Please cite this article in press as: Morel GR et al. Cognitive impairment and morphological changes in the dorsal hippocampus of very old female rats. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.06.050

Neuroscience xxx (2015) xxx–xxx

COGNITIVE IMPAIRMENT AND MORPHOLOGICAL CHANGES IN THE DORSAL HIPPOCAMPUS OF VERY OLD FEMALE RATS

G. R. MOREL,[a] T. ANDERSEN,[a] J. PARDO,[a] G. O. ZUCCOLILLI,[b] V. L. CAMBIAGGI,[b] C. B. HEREÑÚ[a†] AND R. G. GOYA[a*†]

[a] *INIBIOLP-Histology B-Pathology B, School of Medicine, National University of La Plata, La Plata city, Argentina*

[b] *Institute of Anatomy, School of Veterinary Sciences, National University of La Plata, La Plata city, Argentina*

Abstract—The hippocampus, a medial temporal lobe structure necessary for the formation of spatial memory, is particularly affected by both normal and pathologic aging. In previous studies, we observed a significant age-related increase in dopaminergic neuron loss in the hypothalamus and the substantia nigra of female rats, which becomes more conspicuous at extreme ages. Here, we extend our studies by assessing spatial memory in 4–6 month-old (young), 26-month-old (old) and 29–32-month-old (senile) Sprague–Dawley female rats as well as the age-related histopathological changes in their dorsal hippocampus. Age changes in spatial memory performance were assessed with a modified version of the Barnes maze test. We employed two probe trials (PTs), one and five days after training, respectively, in order to evaluate learning ability as well as short-term and longer-term spatial memory retention. A set of relevant hippocampal cell markers was also quantitated in the animals by means of an unbiased stereological approach. The results revealed that old rats perform better than senile rats in acquisition trials and young rats perform better than both aging groups. However, during short-term PT both aging groups showed a preserved spatial memory while in longer-term PT, spatial memory showed deterioration in both aged groups. Morphological analysis showed a marked decrease (94–97%) in doublecortin neuron number in the dentate gyrus in both aged groups and a reduction in glial fibrillary acidic protein-positive cell number in the stratum radiatum of aging rats. Astroglial process length and branching complexity decreased in aged rats. We conclude that while target-seeking activity and learning ability decrease in aged females, spatial memory only declines in the longer-term tests. The reduction in neuroblast number and astroglial arborescence complexity in the dorsal hippocampus are likely to play a role in the cognitive deficits of aging rats. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: aging, female-spatial memory, Barnes maze, hippocampus, doublecortin, GFAP.

INTRODUCTION

The hippocampus, a medial temporal lobe structure necessary for the formation of spatial memory, is particularly affected by both normal and pathologic aging impairment (Scoville and Milner, 1957; Corkin et al., 1997; Smith et al., 1999; Stefanacci et al., 2000).

In rats as in humans, learning and memory performance declines with age which makes this rodent species a suitable model to evaluate therapeutic strategies of potential clinical value for restoring age-related cognitive deficits. In previous studies we have reported that hypothalamic or intracerebroventricular insulin-like growth factor-I (IGF-I) gene therapy is able to reverse or at least attenuate the lactotrophic, reproductive and motor derangements of aging female rats (Hereñú et al., 2007; Nishida et al., 2011; Rodriguez et al., 2013), and are now interested in assessing the restorative potential of this intervention on the spatial memory performance of aging female rats. In most rodent studies, spatial memory has been assessed using the Morris Water Maze (MWM, Morris, 1984) for which a considerable performance database is available both for rats and mice. Most rat studies on the impact of aging on spatial memory have been performed in males (some relevant studies are: Barnes et al., 1980; Frick et al., 1995; Barrett et al., 2009; Bizon et al., 2009; McQuail and Nicolle, 2015), a fact that makes it difficult to establish eventual sex-related differences in the impact of aging on cognitive performance. A potential disadvantage of the MWM in aging studies is that it requires a substantial degree of physical fitness. This has led some investigators to prefer the less physically demanding Barnes maze (Barnes, 1979), especially for aged rats. In both tests, rats learn to use spatial cues to guide them to a hidden platform or tunnel. While the MWM involves immersion in water, a stimulus that provokes considerable corticosterone and corticotropin release (Sternberg et al., 1992), in the Barnes maze animals are placed on an open, unprotected circular platform for them to walk in search of the escape tunnel. Comparison between the Barnes

*Corresponding author. Address: INIBIOLP, School of Medicine, UNLP, Calles 60 y 120, 1900 La Plata, Argentina. Tel: +54-221-425-6735.
E-mail address: goya@isis.unlp.edu.ar (R. G. Goya).
† These two authors contributed equally to this work.

Abbreviations: AT, acquisition trial; DG, dentate gyrus; DH, dentate hilus; DCX, doublecourtin; GFAP, glial fibrillary acidic protein; GS, goal sector; GCL, granular cell layer; IGF-I, insulin-like Growth Factor I; ML, molecular layer; MWM, Morris Water Maze; NGS, non-goal sector; PT, probe trial.

http://dx.doi.org/10.1016/j.neuroscience.2015.06.050
0306-4522/

maze and MWM in mice has shown that the latter induces higher circulating corticosterone concentration than the former and that serum corticosterone levels show an inverse correlation with the performance of the animals in the MWM but not in the Barnes maze (Harrison et al., 2009). Furthermore, even at the tepid temperatures typically used in MWM studies, swim stress also causes sympathetic activation and peripheral adrenaline release, especially in aged rats (Mabry et al., 1995).

In order to generate a reference framework for future therapeutic investigations in female rats, in the present study we undertook to characterize the changes in spatial memory in old and very old (senescent) females using a modified version of the Barnes maze so that nonspecific exploration and target-seeking activity as well as the ability for acquisition and retention of spatial information can be compared in the different age groups. A set of relevant hippocampal cell markers was also quantitated in the animals by means of an unbiased stereological approach.

EXPERIMENTAL PROCEDURES

Animals

Forty-two young (4–6 months), twenty old (26 months) and thirty-seven senile (29–32 months) female Sprague–Dawley (SD) rats weighing 199 ± 1, 271 ± 5 and 267 ± 5 g, respectively were used. Animals were housed in a temperature-controlled room (22 ± 2 °C) on a 12:12-h light/dark cycle. Food and water were available *ad libitum*. All experiments with animals were performed in accordance to the Animal Welfare Guidelines of NIH (INIBIOLP's Animal Welfare Assurance No A5647-01).

Spatial memory assessment

The modified Barnes maze protocol used in this study was based in part on a previously reported procedure (Vargas-López et al., 2011). It consists of an elevated (108 cm to the floor) black acrylic circular platform, 122 cm in diameter, containing twenty holes around the periphery. The holes are of uniform diameter (10 cm) and appearance, but only one hole is connected to a black escape box (tunnel). The escape box is 38.7-cm long × 12.1-cm wide × 14.2-cm in depth and is removable. A white-squared starting chamber (an opaque, 20 cm × 30 cm long, and 15 cm high, open-ended chamber) is used to place the rats on the platform. Four proximal visual cues are placed in the room, 50 cm away from the circular platform. The escape hole was numbered as hole 0 for graphical normalized representation purposes, the remaining holes being numbered 1–10 clockwise, and −1 to −9 counterclockwise (Fig. 1). Hole 0 remained in a fixed position, relative to the cues in order to avoid randomization of the relative position of the escape box. During the tests the platform was rotated daily. A 90-dB white-noise generator and a white-light 500-W bulb provided the escape stimulus from the platform. We used an abbreviated protocol based on three days of acquisition trials (ATs), followed by two probe trials (PT) (1 and 5 days after training) to assess recent and longer-term spatial

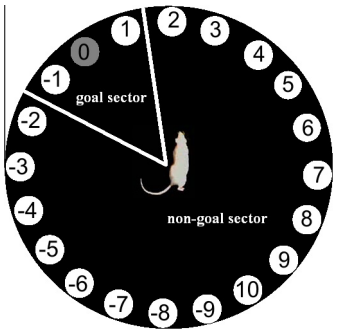

Fig. 1. Barnes Maze design used in the present study. The maze consists of a black acrylic circular platform, 122 cm in diameter, containing twenty holes around the periphery. The holes are of uniform diameter (10 cm) and appearance, but only one hole is connected to a black escape box. An opaque squared starting chamber is used to place the rats on the platform. Four proximal visual cues are located in the room, 50 cm away from the circular platform. The escape hole is numbered as hole 0 for graphical normalized representation purposes, the remaining holes being numbered 1–10 clockwise, and −1 to −9 counterclockwise. Hole 0 remains in a fixed position, relative to the cues in order to avoid randomization of the relative position of the escape box.

memory retention. An AT consists of placing a rat in the starting chamber for 30 s, the chamber is then raised, and the aversive stimuli (bright light and high pitch noise) are switched on and the rat is allowed to freely explore the maze for 120 s. Probe trials are defined as trials where the escape box has been removed, their purpose being to assess the latency to explore the empty escape hole and the error frequency. After the starting chamber is raised, the rat is given 120 s to explore and the number of explorations per hole is recorded. On the day before the first trial (experimental day 0), rats underwent a habituation routine to let them get acquainted with the platform and the escape box. Once AT started, rats were tested (120 s per trial) with the escape box, two times per day for three consecutive days (experimental days 1–3). On day 4, rats were submitted to a first probe trial (PT1) during 120 s without escape box, followed by a 120-s reinforcement trial with the escape box in place. Afterward, rats had a 4-day rest (days 5, 6, 7 and 8) and on day 9 they were submitted to a second probe trial (PT2). In order to eliminate olfactive clues from the maze and the boxes, the surfaces were cleaned with 10% ethylic alcohol solution, after each trial.

The behavioral performances were recorded using a computer-linked video camera mounted 110 cm above the platform. The video-recorded performances of the subjects were measured using the Kinovea v0.7.6 (http://www.kinovea.org) and Image Pro Plus v5.1 (Media Cybernetics Inc., Silver Spring, MD, USA) software. The behavioral parameters assessed were as follows.

(a) **Escape box latency:** time (in s) spent by an animal since its release from the start chamber until it enters the escape box (during an AT) or until the first exploration of the escape hole (during a PT).
(b) **Non-goal hole exploration:** number of explorations of holes different from the escape one. Each exploration of an incorrect hole is counted as an error, provided that the rat lowers its nose below the plane of the table surface.

Please cite this article in press as: Morel GR et al. Cognitive impairment and morphological changes in the dorsal hippocampus of very old female rats. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.06.050