GABA_\text{A} Receptors Reorganize when Layer 4 in Ferret Somatosensory Cortex is Disrupted by Methylazoxymethanol (MAM)

We established a model of cortical development that arrests the birth of layer 4 cells by injecting methylazoxymethanol (MAM) on embryonic day 33 (E33) in ferrets. This leads to adult somatosensory cortex with a very thin layer 4. Earlier, we determined the relative absence of layer 4 changed the growth and differentiation of the somatosensory cortex and the growth of thalamic afferents into the cortical plate. To identify other features of cortical organization that might be altered after MAM treatment, we assessed the distribution of selected excitatory and inhibitory receptors in area 3b of ferret somatosensory cortex. Initial screening revealed the distribution of several excitatory receptors (NMDA, AMPA, kainate) in E33 MAM-treated cortex was similar to that in normal adult animals. In contrast, the binding pattern of inhibitory GABA_\text{A} receptors was altered in MAM-treated cortex. Normally, GABA_\text{A} receptors densely locate in central layers of cortex. In E33 MAM-treated animals, GABA_\text{A} receptor binding extended superficially, covering a broader area of cortex. Further experiments using antibodies directed against GABA_\text{A}_\alpha subunit receptors disclosed that pan GABA_\text{A} receptors strongly localize to layer 4 in normal area 3b. In E33 MAM-treated cortex, however, GABA_\text{A} receptors extend outside and are located above and below the very thin layer 4. The redistribution of inhibitory receptors suggests that layer 4 plays an important role in regulating thalamic terminations and also in the resulting ability to refine processing of incoming stimuli.

Keywords: cerebral cortex, development, GABA_\text{A}_\alpha subunit, glutamate receptors, MAM, receptor binding, thalamus

Introduction

The pathological mechanisms leading to improper migration of cortical neurons and abnormal formation of cortical layers may cause significant functional alterations. We developed a cortical malformation model of epigenetic origin using an antimitotic agent that interrupts normal development of cortical layers (Noctor et al., 1997, 2001). Methylazoxymethanol (MAM) injected into pregnant ferrets prevents mitosis for a short time. When injected on a single day of corticogenesis it produces highly specific deficits in the normal laminar pattern of the neocortex in newborn ferrets (Cattaneo et al., 1995; Noctor et al., 1997, 2001; Palmer et al., 2001). Noctor et al. (2001) established that injection of MAM on embryonic day 33 (E33) in ferrets interferes with neuronal proliferation and results in a reduction of cortical thickness, especially due to a very thin layer 4. Although many features of cortical organization appear relatively normal after this treatment, such as the somatotopic organization and the dimension and cell size of the remaining cortical layers, the distribution of thalamic afferent terminations is severely disrupted in E33 MAM-treated ferret somatosensory cortex (Palmer et al., 2001). The abnormal thalamic terminations suggest that processes of information transmission are impaired throughout cortical layers.

Because of the likely involvement of excitatory and inhibitory receptors in thalamocortical transmission, we examined whether E35MAM treatment also affected the distribution of markers of excitatory and inhibitory systems in cerebral cortex. The distribution of specific receptors in somatosensory cortex is very similar in many adult species. They concentrate in distinct cortical layers with specific patterns, depending on the receptor (Insel et al., 1990; Chalmers and McCulloch, 1991; Jaarsma et al., 1991; Young et al., 1991; Kumar et al., 1994; Glazewski et al., 1995; Gordon et al., 1996). The precise arrangement of excitatory and inhibitory receptors in cerebral cortex suggests that their specific distribution in cortical laminae may play important roles in the processing of information in each layer and the flow of information through the cortex.

To define whether disruption of layer 4, the main target of thalamic input, alters the distribution of receptors involved in transmission of cortical sensory information, we evaluated the laminar distribution of NMDA, AMPA, kainate and GABA_\text{A} receptors. We compared this distribution in the somatosensory cortex of normal and MAM-treated ferrets. Although the distribution of excitatory receptors did not vary substantially between normal and MAM-treated animals, the pattern of GABA_\text{A} receptors was altered in MAM-treated somatosensory cortex and extended outside of layer 4, especially in the upper layers. Our results imply that MAM treatment at embryonic day 33 alters the distribution of inhibitory receptors in somatosensory cortex, leaving excitatory markers unchanged.

Materials and Methods

MAM Treatment and Tissue Preparation

Adult ferrets were obtained from Marshall Farms (New Rose, NY). At E33, pregnant ferrets were anesthetized with 2% halothane and received an i.p. injection of MAM (12 mg/kg). Each animal was monitored carefully until it recovered, when it was returned to its cage. Both normal (n = 12) and MAM-treated (n = 13) animals were used either at various postnatal ages [postnatal day 0 (P0), P14, P28] for immunocytochemistry studies or at P36 for in vitro receptor binding autoradiography. For immunohistochemical experiments, ferrets were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and perfused with normal saline followed by 4% paraformaldehyde with 4% sucrose in 0.1 M sodium phosphate buffer (pH 7.4) at room temperature. The brains were immediately removed, cryoprotected in 20% sucrose overnight at 4°C, then frozen in isopentane and kept in a −80°C freezer. The brains were cut at 30 μm thickness sections on a cryostat. For the animals used in receptor binding studies, each ferret was anesthetized with pentobarbital (50 mg/kg, i.p.) and when insensitive to pain, the brain removed and quickly frozen in isopentane at −35°C. Alternate sections through the somatosensory cortex were cut and saved for Nissl staining or autoradiography.
In Vitro Receptor Binding Autoradiography

Steady-state binding assays were performed by incubating the sections (taken from P36 animals) with the appropriate radioligand to identify NMDA receptors (\(^{[3]H}\)MK-801), kainate receptors (\(^{[3]H}\)kainic acid), AMPA receptors (\(^{[3]H}\)fluorowillardine), or GABA\(_{A}\) receptors (\(^{[3]H}\)musccimol). NMDA and kainate receptor binding were performed as previously described (Jablonska et al., 1996, 1998). Prior to incubation with the appropriate ligand the brain sections were washed with a specific incubation buffer (see below) at 4°C to remove endogenous ligands, for either 10 min with \(^{[3]}\)H-MK-801 binding and \(^{[3]}\)H-musccimol binding or 10 min × 3 for \(^{[3]}\)H-fluorowillardine and \(^{[3]}\)H-kainate binding. After this, the sections used to identify NMDA receptors were incubated with 3 nM \(^{[3]}\)H-MK-801 (32.75 Ci/mmol, Tocris), 5 \(\mu\)M glycine, 50 \(\mu\)M spermidine in 50 mM Tris-HCl buffer (pH 7.4, 20°C) for 1 h. Non-specific binding was determined in the presence of 100 \(\mu\)M MK801. The slides were subsequently rinsed three times with ice-cold Tris-HCl (pH 7.4) for 15 s, twice in ice-cold water (5 s) and finally rapidly dipped in two changes of acetone containing 2.5% glutaraldehyde for 15 s. Kainate receptors were labeled with 5 nM \(^{[3]}\)H-kainate (58 Ci/mmol, Tocris) in 50 mM Tris-acetate (pH 7.0, 4°C) for 30 min. Non-specific binding was defined in the presence of 100 \(\mu\)M unlabelled kainate. Following incubation, the sections were washed three times in ice-cold buffer (50 mM Tris-acetate (pH 7.4) and once in ice-cold water over a total wash time of 5 s. Then the slides were dried in a stream of cold air. For the AMPA and GABA\(_{A}\) receptor binding assays, sections were incubated in 20 mM \(^{[3]}\)H-fluorowillardine (29.08 Ci/mmol, Tocris) in 50 mM Tris-HCl buffer (pH 7.4, 4°C) for 40 min or with 20 nM \(^{[3]}\)H-musccimol (29.50 Ci/mmol, NEN) in the same buffer for 60 min. Non-specific binding was determined in the presence of 0.1 mM fluorowillardine or 0.1 mM musccimol. After incubation with the ligand, the tissue sections were washed in the incubation buffer alone for a total time of either 5 s for \(^{[3]}\)H fluorowillardine binding, or 2 min for \(^{[3]}\)H-musccimol binding, before being blown dry in a stream of cold, dry air. For each radioligand, non-specific binding never exceeded 1% of total binding.

Quantitative Analysis of Autoradiographs

Once the sections were completely dry they were exposed to \(^{[3]}\)H-Hyperfilm (Amersham, UK), together with radioactive standards (\(^{[3]}\)H microscales, Amersham, UK) in X-ray cassettes for 4 weeks for \(^{[3]}\)H-MK801, \(^{[3]}\)H-fluorowillardine and \(^{[3]}\)H-musccimol binding and 3 months for \(^{[3]}\)H-kainate binding. Quantitative analysis of the autoradiographs was performed using a computer-assisted optical densitometry system (Image-Pro Plus; MediaCybernetics, Carlsbad, CA). The cortical layers were identified in adjacent Nissl-stained sections. Optical density measurements were taken through each cortical layer in the hand region of area 3b. Samplings of each layer were obtained from 5–11 different sections using at least three different animals from each condition and the arithmetic mean (±SEM) was calculated from all readings. The concentration of the bound radioligand was calculated from a standard curve created by using the \(^{[3]}\)H microscale references. Receptor density was expressed in femtomoles of specifically bound ligand per milligram of tissue weight (fmol/mg tissue). Differences in binding in each layer between normal and E33 MAM-treated animals were analyzed using two-way t-test.

Immunocytochemistry

To localize GABA\(_{A}\) receptors in somatosensory cortex we applied an antibody directed against all a subunits of GABA\(_{A}\) receptors (mouse anti-GABA\(_{A}\) Receptor, \(\alpha\)-chain monoclonal antibody; Chemicon). To quench endogenous peroxidase, sections were preincubated in Tris-HCl buffer (pH 7.4) with 5% normal horse serum (NHS), 0.3% TritonX-100, 1% H\(_2\)O\(_2\) and 10% methanol for 1 h. To block non-specific binding the slides were transferred to the Tris-HCl buffer (pH 7.4) as above without hydrogen peroxide for 20 min at room temperature. After overnight incubation with the primary antibody to GABA\(_{A}\) receptor (1:10) sections were washed (3 × 10 min in buffer) and then incubated with the secondary antibody (Vectorstain Elite ABC kit). The GABA\(_{A}\) staining was visualized using a Vector VIP peroxidase substrate kit. The sections were then mounted on subbed slides and coverslipped. Each experiment included control sections, processed with omission of the primary antibody.

Analysis of Immunoreactivity

The immuno- and Nissl-stained sections were analyzed using a computerized image acquisition and analysis system. Each image was spatially calibrated using an objective scale. To measure the distribution of GABA\(_{A}\) label in sections of normal and MAM-treated animals, profiles of optical density across the cortical layers (from pia to white matter) were measured. Distributions of GABA\(_{A}\)-IR in the somatosensory cortex of ferret were determined by superimposing immunoreactive sections with adjacent Nissl stained sections. All analysis was done in the region of the hand representation of the somatosensory cortex, area 3b. The location of this region was determined in older animals using cytoarchitecture and previous observations (McLaughlin et al., 1998; Noctor et al., 2001). In younger animals (P0), this region can be identified using established morphologic features, although the cytoarchitecture is not mature at this age (Juliano et al., 1996; Noctor et al., 2001).

Thalamic Injections in Thick Slices of Ferret Somatosensory Cortex

In three brains of normal ferrets obtained at P14, thick slices were prepared using a roughly coronal orientation at an angle that included the ventrobasal thalamus and somatosensory area 3b. Each slice was placed in a chamber prepared to maintain live slices as described previously (Juliano et al., 1996). Injections were made into ventrobasal thalamus using glass pipettes filled with fluororuby (Molecular Probes). The injections were made iontophoretically using positive alternating current at 4 \(\mu\)A through a tip size of ~20 \(\mu\)m. The slices were maintained in the chamber for 8–10 h to allow for transport of the fluorescent dextran. The slices were then briefly fixed and processed for GABA\(_{A}\) receptor immunoreactivity as described above, except that the secondary antibody was conjugated to Cy2 to allow for easier visualization with the fluorescent dextran injections into the thalamus. After the immunohistochemistry, the thick slices were cut on a vibratome at 30 \(\mu\)m thickness.

Results

Distribution of Excitatory and Inhibitory Receptors in Mature Cortex of Normal and E33 MAM-treated Adult Ferrets

To determine if administration of MAM on E35 affected the distribution of excitatory or inhibitory receptors in ferret somatosensory cortex, a preliminary study was conducted to assess the ligand binding pattern of a battery of receptors, specifically in area 3b of mature cortex. The same region was assessed in each animal, corresponding to a site slightly lateral to the postcruciate dimple, in the hand region of area 3b (McLaughlin et al., 1998). Densitometric readings were obtained separately for each layer. One of the effects of treatment with MAM on E35 is to reduce the thickness of neocortical layers 3 and 4 in area 3b of ferret somatosensory cortex, with layer 4 being drastically thinner than normal. A more detailed and quantitative analysis of the distinctions between normal and E35 MAM-treated cortex can be seen in Noctor et al. (2001).

Examination of the autoradiographs and densitometric readings for excitatory receptors revealed only slight distinctions between the laminar pattern for the normal and MAM-treated brains of NMDA (\(^{[3]}\)H-MK-801), AMPA (\(^{[3]}\)H-fluorowillardine) and kainate (\(^{[3]}\)H-kainate) receptors. The NMDA receptors were located most heavily in the superficial layers for both the normal and MAM-treated animals. In contrast, the kainate receptors were most
heavily detected in deeper layers 5 and 6 for both groups (Fig. 1). Although minor variations could be observed between
the normal and MAM-treated patterns of excitatory receptors, overall the distributions were similar.

The laminar pattern of inhibitory receptors (GABA<sub>A</sub>) showed distinctions between the normal and E33 MAM-treated distribution. Generally, GABA<sub>A</sub> receptors are preferentially located in layer 4 of somatosensory cortex (Schwark et al., 1994; Land et al., 1995), which we observed in normal ferret area 3b. After MAM treatment, layer 4 was most strongly affected. In the E33 MAM-treated cortex, although the diminished layer 4 also contained a relatively high level of muscimol binding, layers 2 and 3 contained the highest level of GABA<sub>A</sub> receptors, indicating a shift in the overall distribution pattern (Fig. 2).

**Distribution of GABA<sub>A</sub> Receptors in Somatosensory Cortex of Normal and E33 MAM-treated Animals**

Because the distribution of inhibitory GABA<sub>A</sub> receptors in ferret somatosensory cortex of MAM-treated animals was the only distribution that differed from the normal arrangement, we examined the receptor distribution using antibodies directed against GABA<sub>A</sub> receptors. The receptor binding results were intriguing, but did not reveal enough detail to explain why this pattern differed from the normal. We assessed immunoreactivity to all the α subunits (pan α) of the GABA<sub>A</sub> receptor. This family of subunits is present in high amounts in the cerebral cortex and confers the action of inhibition (Mohler et al., 1995). First, we assessed the distribution of GABA<sub>A</sub> receptors in developing cortex. The same region (i.e. hand region of area 3b) was evaluated.

In normal somatosensory cortex at P0, GABA<sub>A</sub> receptor immunoreactivity shows only slight staining in the deeper portion of the cortical plate. A similar staining pattern can be

---

**Figure 1.** Shown here are digitized autoradiographs of [3H]ligand binding to specific excitatory receptors and histograms indicating optical density values obtained from each layer of somatosensory cortex. In the left column are autoradiographs of sections taken from normal ferret somatosensory cortex with accompanying histograms of mean optical density values obtained through each layer; in the right column are autoradiographs and histograms obtained from somatosensory cortex of ferrets treated with MAM on E33. The error bars represent the standard error; the mean optical density values were obtained from at least three different animals. Illustrated are the distribution of binding patterns to NMDA ([3H]MK-801), AMPA ([3H]fluorowillardine) and kainate receptors ([3H]kainate). The histograms were made by obtaining optical density values (converted to fmol/mg tissue) through each cortical layer (indicated on the x-axis); in the MAM-treated cortex, a value for layer IV was obtained through the thin remaining layer, the layers are indicated with numbers. The autoradiographs and histograms indicate that distributions of specific excitatory receptors through layers of somatosensory cortex are similar when comparing normal and MAM-treated sections. Although the E33 MAM-treated brains show shallower sulci and gyr, the overall pattern of receptor binding does not vary substantially when comparing the two conditions. The histograms also indicate that a similar pattern of density values occurs through each layer of somatosensory cortex. Although the density values are not exactly the same for normal and E33 MAM-treated cortex, they present comparable overall distributions. Scale bar = 1500 μm.

**Figure 2.** Example of autoradiographs showing binding to inhibitory receptors ([3H]muscimol) from normal (left) and E33 MAM-treated somatosensory cortex. This pattern of receptor binding was different in normal and MAM-treated cortex. In normal cortex, the muscimol binding concentrated most heavily in layer 4. In MAM-treated cortex, however, the highest concentration of GABA<sub>A</sub> receptors shifted so that layers 2–3 and the remaining layer 4 had high receptor binding, causing the distribution of GABA<sub>A</sub> receptors to be more spread out than in normal cortex. The density of binding was significantly greater in layers 2/3 of the MAM-treated animals (*P < 0.001). The histograms are prepared as in Figure 1, the error bars represent standard error. Scale bar = 1500 μm.
seen in E33 MAM-treated somatosensory cortex (Fig. 3). On P14, the layers are more distinct in normal cortex, although still not fully mature (Noctor et al., 1997). GABA_A receptor immunoreactivity is stronger at this age and in the normal cortex preferentially labels a thin band corresponding to layer 4. In E33 MAM-treated cortex, the distribution is broader and the heaviest label includes a laminar distribution that extends past the boundaries of layer 4, which is very thin in the MAM-treated animals (Fig. 3). In the adult ferret somatosensory cortex, the normal distribution of GABA_A receptor immunoreactivity remains highly localized to layer 4. In the MAM-treated cortex, however, the heavy distribution of immunoreactivity against GABA_A receptors covers a substantially wider region, extending into layers 3 and 5 (Fig. 3).

To more clearly assess the distribution of GABA_A receptors in relation to cortical layers, optical density measurements of the immunoreactivity were obtained across a trajectory from the pia through layer 6. Although a low level of immunoreactivity exists across all layers, these measurements revealed that on P0, the distribution increases slightly in the immature deeper layers (Fig. 4). On P14 and in the adult distributions of immunoreactivity, the greatest optical density values correlate closely with the borders of layer 4 in normal somatosensory cortex, whereas in the MAM-treated brains, the above background GABA_A receptor immunoreactivity extends substantially beyond the layer 4 borders (Fig. 4). In all of these graphs, the optical density values corresponding to layer 1 were omitted. This is because in many sections the immunoreactivity related to layer 1 or the pia were artificially high.

**Cellular Localization of GABA_A Receptors**

At higher magnification, the precise distribution of GABA_A immunoreactivity across all layers was more easily accessible (Fig. 5). In normal cortex, layer 1 contained fine-labeled punctate structures in the neuropil and fine processes without a clear orientation; this pattern continued into layer 2, which contained labeled processes and puncta. Layer 3 contained

---

**Figure 3.** These are examples of immunoreactivity against GABA_A receptors in ferret somatosensory cortex at different ages. In the left column are sections taken from normal animals and in the right column are sections taken from E33 MAM-treated animals. The approximate laminar locations are indicated in the figure. It should be noted that in the E33 MAM-treated cortex on the right, layer 4 is present, but very thin, so is not given a specific designation. At P0, the pattern of immunoreactivity looks similar in both normal and MAM-treated sections, with very light label occurring in deeper portions of the immature cortex that corresponds to the developing cortical plate (CP). The upper portion, corresponding to the marginal zone (MZ) has almost no immunoreactivity (top panels). By P14 (middle panels) the immunoreactivity is strongly localized to layer 4 in normal cortex, while in E33 MAM-treated cortex, the GABA_A receptor immunoreactivity extends outside the boundaries of layer 4. In normal adult somatosensory cortex, GABA_A receptors are preferentially located in layer 4, while in E33 MAM-treated cortex, the immunoreactivity covers a much wider area, extending into layers 3 and 5, although layer 4 is much thinner than normal in E33 MAM-treated somatosensory cortex. Scale bar = 350 µm

**Figure 4.** Shown are graphs of optical density values taken from a trajectory through somatosensory cortex from layer 1 to layer 6 in normal (left column) and E33 MAM-treated brains (right column) at different ages. The values on the x-axis correspond to distance from the pia (in micrometers), the y-axis values correspond to optical density values. The bars colored gray coincide with regions identified cytoarchitectonically as layer 4 (using Nissl staining on adjacent sections). At P0, the density values indicate slight increases in a region corresponding to deeper cortical layers. At P14 and in the adult, the optical density values increase in the central part of the cortex, corresponding to layer 4 in normal cortex, and extending well beyond cytoarchitectonic layer 4 in E33 MAM-treated cortex. In these sections, optical density (OD) values for layer 1 are omitted (see text).
sparsely labeled processes with densely packed small grains. Many of these labeled processes ran in a radial orientation. Layer 4 was the most densely labeled and contained many tightly packed immunoreactive elements occurring at all orientations. In all layers many small round or fusiform shaped neurons were outlined with immunoreactivity (e.g. arrows in Fig. 5).

The details of the distribution were similar in the E33 MAM-treated cortex. In many layers, particularly layers 5 and 6, neurons could be seen outlined with immunoreactivity. The dense immunoreactive product present in normal layer 4 extended in vertical arrays though layer 3 and into layer 2. The fine punctate structures observed in normal layer 1 and upper layer 2 were similar in normal and MAM-treated sections. The punctate distribution of immunoreactivity in layer 1 appeared slightly denser in the E33 MAM-treated cortex.

Relation of Thalamic Afferent Fibers and GABA A Receptors

An additional set of experiments evaluated the relationship between GABA A receptors in the somatosensory cortex and thalamocortical afferent fibers. In the examples shown in Figure 6, both thalamic afferent fibers and GABA A receptors are visible. Living slices containing the thalamus and somatosensory cortex were injected with fluororuby in the thalamus and then reacted to detect GABA A receptors. The images shown in Figure 6 were taken from upper layer 5 of somatosensory cortex and demonstrate that the thalamic axons (shown alone in Fig. 6A,E) are in very close apposition to GABA A receptors (shown alone in Fig. 6B,F and both fields together in Fig. 6C,D,G,I–K). A single cell immunoreactive for GABA A receptors can be seen in Figure 6H. The label occurs in a punctate form over the somata and extends long distances onto the processes. We infer from the size, that this is a neuron. These images indicate that thalamic afferent fibers are frequently coincident with GABA A receptors, which are positioned intermittently along thalamic axons arriving in the cerebral cortex. Figure 6G and I (at higher power) demonstrate a thalamic axon that appears to be terminating on a GABA A immunopositive cell.

Discussion

Distribution of Glutamatergic and GABAergic Receptors in Somatosensory Cortex

The laminar distribution of excitatory receptors found in this study resembles patterns identified previously in other species. In normal ferret somatosensory cortex, NMDA and AMPA receptors are expressed predominantly in the superficial cortical layers, while kainate binding sites are most intense in layer 5. Receptor binding experiments in somatosensory cortex of mice and rats also find the highest levels of NMDA and AMPA receptors in upper cortical layers (Jaarsma et al., 1991; Blue and Johnston, 1995; Glazewski et al., 1995). The deep cortical localization of kainate receptors agrees with previous autoradiographic data in other areas of cerebral cortex in different species (Miller et al., 1989; Gordon et al., 1991; Kumar et al., 1994; Smith and Thompson, 1994). In the E33 MAM-treated animals, the NMDA, AMPA and kainate receptors were also predominantly located in a pattern similar to the normal distribution. In a different study using rats, Rafiki et al.
(1998) found that MAM treatment did not affect the cortical distribution of NR subunits of NMDA receptors.

The prominence of GABA_A receptors in layer 4 of normal ferret somatosensory cortex is also similar to observations in rat, cat and monkey, which demonstrate higher levels of [3H]muscimol binding in layer 4 than adjacent in layers (Lidow et al., 1989; Schwark et al., 1994). Relatively intense immunostaining and dense GABA_A receptors also occur in layer 4 of rodent somatosensory areas (Land et al., 1995). A high level of GABA_A receptor immunoreactivity was also selectively located in layers 4 and 6 of somatosensory cortex in monkeys (Huntley et al., 1990; Hornung and Fritschy, 1996). Several studies report that the density of GABA_A receptors in layer 4 is paralleled by a high density of GABA containing neurons in that site (Beaulieu, 1993; Land et al., 1995).

In the E33 MAM-treated somatosensory cortex, the distribution of GABA_A receptors was different. After MAM treatment, layer 4 is greatly diminished in thickness (Noctor et al., 2001); the distribution of GABA_A receptors did not parallel this change, however, but expanded over a wider portion of cortex. The heaviest density of GABA_A receptors shifted to the upper layers after MAM treatment on E33 and as shown through immunoreactivity, to lower layers as well. The change in distribution reflects a true increase in layers 2 and 3 because in normal animals, these layers demonstrate much lower GABA_A immunoreactivity than layer 4, which is replaced by a strong increase after E33 MAM treatment. It is unlikely that the increased label reflects staining on ‘displaced’ layer 4 cells, since our earlier quantification of E33 MAM treatment demonstrates that the cells in layers 2–3 are not similar in size or shape to those in layer 4 and are composed primarily of cells morphologically similar to those normally in supragranular layers.

On What Neuronal Elements are GABA_A Receptors Located?

The CNS contains two prevalent types of GABA receptors, GABA_A and GABA_B. Our preliminary investigation of receptor binding assessed the distribution of GABA_A receptors, because they are largely responsible for mediating postsynaptic inhibition. The GABA_A receptors are comprised of several subunits; in the cerebral cortex the most common combinations include alpha, beta and gamma subunits. After finding an altered pattern of GABA_A receptor binding in the MAM-treated somato-
sensory cortex, we evaluated the altered distribution of GABA \(_\alpha\) receptors in more detail, particularly the alpha subunits (i.e. \(\alpha_n\)) since they are predominant in the neocortex and in the somatosensory cortex (Fritschy and Mohler, 1995; Golshani et al., 1997).

The results of our study are not conclusive as to the precise location of these receptors on neural elements. The pattern of immunoreactivity we observed in somatosensory cortex confirms that GABA \(_\alpha\) receptors are located on axons, somata and dendrites. Several researchers report that GABA \(_\alpha\) receptors can be found on both pyramidal and non-pyramidal cells, but in non-pyramidal cells the immunoreactivity is localized to somata, whereas in pyramidal cells the label extends into the dendrites (Gu et al., 1993; Land et al., 1995) and is also found on axons (Volk et al., 2002). In our data, the GABA \(_\alpha_\) immuno-reactivity outlines multiple cell types and extends on dendrites, whether cells are pyramidal or not (e.g. Figures 5 and 6). Evidence also exists indicating that GABA \(_\alpha_\) receptors are present on growth cones (Fukura et al., 1999).

The distribution of GABA \(_\alpha_\) receptors shows they are in close relation to the thalamic afferent fibers in the cortex. It was somewhat surprising to observe GABA \(_\alpha_\) receptors studded along the thalamic axons, as well as clearly outlining cell bodies. The intermittent distribution we observed both on cell bodies and on processes, however, resembles the description of GABA \(_\alpha\) receptors reported by Somogyi et al. (1998), who find multiple types of similarly appearing GABA \(_\alpha\) receptors distributed throughout the cortex. GABA \(_\alpha_\) receptors found by these researchers associate with synapses, but are also in extra-synaptic positions. They additionally report axo-axonic synapses acting exclusively through GABA \(_\alpha\) receptors. Although the findings reported here are not detailed enough to provide the precise relation between thalamic axons and GABA \(_\alpha_\) receptors and they could either be pre or postsynaptic; in many instances the receptors and thalamic axons are in close relation to each other.

The change in the distribution of GABA \(_\alpha_\) receptors after E33 MAM treatment is also compatible with several altered features we detected in ferret somatosensory cortex. After MAM treatment that reduces the dimension of layer 4, the termination of thalamic afferents is no longer focused in the central layers, but distributed throughout the cortical thickness (Noctor et al., 2001; Palmer et al., 2001). In concert with this finding, the more diffuse pattern of GABA \(_\alpha_\) receptor immunoreactivity may be coincident with widespread termination of thalamic afferents. In the MAM model, it is possible that cells originally destined to receive thalamic input are displaced and receive thalamic terminations in unusual locations. Our evidence suggests this is not the case, i.e. thalamic afferents are not terminating on their original misplaced targets, since populations of cells similar to those in normal area 3b (in terms of morphology and cell size) populate the layers remaining after E33 MAM treatment (Noctor et al., 2001). This does not necessarily mean that the thalamic afferents and GABA \(_\alpha_\) receptors are colocalized, but that they are in similar positions and may influence each other.

**What Regulates GABA \(_\alpha_\) Receptor Expression?**

The expression of GABA \(_\alpha_\) receptors appears to be regulated both by intrinsic and extrinsic mechanisms. Cells in culture display a neuron specific pattern of GABA \(_\alpha\) receptor subtype expression and a precise spatial pattern of this receptor is expressed prenatally, suggesting a degree of intrinsic regulation (Paysan et al., 1994; Penschuck et al., 1999). On the other hand, GABA \(_\alpha_\) receptor expression is influenced by external factors such as alcohol consumption and a number of disease conditions (Chen et al., 1998). GABA \(_\alpha\) receptors also seem to be regulated by activity dependent mechanisms. Huntsman and Jones (1998) report that the mRNA of several GABA \(_\alpha\) receptor subunits in visual cortex is differentially regulated by monocular deprivation, suggesting activity driven by the thalamus regulates GABA \(_\alpha\) receptor expression (Jones, 1997; Huntsman and Jones, 1998). Several other studies support this idea by demonstrating the GABA \(_\alpha\) receptors are specifically reduced in layer 4 after reduction of input to the somatosensory cortex (Paysan et al., 1997; Wellman et al., 2002). Poluch (2002) also found that GABA \(_\alpha\) receptors are located on thalamocortical fibers, providing a precedent for an intimate linkage between GABA \(_\alpha\) receptors and thalamic terminations.

**Effects of Altered GABA \(_\alpha\) Receptor Distribution on Cortical Transfer of Information**

In MAM-treated cortex, the pattern of excitatory receptors was similar to normal ferret somatosensory cortex distribution, but the inhibitory pattern was altered. This suggests that the transmission of excitatory impulses remains relatively intact, while inhibitory processing is disrupted. There is substantial evidence that GABAergic mechanisms are important in the transfer of information through the cortex and in the initial sculpting of a response. Inhibitory influences act differently at each level of the cerebral cortex and are crucial at the initial transfer of information in layer 4 (Kyriazi et al., 1996, 1998; Brumberg et al., 1999). GABAergic cells in layer 4 are often targets of axons arriving from the thalamus. In subsequent processing, the neural tuning initiated in layer 4 is projected through other cortical layers. This transmission of information results in electrical coupling between GABAergic cells, which leads to patterns of synchronous activity (Beierlein et al., 2000; Tamás et al., 2000). In MAM-treated cortex, the initial transfer of information from the thalamus is altered because layer 4 is largely missing and the thalamic terminations are not focused on layer 4 but distributed through many cortical layers (Noctor et al., 2001; Palmer et al., 2001). The GABA \(_\alpha\) receptors are also widespread. The abnormal arrangement of thalamic projections and GABA \(_\alpha\) receptors probably leads to axons from the thalamus that terminate on cells different from their normal objectives. As a result, the normal projection pattern from layer 4 is probably lost, as is the resulting synchronous pattern of activity. Taken together, these breakdowns are likely to lead to disruption in the cortical response and loss of ability to appropriately process stimulation.

**Notes**

This work was supported by NIH-NINDS RO1-NS24014 (S.L.J.). We thank Donna Tatham for outstanding technical assistance and Amanda Dempster for excellent assistance with the receptor binding studies. Address correspondence to Dr. Sharon L. Juliano, Department of Anatomy, Physiology and Genetics, USUHS, 4301 Jones Bridge Rd, Bethesda, MD 20814, USA. Email: sjuliano@usuhs.mil.