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Exp Gerontol. Author manuscript; available in PMC 2018 May 01.

Published in final edited form as:

Exp Gerontol. 2017 May ; 91: 99–103. doi:10.1016/j.exger.2017.03.001.

Does Aging Alter the Molecular Substrate of Ionotropic Neurotransmitter Receptors in the Rostral Ventral Lateral Medulla? - A Short Communication

Hitesh N. Pawar[1,2,*], **Sivasai Balivada**[1,2], and **Michael J. Kenney**[1,2]

[1]Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506, USA

[2]Department of Biological Sciences, College of Science, University of Texas at El Paso, El Paso, TX 79968, USA

Abstract

Aging alters sympathetic nervous system (SNS) regulation, although central mechanisms are not well understood. In young rats the rostral ventral lateral medulla (RVLM) is critically involved in central SNS regulation and RVLM neuronal activity is mediated by a balance of excitatory and inhibitory ionotropic neurotransmitters and receptors, providing the foundation for hypothesizing that with advanced age the molecular substrate of RVLM ionotropic receptors is characterized by upregulated excitatory and downregulated inhibitory receptor subunits. This hypothesis was tested by comparing the relative mRNA expression and protein concentration of RVLM excitatory (NMDA and AMPA) and inhibitory (GABA and glycinergic) ionotropic neurotransmitter receptor subunits in young and aged Fischer (F344) rats. Brains were removed from anesthetized rats and the RVLM-containing area was micropunched and extracted RNA and protein were subsequently used for TaqMan qRT-PCR gene expression and quantitative ELISA analyses. Bilateral chemical inactivation of RVLM neurons and peripheral ganglionic blockade on visceral sympathetic nerve discharge (SND) was determined in additional experiments. The relative gene expression of RVLM NMDA and AMPA glutamate-gated receptor subunits and protein concentration of select receptor subunits did not differ between young and aged rats, and there were no age-related differences in the expression of RVLM ionotropic $GABA_A$ and Gly receptors, or of protein concentration of select $GABA_A$ subunits. RVLM muscimol microinjections significantly reduced visceral SND by 70±2% in aged F344 rats. Collectively these findings from this short communication support a functional role for the RVLM in regulation of sympathetic nerve outflow in aged rats, but provide no evidence for an ionotropic RVLM receptor-centric framework explaining age-associated changes in SNS regulation.

[*]Correspondence to: Hitesh N. Pawar, PhD, Department of Biological Sciences, College of Science, University of Texas at El Paso, Biosciences Research Building, Room No. 4.216, El Paso, TX 79968, Phone: 915-747-6876, hnpawar@utep.edu.

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Keywords

RVLM; aging; sympathetic nervous system regulation; neurotransmitter receptors

1. Introduction

Physiological function is altered with advancing age, including changes in sympathetic nervous system (SNS) regulation (Seals and Esler, 2000; Kaye and Esler 2005). Multiple lines of evidence suggest that advanced age is associated with an enhanced state of sympathetic activation. For example, total norepinephrine (NE) spillover, hepatomesenteric NE spillover, muscle sympathetic nerve discharge, and cardiac NE spillover are higher in older compared with young adults (Seals and Esler, 2000; Seals and Bell, 2004). Despite the documentation of marked age-related changes in SNS regulation, little information is available regarding the effect of advanced age on molecular mechanisms regulating central sympathetic neural circuits.

The rostral ventral lateral medulla (RVLM) plays a pivotal role in the regulation of central sympathetic nerve outflow (Horiuchi et al., 2004; Kenney et al., 2011). The activity level of RVLM presympathetic neurons in young animals is mediated by a balance of excitatory and inhibitory states (Ito et al., 2000; Sved et al., 2002), due to glutamatergic excitation mediated by NMDA and AMPA ionotropic receptors, and GABAergic and glycinergic inhibition mediated primarily by $GABA_A$ and glycine (Gly) ionotropic receptors, respectively. Because functional interaction between RVLM excitatory and inhibitory ionotropic receptors play a critical role in determining the level of efferent sympathetic nerve outflow, it is possible to speculate that age-related modifications in RVLM ionotropic receptor may provide the molecular framework underlying changes in SNS regulation with advanced age. Consistent with this notion, previous studies have identified age-associated changes in mRNA and protein expression of ionotropic receptors in central nervous system areas associated with learning and memory (Magnusson et al., 2010; Cantanelli et al., 2014; Ruano et al., 2000; Rissman et al. 2006).

Given the pivotal role of the RVLM in SNS regulation in young rats, the functional balance of RVLM excitatory and inhibitory receptor systems, and the established age-associated changes in SNS regulation, we hypothesized that the molecular substrate of RVLM ionotropic receptors is altered with advanced age. To test this hypothesis we examined the relative gene expression of RVLM excitatory (NMDA and AMPA) and inhibitory (GABA and Gly) ionotropic neurotransmitter receptor subunits in young (3–4 months) and aged (22–24 months) Fischer (F344) rats. Because of the diverse array of RVLM ionotropic receptor subunits a comprehensive profiling of 20 RVLM ionotropic receptor subunits was completed (Table 1). We speculated that changes in the RVLM receptor substrate in aged rats would be represented by one of at least three potential profiles: selective upregulation of excitatory NMDA and AMPA ionotropic receptors, supporting a shift toward an active enhancement of neuronal excitation; selective downregulation of GABA and Gly ionotropic receptor subunits, supporting a role for disinhibition in mediating neuronal excitation; or a combined effect of enhanced excitatory and reduced inhibitory ionotropic receptors. The expected

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findings would be the first to demonstrate that advanced age modulates the balance of RVLM ionotropic receptor subunits, thereby providing a framework for the design of studies to modify age-related changes in central regulation of sympathetic nerve outflow by targeting specific receptor systems. In addition, chemical inactivation of RVLM neurons produced by bilateral Muscimol microinjections in aged rats was completed to assess RVLM functionality.

2. Methods

All procedures and protocols were approved by the Institutional Animal Care and Use Committees and were completed in accordance with the American Physiological Society's guidelines for research involving animals. Experiments were completed in young adult (3–4 months; n=8, 336±6g) (age of sexual maturity is 1.5 months) and aged (22–24 months; n=11, 442±9g) (median survival age is 24–26 months) male Fischer 344 rats (Charles River Laboratories, contracted with National Institute on Aging). F344 rats are a strain of rats that are provided by the National Institute on Aging for studies focused on aging research, and are widely used in this research domain (Mitchell et al., 2015). Many studies that have employed direct sympathetic nerve recordings and central microinjections to determine the effects of advancing age on sympathetic nervous system regulation have utilized F344 rats as the preferred rodent model (Helwig et al., 2006; Kenney, 2010; Kenney et al., 2011).

2.1 Brain sectioning and micro-punching

Rats were deeply anesthetized with 5% isoflurane and sacrificed by decapitation. Immediately after sacrifice brains were removed and snap frozen in liquid nitrogen, then stored at -80^0C until further use. RVLM tissue samples were collected by the modified Palkovit's bilateral micropunch technique (Palkovits and Brownstein, 1983). Anatomical reference points were provided by the rat brain atlas of Paxinos and Watson (2014). The hindbrain was serial sectioned in the coronal plane from rostral to caudal using a cryostat. Two 200μm rostral medullary area brain slices were collected (estimated distance from Bregma −12.2mm). The RVLM containing area was identified using the lateral trigeminal tracts and the dorsal fourth ventricle as landmarks and micro-punched with a 0.5mm diameter Harris micro-punch at −20°C. Tissue collected from the micropunches was pooled in RNase free tubes and stored at −80°C.

2.2 Central Microinjections and Sympathetic Nerve Recordings

RVLM microinjections and sympathetic nerve discharge (SND) recording protocols have been described previously (Kenney et al., 2011). Chemical inactivation of the RVLM was completed by bilateral muscimol microinjections (500 pmol, n=3; microinjectate volume 100 nl for each injection).

2.3 RNA extraction

RNA from RVLM tissue punches was extracted using the RNeasy® Lipid Tissue mini kit (Qiagen, USA) and QIAzol® Lysis reagent. RNA was quantified using the Nanodrop D8000 (Thermo-Scientific, Wilmington, DE). The purity of RNA samples was assessed using the

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260/280 absorbance ratio and samples demonstrating an absorbance ratio of 1.8–2.0 were used for cDNA preparation.

2.4 cDNA preparation and preamplification

Total RNA (100ng) was converted to cDNA using a high capacity RNA to cDNA kit (Applied Biosystems, Foster City, CA). Reverse transcription was performed using the BioRad iQ5TM thermal cycler under standard conditions, and cDNA samples were stored at −200C until further use. The number of reactions per sample was increased by preamplification which was completed using 10ng of cDNA with 2×TaqMan® PreAmp master mix (Applied Biosystems, Foster City, CA). Following the preamplification phase, samples were immediately diluted with 1× TE buffer and stored at -20^0C until further use.

2.5 Quantitative real-time PCR (qRT-PCR)

The qRT-PCR was performed using StepOnePlus™ Mastercycler (Applied Biosystems, CA) and the Taqman™ assay mix (Life technologies, CA) for gene expression studies. Each of the 20 ionotropic receptor subunit genes was probed separately with GAPDH as the endogenous control. The qRT-PCR plates with 96 wells were run on a thermal cycler with standard experimental conditions.

2.6 Protein extraction and Quantitative Sandwich ELISA

Protein extraction was completed using the protein precipitation method from the organic phase remaining after RNA isolation (Simoes et al., 2013). Extracted protein samples were processed using standard procedures, aliquoted, and stored at -20^0C until further use. Protein quantification was completed using the PierceTM BCA® Protein Assay Kit (Thermo Scientific, IL).

Rat quantitative sandwich ELISA kits (MyBioSource, San Diego, CA; www.mybiosource.com) were used to analyze three NMDA receptor subunits (*Grin1*, Cat. #MBS2018972; *Grin2a,* Cat. #MBS2602637; *Grin2c*, Cat #MBS9340550) and three $GABA_A$ receptor subunits (*Gabra1*, Cat. #MBS9342109; *Gabra2*, Cat. #MBS100060; *Gabrg2*, Cat. #MBS9336526). Standards and samples were analyzed in duplicate for each gene and optical density of the protein samples was determined with SpectraMax® i3 Multi-Mode Microplate Reader Detection Platform (Molecular Devices, Sunnyvale, CA) set to a wavelength of 450nm.

2.7 Data and Statistical analysis

Relative changes in gene expression between aged and young rats were analyzed by taking the base 2 logarithm transformed $2^{-\Delta Ct}$ values as parameter (MIQE guidelines). Results are represented as fold change values calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Statistical significance for age-related differences in the expression for each gene was tested using Student's t-tests, and error rates associated with multiple comparisons were corrected using the Benjamini-Hochberg false discovery rate (FDR) procedure. Genes that showed ≤0.05 p-value with ≤0.1 FDR were considered significant. Base 2 logarithm transformed $2^{-\Delta\Delta Ct}$ values for each gene were represented as a data point on dot plot. Protein data are expressed as mean ± SD, whereas SND data is presented as

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mean ± SE. Statistical analysis of protein data between young and aged F344 rats as well as SND data in aged rats compared to basal level were completed using the Student's *t-tests*. The level of statistical significance was $p<0.05$.

3. Results

Relative changes in gene expression of RVLM ionotropic NMDA (*Grin1*, *Grin2a*, *Grin2b*, *Grin2c*), AMPA (*Gria1*, *Gria2*, *Gria3*, *Gria4*), $GABA_A\alpha$ (*Gabra1*, *Gabra2*, *Gabra3*, *Gabra4*, *Gabra5*), $GABA_A\beta$ (*Gabrb1*, *Gabrb2*, *Gabrb3*), $GABA_A\gamma$ (*Gabrg1*, *Gabrg2*), and glycinergic (Glra1, Glrb) receptor subunits between aged (n=8) and young (n=8) F344 rats are illustrated by dot plot in Figure 1, whereas Table 1A shows respective Ct values for each subunit. Dots show relative fold change comparisons between aged and young rats, values above 0 indicate a relative increase in gene expression in aged compared with young rats, whereas values below 0 indicate a relative decrease in gene expression in aged compared with young rats. The x-axis depicts fold change as log2 transformed values, thereby allowing for similar scaling on both sides of the center point (designated as 0). Dotted lines depict 1.5 fold changes in relative gene expression, whereas 2-fold changes are depicted by 1 and −1.

None of the genes sampled from the RVLM demonstrated 2-fold, age-associated differences in relative expression (Figure 1); in fact, only two genes attained a relative difference of 1.5 fold (*Gabra2* and *Gabrb3*). Statistical analysis, which included controlling for multiple comparisons, revealed no significant differences between young and aged rats in the relative RVLM gene expression of specific NMDA, AMPA, $GABA_A$ and Gly receptor subunits (Figure 1).

Figure 2 and Table 1B shows summarized data from ELISA analysis comparing the concentration of RVLM NMDA (*Grin1*, *Grin2a*, *Grin2c*) and $GABA_A$ (*Gabra1*, *Gabra2*, *Gabrg2*) neurotransmitter receptor protein subunits in young (n=8) and aged (n=8) F344 rats. There were no age-dependent differences in the RVLM concentrations of the select NMDA (A, left panels) or $GABA_A$ (B, right panels) receptor protein subunits (Figure 2).

Chemical inactivation of the RVLM produced by bilateral RVLM muscimol microinjections significantly ($p<0.05$) reduced renal (n=3) and splenic (n=2) SND by 70 ± 2% (data combined for renal and splenic SND) from basal levels in aged F344 rats.

4. Discussion

The present study is the first to characterize the genomic and proteomic profiles of excitatory and inhibitory ionotropic receptor subunits in the RVLM of aged and young F344 rats, and was completed based on the hypothesis that age-related modifications in the RVLM composition of ionotropic receptor subunits would provide a critical molecular substrate for mediating alterations in SNS regulation with advanced age. The current results provide experimental support for two new findings. First, the relative gene expression of RVLM NMDA and AMPA glutamate-gated receptor subunits, and protein concentration of select receptor subunits did not differ between young and aged rats. Second, there were no age-related differences in the relative gene expression of RVLM ionotropic $GABA_A$ and Gly receptors, or of protein concentration of select $GABA_A$ subunits. These findings do not

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support an ionotropic RVLM receptor-centric framework explaining age-associated changes in SNS regulation under basal conditions.

Ionotropic receptors are composed of a diverse array of subunits. NMDA receptors form tetramers and are composed of two *Grin1* and two selective *Grin2* subunits. AMPA receptors have a core hetero-tetrameric organization consisting of symmetric 'dimer of dimers' of *Gria2* and either *Gria1*, *Gria3* or *Gria4* subunits. $GABA_A$ receptors are characterized by hetero-pentamer forming multiple different subunits, primarily composed of 2α, 2β and 1γ subunit. Gly receptors are trans-membrane protein complexes composed of 5 subunits, α (1–4) and β. The comprehensive profiling of IRS in the present study, coupled with the finding of no age-associated differences in their RVLM expression, suggests that age-related changes in SNS regulation are likely not mediated solely by changes in the molecular substrate of RVLM ionotropic receptor subunits.

Interpretation of the present data was guided by several analytical and methodological considerations. First, because the quantitative credibility of finding a <2-fold difference in mRNA expression is low and the functional effect is debatable, in the present analysis, and consistent with numerous other studies involving gene expression analysis (Karlen et al., 2007), the initial screen for establishing gene expression differences between aged and young rats was set at 2-fold. None of the genes sampled from the RVLM demonstrated 2-fold age-associated differences in relative expression. Second, statistical analyses were applied to the RVLM expression data, including correcting for error rates associated with multiple comparisons. There were no significant age-related differences in the relative gene expression of the ionotropic receptor subunits tested in the RVLM. Third, it is well-established that multiple mechanisms can modulate the functional processes between transcription and translation such that mRNA levels may not correlate with protein quantity (Gutierrez et al. 1997). With this in mind, several primary RVLM ionotropic receptor subunits were analyzed at the protein level. The consistency of the current data at both mRNA and protein levels provides further support for the present conclusions.

In contrast to the current findings, the results of other studies have identified age-related differences in the expression of genes found in the central nervous system. For example, in studies focused on understanding the effects of age on learning and memory, age-associated alterations in mRNA and protein expression of excitatory NMDA and AMPA receptor were reported in the hypothalamus and hippocampus (Magnusson et al., 2010; Cantanelli et al., 2014). Regarding inhibitory receptor subunits, age-related changes in specific $GABA_A$ subunit mRNA have been identified in the frontal cortex, cerebral cortex, cerebellum, and inferior colliculus (Ruano et al., 2000; Rissman et al. 2006). Finally, Li et al. (2003) reported increased glutamatergic activity associated with upregulation of NMDA R1 receptor subunits in the paraventricular nucleus of the hypothalamus in rats with heart failure, a condition that is associated with SNS activation. The paraventricular nucleus of the hypothalamus is a central nervous system site involved in SNS regulation, and is characterized by a complex profile of excitatory and inhibitory neurotransmitters and receptors, similar to the RVLM (Kenney et al., 2003).

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Previous studies have established a critical role for the RVLM in regulation of sympathetic nerve outflow in young animals by demonstrating that chemical inactivation of this brainstem area, produced via muscimol microinjections, substantially reduces sympathetic nerve activity in young rats (Morrison 1999; Kenney et al., 2011), cats (Barman and Orer, 2010), and rabbits (Horiuchi and Dampney, 1998). In the present study, chemical inactivation of the RVLM produced marked reductions in efferent SND in aged F344 rats, supporting a functional role for this medullary area in SND regulation in aged animals. Future studies comparing RVLM muscimol-induced changes in SND between young and aged F344 rats may provide additional insight regarding the effect of advanced age on central regulation of sympathetic nerve outflow.

It must be considered that other mechanisms or regulatory systems may contribute to possible age-related alterations in the functional capability of RVLM presympathetic neurons. For example, aging may be associated with post-translational modifications in receptor subunits, thereby affecting the function of ionotropic receptors (Vanhooren et al., 2015). In addition, aging may modulate the expression of neurotransmitter transporter proteins, which could influence the concentration of neurotransmitter available to bind to RVLM ionotropic receptor complexes. In a recent study, Kenney (2014) reported that RVLM bicuculline ($GABA_A$ receptor antagonist) microinjections increased the level of basal SND in both young and aged F344 rats, consistent with the idea that endogenous RVLM GABA is present and $GABA_A$ receptors are functional with advancing age. However, this study did not assess the possibility that functional changes may include age-related differences in the basal level of RVLM GABA. Finally, the regulatory substrate of the aged RVLM may be characterized by alterations in several physiological modulators that are known to influence the SNS, including; the renin-angiotensin system, neural-immune interactions, and nitric oxide regulation.

Neurotransmitter receptors and receptor subunits are critically involved in mediating signaling in central neural circuits. The present study was completed based on the idea that age-related modifications in the RVLM composition of ionotropic receptor subunits may provide the substrate for mediating changes in SNS regulation with advanced age. However, the present results indicate neither upregulation of excitatory or downregulation of inhibitory receptor subunits with advanced age in the RVLM, suggesting a role for other mechanisms in mediating age-associated changes in regulation of presympathetic neurons.

Acknowledgments

Supported by NIH grant AG-041948. The authors thank Shawnee Montgomery for experimental assistance.

References

Barman SM, Orer HS. Rostral ventrolateral medullary but not medullary lateral tegmental field neurons mediate sympatho-sympathetic reflexes in cats. Am J Physiol Regul Integr Comp Physiol. 2010; 299:R1269–R1278. [PubMed: 20811005]

Cantanelli P, Sperduti S, Ciavardelli D, Stuppia L, Gatta V, Sensi SL. Age-dependent modifications of AMPA receptor subunit expression levels and related cognitive effects in 3xTg-AD mice. Front Aging Neurosci. 2014; 6:200. [PubMed: 25140151]

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Gutierrez ZU, Khan, Miralles CP, Mehta AK, Ruano D, Araujo F, Vitorica J, De Blas AL. GABAA receptor subunit expression changes in the rat cerebellum and cerebral cortex during aging. Mol Brain Res. 1997; 45:59–70. [PubMed: 9105671]

Helwig BG, Parimi S, Ganta CK, Cober R, Fels RJ, Kenney MJ. Aging alters regulation of visceral sympathetic nerve responses to acute hypothermia. Am J Physiol Regul Integr Comp Physiol. 2006; 291(3):R573–579. [PubMed: 16497812]

Horiuchi J, Killinger S, Dampney RA. Contribution to sympathetic vasomotor tone of tonic glutamatergic inputs to neurons in the RVLM. Am J Physiol Regul Integr Comp Physiol. 2004; 287:R1335–R1343. [PubMed: 15271655]

Horiuchi J, Dampney RA. Dependence of sympathetic vasomotor tone on bilateral inputs from the rostral ventrolateral medulla in the rabbit: role of baroreceptor reflexes. Neurosci Lett. 1998; 248:113–116. [PubMed: 9654355]

Ito S, Komatsu K, Tsukamoto K, Sved AF. Excitatory amino acids in the rostral ventrolateral medulla support blood pressure in spontaneously hypertensive rats. Hypertension. 2000; 35:413–417. [PubMed: 10642334]

Karlen Y, McNair A, Perseguers S, Mazza C, Mermod N. Statistical significance of quantitative PCR. BMC Bioinfo. 2007; 8:131.

Kaye D, Esler M. Sympathetic neuronal regulation of the heart in aging and heart failure. Cardiovasc Res. 2005; 66:256–264. [PubMed: 15820194]

Kenney MJ. Medullary Regulation of Visceral Sympathetic Nerve Discharge at Peak Hyperthermia in Aged F344 Rats. Auton Neurosci. 2014; 186:32–37. [PubMed: 25262958]

Kenney MJ, Meyer CN, Hosking KG, Fels RJ. Is visceral sympathoexcitation to heat stress dependent on activation of ionotropic excitatory amino acid receptors in the rostral ventrolateral medulla? Am J Physiol Regul Integr Comp Physiol. 2011; 301:R548–R557. [PubMed: 21632850]

Kenney MJ. Animal aging and regulation of sympathetic nerve discharge. J Appl Physiol. 2010; 109(4):951–958. [PubMed: 20651223]

Kenney MJ, Weiss ML, Haywood JR. The paraventricular nucleus: an important component of the central neurocirculatory regulating sympathetic nerve outflow. Acta Physiol Scan. 2003; 177:7–15.

Li Y, Cornish KG, Patel KP. Alteration of NMDA NR1 receptors within the paraventricular nucleus of hypothalamus in rats with heart failure. Circ Res. 2003; 93:990–997. [PubMed: 14576197]

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C (T)) method. Methods. 2001; 25:402–408. [PubMed: 11846609]

Magnusson KR, Brim BL, Das SR. Selective vulnerabilities of N-methyl-D-aspartate (NMDA) receptors during aging brain. Front Aging Neurosci. 2010; 2:11. [PubMed: 20552049]

Mitchell SJ, Scheibye-Knudsen M, Longo DL, de Cabo R. Animal models of aging research: implications for human aging and age-related diseases. Annu Rev Anim Biosci. 2015; 3:283–303. [PubMed: 25689319]

Morrison SF. RVLM and raphe differentially regulate sympathetic outflows to splanchnic and brown adipose tissue. Am J Physiol. 1999; 276(4):R962–R973. [PubMed: 10198373]

Palkovits, M., Brownstein, MJ. Microdissection of brain areas by the punch technique. In: Cuello, AC., editor. Brain Microdissection Techniques. Wiley; New York: 1983. p. 1-36.

Paxinos, G., Watson, C. Paxinos and Watson’s the rat brain in stereotaxic coordinates. 7. Elsevier Academic Press; San Diego, CA: 2014.

Rissman RA, Fuller LM, Kordower JH, Armstrong DM. Age-related alterations in GABAa receptor a1 and b2/3 subunits in the non-human primate hippocampus. Brain Res. 2006; 1073:120–130. [PubMed: 16430870]

Ruano D, Araujo F, Revilla E, Vela J, Bergis O, Vitorica J. GABA-A and alpha-amino-3-hydroxy-5-methylsoxazole-4-propionate receptors are differentially affected by aging in the rat hippocampus. J Biol Chem. 2000; 275:19585–19593. [PubMed: 10751391]

Seals DR, Esler MD. Human ageing and the sympathoadrenal system. J Physiol. 2000; 528:407–417. [PubMed: 11060120]

Seals DR, Bell C. Chronic sympathetic activation. Consequence and cause of age-associated obesity? Diabetes. 2004; 53:276–284. [PubMed: 14747276]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Simoes AES, Pereira DM, Amaral JD, Nunes AF, Gomes SE, Rodrigues PM, Lo AC, D'Hooge R, Steer CJ, Thibodeau SN, Borralho PM, Rodrigues CMP. Efficient recovery of proteins from multiple source samples after trizol® or trizol®LS RNA extraction and long-term storage. BMC Genomics. 2013; 14:181. [PubMed: 23496794]

Sved AF, Ito S, Yajima Y. Role of excitatory amino acid inputs to the rostral ventrolateral medulla in cardiovascular regulation. Clin Exp Pharmacol Physiol. 2002; 29:503–506. [PubMed: 12010199]

Vanhooren V, Santos AN, Voutetakis K, Petropoulos I, Libert C, Simm A, Gonos ES, Friguet B. Protein modification and maintenance systems as biomarkers of ageing. Mech Ageing Develop. 2015; 151:71–84.

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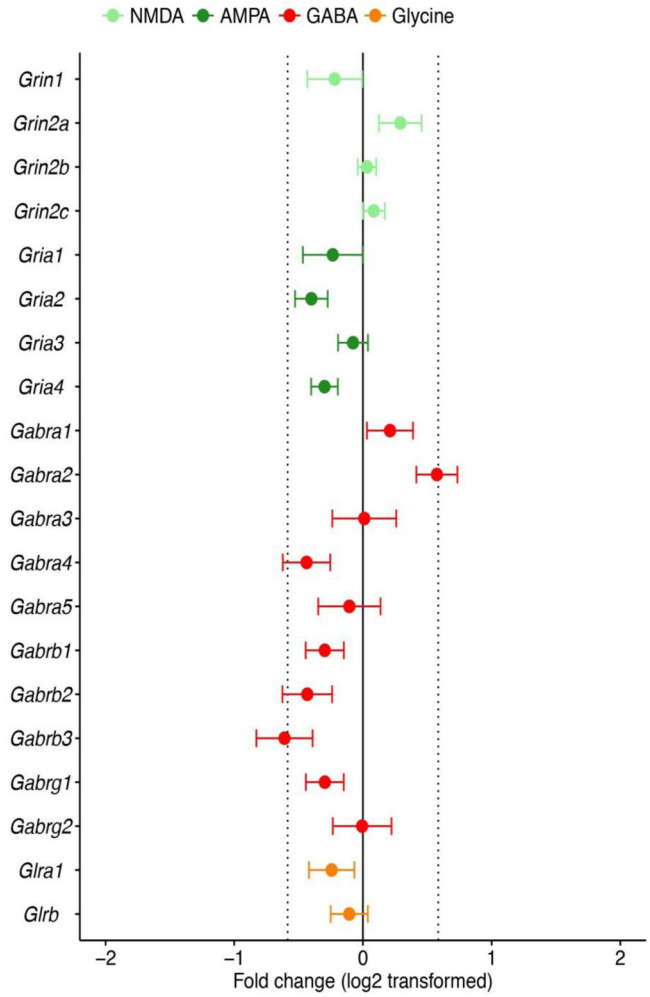

Figure 1.

Relative changes in gene expression of RVLM ionotropic NMDA (*Grin1*, *Grin2a*, *Gri2b*, *Grin2c*), AMPA (*Gria1*, *Gria2*, *Gria3*, *Gria4*), $GABA_A\alpha$ (*Gabra1*, *Gabra2*, *Gabra3*, *Gabra4*, *Gabra5*), $GABA_A\beta$ (*Gabrb1*, *Gabrb2*, *Gabrb3*), $GABA_A\gamma$ (*Gabrg1*, *Gabrg2*), and glycinergic (*Glra1*, *Glrb*) receptor subunits between aged (22–24 months; n=8) and young (3–4 months; n=8) F344 rats. Dots show relative fold change comparisons analyzed using the $2^{-\Delta\Delta Ct}$ method. Values above 0 indicate that gene expression is higher in aged compared with young rats, whereas values below 0 indicate that gene expression is lower in aged compared with young rats. Dotted lines depict 1.5 fold changes in gene expression. Data are represented as Log2 transformed fold change ± SE.

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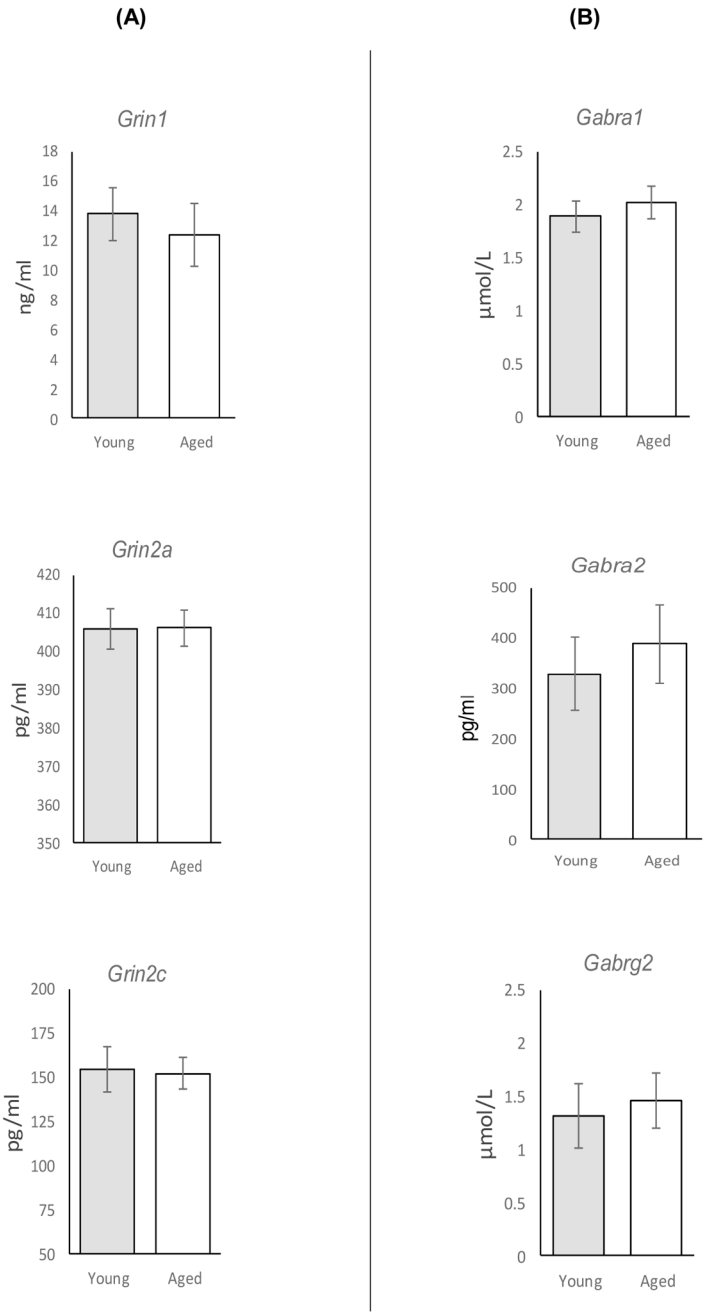

Figure 2.

Protein quantification as determined by sandwich ELISA for (A) NMDA receptor subunits (*Grin1*, *Grin2a*, *Grin2c*) and (B) $GABA_A$ receptor subunits (*Gabra1*, *Gabra2*, *Gabrg2*), in the RVLM of aged (22–24 months; n=8) and young (3–4 months; n=8) F344 rats. Bars show protein concentration of respective subunits in young (closed bars) and aged (open bars) rats. Data are represented as mean ± SD.

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Table 1

(A) Ionotropic neurotransmitter receptor subunit genes included in the qRT-PCR gene expression analysis with respective average Ct values and (B) protein concentrations of select NMDA and $GABA_A$ receptor subunits in young and aged rat groups.

(A)	Ionotropic Neurotransmitter receptor (mRNA)	Subunits	Young (Avg Ct value)	Aged (Avg Ct value)
	NMDA	*Grin1*	21.42	21.67
		Grin2a	24.35	24.33
		Grin2b	24.37	24.74
		Grin2c	23.24	23.62
	AMPA	*Gria1*	23.01	23.18
		Gria2	23.25	23.93
		Gria3	23.07	23.42
		Gria4	21.79	22.36
	$GABA_A\alpha$	*Gabra1*	22.50	22.20
		Gabra2	23.12	22.44
		Gabra3	27.18	27.07
		Gabra4	26.50	26.83
		Gabra5	24.11	24.12
	$GABA_A\beta$	*Gabrb1*	24.59	24.04
		Gabrb2	22.78	23.24
		Gabrb3	22.55	23.15
	$GABA_A\gamma$	*Gabrg1*	22.62	23.05
		Gabrg2	21.75	22.08
	Gly	*Glra1*	21.89	22.27
		Glrab	21.14	21.39
(B)	**Protein Concentration**	**Subunits**	**Young**	**Aged**
	NMDA	*Grin1*	13.77 ± 1.77 ng/ml	12.39 ± 2.11 ng/ml
		Grin2a	405.93 ± 5.23 pg/ml	406.15 ± 4.72 pg/ml
		Grin2c	154.39 ± 12.85 pg/ml	152.12 ± 8.97 pg/ml
	$GABA_A$	*Gabra1*	1.89 ± 0.14 μmol/L	2.02 ± 0.15 μmol/L
		Gabra2	329.06 ± 72.98 pg/ml	388.77 ± 78.35 pg/ml
		Gabrg2	1.32 ± 0.31 μmol/L	1.46 ± 0.25 μmol/L