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Age-Dependent Differences in Behavioral Responses: the Impact of Hsp70 and Hsp90 in the Frontal Cortex

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Age-related neurological complications are common and affect the quality of life. The present study was designed to investigate age-dependent changes in behavioral responses and the role of protective heat shock proteins (Hsp)s 70 and Hsp 90, which expression in the frontal cortex (FC) might be vulnerable to aging processes in rats. We report that 3-month-old rats exhibited the highest motor activity and lowest anxiety (increased distance, time and number of entries in the open arms of the elevated plus maze) compared to 14- and 18-month-old rats. Moreover, old rats showed a decreased level of Hsp 70 and Hsp 90 in the FC compared to young adult rats. These findings suggest that the aging process is accompanied by changes in emotional status that might be associated with a decreased function of protective chaperone proteins in the FC.

Key words: aging, behavioral responses, Hsp70, Hsp 90, rat

Introduction

Ageing is a natural phenomenon that involves many biological changes, including increased oxidative stress, DNA damage and protein misfolding, mitochondrial dysfunction, impaired immune responses, and vascular abnormalities [8]. Literature studies show altered chaperone (Hsp) activity, which in turn leads to oxidative status disturbances, is key to aging-related processes [3,5]. The reduction of inducible Hsp 70 can be a biomarker of altered oxidative status and ROS toxicity [4]. Hsp 90 also has a cellular protective effect as a key molecular chaperone involved in the cytoprotection of eukaryotic cells during stress [2]. In the present study, we aimed to evaluate the role of Hsp 70 and Hsp 90 expression in the frontal cortex (FC) and their link with presumed age-dependent changes in behavioral responses related to anxiety.

Materials and Methods

The procedures used in this study agree with the European Communities Council Directives of 24 November 1986 (86/609/EEC). The experimental design was approved by BFSA (contract # D-65/02.05.2017).

Experimental animals

The experiments were performed on male Wistar rats (Breeding vivarium at INB, BAS), at three ages: 1) young adults, 3-6 months, n= 10; 2) middle-aged, 14-17 months, n= 10; 3) old, 18-21 months, n= 10, kept in standard conditions: temperature: 21 °C, 50-60% humidity.

Behavioural tests

The Elevated plus maze (EPM) test is used to assess anxiety-related behavior. The apparatus consists of a central area, two closed arms and two open arms, positioned perpendicularly to each other. The preference for being in open arms over closed arms (expressed as count of entries, distance and time spent in the open arms) is calculated to measure anxiety-like behavior.

Biochemical analysis of brain homogenates

The frontal cortex was rapidly dissected, inserted in liquid nitrogen and stored at -20 °C until biochemical analysis by ELISA Kit (Rat HSP70, Rat HSP90, Cat: ELK8411) according to the manufacturer's instructions.

Statistical analysis

Data are given as mean ± S.E.M. One-way ANOVA followed by Duncan post hoc test was used. SigmaStat®, GraphPad Prism 6 software were applied for statistical analyses.

Results and Discussion

Total motor activity was significantly decreased with aging. Young adult 3-month-old rats demonstrated the highest motor activity (total distance in closed and open arms) compared to the 18-month-old rat (**Fig. 1A**). Moreover, age-related elevation of the level of anxiety was detected (distance in open arms, time in open arms and number of entries) (**Fig. 1 B, C, D**).

The age-related behavioral changes are reported in animal models and in humans [1,6,7]. Our data confirmed previous reports revealing that aging is associated with decreased motor activity and elevated anxiety level [1,7].

Age-dependent changes in the expression of Hsp 70 and Hsp 90 was detected in the FC. The youngest 3-month-old rats and middle aged rats exhibited the higher expression of Hsp 70 compared to the 18-month-old rats (**Fig. 2A**). In addition, the drop in the level of Hsp 90 was demonstrated in the oldest rat group (**Fig. 2B**). *Post hoc* test confirmed that 3- and 14-month old rats had elevated Hsp 90 compared to 18-month old rats.

The aging process is associated with vulnerability to neurodegeneration and decreased brain activity that neurons should cause misfolded proteins over time. Recently, Yang et al. [9] reported that Hsp70 expression in the brain was closely related to the aging process in mice, while its recovery could alleviate mutant protein toxicity. Our results confirmed previous reports that decreased levels of Hsp 70 and Hsp 90 in the FC are closely associated with rats' aging. Moreover, the age-related changes in emotional responses in rats are suggested to be mediated by decreased function of the Hsp70 and Hsp90 in the FC.

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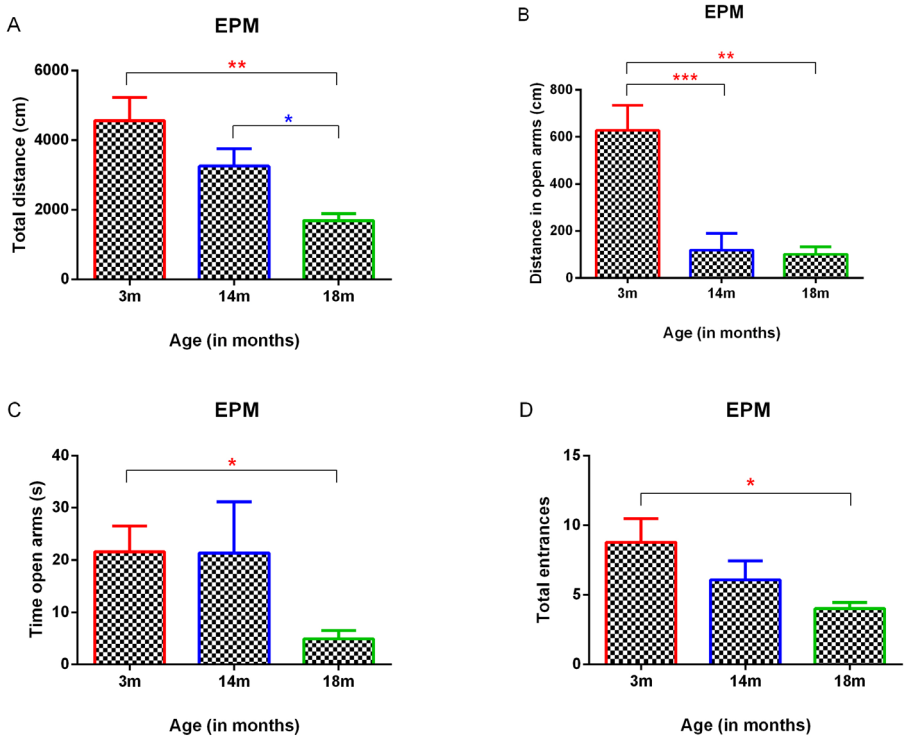

Fig. 1. Effects of aging on motor activity and anxiety parameters in the elevated plus maze test (**A**) total distance; (**B**) distance in the open arms (cm); (**C**) time in the open arms (s); (**D**) number of entries in the open arms. Data are given as mean±SEM. ($F = 4.956$, $p = 0.0147$). Young adult rats demonstrated the highest motor activity (total distance in closed and open arms) compared to aged rat ($p = 0.0091$, 18-month-old rats vs 3-month-old rats ; $p = 0.0458$, 18-month-old rats vs 14-month-old rats) **(Fig. 1A)**. Moreover, age-associated elevation of the level of anxiety was detected (distance in open arms: $F = 13.33$, $p = 0.0002$; $p = 0.0011$, 18-month-old rats vs 3-month-old rats ; $p = 0.0009$, 14-month-old rats vs 3-month-old rats; time in open arms: $p = 0.0295$, 18-month-old rats vs 3-month-old rats; number of entries: $p = 0.0426$, 18-month-old rats vs 3-month-old rats) (**Fig. 1 B, C, D**).

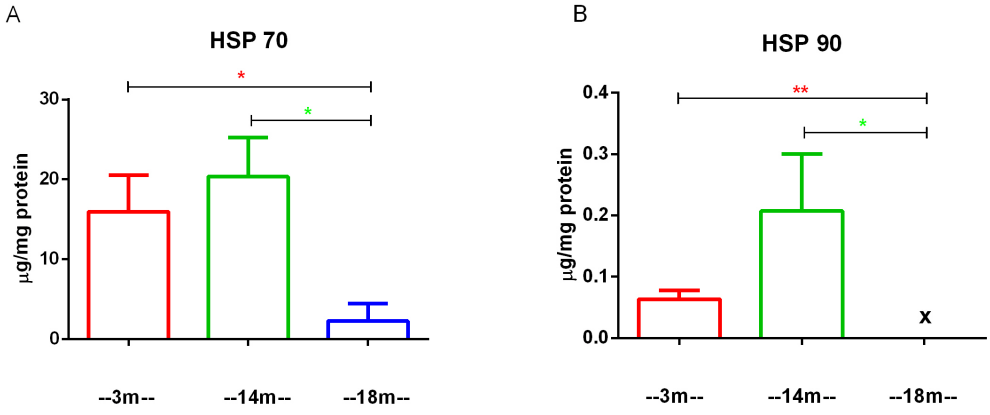

Fig. 2. The aging process affects the expression and function of the chaperone proteins Hsp70 and Hsp 90 in the frontal cortex. Data are given as mean±SEM. p = 0.0333, 3-month-old rats vs 18-month-old rats; p = 0.0106, 14-month-old rats vs 18-month-old rats **(A)**. p = 0.0073, 3-month-old rats vs 18-month-old rats; p = 0.0391, 14-month-old rats vs 18-month-old rats **(B).**

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