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# FURTHER STUDIES OF THE EFFECTS OF AGING ON ARGININE METABOLITES IN THE RAT VESTIBULAR NUCLEUS AND CEREBELLUM

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**Abstract—Some studies have demonstrated that aging is associated with impaired vestibular reflexes, especially otolithic reflexes, resulting in postural instability. However, the neurochemical basis of these age-related changes is still poorly understood. The L-arginine metabolic system has been implicated in changes in the brain associated with aging. In the current study, we examined the levels of L-arginine and its metabolizing enzymes and downstream metabolites in the vestibular nucleus complex (VNC) and cerebellum (CE) of rats with and without behavioral testing which were young (4 months old), middle-aged (12 months old) or aged (24 months old). We found that aging was associated with lower nitric oxide synthase activity in the CE of animals with testing and increased arginase in the VNC and CE of animals with testing. L-citrulline and L-ornithine were lower in the VNC of aged animals irrespective of testing, while L-arginine and L-citrulline were lower in the CE with and without testing, respectively. In the VNC and CE, aging was associated with lower levels of glutamate in the VNC, irrespective of testing. In the VNC it was associated with higher levels of agmatine and putrescine, irrespective of testing. In the CE, aging was associated with higher levels of putrescine in animals without testing and with higher levels of spermine in animals with testing, and spermidine, irrespective of testing. Multivariate analyses indicated significant predictive relationships between the different variables, and there were correlations between some of the neurochemical variables and behavioral measurements. Cluster analyses revealed that aging altered the relationships between L-arginine and its metabolites. The results of this study demonstrate that there are major changes occurring in L-arginine metabolism in the VNC and CE as a result of age, as well as behavioral activity.** 

**Key words: vestibular nucleus, cerebellum, aging, glutamate, GABA, arginine metabolites.**

## INTRODUCTION

Dizziness and vertigo are frequently reported in people aged over 60 years (Iwasaki and Yamasoba, 2014; Fernández et al., 2015). One possible explanation for this is vestibular reflex impairment. While many studies have reported an age-related deterioration of the vestibulo-ocular (VORs), vestibulo-spinal reflexes (VSRs), and vestibulo-sympathetic reflexes (e.g., Baloh, 1992; Paige, 1992; Baloh et al., 1993, 2001; Redfern et al., 2001; Ray and Monahan, 2002; Tian et al., 2002; Baloh et al., 2003; Kuipers et al., 2003; Su et al., 2004; Horak, 2006; Deshpande and Patla, 2007), recently, some inconsistent results have been published. Using the video head impulse test (vHIT), McGarvie et al. (2015) reported that VOR gain was largely unaffected in healthy adults in the 80–89 years' age range, and Matiño-Soler et al. (2015) also demonstrated that the horizontal VOR gain remained the same until 90 years of age. Consistent with these results, perceptual threshold levels related to the horizontal VOR have been reported to be similar for young and older adults (Chang et al., 2014), although the results of some dynamic visual acuity studies are inconsistent (Deshpande et al., 2013). By contrast, animal studies have reported an age-related decline in the VOR gain as well as short-term adaptation (e.g., Stahl et al., 2006; Stahl, 2014; Khan et al., 2016). Despite the conflicting evidence related to the VOR, human otolithic function, measured using ocular and cervical vestibular-evoked myogenic potentials (o- and c-VEMPs), has been reported to decline with age (e.g., Janky and Shepard, 2009; Akin et al., 2011; Chang et al., 2012; Singh et al., 2014; Li et al., 2015).

Evidence suggesting a decline in vestibular reflex function with age has been related to structural studies

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*Abbreviations:* 5-HT, 5-hydroxy-tryptamine; ADC, arginine decarboxylase; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; ANOVA, analysis of variance; CA, cluster analyses; CE, cerebellum; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; HPLC, high performance liquid chromatography; LDA, linear discriminant analysis; MLR, multiple linear regression; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; SD, Sprague–Dawley; VNC, vestibular nucleus complex; VOR, vestibulo-ocular reflex.

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of the peripheral and central vestibular systems in aged animals. There have been many studies that have reported age-related decreases in vestibular hair cells and neurons in Scarpa's ganglion (e.g., Anniko, 1983; Gleeson and Felix, 1987; Merchant et al., 2000; Park et al., 2001; Kevetter and Leonard, 2002; Lopez et al., 2005; see Smith, 2016 for a review), however, other studies have found no difference (e.g., Alidina and Lyon, 1990; Fujii et al., 1990; Gopen et al., 2003; Kevetter et al., 2005). Similarly, while a decrease in the number of neurons has been reported in the vestibular nucleus complex (VNC) of aged humans (Lopez et al., 1997; Alvarez et al., 1998, 2000; Tang et al., 2001) and some animal species (Sturrock, 1989), some findings are inconsistent (Kevetter et al., 2005; Fernandez et al., 2007).

It is conceivable that there might be age-related functional changes in the VNC that are not caused by structural degeneration of the peripheral or central vestibular system and that increased dizziness and vertigo in the elderly could be related to ascending vestibulo-cortical pathways that are independent of the VOR projections (Smith, 2016). However, there is a lack of functional neurophysiological and neurochemical studies of the VNC and cerebellum (CE) related to aging. Changes in noradrenaline, 5-hydroxytryptamine (5-HT) (Cransac et al., 1996), glycine receptor density (Nakayama et al., 1999), the response to GABA (Him et al., 2001) and glutamic acid decarboxylase (GAD) synthesis (Giardino et al., 2002), have been described in the aged VNC. However, since there is evidence for the involvement of nitric oxide (NO) in the normal aging process and in age-related neurodegenerative processes (Law et al., 2001; McCann et al., 2005; Malinski, 2007; Liu et al., 2014), we have been interested in how the levels of L-arginine, its metabolizing enzymes and downstream metabolites change in the aging VNC and CE of the rat.

L-arginine can be metabolized by NO synthase (NOS) to generate L-citrulline and NO, by arginase to generate L-ornithine and urea, and by arginine decarboxylase (ADC) to generate agmatine (Fig. 1; Wu and Morris, 1998). NO produced from neuronal NOS (nNOS) contributes to the synaptic plasticity involved in learning and memory (Susswein et al., 2004; Feil and Kleppisch, 2008; Zhou and Zhu, 2009), while NO produced from endothelial NOS (eNOS) contributes to vascular microenvironment modulation (de la Torre, 2012; Förstermann and Sessa, 2012). Inducible NOS (iNOS) can result in an excessive amount of NO, which can contribute to neurotoxicity (Law et al., 2001; Malinski, 2007). L-ornithine, a product of arginase, is broken down by the enzyme, ornithine decarboxylase (ODC) to generate polyamines (e.g., putrescine, spermidine and spermine), which are necessary for normal cellular growth (Wallace et al., 2003; Alm and Oredsson, 2009; Wallace, 2009; Igarashi and Kashiwagi, 2010). L-ornithine can be used to produce glutamate, which can be further metabolized to γ-aminobutyric acid (GABA) (Wu and Morris, 1998; Wiesinger, 2001; Tapiero et al., 2002). Agmatinase can metabolize agmatine to generate putrescine. Agmatine, potentially a novel neurotransmitter (Reis and Regunathan, 2000), inhibits nNOS and iNOS, stimulates eNOS, and indirectly inhibits ODC and polyamine uptake; therefore, it contributes to the regulation of NO and polyamine generation (Satriano, 2003; Halaris and Piletz, 2007; Joshi et al., 2007; Santhanam et al., 2007). Some recent studies suggest that agmatine may be involved in learning and memory (Liu et al., 2008b, 2009a; Leitch et al., 2011; Seo et al., 2011; Rushaidhi et al., 2013).

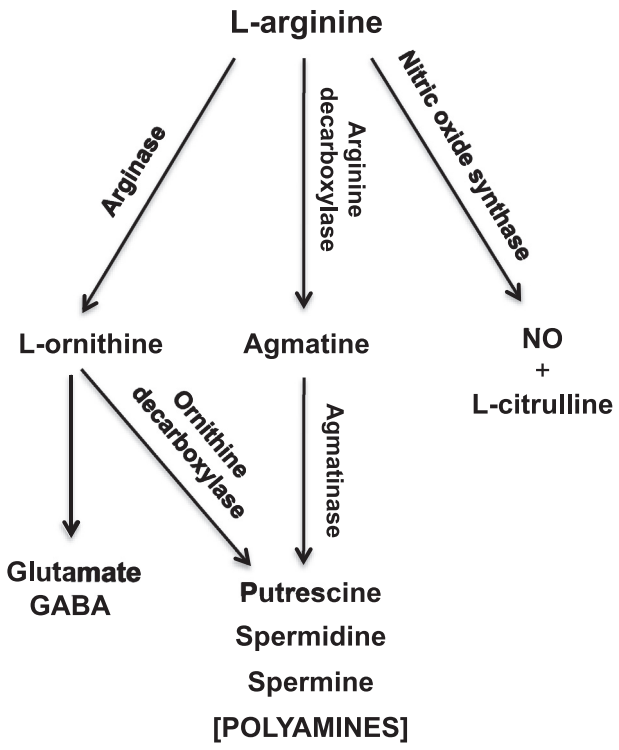


**Fig. 1.** Schematic diagram of the L-arginine metabolic pathways. NO: nitric oxide; GABA: γ–aminobutyric acid.

We previously reported that levels of L-arginine and its metabolites significantly changed in the VNC and CE of aged (24 months old) and young (4 months old) rats (Liu et al., 2010). Since it has been reported that cognitive decline begins in middle-age (Finch, 2009; Salthouse, 2009), in the present study we sought to determine the levels of L-arginine and its eight metabolites, as well as the activity of its metabolizing enzymes NOS and arginase, in the VNC and CE of young (4 months old), middle-aged (12 months old) and aged (24 months old) rats, in order to obtain more detailed information about the arginine metabolic profile changes related to the aging process. As animals' behavioral experience affects NOS and arginase and the concentrations of L-arginine and L-arginine metabolites (Liu et al., 2004, 2005; Gupta et al., 2012), we also investigated whether behavioral testing involving the open field maze, elevated pus maze and water maze, had any effect on arginine and its metabolites in the VNC and CE.

## EXPERIMENTAL PROCEDURES

### Animals

Data were obtained from 57 male Sprague–Dawley (SD) rats at four (young, $n$ = 19), 12 (middle-aged, $n$ = 19)