Behavioural Brain Research 225 (2011) 192–200

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Age increases anxiety and reactivity of the fear/anxiety circuit in Lewis rats

Ksenia Z. Meyza, Pawel M. Boguszewski, Evgeni Nikolaev, Jolanta Zagrodzka*

Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur St. 02-093 Warsaw, Poland

ARTICLE INFO

Article history:
Received 31 August 2010
Received in revised form 5 July 2011
Accepted 6 July 2011
Available online 18 July 2011

Keywords:
Ageing
HPA axis
Amygdala, Hippocampus
c-Fos protein
Lewis rats

ABSTRACT

A growing body of data indicates that changes in emotional behavior occur with age. Young Lewis rats are known to display hypofunction of the HPA axis. With age the reactivity of this axis is thought to increase with a concomitant rise in anxiety. In the current study, we investigate how and if the pattern of neuronal activation (measured as c-Fos protein expression) in Lewis rat brains changes with age and in response to novel environments differing in aversiveness. We found that distinct parts of the fear/anxiety circuit (i.e., the amygdalar complex, hippocampus and hypothalamus) undergo diverse age-related changes in response to behavioral challenges. While in the hypothalamus an increase in responsivity to mild stressors was observed with age, no such effect was present in the hippocampus. The amygdalar complex (especially the medial and cortical nuclei) on the other hand exhibited an age-dependent decrease in neuronal activation to mild stressors. This was accompanied by a marked increase in anxiety not correlated with a decline in locomotor activity.

1. Introduction

It is well documented that ageing is associated not only with a decline in cognitive functions but also with emotional changes. The character of these changes is however ambiguous. Many gerontological surveys indicate an increase in anxiety and depression in the elderly [1,2], yet the impact of risk factors like loneliness or disability as compared to ageing itself remain unknown. On the other hand, psychological research [3–5] suggests that emotion regulation and mood management improve with age and older adults experience less negative affect. Moreover, several recent fMRI studies have shown an ageing-dependent loss of amygdala reactivity and amplified activity in the prefrontal cortex, in response to negative stimuli [6–10]. It should be pointed out however, that the studies mentioned above were all performed with the use of rather low-stressful stimuli (emotion-laden pictures from International Affective Pictures System (IAPS) [11] and words from Affective Norms for English Words (ANEW) database [12] as well as questionnaires with emotionally charged hypothetical problems). The above results are not in line with commonly recognized increases in anxiety among the elderly and with data obtained in animal studies of ageing. Most authors that have addressed this question in rodents have reported an age-related increase in anxiety/hyperemotionality upon exposure to mild stress (e.g., a novel situation) [13–20].

Little is known about the neurobiological mechanisms that underlie the different emotional responses of young and old animals subjected to stressogenic stimuli. Yet there is extensive evidence indicating that various kinds of stressors induce neuronal activation (with an immediate early gene *c-fos* mRNA and protein expression employed as a functional marker) in the brain structures involved in the regulation of emotions [21–23] The most important regions among these structures include the amygdalar complex [24] and the hippocampus [25]. In Lewis rats, used in the current study, behavioral arousal may also be dependent on the activity of their hypothalamo–pituitary–adrenal (HPA) axis [26]. Thus the activity of the hypothalamic regions may also be crucial for the control of the emotional behavior of these rats.

It might be supposed that the increased emotional reactivity of old rats is linked to the higher activation of these key structures in the fear/anxiety circuit. Those few studies that have dealt with the issue report a far more complicated pattern of age-related neuronal changes. While some researchers have observed a decline in the neuronal reactivity of aged rats [27,28], others have shown that distinct parts of the fear/anxiety circuit may be affected diversely by age [29].

The aim of the present experiment is to investigate the effect of age on the behavioral and neuronal response to a wide spectrum of stressful conditions. These range from low aversive spontaneous exploration of the novel environment of the Hole Board arena, through mildly stressful Open Field with Illuminated Center and Elevated Plus Maze tests, to highly stressful Acute Restraint procedure. In a previous study on psychogenetically selected Roman High Avoidance (RHA/Verh) and Roman Low Avoidance (RLA/Verh) rats, we found this set of tests to be a useful and effective measure

* Corresponding author. Tel.: +48 225892246; fax: +48 228225342.
E-mail address: j.zagrodzka@nencki.gov.pl (J. Zagrodzka).

0166-4328/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2011.07.011

of emotional reactivity/anxiety level differences in behavioral and molecular responses [30]. The main advantage of this methodology is that it permits the investigation of (possible) functional heterogeneity within the structures belonging to the fear/anxiety circuit, as a function of a full range of aversiveness of novel behavioral challenge. It also allows for a complex profiling of the emotional reactivity of the animals used in the study. We applied Principal Component Analysis (PCA) on the set of behavioral measures from novel environment tests (HB, OF and EPM) to trace the possible differences in motivation factors driving the behavior of young and old Lewis rats. PCA is considered a particularly beneficial statistical tool for the interpretation of behavioral data since it allows for extraction of presumably independent factors reflecting different drives constituting behavior [31–34]. In our experiment, factor analysis was applied for three main reasons: to identify the relationship between specific test indices and factors such as motor activity, anxiety and exploratory drive, to take account of the individual differences between subjects – it is well known that marked variation in behavioral impairments is seen between individuals of the same aged rat population [35,36], and finally, to assess the applicability of behavioral tests and settings used to the investigation of both young and aged animals behavior.

2. Materials and methods

2.1. Animals

A total of 50 males, inbred Lewis rats from the breeding colony at the Medical University of Warsaw were randomly assigned into two, equally numbered groups. The first group was behaviorally tested at the age of approx. 3 months (Young Adults, YA, $n = 25$, 312 ± 7 g). The second group was kept in the animal house of the Nencki Institute of Experimental Biology, PAS until the age of 20–22 months and then behaviorally tested (Old Adults, OA, $n = 23$, as two animals had to be excluded from the study due to movement impairment, 523 ± 13 g). Both groups were handled and habituated (daily, monday through friday) to the experimental room 3 weeks prior to testing. Additionally, the OA group was handled regularly throughout their lives (twice a week). The animals were housed in groups of 2–3 littermates per cage, with unlimited access to water and standard laboratory rat chow (Labofeed B Standard, Morawski, Kcynia, Poland), in light:dark 12:12 conditions, with lights on at 8:00AM.

2.2. Behavioral testing

At the age of either 3 or 20–22 months the rats were randomly assigned to 5 groups. The control group (YA, $n = 5$ and OA, $n = 4$) was killed directly from their home cages on day 1, before the onset of any other behavioral testing. Rats from the first three experimental groups (each consisting of $n = 5$, named after the last test in the series of three: OF, EPM and HB) were tested in three consecutive tests: Open Field with Illuminated Center (OF), Elevated Plus Maze (EPM) and Hole Board test (HB). The tests had a different order in each group (for experimental design see Fig. 1). To minimize the effect of previous experiences on the behavior in consecutive tests, seven day intervals were applied between them (between the tests 4 habituation sessions took place).

At the end of the behavioral testing animals from the fifth group (YA, $n = 5$ and OA $n = 4$), handled in the same way as the other rats, were subjected to the Acute Restraint procedure. The behavioral data from the first 3 experimental groups was collected (in MPEG-2 format) and analyzed using a video-based, automated Ethovision System (Noldus, Wageningen, NL) and in case of nose-poke activity in the HB test (behavior difficult to assess by means of an automated system) with an observer-based program (BehaView, http://www.pmbogusz.net/).

2.2.1. Open Field with Illuminated Center (OF)

The test arena was a black painted square (90 cm × 90 cm), enclosed by walls (30 cm height) with a 50 W halogen bulb suspended 30 cm above the center (900 lx at the bottom of the cage, directly under the bulb) as the only source of light. The animal was placed in the border zone facing one of the corners and its movements were recorded for 10 min. Two zones, the illuminated center zone (a circle directly corresponding to the brightly lit part of the arena) and the border zone, were drawn according to previously selected criteria [14]. The following parameters were taken into analysis – in the illuminated center: number of entries to zone, time spent, distance moved and movement duration; in the border zone: distance moved and movement duration; in the whole arena: total distance moved, total movement duration and mean velocity.

2.2.2. Elevated Plus Maze (EPM)

A black wooden apparatus [37] consisting of two enclosed arms (10 cm × 50 cm × 30 cm) and two open arms (10 cm × 50 cm) connected with a central platform (10 cm × 10 cm) located 70 cm above the floor was used. The rats were introduced to the closed arm of the maze via a lifted door and their exploration was recorded for 5 min. The testing was done in a dimly lit (30 lx at the maze level) area surrounded with non-transparent, grey curtains in order to limit any additional spatial stimuli. The following parameters were calculated – in closed arms: number of entries, total time spent, distance moved and movement duration; in open arms: number of entries, total time spent, distance moved and movement duration; in the central platform: time spent; in the whole arena: total distance moved, total movement duration, total number of entries to open and closed arm and ratio of entrances to open/closed arms.

2.2.3. Hole Board (HB)

The HB test was performed in a 60 cm × 60 cm × 30 cm box with grey walls and four equidistant, 1 cm deep holes (3 cm in diameter) in the central part of a black-painted floor as previously described [30]. The testing took place in a room lit by two 80 W light bulbs (70 lx). At the beginning of the test the rat was placed in one of the corners of the arena and allowed to explore freely for 10 min. The following parameters were analyzed – in central part of the apparatus: number of entrances, time spent, distance moved and movement duration; in the border zone: distance moved and movement duration; in the whole arena: total distance moved, movement duration, mean velocity and number of nose pokes into holes in the arena floor.

2.2.4. Acute Restraint (immobilization, IM)

The Acute Restraint test was performed using a clear Plexiglas ventilated tube, 20 cm long, 6.5 cm inner diameter, with adjustable length according to the size of the animal and tail protruding. The size of the tube restricted movement in all directions but did not interfere with respiration [29,30]. The animals were kept in the apparatus for 15 min. No behavioral parameters were recorded.

2.3. Immunocytochemistry

Rats from all experimental groups were killed 90 min after the beginning of the final test (OF, EPM, HB or IM) with an overdose of chloride hydrate anesthesia (>360 mg/kg) and perfused transcardially with ice-cold phosphate buffered saline (PBS, pH = 7.4 Sigma) followed by 4% paraformaldehyde (Sigma). The control group was killed directly from their home cages. The brain of each animal was removed from the skull and postfixed as previously described [29,30]. The brains were deep

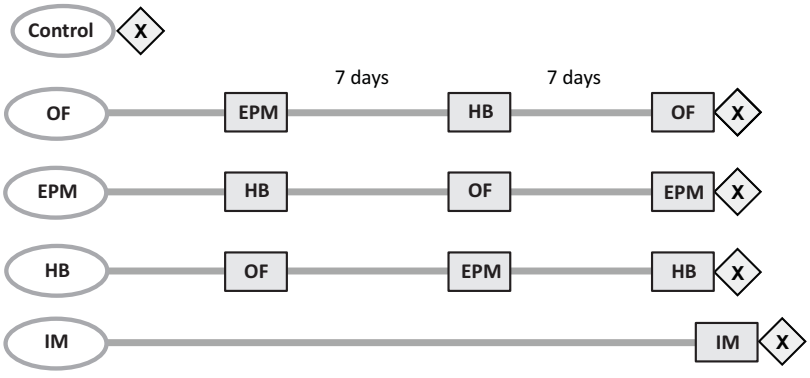

Fig. 1. Experimental design: white ellipses indicate group labeling, grey boxes show behavioral tests. *X* the moment of lethal anesthesia and perfusion.