Neurobiology of Aging 34 (2013) 1486–1496

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Age-related $GABA_A$ receptor changes in rat auditory cortex

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ARTICLE INFO

Article history:
Received 4 June 2012
Received in revised form 13 November 2012
Accepted 16 November 2012
Available online 17 December 2012

Keywords:
Age-related changes
Auditory cortex
$GABA_A$ receptor subunit
Quantitative $GABA_A$ receptor binding

ABSTRACT

Auditory cortex (AI) shows age-related decreases in pre-synaptic markers for gamma-aminobutyric acid (GABA) and degraded AI neuronal response properties. Previous studies find age-related increases in spontaneous and driven activity, decreased spectral and directional sensitivity, and impaired novelty detection. The present study examined expression of $GABA_A$ receptor ($GABA_AR$) subunit message, protein, and quantitative $GABA_AR$ binding in young, middle-aged, and aged rat AI, with comparisons with adjoining parietal cortex. Significant loss of $GABA_AR$ α_1 subunit message across AI layers was observed in middle-aged and aged rats and α_1 subunit protein levels declined in layers II and III. Age-related increases in $GABA_AR$ α_3 subunit message and protein levels were observed in certain AI layers. $GABA_AR$ subunits, including β_1, β_2, γ_1, γ_{2s}, and γ_{2L}, primarily, but not exclusively, showed age-related declines at the message and protein levels. The ability of GABA to modulate [^{3}H]*t*-butylbicycloorthobenzoate binding in the chloride channel showed age-related decreases in peak binding and changes in desensitization kinetics. Collectively, age-related changes in $GABA_AR$ subunit composition would alter the magnitude and temporal properties of inhibitory synaptic transmission and could underpin observed age-related functional changes seen in the elderly.

1. Introduction

Age-related functional changes in a number of sensory systems are strongly suggestive of a loss of normal adult inhibitory amino acid neurotransmission (Angelotti and Macdonald, 1993; Belelli et al., 2005; Burianova et al., 2009; for review, Canlon et al., 2010; Caspary et al., 2008; Gutierrez et al., 1997; Lloyd et al., 1990; Maksay and Ticku, 1985; Malherbe et al., 1990; Mendelson and Rajan, 2011; Olsen et al., 1990; Pinto et al., 2010; Sakurai et al., 1994; Suta et al., 2011; Syka, 2002; Winer, 1992; Wisden et al., 1992; Ymer et al., 1990). In part, these changes in central inhibition likely reflect a compensatory age-related response to decreased peripheral sensory input, reflecting homeostatic plasticity (Noreña, 2011; Oliver et al., 2011; Richardson et al., 2011; Turrigiano and Nelson, 2004). Compensatory age-related changes can result in decreased markers of normal functional inhibition in auditory and visual cortices (Hua et al., 2006; Hughes et al., 2010; Leventhal et al., 2003; Liang et al., 2008; Schmidt et al., 2010). Even when presented with suprathreshold acoustic stimuli, many middle-aged and elderly humans show decreased speech understanding, impaired sound localization, and loss in the ability to extract novel or salient signals from a complex acoustic background (Anderson et al., 2012; Dubno et al., 1984; Fitzgibbons and Gordon-Salant, 1994, 2010; Fogerty et al., 2010; Lui and Mendelson, 2003; Ostroff et al., 2003; Pichora-Fuller et al., 2007; Schneider et al., 1994; Snell, 1997; Strouse et al., 1998; Suta et al., 2011; Tremblay et al., 2002, 2003).

Recent electrophysiologic studies in rat and primate auditory cortex (AI) find age-related increases in spontaneous and sound-evoked discharge rates and less precise directional sensitivity, loss of spectral precision, and impaired novelty detection (de Villers-Sidani et al., 2010; Hughes et al., 2010; Juarez-Salinas et al., 2010; Martin Del Campo et al., 2012). Therefore, human and animal studies suggest that changes in sound processing are consistent with a hypothesis of an age-related loss of normal adult functional inhibition.

Presynaptic markers for gamma-aminobutyric acid (GABA), including GABA levels and levels of the GABA synthetic enzyme glutamic acid decarboxylase are downregulated across aged AI in humans and animal models of aging (Burianova et al., 2009; de Villers-Sidani et al., 2010; Ling et al., 2005; McGeer and McGeer, 1980). Few studies have examined $GABA_A$ receptor ($GABA_AR$) markers across the layers of AI (Pirker et al., 2000; Wisden et al., 1992; Yu et al., 2006). The effect of aging on the subunit makeup of $GABA_ARs$ across AI layers is the focus of the present study. The adjoining parietal cortex was used for comparison. $GABA_ARs$ exist as pentameric subunit complexes which can be allosterically

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http://dx.doi.org/10.1016/j.neurobiolaging.2012.11.009

modulated by numerous pharmacologic agents (Rabow et al., 1995; Sieghart, 1992a, 1992b, 1992c, 1992d, 1995; Sieghart et al., 1992; Wafford et al., 1993; Yu et al., 2006). Molecular cloning has revealed 6-α, 4-β, 3-γ, 1-δ, 1-ε, 1-π, 1-θ, and 3-ρ $GABA_A$ receptor subunits (Olsen and Sieghart, 2008, 2009; Rabow et al., 1995; Rudolph et al., 2001; Sieghart, 1995; Wafford and Ebert, 2006). $GABA_AR$ subunit constructs exhibit specific regional and likely cortical layer specific distributions (Pirker et al., 2000; Wisden et al., 1992; Yu et al., 2006). Altered $GABA_AR$ subunit composition/stoichiometry, potentially in response to age-related presynaptic changes, would affect $GABA_AR$-mediated inhibitory function. Age-related subunit changes would alter the magnitude and temporal precision of inhibitory currents, in turn degrading sensory processing (Angelotti and Macdonald, 1993; Ducic et al., 1995; Macdonald and Olsen, 1994; Takesian et al., 2012; Wafford et al., 1993). The present study examined $GABA_AR$ subunit message, protein, and quantitative $GABA_AR$ binding of selective $GABA_A$ ligands in young, middle-aged, and aged rat AI.

2. Methods

2.1. Animals

Young-adult (4–6 months old), middle-aged (20–22 months old) and aged (30–32 months old) male Fischer Brown Norway (FBN) rats were obtained from Harlan Sprague-Dawley, Inc (Indianapolis, IN, USA). All experiments were carried out under animal use protocols approved by the Southern Illinois University School of Medicine Laboratory Animal Care and Use Committee. Age-related hair cell loss and age-related threshold shifts for this strain have been previously described (Turner and Caspary, 2005; Wang et al., 2009a).

2.2. Sampling criteria for rat AI

Sections were collected through the center of AI from an area at Bregma −4.80 mm (Plate 39) to Bregma −4.16 (Plate 36) (Paxinos and Watson, 1998) identified by measuring 2.25 mm dorsal from the rhinal fissure. The present study used criteria adapted from Winer (1992) and Games and Winer (1988) to define data collection areas sampled from layers II–VI of FBN rat AI. A detailed algorithm for measures used to identify AI layers II–VI was derived from Winer (1992) and Games and Winer (1988) and is detailed in Ling et al. (2005).

2.3. Quantitative in situ hybridization

Eighteen FBN rats (6 young-adult, 6 middle-aged, and 6 aged) were decapitated, brains rapidly removed, rinsed in ice-cold phosphate-buffered saline (PBS) (pH 7.4, diethylpyrocarbonate-treated), frozen in powdered dry ice, and stored at −80 °C. Serial transverse sections (16 μm) through AI were cut using a cryostat (CM1850; Leica Microsystems Nussloch GmbH, Nussloch, Germany) set at −18 °C. Sections were thaw-mounted onto Superfrost/Plus slides (Thermo Fisher Scientific, Pittsburgh, PA, USA) at approximately the same position, 2 sections per slide, and stored at −20 °C (<48 hours) until processed for in situ hybridization.

2.3.1. $GABA_A$ subunit probe preparation

Nine 40–48 mer oligonucleotide probes were synthesized and purified by Sigma Genosys (Woodlands, TX, USA). Sequences selected were based on published sequences: α_1 (Khrestchatisky et al., 1989); α_2 (Pritchett and Seeburg, 1990); α_3 (Malherbe et al., 1990); α_4 (Wisden et al., 1991); β_{1-3} (Ymer et al., 1989); and γ_1, γ_{2s}, and γ_{2L} (Ymer et al., 1990). Procedures for oligonucleotide probe end-labeling, in situ hybridization steps, and data analysis are as described in (Ling et al., 2005) and were modified from Milbrandt et al. (1997). In brief, 5 pM oligonucleotide probes in 50 μL labeling mixture were 3′ end-labeled for 10 minutes at 37 °C with 0.5 μM of ^{35}S-deoxyadenosine triphosphate (PerkinElmer Inc, Downers Grove, IL, USA) using terminal deoxynucleotidyl transferase (16 U/μL) (Fisher Scientific, Pittsburgh, PA, USA). The reaction was halted by addition of 50 μL of Tris-EDTA (TE) buffer. Ten mg/mL transfer RNA (tRNA) was added to enhance recovery of the labeled probe. Labeled probes were extracted using a phenol/chloroform. After hybridization and postwashing steps, slides were dried and dipped in NTB-2 photographic emulsion (VWR, West Chester, PA, USA) and stored in the dark at 4 °C for 4 weeks. Exposed sections were then developed, fixed, and counterstained with thionin for cell identification. Adjacent sections were used as controls for specificity. Competitive blocking of labeled oligonucleotides using excess concentrations (50-fold) of unlabeled oligonucleotide and incubation with labeled sense oligonucleotides were used as controls. Detailed hybridization procedure, consistency, and quality control was described in Ling et al. (2005).

2.3.2. Quantitative analysis of hybridization labeling

Images were captured using a CoolSnap monochrome digital camera connected to an MCID-Elite 6.0 imaging system (InterFocus Imaging Ltd, Cambridge, England) with 40× objective. Accumulation of silver grains over neuronal cell bodies was interpreted as hybridization of the probe to its corresponding messenger RNA (mRNA) (Fig. 1). The identity of sections was concealed/blinded to ensure unbiased quantification. The counting parameters such as threshold, light intensity, and counting area were maintained consistently throughout the counting procedure for a particular subunit. Only grains within the neuronal perimeter were counted by the automated counting system-MCID Elite 6.0 (InterFocus Imaging Ltd) over cells distinguishable from adjacent cells and showing a visible nucleus. Quantitative comparisons were made only within a given subunit probe not across probes. With 2 sections per animal, 2 different fields of fixed size, from each of the layers (II–VI) of AI in each section were digitized and grain counts, neuronal number, size, and area recorded. Background labeling measurements were obtained from 3 random areas located off the tissue sections. Somatic area and number of grains over the somata of at least 10 cells in each of the layers (II–VI) of AI in each section were measured. Data were collected as grain density (number of grains per 100 μm^2 of cell area) and corrected by subtraction of nonspecific hybridization for each layer, subunit, and age group. Analysis of variance was used to determine if differences in background-adjusted mean grain density was attributed to the treatment variables. Tests subsequent to the analysis of variance were carried out using the Bonferroni procedure to control overall type I error rate (Ling et al., 2005).

2.4. Quantitative immunohistochemistry

The methods used for quantitative immunohistochemistry were based on those published by Ling et al. (2005).

2.4.1. Tissue preparation for immunohistochemistry

FBN rats were anesthetized with a mixture of ketamine (105 mg/kg body weight, intraperitonally) and xylazine (7 mg/kg body weight, intraperitonally), and transcardially perfused with 150 mL of physiological saline containing 0.1% of sodium nitrite, followed by 1 L of fixative containing 4% paraformaldehyde in Sorenson's K-Na phosphate buffer (pH 7.4). Brains were removed, postfixed for 1 hour in the same solution, washed in 0.1 M PBS for 30 minutes, and immersed overnight in PBS containing 20% sucrose. Cryoprotected tissues were stored at −80 °C.

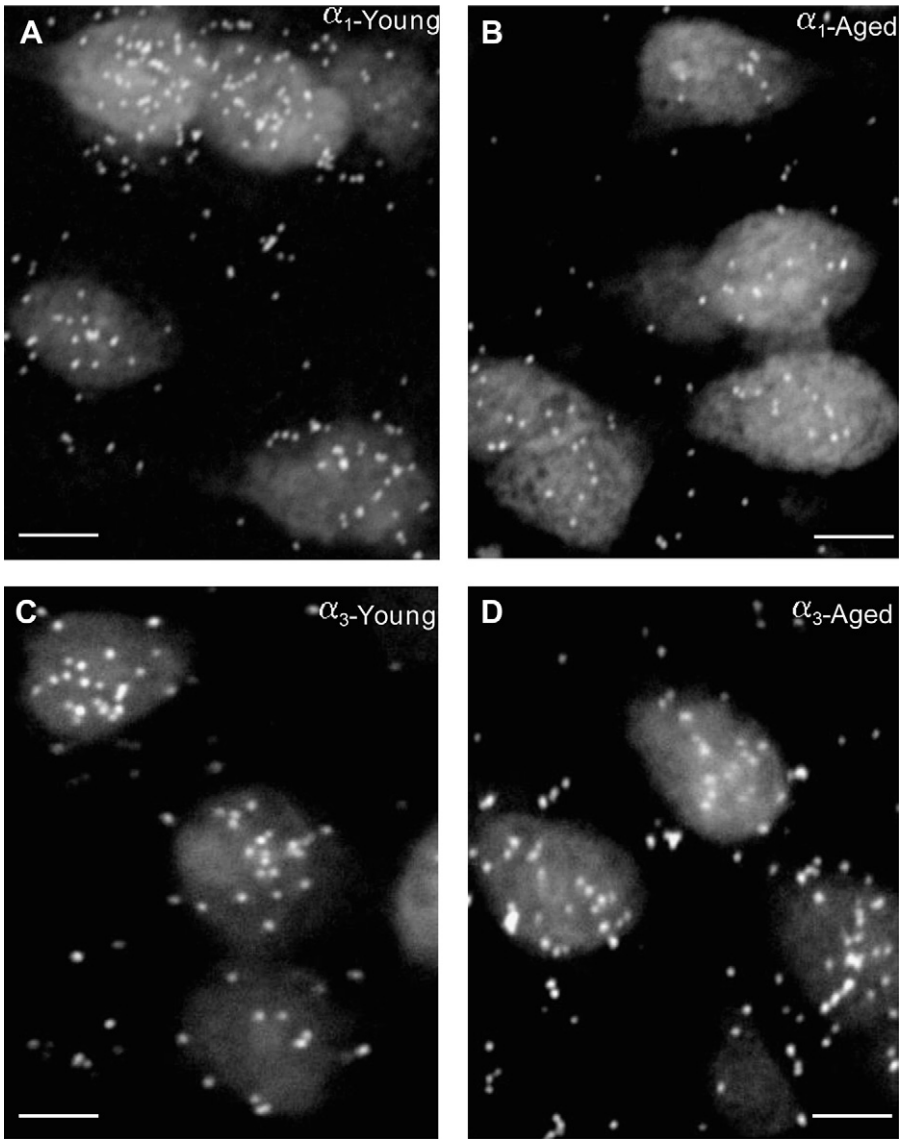

Fig. 1. Distribution of gamma-aminobutyric acid$_A$ receptor ($GABA_AR$) α_1 and α_3 mRNA in layer III neurons of auditory cortex (AI) from young and aged FBN rats. Clusters of silver grains represent hybridization of transcripts of $GABA_AR$ α_1 (A and B) and α_3 (C and D) with ^{35}S-labeled selective oligonucleotide probes. Reduction of silver grains in aged AI neurons of layer III (B), when compared with that in neurons from young adult rat AI (A), indicates the age-related loss of $GABA_AR$ α_1 mRNA. In contrast, $GABA_AR$ α_3 mRNA shows as increased in aged layer III neurons (D). Scale bar = 10 μm.

2.5. Immunohistochemistry

FBN rats used in $GABA_AR$ α_1 and β_1 studies were 4 young, 4 middle-aged, and 4 aged, and in $GABA_AR$ α_3 and β_2 studies there were 6 young, 6 middle-aged, and 6 aged rats. Serial transverse sections through AI were cryostat sectioned at 30 μm and collected as free-floating sections in ice-cold 0.1 M PBS. The sections were rinsed in PBS, transferred to blocking solution (1.5% normal serum and 5% nonfat dry milk in PBS) for 30 minutes and incubated at room temperature in primary antibodies for 1 hour and then at 4 °C overnight with agitation. Polyclonal goat anti-$GABA_AR$ α_1 and β_{1-2} (1:150) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Polyclonal rabbit anti-$GABA_AR$ α_3 (1:500) was obtained from Alomone Labs (Jerusalem, Israel). After rinsing in PBS, sections were processed using Vectastain ABC kits (Vector Laboratories, Burlingame, CA, USA). The labeling was visualized using diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA) and diaminobenzidine reaction time was fixed at 2 minutes. Sections were then mounted onto the Superfrost/Plus slides. When possible, specificity of primary antibodies was tested by preincubation with the control peptide antigens. Secondary antibodies were controlled in cells processed as described above but in the absence of primary antibodies.

To help minimize variability, unrelated to treatments, immunostaining and measurements were carried out in parallel groups, with tissue from 1 young, 1 middle-aged, and 1 aged animal processed at the same time. Sections were blinded so age groups were unknown to the observer. Flat-field correction was performed before digitizing images and held consistent across each group. Digital images of immunoprocessed sections were captured at an objective magnification of 40× as described above. Two fields from each AI layer (LII–LVI) per section and 2–4 sections per animal were analyzed. Relative optical density (ROD) measurements, which are proportional to immunostaining intensity, were measured from all positively stained neurons encountered across layers II–VI of AI. All ROD measurements

were corrected by subtracting background values obtained from the measurements of immunonegative cells in layer V. Only neurons with intact soma outlines and discernible nuclei and nucleoli were measured. All data were expressed as mean ± SD of ROD.

2.6. Quantitative receptor binding autoradiography

RO15-4513 binds both "wild type" ($2\alpha_1 2\beta_2\gamma_2$) and non-"wild type" $GABA_AR$s. Functionally, RO15-4513 acts as a partial inverse agonist at γ_2 subunit containing $GABA_AR$s (Korpi et al., 2002; Luddens and Wisden, 1991; Wisden et al., 1991), while acting as an agonist at α_4 and α_6 subunits containing $GABA_AR$s (Hadingham et al., 1996; Knoflach et al., 1996; Linden et al., 2011; Wafford et al., 1996). Binding of the $GABA_AR$ radioligand *t*-butylbicycloorthobenzoate (TBOB), can be modulated by varying concentrations of GABA, and has been used in picrotoxin ligand binding assays for studying $GABA_AR$ pharmacology and receptor diversity (Lloyd et al., 1990; Maksay and Ticku, 1985; Olsen et al., 1990; Sakurai et al., 1994). [^{3}H] RO15-4513 saturation analysis based on previous studies was used in order to reveal differences in *kd* or Bmax (Braestrup et al., 1983; Niddam et al., 1987; Ruano et al., 1993). Concentrations (1, 3, 5, 8, 10, and 15 nM) of [^{3}H]RO15-4513 (20 Ci/mmol/L, PerkinElmer Inc, San Jose, CA, USA) were added to the incubation buffer and 100 μM of flumazenil was added as a displacer. Modulation of [^{3}H]TBOB binding was carried out with increasing concentrations of GABA from 10 nm to 5 μm (Milbrandt and Caspary, 1995). Autoradiograms were generated by apposing slides to a phosphor screen, and the screen was then scanned using Cyclone phosphor system (PerkinElmer Inc, San Jose, CA, USA). Images were collected at 600 dots per inch and analyzed using OptiQuant image analysis software (version 3.0). The superficial layers II–IV were grouped into 1 box (38% cortical thickness), with layer V (26% cortical thickness), and layer VI (22% cortical thickness) (Games and Winer, 1988) windowed using 1 box for each layer. Digital light units were converted into fmol/mg protein using a standard curve generated from the coexposed ^{14}C-embedded plastic standards (American Radioactive Chemicals, St. Louis, MO, USA) (Pan et al., 1983).

3. Results

The effect of aging on hair cell loss and auditory thresholds (23 dB parallel shift) across frequency have been previously described for the FBN rat aging model used in the present study (Turner and Caspary, 2005; Wang et al., 2009b).

3.1. Age-related $GABA_AR$ subunit message changes

$GABA_AR$ subunit message and protein levels were obtained from primary AI and parietal cortex (PtA) in sections from young, middle-aged, and aged FBN rats. Automated, nonstereological collection of in situ hybridization data provided information regarding neuronal number, size, and area. Consistent with the visual cortical findings of Peters et al. (1983) no significant age-related changes in neuronal number, neuronal size, and neuronal area were observed across AI layers.

Table 1 summarizes results of age-related $GABA_AR$ subunit message changes across AI layers for all subunits examined ($\alpha_{1,2,3,5}$; β_{1-3}, γ_1, γ_{2S}, and γ_{2L}). Age-related subunit message levels for young-adult, middle-aged, and aged rats are presented as raw grain counts over neurons across AI layers II–VI and as percent change from young-adult for middle-aged, and aged AI (Table 1; p values less than 0.05, unless otherwise stated).

Table 1
Changes of gamma-aminobutyric acid $(GABA)_A$ receptor subunits mRNA levels in auditory cortex of FBN rats

		Young	Middle	Percent change from young	Aged	Percent change from young
α_1	LII	5.9 ± 1.9	4.5 ± 2.2	−23.73[a]	3.6 ± 1.9	−38.98[a]
	LIII	5.4 ± 1.8	4.3 ± 2.2	−20.37[a]	3.2 ± 1.7	−40.74[a]
	LIV	5.9 ± 2.0	4.8 ± 2.3	−18.64[a]	4.0 ± 1.7	−32.20[a]
	LV	5.7 ± 1.9	4.6 ± 2.2	−19.30[a]	3.9 ± 1.7	−31.58[a]
	LVI	6.8 ± 2.2	5.4 ± 2.2	−20.59[a]	3.9 ± 2.0	−42.65[a]
α_2	LII	3.8 ± 1.9	3.9 ± 1.9	2.63	3.6 ± 1.6	−5.26
	LIII	3.3 ± 1.4	3.8 ± 1.8	15.15[a]	3.1 ± 1.4	−6.06
	LIV	3.7 ± 1.6	3.9 ± 2.1	5.41	3.8 ± 2.0	2.70
	LV	3.4 ± 1.4	4.0 ± 1.7	17.65[a]	3.3 ± 1.4	−2.94
	LVI	4.1 ± 2.0	5.0 ± 1.8	21.95[a]	3.6 ± 1.5	−12.20[a]
α_3	LII	6.5 ± 2.4	7.1 ± 3.2	9.23	8.5 ± 2.6	30.77[a]
	LIII	6.0 ± 1.8	6.8 ± 3.0	13.33[a]	7.8 ± 2.5	30.0[a]
	LIV	7.2 ± 2.5	7.6 ± 3.2	5.56	8.0 ± 2.6	11.11
	LV	6.7 ± 2.0	7.2 ± 2.9	7.46	7.4 ± 2.7	10.45[a]
	LVI	7.9 ± 2.6	7.6 ± 3.0	−3.80	7.7 ± 2.1	−2.53
α_5	LII	7.2 ± 2.2	6.7 ± 2.4	−6.94	6.7 ± 2.4	−6.94
	LIII	6.0 ± 1.8	6.2 ± 2.5	3.33	6.0 ± 2.0	0
	LIV	6.0 ± 2.4	6.6 ± 2.7	10.0	6.2 ± 2.6	3.33
	LV	5.6 ± 2.0	5.6 ± 2.0	0	6.0 ± 2.2	7.14
	LVI	6.4 ± 2.3	6.3 ± 2.3	−1.56	6.7 ± 2.5	4.69
β_1	LII	5.5 ± 2.1	5.4 ± 2.3	−1.82	4.3 ± 1.9	−21.82[a]
	LIII	5.2 ± 1.8	5.3 ± 2.6	1.92	3.9 ± 1.6	−25.0[a]
	LIV	5.4 ± 2.1	5.5 ± 2.2	1.85	4.6 ± 2.0	−14.81[a]
	LV	5.2 ± 2.0	5.7 ± 2.0	9.62[a]	4.5 ± 1.7	−13.46[a]
	LVI	6.2 ± 2.4	5.6 ± 2.2	−9.68[a]	4.9 ± 2.1	−20.97[a]
β_2	LII	4.4 ± 2.0	4.3 ± 1.9	−2.27	3.1 ± 1.5	−29.55[a]
	LIII	4.1 ± 1.6	3.8 ± 1.7	−7.32	2.8 ± 1.6	−31.71[a]
	LIV	4.1 ± 1.9	4.2 ± 1.7	2.44	3.4 ± 1.6	−17.07[a]
	LV	4.4 ± 1.7	4.2 ± 1.7	−4.55	3.4 ± 1.4	−22.73[a]
	LVI	5.5 ± 2.0	5.0 ± 2.2	−9.09	4.3 ± 1.9	−21.82[a]
β_3	LII	7.5 ± 2.8	8.0 ± 2.3	6.67	6.1 ± 2.5	−18.67[a]
	LIII	6.9 ± 2.5	7.3 ± 2.5	5.80	5.6 ± 2.5	−18.84[a]
	LIV	7.5 ± 2.7	7.6 ± 2.4	1.33	6.4 ± 2.6	−14.67[a]
	LV	7.1 ± 2.7	7.3 ± 1.7	2.82	6.6 ± 2.7	−7.04
	LVI	8.3 ± 2.8	8.2 ± 2.6	−1.20	7.9 ± 3.7	−4.82
γ_1	LII	9.5 ± 3.3	8.6 ± 3.4	−9.47[a]	7.6 ± 2.7	−20.0[a]
	LIII	9.2 ± 3.3	8.4 ± 3.6	−8.70[a]	7.2 ± 2.1	−21.74[a]
	LIV	9.9 ± 3.4	8.6 ± 3.5	−13.13[a]	7.5 ± 2.4	−24.24[a]
	LV	9.9 ± 3.3	9.6 ± 3.1	−3.03	8.6 ± 2.1	−13.13[a]
	LVI	11.0 ± 3.3	10.3 ± 3.5	−6.36[a]	9.2 ± 2.5	−16.36[a]
γ_{2S}	LII	6.0 ± 1.5	6.2 ± 1.8	3.33	5.9 ± 1.7	−1.67
	LIII	5.6 ± 1.5	5.8 ± 1.9	3.57	5.2 ± 1.6	−7.14
	LIV	5.7 ± 1.6	6.3 ± 2.1	10.53[a]	5.6 ± 1.5	−1.75
	LV	5.4 ± 1.2	5.9 ± 1.8	9.26[a]	5.7 ± 1.4	5.56
	LVI	5.8 ± 1.7	6.3 ± 2.0	8.62	6.0 ± 1.9	3.45
γ_{2L}	LII	5.3 ± 1.9	4.2 ± 2.3	−20.75[a]	3.2 ± 1.7	−39.62[a]
	LIII	4.9 ± 1.6	4.1 ± 2.0	−16.33[a]	3.1 ± 1.9	−36.73[a]
	LIV	5.4 ± 1.6	4.2 ± 2.1	−22.22[a]	3.7 ± 2.0	−31.48[a]
	LV	5.1 ± 1.8	4.6 ± 2.0	−9.80[a]	3.9 ± 1.9	−23.53[a]
	LVI	5.7 ± 1.6	4.9 ± 2.1	−14.04[a]	4.4 ± 2.0	−22.81[a]

The data represent mean ± SD (number of grains per 100 μm^2).
[a] Significant difference between the means of young versus aged groups and young versus middle-aged ($p < 0.05$).

3.1.1. Aging and $GABA_AR$ subunit $\alpha_{1,2,3,5}$ message changes

Significant step-wise age-related decreases in $GABA_AR$ α_1 subunit message were observed across layers of AI and PtA (Tables 1 and 2). Images from in situ hybridization show an age-related reduction in number of silver grains, representing $GABA_AR$ α_1 subunit message over AI layer III neurons (Figs. 1A and B). The age-related α_1 subunit message loss was significant across all layers ($p < 0.01$) for young versus aged with percent reductions between 30% and 42% (Table 1, Fig. 2A). Middle-aged animals showed $GABA_AR$ α_1 subunit message levels that were intermediate between young and aged α_1 subunit message levels (Table 1, Fig. 2A). Age-related changes in PtA $GABA_AR$ α_1 subunit message displayed a staircase aging pattern similar to that seen in AI (Table 2, Fig. 2A).

Table 2
Changes of gamma-aminobutyric acid $(GABA)_A$ receptor subunits mRNA levels in parietal cortex of FBN rats

		Young	Middle	Percent change from young	Aged	Percent change from young
α_1	LII	7.1 ± 2.1	4.3 ± 2.3	−39.44[a]	3.7 ± 1.6	−47.89[a]
	LIII	7.0 ± 2.0	4.6 ± 2.0	−34.29[a]	3.8 ± 1.7	−45.71[a]
	LIV	7.7 ± 2.1	4.9 ± 2.3	−36.36[a]	3.9 ± 1.5	−49.35[a]
	LV	6.4 ± 1.7	4.9 ± 2.1	−23.44[a]	3.8 ± 1.5	−40.36[a]
	LVI	7.1 ± 2.1	5.1 ± 2.3	−28.17[a]	4.0 ± 1.4	−43.66[a]
α_3	LII	6.8 ± 2.1	6.9 ± 2.5	1.47	7.3 ± 1.8	7.35
	LIII	6.9 ± 2.3	6.7 ± 2.4	−2.90	8.0 ± 2.1	15.94[a]
	LIV	6.7 ± 2.1	6.8 ± 2.3	1.49	7.5 ± 2.3	11.94[a]
	LV	7.0 ± 2.1	6.6 ± 2.4	−5.71	6.6 ± 1.9	−5.71
	LVI	7.6 ± 2.7	7.7 ± 2.7	1.32	7.3 ± 2.6	−3.95
β_2	LII	5.1 ± 1.8	4.2 ± 1.8	−17.65[a]	3.7 ± 1.5	−27.45[a]
	LIII	4.5 ± 2.1	4.2 ± 1.7	−6.67	3.3 ± 1.4	−26.67[a]
	LIV	4.9 ± 1.8	4.5 ± 1.9	−8.16	4.1 ± 1.7	−16.33[a]
	LV	4.7 ± 1.8	3.9 ± 1.5	−17.02[a]	3.7 ± 1.5	−21.28[a]
	LVI	5.4 ± 1.9	4.8 ± 1.7	−11.11[a]	4.2 ± 1.6	−22.22[a]
γ_{2L}	LII	4.6 ± 1.9	4.6 ± 1.7	−0	3.9 ± 1.7	−15.22[a]
	LIII	4.4 ± 1.8	4.3 ± 1.6	−2.27	4.0 ± 2.1	−9.09
	LIV	4.8 ± 1.7	4.5 ± 1.7	−6.25	4.2 ± 2.0	−12.5
	LV	4.5 ± 1.4	4.3 ± 1.8	−4.44	3.8 ± 2.0	−15.56[a]
	LVI	5.0 ± 1.7	5.1 ± 1.9	2.0	4.4 ± 2.0	−12.0

The data represent mean ± SD (number of grains per 100 μm^2).
[a] Significant difference between the means of young versus aged groups and young versus middle-aged ($p < 0.05$).

While significant age-related declines in α_1 subunit message were observed across layers of AI and PtA, an apparent compensatory age-related increase in α_3 message level was observed for a subset of layers in AI and PtA (Tables 1 and 2; Fig. 2C). $GABA_AR$ α_3 subunit message levels showed significant ($p < 0.001$) age-related increases over neurons in supragranular AI layers II and III, and output layer V with trends toward $GABA_AR$ α_3 subunit message level increases in AI layer IV (Table 1, Fig. 2C). Aged AI layers II and III showed age-related α_3 subunit message level increases near 30% (Fig. 2C) when compared with young layers. Similar, but more modest pattern for $GABA_AR$ α_3 subunit message increases were observed in layers III and IV of PtA (Table 2, Fig. 2C).

No consistent age-related changes were observed for α_2 or α_5 $GABA_AR$ subunit message over neurons in AI layers II–VI (Table 1). However, $GABA_AR$ α_2 subunit showed significant increases in middle-age, (AI layers II, V, and VI) before returning to near young-adult levels in aged rat AI (Table 1).

3.1.2. Aging and $GABA_AR$ β_{1-3} subunit message changes

Significant age-related β_{1-3} $GABA_AR$ subunit message losses were seen across all AI layers (Table 1, Fig. 2E). β_{1-2} subunits showed changes between 17% and 34% across all layers of aged AI when compared with young animals (Table 1, Fig. 2E). AI β_{1-2} subunit changes in middle-aged animals were generally not significantly different from young-adult levels, with the exception of β_1 subunit changes in AI layers V and VI (Table 1). $GABA_AR$ β_3 subunit message showed significant age-related reductions in AI layers II–IV and nonsignificant changes in AI of middle aged animals (Table 1).

3.1.3. Aging and $GABA_AR$ subunit γ_1, γ_{2s}, and γ_{2L} message changes

Significant age-related decreases were seen for γ_1 and γ_{2L} $GABA_AR$ subunit message levels and no age-related γ_{2s} subunit message changes were observed for aged rat AI (Table 1). Only γ_{2L} $GABA_AR$ subunit message levels were examined in PtA. Age-related changes for γ_{2L} $GABA_AR$ subunit message in PtA were smaller than those observed for AI and were significant only in LII and LV of aged PtA (Table 2). Unfortunately, selective antibodies with adequate signal to noise ratios were not available to allow for quantitative immunohistochemistry of γ_1, γ_{2s}, and γ_{2L} $GABA_AR$ subunit proteins.

3.2. Aging and $GABA_AR$ subunit protein changes: α_{1-3}

Densitometric immunohistochemical studies were used to assess $GABA_AR$ subunit protein levels over individual neurons across the layers of AI and PtA for $GABA_AR$ α subunits which showed significant age-related message changes. Age-related protein changes focused on the α_{1-3} $GABA_AR$ subunits, from cortical neurons in AI and PtA (Table 3). Confocal images showed age-related loss of α_1 $GABA_AR$ subunit fluorescence (red) and the apparent compensatory age-related increase in α_3 subunit protein (green) (Fig. 3). Age-related protein changes were, for the most part smaller than, but consistent with α_{1-3} subunit message changes (Fig. 2 and Table 1). Significant age-related reductions in α_1 $GABA_AR$ subunit protein levels (10%–17%) were seen across AI layers reaching significance in superficial layers II and III (Fig. 2B, Table 3). Consistent with the observed increase of α_3 subunit message, there was an age-related upregulation of α_3 $GABA_AR$ subunit protein in AI. $GABA_AR$ α_3 subunit increases ranged between 2% and 13% reaching significance for neurons in layers II–III and layer V (Fig. 2D, Table 3). Similar age-related α_{1-3} subunit message and protein changes were observed in adjoining parietal cortex (Tables 2 and 3). Age-related increases in α_3 subunit protein in PtA exceeded changes observed in AI α_3 subunit protein. However, the general pattern of age-related α_{1-3} subunit changes was similar across the 2 cortical areas.

3.2.1. Aging and $GABA_AR$ β_{1-2} subunit protein changes

Neuronal protein levels were obtained for β_{1-2} $GABA_AR$ subunits but not for the β_3 subunit because of the lack of availability of a specific antibody. Consistent with age-related declines in β_2 subunit message across AI layers, $GABA_AR$ β_2 subunit protein levels declined significantly across AI layers (Table 3, Fig. 2F). Nonsignificant decreases were also observed for β_2 $GABA_AR$ subunit protein in middle-aged AI (Table 3; Fig. 2F). $GABA_AR$ β_2 subunit protein increases in PtA were in sharp contrast to that observed for β_2 $GABA_AR$ subunit protein in neighboring AI and in contrast to β_2 $GABA_AR$ subunit message level decreases observed for PtA and AI (Table 3). Significant increases for β_2 $GABA_AR$ subunit protein were observed across most PtA layers for middle-aged and aged animals when compared with young-adult PtA (Table 3; Fig. 2F).

In contrast to age-related decreases for β_2 subunit protein, β_1 $GABA_AR$ subunit protein decreased significantly only in middle-aged AI with no significant changes observed for aged AI compared with young-adult AI (Table 3). In addition, there were significant increases in β_1 subunit protein levels in LII and LV of middle-aged PtA but no significant changes in aged PtA (Table 3).

3.3. Age-related pharmacological changes of $GABA_A$ receptors in AI

Groups of young (4–6 months old), middle-aged (20–24 months old), and aged (30–34 months old) FBN rats were used to further examine the effect of aging on intact mature $GABA_ARs$ and the ability of GABA to modulate ligand binding at the picrotoxin binding site in the $GABA_AR$ pore of AI neurons (Milbrandt, et al., 1996). Fig. 4 shows higher levels of RO15-4513 binding in the superficial layers of AI. RO15-4513 is thought to be sensitive to the identity of α and γ $GABA_AR$ subunits (Luddens and Wisden, 1991). A significant age-related loss of [^{3}H]RO15-4513 $GABA_AR$ binding sites (Bmax) was found across all layers of aged AI ($p < 0.017$; $n = 8, 8, 8,$) and *kd* values were unaltered (Table 4). In contrast to [^{3}H]RO15-4513 binding, [^{3}H]TBOB binding was highest in the deep layers of AI (Fig. 5). In this assay, in the absence of GABA, $GABA_ARs$ are closed

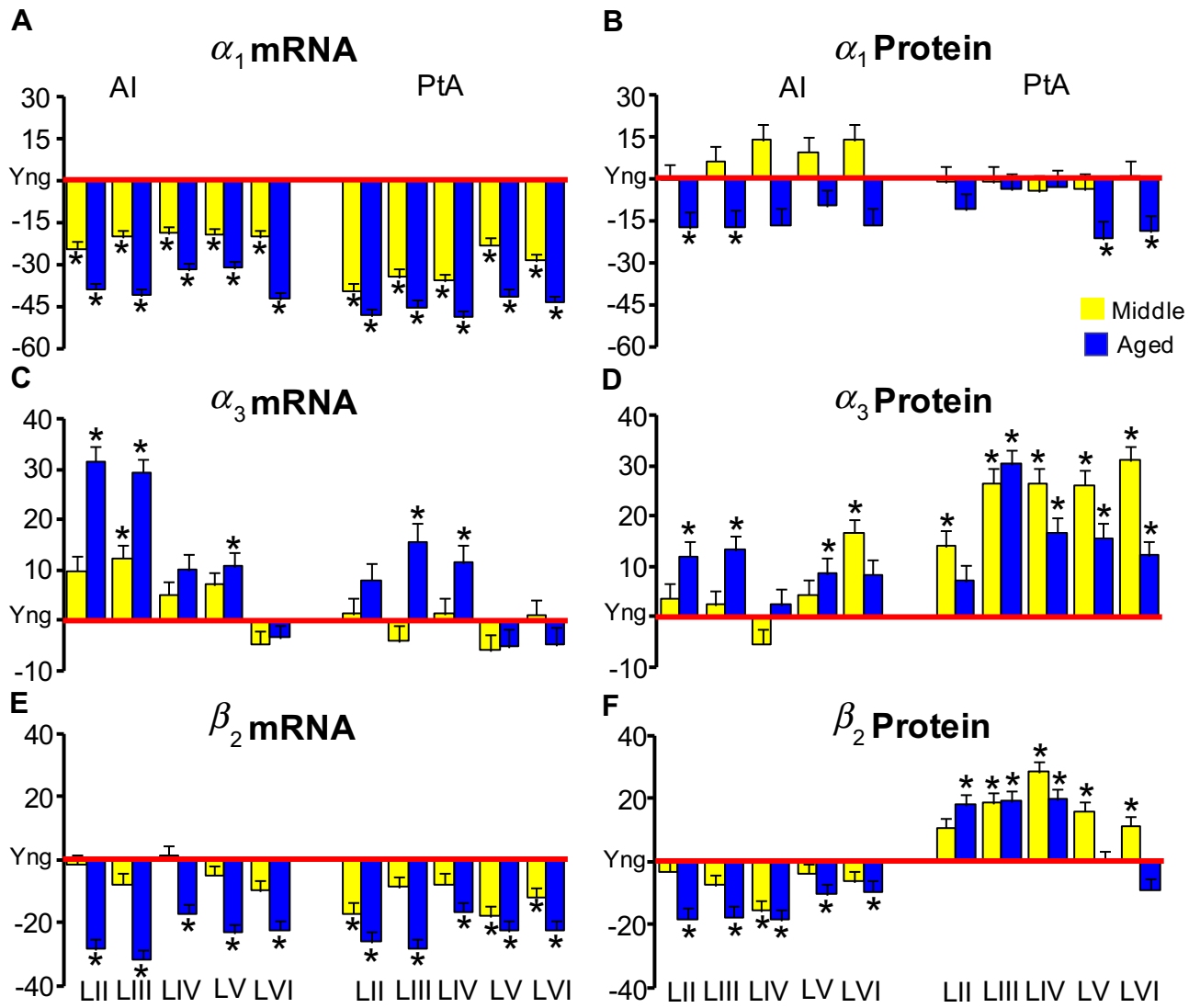

Fig. 2. Age-related changes of gamma-aminobutyric acid$_A$ receptor (GABA$_A$R) subunit α_1, α_3, and β_2 message and proteins in FBN rat auditory cortex (AI) and parietal cortex (PtA). Bar graphs represent the percentage changes of middle-aged ($n = 6$) and aged ($n = 6$) from the young (red x-axis, $n = 6$) for the message and protein levels of GABA$_A$R α_1(A and B), α_3 (C and D), and β_2 (E and F). Error bars represent the standard error of the mean. * $p < 0.05$.

and no [^{3}H]TBOB binding could occur in the chloride channel of either young or aged AI GABA$_A$Rs (Fig. 5). With increasing concentrations of GABA (10 nM–5 μM), AI neuronal GABA$_A$R chloride channels were activated/opened providing access for [^{3}H]TBOB binding at picrotoxin sites. At higher GABA concentrations, GABA$_A$Rs became desensitized and the binding curve began to approximate baseline at the highest concentration of GABA (5 μM). This cycle of events was significantly altered in aged AI. Fig. 5 shows the age-related change in the [^{3}H]TBOB binding curve between young-adult and aged AI layer VI as GABA levels were increased. The observed age-related changes in RO15-4513 binding and TBOB modulation supported subunit message and protein data which indicated an age-related change in the makeup and stoichiometry of GABA$_A$Rs across the layers of aged AI.

4. Discussion

The present findings of age-related GABA$_A$R subunit and pharmacologic changes strongly support previous neurochemical, human psychophysical, and animal physiologic studies suggesting dysfunctional inhibitory processing of acoustic information in aged AI.

4.1. Age-related GABA$_A$R subunit changes and discordance between message and protein changes

The present findings in AI for GABA$_A$R subunit mRNA levels are consistent with cortical changes described by Gutierrez et al. (1997) showing substantial age-related changes for α_1, β_{2-3}, and γ_2 GABA$_A$R subunit messages in other brain areas. In addition, the present study found age-related loss of GABA$_A$R β_1 and γ_1 subunit message across AI layers (Table 1). Changes specific to the GABA$_A$R α_1, the wild type α subunit message were across AI layers and greater than 30%. GABA$_A$R α_1 findings were consistent with previous studies of GABA$_A$R α_1 subunit message changes in aging neocortex, in which Mhatre and Ticku (1992) described an 86% age-related decrease and Gutierrez et al. (1997) found a 29% reduction in the neocortex. In contrast to previous studies which did not observe significant age-related cortical subunit protein changes (Gutierrez, et al., 1996, 1997; Rissman et al., 2007; Yu et al., 2006),

Table 3
Comparison of age-related changes of gamma-aminobutyric acid $(GABA)_A$ receptor subunit protein levels in A1 and PtA

		A1					PtA				
		Young	Middle	Percent change from young	Aged	Percent change from young	Young	Middle	Percent change from young	Aged	Percent change from young
α_1	LII	0.090 ± 0.035	0.090 ± 0.046	0	0.075 ± 0.038	−16.67[a]	0.073 ± 0.026	0.072 ± 0.031	−1.37	0.065 ± 0.036	−10.96
	LIII	0.089 ± 0.034	0.095 ± 0.045	6.74	0.074 ± 0.035	−16.85[a]	0.071 ± 0.027	0.070 ± 0.032	−1.41	0.069 ± 0.039	−2.82
	LIV	0.081 ± 0.033	0.093 ± 0.048	14.81	0.068 ± 0.030	−16.05	0.071 ± 0.027	0.068 ± 0.029	−4.23	0.069 ± 0.039	−2.82
	LV	0.079 ± 0.031	0.087 ± 0.045	10.13	0.071 ± 0.035	−10.13	0.078 ± 0.029	0.075 ± 0.033	−3.85	0.061 ± 0.040	−21.79[a]
	LVI	0.078 ± 0.032	0.089 ± 0.044	14.10	0.066 ± 0.035	−15.38	0.075 ± 0.029	0.075 ± 0.036	0	0.061 ± 0.034	−18.67[a]
α_3	LII	0.136 ± 0.034	0.141 ± 0.030	3.68	0.152 ± 0.039	11.76[a]	0.103 ± 0.020	0.117 ± 0.039	13.59[a]	0.110 ± 0.030	6.80
	LIII	0.134 ± 0.041	0.138 ± 0.034	2.98	0.152 ± 0.034	13.43[a]	0.097 ± 0.025	0.122 ± 0.035	25.77[a]	0.126 ± 0.037	29.90[a]
	LIV	0.122 ± 0.036	0.116 ± 0.032	−4.92	0.125 ± 0.031	2.46	0.093 ± 0.025	0.118 ± 0.030	26.88[a]	0.109 ± 0.034	17.20[a]
	LV	0.125 ± 0.036	0.131 ± 0.036	4.8	0.136 ± 0.031	8.8[a]	0.099 ± 0.028	0.125 ± 0.030	26.26[a]	0.114 ± 0.024	15.15[a]
	LVI	0.096 ± 0.027	0.113 ± 0.028	17.71[a]	0.104 ± 0.027	8.33	0.085 ± 0.026	0.111 ± 0.024	30.59[a]	0.095 ± 0.021	11.76[a]
β_1	LII	0.178 ± 0.037	0.152 ± 0.043	−14.61[a]	0.163 ± 0.044	−8.43	0.104 ± 0.043	0.125 ± 0.055	20.19[a]	0.110 ± 0.049	5.77
	LIII	0.159 ± 0.042	0.139 ± 0.037	−12.58[a]	0.157 ± 0.033	−1.26	0.114 ± 0.045	0.118 ± 0.038	3.51	0.119 ± 0.050	4.39
	LIV	0.141 ± 0.039	0.117 ± 0.031	−17.02[a]	0.138 ± 0.037	−2.13	0.097 ± 0.046	0.112 ± 0.030	15.46	0.107 ± 0.040	10.31
	LV	0.125 ± 0.054	0.128 ± 0.036	2.4	0.123 ± 0.038	−1.6	0.093 ± 0.041	0.120 ± 0.036	29.03[a]	0.109 ± 0.057	17.20
	LVI	0.118 ± 0.041	0.098 ± 0.024	−16.95[a]	0.117 ± 0.042	−0.85	0.090 ± 0.044	0.092 ± 0.029	2.22	0.099 ± 0.053	10.0
β_2	LII	0.128 ± 0.026	0.124 ± 0.044	−3.13	0.105 ± 0.034	−17.97[a]	0.080 ± 0.041	0.089 ± 0.027	11.25	0.095 ± 0.041	18.75[a]
	LIII	0.122 ± 0.024	0.112 ± 0.038	−8.20	0.100 ± 0.030	−18.03[a]	0.078 ± 0.028	0.092 ± 0.035	17.95[a]	0.093 ± 0.035	19.23[a]
	LIV	0.114 ± 0.024	0.096 ± 0.035	−15.79[a]	0.093 ± 0.031	−18.42[a]	0.067 ± 0.021	0.086 ± 0.025	28.36[a]	0.080 ± 0.036	19.40[a]
	LV	0.107 ± 0.026	0.103 ± 0.028	−3.74	0.096 ± 0.030	−10.28[a]	0.085 ± 0.033	0.099 ± 0.030	16.47[a]	0.085 ± 0.038	0
	LVI	0.093 ± 0.028	0.088 ± 0.025	−5.38	0.085 ± 0.026	−8.60[a]	0.083 ± 0.023	0.093 ± 0.027	12.05[a]	0.076 ± 0.030	−8.4

Key: A1, auditory cortex; L, layer; PtA, parietal cortex.
[a] Significant difference between the means of young versus aged groups and young versus middle-aged ($p < 0.05$).

the present study finds $\alpha_{1\ and\ 3}$ $GABA_AR$ subunit protein changes in AI consistent with observed age-related message changes. However, the present study did find substantial quantitative and qualitative discordance between $GABA_AR$ subunit message changes and protein changes as previously noted by Gutierrez et al. (1996, 1997), and Wang et al. (2009b). With the notable exception observed for $GABA_AR$ β subunit changes, the present age-related protein findings were qualitatively consistent with, but more modest than, corresponding subunit message changes in AI and PtA. Relatively smaller age-related percent changes for protein expression, compared with subunit message changes, might reflect posttranslational compensatory mechanisms or perhaps a less sensitive method for assessing cellular protein levels relative to cellular message levels. Contrary to this latter possibility, there were examples of some age-related protein changes which exceeded age-related subunit message changes. One example found that age-related $GABA_AR$ α_3 subunit protein changes in PtA were greater than corresponding $GABA_AR$ α_3 message changes (Tables 1 and 3).

The most striking example of message/protein discordance with aging found significantly increased $GABA_AR$ $\beta_{1\ and\ 2}$ subunit proteins in PtA in the face of dramatically decreased $\beta_{1\ and\ 2}$ subunit message levels (Fig. 2E and F; Tables 1 and 3). It is important to understand how aging affects both expression and post-translational processing of $GABA_AR$ subunits. The presence of age-related discordance between subunit message and protein is emblematic of posttranslational age-related changes. These findings might reflect robust compensatory posttranslational aging mechanisms which will require further study. It is unlikely that these findings are because of experimental error because all measurements were blinded and age-related measures of $\beta_{1\ and\ 2}$ subunit message and protein levels were carried out in different animals, and comparisons between AI and PtA were carried out in the same animals. Immunolabeling over neocortex was not observed to be uneven over PtA and AI, which are adjacent structures. Categorically, similar age-related changes between subunit message and protein have been described for glycine receptor subunits in the aging dorsal cochlear nucleus (Wang et al., 2009b).

4.2. $GABA_AR$ α_1 and α_3 subunit protein changes

The present study examined a subset of $GABA_AR$ subunit proteins partially limited by the availability of high quality subunit antibodies for certain $GABA_AR$ subunits. As noted above, significant age-related $GABA_AR$ subunit protein decreases occurred across layers in AI for α_1

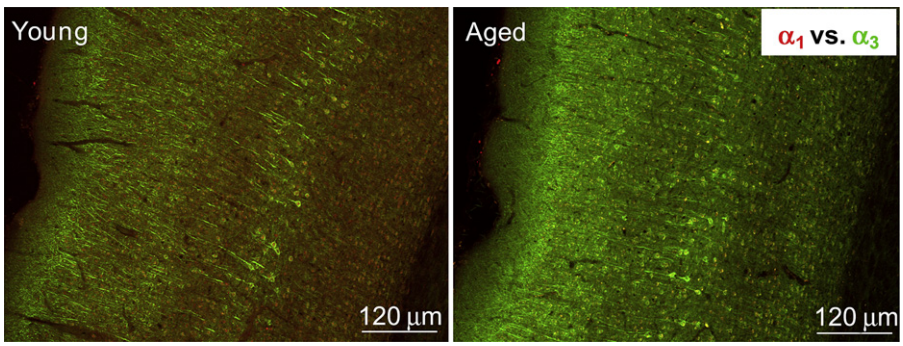

Fig. 3. Confocal images of immuno double-labeling using gamma-aminobutyric $acid_A$ receptor ($GABA_AR$) α_1 (red) and α_3 (green) in auditory cortex (AI) from young adult and aged FBN rat. A clearly increased α_3 immunopositive staining is seen in the aged AI when compared with the young AI. Scale bar = 120 μm.

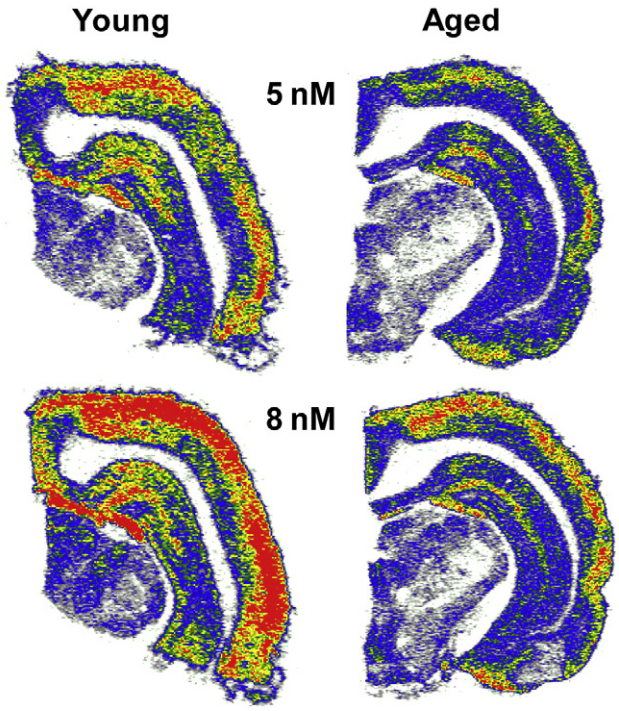

Fig. 4. [^{3}H]RO15-4513 gamma-aminobutyric acid$_A$ receptor (GABA$_A$R) binding in auditory cortex (AI) of young and aged FBN rats. A significant age-related loss of GABA$_A$R binding can be seen across all layers of AI at the concentrations of 5 and 8 nM of ^{3}[H] RO15-4513. Highest binding levels were observed in the superficial layers of AI.

and β_2 with many age-related protein changes approaching 20% (Fig. 2B and F, Table 3). PtA displayed similar GABA$_A$R subunit protein changes with aging with the GABA$_A$R $\beta_{1 \text{ and } 2}$ subunit protein exceptions noted above. Perhaps as a compensatory change for the profound decrease in the GABA$_A$R α_1 subunit protein, GABA$_A$R α_3 subunit proteins tended to increase with age in both AI and PtA. These changes were significant for LII, III, and V in AI and all but LII in PtA. The mechanism for, and the significance of, these age-related compensatory subunit changes are unknown at the present time. A recent aging study in human visual cortex examined GABA$_A$R related protein changes and reported an age-related trend toward increased GABA$_A$R α_3 subunit protein between 20 and 80 years of age (Pinto et al., 2010). The age-related downregulation of α_1 message and protein and the layer selective age-related upregulation of α_3 message and protein are suggestive of a reverse of compensatory changes seen in development and other models of GABA deafferentation (Caspary et al., 2008). Caspary et al. (1990) found an age-related reduction in GABA release in inferior colliculus of aged F344 rats. As reviewed above, models of sensory aging are suggestive of altered GABA inhibitory neurotransmission. The present findings and similar studies of the aged inferior colliculus suggest that this loss of GABA tone is at least in part a result of plastic changes in GABA$_A$R subunit composition (Caspary et al., 2008). Developmental and expression GABA$_A$R subunit studies suggest that observed subunit changes are consistent with smaller peak evoked inhibitory postsynaptic currents (IPSCs) having longer slower time constants, perhaps in an effort to compensate for the loss of inhibitory input (Bosman et al., 2002; Juttner et al., 2001; Wafford et al., 1993). Evidence for age-related compensatory GABA$_A$R subunit changes have been described for inferior colliculus (Caspary et al., 1999) and are implicit in a number of other studies (Rissman et al., 2007; Zhou et al., 2011).

4.3. Age-related receptor binding changes reflect altered GABA$_A$ subunit content of functional receptors

Subunit composition/stoichiometry can dramatically affect receptor pharmacology and channel function (Angelotti and Macdonald, 1993; Caspary et al., 1999; Ducic et al., 1995; Macdonald and Olsen, 1994; Rudolph et al., 2001; Sigel et al., 1990; Wafford et al., 1993). Age-related changes in GABA$_A$R subunit constructs would affect the pharmacology of GABA$_A$Rs (Ebert et al., 1994; Wafford et al., 1993). Age-related changes in GABA$_A$R binding were previously reported for nonauditory cortical structures and hippocampus (Concas et al., 1988; Erdo and Wolff, 1989; Mhatre and Ticku, 1992; Ruano et al., 1992). Previous receptor binding studies have shown age-related changes in GABA$_A$R pharmacology of inferior colliculus using several subunit selective radiolabeled GABA$_A$R ligands (Milbrandt et al., 1994, 1996). RO15-4513 is thought to differentially bind GABA$_A$R constructs containing different α and γ GABA$_A$R subunits (Ebert et al., 1994; Wafford et al., 1993). The literature is not definitive on the binding properties of the benzodiazepine (BDZ) inverse agonist RO15-4513 but strongly suggests a preference for binding constructs containing $\alpha_5 > \alpha_1$ (Lingford-Hughes et al., 2002). The present study found significant RO15-4513 binding in the upper layers of AI in agreement with the description by Pirker et al. (2000) of moderate levels of α_5 subunit containing GABA$_A$Rs in neocortical layers IV and high levels of α_1 GABA$_A$Rs in supragranular layers of the neocortex. Data from the present study finds reduced RO15-4513 binding in aged AI compared with young-adult AI. This age-related change likely reflects the observed α_x and γ_x subunit changes or decreased numbers of functionally assembled and inserted GABA$_A$Rs because of age-related changes in trafficking and or anchoring proteins (Wang et al., 2009a).

The present findings (Fig. 5) are consistent with previous studies showing age-related loss in the ability of GABA to modulate binding at the picrotoxin site with age (Erdo and Wolff, 1989; Mhatre and Ticku, 1992; Milbrandt et al., 1996). TBOB selectively binds to convulsant sites associated with the chloride channel (Olsen et al., 1990). The differential ability of GABA to modulate the binding of picrotoxin analogs, such as TBOB and t-butylbicyclophosphorothionate (TBPS), to the picrotoxin site within the chloride channel of different GABA$_A$ constructs were examined (Im et al., 1994). These authors found that maximal enhancement of t-butylbicyclophosphorothionate (TBPS) binding by GABA (opening of channels to allow binding) in cloned rat GABA$_A$R subtypes varied with the isoforms (153 ± 10%, 438 ± 16%, and 139 ± 29% for $\alpha_1\beta_2$, $\alpha_3\beta_2$, and $\alpha_6\beta_2$, respectively). The present

Table 4
Bmax and *kd* of RO15-4513 saturation analysis

Layer	Bmax (fmol/mg protein)			*Kd* (nM)		
	Young	Middle	Aged	Young	Middle	Aged
II/III/IV	618 ± 44.7	550 ± 25.3[a]	564 ± 34.7[a]	1.03 ± 0.40	0.80 ± 0.22	0.99 ± 0.33
V	545 ± 26.2	485 ± 16.9[a]	510 ± 26.9[a]	0.81 ± 0.23	0.63 ± 0.15	0.81 ± 0.26
VI	505 ± 22.3	465 ± 15.4[a]	472 ± 21.8[a]	0.75 ± 0.21	0.56 ± 0.14	0.64 ± 0.20

[a] Significance from the young ($p < 0.05$).

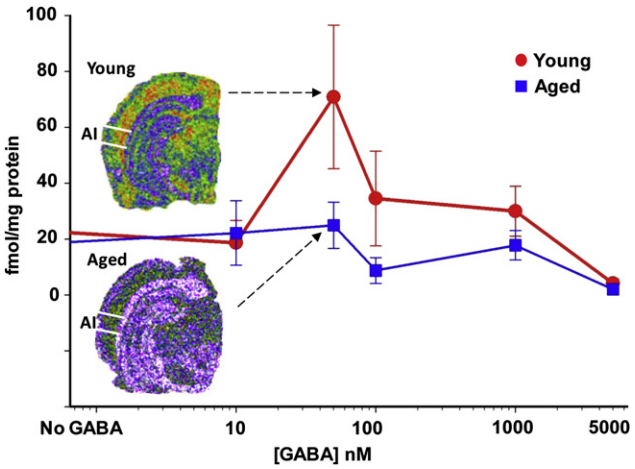

Fig. 5. Gamma-aminobutyric acid (GABA) modulation of ^{3}[H]*t*-butylbicycloorthobenzoate (TBOB) binding in layer VI of young and aged FBN rat auditory cortex (AI). Increasing concentrations of GABA (10 nM–5 μM) were added to the TBOB assay. A significant age-related loss of TBOB binding was observed throughout aged neocortex when compared with young AI (the error bars represent standard error of the mean). The age-related shift in the GABA dose-response curve in layer VI of FBN rat AI suggests a change in GABA's ability to activate/open aged $GABA_A$ receptors ($n = 4$ young and 4 aged).

binding study did not allow us to accurately discriminate individual AI layer changes but the highest levels of TBOB binding was found in infragranular layers of AI.

4.4. Age-related changes of GABA neurotransmission and inhibitory function in AI

An increasing number of studies in AI describe significant age-related losses of presynaptic markers for GABA and functional changes indicative of a loss of normal adult GABAergic function. Glutamic acid decarboxylase, the primary synthesizing enzyme for GABA, is significantly decreased in rat AI (Burianova et al., 2009; Ling et al., 2005) and parallels a significant decrease in the number and optical density of parvalbumin-labeled neurons in rat AI (de Villers-Sidani et al., 2010; Martin Del Campo et al., 2012; Ouda et al., 2008). Human AI shows age-related decreased levels of markers for normal adult GABA function (McGeer and McGeer, 1976; Pinto et al., 2010). Functional loss of adult primate GABAergic function has been described in visual cortex (Betts et al., 2005; Leventhal et al., 2003; Schmolesky et al., 2000). Age-related changes suggestive of a loss of normal young adult GABAergic function in AI have been recently reviewed (Caspary et al., 2008; Mendelson and Rajan, 2011). Young and aged FBN rats show a fairly parallel (15–20 dB) age-related threshold increase across frequencies (Caspary et al., 2005; Wang et al., 2009b). Recent cortical electrophysiology studies are strongly suggestive of an age-related loss of inhibitory function resulting in increased spontaneous and driven activity in the upper layers of rat and primate primary (AI) and secondary AI (Hughes et al., 2010; Juarez-Salinas et al., 2010). Age-related loss in the ability to localize sound in space in primate AI and secondary AI and a negative effect on novelty detection in rats can be directly related to inhibitory changes. We would have preferred if the upper layers showed greater age-related changes in subunit composition than the deeper layers because it appears that some of the greater changes occurred in the layers with the highest levels of $GABA_A$ receptors (Prieto et al., 1994). However, the apical dendrites extending up to LI–III have their cell bodies in IV and V which may well confound the relative distribution of age-related changes because the somatic expression of the subunit markers show somatic expression although receptors might be in the supragranular layers. Behavioral and evoked potential studies are strongly suggestive of reduced temporal processing in aged rat central auditory pathway (Suta et al., 2011). Rat studies describe age-related losses in the ability to detect novel sounds in AI, which was partially reversed by training, resulting in upregulated parvalbumin labeling in aged animals (de Villers-Sidani et al., 2010).

In support of the present observations, a recent study by Schmidt et al. (2010) found significant age-related decreases in paired-pulse inhibition in both auditory and parietal cortices. In contrast to the present findings and those of Gutierrez et al. (1997), this study described $GABA_AR$ α_1 subunit protein increases with aging in parietal cortex for a subset of rats (Schmidt et al., 2010). Studies in the primate visual cortex described age-related changes in the visual receptive fields system (Juarez-Salinas et al., 2010; Leventhal et al., 2003) and Juarez-Salinas et al. (2010) described degraded spatial tuning in unit responses from AI and secondary auditory cortical areas.

Collectively, the present findings are in agreement with the studies reviewed above, by showing significant age-related loss of wild type ($\alpha_1\beta_2\gamma_2$) $GABA_AR$ markers commonly found in young-adult AI. In situ hybridization and immunohistochemistry data showed age-related changes of mRNA and protein within the individual $GABA_AR$ subunits, and receptor binding study revealed the pharmacologic changes when these subunit proteins assembled as functional $GABA_A$ receptors. Loss of wild type $GABA_ARs$ and their replacement by $GABA_AR$ constructs with different subunit combinations would be expected to show slower inhibitory response kinetics and lower peak currents impairing the ability to reliably process temporally demanding stimuli (Richardson et al., 2012; Wafford et al., 1993). It is likely that these age-related changes in normal $GABA_A$ receptor function could affect speech understanding in a subset of the human elderly population.

Disclosure statement

The authors have no conflicts of interest.

All experiments were carried out under animal use protocols approved by the Southern Illinois University School of Medicine Laboratory Animal Care and Use Committee.

Acknowledgements

The authors thank Dr Jennifer Parrish and Judith Bryan for their helpful editing. This research is supported by NIH grant DC000151.

References

Anderson, S., Parbery-Clark, A., White-Schwoch, T., Kraus, N., 2012. Aging affects neural precision of speech encoding. J. Neurosci. 32, 14156–14164.

Angelotti, T.P., Macdonald, R.L., 1993. Assembly of GABAA receptor subunits: alpha 1 beta 1 and alpha 1 beta 1 gamma 2S subunits produce unique ion channels with dissimilar single-channel properties. J. Neurosci. 13, 1429–1440.

Belelli, D., Peden, D.R., Rosahl, T.W., Wafford, K.A., Lambert, J.J., 2005. Extrasynaptic GABAA receptors of thalamocortical neurons: a molecular target for hypnotics. J. Neurosci. 25, 11513–11520.

Betts, L.R., Taylor, C.P., Sekuler, A.B., Bennett, P.J., 2005. Aging reduces center-surround antagonism in visual motion processing. Neuron 45, 361–366.

Bosman, L.W., Rosahl, T.W., Brussaard, A.B., 2002. Neonatal development of the rat visual cortex: synaptic function of GABAA receptor alpha subunits. J. Physiol. 545, 169–181.

Braestrup, C., Nielsen, M., Honore, T., 1983. Binding of [3H]DMCM, a convulsive benzodiazepine ligand, to rat brain membranes: preliminary studies. J. Neurochem. 41, 454–465.

Burianova, J., Ouda, L., Profant, O., Syka, J., 2009. Age-related changes in GAD levels in the central auditory system of the rat. Exp. Gerontol. 44, 161–169.

Canlon, B., Illing, R.B., Walton, J., 2010. Cell biology and physiology of the aging central auditory pathway. In: Gordon-Salant, S., Frisina, R.D., Popper, A.N., Fay, R.R. (Eds.), The Aging Auditory System. Springer, New York, pp. 39–74.

Caspary, D.M., Holder, T.M., Hughes, L.F., Milbrandt, J.C., McKernan, R.M., Naritoku, D.K., 1999. Age-related changes in GABA(A) receptor subunit composition and function in rat auditory system. Neuroscience 93, 307–312.

Caspary, D.M., Ling, L., Turner, J.G., Hughes, L.F., 2008. Inhibitory neurotransmission, plasticity and aging in the mammalian central auditory system. J. Exp. Biol. 211 (pt 11), 1781–1791.

Caspary, D.M., Raza, A., Lawhorn Armour, B.A., Pippin, J., Arneric, S.P., 1990. Immunocytochemical and neurochemical evidence for age-related loss of GABA in the inferior colliculus: implications for neural presbycusis. J. Neurosci. 10, 2363–2372.

Caspary, D.M., Schatteman, T.A., Hughes, L.F., 2005. Age-related changes in the inhibitory response properties of dorsal cochlear nucleus output neurons: role of inhibitory inputs. J. Neurosci. 25, 10952–10959.

Concas, A., Pepitoni, S., Atsoggiu, T., Toffano, G., Biggio, G., 1988. Aging reduces the GABA-dependent 36Cl- flux in rat brain membrane vesicles. Life Sci. 43, 1761–1771.

de Villers-Sidani, E., Alzghoul, L., Zhou, X., Simpson, K.L., Lin, R.C., Merzenich, M.M., 2010. Recovery of functional and structural age-related changes in the rat primary auditory cortex with operant training. Proc. Natl. Acad. Sci. U. S. A. 107, 13900–13905.

Dubno, J.R., Dirks, D.D., Morgan, D.E., 1984. Effects of age and mild hearing loss on speech recognition in noise. J. Acoust. Soc. Am. 76, 87–96.

Ducic, I., Caruncho, H.J., Zhu, W.J., Vicini, S., Costa, E., 1995. Gamma-aminobutyric acid gating of Cl- channels in recombinant GABAA receptors. J. Pharmacol. Exp. Ther. 272, 438–445.

Ebert, B., Wafford, K.A., Whiting, P.J., Krogsgaard-Larsen, P., Kemp, J.A., 1994. Molecular pharmacology of gamma-aminobutyric acid type A receptor agonists and partial agonists in oocytes injected with different alpha, beta, and gamma receptor subunit combinations. Mol. Pharmacol. 46, 957–963.

Erdo, S.L., Wolff, J.R., 1989. Age-related loss of t-[35S]butylbicyclophosphorothionate binding to the gamma-aminobutyric acidA receptor-coupled chloride ionophore in rat cerebral cortex. J. Neurochem. 53, 648–651.

Fitzgibbons, P.J., Gordon-Salant, S., 1994. Age effects on measures of auditory duration discrimination. J. Speech Hear. Res. 37, 662–670.

Fitzgibbons, P.J., Gordon-Salant, S., 2010. Age-related differences in discrimination of temporal intervals in accented tone sequences. Hear. Res. 264, 41–47.

Fogerty, D., Humes, L.E., Kewley-Port, D., 2010. Auditory temporal-order processing of vowel sequences by young and elderly listeners. J. Acoust. Soc. Am. 127, 2509–2520.

Games, K.D., Winer, J.A., 1988. Layer V in rat auditory cortex: projections to the inferior colliculus and contralateral cortex. Hear. Res. 34, 1–25.

Gutierrez, A., Khan, Z.U., Miralles, C.P., Mehta, A.K., Ruano, D., Araujo, F., Vitorica, J., De Blas, A.L., 1997. GABAA receptor subunit expression changes in the rat cerebellum and cerebral cortex during aging. Brain Res. Mol. Brain Res. 45, 59–70.

Gutierrez, A., Khan, Z.U., Ruano, D., Miralles, C.P., Vitorica, J., De Blas, A.L., 1996. Aging-related subunit expression changes of the GABAA receptor in the rat hippocampus. Neuroscience 74, 341–348.

Hadingham, K.L., Garrett, E.M., Wafford, K.A., Bain, C., Heavens, R.P., Sirinathsinghji, D.J., Whiting, P.J., 1996. Cloning of cDNAs encoding the human gamma-aminobutyric acid type A receptor alpha 6 subunit and characterization of the pharmacology of alpha 6-containing receptors. Mol. Pharmacol. 49, 253–259.

Hua, T., Li, X., He, L., Zhou, Y., Wang, Y., Leventhal, A.G., 2006. Functional degradation of visual cortical cells in old cats. Neurobiol. Aging 27, 155–162.

Hughes, L.F., Turner, J.G., Parrish, J.L., Caspary, D.M., 2010. Processing of broadband stimuli across A1 layers in young and aged rats. Hear. Res. 264, 79–85.

Im, W.B., Pregenzer, J.F., Thomsen, D.R., 1994. Effects of GABA and various allosteric ligands on TBPS binding to cloned rat GABA(A) receptor subtypes. Br. J. Pharmacol. 112, 1025–1030.

Juarez-Salinas, D.L., Engle, J.R., Navarro, X.O., Recanzone, G.H., 2010. Hierarchical and serial processing in the spatial auditory cortical pathway is degraded by natural aging. J. Neurosci. 30, 14795–14804.

Juttner, R., Meier, J., Grantyn, R., 2001. Slow IPSC kinetics, low levels of alpha1 subunit expression and paired-pulse depression are distinct properties of neonatal inhibitory GABAergic synaptic connections in the mouse superior colliculus. Eur. J. Neurosci. 13, 2088–2098.

Khrestchatisky, M., MacLennan, A.J., Chiang, M.Y., Xu, W.T., Jackson, M.B., Brecha, N., Sternini, C., Olsen, R.W., Tobin, A.J., 1989. A novel alpha subunit in rat brain GABAA receptors. Neuron 3, 745–753.

Knoflach, F., Reinscheid, R.K., Civelli, O., Kemp, J.A., 1996. Modulation of voltage-gated calcium channels by orphanin FQ in freshly dissociated hippocampal neurons. J. Neurosci. 16, 6657–6664.

Korpi, E.R., Grunder, G., Luddens, H., 2002. Drug interactions at GABA(A) receptors. Prog. Neurobiol. 67, 113–159.

Leventhal, A.G., Wang, Y., Pu, M., Zhou, Y., Ma, Y., 2003. GABA and its agonists improved visual cortical function in senescent monkeys. Science 300, 812–815.

Liang, J., Suryanarayanan, A., Chandra, D., Homanics, G.E., Olsen, R.W., Spigelman, I., 2008. Functional consequences of GABAA receptor alpha 4 subunit deletion on synaptic and extrasynaptic currents in mouse dentate granule cells. Alcohol Clin. Exp. Res. 32, 19–26.

Linden, A.M., Schmitt, U., Leppa, E., Wulff, P., Wisden, W., Luddens, H., Korpi, E.R., 2011. Ro 15-4513 antagonizes alcohol-induced sedation in mice through αβγ2-type GABA(A) receptors. Front. Neurosci. 5, 3.

Ling, L.L., Hughes, L.F., Caspary, D.M., 2005. Age-related loss of the GABA synthetic enzyme glutamic acid decarboxylase in rat primary auditory cortex. Neuroscience 132, 1103–1113.

Lingford-Hughes, A., Hume, S.P., Feeney, A., Hirani, E., Osman, S., Cunningham, V.J., Pike, V.W., Brooks, D.J., Nutt, D.J., 2002. Imaging the GABA-benzodiazepine receptor subtype containing the alpha5-subunit in vivo with [11C]Ro15 4513 positron emission tomography. J. Cereb. Blood Flow Metab. 22, 878–889.

Lloyd, G.K., Danielou, G., Thuret, F., 1990. The activity of zolpidem and other hypnotics within the gamma-aminobutyric acid (GABAA) receptor supramolecular complex, as determined by 35S-t-butylbicyclophosphorothionate (35S-TBPS) binding to rat cerebral cortex membranes. J. Pharmacol. Exp. Ther. 255, 690–696.

Luddens, H., Wisden, W., 1991. Function and pharmacology of multiple GABAA receptor subunits. Trends Pharmacol. Sci. 12, 49–51.

Lui, B., Mendelson, J.R., 2003. Frequency modulated sweep responses in the medial geniculate nucleus. Exp. Brain Res. 153, 550–553.

Macdonald, R.L., Olsen, R.W., 1994. GABAA receptor channels. Annu. Rev. Neurosci. 17, 569–602.

Maksay, G., Ticku, M.K., 1985. GABA, depressants and chloride ions affect the rate of dissociation of 35S-t-butylbicyclophosphorothionate binding. Life Sci. 37, 2173–2180.

Malherbe, P., Sigel, E., Baur, R., Persohn, E., Richards, J.G., Mohler, H., 1990. Functional expression and sites of gene transcription of a novel alpha subunit of the GABAA receptor in rat brain. FEBS Lett. 260, 261–265.

Martin Del Campo, H.N., Measor, K.R., Razak, K.A., 2012. Parvalbumin immunoreactivity in the auditory cortex of a mouse model of presbycusis. Hear. Res. 294, 31–39.

McGeer, E.G., McGeer, P.L., 1980. Aging and neurotransmitter systems. Adv. Biochem. Psychopharmacol. 23, 305–314.

McGeer, P.L., McGeer, E.G., 1976. Enzymes associated with the metabolism of catecholamines, acetylcholine and gaba in human controls and patients with Parkinson's disease and Huntington's chorea. J. Neurochem. 26, 65–76.

Mendelson, J.R., Rajan, R., 2011. Cortical effects of aging and hearing loss. In: Winer, J.A., Schreiner, C.E. (Eds.), The Auditory Cortex. Springer, New York, pp. 493–511.

Mhatre, M.C., Ticku, M.K., 1992. Aging related alterations in GABAA receptor subunit mRNA levels in Fischer rats. Brain Res. Mol. Brain Res. 14, 71–78.

Milbrandt, J.C., Albin, R.L., Caspary, D.M., 1994. Age-related decrease in GABAB receptor binding in the Fischer 344 rat inferior colliculus. Neurobiol. Aging 15, 699–703.

Milbrandt, J.C., Albin, R.L., Turgeon, S.M., Caspary, D.M., 1996. GABAA receptor binding in the aging rat inferior colliculus. Neuroscience 73, 449–458.

Milbrandt, J.C., Caspary, D.M., 1995. Age-related reduction of [3H]strychnine binding sites in the cochlear nucleus of the Fischer 344 rat. Neuroscience 67, 713–719.

Milbrandt, J.C., Hunter, C., Caspary, D.M., 1997. Alterations of GABAA receptor subunit mRNA levels in the aging Fischer 344 rat inferior colliculus. J. Comp. Neurol. 379, 455–465.

Niddam, R., Dubois, A., Scatton, B., Arbilla, S., Langer, S.Z., 1987. Autoradiographic localization of [3H]zolpidem binding sites in the rat CNS: comparison with the distribution of [3H]flunitrazepam binding sites. J. Neurochem. 49, 890–899.

Noreña, A.J., 2011. An integrative model of tinnitus based on a central gain controlling neural sensitivity. Neurosci. Biobehav. Rev. 35, 1089–1109.

Oliver, D.L., Izquierdo, M.A., Malmierca, M.S., 2011. Persistent effects of early augmented acoustic environment on the auditory brainstem. Neuroscience 184, 75–87.

Olsen, R.W., Bureau, M., Khrestchatisky, M., MacLennan, A.J., Chiang, M.Y., Tobin, A.J., Xu, W., Jackson, M., Sternini, C., Brecha, N., 1990. Isolation of pharmacologically distinct GABA-benzodiazepine receptors by protein chemistry and molecular cloning. Adv. Biochem. Psychopharmacol. 46, 35–49.

Olsen, R.W., Sieghart, W., 2008. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol. Rev. 60, 243–260.

Olsen, R.W., Sieghart, W., 2009. GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56, 141–148.

Ostroff, J.M., McDonald, K.L., Schneider, B.A., Alain, C., 2003. Aging and the processing of sound duration in human auditory cortex. Hear. Res. 181, 1–7.

Ouda, L., Druga, R., Syka, J., 2008. Changes in parvalbumin immunoreactivity with aging in the central auditory system of the rat. Exp. Gerontol. 43, 782–789.

Pan, H.S., Frey, K.A., Young, A.B., Penney Jr., J.B., 1983. Changes in [3H]muscimol binding in substantia nigra, entopeduncular nucleus, globus pallidus, and thalamus after striatal lesions as demonstrated by quantitative receptor autoradiography. J. Neurosci. 3, 1189–1198.

Paxinos, W., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego.

Peters, A., Feldman, M.L., Vaughan, D.W., 1983. The effect of aging on the neuronal population within area 17 of adult rat cerebral cortex. Neurobiol. Aging 4, 273–282.

Pichora-Fuller, M.K., Schneider, B.A., Macdonald, E., Pass, H.E., Brown, S., 2007. Temporal jitter disrupts speech intelligibility: a simulation of auditory aging. Hear. Res. 223, 114–121.

Pinto, J.G., Hornby, K.R., Jones, D.G., Murphy, K.M., 2010. Developmental changes in GABAergic mechanisms in human visual cortex across the lifespan. Front. Cell. Neurosci. 4, 1–12.

Pirker, S., Schwarzer, C., Wieselthaler, A., Sieghart, W., Sperk, G., 2000. GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101, 815–850.

Prieto, J.J., Peterson, B.A., Winer, J.A., 1994. Laminar distribution and neuronal targets of GABAergic axon terminals in cat primary auditory cortex (AI). J. Comp. Neurol. 344, 383–402.

Pritchett, D.B., Seeburg, P.H., 1990. Gamma-aminobutyric acidA receptor alpha 5-subunit creates novel type II benzodiazepine receptor pharmacology. J. Neurochem. 54, 1802–1804.

Rabow, L.E., Russek, S.J., Farb, D.H., 1995. From ion currents to genomic analysis: recent advances in GABAA receptor research. Synapse 21, 189–274.

Richardson, B.D., Brozoski, T.J., Ling, L.L., Caspary, D.M., 2012. Targeting inhibitory neurotransmission in tinnitus. Brain Res. 1485, 77–87.

Richardson, B.D., Ling, L.L., Uteshev, V.V., Caspary, D.M., 2011. Extrasynaptic GABA(A) receptors and tonic inhibition in rat auditory thalamus. PLoS One 6, e16508.

Rissman, R.A., De Blas, A.L., Armstrong, D.M., 2007. GABA(A) receptors in aging and Alzheimer's disease. J. Neurochem. 103, 1285–1292.

Ruano, D., Machado, A., Vitorica, J., 1993. Absence of modifications of the pharmacological properties of the GABAA receptor complex during aging, as assessed in 3- and 24-month-old rat cerebral cortex. Eur. J. Pharmacol. 246, 81–87.

Ruano, D., Vizuete, M., Cano, J., Machado, A., Vitorica, J., 1992. Heterogeneity in the allosteric interaction between the gamma-aminobutyric acid (GABA) binding site and three different benzodiazepine binding sites of the GABAA/benzodiazepine receptor complex in the rat nervous system. J. Neurochem. 58, 485–493.

Rudolph, U., Crestani, F., Mohler, H., 2001. GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol. Sci. 22, 188–194.

Sakurai, S.Y., Kume, A., Burdette, D.E., Albin, R.L., 1994. Quantitative autoradiography of [3H]t-butylbicycloorthobenzoate binding to the gamma-aminobutyric acid receptorA complex. J. Pharmacol. Exp. Ther. 270, 362–370.

Schmidt, S., Redecker, C., Bruehl, C., Witte, O.W., 2010. Age-related decline of functional inhibition in rat cortex. Neurobiol. Aging 31, 504–511.

Schmolesky, M.T., Wang, Y., Creel, D.J., Leventhal, A.G., 2000. Abnormal retinotopic organization of the dorsal lateral geniculate nucleus of the tyrosinase-negative albino cat. J. Comp. Neurol. 427, 209–219.

Schneider, B.A., Pichora-Fuller, M.K., Kowalchuk, D., Lamb, M., 1994. Gap detection and the precedence effect in young and old adults. J. Acoust. Soc. Am. 95, 980–991.

Sieghart, W., 1992a. GABAA receptors: ligand-gated Cl- ion channels modulated by multiple drug-binding sites. Trends Pharmacol. Sci. 13, 446–450.

Sieghart, W., 1992b. Heterogeneity of GABAA receptors. Clin. Neuropharmacol. 15 (suppl 1 pt A), 681A–682A.

Sieghart, W., 1992c. Molecular basis of pharmacological heterogeneity of GABAA receptors. Cell Signal. 4, 231–237.

Sieghart, W., 1992d. Pharmacology of benzodiazepine receptors: an update. Clin. Neuropharmacol. 15 (suppl 1 pt A), 523A–524A.

Sieghart, W., 1995. Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. Pharmacol. Rev. 47, 181–234.

Sieghart, W., Fuchs, K., Zezula, J., Buchstaller, A., Zimprich, F., Lassmann, H., 1992. Biochemical, immunological, and pharmacological characterization of GABAA-benzodiazepine receptor subtypes. Adv. Biochem. Psychopharmacol. 47, 155–162.

Sigel, E., Baur, R., Trube, G., Mohler, H., Malherbe, P., 1990. The effect of subunit composition of rat brain GABAA receptors on channel function. Neuron 5, 703–711.

Snell, K.B., 1997. Age-related changes in temporal gap detection. J. Acoust. Soc. Am. 101, 2214–2220.

Strouse, A., Ashmead, D.H., Ohde, R.N., Grantham, D.W., 1998. Temporal processing in the aging auditory system. J. Acoust. Soc. Am. 104, 2385–2399.

Suta, D., Rybalko, N., Pelanova, J., Popelar, J., Syka, J., 2011. Age-related changes in auditory temporal processing in the rat. Exp. Gerontol. 46, 739–746.

Syka, J., 2002. Plastic changes in the central auditory system after hearing loss, restoration of function, and during learning. Physiol. Rev. 82, 601–636.

Takesian, A.E., Kotak, V.C., Sanes, D.H., 2012. Age-dependent effect of hearing loss on cortical inhibitory synapse function. J. Neurophysiol. 107, 937–947.

Tremblay, K.L., Piskosz, M., Souza, P., 2002. Aging alters the neural representation of speech cues. Neuroreport 13, 1865–1870.

Tremblay, K.L., Piskosz, M., Souza, P., 2003. Effects of age and age-related hearing loss on the neural representation of speech cues. Clin. Neurophysiol. 114, 1332–1343.

Turner, J.G., Caspary, D.M., 2005. Comparison of two rat models of aging. In: Syka, J., Merzenich, M.M. (Eds.), Plasticity and Signal Representation in the Auditory System. Springer, US, New York, pp. 217–225.

Turrigiano, G.G., Nelson, S.B., 2004. Homeostatic plasticity in the developing nervous system. Nat. Rev. Neurosci. 5, 97–107.

Wafford, K.A., Bain, C.J., Whiting, P.J., Kemp, J.A., 1993. Functional comparison of the role of gamma subunits in recombinant human gamma-aminobutyric acidA/benzodiazepine receptors. Mol. Pharmacol. 44, 437–442.

Wafford, K.A., Ebert, B., 2006. Gaboxadol–a new awakening in sleep. Curr. Opin. Pharmacol. 6, 30–36.

Wafford, K.A., Thompson, S.A., Thomas, D., Sikela, J., Wilcox, A.S., Whiting, P.J., 1996. Functional characterization of human gamma-aminobutyric acidA receptors containing the alpha 4 subunit. Mol. Pharmacol. 50, 670–678.

Wang, H., Brozoski, T.J., Turner, J.G., Ling, L., Parrish, J.L., Hughes, L.F., Caspary, D.M., 2009a. Plasticity at glycinergic synapses in dorsal cochlear nucleus of rats with behavioral evidence of tinnitus. Neuroscience 164, 747–759.

Wang, H., Turner, J.G., Ling, L., Parrish, J.L., Hughes, L.F., Caspary, D.M., 2009b. Age-related changes in glycine receptor subunit composition and binding in dorsal cochlear nucleus. Neuroscience 160, 227–239.

Winer, J.A., 1992. The functional architecture of the medial geniculate body and the primary auditory cortes. In: Webster, D.B., Popper, A.N., Fay, R.R. (Eds.), The Mammalian Auditory Pathway: Neuroanatomy. Springer-Verlag, New York, pp. 222–409.

Wisden, W., Gundlach, A.L., Barnard, E.A., Seeburg, P.H., Hunt, S.P., 1991. Distribution of GABAA receptor subunit mRNAs in rat lumbar spinal cord. Brain Res. Mol. Brain Res. 10, 179–183.

Wisden, W., Laurie, D.J., Monyer, H., Seeburg, P.H., 1992. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J. Neurosci. 12, 1040–1062.

Ymer, S., Draguhn, A., Wisden, W., Werner, P., Keinanen, K., Schofield, P.R., Sprengel, R., Pritchett, D.B., Seeburg, P.H., 1990. Structural and functional characterization of the gamma 1 subunit of GABAA/benzodiazepine receptors. EMBO J. 9, 3261–3267.

Ymer, S., Schofield, P.R., Draguhn, A., Werner, P., Kohler, M., Seeburg, P.H., 1989. GABAA receptor beta subunit heterogeneity: functional expression of cloned cDNAs. EMBO J. 8, 1665–1670.

Yu, Z.Y., Wang, W., Fritschy, J.M., Witte, O.W., Redecker, C., 2006. Changes in neocortical and hippocampal GABAA receptor subunit distribution during brain maturation and aging. Brain Res. 1099, 73–81.

Zhou, X., Panizzutti, R., de Villers-Sidani, E., Madeira, C., Merzenich, M.M., 2011. Natural restoration of critical period plasticity in the juvenile and adult primary auditory cortex. J. Neurosci. 31, 5625–5634.