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Early detection of age-related memory deficits in individual mice

C. Fouquet[a,1], G.H. Petit[a,b,1,2], A. Auffret[b], E. Gaillard[a], C. Rovira[b], J. Mariani[b,c], L. Rondi-Reig[a,*]

[a] ENMVI team, UMR CNRS 7102, Université P&M Curie, F-75005 Paris, France
[b] DVSN team, UMR CNRS 7102, Université P&M Curie, F-75005 Paris, France
[c] Assistance Publique-Hopitaux de Paris (AP-HP), Hôpital Charles Foix, UEF, F-94200 Ivry-sur-seine, France

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Abstract

To date, no consensus has been reached concerning the age of the earliest onset of age-related cognitive deficits in rodents. Our aim was to develop a behavioral model allowing early and individual detection of age-related cognitive impairments. We tested young (3 months), middle-aged (10 months) and aged (17 months) C57Bl/6 mice in the starmaze, a task allowing precise analysis of the search pattern of mice via standardized calculation of two navigation indices. We performed mouse-per-mouse analyses and compared each mouse's performance to a threshold based on young mice's performances. Using this method we identified impaired mice from the age of 10 months old. Their deficits were independent of any sensorimotor dysfunctions and were associated with an alteration of the maintenance of the hippocampal CA1 late-LTP. This study develops reliable methodology for early detection of age-related memory disorders and provides evidence that memory can decline in some individuals as early as from the age of 10 months.

Keywords: Early detection; Middle-age; Spatial memory; CA1 LTP; Navigation; Starmaze; Cognitive deficit; Strategy

1. Introduction

In humans, improved longevity has increased the importance of understanding the heterogeneity of age-related decline in cognitive abilities. The development of efficient therapeutic interventions relies on the identification of the early key events that trigger the onset of cognitive decline. Our aim was to develop and characterize a behavioral model (Gzil et al., 2009) allowing early, reliable and specific detection of age-related cognitive impairments and associated physiological alterations at the individual level.

In aged rats, the characterization of the cognitive status (impaired or unimpaired) prior to research of neurobiological markers of cognitive aging has proven to be useful for studying the early events triggering these deficits (Baxter and Gallagher, 1996; Gallagher et al., 2003; Tanila et al., 1997) but has not been applied to middle-aged population. Even in a given mouse strain, namely C57Bl/6, no clear consensus has been reached concerning the age of onset of memory alterations, certainly due to differences in detection methods. Studying reference memory of 10–15-month-old mice in the Morris Water Maze, authors concluded to the presence (Magnusson, 1997; Verbitsky et al., 2004) or the absence (Calhoun et al., 1998; Das and Magnusson, 2008; Magnusson, 1998, 2001; Magnusson et al., 2003) of deficits in middle-aged mice. Given the increased inter-individual variability in aged populations (Baxter and Gallagher, 1996; Gage et al., 1984; Gower and Lamberty, 1993), individual screening should shed light on alterations which otherwise would remain undetectable.

We attempt to establish a methodology and analysis regimen that is sensitive to age-related cognitive declines in individual as young as 10 months (middle-aged mice). To avoid any potential bias because of decrease in sensorimo-

* Corresponding author at: ENMVI team, UMR CNRS 7102, UPMC, Boite postale 01, 9 quai Saint Bernard, 75005 Paris, France.
Tel.: +33 144272044; fax: +33 144273252.
E-mail address: laure.rondi@snv.jussieu.fr (L. Rondi-Reig).
[1] Shared first authorship.
[2] Present address: Neuronal Survival Unit, Section for Neuroscience, Department of Experimental Medical Science, Wallenberg Neuroscience Center, 221 84 Lund, Sweden.

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tor skills on learning, we used a series of careful behavioral tests assessing general sensorimotor skills, spatial memory and visuo-motor abilities (Fig. 1A). A behavioral model of age-related memory decline was established with the starmaze task, an aquatic navigation maze which can be learnt using different navigation strategies, i.e. allocentric, sequential egocentric or serial strategies (Rondi-Reig et al., 2006). The starmaze is composed of 10 alleys, 5 forming a central pentagon and 5 alleys radiating from the angles of the central pentagon. The mice not only have to learn to locate a hidden platform but also have to use the shortest path (indicated with an arrow in Fig. 1B). To learn this optimal path, the animal can use different strategies that could be dissociated and identified depending on their trajectories during the probe test performed at the end of the training (see supplementary Fig. IA). A further advantage of this paradigm is that, thanks to the presence of alleys, it allows accurate and standardized analysis of search pattern by means of two complementary navigation scores: the direct path and the localization scores. Both together, these scores precisely assess the individual ability of each mouse to acquire the memory of a spatial trajectory.

We submitted young (3 months), middle-aged (10 months) and aged (17 months) C57Bl/6 mice to the starmaze test. Given that in previous studies (Frick et al., 2003; Rick et al., 1996), massed protocol (characterized by a high number of trials in a short period of time) revealed deficits that remained hidden in a spaced protocol (corresponding to a low number of training trials distributed over a long time period), we tested both types of protocols. Mice were also submitted to a series of control tasks, to check whether deficits in solving the starmaze could be due to non-mnesic impairments. To see if such a behavioral characterization could reveal plasticity alterations which otherwise would have been masked, we assessed late-LTP in the hippocampal CA1 area. Indeed, while making age-by-age comparisons, alterations of the maintenance of the late-LTP were found to be correlated to spatial memory deficits in 18-month-old mice (Bach et al., 1999), but not in younger animals (Bach et al., 1999; Ris and Godaux, 2007).

We identified ‘impaired mice’ among middle-aged and aged groups. Their deficits emerged independently of any sensory-motor abnormality but were associated with an alteration of late-LTP maintenance that was detectable even in 10-month-old mice in our case-by-case analysis.

2. Materials and methods

2.1. Experimental subjects

We used male C57Bl/6J mice. They were housed in groups of 5–8 mice in standard conditions: 12 h light/dark cycle, with water and food *ad libitum*. 7 days prior the beginning of sensory-motor tests, mice were separated and housed individually until the end of the starmaze or electrophysiological experiments, to limit the inter-boxes variability due to social relationships during the behavioral experiments. All behavioral experiments took place during the light cycle of mice. In the spaced protocol, all mice were tested between 9 AM and 1 PM. In the massed protocol, mice were divided in two groups: one tested between 9 AM and 1 PM and another tested between 2 PM and 6 PM. We found no effect of the testing moment on behavioral performances in these experiments [ANOVA $p > 0.05$ for all parameters studied in the starmaze].

For the behavioral study, 80 young (3 months old), 87 middle-aged (10 months old) and 41 aged (17 months old) male C57Bl/6J mice were used. The electrophysiological study has been performed 5 weeks after the end of the starmaze task, on mice from the behavioral study (young ($n = 9$), middle-aged ($n = 14$) and aged ($n = 9$)). A longevity study was performed on 80 other male C57Bl/6J mice bred in the same conditions as mice used for the behavioral study.

2.2. Behavioral studies

All behavioral studies were performed blind to the age of mice. All animals were tested according to the S.H.I.R.P.A. protocol (Crawley, 2000, see also Rondi-Reig et al., 1997; Rondi-Reig and Mariani, 2002) (see Supplementary methods). Data acquisition in the starmaze and in the Cued-MWM was performed by means of a video recording system and tracking software, SMART (BIOSEB, Chaville, France). Data processing (e.g. the computation of the assessed parameters) was automated via a MATLAB batch program developed in our laboratory (Navigation Analysis Tool, NAT).

2.2.1. Spatial navigation task: the starmaze

2.2.1.1. Apparatus. We used a water navigation task: the starmaze (Fig. 1B) (Rondi-Reig et al., 2006). The starmaze consists of five alleys forming a central pentagonal ring and five alleys radiating from the vertices of this pentagonal ring. All of the alleys are filled with water made opaque with an inert nontoxic product (Accuscan OP 301, Brenntage, Lyon, France). The maze is surrounded by a square black curtain with 2D and 3D patterns affixed to provide configurations of spatial cues. To avoid the possible use of a guidance strategy (i.e. relying on the use of a single distal cue), each cue was in duplicate and each copy was grouped with different cues. White noise was used to cover all other sounds that the mice could have used to orientate themselves.

2.2.1.2. Behavioral protocol. To solve the task, animals had to swim to a platform hidden 1 cm below the water surface and located 10 cm from the end of one alley (dotted circle, Fig. 1B). Departure and arrival points were fixed. Separate groups of mice were trained in the starmaze according to two different protocols (Fig. 1A). In the spaced training protocol, they were given 2 trials per day over 25 consecutive days with inter-trial intervals lasting at least 20 min. In the massed training protocol all animals ran 2 sessions per day over 5