Experimental Gerontology 106 (2018) 222–231

Contents lists available at ScienceDirect

Experimental Gerontology

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

Characterizing age-related decline of recognition memory and brain activation profile in mice

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ARTICLE INFO

Section editor: Christian Humpel

Keywords:
Memory
Aging
Novel-object recognition
Object-location recognition
c-Fos
Neuronal networks

ABSTRACT

Episodic memory decline is one of the earlier deficits occurring during normal aging in humans. The question of spatial *versus* non-spatial sensitivity to age-related memory decline is of importance for a full understanding of these changes. Here, we characterized the effect of normal aging on both non-spatial (object) and spatial (object location) memory performances as well as on associated neuronal activation in mice. Novel-object (NOR) and object-location (OLR) recognition tests, respectively assessing the identity and spatial features of object memory, were examined at different ages. We show that memory performances in both tests were altered by aging as early as 15 months of age: NOR memory was partially impaired whereas OLR memory was found to be fully disrupted at 15 months of age. Brain activation profiles were assessed for both tests using immunohistochemical detection of c-Fos (neuronal activation marker) in 3and 15 month-old mice. Normal performances in NOR task by 3 month-old mice were associated to an activation of the hippocampus and a trend towards an activation in the perirhinal cortex, in a way that did significantly differ with 15 month-old mice. During OLR task, brain activation took place in the hippocampus in 3 month-old but not significantly in 15 month-old mice, which were fully impaired at this task. These differential alterations of the object- and object-location recognition memory may be linked to differential alteration of the neuronal networks supporting these tasks.

1. Introduction

Population aging in industrialized countries constitutes one of the most significant public health challenges. Normal aging is associated with cognitive impairments such as memory deficits, episodic memory being the earliest and most strikingly affected by aging (St Jacques and Levine, 2007; Martinelli et al., 2013; see for review, Tromp et al., 2015). Episodic memory refers to the encoding and retrieval of a unique personal experience in terms of "what" happened, as well as "where" and "when" it occurred (Tulving, 2002). Although many investigations postulate that episodic memory gradually deteriorates from the age of 60 years old (Barrash, 1994), this decline may begin earlier in adulthood, *i.e.* at midlife (Salthouse, 2009; Kwon et al., 2016; Nordin et al., 2017). Such age-associated memory impairments can strongly and negatively impact quality of life, making it essential to understand the normal aging process together with the mechanisms underlying these cognitive alterations. Nowadays, the main objectives of aging research are to distinguish between the memory declines attributable to normal aging from those related to pathological aging, especially to Alzheimer's disease (AD), to allow an early diagnosis of these pathologies.

Animal models remain key tools to identify the behavioral and neurobiological bases of cognitive decline. In this context, the development of relevant tasks modelling episodic memory is an important challenge in experimental research to investigate the processes underlying normal, as well as pathological aging (Van der Staay, 2002; Alexander et al., 2012). For this purpose, object recognition memory

Abbreviations: PFC, Prefrontal Cortex; DG, Dentate Gyrus; ISI, Inter-SessionInterval; NOR, Novel Object Recognition; OLR, Object Location Recognition

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https://doi.org/10.1016/j.exger.2018.03.006
Received 15 November 2017; Received in revised form 23 February 2018; Accepted 7 March 2018
Available online 08 March 2018
0531-5565/

task (Ennaceur and Delacour, 1988; Şık et al., 2003), based on the innate tendency of rodents to seek novelty, is commonly used. This behavioral task does not require extensive training, nor rules learning and can assess the three components (*i.e.* "what", "when", "where") of episodic-like memory (ELM) (Ennaceur and Delacour, 1988; Şık et al., 2003; Dere et al., 2005a, 2005b; Belblidia et al., 2015).

The time-course of memory decline of each component during aging remains poorly characterized in rodents. According to Cavoy and Delacour (1993), deficits in object-location recognition (OLR)memory reflecting the spatial component of recognition memory, appear from the age of 18 months in rats, while novel-object recognition (NOR) memory, targeting the object features, was not affected at this age (Cavoy and Delacour, 1993). Similarly, Wimmer and colleagues have shown that recognition memory remains intact for the nature of an object, but not for its location in 22 to 24 months old mice (Wimmer et al., 2012). More recently, impairment of recognition memory for object-location has also been described in mice as soon as 12 months of age (Li et al., 2015). Overall, these investigations suggest that, in rodents, remembering an object-location is more sensitive to aging process than the ability to remember its nature. Nevertheless, the onset of age-related impairments of recognition memory for novel-object and object-location was not compared in these studies. Besides, the underlying mechanisms still remain poorly known. Several mechanisms have been suggested to underlie age-related decline in recognition memory, including hippocampal morphological changes (Nicholson et al., 2004), alteration of firing properties of hippocampal place cells (Wilson, 2005) or reduced synaptic plasticity (Barnes et al., 2000; Samson and Barnes, 2013). In young adult rodents, the neuronal networks underlying memory in object recognition tests have been extensively documented. Measurements of immediate-early gene expression (IEGs), such as c-Fos expression, have reported the engagement of the hippocampus and the perirhinal cortex in recognition tests (Aggleton and Brown, 2005; Albasser et al., 2010; Barbosa et al., 2013; Mendez et al., 2015; Jacklin et al., 2016). Whether the neuronal activation profiles during such recognition memory tests are altered in aged rodents remains an open question.

In this study, we aim to assess the time-course of object- and object location recognition memory decline in mice between 3 and 19 months, as well as the associated neuronal activation profiles. Besides, we aim to determine whether alterations of brain activation profiles are correlated with cognitive performances in the 15 month-old mice.

2. Materials and methods

2.1. Animals

Experiments were performed on a total of 118 young adult (3 months, $n = 24$), adult (7 months, $n = 29$), middle-aged (10 months, $n = 35$), advanced-aged (15 months, $n = 24$) and old (19 months, $n = 24$) female NMRI mice (purchased from Janvier labs, France). A behavioral exclusion criterion (see below) led us to exclude 18 mice as follows: 3 months(2); 7 months (5); 10 months (6); 15 months (1); 19 months (4). This strain of mouse was preferentially chosen in our study because of its short life span (median life span of 17 months, *i.e.* corresponding to the survival of 50% of the population, Gower and Lamberty (1993)). Moreover, memory performances of this strain have been well characterized in our laboratory (Da Silva Costa-Aze et al., 2009, 2011; Freret et al., 2012; Leger et al., 2013). Animals were housed in standard polycarbonate cages (37x23x18cm; $n = 10$ per cage), maintained on a reversed 12 light-dark cycle (20:00–8:00), at constant temperature (21 °C) and humidity (55%).Water and food were available *ad libitum*. All experiments were performed in accordance with the European Community's Council Directive and approved by the regional ethics committee (Comité d'Ethique NOrmandie en Matière d'EXpérimentation Animale, CENOMEXA, agreement number: 03-08-11/16/08-14).

2.2. Behavioral experiments

Behavioral experiments were conducted during the dark phase of the animals' cycle. Mice were placed in the experimental room 30 min before the beginning of experiments. Distal visual cues were available for the mice on the walls.

2.2.1. Apparatus and objects used

The object recognition test was conducted in a grey plastic Y-maze with three arms (33 × 8 × 16 cm each), with a light intensity of 15 lx at the centre of the apparatus, as described in the literature (Leger et al., 2012). Two types of objects (available in quadruplicate) made of plastic were used (Falcon® *versus* assembly Lego®), and randomly used as familiar or novel object to avoid any bias (Leger et al., 2013). For all experimental procedures, objects were placed in two of the three arms of the maze, 5 cm away from the walls and fixed by Patafix® on the maze floor to avoid their displacement by mice. After each session, the maze and the objects were cleaned with diluted ethanol (70%) and dried to prevent any residual olfactory cues.

2.2.2. Experimental procedure

The experimental protocol used to evaluate object recognition memory was based on previously published studies (Leger et al., 2012). Briefly, the task consisted of two 10 min sessions (*i.e.* presentation session and test session) separated by a 24 h inter-session interval (ISI). During each session, the mouse was allowed to freely explore the three arms and the two objects placed within, until a criterion of 20 s of total exploration for the two objects was reached. The time spent to reach the criterion of exploration and the time spent to explore each object during each session was collected manually in a blind manner from video recordings. The time spent to explore the new or the displaced object was used as an index of memory performances by comparison with the chance level (10 s). Mice failing to reach the criterion within 10 min were excluded from data analysis.

Two variants of the object recognition test were used to assess separately the ability of mice to remember the nature (*novel-object recognition test*) or the location of objects presented in session 1 (*object-location recognition test*).

2.2.3. Novel-object recognition test (NOR)

For each of the two sessions, the mouse was placed facing the wall of the starting arm containing no object, and was allowed to freely explore the maze. During the presentation session, two identical objects (A_1 and A_2) were placed in the distal area of two arms of the Y-maze. During the test session, the nature of one of the two previously seen objects remained unchanged (familiar object, A_3); whereas the nature of the other object was changed (novel object, B).

2.2.4. Object-location recognition test (OLR)

For each of the two sessions, the mouse was placed at the centre of the maze, and then was allowed to freely explore the maze. During the presentation session, two identical objects (A_1 and A_2) were placed in the distal area of two arms of the Y-maze. During the test session, the location of one of the two previously seen objects remained unchanged (familiar object location, A_3) whereas the location of the other object was changed, *i.e.* shifted to the previously empty arm (novel object location, A_4).

2.3. c-Fos immunohistochemistry

The neuronal activation profile related to each test was assessed in 3 and 15 month-old mice ($n = 10$/group). These two ages were chosen according to the behavioral results showing that 3 month-old mice successfully performed the two variants of the object recognition test, whereas 15 month-old mice only succeeded the novel-object recognition test. To control for non-memory aspects of the task, such as