Neurobiology of Learning and Memory 109 (2014) 94–103

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

# Changes in the plastic properties of hippocampal dendritic spines underlie the attenuation of place learning in healthy aged rats

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ARTICLE INFO

*Article history:*
Received 25 July 2013
Revised 19 November 2013
Accepted 24 November 2013
Available online 5 December 2013

*Keywords:*
Aging
Hippocampus
Dendritic spines
Plasticity
Morris maze
Rat

ABSTRACT

Normal aging is characterized by slight impairments in spatial memory, and the modification of some electrophysiological parameters that underlie place learning and associated reference memory. However, the morphological mechanisms underlying these impairments remain unknown. In the present study, we analyzed the spine density and the proportion of thin, mushroom, stubby, wide, branched and double spines on pyramidal neuron dendrites in the hippocampal CA1 field of young and aged rats. These parameters were assessed both before and after evaluating place learning and reference memory in the Morris water maze. Aged rats adopted an egocentric strategy to resolve the task, swimming slower and further, and taking longer to locate the sunken platform. While probe trials revealed that aged animals could recall the platform position, these animals spent more time exploring incorrect quadrants than young rats. An increase in spine density was observed after task performance in both young and aged rats, but aging provoked a decrease in the density of thin spines. In addition, there was an increase in the density of mushroom and wide spines in aged animals after task performance as compared with the untested aged counterparts. Moreover, in aged animals there were fewer thin spines and more wide spines after task performance than in the young tested animals. These findings support the view that aging attenuates but does not abolish spatial memory, a process that may be associated with plastic changes in the type of dendritic spines on aged hippocampal CA1 neurons.



## 1. Introduction

Although aging is often accompanied by pathophysiological and/or psychopathological conditions, many individuals age without developing any significant pathology, a process known as "successful aging" (Depp & Jeste, 2006). However, even in these cases there is a relative decline in several psychophysiological functions, including some cognitive processes like learning and memory (Aine et al., 2011), referred to as "normal aging" or "cognitive aging" (Roberson et al., 2012).

Several studies in humans have demonstrated that spatial memory is one of the main cognitive abilities commonly affected during normal aging. Spatial memory serves to situate the individual in relation to their surrounding environment by compiling a cognitive representation of the immediate space. This cognitive map is constructed between the right and left (Burgess, 2002) hippocampus (O'Keefe & Nadel, 1978), incorporating details regarding the speed and direction of movement, and other spatial- and object-related information (Hölscher, 2003). While aging individuals can recall the same number of items as younger individuals in contextual spatial memory tasks, they exhibit deficits in the spatial contextualization of the items recalled (Kukolja, Thiel, Wilms, Mirzazade, & Fink, 2009), which is in line with previous reports showing that activation of the hippocampus and related brain structures during a virtual spatial navigation paradigm is attenuated in aging individuals (Moffat, Elkins, & Resnick, 2006).

The spatial information processing results from the excitatory stimulation of neurons in the hippocampal trisynaptic circuit, which gradually integrates the topological characteristics of objects in the immediate surroundings and the spatial relationships between them by space coding-related firing of hippocampal CA1 pyramidal cells (Goodrich-Hunsaker, Hunsaker, & Kesner, 2008). Electrophysiological experiments have shown that clusters of pyramidal neurons from the hippocampal CA1 subfield fire synchronously when the rat encodes spatial information relating to

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http://dx.doi.org/10.1016/j.nlm.2013.11.017

its particular location (O'Keefe, 1979) in a high theta frequency, which is directly related with place learning (Olvera-Cortés, Cervantes, & González-Burgos, 2002; Olvera-Cortés, Guevara, & González-Burgos, 2004). In this regard, studies carried out on young and aged rats in a water Morris maze paradigm revealed that the high-frequency theta activity of CA1 pyramidal neurons of aged animals during the spatial information integration stage is less than in young animals (Olvera-Cortés et al., 2012).

Trisynaptic circuit-associated excitatory synapses from CA1 neurons are formed mostly with dendritic spines in the CA1 stratum radiatum (Hongpaisan & Alkon, 2007) but also with dendritic spines in the CA1 stratum oriens although in a lesser extent (Leuner, Falduto, & Shors, 2003). Dendritic spines are the point of entry of excitatory synaptic stimulation to postsynaptic neurons, and variations in the content of excitatory information, including that related to cognitive abilities, are regulated by plastic changes in the geometrical structure of dendritic spines (González-Burgos, 2009, 2012). Thus, the spatial navigation-related deficiencies reported during normal aging in both humans and experimental animals may be related to changes in the dendritic spines on hippocampal CA1 pyramidal neurons. To investigate this hypothesis, we conducted a behavioral and morphological study on young and aged rats to evaluate the effect of aging on the ability of dendritic spines to sustain place learning and on memory performance in a spatial reference memory paradigm in the Morris maze.

## 2. Material and methods

### 2.1. Subjects

This study was carried out on 40 male Sprague–Dawley rats proceeding from six different litters. The rats were divided into two main study groups: a control group (Y; $n = 20$) composed of young rats 3–3.5 months-old, and an experimental group of aged rats (A; $n = 20$) 24–25 months-old. All the rats were housed under a regular 12/12 h light/dark cycle at 25 °C with *ad libitum* access to water and food.

### 2.2. Experimental design

The Y and A groups were further divided into two subgroups for behavioral ($n = 14$, each) or morphological ($n = 6$ each) analyses. In addition, animals from the Y and A behavioral subgroups were used for subsequent morphological studies after behavioral testing ($n = 6$ per subgroup, chosen randomly). Rats used for morphological studies after behavioral testing were identified as Yb or Ab, while the Y and A subgroups used for morphological analyses only were identified as Ynb and Anb (Fig. 1).

### 2.3. Behavioral analyses

Behavioral evaluations were performed in a Morris maze, a pool 1.3 m in diameter filled with water (25 °C) that had been dyed blue by the addition of gentian violet. For the place-learning test, a glass platform was placed 1.0 cm below the water level in a given quadrant, and behavioral experiments were performed daily between 16:00 and 18:00 h.

Training was carried out on five consecutive days by the same two researchers in the presence of constant and fixed environmental cues, with all visual stimuli remaining in the same place and position throughout the training. Each animal was challenged to solve the spatial task in four daily trials, starting from a randomly chosen quadrant of the pool. The position of the sunken platform was maintained constant throughout the training sessions. In each session, the animal was placed in the water facing the wall of the pool and 15 s after reaching the platform, the animal was removed. If the rat failed to find the platform within 60 s, it was removed from the water and placed on the platform for 15 s. Trials were performed at 2 min intervals. On the sixth day, the platform was removed from the pool and the animals were challenged to a single search trial for 30 s (probe trial).

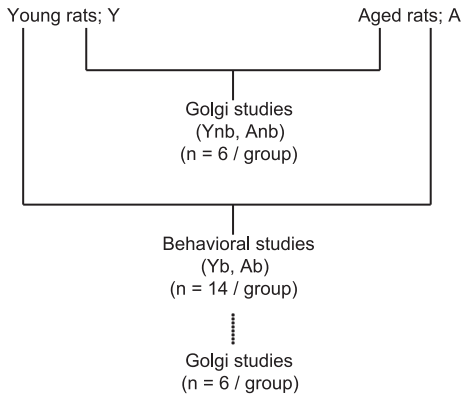


**Fig. 1.** Scheme showing the experimental design. Young (Y) and aged (A) rats were used for Golgi-based morphological studies after the evaluation of place learning/reference memory (Yb, Ab). Two additional sub-groups of young and aged animals were used for Golgi-based morphological study, having not been subjected previously to behavioral study.

The acquisition (training) and retention (probe trial) trials were recorded on video for further off-line analysis using an image analyzer (LAS 4.0). The escape latency, swim velocity and distance traveled were all evaluated during the training, while the number of entries into each of the four quadrants, the time spent in each quadrant, and the number of crosses through the specific area in which the platform was located during training, were evaluated during the probe trial.

### 2.4. Morphological analyses

Morphological studies of the Yb and Ab subgroups began immediately after the probe trial ended. Rats from each of these groups, as well as those from the Ynb and Anb subgroups, were anesthetized with ketamine (30 mg/kg, i.m.) and sodium pentobarbital (50 mg/kg, i.p.), and they were then perfused with 200 ml of a phosphate-buffered solution (pH 7.4; 0.01 M) containing sodium heparin (1000 IU/l) as an anticoagulant and procaine hydrochloride (1 g/l) as a vasodilator (Feria-Velasco & Karnovsky, 1970). The rats were subsequently perfused with 200 ml of a phosphate-buffered 4% formaldehyde fixative solution. Both solutions were perfused at a rate of 11.5 ml/min. The rat's brain was then removed and maintained for at least 48 h in 100 ml of fresh fixative solution. The bilateral dorsal hippocampus (Paxinos & Watson, 1986) was subsequently dissected out and impregnated using a modified version of the Golgi technique (González-Burgos, Tapia-Arizmendi, & Feria-Velasco, 1992). Coronal slices (75 μm thick) were mounted on a slide, one per animal, and six CA1 pyramidal neurons were analyzed from each of the six rats in each subgroup (Ynb, Yb, Anb and Ab). In each neuron, dendritic spines were counted in the stratum radiatum along one 50 μm segment from a secondary oblique dendrite protruding from the apical dendrite distal to the soma, and; in one 50 μm segment of a secondary dendrite from the basilar arborization. In an initial double-blind study, a reliability index was determined for dendritic spine counting (number of agreements – number of disagreements/number of agreements). Once a minimum reliability of 0.95 was reached between at least two experimenters of our Lab, dendritic spines from each of the groups were quantified by only one of them, using a "blind"