ELSEVIER

Neurobiology of Aging 25 (2004) 209–221

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Reduced cortical noradrenergic neurotransmission is associated with increased neophobia and impaired spatial memory in aged rats

Timothy J. Collier[a,*], James G. Greene[b], David L. Felten[c], Suzanne Y. Stevens[d], Kathy Steece Collier[a]

[a] *Department of Neurological Sciences, Research Center for Brain Repair, Rush Presbyterian-St. Luke's Medical Center, Tech 2000, Suite 200, 2242 W. Harrison St., Chicago, IL 60612, USA*

[b] *Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322, USA*

[c] *Samueli Center for Complementary and Alternative Medicine, Irvine College of Medicine, University of California, Irvine, CA 92612, USA*

[d] *Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642, USA*

Received 23 May 2002; received in revised form 27 January 2003; accepted 12 March 2003

Abstract

In the present study, young (5-month-old (mo)) and aged (24 mo) adult male Fischer-344 (F344) rats were assigned to experimental groups based upon their performance of a reference memory task in the Morris water maze and reactivity to a novel palatable taste in a gustatory neophobia task. Levels of norepinephrine (NE) and its metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG) were assayed via high performance liquid chromatography (HPLC) in brain regions associated with the locus coeruleus (LC)–hippocampus–cortex system and A1/A2-hypothalamic system. Binding of ligands specific for alpha-1, alpha-2, beta-1, and beta-2 receptors was assessed in hippocampus and cortex with receptor autoradiography. Impaired acquisition and retention of the water maze task and gustatory neophobia in aged rats was primarily associated with decreased NE activity in cingulate cortex (CC) as indicated by a significant reduction in the MHPG/NE ratio coupled with increased NE content. No significant changes in adrenergic receptor binding were detected in any region sampled. The results suggest that an aging-related reduction in cortical NE neurotransmission is associated with the expression of increased neophobia and deficits in spatial learning and memory performance occurring with advanced age in rats.

Keywords: Norepinephrine; MHPG; Water maze; Locus coeruleus; Hippocampus; Cingulate cortex; Frontal cortex; Hypothalamus; A1/A2 cell groups

1. Introduction

Norepinephrine (NE)-containing neurons of the brain are a target of aging-related changes. Regional changes in NE content [20,33,36,52,59,61,81,82,85,89–91,96], turnover [57,65,69,72,74,88,89,100,101], and interaction with receptors [7,8,17,34,35,37,38,42,53,62,63,67,73,75,87,108,112] have been reported for aging rodents, monkeys, and humans, but the distribution and magnitude of the changes reported is variable. Similarly, function of the NE systems has been associated with arousal [10,22,43,80], attention [2,3,23,51], learning and memory [16,21,27–30,76,93,113], and responses to stress [9,45,47,97], with all of these exhibiting detectable changes with advancing age [1,5,11–15,19,24–28,31,39,40,44,46,48,50,52,54,56,66,68,70,78,79,84,86,94,95,106,107,109,113].

Aging is not a unitary process. Different individuals age at different rates, as do different systems within the body. This disparity between chronological aging and biological/functional aging has been well-documented in studies of rodents [11,24,25,54], and coincides with the concept that human aging can be "usual," "successful," or "unsuccessful" [83]. Consistent with this concept, we demonstrated previously that aged rats comprise a behaviorally heterogeneous population, with some individuals exhibiting behavioral performance on a variety of tasks that is indistinguishable from much younger adult animals, and others exhibiting performance that is severely impaired. In particular, we have found that approximately 50% of a group of 24-month-old (mo) male Fischer-344 (F344) rats exhibited avoidance of a novel palatable gustatory stimulus (gustatory neophobia) [11,12], a response similar to that expressed by young rats depleted of forebrain NE [6,55,98]. In addition, gustatory neophobia was predictive of poor memory performance in a footshock-motivated inhibitory avoidance task [12]. This aging-related deficit was improved by supplementation of

* Corresponding author. Tel.: +1-312-563-3579; fax: +1-312-563-3571.
E-mail address: tcollier@rush.edu (T.J. Collier).

0197-4580/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0197-4580(03)00042-3

NE via intraventricular transplantation of embryonic locus coeruleus (LC) neurons [13].

While these studies suggested a connection between aging-related changes in NE system function, responses to novelty, and memory for a stressful situation, the evidence was indirect. The present study expands this line of investigation. In Experiment 1, young adult and aged rats were tested for performance of the gustatory neophobia task and a reference memory task in the Morris water maze. These animals were analyzed for content of NE and its metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG) in the region of the LC and two of its targets, cingulate cortex (CC) and dorsal hippocampus, as compared to the region of the brainstem A1 and A2 NE cell groups and their primary target the hypothalamus. In Experiment 2, a second group of young and aged rats were screened for performance of the water maze task and analyzed for regional changes in receptor binding via autoradiography in portions of the hippocampus, CC and frontal cortex (FC). Taken together, these assays of pre- and post-synaptic NE system function in brain regions potentially involved in expression of these behaviors indicated that aging-related neophobia and impaired acquisition and retention of the spatial memory task were primarily associated with decreased NE neurotransmission in the CC.

2. Materials and methods

2.1. Animals

Subjects were adult male F344 rats 5 or 24 months of age. Two groups of young and aged animals were studied. In Experiment 1, 10 young and 21 aged rats were screened for performance of a gustatory neophobia task and reference memory task in the Morris swim maze. Samples of brain tissue were collected for determination of regional levels of NE and its metabolite MHPG by high performance liquid chromatography (HPLC). In Experiment 2, 6 young and 13 aged rats were screened for performance of the reference memory task in the Morris swim maze, with brain sections processed for receptor autoradiography for regional binding to alpha- and beta-adrenergic receptor subtypes. Care and use of these animals was in compliance with all applicable laws and regulations as well as principles expressed in the *National Institutes of Health, United States Public Health Service Guide for the Care and Use of Laboratory Animals*. This study was approved by the University Committee on Animal Resources at the University of Rochester School of Medicine, where this work was conducted.

2.2. Behavioral testing: gustatory neophobia task

The gustatory reactivity task was adapted from one described previously [98]. Individually housed rats were adapted for 5 days to drinking water from two water bottles mounted on their cages. Water was available for 30 min each day, during which time the animals learned to drink steadily, consuming their entire daily intake. Bottles were weighed before and after each 30-min presentation to assess the amount of water consumed, and whether each rat preferred one bottle over the other. On day 6, the water in one bottle was replaced with a palatable, but novel, gustatory stimulus: a 0.1% saccharin solution. If a preference for one bottle was documented over the first 5 days, the novel solution was placed in the preferred bottle to increase the likelihood that the stimulus would be sampled. All rats drank from both bottles during each of the 5 adaptation days, but often a preference was detected. On the test day, all rats sampled the novel solution. Reactivity to the novel taste was quantified by weighing both bottles, and expressing the amount of novel solution consumed as percent of total fluid intake (intake from both bottles combined). Previous work indicated that intact young adult rats generally prefer the saccharin solution, despite its novelty, and consume 50% or more of their intake as novel solution [98]. Accordingly, experimental groups were defined for this task based upon relative consumption of the novel solution: >50% of fluid intake as saccharin solution defined as “normal,” <50% consumption of saccharin solution defined as “neophobic.” This definition placed all 10 young rats in the normal category, 11 aged rats in the normal category, and 10 aged rats in the neophobic category. We previously have demonstrated that repeating the presentation of the saccharin solution to aged rats for a second and third day results in aged animals accepting the solution and exhibiting relative consumption similar to young rats (unpublished observation). This finding suggests that neophobic aged rats are not impaired in taste sensation, but exhibit a transient behavioral reaction to the novelty of the solution.

2.3. Behavioral testing: Morris water maze

The task is one described by Morris [64], and takes advantage of the rat’s keen sense of spatial orientation in learning and remembering the location of a non-visible platform in a pool of water. The apparatus consisted of a circular tank, 5 ft in diameter and 2 ft deep, filled with room temperature water (25–27 °C). For analysis of swim-search patterns, the pool is divided into four equal pie-piece shaped quadrants. A 5-in. diameter cylindrical platform served as an escape goal, and was placed in the center of a quadrant, midway between the edge and center of the pool. The platform was submerged, lying 1–2 cm below the surface of the water. The platform and tank were painted black, and indirect lighting was used in the room, yielding a non-visible platform goal when submerged. The test room in which the apparatus was housed was rich in extra-maze visual cues, including four high contrast proximal geometric cues mounted on the outer edge of the tank. Prior to training, all rats were screened for adequate vision by testing for a visual placing response. Rats were held by the experimenter and moved toward a bench top. Animals with adequate visual acuity extend their