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Altered behavioral aspects of aged mice lacking the cellular prion protein

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HIGHLIGHTS

- PrP-KO and control mice were tested at various ages with different behavioral tests.
- No sign of sensory, motor or cognitive impairment was detected in young animals.
- Aged PrP-KO mice reacted to new stressful environments differently than controls.
- Contrary to previous data, PrP-KO mice had normal olfactory sensitivity at all ages.
- Hence, altered performance in behavioral tests may be related to altered reactivity.

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ABSTRACT

The biological function of the prion protein, which is intimately involved in the onset of prion diseases, remains unclear. To understand whether the prion protein could play a role in animal behavior, a battery of tests was applied to young and aged mice that express, or not, the prion protein. In contrast to the similar results obtained in all young animals, we found that aged mice lacking the prion protein reacted to new and stressful environments differently than their wild-type counterparts. This may suggest that, upon aging, the absence of the prion protein results in altered neural processing at the basis of adaptation to new situations.

1. Introduction

It is now widely accepted that an aberrant conformation of the prion protein (PrP^C), a constitutive glycoprotein anchored to the external surface of mammalian cells, originates the prion, the novel infectious particle causing fatal neurodegenerative disorders called transmissible spongiform encephalopathies, or prion diseases [1]. The physiological role of PrP^C is, however, not yet clearly established, in spite of the multiple functions that have been attributed to the protein, among which the involvement in synaptic plasticity, learning, memory and neuroprotection [2–4].

Mice carrying the deletion, also post-natal, of the PrP gene (PrP knockout, PrP-KO) do not show obvious alterations in lifespan and development [5–7]. However, the necessity to understand the cellular role of PrP^C has led to a close inspection of PrP^C involvement in the behavioral domains governing memory, learning, motor coordination and balance [8–11]. Comparative studies on the learning and memory of wild-type (WT) and PrP-KO mice of 3–5 months of age provided conflicting results. While Brentani and co-workers reported that WT and PrP-KO mice had similar short- and long-term fear-motivated memory, anxiety, and exploratory drive [12], others have shown that the absence of PrP^C could generate clear deficits in hippocampal-dependent spatial learning [8]. Alterations were also found in the motor behavior of PrP-KO mice, which displayed impaired locomotor activity following a short, or a forced, swimming trial, or after an acute stress provoked by a foot shock [9,10]. It was also observed that PrP-KO mice had altered anxious

Abbreviations: PrP^C, cellular prion protein; PrP-KO, PrP knockout; WT, wild-type.

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responses to stress of different intensities [9,13], and a depressive-like behavior that was evidenced by increased immobility times during the forced swimming and in the tail suspension test [14]. However, it has also been observed that age could play an important role in modifying the behavior of PrP-KO mice, both in the exploratory drive, in short- and long-term memory [15] and in the motor coordination and balance at the Rotarod test [11].

We report here a detailed study that re-examined the possible influence of PrP^C on mouse behavior. To this end, we subjected both young and aged congenic WT, PrP-KO mice, and transgenic (Tg46) mice (in which PrP^C expression was rescued over a PrP-KO background) to a battery of behavioral tests that included general locomotor activity, learning and memory, social interaction, emotional reactivity in a hostile environment and olfactory-guided behavior. Our results indicate that, contrary to young mice, old PrP-KO mice showed a probable deficit in approaching new situations.

2. Materials and methods

2.1. Animals

Three types of congenic mice were used: WT mice (FVB strain, Harlan, Milan, Italy), PrP-KO mice carrying a FVB genetic background (F10), and Tg46 mice, which were obtained by re-introducing the PrP gene into the F10 background, and which express PrP^C at amounts similar to the natural levels [7]. F10 and Tg46 mice were kindly provided by the MRC Prion Unit, London, UK. All animals were kept in plastic cages at constant temperature (24 °C) and controlled light cycle (light on from 06:00 a.m. to 06:00 p.m.), and, unless otherwise specified, had unrestricted access to water and food, consisting of adult mouse balanced diet pellets (Mucedola, Milano, Italy). Before being tested, each animal was weighted, and analyzed for neurologic symptoms by examining deambulation, posture, righting from the side, placing reaction of hind limbs, geotaxic reaction, avoiding of borders and equilibrium. No anomaly was detected, indicating that all animals lacked major deficits in circuits involved in the different reflexes scheduled for examination.

8 WT and 8 PrP-KO mice were tested at 3 months of age, while 10 mice of each (WT, PrP-KO and Tg46) strain were tested at 15 months of age. 9 animals from each of the latter three groups were also subjected to the cookie-finding test at 18 months of age.

Behavioral tests started at 09:00 a.m. of 5 consecutive days in a testing room separated from the animal house. Each day, animals (kept in their home cage) were transferred to the testing room and left alone for 30 minutes (min) to habituate to the new environment before initiating the tests. These were scheduled as follows: day 1, forced swimming test; day 2, cookie-finding test; day 3, intruder test; day 4, predation test; day 5, open field test. Before performing these tests, in days 1–3 or 1–4 mice were subjected to the Pole test.

All reported experiments were authorized by the local competent authority and conducted under the Italian and European laws on animal experimentation and welfare (D. Lgs. 116/92; 86/609/EEC).

2.2. Pole test

The Pole test evaluates the motor performance of the mouse, and is particularly suited to evaluate bradykinesia [16,17]. The mouse is placed head up on a metallic pole (50 × 1.5 cm) covered with gauze to facilitate grasping. The time necessary to the animal to turn down and reach the floor is then measured, in a 120 second-session. By repeating the test in 3 (with 3 month-old mice), or 4 (with 15 month-old mice) consecutive days, this test is also useful to evaluate the contextual and procedural learning, since a normal mouse is expected to improve the performance from day 1 (in the presence of an unknown testing situation) onwards.

2.3. Forced swimming

This test evaluates the motor performance and emotional reactivity of a mouse placed in a new, hostile environment. Mice, placed at the center of a plastic pool (55 × 35 × 30 cm) filled with 20 cm-deep water (25 °C), are in a restricted space from which they cannot escape, and which induces them to either swim or acquire a characteristic behavior of immobility. A mouse is judged to be immobile when it ceases struggling and remains motionless in the water, making only those movements necessary to keep the head above water. The pool floor is subdivided in 15 squares, and the number of squares that mice cross in a 180 s-session is counted. The swimming time before the mouse reaches the first immobility stage (latency to the first stop) is also measured.

2.4. Cookie-finding test

The cookie-finding test is meant to evaluate olfactory functions [18,19]. Mice are deprived of food during the night between day 1 and 2 of tests. In the morning of day 2, they are placed inside a cage (42 × 26 × 13 cm) containing clean sawdust, in a position opposite to one sawdust-buried food pellet, and the time necessary to the mouse to discover the food pellet (1.5 × 3 cm) is recorded in a 300 s-session. Mice are then returned to their home cage without allowing them to eat the food thus avoiding any reinforcement effect. During the same day, the test is repeated 3 times with at least 1 hour interval, in which the first two times the pellet is buried (invisible), while the third time it is visible (i.e., placed over the sawdust). The position of the pellet is changed between tests. When the test was repeated with 18 month-old mice, we used the same animals that had been tested at 15 months of age. It is important to note that, before the first execution of this test, mice never had the chance to hide the pellet under the sawdust of their housing cage, being the pellet too large (1.5 × 3 cm) to pass through the wire mesh ceiling.

2.5. Intruder test and predatory aggression test

The intruder test evaluates the intra-species competitive aggressiveness towards unrelated mice of the same strain, sex and age. After placing the intruder mouse in the home-cage of the mouse under study, the test consists in recording the latency time to the first aggressive attack, in a 30 min-session. In the predatory aggression test, the predatory aggressiveness towards an animal of another species is assayed. The mouse is placed in a plastic cage (25 × 15 × 13 cm) in which an earthworm (*Lumbricus terrestris*) is released after 10 min. The latency time to the first attack is then recorded in a 20 min-session [19].

2.6. Open field test

This test evaluates the animal's exploration attitude and locomotion in a new environment, under soft light, as already described [19]. The setting is less stressful than the forced-swimming pool, given that in the open field arena the mouse can choose to remain motionless. The mouse is introduced in a plastic cage (55 × 33 × 20 cm) for 10 min, the traveled distance, resting time, and rearings on the walls are recorded by a video camera and then quantified using a suited software (Smart 2.5, 2B Biological Instruments, Varese, Italy). Also the number of fecal boli and urine drops is recorded, as an index of autonomic activation [19].

2.7. Statistical analysis

Data were analyzed with a between subject analysis of variance (ANOVA) for the factor group (WT, Tg46, PrP-KO), or mixed design analysis of variance (group × day), followed by Newman–Keuls post-hoc test using Statistica software (version 5 '97; www.statsoft.com).