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Pre- and Postnatal Development of GABA Receptors in *Macaca* Monkey Visual Cortex

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GABA is a putative inhibitory neurotransmitter in adult mammalian visual cortex but also has been implicated as playing a crucial role in cortical information processing during development. In order to understand better the role of GABA during primate visual cortex development, we have examined the time course of GABA_A and GABA_B receptor ontogenesis in 18 *Macaca nemestrina* monkeys ranging from fetal day 61 (F61d) to adulthood. The GABA and benzodiazepine binding sites of the GABA_A receptor were detected by ³H-muscimol (³H-MS) and ³H-flunitrazepam (³H-FZ), respectively. GABA_B receptors were detected by ³H-baclofen (³H-BA). All ligands were visualized by *in vitro* autoradiography. Quantitative analysis of film density was done to compare laminar changes during pre- and postnatal development. Saturation binding experiments were done for MS and FZ binding sites to determine receptor number (B_{max}) and affinity (K_d) at selected pre- and postnatal ages.

Both MS and FZ binding sites were present at F61d–72d throughout the cortical plate and marginal zone. FZ binding sites were more dense than MS binding sites over the cortical plate at young ages and were especially dense over the marginal zone. FZ binding sites also were present in lesser amounts over the subplate and intermediate zone, but not over the subventricular zone. By F119d–126d, layer 4 could be distinguished by its higher density for both ligands. The basic adult laminar pattern was established for both MS and BZ binding sites by birth (birth = F165d–170d). After birth, MS density increases dramatically in all layers, but layer 4C remains most dense to adulthood. FZ labeling is heavy in both layers 4 and 3 at birth but after 4 weeks after birth (P4 wk) it declines somewhat in the supragranular layers so that layer 4C now predominates. Labeling in layers 5/6 virtually disappears after birth. BA binding sites were present at

F126d, at which time layer 4 was slightly lighter than the remainder of striate cortex; this laminar pattern remained basically the same throughout our series to adulthood.

Competitive binding of agonist and antagonists for the GABA_A receptor showed that MS binding characteristics were similar at F126d and P8.5 years (yr). MS binding site B_{max} was about 8% of adult values at F72d, 24% by F126d, and 56% at F152d. B_{max} then rose rapidly after birth to peak at P18wk at 169% of adult values, and then declined to P1yr. A second peak of 143% was found around P3.5yr, with adult values reached by P8.5yr. K_d values for MS at F126d showed evidence for two binding sites (34 nM and 4 nM) that existed up to P1d, and only one site (26–65 nM) after this age. The B_{max} for FZ was 27% of adult values at F126d, peaked at 113% adult value at P18wk, and declined slowly to adult levels by P8.5yr. K_d values for FZ found one site at all ages. Binding for FZ is proportionately higher in early development with a ratio of 1:2–3 FZ:MS, but in the postnatal cortex MS binding dominates with a ratio of 1:4–6 FZ:MS.

The present study demonstrates the early prenatal occurrence in monkey visual cortex of both the MS and FZ binding sites of the GABA_A receptor and also of the GABA_B receptor. The GABA_A receptor first appears near the age when GABAergic neurons can be detected immunocytochemically (Hendrickson et al., 1988; Meinicke and Rakic, 1989), but before synapses are found in significant numbers (Zielinski and Hendrickson, 1990). Although GABA_A receptors are normally associated with inhibitory synaptic circuits, these data suggest that the initial expression of GABA receptors is not dependent on synaptic contact and that GABA_A and its receptors may play some neurotrophic role in early cortical development. Postnatal changes in receptor distribution and number are more closely correlated with synaptic density changes.

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The macaque monkey striate cortex (primary visual, V1, or area 17) is one of the most widely studied systems in developmental neuroscience. Visual pathways to and from cortex have been mapped (reviewed in Hendrickson et al., 1978; Tigges and Tigges, 1985; Kaas and Huerta, 1988), there is a considerable data base on adult anatomy and physiology (Hubel, 1982; Blasdel and Fitzpatrick, 1984; Horton and Hedley-Whyte, 1984; Sillito, 1984; Hendrickson, 1985; Allman and McGuinness, 1988; Livingstone and Hubel, 1988; Lund, 1988), and the normal development of these parameters is known (reviewed in Rakic, 1977; LeVay et al., 1980; Boothe et al., 1985; Boothe, 1988). Many of these studies indicate that there is a marked similarity between ma-

caque and human visual systems, making the macaque a valuable model for invasive and experimental studies. This similarity is important because modification of retinal input during early postnatal development causes marked abnormalities in macaque cortical structure and function and produces symptoms identical to human visual amblyopias (discussed in LeVay et al., 1980; Wiesel, 1982; Hendrickson et al., 1987; Kiorpes et al., 1987; Harwerth et al., 1989).

Much recent research in this field has focused on defining the chemical circuitry of visual cortex, especially which neurons contain identified neurotransmitters and where receptors for neurotransmitters and/or neuromodulators are localized. Perhaps the best understood neurotransmitter for these studies is the inhibitory amino acid GABA (Houser et al., 1984; Somogyi, 1989). In striate cortex, this neurotransmitter is found within a heterogeneous population of nonpyramidal aspiny stellate neurons intrinsic to the cortex that label both for glutamate decarboxylase, the synthetic enzyme for GABA (Hendrickson et al., 1981; Fitzpatrick et al., 1987), and for GABA itself (Fitzpatrick et al., 1987; Hendry et al., 1987; Somogyi, 1989). GABAergic neurons comprise 15–20% of the neuronal population in all layers except layer 1, in which 90%+ of the neurons are GABAergic. In striate cortex of both cat and monkey, the synapses formed by these neurons are almost exclusively of the symmetric type and terminate on dendritic spines, shafts, cell bodies, and initial axon segments (Ribak, 1978; Houser et al., 1984; Somogyi and Hodgson, 1985; LaVie and Hendrickson, 1986; Somogyi, 1989). There is also abundant electrophysiological evidence that GABA is an inhibitory neurotransmitter in cat striate cortex, heavily influencing directional and orientation specificity but with less influence on ocular dominance (discussed in Sillito, 1984; Wolf et al., 1986). Recent work in brain slices indicates that GABA has an inhibitory function in human cerebral cortex as well (McCormick, 1989). The results of combining Golgi impregnation or intracellular filling with EM immunocytochemical labeling suggest that each morphological type of GABAergic neuron has a characteristic pattern of axon termination that is specific for one part of the postsynaptic neuron (reviewed by Somogyi, 1989). This correlation should help explain the role of GABA neurotransmission in determining physiological specificity when these anatomical circuits are better analyzed.

As would be expected from the large number of GABAergic neurons, the adult primate striate cortex is well endowed with muscimol (MS)-binding sites that label the GABA-binding component of GABA_A receptors, and with benzodiazepine-binding sites that label another component of the GABA_A receptor complex (Braestrup et al., 1977; Shaw and Cynader, 1986; Rakic et al., 1988; McCormick, 1989). Antisera specific for the GABA_A receptor label all cortical layers to varying degrees (Hendry et al., 1990). Baclofen (BA)-binding sites that label the putative GABA_B receptor also are present but are less prominent (McCormick, 1989; Shaw et al., 1989, 1990). GABA_A and GABA_B receptor subtype activation typically causes inhibition via hyperpolarization of target neurons (recently reviewed in Kuman et al., 1987; Bowery, 1989; Matsumoto, 1989; Sieghart, 1989; Olsen and Tobin, 1990). Scharfman and Sarvey (1987) have also shown that GABA_A receptors on distal dendrites mediate depolarizing responses while those on somata and proximal dendrites mediate hyperpolarization. Activation of the MS-binding, bicuculline-sensitive GABA_A receptor leads to an inwardly directed chloride current, while activation of the BA-binding, bicuculline-insensitive GABA_B receptor initiates a

G-protein cascade that results in increased potassium or decreased calcium conductance. Benzodiazepine receptors are usually, but not always, linked to GABA_A receptors (Unnerstall et al., 1981; Richards and Mohler, 1984; Shaw et al., 1987) and appear to modulate their sensitivity (Costa et al., 1978). In order to distinguish between a true receptor and a binding site, it is necessary to identify the pharmacological profile of agonist and antagonist displacement of the labeled ligand, and the receptor affinity (K_d) and number (B_{max}). Because such criteria have not been met by all the studies cited below, we will use the less specific term "binding site" for ³H-MS binding that labels GABA_A receptors and ³H-flunitrazepam (³H-FZ) binding as a marker of benzodiazepine receptors.

Surprisingly little is known about the normal developmental sequence of GABA receptors in monkey striate cortex. Two recent reports using monoclonal antisera to GABA_A and benzodiazepine receptors (Meinicke and Rakic, 1989; Huntley et al., 1990) detect labeling in several areas of macaque cortex at the relatively late age of fetal day 100 (F100d)–131d (birth = F165d–170d). Autoradiographic ligand binding studies (Shaw et al., 1989, 1990) detected FZ and MS binding sites in striate cortex by F72d, which is close to the age when GABAergic neurons are first stained immunocytochemically (Hendrickson et al., 1988; Meinicke and Rakic, 1989). There are several GABA_A receptor membrane binding studies in developing human cortex, but no autoradiographic studies have been reported. Human cerebral cortex at fetal week 15 (F15wk) contains 6% of adult benzodiazepine levels (Aaltonen et al., 1983). In human frontal cortex (Brooksbank et al., 1981, 1982), MS binding was 9% of adult in the F18wk–28wk prenatal group, 47% in the F26wk–42wk perinatal group, and 97% by postnatal (P)4wk–8mo. On the other hand, FZ binding is 9% of adult in early prenatal life but is only 22% in the perinatal and 55% in the postnatal group, indicating that the benzodiazepine component of the GABA_A receptor is somewhat slower to complete development. By the second half of gestation, MS binding is about twice that of FZ, and this ratio remains constant into adulthood, although overall MS binding increases 10-fold and FZ binding increases 6-fold from F18wk to adult. Glutamic acid decarboxylase (GAD) activity in cortical neurons is very slow to develop (Brooksbank et al., 1981), being only 5% of adult in the prenatal group, and still only 38% of adult by P8mo. The age at which GABAergic neurons first appear in human cortex is not known.

The data on GABA_A receptor development in cat and rodent cortex is much more extensive. Whole rat brain demonstrates benzodiazepine binding at 5% of adult levels by F14d (Braestrup and Nielsen, 1978) or F15d (Coyle and Enna, 1976), while mouse brain at F17d contains 10% of adult levels (Regan et al., 1980). On the other hand, MS binding in rat cortex still was less than 10% at P2d, while FZ binding had reached 50% of adult (Palacios et al., 1979). The only study using autoradiographic methods to determine prenatal laminar developmental patterns is that of Schlumpf et al. (1983); postnatal ages were not included. They find a rostral-caudal developmental gradient for FZ binding in that labeling appeared in frontal cortex at F16d but did not reach occipital cortex until F21d. At F16d, only the marginal zone or future layer 1 was labeled, but by F18d the subplate and/or layer 6 was also labeled. The cortical plate was not clearly labeled until F21d, when the deeper older layers were more dense than the upper younger layers. Zones containing proliferating stem cells were never labeled. Neurons immunocytochemically labeled for GABA are found within the developing

rat marginal zone at F16d and in cortical plate by F18d–21d (Lauder et al., 1986; Van Eden et al., 1989), suggesting in rodents that GABAergic neurons and receptors appear at the same time. Prenatal studies of receptor development have not been performed in the cat. Postnatally, MS binding is 41% of adult levels by P3d and reaches 131% of adult at P14wk, while FZ binding is 18% at P3d and peaks at 133% at P8wk. Both decline by P1yr to adult levels (Shaw et al., 1984, 1986, 1987). GABA neurons are present in the subplate of cat visual cortex by 1 week before birth (Chun and Shatz, 1989), but when they first appear in the cat cortical plate is not yet clear.

These data indicate that both GABA neurons and GABA_A receptors are found prenatally in several mammalian species. For monkeys, the exact developmental sequence of GABA_A receptors has yet to be determined, there is little data on laminar sequences, and GABA_B receptor development has not been studied. It is important to determine this sequence more accurately because GABAergic neurotransmission is altered in some forms of visual deprivation and postnatal changes in layer 4 could play a role in postnatal visual deprivation syndromes (Hendry and Jones, 1986; Skangiel-Kramska and Kossut, 1984; Shaw and Cynader, 1988; Mower and Christen, 1989; Hendry et al., 1990). The present article will describe GABA_A receptor characteristics and the laminar distributions for MS, BA, and FZ binding sites in *Macaca nemestrina* striate cortex from early gestation to adulthood. Because the time of cell generation (Rakic, 1977) and the time of the appearance of GABA immunoreactivity in neurons is known (Hendrickson et al., 1988; Meineicke and Rakic, 1989) and there are quantitative data regarding synaptic development at the same ages in macaque monkey (O'Kusky and Colonnier, 1982; Rakic et al., 1986; Zielinski and Hendrickson, 1990), it is possible to compare these receptor data with neuronal and synaptic development to reveal the sequence of appearance and possible interdependencies for the GABAergic neurotransmitter system.

Materials and Methods

Tissue preparation. For receptor studies, 18 normal colony-bred *Macaca nemestrina* monkeys were used. They were killed at prenatal F61d, 72d, 119d, 126d, and 152d; postnatal P1d and 1.5d; 4, 7, 18, 32, 36, and 58 weeks; juveniles at 2.6, 3.3, and 3.5 years; and full adults of 8.5 and 11.3 years. The gender of the two youngest animals was not determined, the F119d, P7wk, one P36wk, and the 2.6yr and 8.5yr animals were males, and the remainder were females. Prenatal animals were the result of timed matings such that the ages were known within ± 1 d. In this colony, birth occurs at F165d–170d. Postnatal animals were born spontaneously and were at or above the norms for birth weights in the Regional Primate Center colony to exclude premature animals. Because of the difficulty and expense in obtaining large numbers of fetal and infant monkeys, we chose to have our series cover as many time points as possible along the prolonged period of primate visual development. However, the variation in our data can be compared in closely matched pairs at several ages (F119d–126d; P1d–1.5d; P32wk–36wk, and P3.3yr–3.5yr).

For cesarian section, the pregnant female monkey was tranquilized with ketamine, intubated, and then deeply anesthetized with halothane/O₂ mixture. The fetus was delivered under aseptic conditions and given a lethal intraperitoneal dose of barbiturate. Postnatal animals were killed with intravenous barbiturate. The brains were removed immediately after death, and striate cortex blocks from known striate cortical areas were oriented in cryostat freezing compound in disposable paper beakers and rapidly frozen in liquid Freon at -45°C . All frozen blocks were stored at -65°C for 1–12 weeks before the first assays for receptor binding were done. Because of the difficulties of obtaining a series of monkey fetuses, initial storage times varied between age, but in no systematic way. No variations in the distribution of binding sites could be detected that correlated with differences in storage times, nor has a

recognizable change occurred in binding characteristics over time. All assays were performed on 20- μm -thick frozen sections that were cut on a cryostat, thaw mounted onto subbed glass slides, and stored for 1–7 d at -20°C until assayed. The storage of visual cortex tissues at -25°C in the original blocks or as cut sections does not appear to alter significantly either ^3H -MS or ^3H -FZ binding (see Shaw and Cynader, 1988, and Shaw et al., 1987, respectively.) Nevertheless, quantitative binding assays in the present experiments were performed on tissue that was sectioned within the same 48 hr period and stored for no more than 1 week. Although some saturation binding assays were run separately, the sections from all animals were treated equivalently. Preliminary studies showed that extraction of the sections to remove lipids (myelin artifact) had little effect on the resulting film density pattern. Because lipid extraction caused deterioration of morphological integrity, especially in prenatal tissue, it was not used for the autoradiographs presented in this article.

Binding assays. Each binding assay included sections from all ages. These assays follow the protocols used previously to characterize GABA_A receptors in cat (Needler et al., 1984; Shaw et al., 1987) and adult monkey striate cortex (Shaw and Cynader, 1986). GABA_B protocols were based on our characterization of these receptors in cat visual cortex (C. Shaw and L. Cameron, unpublished observations). Details of each binding protocol are given in Table 1. Following the postincubation rinse, the sections were rapidly dried under a stream of cool air and stored overnight with desiccant. Slide-mounted sections destined for autoradiography were taped onto heavy cardboard and overlain with tritium-sensitive film (Amersham Hyperfilm); a slide containing ^3H standards (Amersham) was included on each film. At least two different exposures were made from each assay. Once suitable autoradiograms had been generated, the original sections were then stained with cresyl violet. Both the film and corresponding stained section were photographed at the same magnification, and photographic prints of the two were aligned to determine laminar boundaries. For each receptor population, sections from animals of different ages were exposed to Hyperfilm for the same period, usually on the same sheet of film.

Characterization and quantitative determinations of receptor number (B_{max}) and affinity (K_d) were made by saturation binding assays using ^3H -MS and ^3H -FZ (New England Nuclear) as described in detail elsewhere (Needler et al., 1984; Shaw et al., 1987). In brief, for each animal, total binding at a range of ligand concentrations was determined in three to four sections. Nonspecific binding was determined in two sections by incubation with the appropriate displacer (Table 1). In each individual section, the same region of layers 1–6 from striate cortex was scraped from the slide onto filter paper and placed in scintillation fluid (New England Nuclear, Formula 963). Quantitative binding in the youngest animal, F72d, was obtained from the cortical plate and marginal zone only. Bound radioactivity was counted in a scintillation counter, and Eadie–Hofstee analysis for a single site was performed by the methods of Zivin and Waud (1982). Multiple binding site curve fitting was performed using best-fit correlation coefficient values for a series of separately determined partial binding curves (Hunston, 1975). Matching alternate sections from each cortical block were assayed for protein content (Lowry et al., 1951), and the number of receptors is expressed as fmol/mg protein. See captions of Figures 4–6 for further details.

Quantitative analysis of laminar binding density on film autoradiograms was done using an Imaging Research Inc. (St. Catherine's, Ontario, Canada) image analysis system calibrated with the included ^3H standards (Amersham Microscales; 3.03–109.08 nCi/mg actual polymer activity). A density profile was constructed across all cortical layers in one representative section of striate cortex from each animal. The calculated values are given in Table 3.

Results

Qualitative changes with age

GABA_A receptors in the prenatal monkey striate cortex. Figure 1 shows cresyl violet-stained sections in the left column, ^3H -MS binding sites in the center column, and ^3H -FZ binding sites in the right column. Ages are F61d, F72d, F126d, and F152d from top to bottom, respectively. Figure 2 continues this developmental sequence after birth, with P1.5d, P4wk, P18wk, and P3.5yr shown from top to bottom, respectively. In Figures

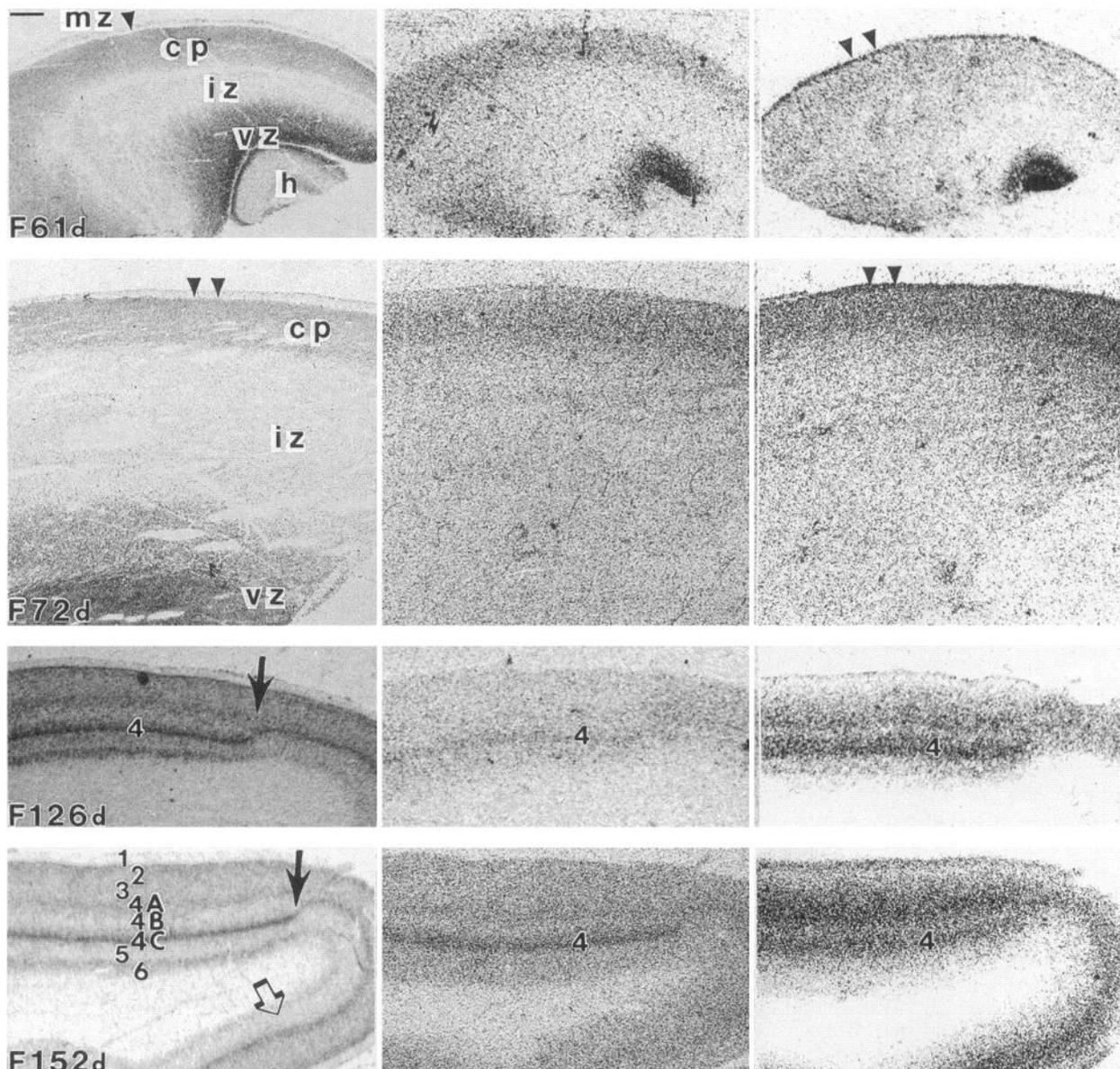


Figure 1. Autoradiographic distribution of GABA_A receptors before birth in *Macaca nemestrina* visual cortex. This plate shows sections of macaque visual cortex at F61d (top row), F72d (second row), F126d (third row), and F152d (bottom row). The left column shows sections stained for cresyl violet; the middle column, sections labeled by ³H-MS and the right column, sections labeled by ³H-FZ. Frozen sections were cut from unfixed tissue, and these slide-mounted sections were incubated as described in Table 1. In the youngest cortex, the marginal zone (mz), cortical plate (cp), subplate-intermediate zone (iz), and ventricular zone (vz) are indicated in the cresyl violet-stained section. The mz is marked by arrowheads in the FZ section. In the F61d brain, the underlying hippocampus (h) is heavily labeled and should not be confused with the overlying unlabeled vz. The high level of ³H-MS labeling over iz at the two youngest ages is background that was created in printing the relatively lightly labeled films at this age. In F152d, all layers are marked on the cresyl violet section; in other photos layer 4C is marked by 4. The striate-prestriate border is indicated by a solid arrow, and in the bottom row, prestriate area 18 is marked by an open arrow. All photos are 10×; scale bar in upper left corner equals 500 μm.

1 and 2, reading across the rows allows a comparison of ligands at the same age, while reading down the rows compares age-related changes. In the following description, the terms “labeling density” or “heavy versus light” will be used to indicate qualitative differences in film density for a single section. Because film density is related to binding-site number (Baskin and Dorsa, 1986), computer-assisted quantitative determinations of age-related changes in receptor density within individual layers were done and are discussed below.

At the youngest age examined thus far, F61d (Fig. 1, top row),

cresyl violet staining shows a dense band of neurons consisting of the superficial marginal zone and a thin cortical plate on the surface of the cerebral vesicle. This band is underlain by the scattered neurons and fibers of the subplate/intermediate zone and even deeper by the subventricular zone that gives rise to neurons that migrate across the intermediate zone into the cortical plate. The cortical plate forms the adult cerebral cortex by progressive sequential addition of neurons, beginning with layer 6 and ending with layer 2. The marginal zone becomes layer 1 of adult cortex (Sidman and Rakic, 1973). At F61d, the cortical

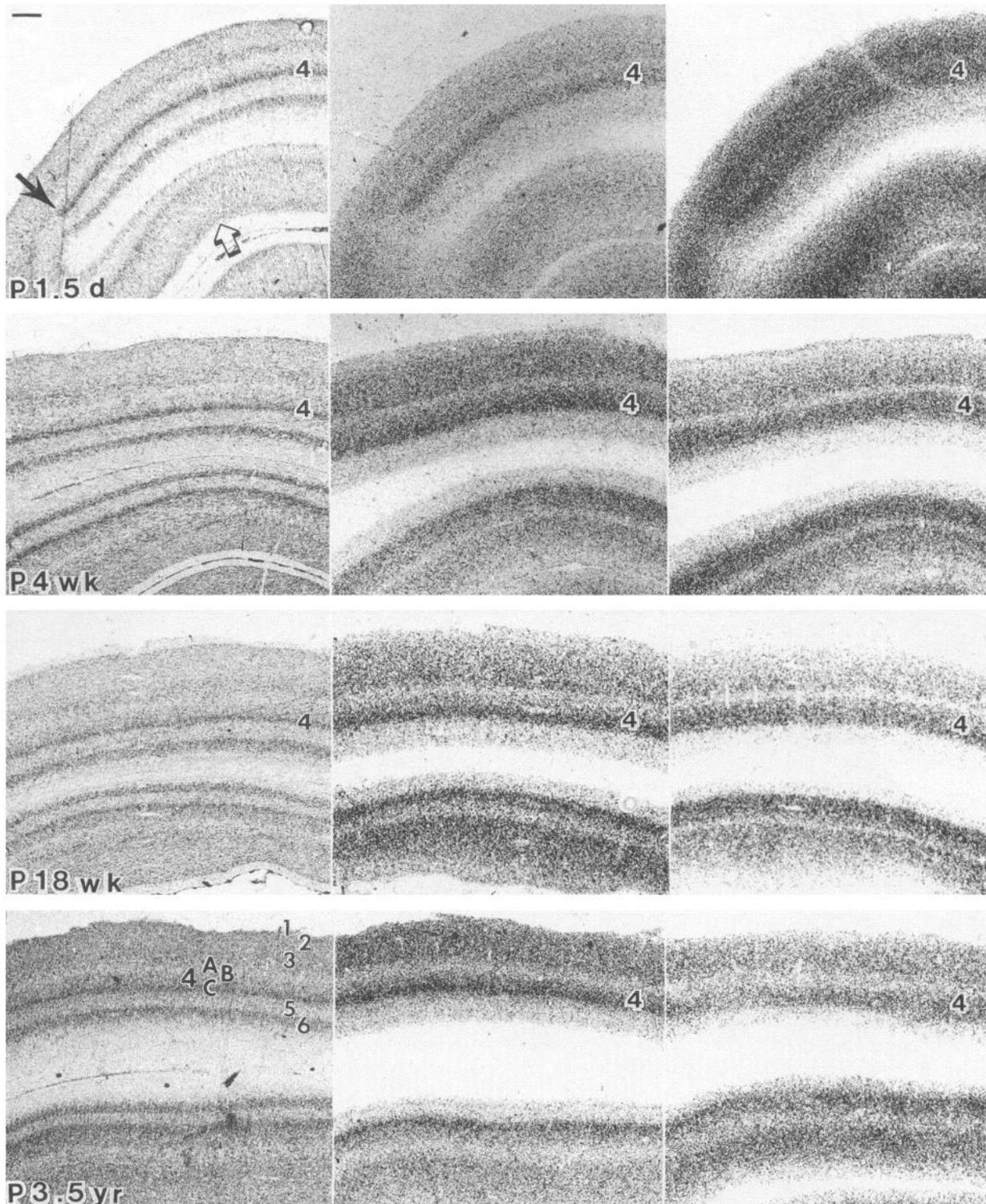


Figure 2. Autoradiographic distribution of GABA_A receptors after birth in *Macaca nemestrina* visual cortex. This plate shows sections of macaque visual cortex at P1.5d (top row), P4wk (second row), P18wk (third row), and P3.5yr (bottom row). The cresyl violet section of P3.5yr has all layers marked; labels are otherwise the same as Figure 1. Scale bar, 500 μ m.

plate contains mainly neurons that will form adult layers 6 and 5 (Rakic, 1977). Even at this young age, both MS and FZ binding sites are present. MS binding sites are confined to the cortical plate and marginal zone. FZ binding sites are heavy in the cortical plate but have the highest density in the outer portion of the marginal zone and also extend into the subplate/inter-

laminar zone. The ventricular zone is not labeled for either ligand, although the underlying hippocampus is very heavily labeled for both ligands. At F72d, the binding patterns are similar, but FZ binding sites are somewhat more confined to the cortical plate.

By F126d, the cortex contains its full complement of neurons

Table 1. Receptor assays

Receptor	Ligand	Displacer	Conditions
GABA _A	³ H-MS 100 nM ^a 0.2–400 nM ^b 25 and 50 nM ^c	10 ⁻⁴ M GABA	50 mM Tris-citrate buffer, pH 7.0, at 4°C; preincubation wash w/cold 0.2% formaldehyde buffer for 10 min; 2 × 5 min wash in buffer alone; 30 min incubation at 4°C w/100 nM ³ H-MS; 3 × 5 sec rinse in cold buffer.
Benzodiazepine	³ H-FZ 10 nM ^a 0.1–26 nM ^b	3 × 10 ⁻⁶ M Clonazepam	50 mM Tris-HCl buffer, pH 7.0, at 4°C; preincubation wash w/cold 0.2% formaldehyde buffer for 10 min; 2 × 5 min wash in buffer alone; 60 min incubation at 4°C w/10 nM ³ H-FZ; 3 × 30 sec rinse in cold buffer.
GABA _B	³ H-BA 50 nM ^a	10 ⁻⁴ M GABA	50 mM Tris-HCl buffer w/2.5 mM CaCl ₂ , pH 7.4, at 20°C; preincubation wash w/cold 0.2% formaldehyde buffer for 10 min; 2 × 5 min wash in buffer alone; 60 min incubation at 20°C w/50 nM ³ H-BA + 40 μM isoguvacine; 3 × 5 sec rinse in cold buffer.

^a Ligand concentration for film autoradiography.

^b Ligand concentrations for B_{max} and K_d determinations.

^c Ligand concentrations for displacement studies. Radioligand concentrations for the displacement curves were chosen to be near predicted K_d values, especially for the younger animal (F72d) where B_{max} was relatively small, in order to ensure dpm levels well above background.

(Rakic, 1977) and the adult striate cortex layers can be identified (Fig. 1, third row). The border between striate (to left) and prestriate (to right) cortex is indicated by a solid arrow. MS labeling still is quite sparse but is present in all layers, although now it is somewhat denser in layer 4C of striate cortex (marked by "4" in Figs. 1 and 2). Prestriate cortex is more densely labeled than striate at this age, particularly in the upper layers. FZ binding in cortex is strikingly increased in density and shows a clearly laminated distribution, with layers 4A/deep 3 and 4C heavier than the other layers, while layer 4B stands out as a pale band between these layers. The band of FZ labeling over layer 4C is much wider than for MS, and covers 4C α and 4C β . Comparison with cresyl violet-stained sections indicates that FZ binding sites extend somewhat below layer 6 into the developing white matter, but deeper white matter is now only lightly labeled. Prestriate cortex is less obviously laminated, but upper layers are much darker than lower.

At F152d, the cortex has increased in thickness, but lamination has changed relatively little from F126d. Overall MS binding site density has increased so that layers deep 3/4A can be detected by an increase in density compared to 4B, which remains light. Layer 4C, particularly its deeper subdivision 4C β , remains the layer with the heaviest MS labeling. FZ labeling is still much heavier than MS overall, with a thick band over both 4C α and 4C β and an extension of heavy labeling into layer 3. Prestriate cortex (open arrow) also shows increased FZ labeling, with a darker band now present over layer 4/deep 3.

After birth (Fig. 2), cortical lamination as shown by cresyl violet staining changes relatively little. In the first months after birth, there appears to be a general trend in which MS labeling density increases with age, while FZ labeling density stabilizes or decreases slightly. Shortly after birth at P1.5d, MS labeling

is somewhat heavier in striate layers 4C and 2/3, and in prestriate cortex middle layers. A comparison of Figures 1 and 2 suggests that before birth MS binding sites are found mainly over 4C β , but after birth extend into or increase over 4C α . Layer 4C β predominates for MS labeling at all older ages, in substantial agreement with Shaw and Cynader (1986) and Rakic et al. (1988). At P4wk, there is a dramatic increase in MS labeling density in layers 2/3 to a level similar to layer 4C. At later ages, MS labeling in the upper layers is somewhat variable, but deep 4C is always the most heavily labeled layer after birth.

Just after birth, FZ labeling density is high in all layers except over layer 1. With age, it decreases in the upper layers relative to layer 4C, and it is not until P4wk that FZ labeling is heaviest in 4C α / β . This progression continues so that by P18wk layers 1–3 are light relative to 4C, although they are somewhat darker again in the adult. FZ labeling in layers 5/6 is barely detectable by P4wk and remains light into adulthood.

In the age groups with two or more animals (F119d–126d, P1d–1.5d, P32wk–36wk, juvenile, and adult), there was no difference between animals of the same age in the laminar distribution of MS or FZ labeling. Quantitative measures also were very similar except for one juvenile female (see below).

Throughout this developmental sequence there is no consistent evidence for "patchiness" in the binding pattern in any layer for either MS or FZ that would suggest a correlation with developing ocular dominance columns. The blocks at F126d, F152d, and P1.5d in Figures 1 and 2 were cut parallel to the long axis of the ocular dominance columns as they terminate at the striate–prestriate border (Hubel, 1982), an orientation that is not optimal to visualize columns. However, the older cortex blocks in Figure 2 were from calcarine cortex on the horizontal meridian, and they were oriented such that ocular dominance

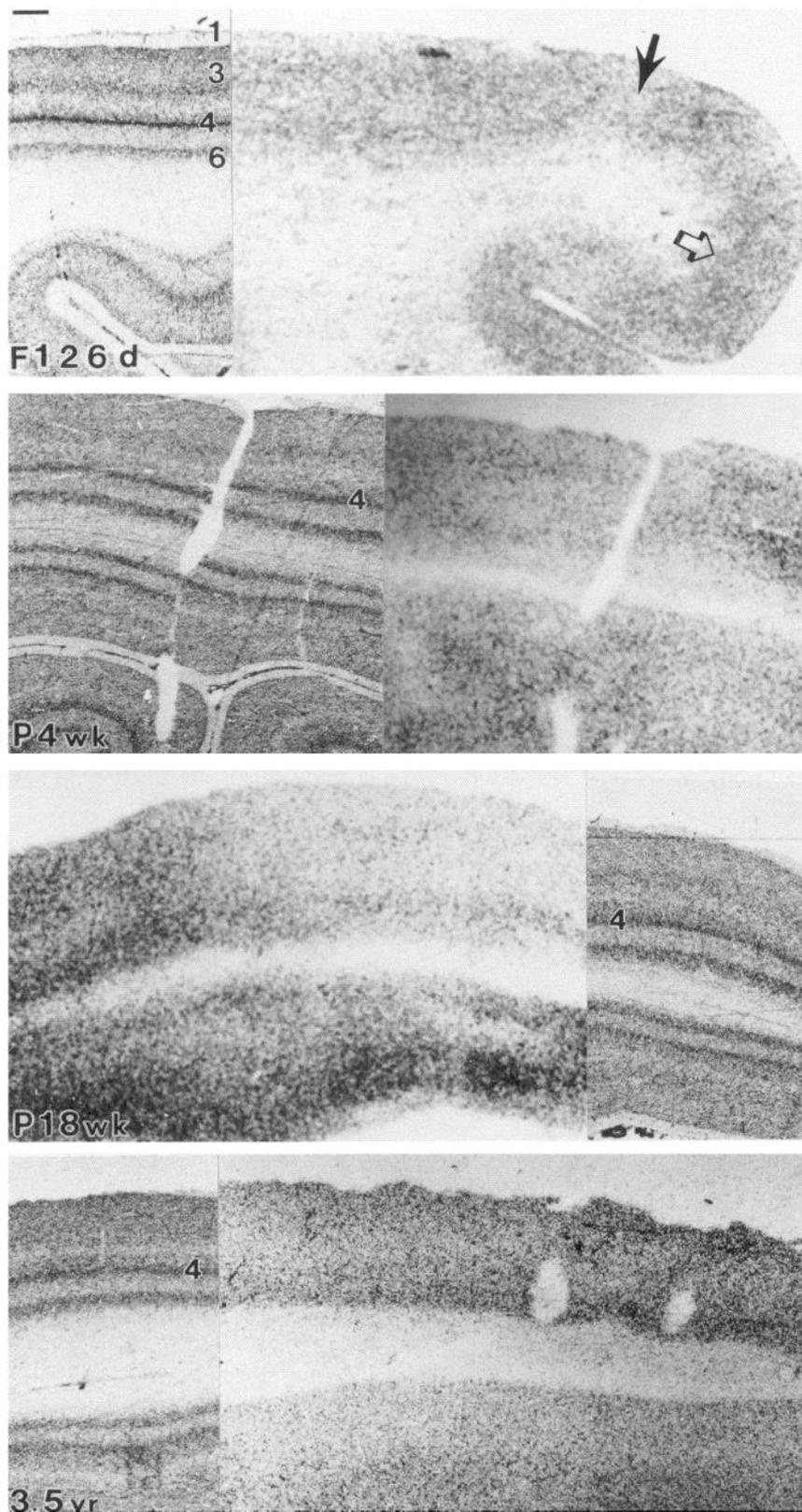


Figure 3. Autoradiographic distribution of GABA_B receptors in *Macaca nemestrina* visual cortex. This plate shows sections of striate and prestriate cortex at F126d (*top row*), and striate cortex at P4wk (*second row*), P18wk (*third row*), and P3.5yr (*bottom row*) stained for cresyl violet or labeled for ³H-BA binding sites. The cresyl violet sections are aligned with the BA-labeled sections. Layer 4C is marked by 4 and layers 1, 3, and 6 are also marked in the panel from F126d. In the *top row*, the striate-prestriate border is marked by a *solid arrow*, and area 18, by an *open arrow*. In the postnatal ages, both cortical layers are striate. Frozen sections were cut from unfixed tissue, and these slide-mounted sections were incubated as described in Table 1. All photos are 10×; scale bar in upper left corner equals 500 μm.

columns were sectioned perpendicularly. This issue of “patchiness” is currently under study in blocks sectioned parallel with the cortical surface, a method that should optimize visualization of any possible difference in binding pattern that would correlate

with the ocular dominance, color, and orientation module sub-organization of primate striate cortex (reviewed in Hendrickson, 1985).

³H-BA binding for GABA_B receptors shows light labeling

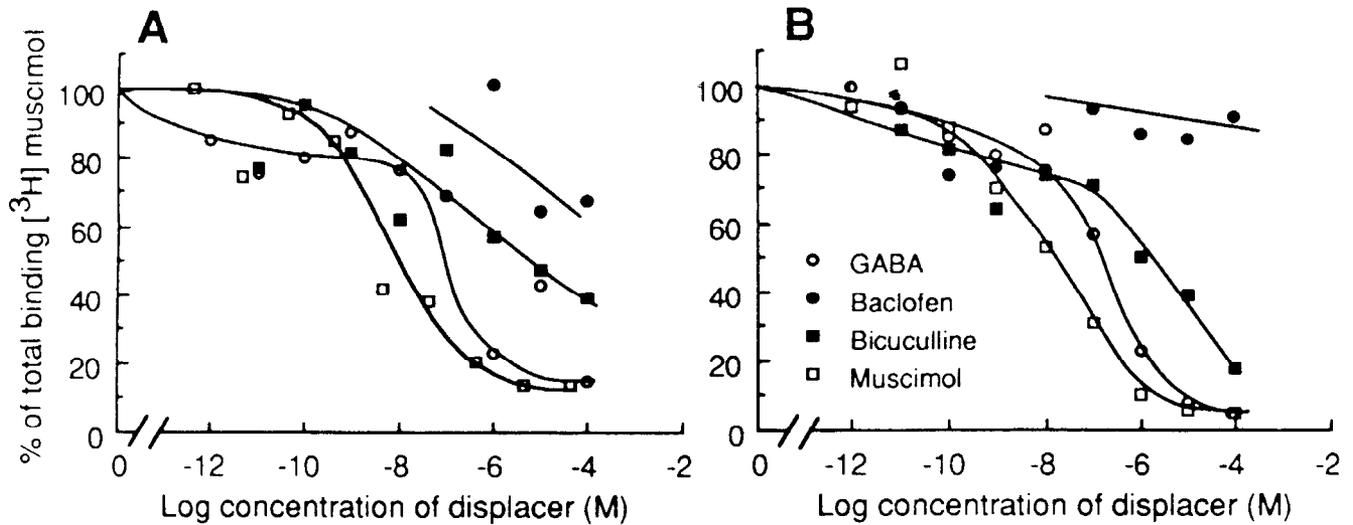


Figure 4. Characterization of the ^3H -MS binding site in macaque striate cortex. Displacement of ^3H -MS by other ligands in the F72d prenatal (A) and P8.5yr adult (B) monkey striate cortex is shown. All preincubation and incubation conditions are as described in Table 1, with a minimum of four sections used at each data point. Curves were fit by eye. Note that the relative potencies of the displacing ligands were similar in the two ages such that the GABA_A agonists MS and GABA were more effective displacers of ^3H -MS than the GABA_A antagonist bicuculline. The GABA_B agonist BA was almost without effect.

throughout all layers in both striate and prestriate cortex from F126d onward (Fig. 3). With increasing age, a slightly lighter diffuse band appears in the middle of striate cortex that seems to overlap most or all of layer 4 and the overall density of BA binding shows a modest increase. Laminal labeling for BA is very diffuse compared to MS or FZ at all ages.

Quantitative changes with age

In order to establish that the binding sites labeled with ^3H -MS are indeed GABA_A receptors at the various ages, we have per-

formed displacement experiments on visual cortex sections from the F72d (Fig. 4A) and the P8.5yr cortex (Fig. 4B) using the ligands GABA, MS, bicuculline methiodide, and BA. At both ages, the GABA_A agonists GABA and MS and the GABA_A antagonist bicuculline were effective displacers of MS binding, whereas BA, a GABA_B agonist, had little effect. IC₅₀ values for the F72d striate cortex were, in order of potency, MS > GABA > bicuculline ≫ BA, and in the adult, were MS > GABA > bicuculline ≫ BA. These data are consistent with the view that ^3H -MS is selectively labeling a GABA_A receptor population at

Table 2. ^3H -MS binding site characterization

Age	B_{max} (fmol/mg protein)		Total [Total] ^a	% Adult ^b	Affinity (K_d in nM)		
	Low	High			Low	High	Single site fit
F72d	197 ± 29	108 ± 18	305 [148]	8% [4%]	26 ± 12	4 ± 1	7 ± 1
F119d	538 ± 103	164 ± 2	702 [336]	19% [10%]	67 ± 24	7 ± 0	19 ± 3
F126d	1046 ± 118	249 ± 42	1295 [844]	34% [24%]	34 ± 6	4 ± 1	21 ± 5
F152d	903 ± 85	602 ± 25	1505 [765]	40% [22%]	62 ± 17	22 ± 2	31 ± 4
P1d	1366 ± 119	183 ± 19	1549 [1134]	41% [33%]	70 ± 12	7 ± 1	50 ± 13
P1.5d	1825 ± 58	867 ± 57	2692 [1324]	71% [38%]	100 ± 7	17 ± 2	30 ± 5
P4wk	—	3011 ± 411	—	86%	—	30 ± 8	—
P7wk	—	3192 ± 407	—	92%	—	43 ± 8	—
P18wk	—	5869 ± 723	—	169%	—	65 ± 10	—
P34wk	—	4020 ± 629	—	116%	—	53 ± 10	—
P36wk	—	3948 ± 646	—	114%	—	60 ± 13	—
P58wk	—	2988 ± 336	—	86%	—	26 ± 5	—
P2.6yr	—	785 ± 125 ^c	—	—	—	41 ± 10	—
P3.3yr	—	4865 ± 503	—	140%	—	70 ± 10	—
P3.5yr	—	5065 ± 506	—	146%	—	38 ± 5	—
P8.5yr	—	4079 ± 273	} avg 3467	100%	—	30 ± 3	—
P11.3yr	—	2854 ± 261			—	49 ± 6	—

^a Numbers inside brackets indicate B_{max} values determined for a single site fit to the Eadie-Hofstee plots. Such determinations would underestimate B_{max} values for multiple binding sites.

^b Percent of average of two oldest animals B_{max} values determined for a single site fit to the Eadie-Hofstee plots. The numbers in brackets are % adult for B_{max} values determined for a single site as explained above.

^c Samples mistakenly included areas 17 and 18; not plotted in Figure 5.

both ages and that the same pharmacologic receptor profile is present in fetal and adult cortex.

In Figure 5 and Table 2, we give the results of ^3H -MS saturation binding assays to determine GABA_A receptor number (B_{max}). Figure 5A shows all developmental ages, and Figure 5B, up to P18wk only; specific numbers are given in Table 2. At F72d, B_{max} is at the lowest level of any age examined and is 8% of adult density, but less than 4% if a single site is assumed. B_{max} values increase steadily to an average of 56% (35% for a single site) of adult at P1d–1.5d and 86% at P4wk and reach an apparent peak at P18wk of 169% adult density. Values then decrease to 1 year (86%), rise again in the juvenile animals to an average of 143%, and finally decline to adult levels by P8.5yr or later. Thus GABA_A B_{max} has a bimodal developmental sequence. The close agreement of paired age animals supports that this is not due to individual variation.

In Figure 6, we show Eadie–Hofstee plots of saturation binding experiments to determine GABA_A receptor affinity (K_d) at F126d and P8.5yr. In the adult (Fig. 6A), the resulting graph shows a straight line with a K_d of 30 nM and a correlation coefficient of 0.96, but at F126d (Fig. 6B), the Eadie–Hofstee plot has a curvilinear shape with a correlation coefficient of 0.67, suggesting multiple sites. A curvilinear Eadie–Hofstee plot is not in itself indicative of multiple binding sites because poor-quality data, as well as multiple binding sites, will give large SD(erad) (SD of background error) values (Zivin and Waud, 1982). In such instances, Hill plots, as well as the use of a “limiting slopes” technique (Hunston, 1975), increase confidence that such curvilinear plots do comprise multiple binding sites. A Hill plot of the adult data (Fig. 6C) gives a coefficient of 1.03, indicating a single binding site. At F126d (Fig. 6D), the data have a Hill coefficient of greater than 1.42, and multiple site analysis reveals the presence of two binding sites, with K_d values of 34 nM and 4 nM. Data from F72d, F119d, F152d, and P1d also show two binding sites, but older animals all show a single binding site (Table 2). In Figure 6E, GABA_A receptor K_d is plotted using a best fit to a single binding site for all ages. K_d values peaked at P18wk, declined to P58wk, and then remained relatively constant into adulthood. One female at P3.3yr showed a high K_d value compared to all other postnatal animals. The SD(erad) value for this animal was acceptable, and the reason for this discrepant point is not clear. Affinity changes associated with the estrous cycle have been reported (Van Huizen et al., 1988), and this monkey would have been at or near the onset of puberty in our colony (G. Ruppenthal, personal communication). With the exception of this one point, the curve in Figure 6E is in good agreement with the development profile for receptor number (Fig. 5A). The affinity changes over the entire developmental period are of a smaller magnitude than the corresponding B_{max} changes. The presence of multiple binding sites before birth would raise the number of receptors at these ages (see Table 2), so the points are likely to be the minimum prenatal values for the number of GABA_A receptors. Our data indicate a shift near birth from multiple to single binding sites in the GABA_A receptor population, as well as a striking increase in receptor number at the same age.

Due to scarcity of tissue, benzodiazepine receptors were studied using ^3H -FZ binding at selected ages only. The resulting B_{max} values are F126d = 254 ± 16 , P1.5d = 1067 ± 60 , P18wk = 701 ± 58 , and P8.5yr = 946 ± 133 . K_d values are F126d = 2.9 ± 0.8 nM, P1.5d = 1.8 ± 0.2 nM, P18wk = 2.3 ± 0.2 nM, P8.5yr = 5.4 ± 1.1 nM, with only a single site detected at each age.

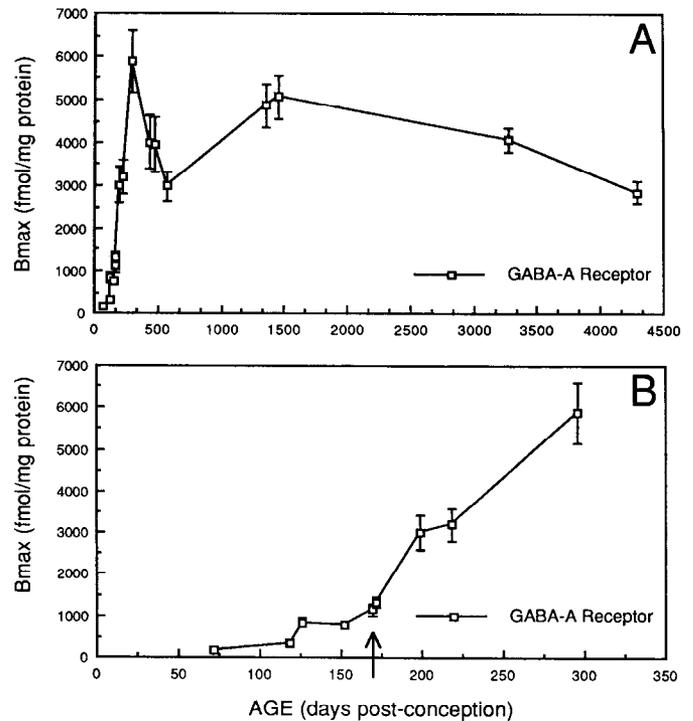


Figure 5. B_{max} determination for GABA_A receptors in macaque striate cortex during development. The number of GABA_A receptors (B_{max}) labeled with ^3H -MS was determined from scraped sections by saturation binding assays (see Materials and Methods) and expressed as fmol/mg protein. Section assays were carried out as described in Table 1, and the resulting data were analyzed using Eadie–Hofstee plots as described in Zivin and Waud (1982). In all cases, SD(erad) values were very good to acceptable, ranging from 0.07 to 0.16. In A, B_{max} values for F72d to P11.3yr are plotted. In B, the ages up to P18wk are shown on an expanded time scale. Birth is indicated by an arrow in B. In both graphs, error bars indicate SEM for values determined in one individual experiment only. Note the good agreement between animals at similar ages. The lines were fit by eye, although the quantitative determination of B_{max} and K_d values were obtained from the LIGAND binding program used to generate Eadie–Hofstee plots, which some authors consider to be more accurate, with B_{max} being determined by the y-axis intercept (Zivin and Waud, 1982). In ages with apparent multiple binding sites, we felt confident enough both in the range and number of concentrations and in the quality of the resulting data to attempt a “limiting slopes” method for calculating B_{max} and K_d values separately for each of two binding sites (see Hunston, 1975; Bylund, 1980). Such separately determined B_{max} and K_d values had SD(erad) values below 0.2, thus satisfying the criteria of Zivin and Waud (1982) for acceptable error.

Figure 7 shows a comparison of B_{max} values for ^3H -FZ and ^3H -MS binding at the selected ages for which both were determined. First, note that at all ages the absolute B_{max} value is at least three times higher for the GABA_A receptor (left ordinate) compared to the benzodiazepine receptor (right ordinate). Second, the two B_{max} values have different developmental profiles. There is a large prenatal increase in the benzodiazepine receptor from 27% of adult values at F126d to 74% at P1.5d, relative to a much smaller increase in the GABA_A receptor from 21% versus 33% over the same period. In contrast, although postnatal ^3H -FZ binding increases by P18wk to 113% of adult value, this increase is less than the 144% postnatal increase for ^3H -MS binding. Third, the B_{max} ratio of GABA_A to benzodiazepine is not constant with age. At F126d, it is 1:3 and drops to 1:2 at birth, but then the ratio rises to 1:6 at P18wk and levels out at 1:4 in the adult. This suggests that MS and FZ binding sites are

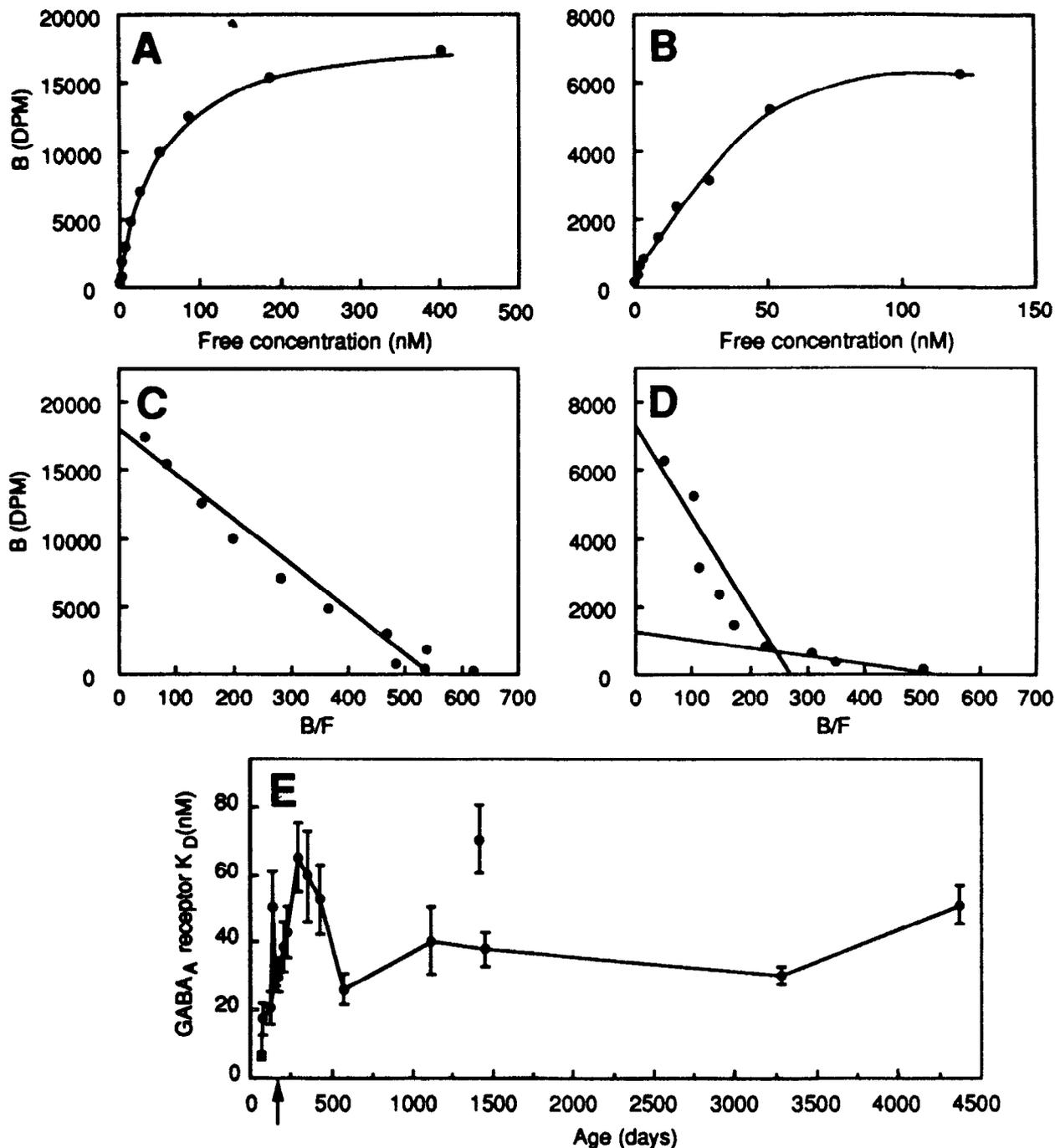


Figure 6. K_d determinations for GABA_A receptors in macaque striate cortex during development. Saturation binding assay for ³H-MS done as in Table 1 in the P8.5yr (A) and F126d (B) visual cortex, plotting specific binding in dpm versus free concentration of ³H-MS. In C and D, these data are replotted using Eadie–Hofstee methods. In the adult (C), the best fit of these data is a straight line, indicating a single ³H-MS binding site. The F126d data (D) shows a curvilinear shape, which is best fit by two straight lines, indicating the presence of a high- and a low-affinity binding site. E, K_d values plotted as a function of days postconception. For each animal, the best fit for a single binding site was made (see Materials and Methods). Error bars indicate individual experiment SEM based on multiple data points. Birth is marked by an arrow in E.

differentially altered during development. These ratio changes further suggest that the two GABA_A receptor components are not always linked in the same ratio and may not even share the same neuronal sites.

The percentage of nonspecific to total binding at all ages was low for both ligands. For ³H-MS, the highest nonspecific binding of 26% was found at F72d and was only 8% in the adult. Con-

versely, ³H-FZ nonspecific binding was 2% at F126d, increased to 11% at P18wk, and was 17% in the adult. This relatively low level at all ages justifies our conclusions of high specific binding during development, and supports the qualitative autoradiographic film pictures discussed earlier.

Quantitative densitometric analysis of layers 4C and 2/3 from films at F126d to adult gave a slightly more complex picture of

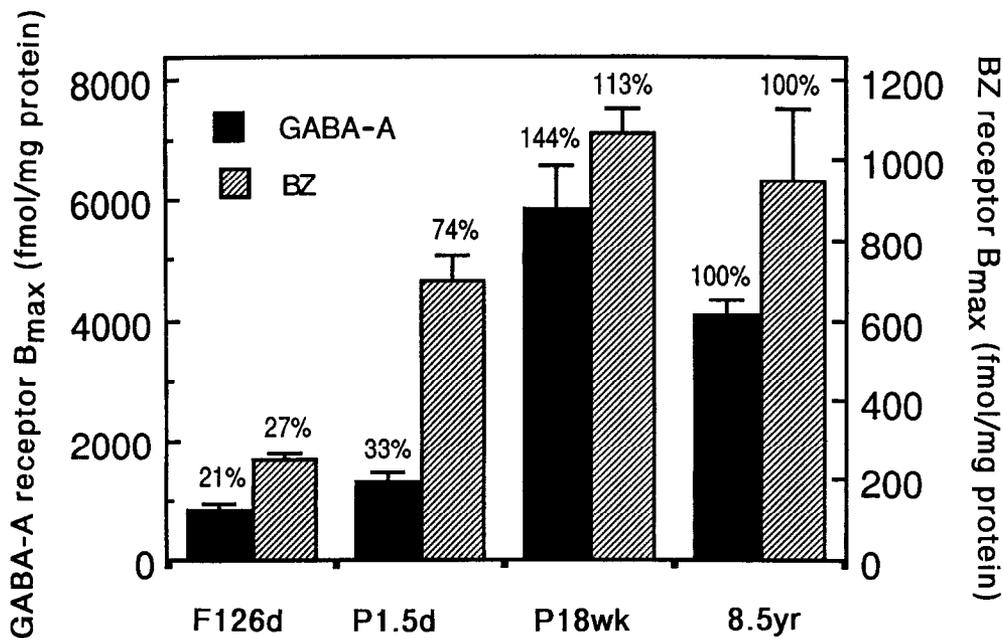


Figure 7. Comparison of B_{\max} values for MS and FZ binding sites at selected ages in macaque striate cortex. Saturation binding receptor assays were performed as described in Table 1. The number of fmol/mg protein was determined from scraped sections, and Eadie-Hofstee analysis was used to determine B_{\max} values as described in Materials and Methods and Figure 5. The left y-axis gives B_{\max} values for binding site receptors, and the right y-axis gives B_{\max} values of FZ binding sites. Note that GABA_A receptors have a maximum about four times higher than FZ receptors. The percentage values above the bars are percent adult B_{\max} values. SD(erad) values ranged from 0.08 to 0.15.

GABA_A receptor development. The absolute values are given in Table 3, and the percent highest value for each layer is plotted in Figure 8. MS binding sites are quite sparse before birth, but layer 4C still is 30% higher than the upper cortical layers. Overall, MS binding values increase after birth, but layer 4C always has the highest concentration, although the difference is small at P49d and P18wk. When MS binding site density is plotted as a percentage of its highest value for each layer (Fig. 8A), it can be seen that layer 2/3 actually overshoots layer 4C after birth and then declines somewhat by adulthood. The MS binding site density in layer 4C continues to rise steadily after birth to adulthood. FZ binding site density shows relatively little difference between layers 4C and 2/3 before birth, and then a larger separation appears at P49d and P18wk when layer 4C has twice the density of layer 2/3. When FZ laminar density is plotted for percentage of its highest value (Fig. 8B), layer 4C actually peaks at P49d and then declines sharply to P58wk. Layer 2/3 rises steadily after birth to P18wk and changes little thereafter. In Figure 8C, we plot the ratios of MS and FZ binding site density in layers 2/3 compared to layer 4C. Notice that the two ligands show opposite patterns in that just after birth, MS binding is relatively enriched in the upper layers while layer 4 is relatively enriched for FZ binding. In older animals, the opposite relationship was found.

Discussion

There are several notable results in this study of the development of GABA_A and GABA_B receptors in monkey visual cortex: (1) Significant levels of MS and FZ binding sites are found at F61d–72d, more than 3 months before birth. (2) There is a direct correlation between prenatal age and number of MS binding sites. The youngest cortex had the lowest number, followed by a steady increase up to birth, a rapid increase in the 4 months after birth, and a long decline into adulthood. (3) FZ binding site number is relatively high before birth, shows a small increase in the 4 months after birth, but declines relatively little into adulthood. (4) These two patterns are reflected in a ratio of FZ to MS binding site number, which is 1:2–3 before birth but rises

to 1:4–6 after birth. (5) Laminar patterns for the two ligands differ during development. MS density in layer 4C increases up to P13mo but plateaus in layer 2/3 by P18wk. FZ density in layer 4C drops sharply after P49d, while layer 2/3 remains high into adulthood. (6) GABA_B binding was present before birth in all layers and showed little change with age for either density or laminar distribution.

An unexpected result in this study is the presence of significant levels of both MS and FZ binding sites in the cortical plate early in development. Our saturation binding analysis has shown that at F72d, binding site number is 4–8% of adult levels. Many cortical plate neurons also are immunocytochemically stained for GABA by F60d–72d (Hendrickson et al., 1988; Meinicke and Rakic, 1989). In contrast, at F72d synaptic contacts are

Table 3. Densitometry of laminar receptor binding

Age	³ H-MS		³ H-FZ	
	Layer 4C	Layer 2/3 ^a	Layer 4C	Layer 2/3 ^a
F126d	0.9	0.6	—	—
F152d	2.6	1.6	6.2	5.8
P1.5d	8.2	5.1	9.5	9.1
P4wk	14.4	11.2	12.3	10.2
P7wk	13.5	11.9	25.3	12.4
P18wk	15.8	13.9	23.1	14.3
P58wk	19	13.8	15.8	14.5
P3.5yr	19	12.4	19	15.9

Peak densities for receptor binding in the different layers were determined by calibration to measured film values of Amersham ³H-Microscales in the range of actual polymer activity (nCi/mg) from 3.03 to 109.08 nCi/mg. Because K_d values are age dependent and the measured density values were determined from film autoradiograms of binding experiments using a single concentration of radioligand, these values do not necessarily represent receptor B_{\max} for that layer at each age. Further, from the values given in the table, it is not possible to compare actual receptor density for MS versus FZ binding sites since the values given are in relative units. For overall B_{\max} values see Figures 5 and 7 and Table 2.

^a Measurements for layers 2/3 include a variable amount of layer 4A that could not be separated in the films from layer 3.

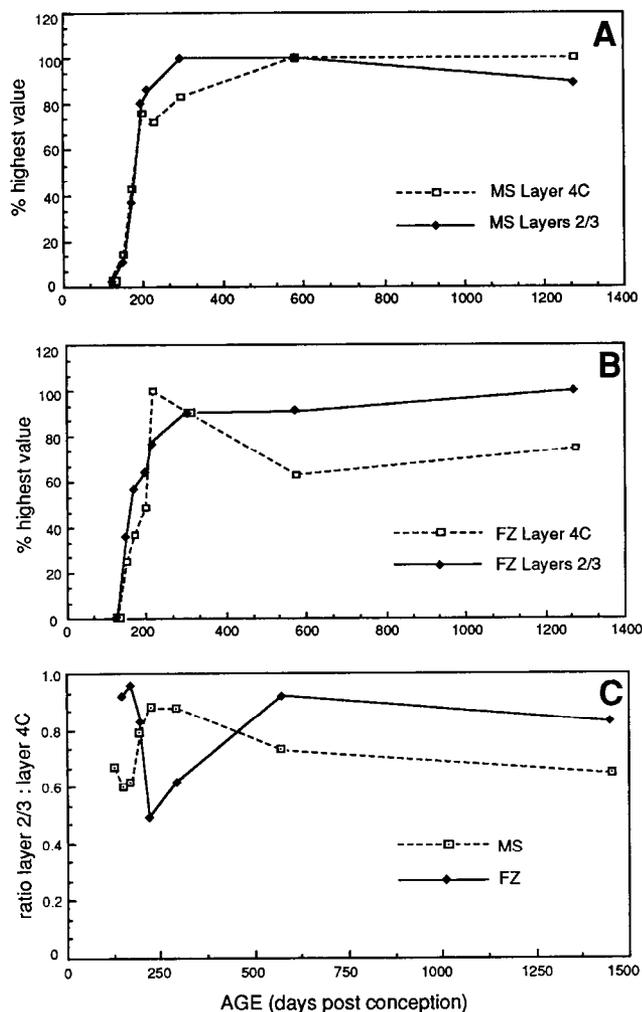


Figure 8. Plots of the change in binding site density with age in layer 4C and layers 2/3 of macaque striate cortex for $^3\text{H-MS}$ (A) and $^3\text{H-FZ}$ (B). Binding site density was determined from autoradiograms films using an Imaging Research Inc. system (see Materials and Methods and values in Table 3). The percent highest value in layer 4C and layers 2/3 is plotted for each age analyzed. C, The density in layers 2/3 is compared to density in layer 4C, and this ratio is plotted for MS and FZ. Age is given in days postconception (approximately 165–170 d).

virtually absent from the cortical plate, although a very few are found in the marginal zone and subplate (Rakic et al., 1986; Zielinski and Hendrickson, 1990). By F72d, the axons from the dorsal lateral geniculate nucleus have reached the deep intermediate zone but still have not penetrated the cortex in any numbers (Rakic, 1977). Thus, both the MS and FZ binding sites of the GABA_A receptor and GABAergic neurons are clearly present well before most synaptic development begins in either intrinsic or thalamic axon systems. Film-based, receptor-binding autoradiography does not allow cellular resolution, so light microscopic and EM immunocytochemical methods currently are being applied to identify exactly where these early GABA_A receptors are located.

Although both FZ and MS labeling are coincident with the cortical plate, FZ binding sites are also present over the subplate/intermediate zone and in high amounts over the marginal zone in early prenatal cortex. As well, overall FZ binding site numbers are relatively high prenatally compared to MS binding site num-

bers. One explanation of this discrepancy is that benzodiazepine receptors may exist that are not coupled to GABA_A receptors (Unnerstall et al., 1981; Richards and Mohler, 1984; Shaw et al., 1987). However, recent molecular biology analysis of the GABA_A receptor has revealed an unexpected structural complexity in that it is composed of four types of subunits, each subunit has several different forms, and the subunits can occur in different combinations (Kuman et al., 1987; Sieghart, 1989; Malherbe et al., 1990; Olsen and Tobin, 1990). In immature rodent cortex, protein gels and immunoprecipitation studies indicate several different but closely related proteins are found that bind $^3\text{H-FZ}$, while in the adult only a single protein is found (Eichinger and Sieghart, 1986; Sato and Neale, 1989a,b; Vitorica et al., 1990). Moreover, electrophysiological recording of cortical slices *in vitro* has found that most GABA_A receptors mediate depolarization in neonatal rat cortex, but this changes to a preponderance of hyperpolarization responses as the cortex matures (Kriegstein et al., 1987; McCormick and Prince, 1987; Scharfman and Sarvey, 1987; Luhmann and Prince, 1991). Thus, during development there may be a shift in the subunit type and composition of the GABA_A receptor, giving rise to different receptor characteristics that may modify not only FZ and MS binding, but GABA_A receptor function as well. Our results in monkey are in keeping with this view, showing the existence of at least two different $^3\text{H-MS}$ binding sites early in development, but only one after birth. There is also an age-related change in affinity in monkey cortex, with the lowest affinity (highest K_d) coincident with the highest number of MS binding sites at P18wk. These changes are similar to GABA_A receptor affinity/number changes in cat visual cortex during postnatal development (Shaw et al., 1984, 1987). Although our quantitative analysis of FZ binding sites did not include all prenatal ages because of the scarcity of tissue, our K_d values suggest a decrease in affinity with age, also similar to cat visual cortex (Shaw et al., 1987). Thus, monkey striate cortex GABA_A receptors go through a stage shortly after birth in which a larger number of receptors exist that bind GABA relatively easily at low concentration (low affinity). With age the number of receptors decreases and the specificity of binding increases; both changes suggest that maturation involves the emergence of highly selective GABA binding at developmentally selected postsynaptic sites.

How does our monkey data compare with GABA receptor development in other species? Several problems arise when attempting such a cross-species comparison. The first is the difficulty of correcting for differing gestation lengths and maturity at birth. Rat cortical development at birth is equal to that of human at embryonic (E)20wk, which is 50% of human gestation (Kellogg, 1988). This allows an equivalent prenatal scale to be constructed to correct for different lengths of gestation and different degrees of maturity at birth in rat, monkey, and human, if we assume that monkey and human cortical development is equal at birth (Boothe et al., 1985; Boothe, 1988). In the following discussion, we have made these calculations and the percentages given are for "corrected gestation." Second, many studies use whole brain, rather than cerebral cortex, and of those using cortex, few sample only visual cortex. Given the very different rates of regional development within the brain and the known regional receptor heterogeneity, this limits direct numerical comparisons. Finally, few laboratories have studied more than one receptor, or more than one species, so interlaboratory variability in methods can be a problem. Given these limitations, we can point out the following comparisons of our monkey

developmental data for MS and FZ binding sites with that available from rat, cat, and human studies.

FZ binding sites detected by ligand binding appear at similar prenatal ages; in rat frontal cortex at 32% of gestation (Schlumpf et al., 1983), in human at 37% (Aaltonen et al., 1983), and in this study at 37%, both FZ and MS binding sites are present in monkey visual cortex. The first GABAergic neurons are found in marginal and subplate/intermediate zones of rat frontal cortex at 32% of gestation and in cortical plate at 36% (Lauder et al., 1986; Van Eden et al., 1989). Numerous GABA⁺ cells are present in all these areas by F61d in monkey (Hendrickson et al., 1988; Meinicke and Rakic, 1989), which is 37% of gestation. Although monkey fetuses need to be examined at even earlier ages, these data in both rat and monkey suggest that GABAergic neurons appear about the same time as GABA_A receptors. GABA_A receptors detected immunocytochemically by one antiserum do not appear until 75% of gestation in monkey visual cortex (Meinicke and Rakic, 1989) or at 71% in somatosensory cortex using another antiserum (Huntley et al., 1990). The reason for this late immunocytochemical appearance is not clear and needs to be examined with other antisera to the wide variety of GABA_A receptor subunits that have been recently discovered (reviewed by Kuman et al., 1987; Sieghart, 1989; Malherbe et al., 1990; Olsen and Tobin, 1990).

Synaptic development occurs much later than GABA_A receptor appearance in both rat and monkey. At 44% gestation in monkey, synaptic density in striate cortex is almost nil (Rakic et al., 1986; Zielinski and Hendrickson, 1990). Even at 68% gestation, the upper cortical layers have few synapses but are well endowed with FZ binding sites and, to a lesser degree, with MS binding sites (see below). In rat occipital cortex, synaptogenesis does not begin until after birth (Blue and Parnavalas, 1983) but both FZ and MS binding sites are detectable well before birth (Coyle and Enna, 1976; Braestrup and Nielsen, 1978), although Schlumpf et al. (1983) point out that the cortical plate does not contain FZ binding sites until F21. E16d rat cortical neurons cultured *in vitro* have functional GABA_A receptors in the total absence of synapses (Köller et al., 1990), and it is interesting that in this *in vitro* system GABA_A receptors appear 4 d earlier than the first functional glutamate receptors. Human cortex has 6% of adult FZ labeling by 38% gestation (Aaltonen et al., 1983), well before synapses are described (Boothe, 1988). Thus, both rats and primates show similar developmental sequences, with GABA_A receptors appearing earlier than morphological synapses.

Our monkey data show a rapid postnatal increase of GABA_A receptors to a peak around P18wk, which is particularly clear for MS binding sites but also is true to a lesser degree for FZ binding sites. Both eventually decline by adulthood. In rat brain, FZ binding sites reach 35–50% of adult levels by birth and adult levels by P4wk–5wk (Braestrup and Nielsen, 1978; Palacios et al., 1979; Aldinio et al., 1981). The only rat study to compare FZ and MS binding directly (Palacios et al., 1979) found that MS levels are less than 10% of adult at birth, which is significantly lower than FZ levels. MS binding then shows a rapid postnatal rise to also reach adult levels by P4wk. Aldinio et al. (1981) and Eichinger and Sieghart (1986) also find a postnatal decline for FZ binding in rat; Aldinio et al. (1981) report a peak at P35d followed by a 15% decrease to P12wk, while the latter authors find a peak at P22d and a similar decline. Most rodent studies utilize P4wk–6wk animals as “adult,” so it is possible that the failure to extend the age range may have caused others

to miss a postnatal peak and subsequent decline. The cat visual cortex is quite immature at birth and is more similar to rat than to monkey in this regard. At P3d in cat striate cortex, FZ binding levels are 27% (Shaw et al., 1987) and MS 59% (Shaw et al., 1984) of adult. FZ binding site numbers peak at P8wk, and MS binding site numbers peak at P14wk, followed by a decline of about 30% to adulthood for both. Human frontal cortex has 47% of adult MS binding and 22% of adult FZ binding near birth (Brooksbank et al., 1981, 1982), with no postnatal decline noted. The Brooksbank et al. perinatal group was composed of premature plus full-term infants, and given the rapid rise in both MS and FZ labeling that we found in monkeys in the latter part of gestation, mixing these critical ages could lower their final number and mask a peak. Thus, monkeys, cats, and perhaps rats show a postnatal peak followed by a decline in one or both GABA_A receptor binding sites.

Is there any correlation with synaptic density changes and GABA_A receptor number in monkey striate cortex? To test this, we plotted synaptic density data from Zielinski and Hendrickson (1990) against our B_{max} data for MS binding sites (Fig. 9A). Both curves show a similar overall shape, a peak density around 3 months, and a long decline. In early development (Fig. 9B), MS binding site numbers lead synaptic density, as would be predicted from the species comparisons discussed above. By F126d in macaque striate cortex, all neurons are in place, all of the layers can be recognized, and LGN axons are present in layer 4 (Rakic, 1977). Synapses are found in all layers, although still at a relatively low density of 15% adult levels. By F152d, ocular dominance columns are beginning to appear (Rakic, 1977), and many synapses are found in all layers of cortex with a density of 64% of adult. MS binding site numbers now lag behind synaptic density, being only 22% of adult levels. After birth, both synaptic density and MS binding site numbers rise very rapidly to an apparent peak of 160% adult at P12wk for synaptic density and 169% adult at P18wk for MS binding site number. Both decline to reach approximate adult levels by P1yr–2yr, although MS binding site number shows a second late peak around P3.5yr. This time point was not included in our study, but the data of Rakic et al. (1986) do not show a second peak at this time for synaptic development.

Although the correlation in shape and peak density between MS binding sites and synaptic density is intriguing, this should be treated with caution. Because GABAergic synapses are no more than 25% of the total average density (V. LaVie and A. Hendrickson, unpublished observations), they may have a different developmental profile that is masked by the larger number of asymmetric contacts. On the basis of morphological criteria, Zecevic et al. (1989) found that symmetric contacts, presumably GABAergic, constitute a relatively constant <10% throughout monkey motor cortex development and do not show either a peak after birth or a massive decline postnatally. If true for monkey striate cortex, this would mean that there is little correlation between GABAergic synaptic number and receptor number. On the other hand, Blue and Parnavalas (1983) found a dramatic postnatal increase in symmetric contacts in rat striate cortex followed by a marked decline between P20d–90d. This is similar to our overall synaptic density curve and the rat cortex FZ and MS binding studies discussed earlier (Braestrup and Nielson, 1978; Palacios et al., 1979; Aldinio et al., 1981; Eichinger and Sieghart, 1986). We have found that EM morphological criteria fail to differentiate asymmetric from symmetric synapses in more than 80% of prenatal synaptic contacts (Zie-

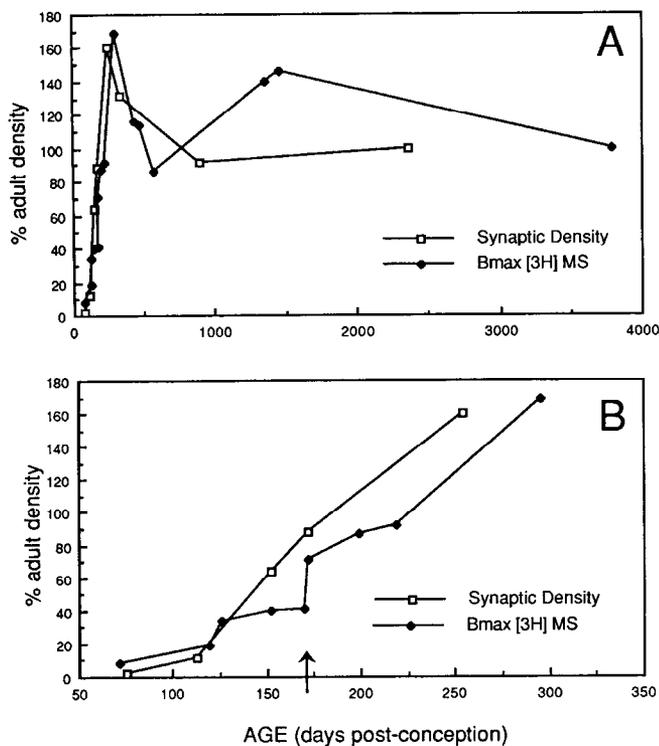


Figure 9. A comparison of age-related changes between the number of ^3H -MS binding sites and the average synaptic density in macaque striate cortex. Each point represents one animal whose receptor number (B_{\max} ^3H -MS) or synaptic density averaged for all cortical layers was determined. Values are plotted as percentages of adult values. For synaptic counts, all animals were perfused with 4% paraformaldehyde, 0.05% glutaraldehyde, 0.2% picric acid in 0.1 M phosphate buffer, pH 7.3. Blocks of opercular striate cortex over the calcarine sulcus were postfixed in 2% osmium tetroxide and embedded in plastic. Thin sections were cut that included all layers of the cortical plate or cortex, and EM photographic montages were made at a final magnification of 24,000 \times . Synapses (defined as three or more vesicles in the presynaptic process opposite an obvious postsynaptic density) were counted and the amount of neuropil determined using a grid overlay in every other photograph of the montage. Synaptic density is expressed as synapses/100 μm^2 neuropil. *A* covers the entire age range from F72d to adult, and *B* is an expanded view of F72d to P18wk (birth is marked by an arrow).

linski and Hendrickson, 1990), and so studies using EM immunogold to label GABA synapses are currently under way to detect directly this population and allow more accurate counts of its development in monkey visual cortex.

What might be the role for GABAergic nonsynaptic interactions early in development? One possibility is that GABA and its receptors may serve a mitogenic or growth-related role in early neural development (reviewed in Spoerri, 1987; Wolff et al., 1987; Lauder, 1988; Belhage et al., 1990). These studies have shown that neurons *in vivo* and *in vitro* respond to the application of GABA by enhanced neurite outgrowth and branching, accelerated organelle development, synaptogenesis, or increased protein synthesis. In some of these studies, this action is blocked by the GABA_A antagonist bicuculline, indicating that the transmitter/receptor interactions are involved. Recently, it has been shown in adult monkey cerebellum that both MS and FZ binding sites as detected by subunit specific antibodies are found on membrane surfaces of postsynaptic cell bodies and dendrites, as well as at symmetric synaptic contacts (Somogyi, 1989; Somogyi et al., 1989). This suggests that GABA

acting via GABA_A receptors may subserve a modulatory role in addition to neurotransmission, even in adult neurons. Another possibility is that GABA_A receptors may serve as targets for incoming axons, particularly because some growth cones release GABA (Gordon-Weeks et al., 1984). Subsequent interactions with the GABA receptor could act to guide the axon to its correct target and increase the likelihood of synaptic formation.

A third role for GABA, particularly in the postnatal period, may be in acting via GABA_A receptors to interact with other neurotransmitter receptors in order to control rates of neuronal differentiation or to enhance specific axon pathway synaptogenesis. Evidence has accumulated recently that the NMDA glutamate receptor subtype is of primary importance in activity-dependent segregation of geniculate afferents in cat striate cortex that occurs between P2wk and P6wk (Tsumoto et al., 1986; Kleinschmidt et al., 1987; Rauschecker and Hahn, 1987; Fox et al., 1989). If one eyelid is closed during this period, most neurons in cat layer 4 are driven by the open eye, but if NMDA receptors are blocked at the time the eyelid is closed, no such shift takes place (Kleinschmidt et al., 1987; Rauschecker and Hahn, 1987). The NMDA receptor is voltage gated and is rendered ineffective if the neuron is hyperpolarized by GABA (Mayer et al., 1984), so GABA could exert an indirect developmental effect by setting the activity level of the neuron carrying the NMDA receptor. Application of MS *in vivo* to visual cortex not only prevents the expected ocular dominance shift to the open eye when one eyelid is closed but causes a shift in favor of the closed eye (Reiter and Stryker, 1988). This suggests that GABA and/or postsynaptic activity could have some direct action during these developmental interactions, in addition to affecting NMDA receptors indirectly.

The computation of FZ:MS binding site ratios in different species suggests an evolutionary shift away from FZ binding sites and toward MS binding sites in mammalian cerebral cortex. We found that the monkey ratio is 1:2–3 before birth and then rises to 1:4–6 after birth. In human frontal cortex, MS binding also predominates throughout life, with a ratio of 1:2 in fetuses and adults, and a peak of 1:4 just before and after birth (Brooksbank et al., 1981, 1982). In adult rat whole brain assays, FZ binding sites dominate those for MS with a ratio of 2:1 (Palacios et al., 1979) and 4:1 in adult rat neocortex (Shaw and Scarth, 1991). Cat visual cortex is intermediate with a ratio of 1.5:1 at both P30d and P1yr (Shaw et al., 1987). Our B_{\max} studies in monkey show that in early striate cortex development the FZ:MS binding site ratio is at its highest. If these FZ binding sites are linked to GABA_A receptors, this suggests greater modulation of GABA binding at early developmental stages. If they are not linked, this suggests the occurrence of a prenatal receptor population that might have a very different function from the classic GABA_A receptor. Protein analysis and binding of benzodiazepine ligands with different specificities have shown the presence of several peptides in immature rat cortex, but only a single peptide in adult, that bind or are precipitated by ^3H -FZ (Eichinger and Sieghart, 1986; Sato and Neale, 1989a,b; Vitorica et al., 1990). These results also suggest a shift during cortical development from multiple to single forms of the GABA_A receptor.

Quantitative analysis of film autoradiographs found no changes in MS binding over the first P18wk that could be related to monkey critical period visual developmental changes in layer 4C (Blakemore et al., 1978; LeVay et al., 1980; Wiesel, 1982).

MS labeling increased rapidly over this period in both layers 4C and 2/3, and showed no "patchy" patterns. On the other hand, FZ density in layer 4C showed a peak at P49d and then sharply declined, coincident with the end of the most critical period for ocular dominance development. What role, if any, the FZ binding site plays in layer 4C thalamocortical interactions remains to be determined. We did note several interesting differences in peak binding measured in layers 2/3 or layer 4 versus whole cortex determinations. First, MS binding in whole cortex shows a greater decline in adulthood than in either layers 2/3 or layer 4. This probably is due to the marked loss of MS binding sites in infragranular layers seen in the film autoradiographs. FZ binding sites in whole cortex show a slight decline in adulthood that may be accounted for by a marked decline in layer 4C coincident with an increase in layers 2/3. Second, we note that the ratios of MS and FZ binding in layers 2/3 versus layer 4 change in a reciprocal fashion during development; for instance, between P7wk and P18wk, the highest ratio for MS is coincident with the lowest for FZ. This suggests very different changes in receptor subtypes in the same layer at the same age that may be due to developmental modifications of the receptor subunit composition (Sieghart, 1989; Olsen and Tobin, 1990; Vitorica et al., 1990).

In this article, we have presented the first complete developmental sequence for GABA_A and GABA_B receptors in monkey striate cortex. MS and FZ binding sites appear early in development, show a high level in layer 4 as soon as it is formed, and have an adult laminar distribution shortly after birth. BA binding sites are present by midgestation, become slightly lighter in layer 4 with age, and do not change their laminar distribution throughout development. GABA also appears in neurons at the same time that MS and FZ binding sites are detected, but before synapses are present in significant numbers. This suggests a role for GABA and GABA_A receptors that is not linked to synaptic activity in the initial stages of cortical development. In later prenatal and in postnatal development, GABA_A receptor numbers closely follow synaptic density changes, suggesting a closer link to neurotransmission.

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