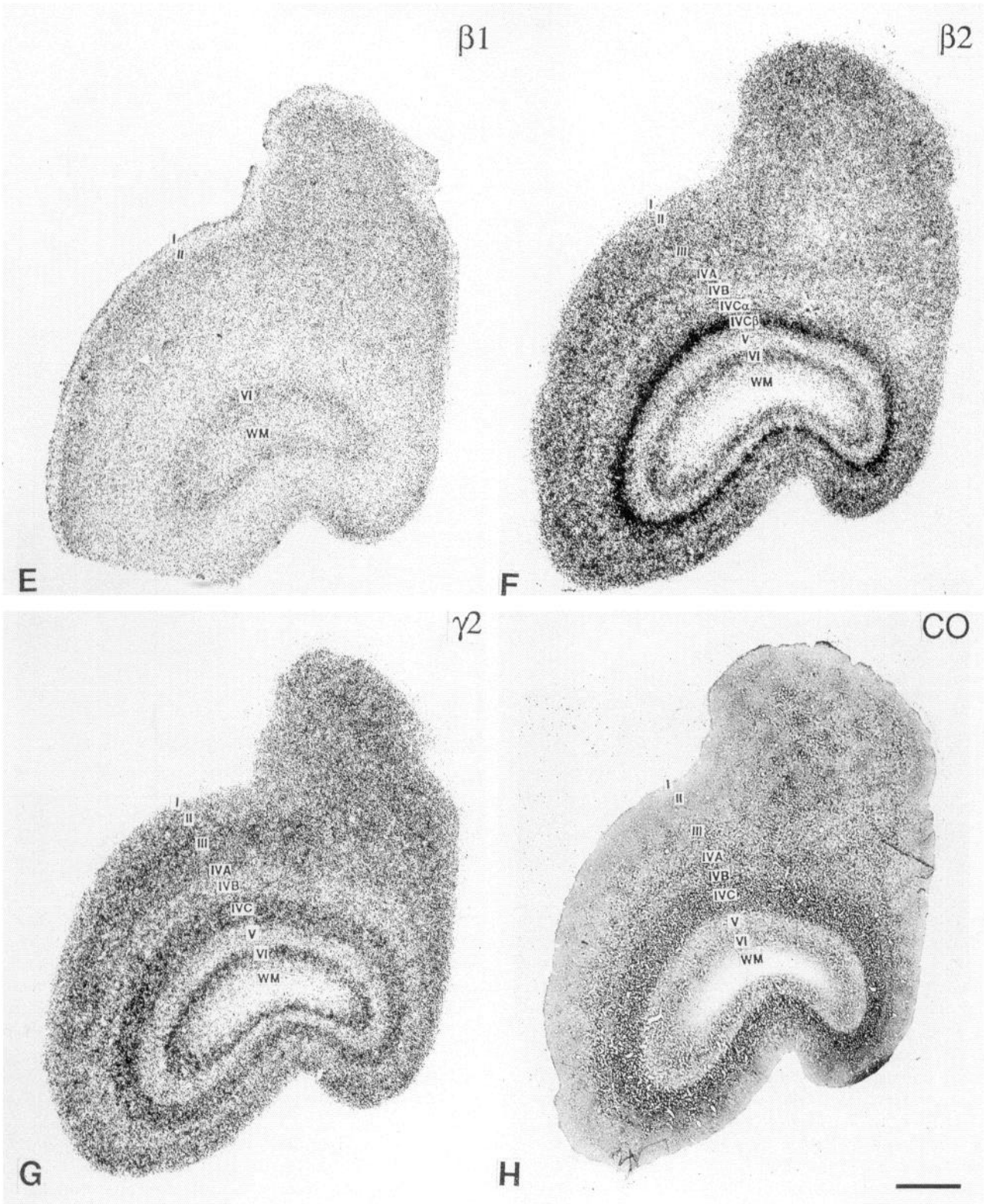


Figure 4. Continued.

In layer V there was a dramatic reduction in density of label, approaching that seen over the white matter. In layer VI there was an increase in label density to levels similar to that in layer IVC, with a sharp drop-off at the border with the white matter. In emulsion autoradiographs, most grain clusters in layers V and VI were of large size (~25 μm in diameter; Fig. 3*D*).

α2 subunit. Autoradiographic labeling for α2 subunit mRNA showed a laminar distribution pattern dramatically different from that seen with α1 riboprobes (Figs. 4*B*, 5). Labeling in all layers was relatively weak but denser hybridization signal appeared in two bands coextensive with layers II and VI.

Layer I showed the weakest labeling density, only slightly

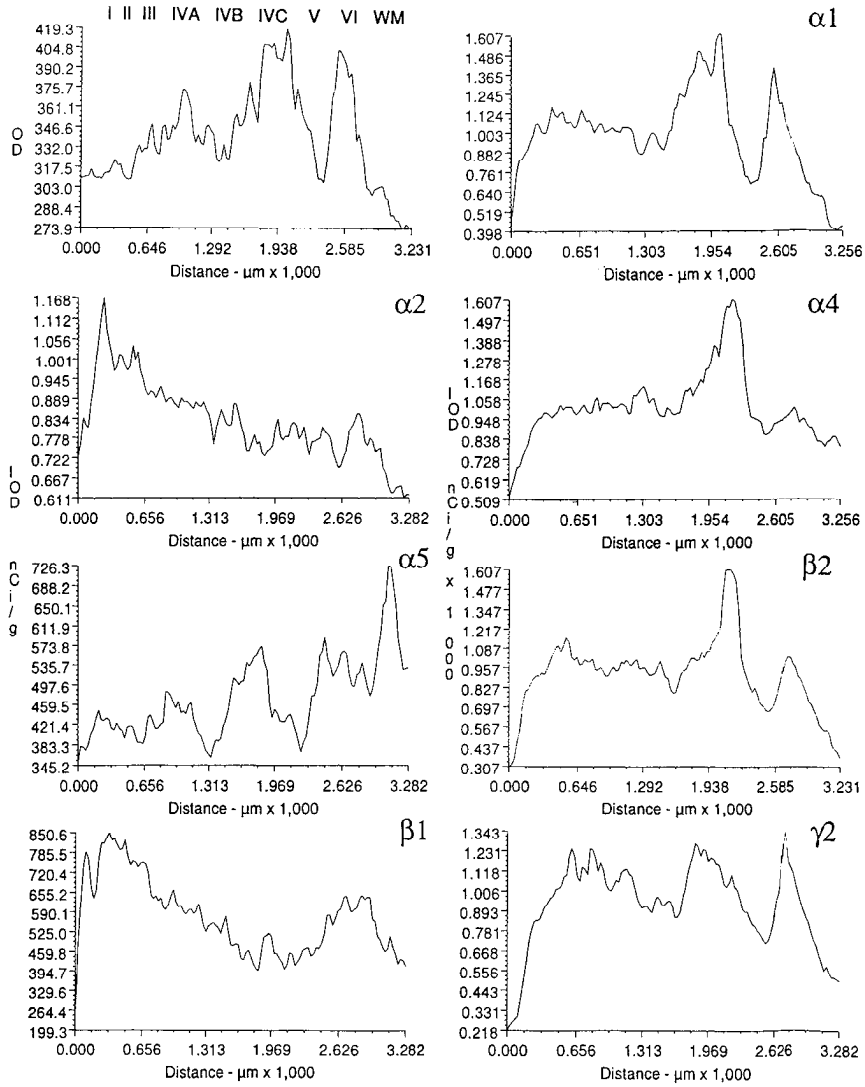


Figure 5. Optical density readings taken in traverses across the full thickness of the cortex approximately in the position of the line of labels marking the cortical layers in Figure 4*A–G*. Density readings have been converted to measures of radioactivity by reference to standards exposed on the same sheet of film. Layers have been added by matching to a similarly digitized image (*top*) of a Nissl-stained section adjacent to Figure 4*H*.

greater than that over the white matter. Layer II showed enhanced labeling. The greater part of layer III, although more weakly labeled than layer II, showed a moderate increase in label in comparison with layer I. Labeling in the deepest 100–200 μm of layer III became reduced again, only to return to the levels of the upper parts of layer III in a region coextensive with layer IVA. Labeling in layer IVB returned to the low levels of layer III, but increased slightly in a region coextensive with layer IVCα. Most of layer IVCβ showed low levels of label except for a moderately dense line at its deep border coextensive with the line of small, darkly stained cells found prominently in the Japanese monkey (see Fig. 7*E*). Layer V had a very low level of label but that in layer VI formed a wide band of equally high density in both the superficial and deep subdivisions of layer VI and extending for approximately 100 μm into the white matter. All grain clusters in emulsion autoradiographs were in the 12–15 μm range and thus overlay small or relatively small neurons.

α4 subunit. The densest labeling with α4 subunit riboprobes was in layer IV. There was a clear bilaminar pattern with a dense band, coextensive with layer IVCβ, that approached in density that seen with the α1 subunit probe (Figs. 4*C*, 5). Layer IVCα was also relatively densely labeled but less intensely than layer IVCβ. Elsewhere, layer I showed virtually no labeling above background and layers II and III appeared as a homogeneous zone of moderately dense labeling. In comparison, there was slightly enhanced labeling in layer IVA. Layer IVB showed weak labeling, less intense than that in layers II–III. Layer V labeling was also weak. Layer VI labeling was enhanced and approached that found in layers II–III. In emulsion autoradiographs, grain clusters representing labeled cells were mostly 12–15 μm in diameter.

α5 subunit. Hybridization of cRNA probes specific for α5 subunit mRNA showed the most unique pattern of laminar distribution, since it was difficult to associate with many of the standard Nissl- or CO-stained layers of area 17 (Figs. 4*D*, 5). The densest band of label was coextensive with a deep zone of layer VI and 100–200 μm of the underlying white matter. Layer I showed the weakest labeling. Layer II showed enhanced labeling in comparison with layer III. Layer III was weakly labeled but showed slightly enhanced labeling in its deeper parts. Layer IVA showed reduced labeling approaching that seen in layer I. Layer IVB showed slightly enhanced labeling equivalent in density to that in layer II. It was difficult to be confident that this

label was confined to layer IVB; it may have extended into the superficial part of layer IVCα. Most of layer IVCα and all of layer IVCβ, including its deep row of cells in the Japanese monkeys, had weak labeling, equivalent in density to that in layers I and IVA. Labeling increased in density again in layer V to a level only a little less than that in layers II and deep III and continued at this level throughout layer V and the superficial two-thirds of layer VI. Then commenced the densely labeled band of deep layer VI and subjacent stratum of white matter. Grain clusters representing labeled cells were mostly 12–15 μm in diameter. Clustered labeling in the white matter was restricted to neuronal profiles.

β1 subunit. Hybridization of β1 subunit probes led to labeling that was only a little greater than background throughout most of area 17. There was a modest increase in density in layers II and VI that was only clearly visible in autoradiographs exposed for at least twice as long as those labeled with other subunit probes. The longer exposures also revealed some slightly enhanced labeling in layer V in comparison with layers III and IV (Figs. 4*E*, 5). In emulsion autoradiographs, there were no obvious grain clusters that could be associated with single cells.

β2 subunit. Hybridization of β2 subunit probes gave a pattern of autoradiographic labeling that resembled that seen with α1 probes (Figs. 4*F*, 5). The densest label was over layer IVC, which showed a distinct bilaminar pattern of labeling. Layer I showed weak labeling only slightly greater than that over the white matter. Layers II–IVA were merged as a single zone of moderate to dense label with slightly increased density over layer IVA. Layer IVB labeling was almost as weak as that over layer I. In layer IVC, labeling of layer IVCα was weak in comparison with layer IVCβ and approximately equal to that in layers II–IVA. Layer IVCβ showed very dense labeling in its upper half and slightly weaker labeling in its deeper half. Layer V had the weakest labeling of all layers. Layer VI labeling was relatively dense at a level slightly greater than that over layer IVA. Labeling in its upper half was less dense than in its deeper half. In emulsion autoradiographs, small grain clusters 10–15 μm in diameter were widely distributed through all layers and were densely packed in layer IVCβ. Larger grain clusters 20–25 μm in diameter were scattered through layers II–III and increased in number in layers V and VI.

γ2 subunit. The main distinguishing features of hybridization for γ2 subunit mRNA was dense labeling overall, only slightly less than with α1 probes, and homogeneous labeling of layer IVC (Figs. 4*G*, 5). Layer I showed a low intensity of labeling. Layers II–IVA showed homogeneous labeling only slightly less dense than that of layer IVC. Layers II, III, and IVA were not separable in the labeling pattern. Layer IVB showed weak labeling approximately equal in density to that of layer I. Layer IVC was homogeneously and densely labeled with no obvious distinction between layers IVCα and IVCβ. With ^{33}P-labeled probes, there tended to be a slight increase in labeling density in the deepest line of cells in layer IVCβ, especially in the Japanese monkeys (Fig. 7*C*). Layer V was labeled at a level comparable to layer I and only slightly greater than white matter levels. Layer VI formed a wide band of labeling incorporating both its superficial and deep parts. Label density was approximately equal to that in layers III–IVA. Emulsion autoradiographs were characterized by many small grain clusters 10–15 μm in diameter through all layers, with high concentrations in layer IVCβ. Larger grain clusters 15–20 μm in diameter were mainly found in layers II–III.

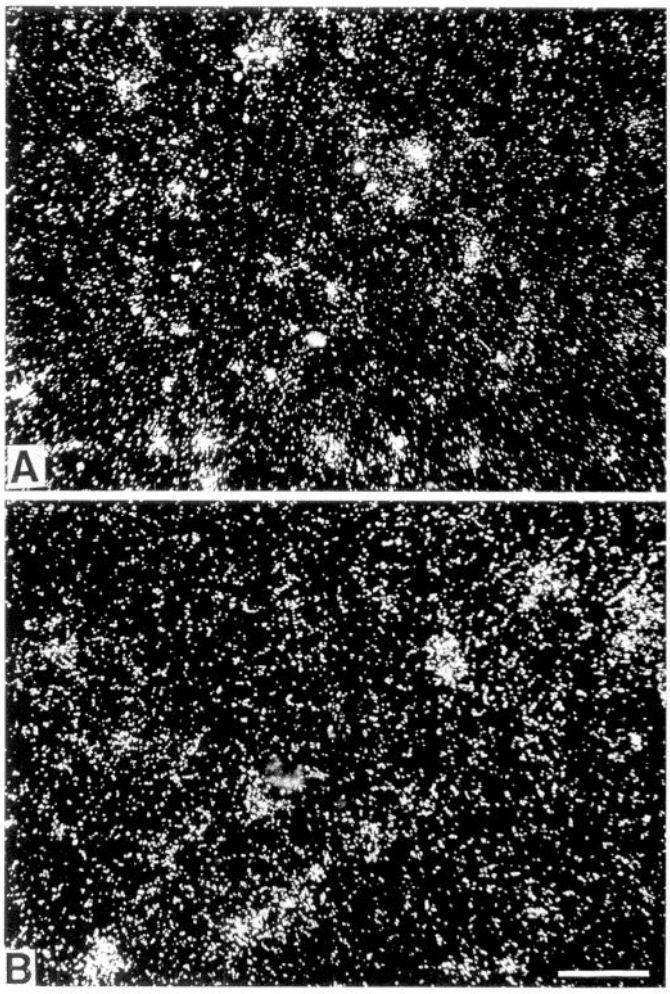


Figure 6. Dark-field photomicrographs at same magnification from an emulsion autoradiograph, showing small grain aggregations in layer IVC (*A*) and large grain aggregations in layer III (*B*), indicative of labeling of neurons of different sizes with a cRNA probe specific for β2 $GABA_A$ receptor subunit mRNAs. Scale bar, 25 μm.

Area 18

In situ hybridization histochemistry also revealed distinct laminar patterns of localization for each of the $GABA_A$ receptor subunit mRNAs in area 18. In the majority of cases, these could be assessed in the same sections as those used in the examination of area 17, so that changes occurring at the border between the two areas could be detected (Fig. 7*A–I*). In no case could enhancements or reductions of labeling be detected that corresponded to the various CO-stained stripes found in area 18 (Fig. 7*D*).

α1 subunit. The most distinctive feature was the presence in the middle layers of two thin bands of intense hybridization separated by a third band of much weaker hybridization (Fig. 7*A*). The deepest of these three bands showed the densest labeling and coincided with the deeper half of layer IV. The labeling was approximately equal in intensity to the densest labeling found in area 17, that is, in layer IVCα. The most superficial of the three bands of label showed slightly less intense labeling and coincided with the layer III/IV border region. Labeling of the remainder of layer III and of layer II was moderately dense

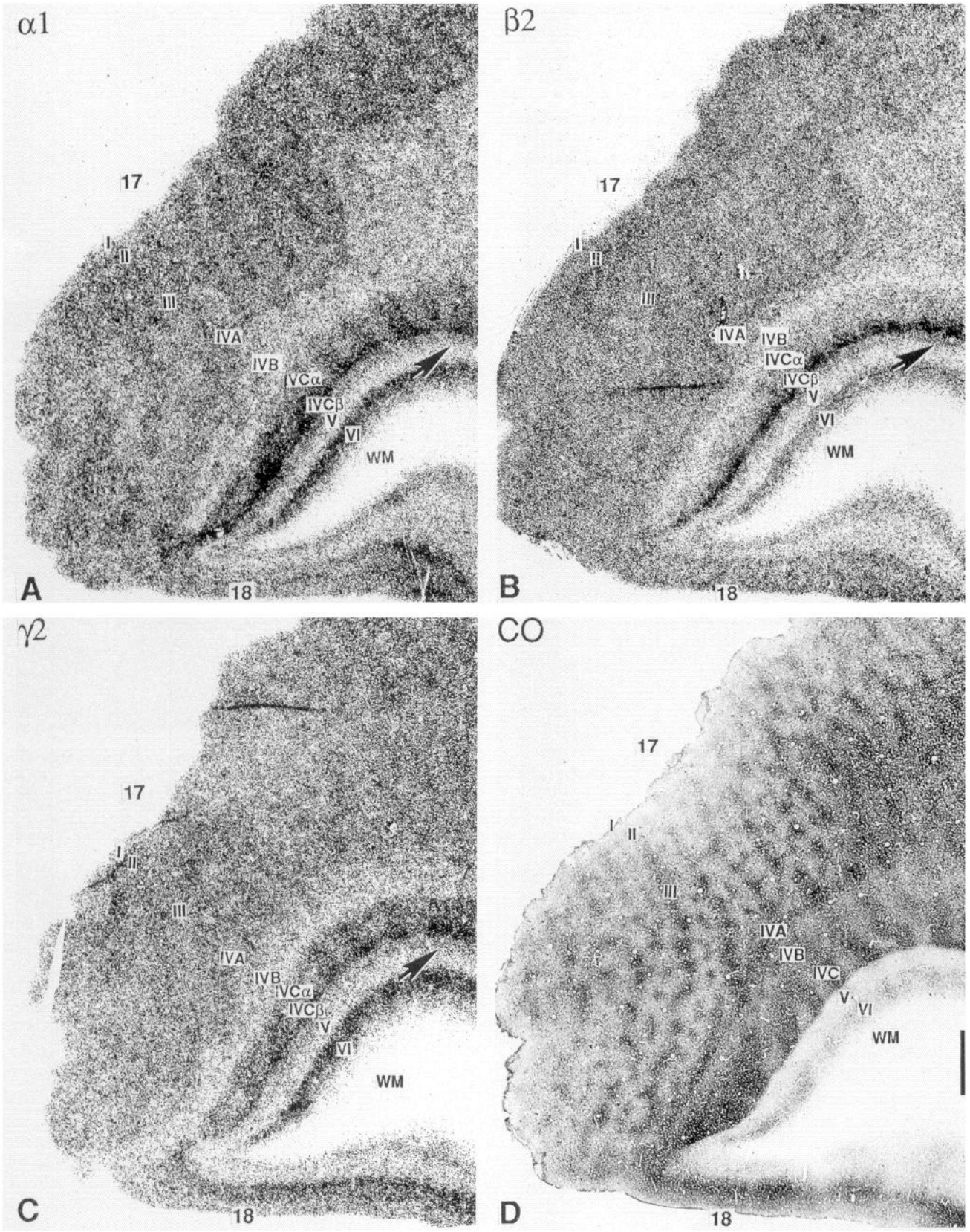
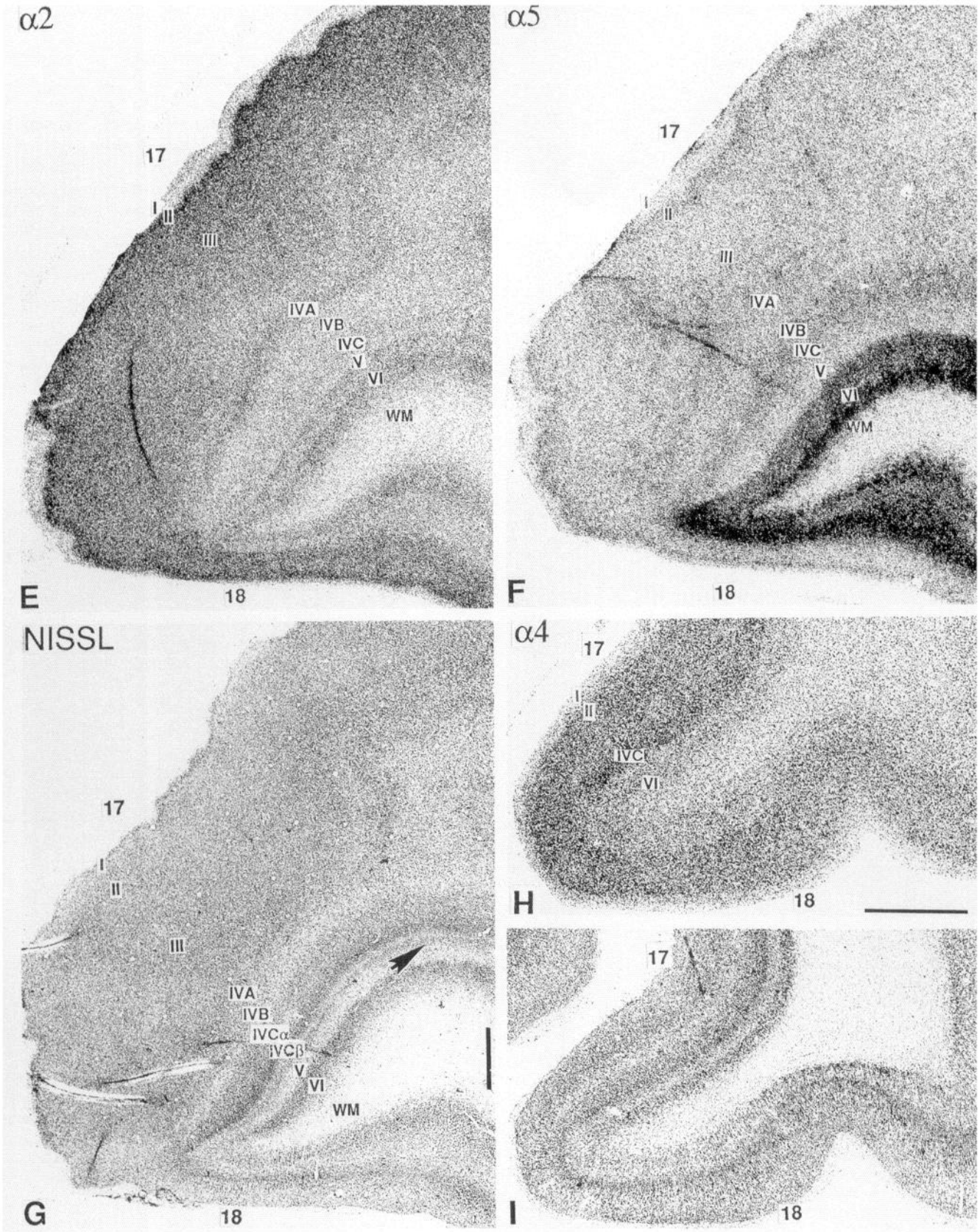


Figure 7. Photomicrographs from film autoradiograms (*A–C, E, F*) of adjacent sections through areas 17 and 18, hybridized with $GABA_A$ receptor subunit–specific cRNA probes as indicated, and from further adjacent sections stained for CO (*D*) or with thionin (*G*), from a monkey, monocularly deprived for 8 d. Alternating zones of low and high density of hybridization or of CO staining indicative of deprived and undeprived ocular dominance columns, respectively, can be seen in layer IVC in *A–D*. The effect is weaker with the β2 cRNA probe (*B*) than with α1 and γ2 cRNA



probes; $\alpha 2$ and $\alpha 5$ cRNA probes (*E, F*) show little or no deprivation effect, except for layer VI in *F* (from *Macaca fuscata*). *Arrow* in *A–C* and *G* indicates thin line of cells and label characteristic of deep layer IVCβ in this species. These sections also serve to illustrate the normal patterns of subunit-specific mRNA localization seen in area 18 that are not affected by monocular deprivation. *H* and *I*, from *Macaca mulatta*, show area 18 localization pattern for the $\alpha 4$ subunit mRNA (*H*) and the adjacent Nissl-stained section (*I*). Scale bars, 1 mm.

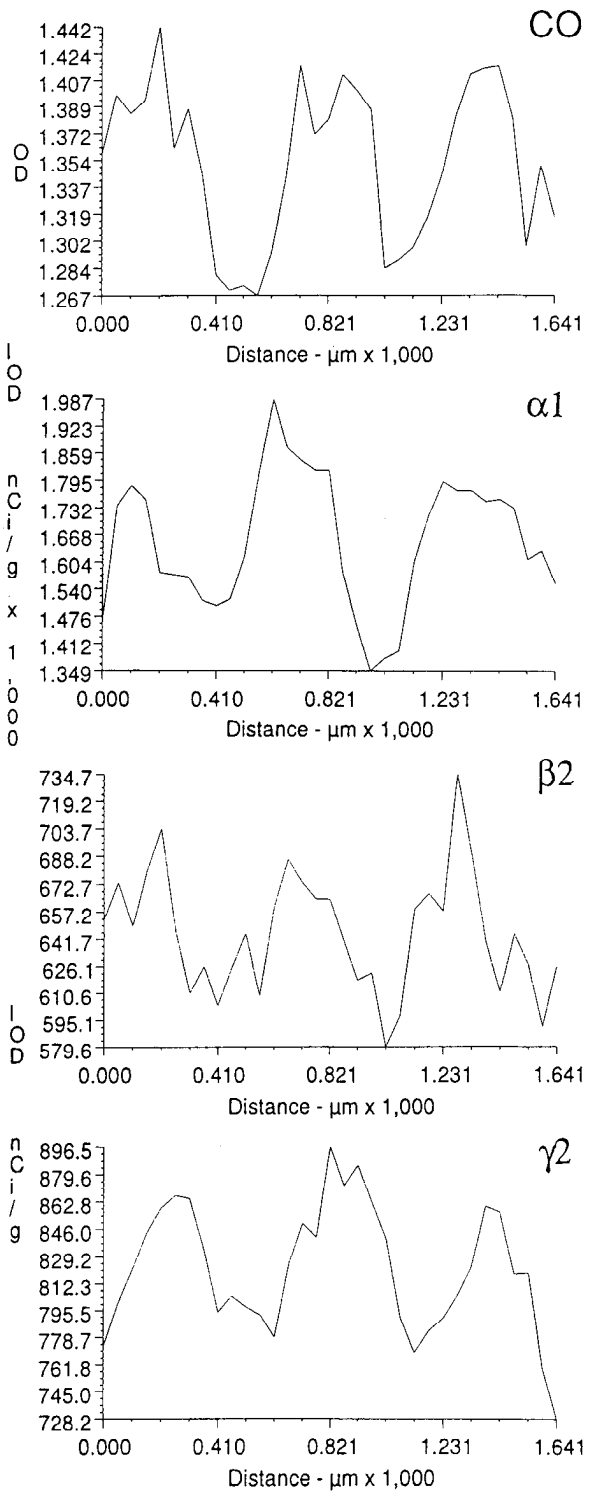


Figure 8. Optical density readings taken from traverses across five alternating nondeprived and deprived ocular dominance columns in layer IVC from a monocularly deprived monkey, showing reduction in CO staining (*top*) in the two deprived columns and comparable reductions in radioactivity in the same columns seen in autoradiographs of matched sections hybridized with α1, β2, and γ2 subunit–specific cRNA probes.

with slightly increased density in layer II. Layer VI showed a density approximately equal to that in the band in the deep aspect of layer III. Layers I and V had the weakest labeling.

α2 subunit. Labeling with the α2 riboprobes was relatively weak throughout area 18 with moderate increases in density corresponding to layers II, IV, and VI (Fig. 7*E*).

α4 subunit. Labeling with α4 subunit probes was very weak in layers I, V, and VI and moderately dense in layers II–IV, with a modest enhancement in layer IV (Fig. 7*H*).

α5 subunit. Labeling with α5 riboprobes was distinguished by the presence of two very intense bands of hybridization, one of which coincided with layer VI and the adjacent white matter and the other with layer IV and adjacent parts of layers III and V (Fig. 7*F*). The deep band was directly continuous with the similar band in area 17. The two dense bands in area 18 were separated by a zone of moderately intense labeling corresponding to the superficial half of layer VI and the adjacent part of layer V. Labeling was also moderately enhanced in layer II. That in layer I and in the rest of layer III was weak.

β1 subunit. Labeling with β1 subunit riboprobes was very weak overall and approximately equal in intensity to that seen in area 17 (not shown). As in area 17, there was a deep zone of slightly enhanced labeling that coincided with layer VI.

β2 subunit. Labeling with β2 subunit riboprobes was relatively weak overall but showed enhanced density in layers IV and VI and a weaker enhancement in layer II (Fig. 7*B*).

γ2 subunit. Labeling with γ2 subunit riboprobes was similar to that found with α1 riboprobes. A similar trilaminar pattern of labeling was observed in the middle layers (Fig. 7*C*). The deep band, corresponding to the deep half of layer IV, was the most intensely labeled. The superficial band, corresponding to the deep aspect of layer III, was slightly less intensely labeled and the intervening band showed weak labeling. Layer II showed labeling approximately equal to that in the deep layer III band. The remainder of layer III showed moderately dense labeling. Layers I, V, and VI were weakly labeled, layer VI labeling showing some enhancement in comparison with layer V.

Effects of monocular deprivation

All monkeys subjected to monocular deprivation showed the same effects in area 17 (Figs. 7–9). No variations that could be attributed to differences in the duration of the deprivation could be detected. The effects were most overt on the patterns of α1, β2, and γ2 subunit mRNA localization and less evident or absent in those of the other subunit mRNAs. No changes could be detected in area 18.

α1 subunit. The effects were most evident in layer IVC (Fig. 7*A*). There, alternating columns or stripes (depending on the plane of section) of high and low density of labeling could be seen. The low-density columns or stripes corresponded to the weakly stained, deprived ocular dominance columns found in the matched CO-stained sections, and the high-density stripes or columns, to the densely stained, undeprived ocular dominance columns. The densely hybridized, undeprived stripes tended to be somewhat wider than the weakly hybridized, deprived stripes. The undeprived were on average 500–600 μm wide and the deprived 300–400 μm wide. The deprived columns or stripes extended through the full thickness of layer IVCα and through much of layer IVCβ. In the deepest zone of layer IVCβ, corresponding to the thin line of densely Nissl-stained cells best seen in the Japanese macaque, there was a less severe reduction in the labeling of the deprived columns so that the contrast between deprived and nondeprived columns was less distinct.

Alternating columns or stripes of higher and lower hybridization, each approximately 500 μm wide, were also present in layer VI, corresponding to dense, undeprived and light, deprived

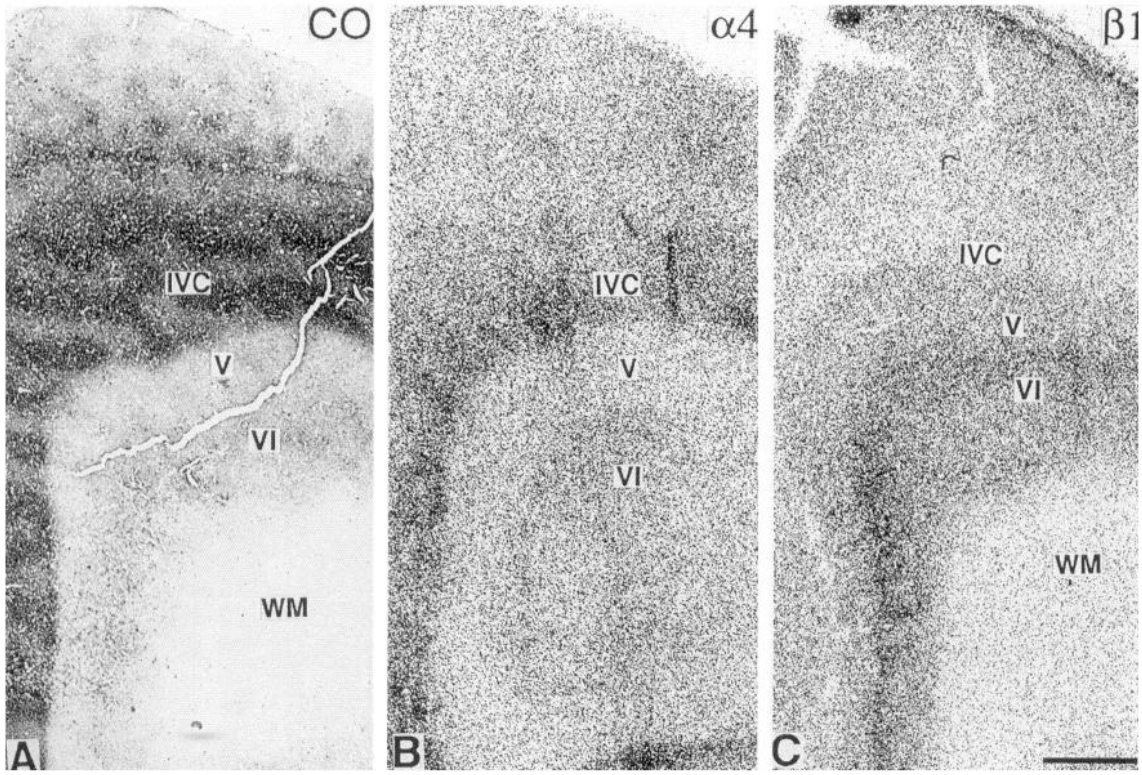


Figure 9. Photomicrographs from a CO-stained section (*A*) through area 17 of a monocularly deprived monkey and from autoradiograms of adjacent sections hybridized with $\alpha 4$ or $\beta 1$ subunit–specific cRNA probes (*B, C*). There are hints of alternating high and low density in layer IVC in *B*, suggesting a mild deprivation effect, but there are no obvious changes in *C*. (*C* is heavily overexposed in comparison with Fig. 4*E*).

columns, respectively, in the matched, CO-stained sections. The distinction between the two was not as clear as in layer IVC, however, because of the heavy overall mRNA labeling. Similar alternating stripes of enhanced and weaker labeling was found in layers IVB and IVA, although the overall level of labeling was much weaker than in other layers. None were observed in layer V.

In layers II and III a distinct pattern of alternating high- and low-density stripes was detected in surface-parallel sections (Figs. 7*A*, 10*A*). The stripes of denser autoradiographic labeling were wider than the alternating more weakly labeled stripes, measuring 350–400 μm in width, in comparison with 250–300 μm for the low-density stripes. When overlain with adjacent CO-stained sections, the narrower stripes of less dense labeling coincided with rows of shrunken (i.e., deprived) CO periodicities ("blobs"). The wider, densely stained rows corresponded to rows of unshrunken CO periodicities. There was some indication of periodic patchiness in the hybridization labeling in the dense, undeprived stripes (Fig. 7*A*), but it was not as distinct as in the CO-stained pattern.

$\alpha 5$ subunit. The clearest evidence of a deprivation effect was seen in layer VI, especially in the zone of very intense labeling in its deepest part (Fig. 7*F*). There, alternating columns or stripes of higher and lower density, and of approximately equal width, corresponded to undeprived and deprived columns or stripes seen in the matched, CO-stained sections. The degree of reduction of labeling in the deprived columns or stripes was not great, however, so the contrast between these and the undeprived columns was not dramatic. There were no overt changes in other layers.

$\alpha 2$, $\alpha 4$, and $\beta 1$ subunits. No changes could be detected in the pattern of hybridization for $\alpha 2$ (Fig. 7*E*) and $\beta 1$ (Fig. 9*C*) subunit mRNAs. There were some hints of a periodic pattern of reduced labeling in layer IVCβ with $\alpha 4$ probes (Fig. 9*B*).

$\beta 2$ subunit. A distinct deprivation pattern was seen in layer IVCβ (Figs. 7*B*, 8). There, alternating stripes or columns of dense hybridization signal, approximately 500 μm wide, alternated with stripes or columns of less intense labeling approximately 400 μm wide. These corresponded to deprived and nondeprived ocular dominance stripes or columns when matched to the adjacent CO-stained section (Figs. 7*D*, 8). The distinction between less densely labeled, deprived, and more intensely labeled, undeprived, ocular dominance stripes was only clear-cut in the superficial and deeper aspects of layer IVCβ. In the middle of this sublayer, the reduction in hybridization signal in the deprived columns was far less severe and the denser labeling of the adjacent, nondeprived columns tended to merge across the deprived column. Columns or stripes of weaker labeling corresponding in the CO-stained sections to deprived ocular dominance columns could be detected in the deep line of cells in layer IVCβ of the Japanese monkey and in the deeper half of layer IVCα, but the changes were very faint in comparison with those in the greater part of layer IVCβ.

In layer III, very faint stripes of enhanced label, similar in width to those seen with $\alpha 1$ riboprobes, could be detected mainly in overexposed autoradiograms (Figs. 7*B*, 10*D*). Even these, however, were never as distinct as with $\alpha 1$ or $\gamma 2$ riboprobes (Fig. 8). No changes were seen in other layers.

$\gamma 2$ subunit. The deprivation effect on $\gamma 2$ subunit mRNA hybridization was similar in severity to that found with the $\alpha 1$

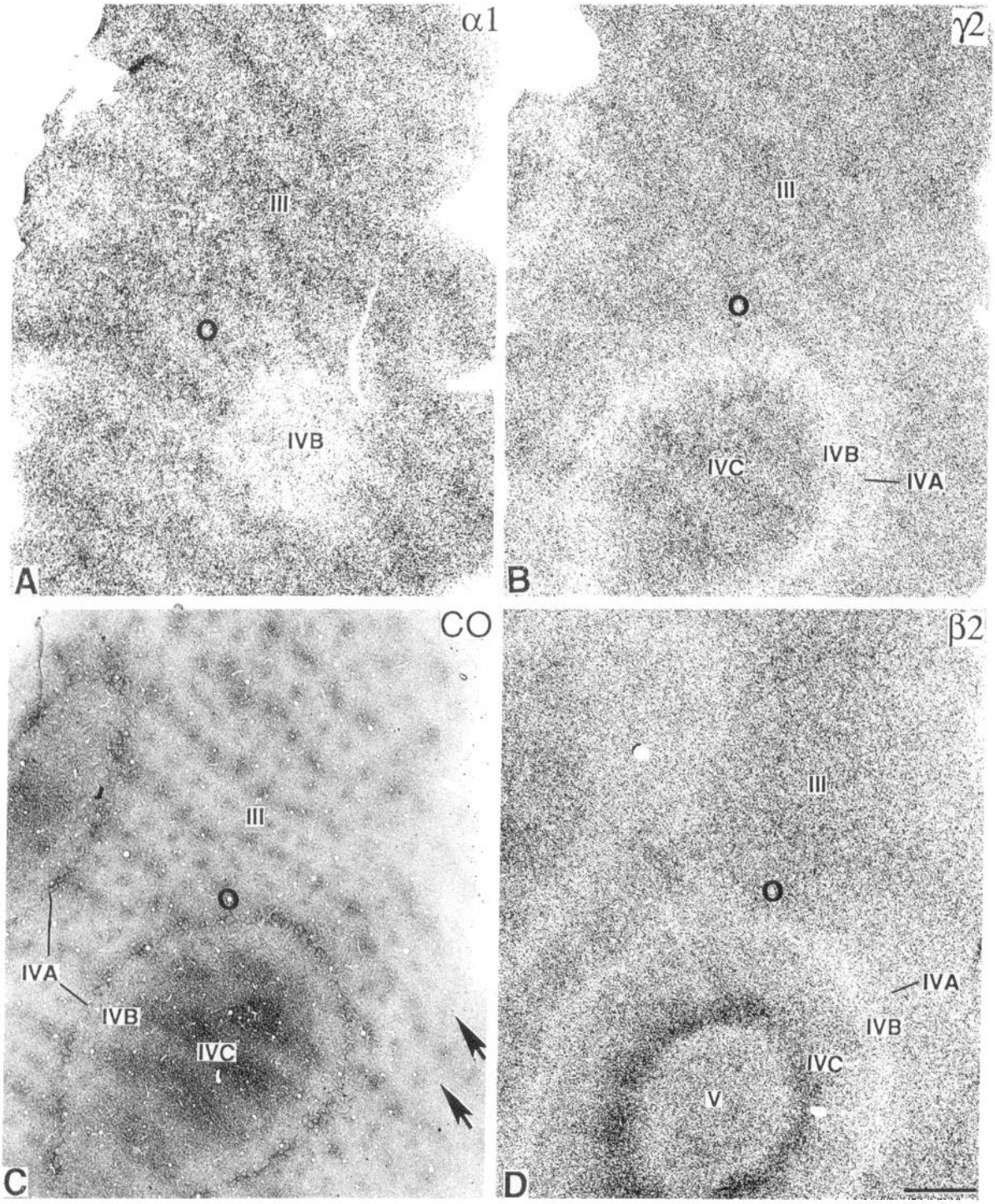


Figure 10. Photomicrographs in superficial to deep order from autoradiograms (*A, B, D*) of sections through area 17, hybridized with α1, β2, or γ2 subunit–specific cRNA probes and from a further section stained for CO (*C*). *Circles* enclose profiles of same blood vessel (from a monocularly deprived monkey). Stripes of reduced hybridization signal in *A* and *B* match rows of shrunken CO-stained periodicities (*arrows, C*) indicative of deprived ocular dominance domains. Stripes are not evident in layer III in *D* (from *Macaca mulatta*). Scale bar, 1 mm.

and β2 riboprobes (Figs. 7*C*, 8). Alternating columns or stripes of higher- and lower-intensity labeling, corresponding to undeprived and deprived ocular dominance stripes or columns in the matched CO-stained sections, were very clearly evident in layers IVCα, IVCβ (including in the deep line of cells of the Japanese monkey), and VI. In layer IVC, the higher-density, undeprived stripes measured 500–600 μm in width and the lower-density deprived stripes measured 350–400 μm in width.

There were alternating stripes of high and low density corresponding to rows of normal and shrunken CO periodicities, respectively, in layers II and III (Figs. 7*C*, 10*B*). Similar alternating stripes were seen in layers IVA, IVB, and V. They were

less distinct than those seen with $\alpha 1$ riboprobes but more distinct than those seen with $\beta 2$ riboprobes.

Quantification

Optical density scans were made across five or more adjacent layer IVC normal and deprived ocular dominance columns in CO-stained sections and matched to scans of comparable parts of adjacent sections hybridized with $\alpha 1$, $\beta 2$, or $\gamma 2$ cRNA probes (Fig. 8). There was a precise match of deprived, CO-weak columns with columns showing reduced levels of hybridization. Reductions in levels of radioactivity over the deprived columns had ranges of 20–30% for $\alpha 1$ subunit probes, 13–17% for $\beta 2$ subunit probes, and 9–12% for $\gamma 2$ subunit probes.

Discussion

In the present study, monkey-specific $GABA_A$ receptor cDNAs were subcloned and used as probes for Northern blot and *in situ* hybridization analysis. The individual cDNA sequences of the $\alpha 1$, $\alpha 2$, $\alpha 4$, $\alpha 5$, $\beta 1$, $\beta 2$, and $\gamma 2$ subunits are unique to the monkey but recognized mRNAs of distinct molecular weights that are consistent with previously published data from other species (Garrett et al., 1988, 1990; Khrestchatisky et al., 1989, 1991; Lolait et al., 1989b; Ymer et al., 1990). mRNAs for the $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits, in particular, are expressed at much higher levels in the primary visual cortex than in the other three cortical areas examined. In the visual cortex, the seven $GABA_A$ receptor subunit mRNAs displayed overlapping yet distinct patterns of expression that were layer specific and, for some, correlated with previous studies of receptor localization by radioactive ligand binding or immunocytochemistry. Others showed unique patterns not anticipated from studies of receptor localization. The findings in area 17 of monocularly deprived animals indicated that expression of four of the mRNAs is regulated by neural activity.

Northern blot analysis

Since the $GABA_A$ receptor subunits share a high degree of sequence homology, the specificity of mRNA localization is important. In the absence of information about monkey subunit sequences, it was decided to use riboprobes generated from monkey cDNAs in order to ensure specificity. Northern blots showed that the probes used had a high degree of specificity detecting mRNA transcripts as distinct bands of different molecular weights corresponding to those in other species. The monkey $\alpha 1$ subunit mRNA is similar to known rat (Khrestchatisky et al., 1989; Lolait et al., 1989a,b) and human transcripts (Garrett et al., 1988) in that the monkey $\alpha 1$ probe recognized major (4.3 kb) and possibly minor (3.9 kb) transcripts. The $\alpha 2$ subunit probe recognized transcripts at 8.5 and 3.6 kb, the former of which is similar to that found in the cow (Wisden et al., 1988), and the latter corresponds to one of two (3.6 and 6.6 kb) transcripts found in the rat (Khrestchatisky et al., 1991). The $\alpha 4$ subunit probe recognized major transcripts at 11 and 4 kb, of which the former corresponds to that found in the cow and the latter to a 4 kb transcript found in the rat (Ymer et al., 1989b; M. M. Huntsman, P. J. Jackson, and E. G. Jones, unpublished observation). The $\alpha 5$ subunit probe recognized a single transcript at 2.8 kb, which is the same as that described as $\alpha 4$ in the rat (Khrestchatisky et al., 1989). The monkey $\beta 1$ subunit probe recognized a single transcript at 13 kb, which is similar to the 12 kb transcript of the rat (Ymer et al., 1989a) and the 13 kb transcript of the cow (Ymer et al., 1989a). Both the $\beta 2$ and $\gamma 2$ subunit probes recognized single transcripts, at 8 and 3.9 kb, respectively, as found in the rat (Ymer et al., 1989b; Malherbe et al., 1990c).

High levels of subunit expression in visual cortex

The higher expression of all $GABA_A$ receptor subunit mRNAs in the primary visual cortex in comparison with other areas of monkey cerebral cortex is likely to reflect the greater density of neurons in the primary visual area. Neuronal density in area 17 is reported to be 116,000–120,000 cells per mm^3 (Rockel et al., 1980; O'Kusky and Colonnier, 1982; Hendry et al., 1987; Beaulieu et al., 1992). This is approximately double the density found in somatosensory and motor cortex and in parietal, temporal, and frontal association areas. Approximately 20% of the neurons (~24,000 per mm^3) in area 17 and 25% (11,000–16,000 per mm^3) in the other areas are GABAergic (Hendry et al., 1987). Approximately 17% of the synapses in monkey striate cortex are reported to be GABAergic, with an estimated density of approximately 76×10^6 GABA synapses per mm^3 (Beaulieu et al., 1992). The higher level of $GABA_A$ receptor subunit gene expression in area 17 may thus reflect both the larger number of neurons overall and the larger number of GABA neurons whose axon terminals and their synaptic targets will be the major sites of insertion of pre- and postsynaptic $GABA_A$ receptors into neuronal membranes. The higher expression of $\alpha 1$, $\beta 2$, and $\gamma 2$ transcripts in comparison with the other $GABA_A$ receptor subunit transcripts probably reflects the fact that a combination of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits confers the full range of classical $GABA_A$ receptor properties (Sigel et al., 1990; Verdoorn et al., 1990), as well as the predominant expression of these subunits in the cerebral cortex and many other brain regions of adult animals (Shivers et al., 1989; Laurie et al., 1992a; Poulter et al., 1992).

Differential laminar distribution of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit mRNAs

$GABA_A$ receptors have been localized in the adult monkey visual cortex by radioligand binding (Shaw and Cynader, 1986; Rakic et al., 1988; Hendry et al., 1990; Shaw et al., 1991), and by immunocytochemistry (Hendry et al., 1990, 1993; Huntsman et al., 1991). These studies revealed the highest densities of binding or of immunocytochemical reaction product in layers II–III, IVA, IVCβ, and VI of area 17 and in layers II–III, IV, and VI of area 18.

Comparison of the results of $\beta 2/\beta 3$-specific immunostaining with that obtained using antibodies specific for the $\alpha 1$ and $\gamma 2$ subunit polypeptides (Huntsman et al., 1991; Hendry et al., 1993) shows a pattern of differential distribution primarily in layer IVC that has a close resemblance to the patterns of gene expression for the $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits revealed by mRNA localization. Although showing individual differences that will be discussed below, the highest levels of expression of three "adult" transcripts ($\alpha 1$, $\beta 2$, and $\gamma 2$) found in the present study were in layer IVC, with secondary peaks in layers IVA and VI and a generally high level in layers II–III.

The high density of the $\alpha 1$, $\beta 2$, and $\gamma 2$ transcripts in layer IVC is at variance with findings reported in rats by Wisden et al. (1992), who emphasize the lack of these three in layer IV. Unfortunately, their delineation of the cortical layers appears inaccurate, probably stemming from the reliance on film autoradiograms examined at low magnification and from the common failure to recognize the greater relative thickness of layers V and VI in the cerebral cortex of rodents in comparison with

other species. The barrel field, a component of layer IV in the somatic sensory cortex, appears, for example, to be enriched in $\alpha 1$ subunit transcripts in their Figures 1, 3, 5, and 11. The localization of $\alpha 1$, $\beta 2$, and $\gamma 2$ transcripts by Persohn et al. (1992) in rat cerebral cortex also shows high densities in layer IV. The apparent species difference between rat and monkey is, thus, probably not significant.

The localization of mRNAs by *in situ* hybridization histochemistry in most cases results in labeling of neuronal somata only, and the results of the present study showed no exception to this. Thus, the laminar distribution of $GABA_A$ receptor subunit transcripts is not necessarily a reflection of the sites of concentration of the receptors of which the translated polypeptides form a component and which would be revealed by immunocytochemistry. This is a point of some significance in the cerebral cortex in which neurons in most layers have dendrites and axon branches that are not constrained by laminar borders and in the case of deeper pyramidal cells can extend through all cortical layers. Radioligand binding and immunocytochemical staining for $\alpha 1$, $\beta 2/\beta 3$, and $\gamma 2$ subunits (Shaw and Cynader, 1986; Hendry et al., 1987, 1993; Rakic et al., 1988), however, present a picture of receptor localization that does not differ greatly from the laminar pattern of localization of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit mRNAs. The immunocytochemistry stains both cell somata and elements of the neuropil (see also Huntley et al., 1990, in monkey sensory-motor cortex, Gu et al., 1993, in cat visual cortex, and Fritschy et al., 1992, in subcortical regions of the rat). This suggests that it identifies both expressing cells and the location of receptor complexes. From the present study, then, cells of layers II–III, IVA, IVC, and VI would be those most richly endowed with receptors made up of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits. Layer V pyramidal cells, although having apical and basal dendrites extending into some or all of these layers, because of low levels of expression in layer V might be expected to show a lower density of $GABA_A$ receptors assembled from these subunits.

Although the high density of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit mRNAs in layer IVC has been emphasized, these three important subunits do not show identical patterns of localization in that layer. $\alpha 1$ subunit transcripts are highly expressed in both sublayers IVCα and IVCβ, with an increase in density in IVCβ but no clear-cut line of demarcation between the two sublayers. By contrast, $\beta 2$ transcript localization shows a very high density in sublayer IVCβ and a much lower density than $\alpha 1$ and $\gamma 2$ in IVCα; the density of $\gamma 2$ transcripts is equal in IVCα and IVCβ, but with a thin line of weak hybridization separating them. In the Japanese macaque, which shows a more clearly demarcated line of densely stained cells in the deepest aspect of sublayer IVCβ, a zone characterized mainly by decreased cell density in other macaques (see, e.g., Lund, 1973), there are also differences in the density of $\alpha 1$, $\beta 2$, and $\gamma 2$ transcript labeling and in the clarity with which labeling in this line of cells is dissociated from that in layer IVCβ proper.

Differences in $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\beta 1$ subunit expression

The laminae and sublaminae showing the highest densities of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit transcript localization, on the whole, match quite closely the patterns of receptor localization seen with receptor autoradiography and immunocytochemistry. By contrast, the localization of the $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\beta 1$ subunit transcripts in relation to the cortical layers bears little resemblance to the autoradiographic and immunocytochemical localization patterns. For all of these transcripts, not only are levels relatively low, some requiring almost double exposure time to elicit labeling suitable for anatomical localization on film autoradiograms, but also the major concentration of most is found in layer VI. The density of transcript localization in layer VI differs among all the subunits. It is highest for the $\alpha 5$ subunit transcript, exceeding that of $\alpha 1$, $\beta 2$, and $\gamma 2$ transcript labeling in layer VI, and approaches the density seen for labeling of these transcripts in layer IVC. Density of layer VI labeling for the $\alpha 4$ subunit is next greatest, approaching that seen for $\alpha 1$, $\beta 2$, and $\gamma 2$ transcripts in that layer, while labeling density of $\alpha 2$ and $\beta 1$ transcripts is lowest but still significant.

In other layers, patterns of $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\beta 1$ subunit transcript localization also differ, with modest concentrations of $\alpha 4$ transcripts in layers IVCα and IVCβ. $\alpha 5$ transcript labeling outside layer VI shows a complex pattern of alternating higher- and lower-density labeling that is not readily matched to the cortical laminae, and the dense labeling in layer VI extends well into the white matter. $\alpha 2$ and $\beta 1$ transcript labeling outside layer VI is low, but slight enhancements of labeling are seen in layers II and V.

The highly specific patterns of laminar and sublaminar concentration exhibited by each of the $GABA_A$ subunit–specific mRNAs in area 17 are a reflection of specific patterns of subunit gene expression exhibited by visual cortical neurons. Layer VI shows the widest range of subunit gene expression, with all six of the subunit mRNAs being located at high or moderate density in it. Layer IVC is next, containing high levels of $\alpha 1$, $\beta 2$, and $\gamma 2$ transcripts and modest levels of $\alpha 4$ transcripts. Layers II, III, and IVA follow with high levels of $\alpha 1$, $\beta 2$, and $\gamma 2$ transcripts and low levels of the others. Layers I, IVB, and V are the most impoverished, with low levels of all the transcripts examined. These differential laminar patterns of localization have a number of functional implications.

Functional consequences of differential subunit expression

It is agreed that a functionally active $GABA_A$ receptor is most likely to be assembled from a combination of α, β, and γ subunits, probably in a pentameric array (Pritchett et al., 1989a,b; Malherbe et al., 1990c; Olsen and Tobin, 1990; Wieland et al., 1992). The known physiological diversity of $GABA_A$ receptors throughout the nervous system, however, is probably a reflection of the assembly of functional receptors from different combinations of subunit classes (reviewed in Olsen et al., 1990a; Wisden and Seeburg, 1992). Recombinant receptors composed of α and β subunits form GABA-gated chloride channels that can be blocked by bicuculline and picrotoxin and facilitated by barbiturates (Levitan et al., 1988; Pritchett et al., 1988; Puia et al., 1990, 1991; Verdoorn et al., 1990; Von Blankenfeld et al., 1990; Sigel et al., 1990; Knoflach et al., 1992). Recombinant receptors consisting of $\alpha\beta$, $\alpha\gamma$, and $\alpha\beta\gamma$ subunits exhibit different single-channel conductances (Verdoorn et al., 1990; Moss et al., 1991; Angelotti and MacDonald, 1993; Angelotti et al., 1993), which may reflect variations found in cultured mammalian neurons (MacDonald et al., 1989). Different members of the α subunit class in combination with β and γ subunits affect the sensitivity of the receptor to GABA (Levitan et al., 1988; Malherbe et al., 1990c). α subunits also confer different binding affinities for various types of benzodiazepines upon which the original classification of $GABA_A$ receptors into type I benzodiazepine (BZI) and type II benzodiazepine (BZII) classes was based (Pritchett et al., 1989a,b; Sato and Neale, 1989; Lüddens et al.,

1990; Pritchett and Seeburg, 1990; Lüddens and Wisden, 1991; for a redefinition of these classes, see Olsen et al., 1990b; Lüddens and Wisden, 1991). β subunits appear to affect current amplitudes in recombinant receptors (Sigel et al., 1990) and influence barbiturate binding (Bureau and Olsen, 1990). γ subunits determine the level of benzodiazepine response when in association with α and β subunits (Pritchett et al., 1989b; Malherbe et al., 1990c; Ymer et al., 1990; Herb et al., 1992) and in these combinations a $\gamma 2$ subunit needs to be expressed to ensure a fully operative benzodiazepine binding site (Pritchett et al., 1989a,b; Malherbe et al., 1990c). γ subunits also affect the affinity of the receptor for antagonists and inverse agonists (Pritchett et al., 1989a,b; Ymer et al., 1990; Herb et al., 1992).

Based on this evidence, the differential expression of receptor subunits in relation to the layers of the visual cortex, as found in the present study, could confer distinct functional properties on the $GABA_A$ receptors of neurons in those layers. Apart from differences in allosteric binding of benzodiazepines, barbiturates, and steroids, properties that might be expected from different combinations of subunits would be variations in GABA affinity, in mean channel opening time, and in desensitization rate, all of which would affect how a visual cortical neuron responds to GABA. Further functional differences could be conferred by the insertion of β or γ subunits (e.g., $\gamma 2S$ and $\gamma 2L$) formed from alternatively spliced mRNAs (Whiting et al., 1990; Bateson et al., 1991b; Kofuji et al., 1991) or by insertion of subunits that are preferentially phosphorylated by different protein kinases (Sweetnam et al., 1988; Sigel and Baur, 1988; Harrison and Lambert, 1989a,b; Kirkness et al., 1989; Browning et al., 1990; Leidenheimer et al., 1991). Protein kinase A, for example, probably by phosphorylating the intracellular domain of the $\beta 1$ subunit, can lead to a reduction in GABA-mediated receptor currents due to reduced channel opening frequency (Porter et al., 1990). It is also possible that differential protein trafficking in a neuron could result in specific combinations of subunit polypeptides being directed to proximal and more distal dendrites and/or dendrites and soma, with physiological consequences for the receptor macromolecule assembled at each site. The observations of Fritschy et al. (1992) on subcortical structures in the rat brain, however, would tend to argue against this: in double and triple immunofluorescent staining, using $\alpha 1$, $\alpha 3$, $\gamma 2$, and $\beta 2/\beta 3$ subunit–specific antisera, they found that in most cases the same combinations of immunostaining appeared in identical cell types and, moreover, that the same combinations usually appeared together at the same "hot spots" of immunostaining on an individual neuron. These findings and those of Angelotti and MacDonald (1993), showing selective assembly of subunit combinations in tranfected cells, also tend to rule out the possibility raised by Burt and Kamachi (1991) that assembly of $GABA_A$ receptors in a neuron might occur randomly from a set of available subunits to form a heterogeneous mixture of receptor subtypes.

Nature of the expressing cells

Immunocytochemistry in monkey and cat cerebral cortex has revealed the presence of $GABA_A$ receptor immunoreactivity in both pyramidal and nonpyramidal neurons (Hendry et al., 1990; Huntley et al., 1990; Gu et al., 1993). Double labeling studies involving immunostaining for the $\gamma 2$ subunit polypeptide and for parvalbumin, a calcium-binding protein found in the neocortex only in GABAergic neurons (Hendry et al., 1989), show coexistence of the two and thus the presence of at least the $\gamma 2$ subunit in GABAergic cells (Hendry et al., 1993). Pyramidal cells are major targets of GABAergic synapses and much of their soma-dendritic membrane is contacted by terminals of cortical GABA cells (Hendry et al., 1983; Houser et al., 1983, 1984; Kisvarday et al., 1986). GABA cells in monkey cerebral cortex are also contacted by GABAergic terminals (DeFelipe et al., 1986; Kisvárday et al., 1986). $GABA_A$ receptors can be expected to be located at all these synapses as well as on the axon terminals of the GABA cells where they may serve to regulate GABA release.

In view of the large number of GABA synapses on cortical pyramidal cells, it is perhaps remarkable that the highest concentrations of $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit mRNAs, from whose translated products "classical" $GABA_A$ receptors appear to be assembled in adults, are found in layer IVC, the layer in which GABA cells are most highly concentrated (Hendry et al., 1987) and in which pyramidal cells are largely lacking. Conversely, expression of these three subunits is relatively lacking in layer V cells, which constitute the largest-sized, and one of the largest populations of pyramidal neurons. Apart from the consideration that GABA neurons may have a requirement for producing potentially large numbers of presynaptic $GABA_A$ receptors, which pyramidal cells do not, there may be a greater requirement for GABA-mediated inhibition and, thus, for $GABA_A$ receptors on layer IVC GABA and non-GABA cells, which are the major recipients of thalamic axon terminations in the visual cortex and which may be subjected to high levels of excitation from incoming afferent volleys.

$GABA_A$ receptors in cortical development

The high levels of expression of $\alpha 2$ and $\alpha 5$ subunit transcripts in layer VI and especially in the immediately underlying stratum of white matter may reflect the early developmental history of these zones and the more predominant expression of $\alpha 2$ and $\alpha 5$ transcripts in cortical development (Laurie et al., 1992b; Poulter et al., 1992).

Layer VI is the first of the definitive cellular cortical layers to be formed (Rakic, 1972, 1977) and is from the earliest period closely associated with a transient underlying zone of neurons and synaptic neuropil termed the cortical subplate (Shatz et al., 1988). The subplate neurons are generated in large numbers and settle in the subplate before the arrival of the earliest cortical neurons (Marin-Padilla, 1970; Luskin and Shatz, 1985). Eventually, a large number succumb to a pattern of preprogrammed cell death (Chun and Shatz, 1989), although in monkeys and humans, the number remaining in the white matter within 200–300 μm of the overlying cortex is substantial (Kostovic and Rakic, 1980, 1990; Hendry et al., 1984; Sandell, 1986; Akbarian et al., 1993; Jones et al., 1994). It is this stratum and the overlying part of layer VI that show the highest gene expression for $\alpha 2$ and $\alpha 5$ subunit mRNAs in the present study.

Experimental studies in carnivores and rodents suggest that the subplate plays a key role in helping establish normal patterns of afferent fiber terminations in the cerebral cortex (Ghosh et al., 1990) and in guiding the earliest cortical efferent axons toward their subcortical targets (McConnell et al., 1989; DeCarlos and O'Leary, 1992). It has been conjectured that early recognition in the subplate of growing afferent fibers by migrating neurons may facilitate the later identification of target cells by the afferents in the overlying cortex (see Shatz et al., 1988). The early recognition process in the subplate may depend upon the presence of rudimentary forms of synapse—like membrane con-

tacts (Chun and Shatz, 1988; Friauf et al., 1990; Kostovic and Rakic, 1990), and on the presence of a number of neurotransmitter and neurotrophin receptors, including $GABA_A$ receptors, that have been localized in the subplate in fetal monkeys (Huntley et al., 1990; Shaw et al., 1991; Meinecke and Rakic, 1992).

In the hippocampal formation of rats, the high expression of $\alpha 2$ and $\alpha 5$ transcripts found during the fetal period persists into adulthood, unlike in other regions, in which there is a decline after birth (Laurie et al., 1992b). In rat hippocampus up to the second postnatal week, $GABA_A$-mediated neuronal responses are primarily depolarizing ones (Ben-Ari et al., 1989; Cherubini et al., 1991). After this time, both depolarizing and the more conventional hyperpolarizing responses are obtained (Ben-Ari et al., 1989; Swann et al., 1989; Michelson and Wong, 1991; Pearce, 1993). The depolarizing response may reflect the early expression and maintenance of $\alpha 2$ and $\alpha 5$ receptor subunits (Laurie et al., 1992b). It would therefore be interesting to determine if $GABA_A$-mediated depolarization could be located in layer VI neurons of the adult monkey visual cortex. Populations of native $GABA_A$ receptors containing $\alpha 5$ subunits and immunoprecipitated from brain extracts are characterized by ligand binding affinities that distinguish them from all other known $GABA_A$ receptor populations (Mertens et al., 1993). These, too, could result in demonstrable differences in layer VI neurons.

GABA-immunoreactive neurons are found in fetal monkey visual cortex as early as 110 d of gestation, when they are concentrated in layer I and in the layer VI/subplate junctional region (Huntley et al., 1988). The predominant influence exerted by GABA in this early period, before major synaptogenesis has occurred (Rakic et al., 1986), may therefore be primarily a neurotrophic one, since GABA applied to cultures of immature CNS neurons can induce neurite outgrowth, receptor synthesis, and synaptogenesis (Wolff, 1981; Hanson et al., 1987; Meier et al., 1987; Wolff et al., 1987; Kater and Guthrie, 1990). This might depend upon selective expression of unique $GABA_A$ subunit combinations that leads to GABA-induced depolarization followed by calcium entry via voltage-gated calcium channels, and Ca^{2+}-mediated trophic effects (Meier et al., 1987; Laurie et al., 1992b). They could affect trophic interactions in the subplate and a change in subunit expression leading to enhanced calcium entry, in the absence of protective mechanisms, could be a trigger in setting off the program of physiological cell death in the subplate.

Activity-dependent regulation

$GABA_A$ receptors in the visual cortex of adult monkeys have previously been shown to be under activity-dependent control and to be downregulated by brief periods of total monocular deprivation (Hendry et al., 1990). Five days after silencing ganglion cell activity in one retina by an intravitreal injection of TTX, the binding of ^{3}H-muscimol and of ^{3}H-flunitrazepam is reduced by approximately 25% in the deprived eye dominance columns of layer IV$C\beta$. There are comparable reductions in immunostaining for the $\alpha 1$, $\beta 2/\beta 3$, and $\gamma 2$ subunits (Hendry et al., 1990, 1993; Huntsman et al., 1991). The present results extend these investigations to a new level by showing that this downregulation is dependent upon reductions in mRNA levels for the $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits. The most likely cause of this is an activity-dependent regulation of subunit gene transcription, although changes in mRNA stability cannot be completely ruled out by the methods used. Monocular deprivation by TTX injection in adult monkeys also leads to reductions in immunocytochemically detectable levels of GABA and its synthesizing enzyme, GAD, in deprived eye dominance columns (Hendry and Jones, 1986). This effect, which is reversible (Hendry and Jones, 1988), also depends upon reductions in GAD mRNA levels, albeit over a longer time course than that seen for the $GABA_A$ receptor subunit mRNAs or for a number of other neuronal mRNAs such as β-preprotachykinin (Benson et al., 1991a, 1993).

The reductions in $GABA_A$ receptor subunit mRNAs in deprived ocular dominance columns did not affect each receptor subunit mRNA equally. The failure to observe changes in the $\alpha 2$ and $\beta 1$ subunit mRNAs, the equivocal changes in $\alpha 4$ mRNAs in layer IVC, and the restriction of changes in $\alpha 5$ mRNAs to layer VI are perhaps explicable, given the relatively low levels of expression of these subunit mRNAs in layer IVC, where deprivation effects are customarily best revealed. Potentially more important are the differential effects upon mRNAs for the $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit polypeptides, which probably form the basis for the majority of functional $GABA_A$ receptors in the adult cerebral cortex (Wisden et al., 1992). In layer IVC, changes were most pronounced for $\alpha 1$ subunit mRNA, with reductions of 20–30% in the deprived ocular dominance columns. Reductions in $\beta 2$ and $\gamma 2$ subunit mRNAs, although clearly evident, were far less severe, amounting to 13–17% and 9–12%, respectively. Outside layer IVC, the most dramatic difference occurred in layers II and III, where alternating stripes of high- and low-intensity hybridization signal were revealed for $\alpha 1$ and $\gamma 2$ subunit mRNAs but far less clearly or not at all for $\beta 2$ subunit mRNA. These stripes appeared to depend upon reductions in $\alpha 1$ and $\gamma 2$ subunit mRNAs in the rows of CO-stained periodicities related to the deprived eye. The periodicities themselves were not selectively outlined by enhanced *in situ* hybridization in layers II–III of undeprived animals, although traces of patches comparable to the CO-stained patches are found in the stripes of enhanced mRNA labeling in the deprived animals. The shrinkage of the rows of CO-stained layer II–III patches related to a deprived eye is well known (Horton and Hubel, 1981), and our earlier studies have shown that this is accompanied by reductions in GAD and preprotachykinin mRNA levels and increases in mRNA levels for the α subunit of the multifunctional protein kinase, type II calcium/calmodulin-dependent protein kinase in the rows of shrunken periodicities (Benson et al., 1991a, 1993). The effects on layers II and III and the similar effects, including very robust changes in $\alpha 5$ subunit mRNAs, in infragranular layers of area 17 indicate that the deprivation effect extends along the well-known vertical lines of cortical connectivity leading out of layer IVC, the major layer of termination of incoming thalamic afferent fibers.

The significance of the differential effects upon $\alpha 1$, $\beta 2$, and $\gamma 2$ subunit mRNAs is less easy to predict, assuming the differences reflect differential changes in expression in the same cells, not selective alterations in different cells. They may be highly significant in determining the nature of the responses deprived cortical neurons are capable of generating to a GABA signal, particularly in the presence of benzodiazepine anxiolytics or barbiturates, whose binding will be modified depending upon the relative abundance of the subunits (see above).

References

Akbarian S, Bunney WE Jr, Potkin SG, Wigal SB, Hagman JO, Sandman CA, Jones EG (1993) Altered distribution of nicotinamide-adenine dinucleotide phosphate-diaphorase cells in frontal lobe of schizo-

phrenics implies disturbances of cortical development. Arch Gen Psychiatry 50:169–177.

Alloway KD, Burton H (1986) Bicuculline-induced alterations in neuronal responses to controlled tactile stimuli in the second somatosensory cortex of the cat: a microiontophoretic study. Somatosens Res 3:107–211.

Angelotti TP, MacDonald RL (1993) Assembly of $GABA_A$ receptor subunits: $\alpha 1$, $\beta 2$ and $\gamma 2$ subunits produce unique ion channels with dissimilar single-channel properties. J Neurosci 13:1429–1440.

Angelotti TP, Uhler MD, MacDonald RL (1993) Assembly of $GABA_A$ receptor subunits: analysis of transient single-cell expression utilizing a fluorescent substrate/marker gene technique. J Neurosci 13:1418–1428.

Aram JA, Michelson HG, Wong RKS (1991) Synchronized GABAergic IPSPs recorded in the neocortex after blockade of synaptic transmission mediated by excitatory amino acids. J Neurophysiol 65: 1034–1041.

Avoli M (1986) Inhibitory potentials in neurons of the deep layers of the *in vitro* neocortical slice. Brain Res 370:165–170.

Barnard EA, Darlison MG, Seeburg P (1987) Molecular biology of the $GABA_A$ receptor: the receptor/channel superfamily. Trends Neurosci 10:502–509.

Bateson AN, Harvey RJ, Wisden W, Glencorse TA, Hicks AA, Hunt SP, Barnard EA, Darlison MG (1991a) The chicken $GABA_A$ receptor $\alpha 1$ subunit: cDNA sequency and localization of the corresponding mRNA. Mol Brain Res 9:333–339.

Bateson AN, Lasham A, Darlison MG (1991b) γ-Aminobutyric $acid_A$ receptor heterogeneity is increased by alternative splicing of a novel β-subunit gene transcript. J Neurochem 56:1437–1440.

Beaulieu C, Kisvárday Z, Somogyi P, Cynader M, Cowey A (1992) Quantitative distribution of GABA-immunopositive and -immunonegative neurons and synapses in the monkey striate cortex. Cereb Cortex 2:295–309.

Ben-Ari Y, Cherubini E, Coradetti R, Gairrsa J-L (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurons. J Physiol (Lond) 416:303–325.

Benke D, Mertens S, Trzeciak A, Gillessen D, Möhler H (1990) Identification of the $\gamma 2$-subunit in native $GABA_A$-receptors in brain. Eur J Pharmacol 189:337–340.

Benke D, Cicin-Sain S, Mertens S, Möhler H (1991a) Immunochemical identification of the $\alpha 1$- and $\alpha 3$-subunits of the $GABA_A$ receptor in rat brain. J Recept Res 11:407–424.

Benke D, Mertens S, Möhler H (1991b) Ubiquitous presence of $GABA_A$ receptors containing the $\alpha 1$-subunit in rat brain demonstrated by imunoprecipitation and immunohistochemistry. Mol Neuropharmacol 1:103–110.

Benke D, Mertens S, Trzeciak A, Gillessen D, Möhler H (1991c) $GABA_A$- receptors display association of the $\gamma 2$-subunit with the $\alpha 1$-subunit and $\beta 2/\beta 3$ subunits. J Biol Chem 266:4478–4483.

Benke D, Mertens S, Trzeciak A, Gillessen D, Möhler H (1991d) Identification and immunohistochemical mapping of $GABA_A$ receptor subtypes containing the δ-subunit in rat brain. FEBS Lett 2893:145–149.

Benson DL, Isackson PJ, Gall CM, Jones EG (1991a) Differential effects of monocular deprivation on glutamic acid decarboxylase and type II calcium-calmodulin–dependent protein kinase gene expression in the adult monkey visual cortex. J Neurosci 11:31–47.

Benson DL, Isackson PJ, Hendry SHC, Jones EG (1991b) Differential gene expression for glutamic acid decarboxylase and type II calcium-calmodulin–dependent protein kinase in basal ganglia, thalamus and hypothalamus of the monkey. J Neurosci 11:1540–1564.

Benson DL, Huntsman MM, Jones EG (1993) Activity dependent changes in GAD and preprotachykinin mRNAs in visual cortex of adult monkeys. Cereb Cortex, in press.

Bolz J, Gilbert CD (1986) Generation of end-inhibition in the visual cortex via intralaminar connections. Nature 320:362–364.

Bowery NG (1983) Classification of GABA receptors. In: The GABA receptors (Enna SJ, ed), pp 177–213. Clifton: Humana.

Bowery NG (1989) $GABA_B$ receptors and their significance in mammalian pharmacology. Trends Pharmacol Sci 10:401–407.

Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J, Turnbull M (1980) (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature 283:92–94.

Bowery NG, Price GW, Hudson AL, Hill DR, Wilkin GP, Turnbull MJ (1984) GABA receptor multiplicity. Visualization of different receptor types in the mammalian CNS. Neuropharmacology 23:219–231.

Bowery NG, Pratt GD, Knott C (1990) $GABA_B$ receptors: past, present and future. In: $GABA_B$ receptors in mammalian function (Bowery NG, Bittiger H, Olpe H, eds), pp 3–28. New York: Wiley.

Browning MD, Bureau M, Dudek EN, Olsen RW (1990) Protein kinase C and cAMP-dependent protein kinase phosphorylate the β-subunit of the purified $GABA_A$ receptor. Proc Natl Acad Sci USA 87:1315–1318.

Buchstaller A, Admiker D, Fuchs K, Sieghart W (1991a) *N*-deglycosylation and immunological identification indicates the existence of α-subunit isoforms of the rat $GABA_A$ receptor. FEBS Lett 287:27–30.

Buchstaller A, Fuchs K, Sieghart W (1991b) Identification of $\alpha 1$-, $\alpha 2$- and $\alpha 3$-subunit isoforms of the $GABA_A$-benzodiazepine receptor in the rat brain. Neurosci Lett 129:237–241.

Bureau M, Olsen RW (1990) Multiple distinct subunits of the γ-aminobutyric acid-A receptor protein show different ligand-binding affinities. Mol Pharmacol 37:497–502.

Burt DR, Kamachi GL (1991) $GABA_A$ receptor subtypes: from pharmacology to molecular biology. FASEB J 5:2916–2923.

Chagnac-Amitai Y, Connors BW (1989a) Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition. J Neurophysiol 61:747–758.

Chagnac-Amitai Y, Connors BW (1989b) Synchronized excitation and inhibition driven by intrinsically bursting neurons in neocortex. J Neurophysiol 62:1149–1162.

Cherubini E, Gaiarsa J-L, Ben-Ari Y (1991) GABA: an excitatory transmitter in early postnatal life. Trends Neurosci 14:515–519.

Chirgwin JM, Przybyla AE, McDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Chun JM, Shatz CJ (1989) Interstitial cells of the adult neocortical white matter are the remnant of the early-generated subplate neuronal population. J Comp Neurol 282:555–569.

Churchill L, Bourdelais A, Austin MC, Lolait SJ, Mahan LC, O'Carrol A-M, Kalivas PW (1991) $GABA_A$ receptors containing $\alpha 1$ and $\beta 2$ subunits are mainly localized on neurons in the ventral pallidum. Synapse 8:75–85.

Connors BW, Malenka RC, Silva LR (1988) Two inhibitory postsynaptic potentials, and $GABA_A$ and $GABA_B$ receptor-mediated responses in neocortex of rat and cat. J Physiol (Lond) 406:443–468.

Crook JM, Eysel UT (1992) GABA-induced inactivation of functionally characterized sites in cat visual cortex (area 18): effects on orientation tuning. J Neurosci 12:1816.

Cutting GR, Lu L, O'Hara BF, Kasch LM, Montrose-Rafizadeh C, Donovan DM, Shimada S, Antonarakis SE, Guggino WB, Uhl GR, Kazazian HH Jr (1991) Cloning of the γ-aminobutyric acid (GABA) ρl cDNA: a GABA receptor subunit highly expressed in the retina. Proc Natl Acad Sci USA 88:2673–2677.

Danielson PE, Forss-Petter S, Brow MA, Calavetta L, Douglas J, Milner RJ, Sutcliffe JG (1988) p1B15: a cDNA clone of the rat mRNA encoding cyclophilin. DNA 7:261–267.

de Blas AL, Victorica J, Friedrich P (1988) Localization of the $GABA_A$ receptor in rat brain with a monoclonal antibody to the 57,000 M_r peptide of the $GABA_A$ receptor/benzodiazepine receptor/Cl-channel complex. J Neurosci 8:602–614.

DeCarlos JA, O'Leary DDM (1992) Growth and targeting of subplate axons and establishment of major cortical pathways. J Neurosci 12: 1194–1211.

DeFelipe J, Hendry SHC, Jones EG (1986) A correlative electron microscopic study of basket cells and large GABAergic neurons in the monkey sensory-motor cortex. Neuroscience 17:991–1009.

DeLorey TM, Olsen RW (1992) γ-Aminobutyric acid receptor structure and function. J Biol Chem 267:16747–16750.

Duggan MJ, Stephenson FA (1989) Bovine γ-aminobutyric $acid_A$ receptors. J Neurochem 53:132–139.

Duggan MJ, Stephenson FA (1990) Biochemical evidence for the existence of γ-aminobutyrate$_A$ receptors iso-oligomers. J Biol Chem 265:3831–3835.

Duggan MJ, Pollard S, Stephenson FA (1992) Quantitative immunoprecipitation studies with anti-$GABA_A$ receptor $\gamma 2$ I-15 cys antibodies. J Neurochem 58:72–77.

Endo S, Olsen RW (1992) Preparation of antibodies to β subunits of γ-aminobutyric $acid_A$ receptors. J Neurochem 59:1444–1451.

Endo S, Olsen RW (1993) Antibodies specific for α-subunit subtypes of GABA receptors reveal brain regional heterogeneity. J Neurochem 60:1388–1398.

Fitzpatrick D, Lund JS, Schmechel DE, Towles AC (1987) Distribution of GABAergic neurons and axon terminals in the macaque striate cortex. J Comp Neurol 264:73–91.

Friauf E, McConnell SK, Shatz CJ (1990) Functional synaptic circuits in the subplate during fetal and early postnatal development of cat visual cortex. J Neurosci 10:2601–2613.

Fritschy J-M, Benke D, Mertens S, Oertel WH, Bachi T, Möhler H (1992) Five $GABA_A$-receptor subtypes identified in neurons by double and triple immunofluorescence staining with subunit-specific antibodies. Proc Natl Acad Sci USA 89:6726–6730.

Fuchs K, Sieghart W (1989) Evidence for the existence of several different α- and β-subunits of the GABA/benzodiazepine receptor complex from rat brain. Neurosci Lett 97:329–333.

Fuchs K, Adamiker D, Sieghart W (1990) Identification of α2- and α3-subunits of the $GABA_A$-benzodiazepine receptor complex purified from the brains of young rats. FEBS Lett 261:52–54.

Gambarana C, Beattie CE, Rodriguez ZR, Sigel RE (1991) Region-specific expression of messenger RNAs encoding $GABA_A$ receptor subunits in the developing rat brain. Neuroscience 45:423–432.

Garrett KM, Duman RS, Saito N, Blume AJ, Vitek MP, Tallman JF (1988) Isolation of cDNA clone for the α subunit of the human $GABA_A$ receptor. Biochem Biophys Res Commun 156:1039–1045.

Garrett KM, Saito N, Duman RS, Abel MS, Ashton RA, Fujimori S, Beer B, Tallman JF, Vitek MP, Blume AJ (1990) Differential expression of γ-aminobutyric $acid_A$ receptor subunits. Mol Pharmacol 37:652–657.

Ghosh A, Antonini A, McConnell SK, Shatz CJ (1990) Requirement for subplate neurons in the formation of thalamocortical connections. Nature 347:179–181.

Gu QG, Perez-Valazquez L, Angelides KJ, Cynader MS (1993) Immunocytochemical study of $GABA_A$ receptors in the cat visual cortex. J Comp Neurol 333:94–108.

Hanson GH, Meier E, Abraham J, Schousboc A (1987) Trophic effects of GABA on cerebellar granule cells in culture. In: Neurology and neurobiology, Vol 32, Neurotrophic activity of GABA during development (Redburn DA, Schousboe A, eds), pp 109–138. New York: Liss.

Harrison NL, Lambert NA (1989a) The effects of protein kinase activators on $GABA_A$ receptor function in cultured rat hippocampal neurons. J Physiol (Lond) 412:17–26.

Harrison NL, Lambert NA (1989b) Modification of $GABA_A$ receptor function by an analog of cyclic AMP. Neurosci Lett 105:137–142.

Hatta Y, Tsumoto T, Sato H, Hagihara K, Amaura H (1988) Inhibition contributes to orientation selectivity in visual cortex of cat. Nature 338:815–817.

Hendry SHC, Jones EG (1986) Reduction in number of GABA immunostained neurons in deprived-eye dominance columns of monkey area 17. Nature 320:750–753.

Hendry SHC, Jones EG (1988) Activity-dependent regulation of GABA expression in the visual cortex of adult monkeys. Neuron 1:701–712.

Hendry SHC, Houser CR, Jones EG, Vaughn JE (1983) Synaptic organization of immunocytochemically identified GABAergic neurons in the monkey sensory-motor cortex. J Neurocytol 12:639–660.

Hendry SHC, Jones EG, Emson PC (1984) Morphology, distribution and synaptic relations of somatostatin and neuropeptide Y-immunoreactive neurons in rat and monkey neocortex. J Neurosci 4:2497–2517.

Hendry SHC, Schwark HD, Jones EG, Yan Y (1987) Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. J Neurosci 7:1503–1519.

Hendry SHC, Jones EG, Emson PC, Lawson DEM, Heizmann CW, Streit P (1989) Two classes of cortical GABA neurons defined by differential calcium binding protein immunoreactivities. Exp Brain Res 76:467–472.

Hendry SHC, Fuchs J, de Blas AL, Jones EG (1990) Distribution and plasticity of immunocytochemically localized $GABA_A$ receptors in adult monkey visual cortex. J Neurosci 10:2438–2450.

Hendry SHC, Huntsman MM, Viñuela A, Möhler H, de Blas AL, Jones EG (1993) $GABA_A$ receptor subunit immunoreactivity in primate visual cortex: distribution in macaques and humans and regulation by visual input in adulthood. J Neurosci, in press.

Herb A, Wisden W, Lüddens H, Puia G, Vicini S, Seeburg PH (1992) The third γ subunit of the $GABA_A$ receptor family. Proc Natl Acad Sci USA 89:1433–1437.

Hicks TP, Dykes RW (1983) Receptive field size for certain neurons in primary somatosensory cortex is determined by GABA-mediated intracortical inhibition. Brain Res 274:160–164.

Hironaka T, Morita Y, Hagihira S, Tateno E, Kita H, Tohyama M (1990) Localization of $GABA_A$-receptor α1 subunit mRNA-containing neurons in the lower brainstem of the rat. Mol Brain Res 7:335–345.

Hironchi M, Kuwano R, Katagiri T, Takahashi Y, Kuriyama K (1989) Nucleotide and deduced amino acid sequences of the $GABA_A$ receptor α-subunit from human brain. Neurochem Int 15:33–38.

Horton JC, Hubel DH (1981) Regular patchy distribution of cytochrome oxidase staining in primary visual cortex of macaque monkey. Nature 292:762–764.

Houser CR, Hendry SHC, Jones EG, Vaughn JE (1983) Morphological diversity of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. J Neurocytol 12:617–638.

Houser CR, Vaughn JE, Hendry SHC, Jones EG, Peters A (1984) GABA neurons in the cerebral cortex. In: Cerebral cortex, Vol 1, Functional properties of cortical cells (Jones EG, Peters A, eds), pp 63–89. New York: Plenum.

Huntley GW, Hendry SHC, Killackey HP, Chalupa LM, Jones EG (1988) Temporal sequence of neurotransmitter expression by developing neurons of fetal monkey visual cortex. Dev Brain Res 43:69–96.

Huntley GW, de Blas AL, Jones EG (1990) $GABA_A$ receptor immunoreactivity in adult and developing monkey sensory-motor cortex. Exp Brain Res 82:519–535.

Huntsman MM, Jones EG, Möhler H, Hendry SHC (1991) Distribution of immunocytochemically localized $GABA_A$ receptor subunits in the monkey and human visual cortex. Soc Neurosci Abstr 17:115.

Huntsman MM, Isackson PJ, Jones EG (1992) Differential regulation of $GABA_A$ receptor subunit mRNAs in adult monkey visual cortex by monocular deprivation. Soc Neurosci Abstr 18:299.

Jacobs KM, Donoghue JP (1991) Reshaping the cortical motor map by unmasking latent intracortical connections. Science 251:944–945.

Jones EG, Huntley GW, Benson DL (1994) α Calcium/calmodulin-dependent protein kinase II selectively expressed in a subpopulation of excitatory neurons in monkey sensory-motor cortex: comparison with GAD-67 expression. J Neurosci, in press.

Kater SB, Guthrie PB (1990) Neuronal growth cone as an integrator of complex environmental information. Cold Spring Harb Symp Quant Biol 55:359–370.

Kato K (1990) Novel $GABA_A$-receptor α subunit is expressed only in cerebellar granule cells. J Mol Biol 214:619–624.

Kawaguchi Y (1993) Groupings of non-pyramidal and pyramidal cells with specific physiological and morphological characteristics in rat frontal cortex. J Neurophysiol 69:416–431.

Khrestchatisky M, MacLennan AJ, Chiang M-Y, Xu W, Jackson MB, Brecha N, Sternini C, Olsen RW, Tobin AJ (1989) A novel α subunit in rat brain $GABA_A$ receptors. Neuron 3:745–753.

Khrestchatisky M, MacLennan AJ, Tillakaratne NJK, Chiang M-Y, Tobin AJ (1991) Sequence and regional distribution of the mRNA encoding the α2 polypeptide of rat γ-aminobutyric $acid_A$ receptors. J Neurochem 56:1717–1722.

Kirkness EF, Bovenkirk CF, Ueda T, Turner AJ (1989) Phosphorylation of γ-aminobutyrate (GABA)/benzodiazepine receptors by cyclic AMP-dependent protein kinase. Biochem J 259:613–616.

Kisvárday ZF, Cowey A, Somogyi P (1986) Synaptic relationship of a type of GABA-immunoreactive neuron (clutch cell), spiny stellate cells and lateral geniculate nucleus afferents in layer IVC of the monkey striate cortex. Neuroscience 19:741–761.

Knoflach F, Rhyner T, Villa M, Kellenberger S, Drescher U, Malherbe P, Sigel E, Möhler H (1991) The γ3-subunit of the $GABA_A$ receptor confers sensitivity to the benzodiazepine receptor ligands. FEBS Lett 293:191–194.

Knoflach F, Backus KH, Giller T, Malherbe P, Pflimlin P, Möhler H, Trube G (1992) Pharmacological and electrophysiological properties of recombinant $GABA_A$ receptors comprising α3, β1, and γ2 subunits. Eur J Neurosci 4:1–9.

Kofuji P, Wang JB, Moss SJ, Huganir RL, Burt DR (1991) Generation of two forms of the γ-aminobutyric acid$_A$ receptor γ2 subunit in mice by alternative splicing. J Neurochem 56:713–715.

Kostovic I, Rakic P (1980) Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. J Neurocytol 9:219–242.

Kostovic I, Rakic P (1990) Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. J Comp Neurol 297:441–470.

Kriegstein AR, Suppes T, Prince DA (1987) Cellular and synaptic physiology and epileptogenesis of developing rat neocortical neurons *in vitro*. Dev Brain Res 34:161–171.

Krnjević K (1987) GABAergic inhibition in neocortex. J Mind Behav 8:537–547.

Lasham A, Vreugdenhil E, Bateson AN, Barnard EA, Darlison MG (1991) Conserved organization of γ-aminobutyric acid$_A$ receptor genes: cloning and analysis of the chicken β4-subunit gene. J Neurochem 57:352–355.

Laurie DJ, Seeburg PH, Wisden W (1992a) The distribution of 13 GABA$_A$ receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. J Neurosci 12:1063–1076.

Laurie DJ, Seeburg PH, Wisden W (1992b) The distribution of 13 GABA$_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci 12:4151–4172.

Leidenhemer NJ, Browning MD, Harris RA (1991) GABA$_A$ receptor phosphorylation: multiple sites, actions and artifacts. Trends Pharmacol Sci 12:84–87.

Levitan ES, Schofield PR, Burt DR, Rhee LM, Wisden W, Köhler M, Fijita N, Rodriguez HF, Stephenson A, Darlison MG, Barnard EA, Seeburg PH (1988) Structural and functional basis for GABA$_A$ receptor heterogeneity. Nature 335:76–79.

Lolait SJ, O'Carroll A-M, Kusano K, Muller JM, Brownstein MJ, Mahan LC (1989a) Cloning and expression of a novel rat GABA$_A$ receptor. FEBS Lett 246:145–148.

Lolait SJ, O'Carroll A-M, Kusano K, Mahan LC (1989b) Pharmacological characterization and region-specific expression in brain of the β2- and β3-subunits of the rat GABA$_A$ receptor. FEBS Lett 258: 17–21.

Lüddens H, Wisden W (1991) Function and pharmacology of multiple GABA$_A$ receptor subunits. Trends Pharmacol Sci 12:49–51.

Lüddens H, Pritchett DB, Köhler M, Killisch L, Keinänen H, Möhler H, Sprengel R, Seeburg PH (1990) Cerebellar GABA$_A$ receptor selective for a behavioral alcohol antagonist. Nature 346:648–651.

Lüddens H, Killisch I, Seeburg PH (1991) More than one α variant may exist in a GABA$_A$/benzodiazepine receptor complex. J Recept Res 11:535–551.

Luhmann HJ, Prince DA (1990) Control of NMDA receptor-mediated activity by GABAergic mechanisms in mature and developing rat neocortex. Dev Brain Res 54:287–290.

Lund JS (1973) Organization of neurons in the visual cortex area 17, of the monkey (*Macaca mulatta*). J Comp Neurol 147:455–496.

Luskin MB, Shatz CJ (1985) Studies of the earliest generated cells of the cat's visual cortex: cogeneration of subplate and marginal zones. J Neurosci 5:1062–1075.

MacDonald RL, Rogers CJ, Twyman RE (1989) Kinetic properties of the GABA$_A$ receptor main conductance state of mouse spinal cord neurons in culture. J Physiol (Lond) 410:479–499.

MacLennan AJ, Brecha N, Khrestchatisky M, Sternini C, Tillakaratne NJK, Chang M-Y, Anderson K, Lai M, Tobin AJ (1991) Independent cellular and ontogenetic expression of mRNAs encoding three polypeptides of the rat GABA$_A$ receptor. Neuroscience 43:369–380.

Malherbe P, Sigel E, Bauer R, Persohn E, Richards JG, Möhler H (1990a) Functional characteristics and sites of gene expression of the α1, β1, γ2-isoform of the rat GABA$_A$ receptor. J Neurosci 10:2330–2337.

Malherbe P, Draguhn A, Multhaup G, Beyreuther K, Möhler H (1990b) GABA$_A$-receptor expressed from rat brain α- and β-subunit cDNAs displays potentiation by benzodiazepine receptor ligands. Mol Brain Res 8:199–208.

Malherbe P, Sigel E, Baur R, Persohn E, Richards JG, Möhler H (1990c) Functional expression and sites of gene transcription of a novel α-subunit of the GABA$_A$-receptor in rat brain. FEBS Lett 260:261–265.

Marin-Padilla M (1970) Prenatal and early postnatal ontogenesis of the human motor cortex. a Golgi study. I. The prenatal sequential development of the cortical layers. Brain Res 23:167–183.

Matsumoto RR (1989) GABA receptors: are cellular differences reflected in function? Brain Res Rev 14:203–225.

McConnell SK, Ghosh A, Shatz CJ (1989) Subplate neurons pioneer the first axon pathway from the cerebral cortex. Science 245:978–982.

McCormick DA (1989) GABA as an inhibitory neurotransmitter in human cerebral cortex. J Neurophysiol 62:1018–1027.

McKernan RM, Quick K, Prince R, Cox PA, Gillard NP, Ragen CI, Whiting P (1991) GABA$_A$ receptor subtypes immunopurified from rat brain with α subunit-specific antibodies have unique pharmacological properties. Neuron 7:667–676.

Meier E, Jorgensen OS, Schousboe A (1987) Effect of repeated treatment with a γ-aminobutyric acid receptor agonist on postnatal neuronal development in rats. J Neurochem 49:1462–1470.

Meinecke DL, Rakic P (1992) Expression of GABA and GABA$_A$ receptors by neurons of the subplate zone in developing primate occipital cortex: evidence for transient local circuits. J Comp Neurol 317:91–101.

Mertens S, Benke D, Möhler H (1993) GABA$_A$ receptor populations with novel subunit combinations and drug binding profiles identified in brain by α5- and γ-subunit-specific immunopurification. J Biol Chem 268:5965–5973.

Michelson HB, Wong RKS (1991) Excitatory synaptic responses mediated by GABA$_A$ receptors in the hippocampus. Science 253:1420–1423.

Möhler H, Sigel E, Malherbe P, Richards JG, Persohn E, Mertens S, Benke D (1991) GABA$_A$-receptor heterogeneity: functional architecture of receptors and sites of subunit-expression. In: Fidia Research Foundation symposium series, Vol 6, Amino acid receptors: structures, transduction (Costa E, Barnard EA, eds), pp 23–35. New York: Raven.

Montpied P, Martin BM, Cottingham SL, Stubblefield BK, Ginns EI, Paul SM (1988) Regional distribution of the GABA$_A$/benzodiazepine receptor (α subunit) mRNA in rat brain. J Neurochem 51:1651–1654.

Moss S, Ravindran A, Mei L, Wang JB, Kofiji P, Huganir R, Burt D (1991) Characterization of recombinant GABA$_A$ receptors produced in transfected cells from murine α1, β1 and γ2 subunit cDNAs. Neurosci Lett 123:265–268.

O'Kusky J, Colonnier M (1982) Postnatal changes in the number of neurons and synapses in the visual cortex (area 17) of the macaque monkey: a stereological analysis in normal and monocularly deprived animals. J Comp Neurol 210:291–306.

Olsen RW (1991) GABA and inhibitory synaptic transmission in the brain. Semin Neurosci 3:175–181.

Olsen RW, Tobin AJ (1990) Molecular biology of GABA$_A$ receptors. FASEB J 4:1469–1480.

Olsen RW, Bureau M, Khrestchatisky M, MacLennan AJ, Chiang M-Y, Tobin AJ, Xu W, Tackson M, Sternini C, Brecha N (1990a) Isolation of pharmacologically distinct GABA–benzodiazepine receptors by protein chemistry and molecular cloning. In: GABA and benzodiazepine receptor subtypes (Biggio G, Costa E, eds), pp 35–49. New York: Raven.

Olsen RW, McCabe RT, Wamsley JK (1990b) GABA$_A$-receptor subtypes: autoradiographic comparison of GABA, benzodiazepine and convulsant binding sites in the rat central nervous system. J Chem Neuroanat 3:59–76.

Olsen RW, Bureau M, Endo S, Smith G, Sapp D, Tobin AJ (1991a) GABA$_A$-receptor subtypes: comparison of regional variation in binding heterogeneity with subunit subtype polypeptides and mRNAs. Paper presented at the Annual Meeting of the Society for Neuroscience, New Orleans, LA, November.

Olsen, RW, Bureau MH, Endo S, Smith G (1991b) The GABA$_A$ receptor family in the mammalian brain. Neurochem Res 16:317–325.

Park D, de Blas AL (1991) Peptide subunits of γ-aminobutyric acid$_A$/benzodiazepine receptors from bovine cerebral cortex. J Neurochem 56:1972–1979.

Park D, Victorica J, Tous G, de Blas AL (1991) Purification of the γ-aminobutyric acid$_A$/benzodiazepine receptor complex by immunoaffinity chromatography. J Neurochem 56:1962–1971.

Pearce RA (1993) Physiological evidence for two distinct GABA$_A$ responses in rat hippocampus. Neuron 10:189–200.

Persohn E, Malherbe P, Richards JG (1991) *In situ* hybridization histochemistry reveals a diversity of $GABA_A$ receptor subunit mRNA in neurons of the rat spinal cord and dorsal root ganglia. Neuroscience 42:497–507.

Persohn E, Malherbe P, Richards JG (1992) Comparative molecular neuroanatomy of cloned GABA receptor subunits in the rat CNS. J Comp Neurol 326:193–216.

Porter NM, Twyman RE, Uhler MD, MacDonald RL (1990) Cyclic AMP-dependent protein kinase decreases $GABA_A$ receptor current in mouse spinal neurons. Neuron 5:789–796.

Poulter MO, Barker JL, O'Carroll A-M, Lolait SJ, Mahan LC (1992) Differential and transient expression of $GABA_A$ receptor α subunits mRNAs in the developing rat CNS. J Neurosci 12:2888–2900.

Prince DA, Tseng G-F (1993) Epileptogenesis in chronically injured cortex: *in vitro* studies. 69:1276–1291.

Pritchett DB, Seeburg PH (1990) γ-Aminobutyric $acid_A$ receptor α5-subunit creates novel type II benzodiazepine receptor pharmacology. J Neurochem 54:1802–1804.

Pritchett DB, Sontheimer H, Gorman CM, Kettenmann H, Seeburg PH, Schofield PR (1988) Transient expression shows ligand gating and allosteric potentiation of $GABA_A$ receptor subunits. Science 242: 1306–1308.

Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, Seeburg PH (1989a) Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature 338:582–585.

Pritchett DB, Lüddens H, Seeburg PH (1989b) Type I and type II $GABA_A$ benzodiazepine receptors produced in transfected cells. Science 245:1389–1392.

Puia G, Santi M, Vicini S, Pritchett DB, Purdy RH, Paul SM, Seeburg PH, Costa E (1990) Neurosteroids act on recombinant human $GABA_A$ receptors. Neuron 4:759–765.

Puia G, Vicini S, Seeburg PH, Costa E (1991) Influence of recombinant γ-aminobutyric $acid_A$ receptor subunit composition on the action of allosteric modulators of γ-aminobutyric acid-gated Cl^- currents. Mol Pharmacol 39:691–696.

Rakic P (1972) Mode of cell migration to the superficial layers of fetal monkey neocortex. J Comp Neurol 145:61–84.

Rakic P (1977) Prenatal development of the visual system in the rhesus monkey. Philos Trans R Soc Lond [Biol] 278:245–260.

Rakic P, J-P, Eckenhoff MF, Zecevic N, Goldman-Rakic P (1986) Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. Science 232:232–235.

Rakic P, Goldman-Rakic PS, Gallagher D (1988) Quantitative autoradiography of major neurotransmitter receptors in the monkey striate and extrastriate cortex. J Neurosci 8:3670–3690.

Rakic P, Bourgeois J-P, Eckenhoff MF, Zecevic N, Goldman-Rakic P (1986) Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. Science 232:232–235.

Ramoa AS, Paradiso MA, Freeman RD (1988) Blockade of intracortical inhibition in kitten striate cortex: effects on receptive field properties and associated loss of ocular dominance plasticity. Exp Brain Res 73:285–298.

Reiter HO, Stryker MP (1988) Neural plasticity without postsynaptic action potentials: less active inputs become dominant when kitten visual cortical cells are pharmacologically inhibited. Proc Natl Acad Sci USA 85:3623–3627.

Richards JG, Schoch P, Haefely W (1991) Benzodiazepine receptors: new vistas. Semin Neurosci 3:191–203.

Rockel AJ, Hiorns RW, Powell TPS (1980) The basic uniformity in structure of the neocortex. Brain 103:221–224.

Sambrook J, Fritsch EF, Maniatus T (1989) Molecular cloning, a laboratory manual, 2d ed. New York: Cold Spring Harbor Laboratory.

Sandell JH (1986) NADPH-diaphorase histochemistry in the macaque striate cortex. J Comp Neurol 251:388–397.

Sanger F, Nicklin S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467.

Sato N, Neale JH (1989) Type I and type II γ-aminobutyric acid/benzodiazepine receptors: purification and analysis of novel receptor complexes from neonatal cortex. J Neurochem 52:1114–1122.

Scharfman HE, Sarvey JM (1985) Responses to γ2-aminobutyric acid applied to cell bodies and dendrites of rat visual cortical neurons. Brain Res 358:385–389.

Scharfman HE, Sarvey JM (1987) Responses to GABA recorded from identified rat visual cortical neurons. Neuroscience 23:407–422.

Schofield PR (1989) The $GABA_A$ receptor: molecular biology reveals a complex picture. Trends Pharmacol Sci 10:476–478.

Schofield PR (1990) The role of receptor subtype diversity in the CNS. Trends Neurosci 13:8–13.

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA, Seeburg PH, Barnard EA (1987) Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. Nature 328:221–227.

Schofield PR, Pritchett DB, Sontheimer H, Kettenmann H, Seeburg PH (1989) Sequence and expression of human $GABA_A$ receptor α1 and β1 subunits. FEBS Lett 244:361–364.

Seeburg PH, Wisden W, Vendoorn TA, Pritchett DB, Werner P, Herb A, Lüddens H, Sprengel R, Sakmann B (1990) The $GABA_A$ receptor family: molecular and functional diversity. Cold Spring Harb Symp Quant Biol 55:29–40.

Séquier JM, Richards JG, Malherbe P, Price GW, Mathews S, Möhler H (1988) Mapping of brain areas containing RNA homologous to cDNAs encoding the α and β subunits of the rat $GABA_A$ γ-aminobutyrate receptor. Proc Natl Acad Sci USA 85:7815–7819.

Shatz CJ, Chun JJM, Luskin MB (1988) The role of the subplate in the development of the mammalian telencephalon. In: Cerebral cortex, Vol 7, Development and maturation of cerebral cortex (Peters A, Jones EG, eds), pp 35–58. New York: Plenum.

Shaw C, Cynader M (1986) Laminar distribution of receptors in monkey (*Macaca fascicularis*) geniculostriate system. J Comp Neurol 248: 301–312.

Shaw C, Cameron L, March D, Cynader M, Zielinski B, Hendrickson A (1991) Pre- and postnatal development of GABA receptors in *Macaca* monkey visual cortex. J Neurosci 11:3943–3959.

Shivers DB, Killisch I, Sprengel R, Sontheimer H, Köhler M, Schofield PR, Seeburg PH (1989) Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. Neuron 3:327–337.

Sieghart W (1989) Multiplicity of $GABA_A$-benzodiazepine receptors. Trends Pharmacol Sci 10:407–411.

Sieghart W (1991) Heterogeneity of $GABA_A$-benzodiazepine receptors. Biochem Soc Trans 10:129–132.

Sieghart W, Schlerka W (1991) Potency of several type I-benzodiazepine receptor ligands for inhibition of [^{3}H] flunitrazepam binding in different rat brain tissues. Eur J Pharmacol 197:103–107.

Sigel RE (1988) The mRNAs encoding $GABA_A$/benzodiazepine receptor subunits are localized in different cell populations of the bovine cerebellum. Neuron 1:579–584.

Sigel RE, Baur R (1988) Activation of protein kinase C differentially modulates neuronal Na^+, Ca^{2+}, and γ-aminobutyrate type A channels. Proc Natl Acad Sci USA 85:6192–6196.

Sigel RE, Baur R, Trube G, Möhler H, Malherbe P (1990) The effect of subunit composition of rat brain $GABA_A$ receptors on channel function. Neuron 5:703–711.

Sillito AM (1975) The contribution of inhibitory mechanisms to the receptive field properties of neurons in the striate cortex of the cat. J Physiol (Lond) 250:305–329.

Sillito AM (1977) Inhibitory mechanisms influencing complex cell orientation selectivity and their modification at high resting discharge levels. J Physiol (Lond) 289:33–53.

Sillito AM (1984) Functional considerations of the operation of GABAergic inhibitory processes in the visual cortex. In: Cerebral cortex, Vol 2, Functional properties of cortical cells (Jones EG, Peters A, eds), pp 91–117. New York: Plenum.

Sillito AM, Kemp JA, Wilson JA, Berardi N (1980) A reevaluation of the mechanisms underlying simple cell orientation selectivity. Brain Res 194:517–520.

Smart TG, Moss SJ, Xie X, Huganir RA (1991) $GABA_A$ receptors are differentially sensitive to zinc-dependence on subunit comparison. Bri J Pharmacol 103:1837–1839.

Stephenson FA (1988) Understanding the $GABA_A$ receptor: a chemically gated in ion channel. Biochem J 249:21–32.

Stephenson FA, Duggan MJ, Casalotti SO (1989) Identification of the α3-subunit in the $GABA_A$ receptor purified from bovine brain. FEBS Lett 243:358–362.

Stephenson FA, Duggan MJ, Pollard S (1990) The γ2 subunit is an integral component of the γ-aminobutyric acid A receptor but the α polypeptide is the principal site of the agonist benzodiazepine photoaffinity labeling reaction. J Biol Chem 265:21160–21165.

Swann JW, Brady RJ, Martin DL (1989) Postnatal development of

GABA-mediated synaptic inhibition in rat hippocampus. Neuroscience 28:551–561.

Sweetnam PM, Lloyd J, Gallombardo P, Malison RT, Gallager DW, Tallman JF, Nester EJ (1988) Phosphorylation of the $GABA_A$/benzodiazepine receptor α subunit by a receptor-associated protein kinase. J Neurochem 51:1275–1284.

Tsumoto T, Eckart W, Creutzfeldt OD (1979) Modification of orientation sensitivity of cat visual cortex neurons by removal of GABA mediated inhibition. Exp Brain Res 34:351–365.

Verdoorn TA, Draguhn A, Ymer S, Seeburg PH, Sakmann B (1990) Functional properties of recombinant rat $GABA_A$ receptors depend upon subunit composition. Neuron 4:919–928.

Vicini S (1991) Pharmacologic significance of the structural heterogeneity of the $GABA_A$ receptor-chloride ion channel complex. Neuropsychopharmacology 4:9–15.

Von Blankenfeld G, Ymer S, Pritchett DB, Sontheimer H, Ewert M, Seeburg PH, Kettenmann H (1990) Differential benzodiazepine pharmacology of mammalian recombinant $GABA_A$ receptors. Neurosci Lett 115:269–273.

Whiting P, McKernan RM, Iversen LI (1990) Another mechanism for creating diversity in γ-aminobutyrate type A receptors; RNA splicing directs expression of two forms of $\gamma 2$ subunit, one of which contains a protein kinase C phosphorylation site. Proc Natl Acad Sci USA 87:9966–9970.

Wieland HA, Lüddens H, Seeburg PH (1992) Molecular determinants in $GABA_A$/BZ receptor subtypes. In: GABAergic synaptic transmission (Biggio G, Concas A, Costas E, eds), pp 27–41. New York: Raven.

Wisden W, Seeburg PH (1992) $GABA_A$ receptors: from subunits to functional entities. Curr Opin Neurobiol 2:263–269.

Wisden W, Morris BJ, Darlison MG, Hunt SP, Barnard EA (1988) Distinct $GABA_A$ receptor α subunit mRNAs show differential patterns of expression in bovine brain. Neuron 1:937–947.

Wisden W, McNaughton LA, Darlison MG, Hunt SP, Barnard EA (1989a) Differential distribution of $GABA_A$ receptor mRNAs in bovine cerebellum-localization of $\alpha 2$ mRNA in Bergmann glia layer. Neurosci Lett 106:7–12.

Wisden W, Morris BJ, Darlison MG, Hunt SP, Barnard EA (1989b) Localization of $GABA_A$ receptor-α-subunit mRNAs in relation to receptor subtypes. Mol Brain Res 5:305–310.

Wisden W, Herb A, Wieland H, Keinänen K, Lüddens H, Seeburg PH (1991a) Cloning, pharmacological characteristics and expression pattern of the rat $GABA_A$ receptor $\alpha 4$ subunit. FEBS Lett 289:227–230.

Wisden W, Gundlach AL, Barnard EA, Seeburg PH, Hunt SP (1991b) Distribution of $GABA_A$ receptor subunit mRNAs in rat lumbar spinal cord. Mol Brain Res 10:179–183.

Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J Neurosci 12:1040–1062.

Wolf W, Hicks TP, Albus K (1986) The contribution of GABA-mediated inhibitory mechanisms to visual response properties of neurons in the kitten's striate cortex. J Neurosci 6:2779–2796.

Wolff JR (1981) Evidence for a dual role of GABA as a synaptic transmitter and a promotor of synaptogenesis. In: Amino acid neurotransmitters (DeFeudis FV, Mandel PM, eds), pp 459–465. New York: Raven.

Wolff JR, Joo F, Kasa P (1987) Synaptic, metabolic and morphogenetic effects of GABA in the superior cervical ganglion of rats. In: Neurotrophic activity of GABA during development (Redburn DA, Schousboe A, eds), pp 221–252. New York: Liss.

Wong-Riley M (1979) Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. Brain Res 171:11–28.

Ymer S, Schofield PR, Draguhn A, Werner P, Kohler M, Seeburg PH (1989a) $GABA_A$ receptor $\beta 1$ subunit heterogeneity: functional expression of cloned cDNAs. EMBO J 8:1165–1670.

Ymer S, Draguhn A, Köhler M, Schofield PR, Seeburg PH (1989b) Sequence and expression of a novel $GABA_A$-receptor α subunit. FEBS Lett 253:119–122.

Ymer S, Draguhn A, Wisden W, Werne P, Keinänen K, Schofield PR, Sprengel R, Pritchett DB, Seeburg PH (1990) Structural and functional characterization of the $\gamma 1$ subunit of $GABA_A$/benzodiazepine receptors. EMBO J 9:3261–3267.

Zezula J, Sieghart W (1991) Isolation of type I and type II $GABA_A$-benzodiazepine receptors by immunoaffinity chromatography. FEBS Lett 284:15–18.

Zezula J, Fuchs K, Sieghart W (1991) Separation of $\alpha 1$, $\alpha 2$ and $\alpha 3$ subunits of the $GABA_A$-benzodiazepine receptor complex by immunoaffinity chromatography. Brain Res 563:325–328.

Zhang J-H, Sato M, Noguchi K, Tohyama M (1990) The differential expression patterns of the mRNAs encoding β subunits ($\beta 1$, $\beta 2$ and $\beta 3$) of $GABA_A$ receptor in the olfactory bulb and its related areas in the rat brain. Neurosci Lett 119:257–260.

Zhang J-H, Sato M, Tohyama M (1991a) Differential postnatal ontogenic profiles of neurons containing β ($\beta 1$, $\beta 2$ and $\beta 3$) subunit mRNAs of $GABA_A$ receptor in the rat thalamus. Dev Brain Res 58:289–292.

Zhang J-H, Sato M, Tohyama M (1991b) Different postnatal development profiles of neurons containing distinct $GABA_A$ receptor β subunit messenger RNAs ($\beta 1$, $\beta 2$ and $\beta 3$) in the rat forebrain. J Comp Neurol 308:586–613.

Zhang J-H, Sato M, Tohyama M (1991c) Region-specific expression of the mRNAs encoding β subunits ($\beta 1$, $\beta 2$ and $\beta 3$) of $GABA_A$ receptor in the rat brain. J Comp Neurol 303:637–657.

Zimprich F, Zezula J, Sieghart W, Lassmann H (1991) Immunohistochemical localization of the $\alpha 1$, $\alpha 2$ and $\alpha 3$ subunit of the $GABA_A$ receptor in the rat brain. Neurosci Lett 127:125–128.