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Differential Downregulation of $GABA_A$ Receptor Subunits in Widespread Brain Regions in the Freeze-Lesion Model of Focal Cortical Malformations

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Focal cortical malformations comprise a heterogeneous group of disturbances of brain development, commonly associated with drug-resistant epilepsy and/or neuropsychological deficits. Electrophysiological studies on rodent models of cortical malformations demonstrated intrinsic hyperexcitability in the lesion and the structurally intact surround, indicating widespread imbalances of excitation and inhibition. Here, alterations in regional expression of $GABA_A$ receptor subunits were investigated immunohistochemically in adult rats with focal cortical malformations attributable to neonatal freeze-lesions. These lesions are morphologically characterized by a three- to four-layered cortex with microsulcus formation. Widespread regionally differential reduction of $GABA_A$ receptor subunits $\alpha1$, $\alpha2$, $\alpha3$, $\alpha5$, and $\gamma2$ was observed. Within the cortical malformation, this downregulation was most prominent for subunits $\alpha5$ and $\gamma2$, whereas medial to the lesion, a significant and even stronger decrease of all subunits was detected. Lateral to the dysplastic cortex, the decrease was most prominent for subunit $\gamma2$ and moderate for subunits $\alpha1$, $\alpha2$, and $\alpha5$, whereas subunit $\alpha3$ was not consistently altered. Interestingly, the downregulation of $GABA_A$ receptor subunits also involved the ipsilateral hippocampal formation, as well as restricted contralateral neocortical areas, indicating widespread disturbances in the neocortical and hippocampal network. The described pattern of downregulation of $GABA_A$ receptor subunits allows the conclusion that there is a considerable modulation of subunit composition. Because alterations in subunit composition critically influence the electrophysiological and pharmacological properties of $GABA_A$ receptors, these alterations might contribute to the widespread hyperexcitability and help to explain pharmacotherapeutic characteristics in epileptic patients.

Key words: cortical dysplasia; GABA; epilepsy; hyperexcitability; receptors; immunohistochemistry; developmental lesion

Cortical malformations comprise a heterogeneous group of genetic or acquired disturbances of cortical development that are frequently associated with drug-resistant epilepsies and/or neuropsychological deficits (Palmini et al., 1991; Raymond et al., 1995; Guerrini et al., 1999). Although recent research led to a better understanding of the pathogenesis (Gressens, 1998; Walsh, 1999), the pathophysiology of the resulting neurological abnormalities is only poorly understood. Electrophysiological studies on humans, as well as *in vitro* studies on rodent models of cortical dysgenesis, revealed an intrinsic epileptogenicity within the malformation and in widespread surrounding areas (Palmini et al., 1995; Jacobs et al., 1996; Luhmann and Raabe, 1996; Luhmann et al., 1998a; Redecker et al., 1998a). Altered intrinsic membrane properties or changes in network characteristics may contribute to this hyperexcitability. It is still debated to what extent and even in which direction the inhibitory function is altered by cortical malformations (Prince et al., 1997). An increase in GABA-mediated inhibitory efficacy was observed in layer V neurons within the paralesional zone (Prince et al., 1997; Jacobs and Prince, 1999). In pharmacological studies using 4-aminopyridine to induce epileptiform discharges, the inhibitory systems were at least not grossly impaired in the surround of cortical malformations (Hablitz and DeFazio, 1998). However, close to the dysplastic cortex, intracellular recordings in upper layers revealed a decrease in GABA-mediated inhibition (Luhmann et al., 1998b). Recent autoradiographic studies disclosed a significant reduction of binding to $GABA_A$ and $GABA_B$ receptors within the malformation and the surrounding neocortex (Zilles et al., 1998), pointing toward widespread changes in GABA receptor function.

To analyze whether alterations in distribution of specific $GABA_A$ receptor subunits help to understand the changes in GABAergic function, the expression of five major subunits were immunohistochemically investigated in adult rats with focal cortical malformations after neonatal freeze-lesions (Jacobs et al., 1996; Luhmann and Raabe, 1996; Luhmann et al., 1998a). So far, at least 19 different subunit subtypes of the pentameric $GABA_A$ receptors have been sequenced from the mammalian nervous system, comprising six α, three β, three γ, one δ, one ϵ, one θ, one π, and three ρ subunits (Barnard et al., 1998; Whiting et al., 1999). The majority of $GABA_A$ receptors contain a variable combination of α, β, and γ subunits, showing a specific regional and cellular distribution (Fritschy and Mohler, 1995). Although little is known about the specific properties of single subunits, functional studies demonstrated that the subunit composition of receptor subtypes determines their electrophysiological and pharmacological properties (Barnard et al., 1998; Narahashi, 1999),

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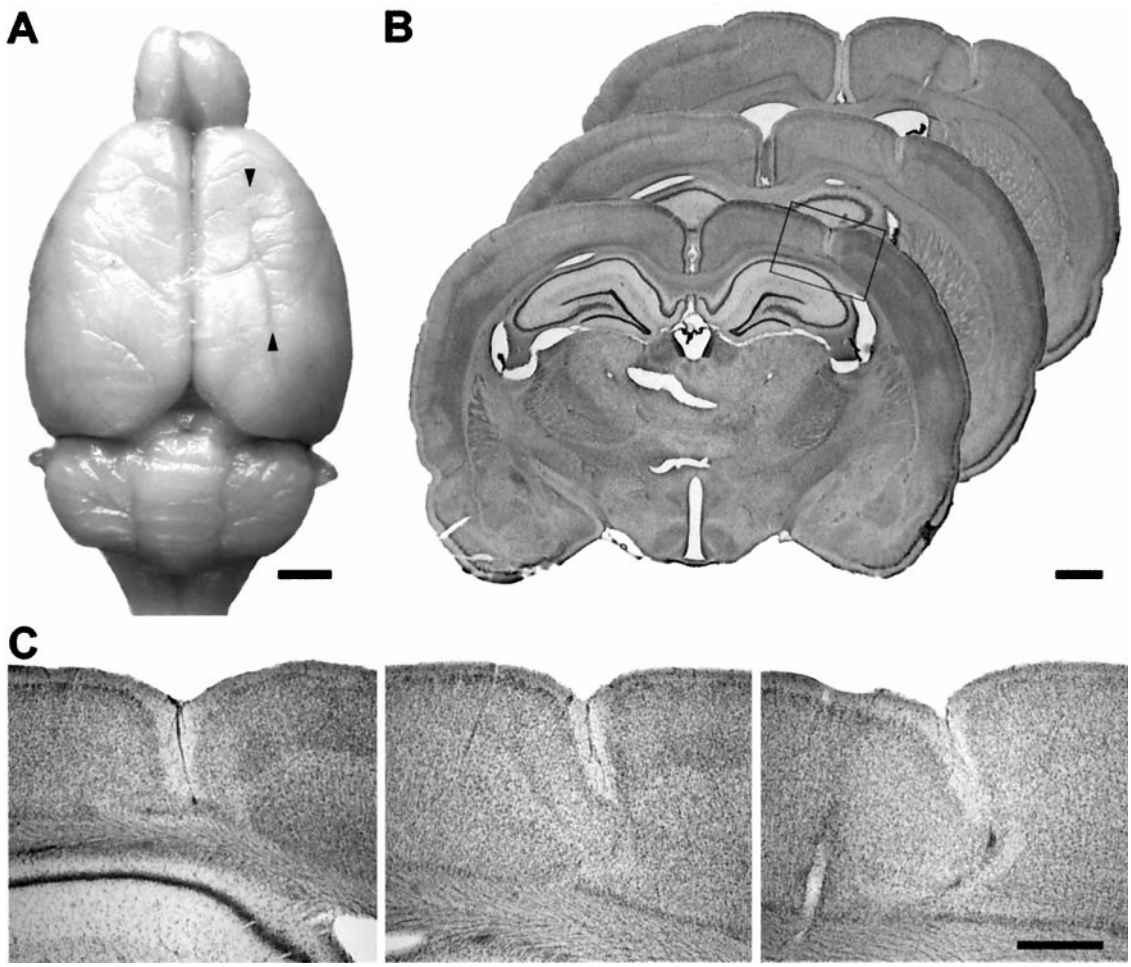

Figure 1. Morphology of freeze-lesion-induced focal cortical malformations. *A*, Adult rat brain that received a freeze-lesion at the day of birth, resulting in a longitudinal microgyrus. The microgyrus is macroscopically characterized by an infolding of the brain surface (*arrowheads*). *B*, Cresyl violet-stained coronal sections through the cortical malformation associating a loss of deep cortical layers and formation of a microsulcus (*frame*). The depth of the microsulcus increased in the anteroposterior direction. *C*, Higher magnifications of the sections displayed in *B*. In rostral parts of the brain, the dysplastic cortex is typically characterized by a three- to four-layered cortex. Because of the increase in depth, a nearly complete division of the neocortex is observed more occipitally. Scale bars: *A*, 2 mm; *B*, 1 mm; *C*, 500 μm.

thus allowing a variety of adaptive changes (Olsen et al., 1999). Because different α subunits correspond to primarily distinct receptor subtypes (Fritschy and Mohler, 1995; Sieghart et al., 1999), this study concentrated on the regional distribution of four α subunits and one γ subunit. Hereby, a widespread regionally differential downregulation of $GABA_A$ receptor subunits was observed involving not only the cortical malformation but also structurally intact surrounding and remote brain regions.

MATERIALS AND METHODS

Lesion induction. Several litters of newborn Wistar rats (Experimental Animal Laboratory of the Heinrich-Heine-University, Düsseldorf, Germany) were used for the experiments. Focal freeze-lesions were induced at the day of birth (postnatal day 0, <24 hr) using a modification of the method of Dvorak and Feit (1977), as described previously in detail (Luhmann and Raabe, 1996; Luhmann et al., 1998a). In brief, newborn rats (n = 12; 8 females and 4 males) were anesthetized by hypothermia, and a liquid nitrogen cooled copper cylinder (diameter of 1 mm) was placed for 8 sec on the calvarium above the frontoparietal cortex. To create a longitudinal freeze-lesion, three identical freeze-lesions were placed in line parallel to the midline with a distance of 1.5 mm between the lesions. These lesions resulted in a 3- to 5-mm-long microsulcus in the rostrocaudal direction (Fig. 1*A*). The wound was closed with histoacryl tissue glue (Braun-Dexon, Melsungen, Germany). Sham-operated rats (n = 7; 6 females and 1 male 1) were treated in the same way without cooling the copper cylinder. Freeze-lesioned and sham-operated rats were allowed to survive for 10–16 weeks before further immunohistochemical studies.

Immunohistochemistry. Adult rats were deeply anesthetized with diethylether and perfused through the ascending aorta with 4% paraformaldehyde and 15% saturated picric acid solution in phosphate buffer (0.15 M), pH 7.4 (Fritschy and Mohler, 1995). Brains were removed immediately after the perfusion and post-fixed for 3 hr in the same fixative at 4°C. All samples were then cryoprotected in PBS containing 30% sucrose for 24 hr and stored at −75°C for further processing. To enhance the detection of synaptic receptor proteins in the subsequent immunohistochemical staining, the brains were processed with a modified antigen-retrieval procedure (Bohlhalter et al., 1996; Fritschy et al., 1998). The brains were incubated overnight at room temperature in 0.1 M sodium citrate buffer, pH 4.5, and irradiated with microwaves (650 W, 135 sec) in the same buffer. Coronal sections were cut at 50 μm with a freezing microtome and collected in ice-cold 0.1 M PBS. A series of sections was Nissl-stained with cresyl violet for histological analysis of the freeze-lesion-induced cortical malformations.

The $GABA_A$ receptor subunits α1, α2, α3, α5, and γ2 were visualized using subunit-specific antisera raised in guinea pigs against synthetic peptides derived from rat subunit cDNA. These subunit-specific antisera have been extensively characterized biochemically by Western blotting and immunoprecipitation (for additional details, see Fritschy and Mohler, 1995), and their suitability for immunohistochemistry has been

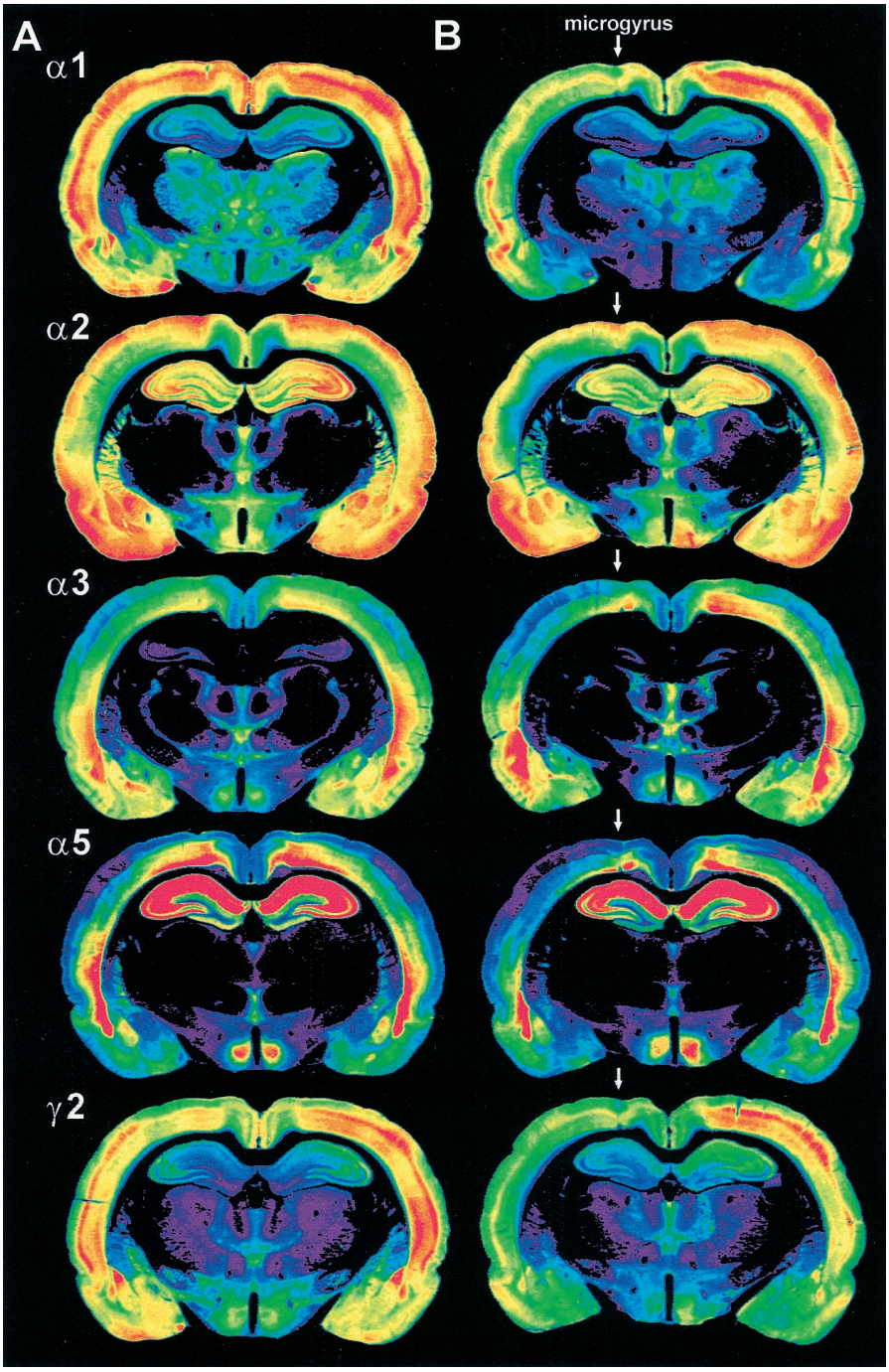

Figure 2. Distribution of $GABA_A$ receptor subunits α1, α2, α3, α5, and γ2 in sham-operated (*A*) and freeze-lesioned (*B*) rats. Color-coded images from immunohistochemically processed sections. For each subunit, the optical density of the immunoreactivity product was color-coded using a standard 256-level scale, ranging from *black* for background to *violet*, *blue*, *green*, *yellow*, and *red* for the most intense signals. Sham-operated rats (*A*) show the typical distribution pattern of subunits α1, α2, α3, α5, and γ2 with symmetric intensities on both hemispheres. Animals with freeze-lesion-induced cortical malformations (*B*, microgyrus marked with an *arrow*) display widespread reduction in immunoreactivity for all subunits, most prominently for subunits α1 and γ2, involving the area of the dysplastic cortex, but also surrounding neocortical areas and the ipsilateral hippocampal formation (see Fig. 6).

documented in several previous reports (Fritschy and Mohler, 1995; Fritschy et al., 1998; Neumann-Haefelin et al., 1998).

Free-floating sections were washed three times in Tris buffer (Tris saline, pH 7.4, and 0.05% Triton X-100) and incubated at 4°C overnight in primary antibody solution diluted in Tris-buffer containing 2% normal goat serum (NGS). The following dilutions of the antisera were used: $GABA_A$ receptor subunit α1, 1: 20,000; subunit α2, 1: 2000; subunit α3, 1: 2000; subunit α5, 1: 4000; and subunit γ2, 1: 3000. Sections were then washed three times in Tris buffer and incubated in biotinylated secondary antibody solution (Jackson ImmunoResearch, West Grove, PA) diluted 1: 300 in Tris buffer containing 2% NGS for 30 min at room temperature. After additional washing, sections were transferred to the avidin-peroxidase solution (Vectastain Elite kit; Vector Laboratories, Burlingame, CA) for 25 min, washed, and processed using diaminobenzidine hydrochloride (Sigma, St. Louis, MO) as chromogen. Sections were mounted onto gelatin-coated slides, air-dried, dehydrated with ascending series of ethanol, cleared, and coverslipped with toluene (Entellan; Merck, Darmstadt, Germany).

Changes in the regional and laminar distribution of $GABA_A$ receptor subunits were analyzed by light microscopy. For semiquantitative image analysis, sections were digitized with a charge-coupled device camera and processed with an imaging program (NIH Image). Measures of relative optical density of $GABA_A$ receptor subunit staining were performed on one section per animal and per antibody. The following brain regions were evaluated on both hemispheres (a scheme is illustrated in Fig. 4): the area of the cortical malformation, the frontal cortex (Fr), the

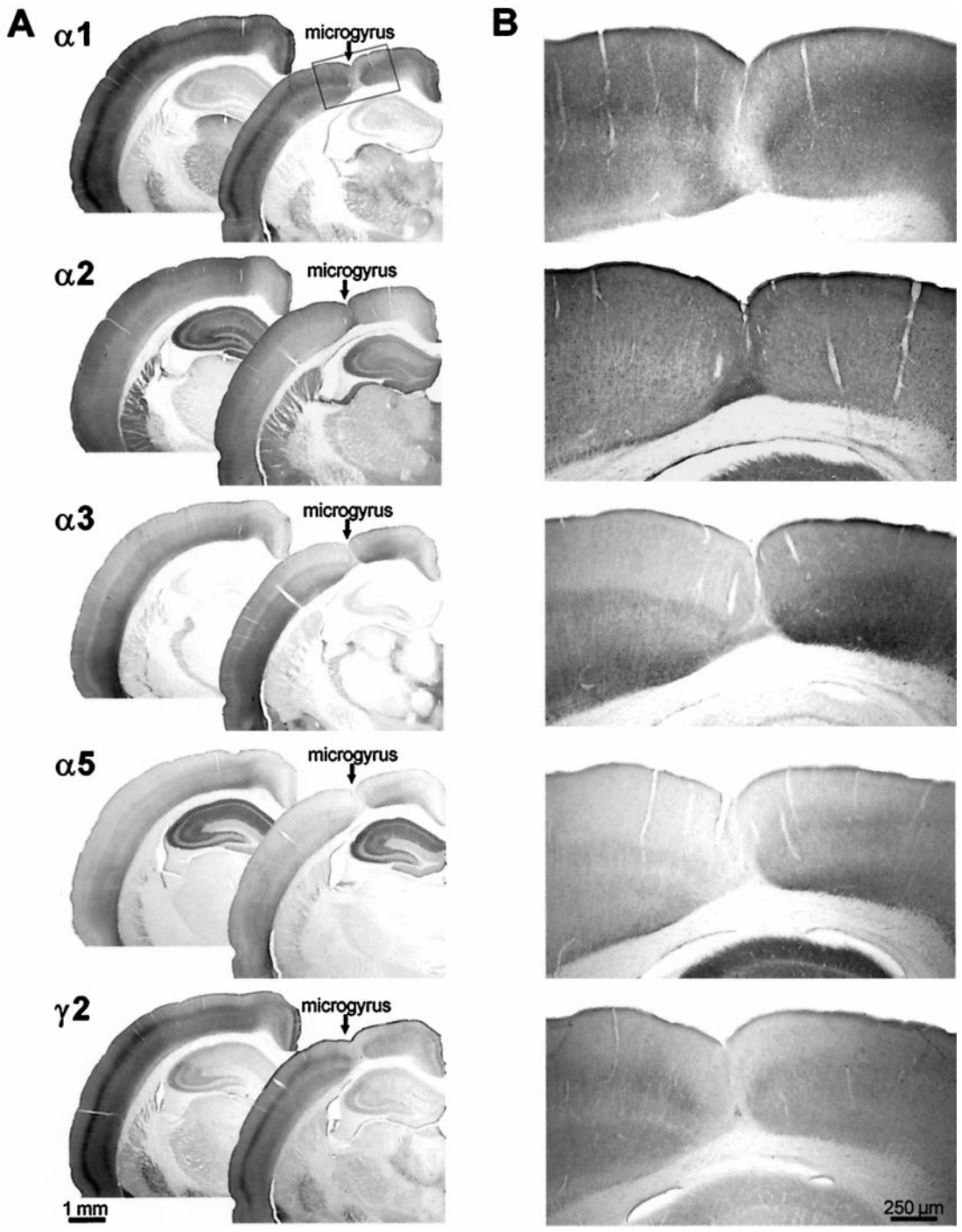

Figure 3. Comparison of neocortical distribution of $GABA_A$ receptor subunits α1, α2, α3, α5, and γ2 in sham-operated and freeze-lesioned rats. *A*, Photomicrographs of coronal sections at low magnification showing a freeze-lesion-induced cortical malformation (microgyrus marked with an *arrow*) and corresponding sections from a sham-operated animal. Animals with cortical malformations show a decreased immunoreactivity in the lesioned area and in adjacent neocortical areas, whereas the characteristic laminar distribution pattern of the different subunits is conserved. The reduction in staining intensity is most prominent for subunits α1 and γ2, moderate for subunits α2 and α5, and only mild for subunit α3. *B*, Higher magnification of the cortical malformation (*frame* in *A*) showing the laminar distribution of $GABA_A$ receptor subunits within the lesion. Note the differential immunoreactivity for subunits α3 and α5 on the lateral and medial wall of the microgyrus.

hindlimb representation cortex (HL), the primary and secondary somatosensory cortex (Par1, Par2), the hippocampal formation, and the thalamus (Zilles, 1992). For background correction, the signal obtained in the corpus callosum was subtracted. Statistical significance of differences in mean optical densities between the experimental and control group was assessed using a two-sample *t*-test ($p < 0.05$). For display, images were contrast-enhanced and color-coded on a 256-level.

RESULTS

Morphology of cortical malformations

All freeze-lesioned animals ($n = 12$) displayed typical cortical malformations consisting of a longitudinal microgyrus located in parallel to the midline with a length of 3–5 mm (Fig. 1*A*). The

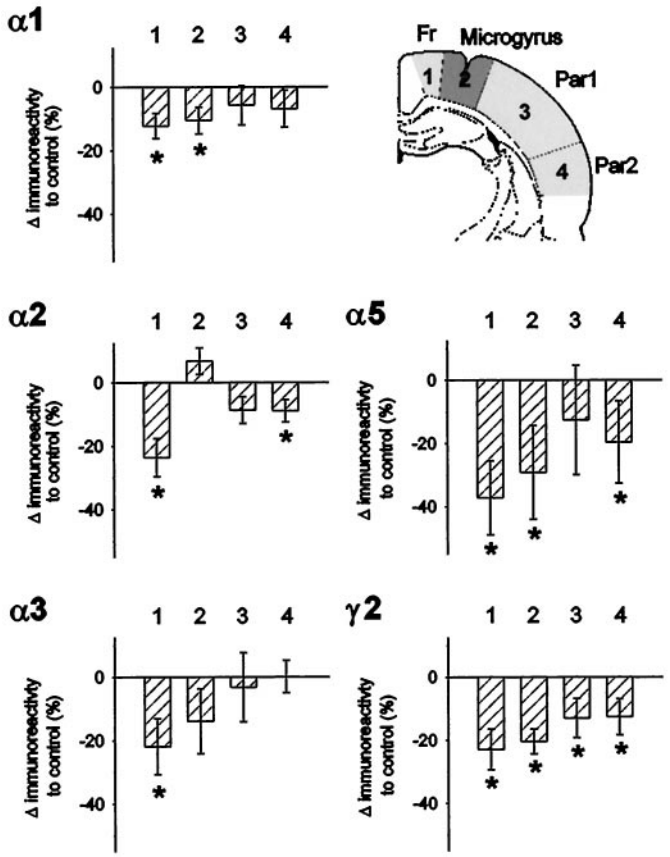

Figure 4. Semiquantitative analysis of regional alterations in immunoreactivity of $GABA_A$ receptor subunits. The relative differences of staining intensities measured as optical densities in sections from animals with focal cortical malformations compared with sham-operated animals are displayed for different neocortical areas. A schematic drawing of the evaluated regions is shown in the *inset*. Significant differences ($p < 0.05$) are indicated by *asterisks*. Within the microgyrus, receptor subunits α1, α5, and γ2 showed a significant decrease in immunoreactivity compared with sham-operated animals. In the adjacent Fr, a significant reduction is measured for all subunits, whereas in Par1 and Par2, subunits α2 and α5 were significantly decreased in Par2 and subunit γ2 was reduced in both areas.

microgyrus had a distance to midline of 1.5–3.5 mm (mean of 2.5 mm) and involved the forelimb (FL) and hindlimb representation cortex, as well as the secondary occipital cortex (Oc2) in most cases (Zilles, 1992). The laminar architecture of the cortical malformation varied to some extent. Most animals showed the formation of a small sulcus with an underlying three- to four-layered cortex as reported previously (Rosen et al., 1992, 1998; Jacobs et al., 1996; Luhmann et al., 1998a; Zilles et al., 1998). The depth of the microsulcus increased in the anteroposterior direction, extending to layer IV or V frontally and resulting in a complete division of the gray matter more occipitally (Fig. 1). In two animals, a complete division of the cortex throughout the microsulcus without a thin layer of remaining cells was observed, resembling the pathology of schizencephaly in humans. In addition to the longitudinal microgyrus, small clusters of ectopic cells were found in 4 of 12 freeze-lesioned animals in the molecular layer adjacent to the microsulcus. No structural change was observed in the hippocampal formation or other remote brain regions. In sham-operated animals, no abnormalities in cortical structure or lamination were detected.

Widespread dysregulation of $GABA_A$ receptor subunits

The distribution of the $GABA_A$ receptor subunits α1, α2, α3, α5, and γ2 in sham-operated animals showed the same pattern as described previously (Fritschy and Mohler, 1995) (Fig. 2). Briefly, the most abundant subunits in the cerebral cortex were α1 and γ2, which displayed a nearly identical distribution pattern. In neocortex, these subunits showed particularly intense staining in layers III – IV, whereas the remaining layers exhibited a slightly lighter immunoreactivity. Subunits α2, α3, and α5 showed a more restricted distribution in the cerebral cortex, being primarily confined to certain layers. Whereas subunit α2 was strongly expressed in the upper layers (I–IV) and showed only slight staining in layers V–VI, subunits α3 and α5 were most abundant in deeper cortical layers and were almost absent in the outer layers. Subunit α3, and with a weaker staining also subunit α5, revealed a certain regional selectivity with a particularly intense immunoreactivity in deep layers of the Fr, the HL/FL, as well as the Oc1/Oc2. In contrast to the cerebral cortex, the hippocampal formation showed a differential pattern of $GABA_A$ receptor subunit expression with a particularly strong immunoreactivity of the subunits α2, α5, and to lesser extent of γ2. In the hippocampal formation, subunit α1 revealed a moderate and subunit α3 only a very weak staining.

In all animals with freeze-lesion-induced cortical malformations, the distribution pattern of $GABA_A$ receptor subunits was different compared with sham-operated controls (Fig. 2). Although the extent of changes in subunit distribution varied to some degree, a consistent pattern of alterations was observed in all freeze-lesioned animals. In the dysplastic cortex, qualitative and semiquantitative evaluation of the sections revealed a remarkable decrease in staining for all receptor subunits, with exception of subunit α2 (Figs. 2, 3). Within the lesion, this downregulation was most prominent for subunits γ2 (−20%, $p < 0.001$) and α5 (−29%, $p < 0.05$), and less clear for subunits α1 (−11%, not significant) and α3 (−14%, not significant). In contrast, immunoreactivity of subunit α2 was slightly increased in the microgyrus. This increase was attributable to the infolding of superficial cortical layers that formed the microgyrus and also showed an intense staining in the surrounding cortex. Alterations in $GABA_A$ receptor subunit distribution also involved widespread neocortical areas surrounding the dysplastic cortex. In the adjacent frontal cortex, a strong decrease in immunoreactivity was observed for all receptor subunits. Interestingly, the degree of this decrease was more pronounced in the structurally mostly intact frontal cortex than in the dysplastic lesion itself (Fig. 4), showing the most prominent decrease in immunoreactivity for subunit α5 (−37%, $p < 0.05$) but also a clear decrease in staining for subunits α1 (−12%, $p < 0.05$), α2 (−24%, $p < 0.001$), α3 (−22%, $p < 0.05$), and γ2 (−23%, $p < 0.05$). In neocortical regions lateral to the microgyrus, in the Par1/Par2, the pattern of alterations was different compared with the lesion and medially adjacent areas. Whereas subunits α1, α2, α5, and γ2 showed a differentially intense reduction in immunoreactivity in these areas, subunit α3 revealed no consistent changes (Fig. 4). In these areas, the most prominent decrease was observed for subunits γ2 (Par1, −13%, $p < 0.05$; Par2, −13%, $p < 0.05$) and α5 (Par1, −13%, not significant; Par2, −20%, $p < 0.05$). In most animals, the alterations in subunit distribution did also involve the contralateral frontal cortex in which a decrease in immunoreactivity was found for all subunits that was slightly less pronounced than in ipsilateral frontal cortex (subunit α1, −13%, $p < 0.05$; subunit α2, −15%, $p < 0.05$; subunit α3, −15%, not significant; subunit

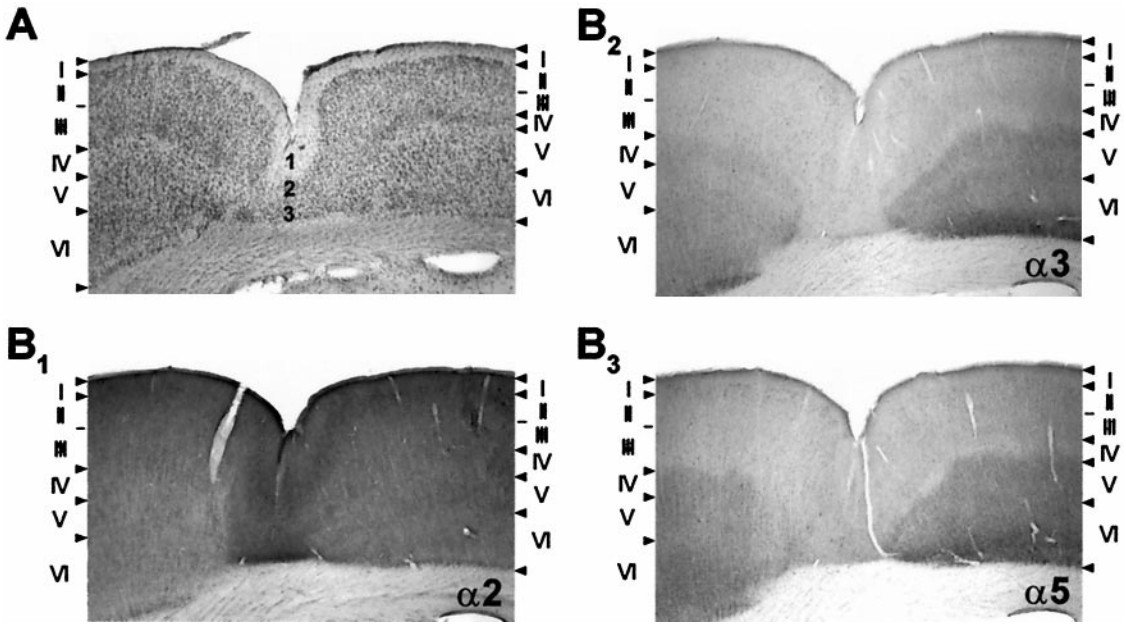

Figure 5. Laminar distribution of $GABA_A$ receptor subunits $\alpha 2$, $\alpha 3$, and $\alpha 5$ within the cortical malformation compared with the underlying histology. *A*, Freeze-lesion-induced microgyrus showing a deep infolding of the cortex with an underlying three-layered cortex. *B*, The microgyrus is lined with an intense staining of subunit $\alpha 2$ reflecting a continuation of the superficial layers I, II, and III of adjacent cortex. Subunits $\alpha 3$ and $\alpha 5$ within the microgyrus also display a very similar immunoreactivity within the microgyrus compared with superficial layers of surrounding neocortical areas.

$\alpha 3$, -31%, $p < 0.05$; subunit $\gamma 2$, -21%, $p < 0.05$). Furthermore, in some animals, a mild reduction in staining was also observed in the contralateral area homotopic to the lesion, as well as in the contralateral somatosensory cortex, but these effects were neither as consistent nor as prominent as the changes found ipsilaterally.

In the area of the cortical malformation, the laminar distribution of $GABA_A$ receptor subunits was conserved, showing consistent similarities with adjacent structurally intact neocortex (Figs. 3, 5). The most superficial layer of the three- to four-layered microgyrus (Fig. 5*A*, *layer 1*) displayed the same distribution of $GABA_A$ receptor subunits compared with the corresponding layer I of the surrounding cortex, and the second and third layer of the dysplastic cortex (Fig. 5*A*, *layers 2*, *3*) revealed very similar immunoreactivity as found in layers II–III of adjacent neocortical areas (Fig. 5). In addition, regional selectivity in distribution of certain $GABA_A$ receptor subunits was also conserved in the dysplastic cortex. In animals in which the microgyrus was located at the border between the sensorimotor hindlimb and motor frontal cortex ($n = 3$) (Fig. 3), the medial and lateral wall of the microgyrus exhibited a differential immunoreactivity of $GABA_A$ receptor subunits. In particular, the staining of subunits $\alpha 3$ and $\alpha 5$ showed a clear difference in staining intensity between the lateral and medial wall of the microgyrus corresponding to layers II–III of adjacent frontal cortex for the medial part and to layers II–III of hindlimb cortex on the lateral part of the microgyrus (Figs. 3, 5*B*).

Alterations in distribution of $GABA_A$ receptor subunits in animals with freeze-lesion-induced cortical malformations were not restricted to the neocortex but also involved the ipsilateral hippocampal formation (Figs. 2, 3, 6). Qualitative and semiquantitative evaluation of the ipsilateral hippocampal formation revealed a clear reduction in immunoreactivity for subunits $\alpha 1$ (-28%, $p < 0.05$), $\alpha 2$ (-12%, $p < 0.05$), $\alpha 5$ (-12%, $p < 0.05$), and most strikingly for subunit $\gamma 2$ (-39%, $p < 0.05$). This decrease in $GABA_A$ receptor subunit immunoreactivity appeared to be pronounced in the CA1 region, as well as the dentate gyrus, but was less marked in the regions CA2 and CA3 (Fig. 6*B*). Subunit $\alpha 3$ also showed some decrease in staining in the hippocampal region, but the weak expression of this subunit in control and freeze-lesioned animals resulted in a poor signal-to-noise-ratio, which did not allow a reliable assessment of changes in this area. Within the thalamus and the contralateral hippocampal formation, no consistent alteration of $GABA_A$ receptor subunit immunoreactivity was found.

DISCUSSION

The present study clearly demonstrates that experimentally induced cortical malformations induce a widespread dysregulation in the distribution of $GABA_A$ receptor subunits in adult animals. These alterations comprise a regionally differential reduction of $GABA_A$ receptor subunits in the dysplastic cortex but also in extended structurally intact brain regions, involving adjacent and remote neocortical areas of the ipsilateral hemisphere and surprisingly also the ipsilateral hippocampus and the contralateral frontal neocortex. This pattern of changes points toward a considerable modulation of $GABA_A$ receptor subunit composition, and taking into account that five major subunits were investigated, these changes might also allude to an absolute reduction of $GABA_A$ receptors.

Topography of alterations in $GABA_A$ receptor subunit distribution

Evidence from receptor autoradiographic studies indicated that binding of the GABA analog muscimol, which selectively binds to $GABA_A$ receptors, is reduced in the dysplastic cortex and widespread surrounding and remote brain regions (Zilles et al., 1998). Reduction in binding to $GABA_A$ receptors can be interpreted as decreases in density and/or changes in affinity of the receptor. The prominent downregulation of neocortically most abundant $GABA_A$ receptor subunits $\alpha 1$ and $\gamma 2$ described here strongly points toward a reduction of receptor density. However, subunits $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 5$ showed a regionally differential downregula-

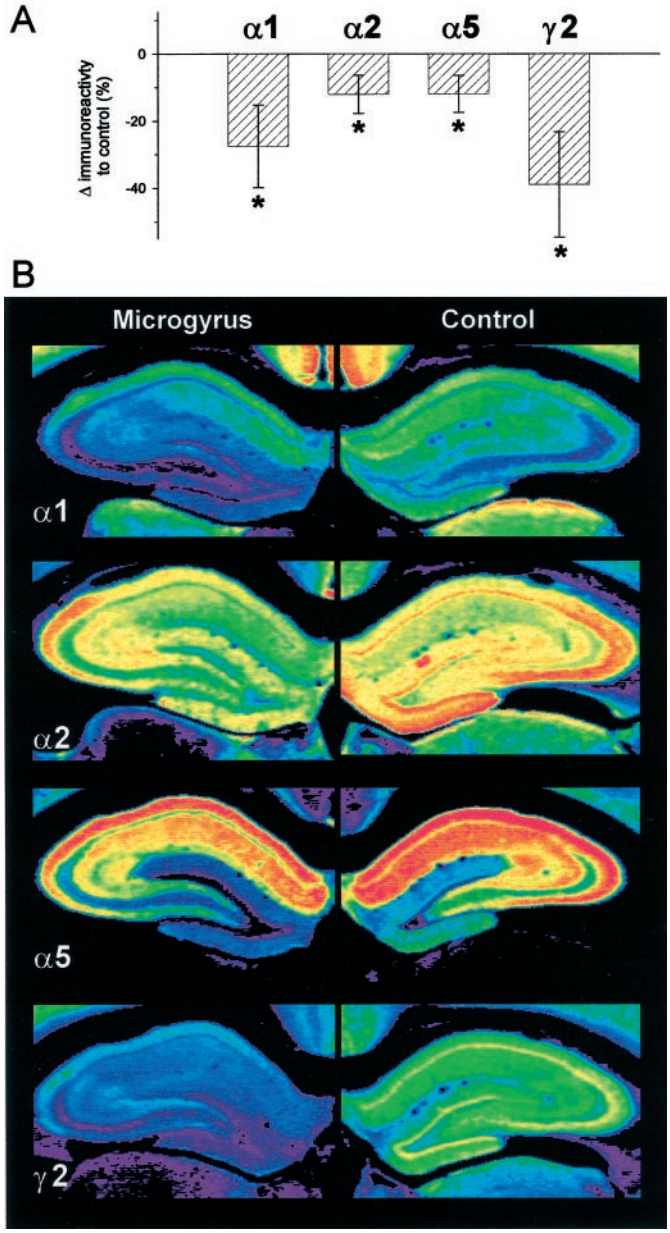

Figure 6. Alterations in the distribution of $GABA_A$ receptor subunits $\alpha1$, $\alpha2$, $\alpha5$, and $\gamma2$ within the ipsilateral hippocampal formation in animals with focal cortical malformations. *A*, The relative differences of staining intensities measured as optical densities in sections from animals with microgyri compared with sham-operated animals. Significant differences ($p < 0.05$) are indicated by *asterisks*. *B*, Color-coded images of the hippocampal formation from an animal with a freeze-lesion-induced microgyrus and a sham-operated control using a standard 256-level scale, ranging from *black* for background to *violet*, *blue*, *green*, *yellow*, and *red* for the most intense signals. Note the prominent downregulation for subunits $\alpha1$ and $\gamma2$.

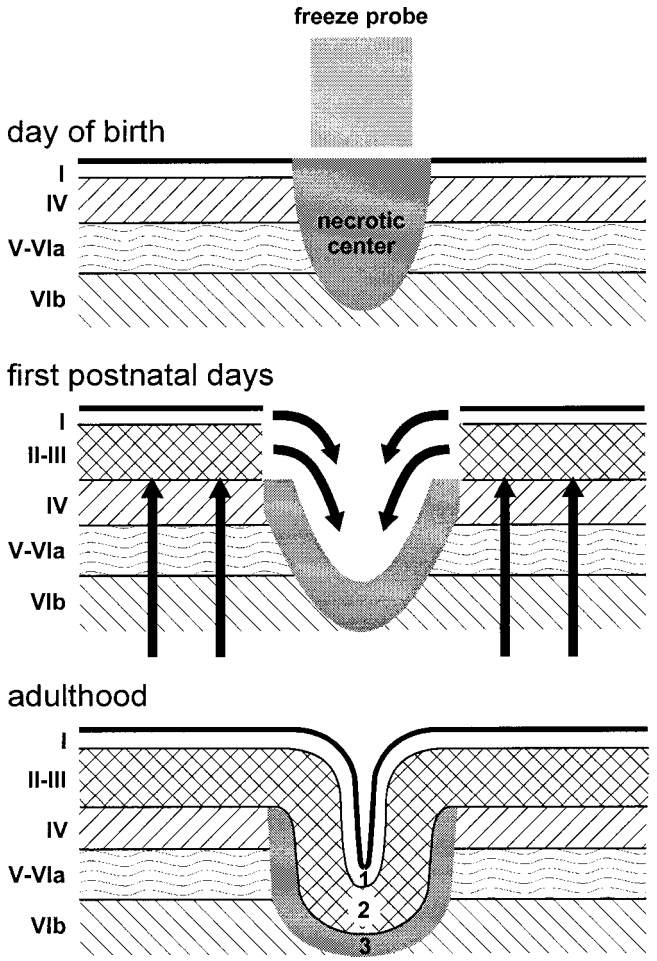

Figure 7. Schematic illustration of the development of a freeze-lesion-induced microgyrus (modified from Zilles et al., 1998). Cytoarchitectonical layers of the adjacent neocortex are specified with *roman numerals* and with *arabic numerals* within the dysplastic cortex. *Arrows* indicate the direction of migration of layer II–III neurons during the first postnatal days. For details, see Discussion.

tion, indicating changes in subunit composition that are likely to alter the affinity of the receptor.

Interestingly, alterations in distribution of $GABA_A$ receptor subunits were not restricted to the ipsilateral hemisphere but also involve remote brain regions, including the contralateral frontal cortex and the ipsilateral hippocampal formation. These findings indicate widespread disturbances of the neocortical and hippocampal network. Similar remote alterations of $GABA_A$ receptor function have been reported after acute focal cortical lesions in adult rat brain using receptor autoradiographic techniques (Witte et al., 1997; Qu et al., 1998; Que et al., 1999).

The pattern of $GABA_A$ receptor subunit distribution found within the microgyrus, especially the finding that the lateral and medial wall of the microgyrus, when located between two distinct cortical regions showed a differential distribution of subunits, is in agreement with the hypothesis of microgyrus development proposed by Zilles et al. (1998), which is schematically illustrated in Figure 7. Initially, neonatal freeze-lesions induce a focal destruction of all layers of the developing cortex present at the cortical surface at day of birth. During the first postlesional days, migration of layer II–III neurons into adjacent parts of intact cortex continues and replaces, together with layer I and the pial surface, the lesioned area by tangential expansion (Suzuki and Choi, 1991). Layer 2 of the microgyrus therefore results from tangential growth of adjacent layers II–III.

Functional consequences of alterations in $GABA_A$ receptor subunit distribution

In contrast to the widespread changes in $GABA_A$ receptor distribution and binding, electrophysiological investigations did not disclose a general impairment of GABAergic inhibition. In intra-

cellular recordings, the conductance of stimulus-evoked polysynaptic $GABA_A$-mediated IPSPs was reduced in layer II–III neurons close to the microgyrus (Luhmann et al., 1998b). However, pharmacologically isolated monosynaptic $GABA_A$-mediated IPSPs were similar in dysplastic and control cortex in this study. More laterally in the paramicrogyral cortex, layer V neurons revealed evoked and spontaneous IPSCs with significant larger amplitudes (Prince et al., 1997; Jacobs and Prince, 1999). These changes of inhibitory function were interpreted as alterations in excitatory innervation of GABAergic neurons in both studies, causing a decrease or loss of excitatory drive on inhibitory interneurons close to the microgyrus and an increase of these inputs in the paramicrogyral zone. However, these studies concentrated on two different areas and cortical laminae. The prominent downregulation of $GABA_A$ receptor subunits within the cortical malformation might contribute to the decrease in GABAergic function in upper layers close to the microgyrus, but the increase in inhibitory efficacy described in deep layers of the paramicrogyral zone coinciding with a downregulation of $GABA_A$ receptor subunits remains elusive. However, evidence for an increase in inhibitory efficacy also comes from studies on ibotenate-induced focal cortical malformations, showing very similar lesions and changes in cortical excitability (Redecker et al., 1998a,b). Ibotenate-induced cortical malformations induce a very similar reduction of $GABA_A$ receptor subunit distribution (Redecker et al., 1999), whereas *in vitro* extracellular recordings using the paired-pulse paradigm as a measure of functional inhibition reveal no significant impairment throughout the ipsilateral neocortex (Hagemann et al., 2000).

The reduction of the neocortically abundant receptor subunits $\alpha 1$ and $\gamma 2$ in the paramicrogyral zone might represent a compensatory downregulation to account for the increased excitatory drive on GABAergic neurons (Prince et al., 1997; Jacobs and Prince, 1999). Interestingly, also less abundant subunits $\alpha 2$ and $\alpha 5$ were decreased in the paramicrogyral zone, whereas subunit $\alpha 3$ was not altered. This differential downregulation might represent modifications in subunit composition of the remaining receptors changing the function of the receptor and contributing to the increased GABAergic efficacy in this region. This hypothesis is supported by an increase in miniature IPSCs (Jacobs and Prince, 1999), likely reflecting a modification of the $GABA_A$ receptor. In addition, pharmacological studies point toward a switch in subunit composition in the paramicrogyral zone, showing a reduced sensitivity to the benzodiazepine receptor agonist zolpidem (DeFazio and Hablitz, 1999). $GABA_A$ receptors containing the $\alpha 1$ subunit exhibit a high affinity for zolpidem, whereas expression of subunits $\alpha 2$ or $\alpha 3$ give rise to less sensitive receptors (Luddens et al., 1995). The selective decrease of subunits $\alpha 1$, $\alpha 2$, and $\alpha 5$ indicates that the balance between α subunits is relatively changed in favor of subunit $\alpha 3$, likely decreasing the sensitivity to zolpidem. Because subunit $\alpha 3$ is more abundant during early postnatal development, even eclipsing the expression of subunit $\alpha 1$ (Laurie et al., 1992), this imbalance might reflect a delay in maturation of $GABA_A$ receptors.

Additional mechanisms

The underlying events causing these multiple modifications of GABAergic functions still warrant additional studies. Keeping in mind the complex subunit architecture of $GABA_A$ receptors, changes of subunits not analyzed here are likely to be present, making the picture even more complicated. Furthermore, GABAergic transmission is regulated by a variety of additional factors affecting presynaptic GABA release and postsynaptic GABA efficacy. In particular, alterations in GABA synthesis and transport might contribute to an increased efficacy in the paramicrogyral zone. In this context, it has to be mentioned that the release of GABA from inhibitory terminals can be modulated by presynaptic $GABA_B$ autoreceptors (Bowery et al., 1980), which can inhibit the GABA release by as much as 40–60% (Davies et al., 1991; Mott et al., 1993; Thompson et al., 1993). Reduction in $GABA_B$ receptor binding in the surround of cortical malformations has been demonstrated recently (Zilles et al., 1998), probably contributing in part to the increase in GABAergic efficacy. Furthermore, additional alterations in density and function of GABAergic interneurons have to be considered. In the freeze-lesion model of cortical malformations, a reduction of parvalbumin-positive interneurons was described only temporarily in young animals (Jacobs et al., 1996; Rosen et al., 1998), whereas older animals, such as those used in this study, did not reveal a reduction of parvalbumin or calbindin immunoreactivity (P. Schwarz, C. C. Stichel, H. J. Luhmann, unpublished observations). Furthermore, the function of $GABA_A$ receptors is also modified via phosphorylation–dephosphorylation of specific subunits and a variety of endogenous and exogenous modulators, such as benzodiazepines, barbiturates, and neurosteroids, which potentiate the effects of GABA (Puia et al., 1990; Ito et al., 1996; Hevers and Luddens, 1998), as well as polyvalent cations like zinc, which diminishes the GABAergic efficacy (Buhl et al., 1996; Huang, 1997).

Clinical implications

The pattern of alterations in $GABA_A$ receptor subunit distribution corresponds well with clinical data on epileptic patients with focal cortical dysgenesis. In these patients, positron emission tomography was performed using ^{11}C-flumazenil as a tracer. ^{11}C-flumazenil is a neutral antagonist binding to the central benzodiazepine receptor, an allosteric modulatory site depending on the presence of both an α and a γ subunit. Using this method, widespread abnormalities in receptor binding were detected, involving not only the dysplastic area but also the structurally intact surround (Richardson et al., 1996).

The present findings strongly point toward widespread alterations in $GABA_A$ receptor subunit distribution. It has been shown recently using single-cell PCR techniques that, in a chronic model of mesial temporal lobe epilepsy, the development of behavioral seizures coincides with a decrease in mRNA for subunits $\alpha 1$ and $\gamma 2$, no change for subunit $\alpha 3$, and an increase for subunits $\alpha 4$, $\beta 3$, δ, and ϵ in dentate gyrus neurons. This switch in subunit composition altered the zinc sensitivity, providing therefore a possible mechanism for the generation of epileptic seizures (Buhl et al., 1996; Brooks-Kayal et al., 1998). A similar mechanism could play a role in this model; changes in $GABA_A$ receptor subunit distribution in widespread brain regions might aberrantly sensitize the receptors to endogenous modulators and therefore contribute to epileptogenesis. Alterations in $GABA_A$ receptor subunit distribution also have implications for antiepileptic drug therapy in patients with cortical malformations and help to explain the clinical finding that patients with cortical malformations frequently suffer from drug-resistant epilepsies (Palmini et al., 1994; Raymond et al., 1995).

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