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GABA receptor subunits in human auditory cortex in normal and stroke cases

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GABA receptors are ubiquitous in the cerebral cortex and play a major role in shaping responses of cortical neurons. $GABA_A$ and $GABA_B$ receptor subunit expression was visualized by immunohistochemistry in human auditory areas from both hemispheres in 9 normal subjects (aged 43–85 years; time between death and fixation 6–24 hours) and in 4 stroke patients (aged 59–87 years; time between death and fixation 7–24 hours) and analyzed qualitatively for $GABA_A$ and semi-quantitatively for $GABA_B$ receptor subunits. In normal brains, the primary auditory area (TC) and the surrounding areas TB and TA displayed distinct $GABA_A$ receptor subunit labeling with differences among cortical layers and areas. In postacute and chronic stroke we found a layer-selective downregulation of the α2 subunit in the anatomically intact cerebral cortex of the intact and of the lesioned hemisphere, whereas the α1, α3 and $\beta_{2/3}$ subunits maintained normal levels of expression. The $GABA_B$ receptors had a distinct laminar pattern in auditory areas and minor differences among areas. Unlike in other pathologies, there is no modulation of the $GABA_B$ receptor expression in subacute or chronic stroke.

Key words: GABA, human, postlesional plasticity, diaschisis, auditory

INTRODUCTION

The GABA (γ-aminobutyric acid) family of receptors, whose primary ligand is GABA, the main inhibitory neurotransmitter of the cerebral cortex, includes the $GABA_A$ receptor, the $GABA_B$ receptor and the recently described $GABA_C$ receptor (Beleboni et al. 2004, Chebib 2004). Functional $GABA_A$ receptors are fast-acting ligand-gated ion channels that gate a Cl^- channel and are formed in a pentameric structure composed of several subunits: α 1–6, β 1–3, γ 1–3, δ, ε, Φ, π, and ρ 1–3 (Burt 2003). The most frequent combination is α1 β2 γ2, which represents 60% of $GABA_A$ receptors and is found, among other structures, in all layers of the cerebral cortex. Two less frequent combinations are α2 β3 γ2 (15–20% of $GABA_A$ receptors) and α3 βn γ2 (10–15%), occurring in cortical layers I–VI and V–VI, respectively. All three combinations are localized in synapses. A fourth combination, α5 β3 γ2 (5% of $GABA_A$ receptors), has been found in the cerebral cortex, located extrasynaptically. Two other combinations, α4 βn δ (5%) and α6 $\beta_{2,3}$ γ2 (5%), have been found in brain structures other than the cerebral cortex (Fritschy and Brunig 2003, Mohler 2006).

In normal human brains $GABA_A$ receptors were investigated in visual (Albin et al. 1991, Hendry et al. 1994, Zilles et al. 2002, Eickhoff et al. 2007), prefrontal (Ishikawa et al. 2004, Loup et al. 2006) and non-auditory temporal cortices (Loup et al. 2006) but not in the auditory cortex. The latter is of particular interest because of its involvement in high level auditory analysis, including that of speech (Scott and Johnsrude 2003). Furthermore, the auditory cortex is the seat of plastic changes that occur, for example, when auditory discrimination is improved by training (Recanzone et al. 1993, Jancke et al. 2001, Ohl and Scheich 2005,

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Spierer et al. 2007). Evidence from electrophysiological studies in animal models suggests an important role of GABAergic inhibition in shaping the receptive fields of auditory cortex neurons, for example, frequency (Kaur et al. 2004, Chang et al. 2005).

Unlinke ionotropic $GABA_A$ receptors, the metabotropic $GABA_B$ receptors are slow long-lasting G-protein coupled receptors that activate a second messenger cascade and are indirectly linked to calcium and potassium channels (Beleboni et al. 2004, Bettler and Tiao 2006). They are composed of two heterodimers, $GABA_{B1}$ and $GABA_{B2}$, that must be bound in order to form a fully functional receptor (Marshall et al. 1999, Bettler and Tiao 2006). $GABA_{B1}$ contains the GABA-binding site and $GABA_{B2}$ contains the G-protein-coupling domain (Robbins et al. 2001, Bowery 2006). Presynaptically occurring $GABA_B$ receptors modulate neurotransmitter release by depressing Ca^{2+} influx *via* voltage-activated Ca^{2+} channels whereas postsynaptically occurring $GABA_B$ receptors predominantly modulate inwardly rectifying K^+ channels and mediate slow inhibitory postsynaptic potentials (Bowery et al. 2002, Cryan and Kaupmann 2005).

The highest concentration of $GABA_B$ binding sites in the mammalian brain was found in the thalamic nuclei, the molecular layer of the cerebellum, the cerebral cortex, the interpeduncular nucleus and the dorsal horn of the spinal cord (Bowery et al. 1987, Chu et al. 1990). In human and non-human primate cerebral cortex, $GABA_B$ receptor expression has been investigated in visual (Munoz et al. 2001, Eickhoff et al. 2007), somatosensory (Scheperjans et al. 2005, Garraghty et al. 2006), parietal (Scheperjans et al. 2005) and entorhinal areas (Mizukami et al. 2002). Further human studies were carried out in the cerebellum (Billinton et al. 2000) and the basal ganglia (Waldvogel et al. 2004) and displayed the co-occurrence of $GABA_{B1}$ and $GABA_{B2}$ subunits.

$GABA_B$ expression was shown to be modulated in chronic diseases such as epilepsy (Billinton et al. 2001a,b, Furtinger et al. 2003, Princivalle et al. 2003), Alzheimer's disease (Chu et al. 1987), schizophrenia (Mizukami et al. 2000, 2002), and bipolar disorders (Ishikawa et al. 2005). None of the human studies investigated postlesional changes in $GABA_B$ receptors associated with stroke.

The human auditory cortex is located on the supratemporal plane and consists of several cytoarchitectonic areas. The primary auditory area is believed to be located on Heschl's gyrus and to correspond to area 41 of Brodmann (1909); area TC of von Economo and Koskinas (1925); areas KAm plus KAlt of Galaburda and Sanides (1980); and areas Te1.0, 1.1 and 1.2 of Morosan and others (2001). It is surrounded by non-primary cortex, which is subdivided into two (areas 42 and 22; Brodmann 1909); three (Te 2.1, 2.2 and 3; Morosan et al. 2005); four (TB, TA, TD and TG; von Economo and Horn 1930); or six areas (PaAi, PaAc/d, PaAe, PaAr, ProA, and Tpt; Galaburda and Sanides 1980). Cytochrome oxidase, acetylcholinesterase, calbindin, calretinin and parvalbumin staining revealed additional subdivisions (Rivier and Clarke 1997, Clarke and Rivier 1998, Wallace et al. 2002, Chiry et al. 2003) that correlate, at least partially, with functional specialization for speech and non-speech sounds (Scott and Johnsrude 2003, Viceic et al. 2006).

Imaging studies have shown that brain damage may alter the physiological responsiveness within the auditory cortex, even if the lesion does not encroach upon it. A striking example is the loss of parallel processing of information relevant to sound recognition and to sound localization within the auditory "What" and "Where" streams, which has been reported in intact hemispheres of patients who sustained unilateral hemispheric stroke (Adriani et al. 2003a). Similarly, the auditory regions of the intact right hemisphere of aphasic patients have been found to change their response characteristics and increase their activation in response to speech stimuli (e.g., Leff et al. 2002, Crinion and Price 2005). Downregulation in GABAergic transmission in an anatomically intact auditory cortex may be the origin of these changes, as suggested by investigations in an animal model of stroke (Qu et al. 1998a,b, Que et al. 1999). For this reason we investigated the distribution of $GABA_A$ and $GABA_B$ receptor subunits within the primary and non-primary auditory areas in the normal human brain and in stroke cases.

METHODS

Cases

Brain tissue was obtained from 9 normal subjects, aged between 43 and 85 years (cases 1–9), and from 4 patients, aged between 59 and 87 years, who sustained stroke (Cases 10–13, Table I). Two subjects participated in a donor program (cases 8 and 11) and 11 underwent autopsy with consent from the patients'

relatives (Cases 1–7, 9, 10, 12, 13). The study was approved by the Ethical Committee of the Faculty of Biology and Medicine at the University of Lausanne. None of the normal subjects (cases 1–9) had a history of neurological and psychiatric diseases or auditory complaints and post mortem macro- and microscopic examination did not reveal the existence of brain lesions. All patients suffered stroke and died, according to clinical records and neuropathological examination of the lesions, more than 30 days later. Patient 10 sustained, 17 years prior to death, hemorrhagic stroke in the right basal ganglia, which left him with a left spastic hemisyndrome, a left homonymous hemianopsia, visuo-spatial memory deficits (whereas verbal memory was within normal limits) and signs of moderate executive dysfunction. Two years after stroke an audiogram was performed and was within normal limits. Patient 11 suffered, 16 years before death, a stroke with reported aphasic/dysarthric symptoms; no brain imaging was available from this period. Patient 12 suffered, 5 weeks before death, a right hemispheric ischemic stroke with a left sensorimotor hemisyndrome; a CT scan performed upon hospital admission visualized diffuse leucoencephalopathy but not the acute lesion. Patient 13 suffered a subacute stroke in the frontal white matter on the left side, which was diagnosed only during autopsy.

The control and stroke populations did not differ in age, time between death and fixation or postfixation time (Table I). However, there was a sex bias, only 2 out of the 9 control cases being female *versus* 3 out of the 4 stroke cases. To our knowledge, there is no evidence as to sex-related differences in $GABA_A$ receptors. Diagnosed diabetes was distributed unequally in the two populations; it was reported in 4 out of the 9 control cases versus only in 1 out of the 4 stroke cases. This is, however, very unlikely to account for the downregulation of the $GABA_A$ subunit which we report for stroke cases; the only current evidence being circumstantial and suggesting a decrease in $GABA_A$ receptor function in pancreatomized (i. e. potentially diabetic) rats (Balarama Kaimal et al. 2007).

Tissue processing

Whole brains were removed 7–24 h after death and perfused through the basilar and the two internal carotid arteries in a skull-shaped perforated plastic container to preserve shape and prevent distortion of the brain during perfusion. First, 5 ml of heparin (Liquemin, 25 000 UI; Roche Pharma, Switzerland) were injected into each artery followed by 1–2 l of isotonic saline solution to wash out residual blood and clots. This was followed with 1–2 l of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB, pH 7.4) at 4°C for approximately 30 minutes. The brains were then cut into 1 or 2 cm thick coronal slices; postfixed by immersion in 4% PFA for 24–144 hours at 4°C; cryoprotected in graded concentrations of sucrose solution (10–30% in PB) at 4°C; rapidly frozen; and stored at –20°C. Blocks containing the regions of interest were cut frozen into serial, 40 μm-thick sections; the first sections of each set of 25 sections, corresponding to a 1 mm slab of the block; were stained with Cresyl violet or thionin while the remaining sections were stored, in batches of five, at −20°C in cryoprotectant solution until processed for immunohistochemistry. Detailed photographic documentation was carried out throughout the processing of the brains (same procedure as Rivier and Clarke 1997). In cases 10–13 sections containing lesions were also stained for haematoxylin and eosin, GFAP and CD68. Chronic lesions were demonstrated in cases 10, 11 and 12 (oligodendrocytic gliosis in perilesional white matter indicating Wallerian degeneration; CD68 positive macrophages absent in the vicinity of lesions, present only in perivascular spaces, which is compatible with the history of arterial hypertension), whereas additional subacute lesions (characterized by the presence of CD68 positive macrophages at lesion border) were found in cases 11 and 12 and only subacute in case 13.

Immunohistochemistry

In a preliminary study we assessed the reliability of the commercially available antibodies against human $GABA_A$ subunits α1, α2, α3, $\beta_{2/3,}$ γ2, δ and π in the normal cases of this study and in particular the consistency of staining in cases with different times between death and fixation and different postfixation durations (Table I). This preliminary investigation showed that the staining with antibodies against α1, α2, α3 and $\beta_{2/3}$ subunits was consistent, whereas that with antibodies against γ2 or δ showed loss of neuronal staining with prolonged time-between-death-and-fixation and/or postfixation duration. The π subunit did not yield any neuronal labeling in the auditory cortex either in nor-

Table I

Description of cases

Case	Age (years)	Sex	TDF (h)	PF (h)	Cause of death; concurrent disease [1]	Location of lesion	Size of lesion (cm)	Stage of lesion
1	43	M	10	65	Acute myocardial infarction; arterial hypertension, diabetes	–	–	–
2	46	M	12	24	Septic shock; chronic myeloid leukaemia	–	–	–
3	61	M	22	24	Cardiac failure; acute mesenterial infarction; cardiovascular disease	–	–	–
4	70	F	15	24	Cardiac failure; diabetes, arterial hypertension, cardiovascular disease	–	–	–
5	72	M	6	73	Cardiac failure; lung fibrosis, previous myocardial infarctions	–	–	–
6	73	M	7	120	Cardiac failure; diabetes, arterial hypertension, cardiovascular disease	–	–	–
7	79	M	17	45	Hepatic failure; metastatic prostatic cancer, arterial hypertension, diabetes, cardiovascular disease	–	–	–
8	80	F	9	24	Acute kidney failure; metastatic uterine carcinosarcoma	–	–	–
9	85	M	24	48	Cardiac failure; hypertensive cardiopathy	–	–	–
10	68	M	24	48	Broncopneumonia;	R parietal white matter	2 × 3 × 3	Chronic
					arterial hypertension, nephropathy	R internal capsula	0.5 × 2 × 1	chronic
					myocardial infarction (7 years prior to death)	R lenticular nucleus	lacunes	Chronic
						R medio-inferior occipital	2 × 4 × 7	Chronic

Case	Age (years)	Sex	TDF (h)	PF (h)	Cause of death; concurrent disease [1]	Location of lesion	Size of lesion (cm)	Stage of lesion
						L parietal white matter	lacunes	Chronic
						L lenticular nucleus	lacunes	Chronic
11	70	F	7	111	Acute cardiac failure; arterial hypertension	L temporal stem	0.5 × 1.5 × 1.5	Chronic
					asthma	R lateral occipital white matter	1 × 1.5 × 1	Subacute
						R basal ganglia	lacunes	Subacute–chronic
12	87	F	24	141	Intestinal haemorrhage; arterial hypertension,	R parietal	1 × 1 × 1	Chronic
					atrial fibrillation (for 10 years)	R internal capsule	0.3 × 0.5 × 0.5	Subacute
						R lenticular nucleus	lacunes	chronic
						R insula	0.5 × 0.8 × 0.5	Subacute
13	59	F	18	96	Diabetic failure and septic shock: arterial hypertension, hepatic adenocarcinoma	R prefrontal	1 × 1 × 1	subacute

Location, size and stage of brain lesions were dated at autopsy by macro- and microscopic examination; the maximal extent of lesion is indicated along the axes: medio-lateral × supero-inferior × antero-posterior axes. [1]For neurological affections see case descriptions in Methods. (F) female; (M) male; (R) right; (L) left; (PF) postfixation; (TDF) time between death and fixation.

mal or stroke cases as expected from previous animal studies (Mohler et al. 2002). Antibodies against α 4–6, β1, γ 1,3, ε, Φ, and ρ 1–3 subunits that would be suitable for human tissue were not commercially available at the time of this study.

Serial sections from the supratemporal region and the superior temporal gyrus from both hemispheres of all cases were labeled immunohistochemically for the subunits α1, α2, α3, $\beta_{2/3}$ and π of the $GABA_A$ receptor and for the $GABA_{B1}$ and $GABA_{B2}$ subunits with an adaptation of a previously described protocol (Armstrong et al. 1994). Briefly, free-floating tissue sections were sequentially processed at room temperature, unless otherwise noted, as follows: (1) washed in 0.3% hydrogen peroxide in 0.25% Triton X-100 in Tris-buffered saline (TBS, pH 7.4, 45 min); (2) rinsed with 0.25% Triton X-100 in TBS (TBST); (3) blocked with 3% serum (in agreement with the secondary antibody host) in TBST (30 minutes); (4) blocked with 1% serum in TBST (2 × 10 min); (5) incubated overnight at 4°C in primary antibodies diluted in 1% serum in TBST as follows: α1 subunit (1:2 000, Chemicon: MAB339), α2 subunit (1:4 000, gift from J. M. Fritschy) (Loup et al. 2006), $\beta_{2/3}$ subunit (1:20 000, gift from J. M. Fritschy)

(Loup et al. 2006); π subunit (1:100, Abcam: ab26055), $GABA_{B1}$ (1:1 000, Chemicon, AB1531), $GABA_{B2}$ (1:500, Chemicon, AB5394); (6) rinsed in TBST with 1% serum (2 × 10 minutes); (7) incubated in biotinylated secondary antibody (Vector Labs, Burlingame, CA, USA) diluted 1:200 in TBST with 1% serum: goat anti-mouse for α1 and $\beta_{2/3}$ subunits, goat anti-guinea pig for the α2, $GABA_{B1}$, and $GABA_{B2}$ subunits, or goat anti-rabbit for π subunit); (8) rinsed with TBST (3 × 5 min); (9) blocked in avidin-biotin complex (as recommended in the ABC kit elite, Vector Labs); (10) rinsed in TBST (3 × 5 min); (11) rinsed in imidazole acetate buffer (IAB, pH 7.4, 3 × 5 min); (12) treated for 4 min with IAB (pH 9.6) containing 0.05% diaminobenzidine, 2.5% nickel ammonium sulfate and 0.005% hydrogen peroxide; (13) rinsed in IAB (pH 7.4, 3 × 5 min).

For $GABA_A$ α3, sections followed steps (1) and (2), were microwaved for 5 minutes in 500 ml of 0.01 M sodium citrate buffer, cooled in solution for 1 h, and followed the remaining steps of the protocol [step (5) 1:100, Chemicon: AB5594, step (7) goat anti-rabbit]. Sections were mounted on chrom-alum slides. For controls, adjacent sections were processed without the primary antibody to detect non-specific labeling.

Photomicrographs were taken with a Zeiss AxioPlan 2 microscope and Axiocam camera. For the high-magnification photos, stacks of 1 µm spaced photomicrographs were taken through the section and projected on a single plane. Images were assembled and corrected for contrast with Adobe Photoshop (USA). To assess layer profiles of $GABA_A$ receptor subunit expression, low magnification photomicrographs of areas TC, TB or TA including all cortical layers were taken, with the same microscope light settings for all samples. The density of the neuropil labeling was measured by Scion Image for Windows (Scion Corporation, Frederick, Maryland, USA). Plot profiles (glass slide, mounting medium plus cover slip = 0, darkest immunohistochemical staining within a given profile = maximum) were imported into Photoshop; the border between the cortex and the white matter and borders between cortical layers were determined in the same regions from adjacent Nissl stained sections and then transferred onto the densitometric profiles.

Integrity of the tissue as assessed by electron microscopy

The integrity of the auditory cortex was assessed by electron microscopy. After perfusion, a 10 × 8 × 1.5 mm block of cortical tissue was removed from the right Heschl's gyrus of case 6 and left in fixative (2.5% glutaraldehyde, 2% paraformaldehyde in cacodylate buffer 0.1 M, pH 7.4) for 12 hours. Sixty µm-thick coronal sections were then cut with a vibratome (Leica VT100; Wetzlar, Germany), washed in cacodylate buffer (0.1 M, pH 7.4), dehydrated in alcohol and embedded in Durcupan resin (Fluka, Buchs, Switzerland) between silicon coated glass slides. Once the resin cured after 24 h at 60°C, a trapezoid block was prepared encompassing layer III of auditory area TC. Series of 60–100 silver/grey (60 nm thick) thin sections were cut on single-slot grids bearing a Formvar support film that had been lightly carbon coated. Imaging of the neuropil with a digital camera (Mega View III, SIS, Munich, Germany) in a Philips CM12 electron microscope using an accelerating filament voltage of 80 kV, showed the well-preserved symmetrical and asymmetrical synapses (Fig. 1), the former of which are most likely GABAergic.

Quantitative study and statistical analysis

Numerical densities of immunopositive neurons were quantified using a DMR microscope (Zeiss, Oberkochen, Germany) equipped with a motorized stage and Stereo Investigator software-controlled computer system (MicroBrightField, Inc, USA). The procedure was as described previously (Leuba et al. 2004). Briefly, the total cortical area of interest was delineated using a 1.25× magnification on live microscopic images displayed on a monitor. Counting frames were created by the software and placed at the intersections of a grid that had been randomly placed over the section. The counting frames were replaced systematically by the stepwise movements in x- and y-directions. The parameters for the quantitative analysis were counting height 15 µm, guard space depth 1 µm, distance between the optical dissectors varied from 300 µm to 500 µm according to the size of the counting area.

Data were analyzed using the SAS package (SAS Institute Inc., Cary, NC, USA). The distribution of data values was tested for normality with the UNIVARIATE procedure and data were rank transformed with the PRINQUAL procedure in order to obtain Gaussian distributions. The effect of group, area and layer was tested using a three way ANOVA with a general linear model procedure. Multiple comparisons between means were done using the *post-hoc* Tukey test to check for significant differences at $P \leq 0.05$.

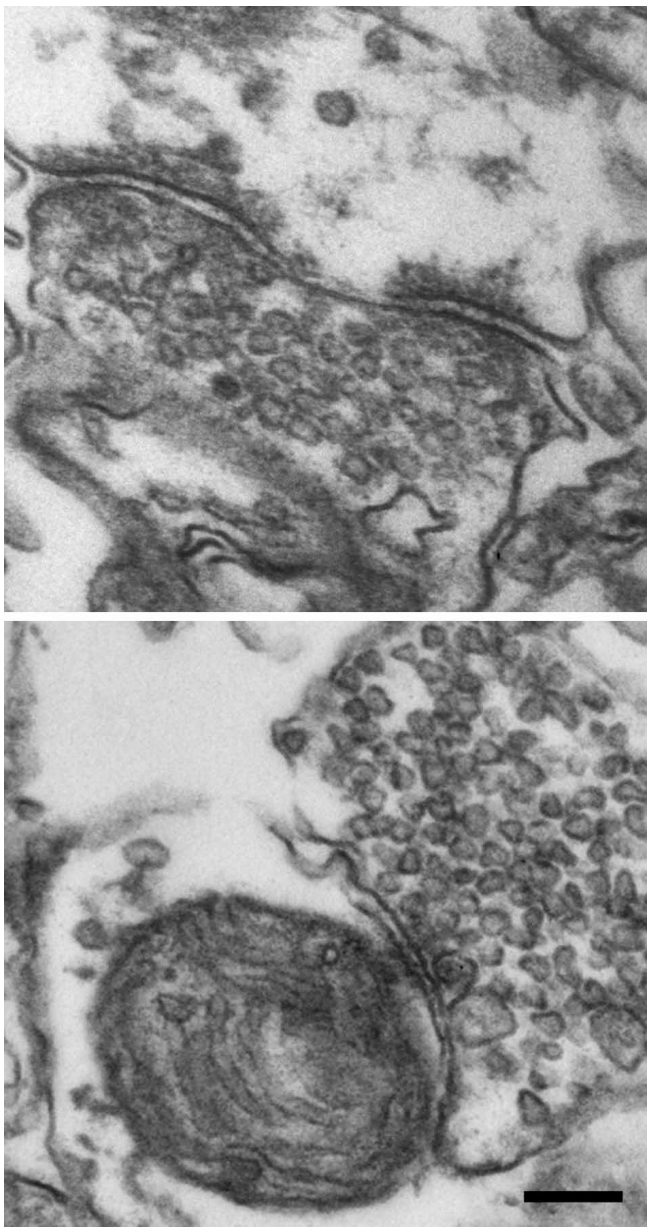

Fig. 1. Electron microscope images of asymmetric (top) and symmetric (bottom) synapses located in layer III of Heschl's gyrus (area TC) from the right hemisphere of case 6. Scale bar is 200 nm.

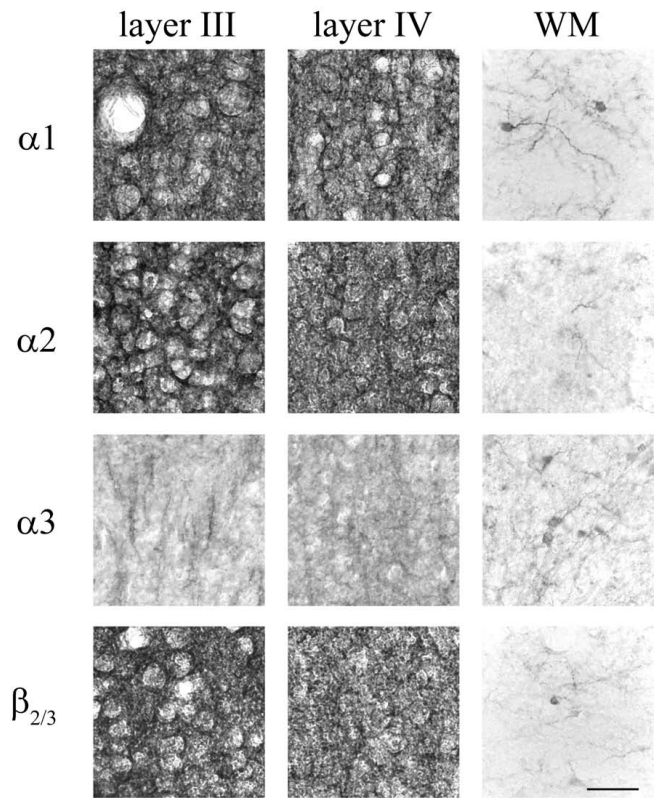

Fig. 2. High magnification photomicrographs for the $GABA_A$ subunits α1, α2, α3 and $\beta_{2/3}$ in layers III and IV of area TC and in the underlying white matter (WM) of a normal brain (case 8). Scale bar is 50 μm. Note the dense neuropil labeling in the grey matter. The honeycomb pattern for α1, α2 and $\beta_{2/3}$ is due to unlabeled neuronal cell bodies. In the white matter occasional axons and neuronal somata were labeled for all 4 subunits, whereas α3 produced labeled dendritic processes in lower layer III.

RESULTS

The supratemporal plane contains distinct cytoarchetectonic areas TC (most often situated on Heschl's gyrus), TB and TA (von Economo and Horn 1930, Morosan et al. 2001). Area TC, corresponding to the primary auditory cortex, is characterized by a thick and granular layer IV; area TB by the presence of large and darkly stained pyramidal neurons in layer III; and area TA by the presence of large pyramidal neurons in layers III and V [see also Table II in Rivier and Clarke (1997)]. In the present study we have used cytoarchitectonic criteria to localize these three areas and analyzed, in adjacent or nearby sections, the labeling patterns of $GABA_A$ (α1, α2, α3 and $\beta_{2/3}$) and $GABA_B$ subunit immunoreactivity.

$GABA_A$ receptor subunits in normal auditory cortex

The $GABA_A$ receptor subunits α1, α2, α3 and $\beta_{2/3}$ were expressed throughout the neuropil of all cortical levels as well as in individual neuronal somata and fibers in the white matter (Fig. 2). In layers II–VI the labeling displayed a honeycomb pattern around neuronal somata.

For the α1, α2, α3 and $\beta_{2/3}$ subunits the overall labeling intensity was roughly similar all over the supratemporal plane, the superior temporal gyrus, and the upper bank of the superior temporal sulcus (Fig. 3). In particular, none of the areas or their subparts stood out as more intensely labeled for any of the subunits. However, there were layer-specific differences among subunits and, for a given subunit, among cytoarchitectonic areas.

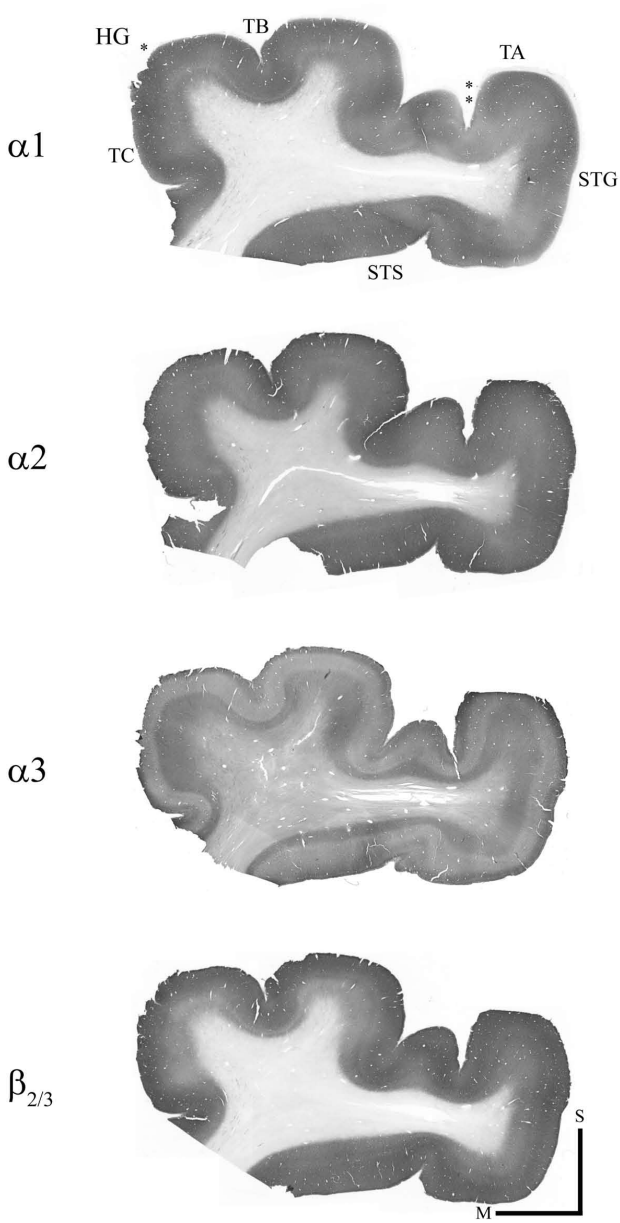

Fig. 3. Low magnification photomicrographs of adjacent coronal sections through the supratemporal plane, the superior temporal gyrus (STG), and the upper bank of the superior temporal sulcus (STS) at the level of Heschl's gyrus (HG), labeled for the $GABA_A$ subunits α1, α2, α3 and $\beta_{2/3}$ in a normal brain (case 8). The position of areas TC, TB and TA and their respective boundaries (* TC/TB; ** TB/TA) are indicated as determined on an adjacent Nissl-stained section. (M) medial; (S) superior. Scale bar is 5 mm.

$GABA_A$ α1 subunit

The intensity of the $GABA_A$ receptor α1 subunit varied partially among layers and the layer-specific profiles differed to a certain extent among the three areas (Fig. 4). Area TC had darkly labeled supragranular (I–III) and granular (IV) layers with less intensely labeled infragranular layers (V–VI; Figs 4, 5). Area TB, in comparison, had a relatively darkly labeled layer IV and upper supragranular layers with lighter bands in lower layer III and upper layer V. Area TA had a banding pattern similar to that of area TB. The transitions between TC/TB and TB/TA did not occur abruptly, but gradually over a few hundred microns (Fig. 3).

$GABA_A$ α2 subunit

The $GABA_A$ α2 subunit also displayed laminar and area specific labeling. The α2 pattern in area TC resembled that of α1, with layers I–IV labeled more darkly than the infragranular layers (Figs 4, 5). Area TB had a more striking banding pattern for α2 than for α1. The upper supragranular layers and layer IV were labeled very darkly with lighter bands flanking layer IV. Lower layer V and VI were darker than the light bands in lower layer III and upper layer V, but were still lighter than the upper supragranular layers (Figs 4, 5). The α2 pattern in area TA resembled that in area TC with darkly labeled layers I–IV and lighter infragranular layers. The transition between TC and TB occurred gradually over ~500 μm from lightly reactive infragranular layers in TC to striped infragranular layers in TB. As seen with the α1 staining, high magnification photos revealed a high level of neuropil labeling (Fig. 2).

$GABA_A$ α3 subunit

The $GABA_A$ α3 subunit labeling showed a strong laminar specificity. The immunoreactivity was most intense in layer I and the upper part of layer II, relatively light in layer III and moderate in layers IV, V and VI and did not vary among auditory areas TC, TB and TA (Fig. 3). In layer III of all areas, lightly labeled soma were visible as were moderately labeled dendritic pro-

cesses. These processes were more numerous and more densely packed in area TC, with fewer in area TA, and the fewest and longest processes in area TB.

$GABA_A$ $\beta_{2/3}$ subunit

The $GABA_A$ $\beta_{2/3}$ subunit also strongly labeled the neuropil. As with the previous two subunits, the supragranular layers and layer IV of area TC were darkly labeled with a lighter infragranular layer (Figs 3, 4, 5). The infragranular layers of TC were labeled more lightly with $\beta_{2/3}$ than with either α1 or α2. Area TB followed the same banding pattern seen with α1 and α2, but the band in lower layer III was only marginally lighter than layer IV and the upper supragranular layers. Upper layer V in TB was clearly lighter than the adjacent layer IV and the lower infragranular layers. Area TA had a banding pattern that was similar to the one seen with α1. The transition between TC and TB gradually changed from a lightly labeled infragranular layer in TC to a striped infragranular layer in TB, similar to α1 and α2. High magnification photos revealed a high level of neuropil staining, as seen with both α1 and α2 staining (Fig. 2).

$GABA_A$ receptor subunits in the auditory cortex of stroke cases

In cases of subacute and/or chronic stroke, the expression of the $GABA_A$ receptor subunits α1, α3 and $\beta_{2/3}$ were similar to that of normal brains (Figs 6, 7). In particular, the intensity of the $GABA_A$ α1 subunit varied among layers and the layer-specific profiles differed to a certain extent among the three areas. As in normal brains, area TC had darkly labeled supragranular (I–III) and granular (IV) layers with less intensely labeled infragranular layers (Figs 5, 7) whereas areas TB and TA had a relatively darkly labeled layer IV and upper supragranular layers with lighter bands in lower layer III and upper layer V. The transition areas between TC/TB and TB/TA do not occur abruptly, but gradually over a few hundred microns (Fig. 6).

The $GABA_A$ α3 subunit showed the same laminar variations as in normal brains, with dark staining in layer I and the upper part of layer II, relatively light in layer III and moderate in layers IV, V and VI (Figs 6, 7). As in normal cases this pattern was the same in all three auditory areas.

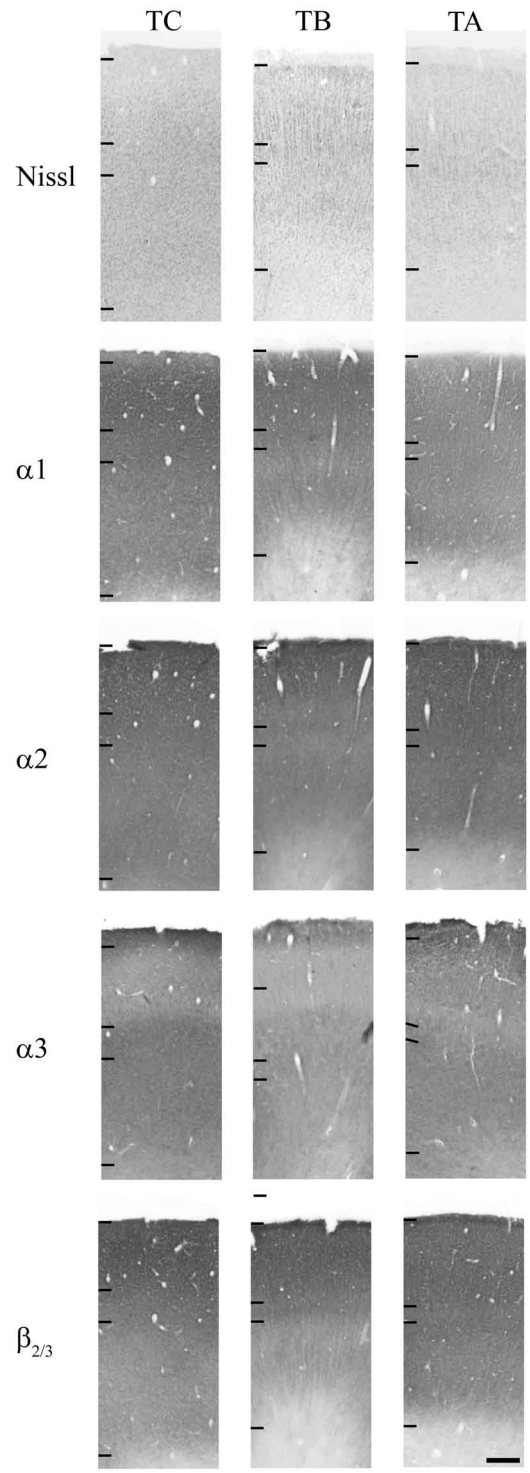

Fig. 4. Photomicrographs of Nissl stained (top row) and $GABA_A$ subunit α1, α2, α3 and $\beta_{2/3}$ labeled sections through areas TC, TB and TA of a normal brain (case 8). Pia is up, white matter down; short lines on the left side of photographs denote limits between layers I/II, III/IV, IV/V and VI/white matter. Scale bar is 0.5 mm.

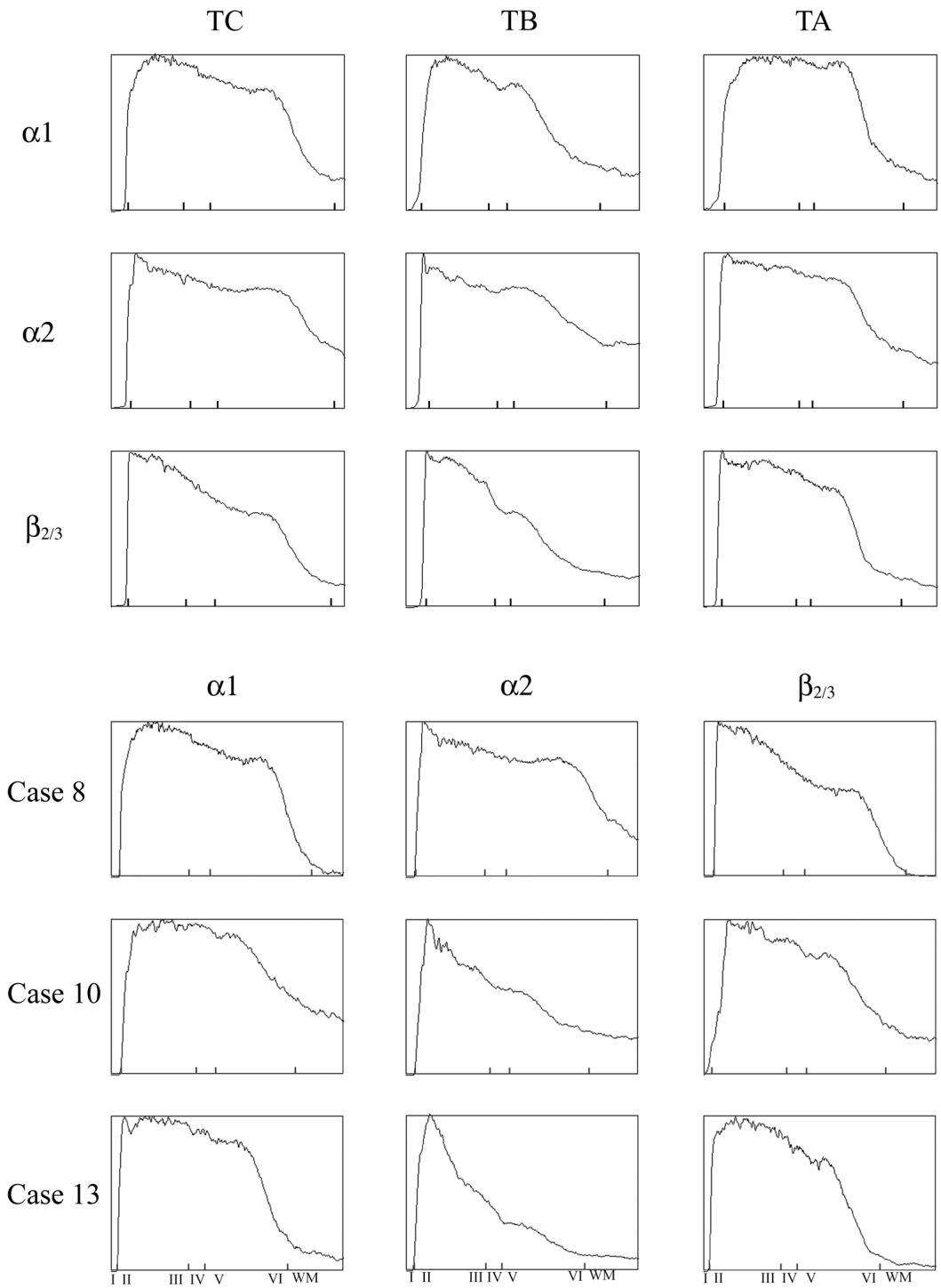

Fig. 5. Densitometric profiles of immunohistochemical staining for α1, α2 and $\beta_{2/3}$ subunits comparing TC, TB and TA in normal cases (top panel); and comparing area TC in normal vs. stroke cases (bottom panel; case 8 = normal; cases 10 and 13 = stroke). For each profile, layer I is to the left, the white matter to the right; the five short lines along the x-axis indicate the limits between layers I/II, III/IV, IV/V and the limit between layer VI and the white matter, respectively. The density is indicated in arbitrary units along the y-axis; the minimum corresponds to the density of the glass slide plus mounting medium plus cover slip, the peak to the maximum within the region measured. Note the loss of α2 expression in granular and infragranular layers in stroke as compared to normal cases.

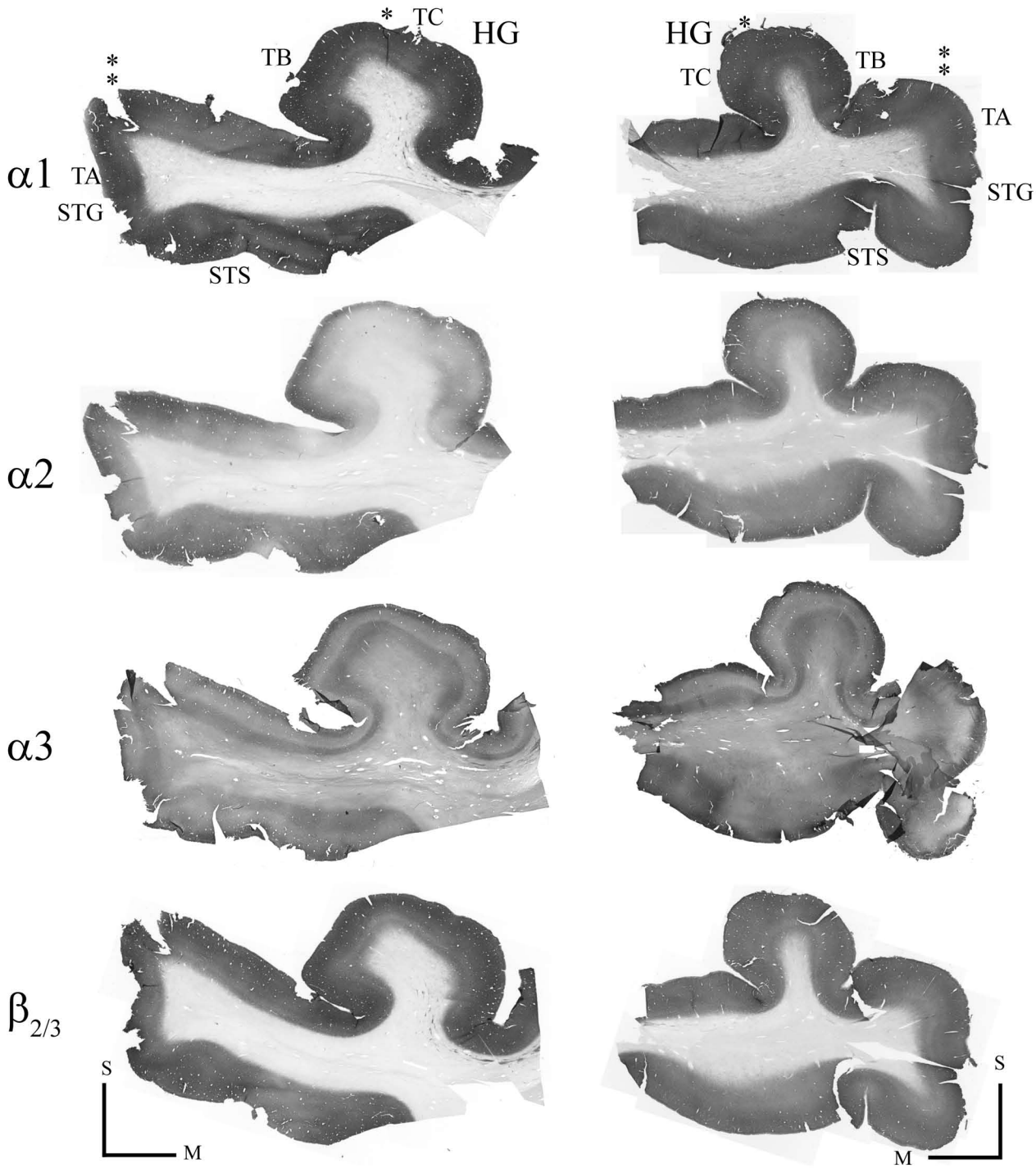

Fig. 6. Low magnification photomicrographs of adjacent coronal sections through the supratemporal plane, the superior temporal gyrus (STG), and the upper bank of the superior temporal sulcus (STS) at the level of Heschl's gyrus (HG), labeled for the $GABA_A$ subunits α1, α2, α3 and $\beta_{2/3}$ in two stroke cases (left hemisphere of case 13 on the left; right hemisphere of case 10 on the right). Same conventions as Fig. 3. Scale bar is 5 mm. Note the weak labeling, predominant in deep layers, of the α2 subunit, as compared to a normal case (Fig. 3).

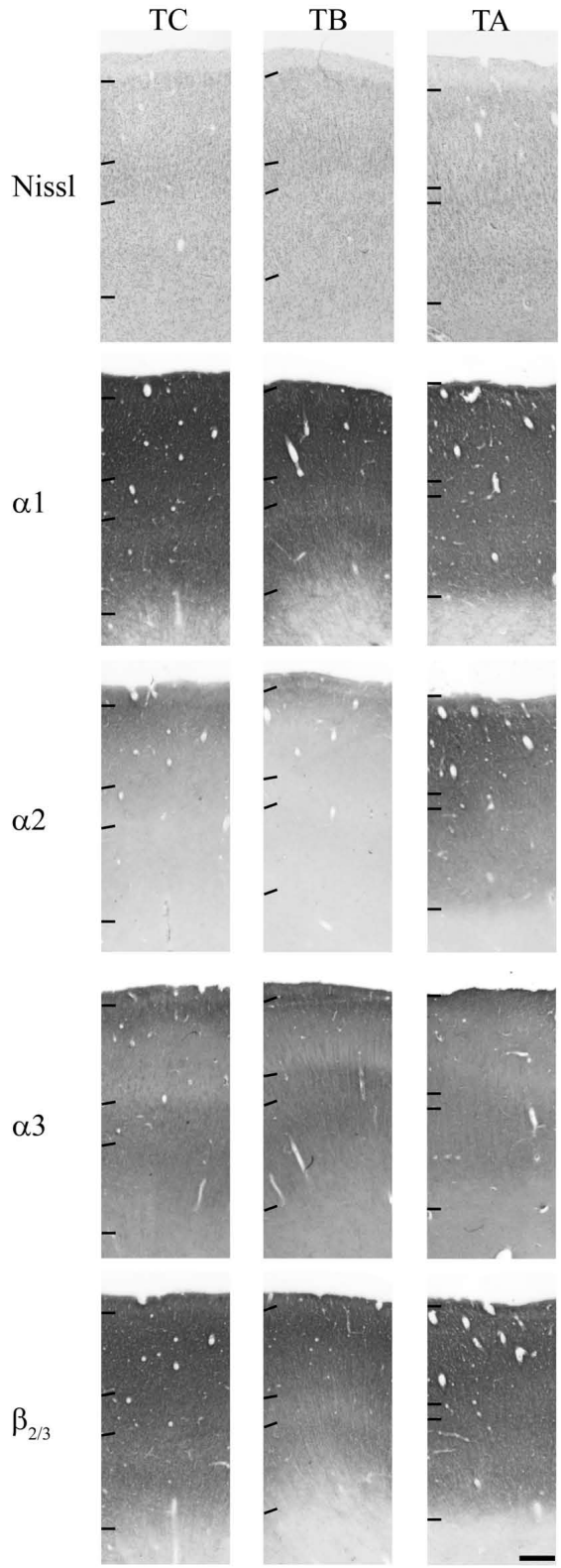

Fig. 7. Photomicrographs of Nissl stained (top row) and $GABA_A$ subunit α1, α2, α3 and $\beta_{2/3}$ labeled sections through areas TC, TB and TA of a stroke case (case 13). Same conventions as in Fig. 4. Scale bar is 0.5 mm.

The $GABA_A$ $\beta_{2/3}$ subunit showed the same areal and laminar variations as in normal brains. In TC, the supragranular layers and layer IV were darkly labeled with a lighter lower layer III and upper layer V (Figs 5, 6, 7). Area TA had a banding pattern that was similar to the one seen with α1. As in normal brains, the transition between TC and TB gradually changed from a lightly labeled infragranular layer in TC to a striped infragranular layer in TB (Fig. 6).

The staining pattern of the $GABA_A$ α2 subunit was very different in stroke cases from that in normal brains. In stroke cases, the α2 subunit showed a marked decrease in the granular and infragranular layers in area TC and a smaller decrease in TB (Figs. 5, 6, 7, 8) when compared to normal brains. This decrease was specific for the α2 subunit, since, as described above, no such downregulation occurred for the α1, α3 and $\beta_{2/3}$ subunits. In contrast to normal cases, no α2 banding pattern was observed in TB (Fig. 5, 7, 8).

$GABA_{B1}$ subunit

The $GABA_{B1}$ subunit labeled numerous pyramidal neurons in supra- and infragranular layers, and to a lesser degree in the granular layer of areas TC, TB and TA (Fig. 9, 10). Multipolar neurons were intensely labeled in layers III and IV in TC, TB and TA, but were relatively scarce. Bipolar and bitufted neurons were moderately numerous in all auditory areas, but were restricted to layers III and VI. They were more numerous than the multipolar neurons, but were still infrequent when compared to the pyramidal neurons. Also visualized throughout layers II through VI were lightly labeled non-identified neurons. The same neuronal types, with roughly the same layer distribution, were labeled in normal and in stroke cases. Both in normal and in stroke cases, the labeling was stronger in supra- then in infragranular layers (Fig. 10).

The density of $GABA_{B1}$ positive neurons varied partially among layers and areas, but not between normal and stroke cases (Figs 10, 11, 12). The three way ANOVA (group: normal vs. stroke; area: TC, TB, TA; layer: II–III, IV, V–VI) for the rank transformed all, pyramidal or non-pyramidal $GABA_{B1}$ positive neurons gave the following results. For all $GABA_{B1}$ positive neurons, there was a significant effect for area ($P<0.0001$), but not for group or layer. A *post-hoc* Tukey test did not show, however, any significant differences for comparisons between means.

For $GABA_{B1}$ positive pyramidal neurons, the three way ANOVA showed a significant effect for area (P=0.021) and for layer (P<0.0001), but not for group. A *post-hoc* Tukey test has shown, in normal and in stroke cases, statistically significant differences among the densities in layers II–III vs. IV and in layers II–III vs. V–VI in each of the areas investigated (i.e., TC, TB, TA); layers II–III has on average significantly higher densities than layers IV or V–VI. In stroke, but not in normal cases, areal differences were demonstrated; the density of $GABA_{B1}$ positive pyramidal neurons in layers V–VI was significantly lower in area TA than in areas TC or TB.

For $GABA_{B1}$ positive non-pyramidal neurons, the three way ANOVA showed a significant effect for area (P<0.0001), but not for group or layer. A *post-hoc* Tukey test revealed statistically significant differences among areas; the density of $GABA_{B1}$ positive non-pyramidal neurons in layers II–III was significantly lower in area TA than in areas TC or TB, both in normal and in stroke cases. Furthermore, their density in layers V–VI was significantly lower in area TA than in

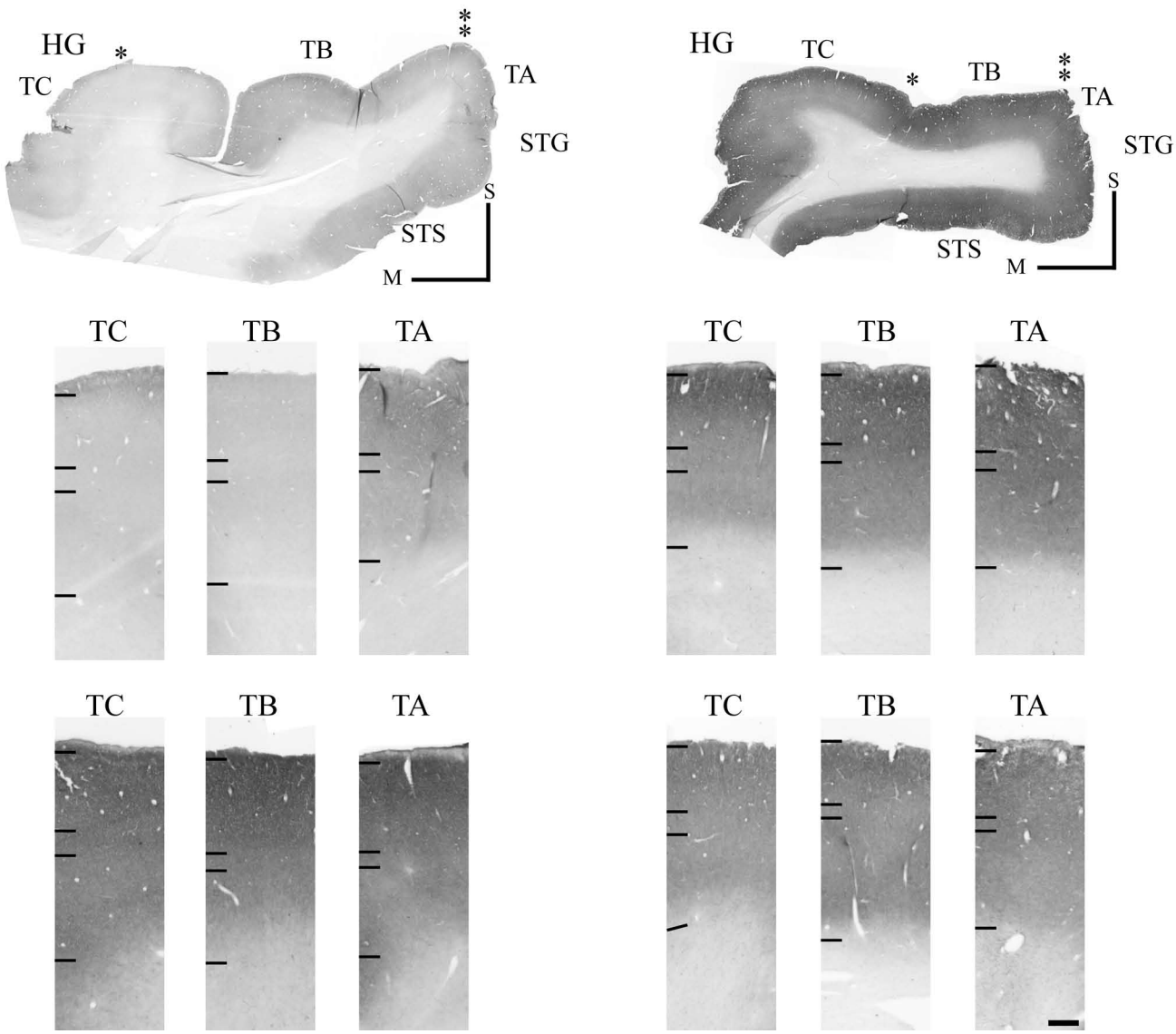

Fig. 8. Low magnification photomicrographs of α2 labeled sections through the supratemporal plane (top row – right hemisphere of case 13 on the left; right hemisphere of case 12 on the right; same conventions as in Fig. 3) and higher magnification photomicrographs of areas TC, TB and TA (middle row – right hemisphere of case 13 on the left; right hemisphere of case 12 on the right; bottom row – left hemisphere of case 10 on the left and left hemisphere of case 12 on the right). Same conventions as in Fig. 4.

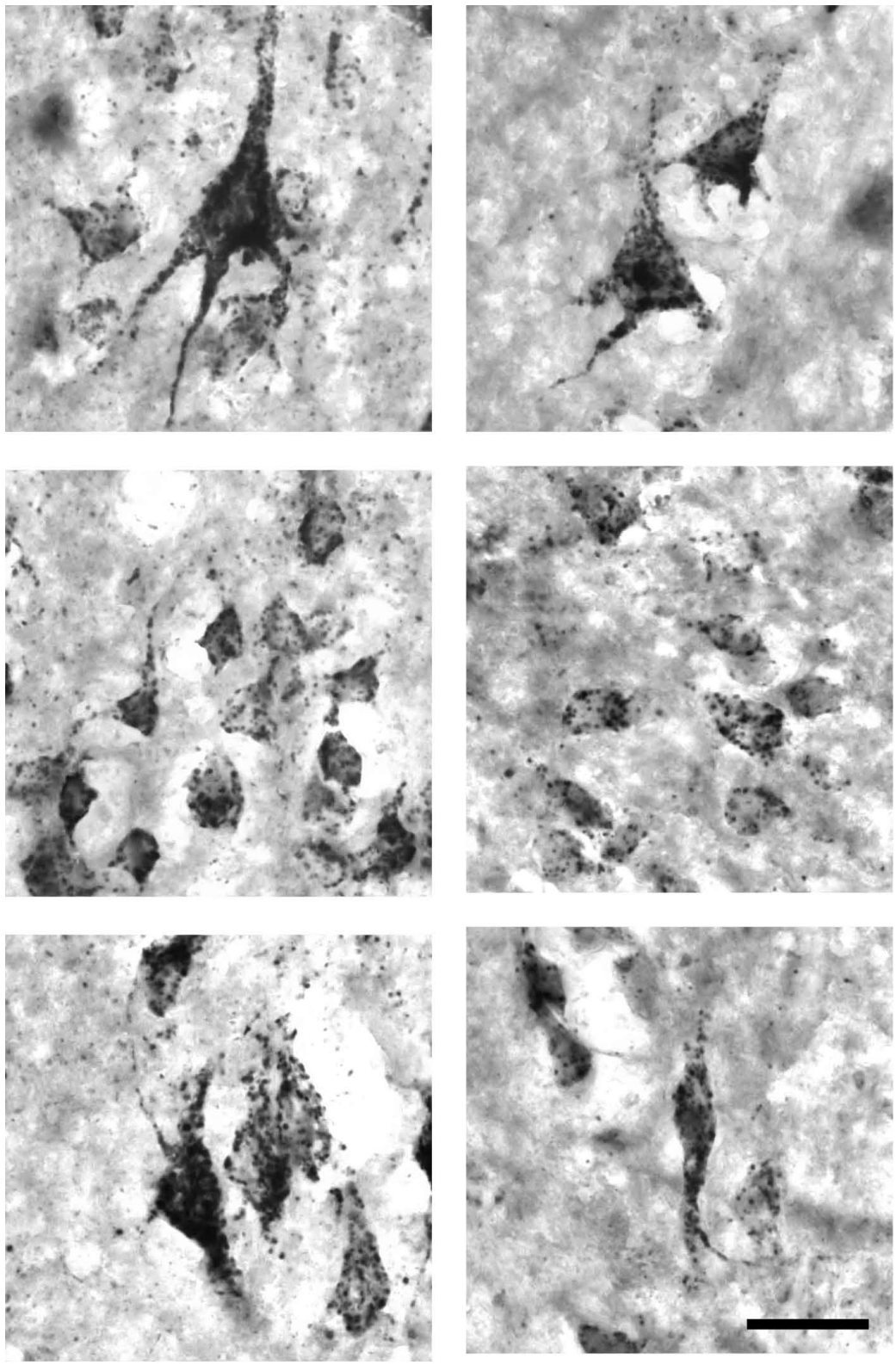

Fig. 9. High magnification photomicrographs of $GABA_{B1}$ (left column) and $GABA_{B2}$ labeling (right column) in supragranular (top row), granular (middle row) and infragranular layers (bottom row) from area TB of a normal brain (right hemisphere of case 8). Scale bar is 20 μm. Note the strong expression on somata and proximal dendritic shafts of pyramidal and non pyramidal neurons as well as the less strong expression limited to the somata of other neurons.

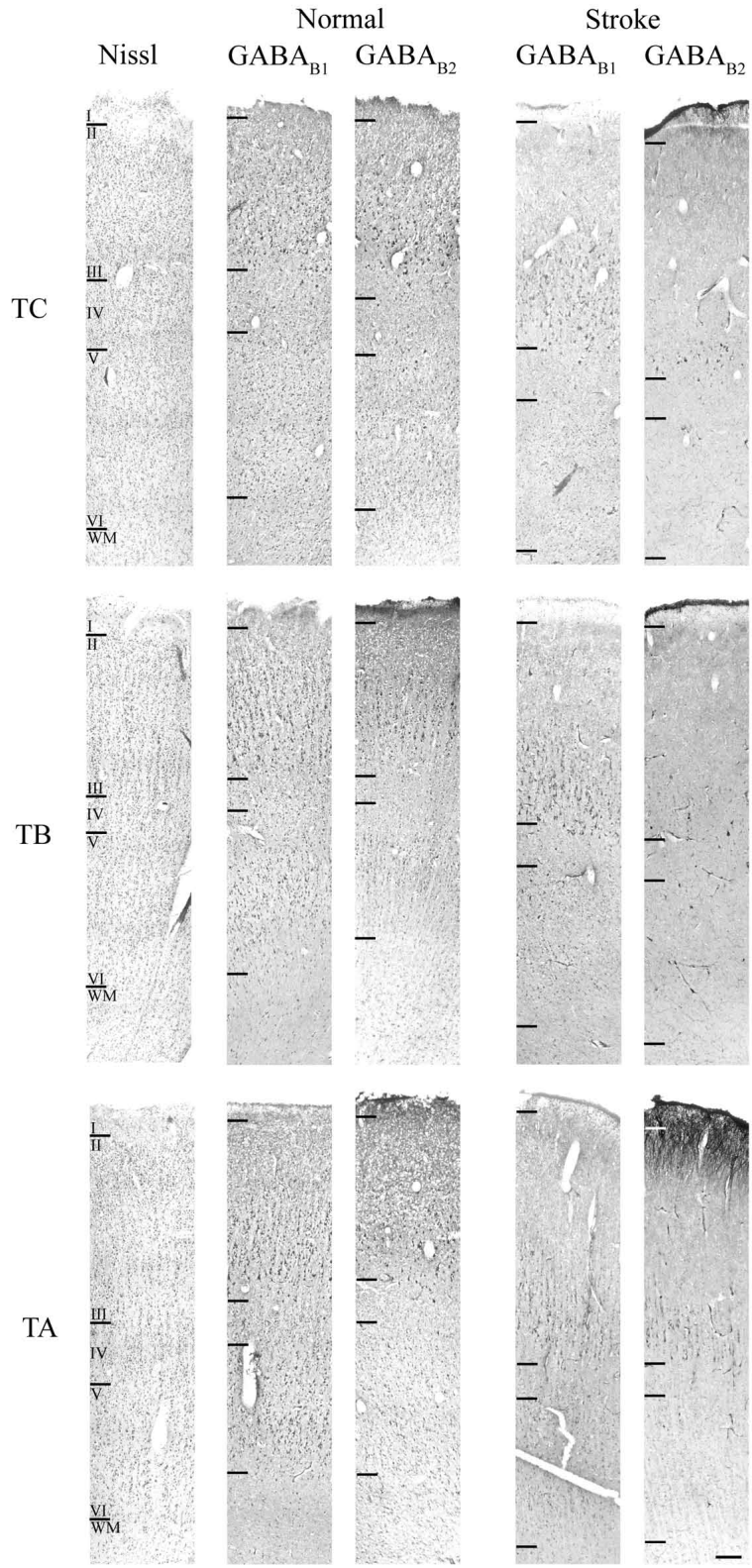

Fig. 10. Photomicrographs of Nissl stained or GABA$_{B1}$ or GABA$_{B2}$ receptor labeled sections through TC (top row), TB (middle row) and TA (bottom row) of a normal brain (right hemisphere of case 8, left three columns) and a stroke case (right hemisphere of case 10, right two columns). Pia is up, white matter down; short lines on the left side of photographs denote limits between layers I/II, III/IV, IV/V and VI/white matter. Scale bar is 0.2 mm.

areas TC or TB in normal cases and than in area TC in stroke cases.

$GABA_{B2}$ subunit

The $GABA_{B2}$ subunit intensely labeled numerous pyramidal neurons in layers II–VI, as well as relatively rare multipolar neurons in layer IV and bipolar and bitufted neurons in layers III and VI of areas TC, TB and TA. In addition to the darkly labeled, above described neurons, layers II through VI contained lightly labeled non-identifiable neurons. Both in normal and in stroke cases, the labeling was stronger in supra- than in infragranular layers (Fig. 10).

The density of $GABA_{B2}$ positive neurons varied among layers and partially among areas, but not between normal and stroke cases (Figs 10, 11, 12). The three way ANOVA (group: normal vs. stroke; area: TC, TB, TA; layer: II–III, IV, V–VI) for the rank transformed all, pyramidal and non-pyramidal $GABA_{B2}$ positive neurons gave the following results. For all $GABA_{B2}$ positive neurons, there was a significant effect for group (P=0.0006) and area (P=0.038), but not for layer. A *post-hoc* Tukey test did not show, however, any significant differences for comparisons between means.

For $GABA_{B2}$ positive pyramidal neurons, the three way ANOVA showed a significant effect for layer (P<0.0001), but not for group or area. A *post-hoc* Tukey test has shown, in normal but not in stroke cases, statistically significant differences between the densities in layers II–III vs. IV in areas TB and TA, and in layers II–III vs. V–VI in areas TC, TB and TA; layers II–III has on average higher densities than layers IV or V–VI. The only statistically significant difference among areas was observed for densities in layers V–VI of normal areas TB vs. TA; layers V–VI had on average higher density in TB than in TA. Two way comparisons did not show any statistically significant differences for densities among the layers of the same area in stroke cases, or for densities among the same layers of different areas either in normal or in stroke cases or among the same layers in the same area

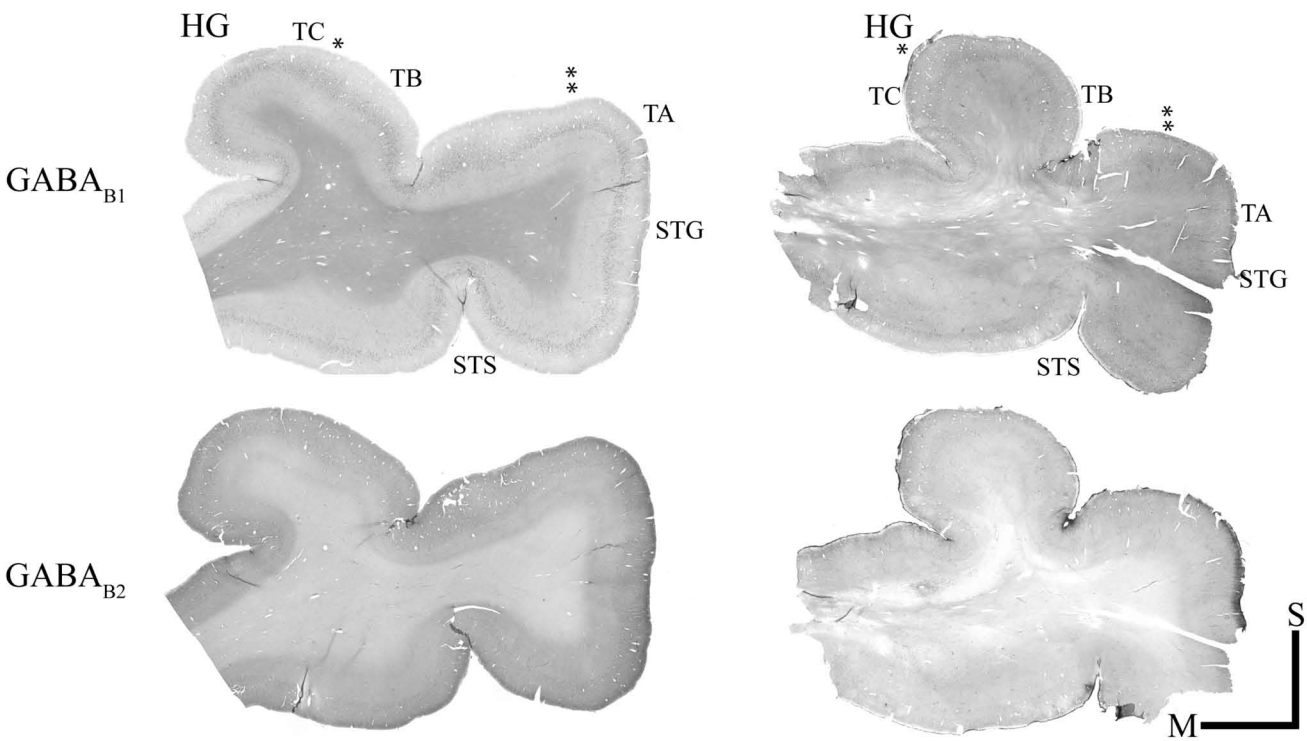

Fig. 11. Low magnification photomicrographs of adjacent coronal sections through the supratemporal plane, the superior temporal gyrus (STG), and the upper bank of the superior temporal sulcus (STS) at the level of Heschl's gyrus (HG), labeled for the $GABA_B$ subunits $GABA_{B1}$ or $GABA_{B2}$ in a normal (right hemisphere of case 5; left column) and a stroke case (right hemisphere of case 10; right column). The position of areas TC, TB and TA and their respective boundaries (* TC/TB; ** TB/TA) are indicated as determined on an adjacent Nissl-stained section. (M) medial; (S) superior. Scale bar is 5 mm.

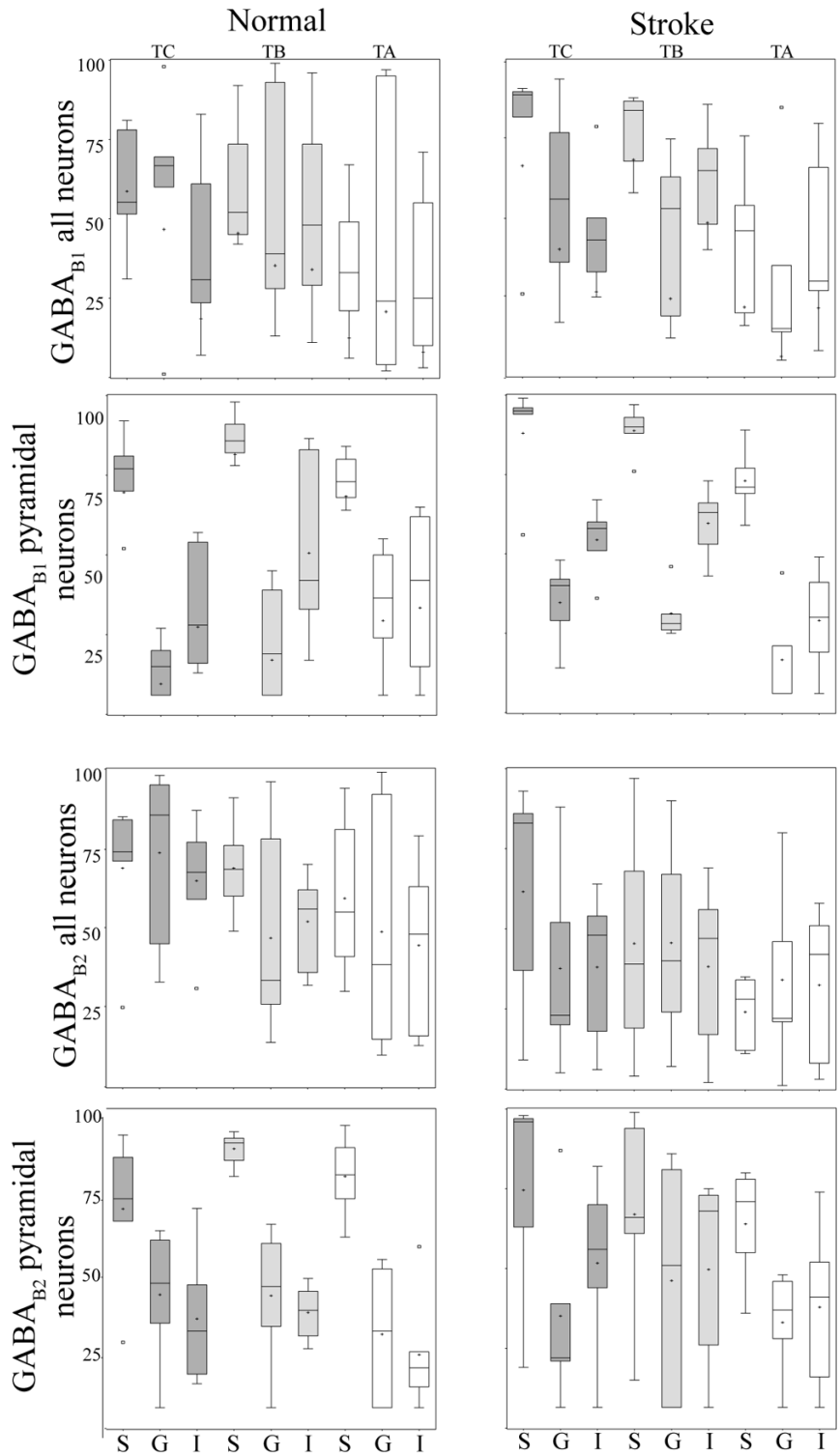

Fig. 12. Boxplot representation of rank transformed densities of $GABA_{B1}$ (top half) and $GABA_{B2}$ positive neurons (bottom half) in normal brains (left column) and stroke cases (right column). Densities are represented in layers II–III (S), IV (G) and V–VI (I) of areas TC (dark grey), TB (light grey) and TA (white). Within the boxplot, the box itself represents the interquartile range (inferior quartile range is 25%; superior quartile range is 75%), the horizontal line – the median value, and the dot – the mean; the vertical lines (whiskers) indicate values laying outside the box within a range of 1.5× that of interquartiles and the square symbol represents values out of the whisker range.

in normal vs stroke cases. $GABA_{B2}$ positive non-pyramidal neurons were present mainly in layer IV of normal cases; they could not be identified as such in stroke cases.

DISCUSSION

Technical considerations

Current knowledge of the intrinsic organization of the human cerebral cortex and of subcortical structures is often derived, by analogy, from animal studies, but is also based on a large number of *post-mortem* investigations in human brain tissue. As in our previous histochemical (Clarke 1994b, Rivier and Clarke 1997, Clarke and Rivier 1998, Eskenasy and Clarke 2000), immunohistochemical (Chiry et al. 2003, 2006, 2008, Tardif et al. 2003) and tracing studies (Clarke and Miklossy 1990, Clarke 1994a, Di Virgilio 1997, Clarke et al. 1999, Di Virgilio et al. 1999, Tardif and Clarke 2001, 2002, Wiesendanger et al. 2004, Tardif et al. 2005, 2007), we have controlled for artifacts due to the conditions of human tissue. First, the electron microscopic investigation showed well preserved tissue, including symmetrical and asymmetrical synaptic clefts, the former being putatively GABAergic (see below). Second, we used in this study only antibodies which yielded, in normal cases, a constant labeling independent of the time-between-death-and-fixation and the postfixation time of our cases. Third, we have ascertained that the time-between-death-and-fixation and the postfixation time were in the same range for normal and stroke cases. Fourth, the study involved the processing and analysis of serial sections through both hemisphere of all cases. The cases which are included here are only those which yielded consistent labeling between adjacent sections, among sections from the same block and among sections from the same region, but processed in different blocks (i. e. from either side of the cut that separated the two blocks). Fifth, during the histological procedure, sections from normal and stroke cases were mostly processed in the same session.

$GABA_A$ receptor subunits in normal auditory cortex

The immunohistochemical staining for $GABA_A$ receptor subunits α1, α2, α3 and $\beta_{2/3}$ yielded distinct labeling patterns in auditory areas of normal human brains with differences among cortical layers and areas in normal brains. The layer differences of $GABA_A$ subunit expression that we have observed in the primary auditory area were relatively minor when compared to layer differences in the primary visual cortex described for subunits α1, $\beta_{2/3}$ and γ2 of both human (Hendry et al. 1994) and non-human primates (Hendry et al. 1990, 1994) or with $GABA_A$/benzodiazepine receptor or muscimol binding in human material (Albin et al. 1991, Zilles et al. 2002, Eickhoff et al. 2007). At the boundary between V1 and V2, the change in the banding pattern of $GABA_A$ receptor expression changed abruptly (Albin et al. 1991, Eickhoff et al. 2007); which is not the case of the boundaries between auditory areas (here) or other cortical areas. Light banding patterns have been described in the somatosensory and non-auditory temporal cortices (Huntley et al. 1990, Loup et al. 2006), and absence of a banding pattern was reported in the human prefrontal cortex (Ishikawa et al. 2004).

Differential distribution of $GABA_A$ and $GABA_B$ receptors

GABA is likely to play a prominent role in human auditory cortex, since both $GABA_A$ and $GABA_B$ receptors are abundantly expressed in the primary auditory area and the surrounding cortex. Immunohistochemical revelation of $GABA_A$ subunits α1, α2 and $\beta_{2/3}$ in normal brains yielded mainly neuropil labeling, with only few faintly labeled neuronal somata. Both $GABA_{B1}$ and $GABA_{B2}$ subunits were also strongly expressed in primary and non-primary auditory areas, but were localized specifically on neuronal somata and proximal dendritic shafts of pyramidal and non-pyramidal neurons, the latter including bipolar, bitufted and multipolar neurons. Immunohistochemical studies of other human cerebral cortices have shown a similar dichotomy between predominant neuropil labeling for $GABA_A$ (Albin et al. 1991, Hendry et al. 1994, Zilles et al. 2002, Ishikawa et al. 2004, Loup et al. 2006, Eickhoff et al. 2007) and soma labeling for $GABA_B$ (Billinton et al. 2000, Munoz et al. 2001, Mizukami et al. 2002, Ishikawa et al. 2005). This dichotomy is sustained by electrophysiological data; patch-clamp recordings in combination with local release of GABA demonstrated, in rat cortex, $GABA_A$ receptors on apical dendrites and relatively more $GABA_B$ receptors near the soma of layer V pyramidal neurons (Eder et al. 2001).

$GABA_B$ receptor subunits in normal auditory cortex

Previous studies on $GABA_B$ receptor binding (Zilles et al. 2002, Scheperjans et al. 2005, Garraghty et al. 2006, Eickhoff et al. 2007), mRNA hybridization (Munoz et al. 2001) or $GABA_B$ subunit immunohistochemistry (Billinton et al. 2000, Munoz et al. 2001, Mizukami et al. 2002, Ishikawa et al. 2005) reported areal differences between visual (Munoz et al. 2001, Eickhoff et al. 2007) and parietal areas (Scheperjans et al. 2005), but not between prefrontal (Ishikawa et al. 2005) or entorhinal and inferior temporal cortices (Mizukami et al. 2002). When present, the laminar patterns differed among cortical areas. In the primary visual area of human and non-human primates the strongest $GABA_B$ binding (Zilles et al. 2002, Eickhoff et al. 2007) or the strongest $GABA_{B1}$ and $GABA_{B2}$ expression (Billinton et al. 2000, Munoz et al. 2001) were in layer IV. In the somatosensory areas 3a and 3b and in parietal area 5 this was the case in layers II and III (Scheperjans et al. 2005, Garraghty et al. 2006). In the entorhinal cortex the most intense neuronal labeling was found in layer III (Mizukami et al. 2002). No layer differences for $GABA_{B1}$ or $GABA_{B2}$ expression were found in prefrontal area 9 (Ishikawa et al. 2005).

Changes of $GABA_A$ receptors in stroke

A decrease of $GABA_A$ receptors, as revealed by benzodiazepine binding, has been described in previous radiological studies of acute and chronic stroke, but this finding was generally interpreted as a correlate of neuronal loss (e. g. review by Heiss and Herholz 2006) in view of the fact that 25 to 30% of neurons in primate neocortex are GABAergic (Jones 1993). Animal studies suggest, however, a more specific $GABA_A$ receptor effect. Focal lesions in rat neocortex were shown to be associated with an ipsi- and contralesional decrease in $GABA_A$ receptor binding 7 to 30 days after lesion (Qu et al. 1998a,b, Que et al. 1999). This reduction was more intense in ipsi- than contralateral regions and was predominant in layers II and III. Immunohistochemical visualisation of individual subunits demonstrated that this downregulation concerned the $GABA_A$ receptor subunits α1, α2, α5 and γ2, whereas subunit α3 was upregulated (Redecker et al. 2002). Our study, which investigated cases for which the time between stroke and death exceeded 30 days, demonstrated an isolated downregulation of the α2 subunit, whereas the expression of the subunits α1, α3 and $\beta_{2/3}$ remained unchanged.

The widespread functional effect of relatively small lesions both in human and animal studies may be linked to the crucial role GABAergic transmission plays in cortical efferent connections, including callosal ones. A recent electrophysiological and anatomical study in rat has shown that GABAergic and pyramidal neurons of deep cortical layers receive direct callosal input (Karayannis et al. 2007) and the lack of callosal input due to a contralateral lesion may thus profoundly change the GABAeric contribution to contralesional neuronal circuits. $GABA_A$ receptors, and in particular those containing the α2 subunit are prominently localized at the axon initial segment of pyramidal neurons, as also shown in human tissue (Loup et al. 1998). In case of an α2 downregulation, as shown here in stroke, the major inhibitory influence on the output of pyramidal neurons is removed or altered and may explain the profound functional changes that have been described in association with focal hemispheric lesions.

In non-human primates, focal lesions in the motor and/or somatosensory cortex have been accompanied by changes of ipsilesional somatotopic representations during recovery (Nudo et al. 1996, Friel and Nudo 1998, Xerri et al. 1998), which were interpreted in the context of neural hyperexcitability that occurred in the vicinity of the cortical lesion and contralateral to it (Buchkremer-Ratzmann et al. 1996, Reinecke et al. 1999), and was associated, in the acute stage, with decreased GABAergic inhibition (Neumann-Haefelin et al. 1995) and increased long-term potentiation in the surround of focal cortical lesions (Hagemann et al. 1998). In patients with unilateral stroke, intracortical inhibition was significantly reduced in the anatomically intact motor cortex of both the affected and intact hemispheres (Liepert et al. 2000a,b). Contralesional hyperexcitability within the motor cortex can last as long as 4 months (Shimizu et al. 2002) and is generally associated with good recovery of motor function (Butefisch et al. 2003).

Changes in cortical processing also occur in the auditory cortex contralateral to lesions. Functional imaging studies have demonstrated that in normal subjects information relevant to sound recognition and sound localization is processed in anatomically distinct cortical networks, referred to as auditory "What" and "Where" processing streams. The specialization

is already present in early stage, non-primary auditory areas on the supratemporal plane (Viceic et al. 2006), which correspond to the cytoarchitectonic areas TB and TA of this study (Rivier and Clarke 1997). On the convexity, sound attributes that convey information about sound source identity are selectively processed within a network that involves the temporal convexity bilaterally as well as a part of the left inferior frontal gyrus, while spatial sound attributes are processed within a bilateral network comprising parietal and prefrontal cortices (Alain et al. 2001, Maeder et al. 2001, De Santis et al. 2007). Unilateral hemispheric lesions, which have damaged greater parts of one or both specialized auditory networks, were found to be associated, in the chronic stage, with deficient performance in one or both functions (Clarke et al. 2000, 2002). In these cases, the parallel processing of auditory information within the "What" and "Where" streams was disrupted; sound recognition and sound localization were processed within the same parts of the auditory cortex (Adriani et al. 2003a). These lasting changes in the functional specificity of the anatomically intact auditory cortex may reflect long-term changes in GABAergic transmission as demonstrated here. Even more striking functional changes occur in the acute stage. In the second week post-lesion, i.e. after the penumbra has been resolved, small focal lesions that only marginally damaged the auditory "What" and/or "Where" processing streams caused sound recognition and/or sound localization deficits in a non-specific way (Adriani et al. 2003b). These non-specific deficits were no longer present in the chronic stage (Rey et al. 2007) and may reflect effects of a wide spread transient and more pervasive GABA downregulation, as demonstrated in animal models before the end of the first post-lesion months (Redecker et al. 2002).

The effects of brain lesions on $GABA_A$ receptors can be long-lasting, as documented in cases of pre- or neonatally occurring brain damage. The spastic type of cerebral palsy, which is often due to hypoxic-ischemic insults to periventricular white matter occurring at 26–34 weeks of gestation, was shown to be associated, 6 to 20 years later, with increased $GABA_A$ receptor density in motor and visual cortices, as visualized by F-Fluoroflumazenil binding (Lee et al. 2007). In another study, changes in flumazenil binding have been demonstrated in adult patients with longstanding epilepsy associated with development malformations of the cerebral cortex (Richardson et al. 1997). A long-term effect was also observed in a rat model of focal cortical malformation; neonatal local freeze-lesions of the cerebral cortex were associated with a widespread reduction of $GABA_A$ subunits α1, α2, α3, α5 and γ2 in the adult animal (Redecker et al. 2000).

The $GABA_A$ α2 subunit

The α2 subunit is prominently located on the axon initial segment of cortical pyramidal neurons (Loup et al. 1998). Its downregulation, as here in stroke, may change the major inhibitory influence on the output of pyramidal neurons and be at the origin of the functional changes which were observed in the auditory cortex after acute or chronic stroke (Adriani et al. 2003a,b, Rey et al. 2007). Another specific effect on the α2 subunit has been demonstrated in schizophrenia, which was reported to be associated with an increased number of α2 labeled terminals on the axon initial segment in the prefrontal cortex (Volk et al. 2002).

Several lines of evidence indicate that the anxiolytic action of benzodiazepines is mediated by the α2 and/or the α3 subunit of $GABA_A$ receptors (Low et al. 2000, Atack et al. 2006); for review see e.g. Rudoph and Möhler (2006). The selective downregulation of the $GABA_A$ α2 subunit that we have observed in subacute and chronic stroke may thus change the responsiveness to benzodiazepines and notably to their anxiolytic action.

The selective downregulation of a single $GABA_A$ subunit in a neurological disorder is not exceptional. In temporal lobe epilepsy, the $GABA_A$ α3 subunit was shown to be downregulated in supragranular cortical layers, but not the α1, α2, $\beta_{2/3}$ or γ2 subunits; furthermore this down regulation was specific for temporal lobe epilepsy and was absent in frontal lobe epilepsy (Loup et al. 2006).

$GABA_B$ receptor distribution in stroke

This is the first human study describing $GABA_B$ receptor expression in stroke and reports absence of major changes in both $GABA_{B1}$ and $GABA_{B2}$ subunit expression in subacute and chronic cases. Changes in $GABA_B$ receptor binding have been described in animal models of stroke, but at much earlier stages than

considered here. In rat, a focal cortical lesion was associated with an upregulation, as revealed by baclofen binding autoradiography, in the intact extralesional ipsi- and contralateral cortex (Que et al. 1999), which reached its maximum at day 7 post-lesion and returned to normal values by day 30.

The absence of major changes in the expression of $GABA_B$ receptors in such a pervasive disorder as stroke (and even repeated stroke in several of our patients) is in contrast with changes reported in epilepsy (Billinton et al. 2001a,b, Furtinger et al. 2003, Princivalle et al. 2003), schizophrenia (Mizukami et al. 2000) or bipolar patients (Ishikawa et al. 2005) or in rat models of seizures (Furtinger et al. 2003, Straessle et al. 2003).

CONCLUSIONS

Our results demonstrate a strong presence of $GABA_A$ and $GABA_B$ receptors in the human auditory cortex, suggesting a crucial role of GABA in shaping auditory responses in the primary and non-primary auditory areas. The differential laminar and areal expression of $GABA_A$ subunits that we have found in the auditory areas, and which is partially different from that in other cortical areas, speaks in favor of a fine turning of GABA-ergic transmission in these different compartments. In contrast, $GABA_B$ expression displayed laminar, but not areal differences; its basic pattern was also very similar to that of other cortical areas, suggesting a more uniform role within the cerebral cortex. In subacute and chronic stroke, the selective $GABA_A$ α2 subunit downregulation is likely to influence postlesional plasticity and susceptibility to medication. The absence of changes in the $GABA_B$ receptors suggests different regulation than in other pathological conditions, such as epilepsy, schizophrenia or bipolar disorder, in which a downregulation has been reported.

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