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Bizarre behaviors limit exploratory activity and impair spontaneous gait performance in aged mice with Alzheimer's disease Lidia Castillo-Mariqueo1,2 and Lydia Giménez-Llort1,2*

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Abstract: The shorter life spans of mice provide an exceptional experimental gerontology scenario. We previously described increased bizarre (disruptive) behaviors in the 6-month-old 3xTg-AD mice model for Alzheimer's disease (AD), compared to C57BL/6J wildtype, when confronting new environments. In the present work, we evaluated spontaneous gait and exploratory activity at old age, using 16-month-old mice. Male sex was chosen since sex-dependent psychomotor effects of aging are stronger in C57BL/6J males than females and, at this age, male 3xTg-AD mice are close to an end-of-life status due to increased mortality rates. Mice's behavior was evaluated in a transparent test box during the neophobia response. Stretching, jumping, backward movements and bizarre circling were identified during the gait and exploratory activity. The results corroborate that in the face of novelty and recognition of places, old 3xTg-AD mice exhibit increased bizarre behaviors than mice with normal aging. Furthermore, bizarre circling and backward movements delayed the elicitation of locomotion and exploration, in an already frail scenario, as shown by highly prevalent kyphosis in both groups. Thus, the translational study of co-occurrence of psychomotor impairments and anxiety-like behaviors can be helpful for understanding and managing the progressive functional deterioration shown in older people, especially those with AD.

Keywords: Alzheimer's disease; bizarre; exploratory activity; gait; 3xTg-AD mice; kyphosis; circling

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive loss of cognitive, language, and behavioral functions [1]. In addition, dementia can have multifaceted clinical presentations [2]. Thus, a wide range of behavioral and psychological symptoms of dementia (BPSD) can manifest, reaching more than 90% in most patients [3]. Therefore, BPSD requires great efforts for caregivers and society in general [4].

The gap between the clinical characteristics of this disease and its elicitation in animal models entails great efforts by researchers to achieve a replicable approach. However, they have begun to be addressed in some of these models in the last decade [5]. In this way, we described early symptoms similar to BPSD in the 3xTg-AD mouse model for the first time at 2.5 months of age [5], and bizarre behaviors at 6 months of age [6]. Subsequently, we described bizarre behaviors in swimming performance in the Morris water maze [7]. Thus, at 13 months of age, it was possible to identify non-seeking, floating, and circling behaviors among genotypes more precisely, the latter group being the character-

istic behavior of 3xTg-AD animals [7]. These findings were later confirmed in a study conducted on 6-month-old male animals [8]. Furthermore, these behaviors are sensitive to environmental factors. Thus, we recently reported that naturally isolated 13-month-old 3xTg-AD male animals, as a social isolation model, exhibit bizarre behaviors that interfere with exploratory activity and locomotion in gait, with stretching and circling behaviors being the most sensitive behaviors exhibited by isolated animals [9].

The present work aimed to identify distinctive patterns of bizarre behaviors related to deficiencies and functional limitations of spontaneous gait and exploratory activity in 16-month-old male 3xTg-AD mice in an advanced AD stage compared to non-transgenic (NTg) mice with normal aging.

2. Materials and Methods

2.1. Animals

A total of twenty-one homozygous 3xTg-AD (n = 11) and non-transgenic (NTg, n = 10) male mice of 16 months of age in a C57BL/6J background (after embryo transfer and backcrossing of at least ten generations) established at the Universitat Autònoma de Barcelona were used in this study. The 3xTg-AD mice harboring transgenes were genetically modified at the University of California at Irvine, as previously described [10]. Animals were kept in groups of 3-4 mice per cage (Macrolon, 35 × 35 × 25 cm3) filled with 5 cm of clean wood cuttings (Ecopure, Chips6, Date Sand, UK; uniform cross-sectional wood granules with 2.8-1.0 mm chip size) and nesting materials (Kleenex, Art: 08834060, 21 cm x 20 cm, White). All animals were kept under standard laboratory conditions of food and water ad lib, 20 ± 2◦C, 12 h light cycle: dark with lights on at 8:00 a.m. and 50-60% relative humidity. The study complies with the ARRIVE guidelines developed by the NC3Rs and aims to reduce the number of animals used [11].

2.2. Behavioral assessment

Behavioral evaluations were carried out in a single day and balanced by observing two independent observers blind to the genotype. During the morning, the tests were carried out; 30 minutes were allowed to habituate the animals in the test room before starting the measurements. The evaluation protocol, bizarre behaviors registered, and physical phenotype of frailty used here are recently reported in Castillo-Mariqueo and Gimenez-Llort's 2021 study [9]. In addition, videos of gait were taken for posterior analysis with KINOVEA 0.8.15 free software.

2.3. Statistics

Statistical analyses were performed using SPSS 23.0 software. Results were expressed as the mean ± standard error of the mean (SEM) for each task and trial or incidence in percentage. The factors were analyzed using the Student's t-test or U-Mann Whitney test and Chi-square or Fisher's exact test. In all cases, $p<0.05$ was considered statistically significant.

3. Results

The animals presented a similar body weight in both groups, reaching 27.8 $\pm$ 1.0 g in the NTg animals and 27.1 $\pm$ 1.1 g in the 3xTg-AD mice. The variable of interest of the physical frailty phenotype, kyphosis, was present in 91% (10/11) of the transgenic animals and 80% (8/10) in the NTg group.

In the exploratory activity, genotype-dependent differences were found where 3xTg-AD animals take longer to start locomotion [Mann-Whitney U p = 0.016]. In the same way, horizontal and vertical activity is decreased compared to the non-transgenic group, which is significant in horizontal activity [Mann-Whitney U p = 0.008].

On the other hand, the incidence of bizarre behaviors exhibited in the 3xTg-AD group reached 82% (9/10), and those in the NTg group 40% (4/10) with a high incidence in circling that reached 36% (4/11) followed by backward movement with 18% (2/11) in transgenic animals. In the case of NTg, the behavior with the highest incidence was stretching at 20% (2/10).

Similar to what occurs in exploratory activity, a decrease in stride length was observed during gait with a low speed of the steps in 3xTg-AD mice [stride length: Mann-Whitney U $p = 0.024$; speed: Student's *t*-test $p = 0.041$].

4. Discussion and conclusions

In this research, bizarre behaviors and the functional limitations of the exploratory activity and gait performance of male 3xTg-AD and NTg mice at the age of 16 were studied. Although these behaviors have been previously reported in the open field, gait tests, and the Morris water maze [6–9], this is the first time confirmed in 16-month-old animals. In this way, the results corroborate that in the face of a novelty situation and the recognition of place, old 3xTg-AD mice exhibit bizarre behaviors, and most importantly, they interfere with their locomotion and spontaneous exploration, in an aging scenario that already includes kyphosis, an indicator of frailty with high incidence in both groups.

The manifestation of bizarre behaviors in anxiety tests suggests that these behaviors could be related to coping with stress [6]. The bizarre behavior patterns in 3xTg-AD mice from 6 to 13 months of age differ from NTg animals and correlate with other anxiety behaviors, locomotion, and emotionality [6,7]. However, bizarre behaviors can be very varied and heterogeneous, which is a limitation to detect genotype differences. Also, most of them depend on sex, with females being the ones that exhibited these behaviors to a greater extent [6]. Therefore, it is relevant that in the present study using the male sex, we detected circling and backward movements as the behaviors with the highest incidence in old transgenic animals mimicking very advanced stages of the disease.

On the other hand, previous gait studies of 13-month-old male 3xTg-AD mice indicate deficits that coincide with an aging pattern, accompanied by a series of bizarre behaviors that can interfere with trajectory and movement [9]. A high period of freezing at the beginning of the test accompanied by a high latency in the exploratory activity that interferes in the horizontal and vertical activity is consistent with our findings at 16 months, making us note that once these behavioral patterns appear, they persist as the disease progresses.

The study of co-occurrence of psychomotor impairments and anxiety-like behaviors can be helpful for understanding and managing the progressive functional deterioration related to aging and the nuances in AD-scenario, with a translational value for older people, especially those with AD.

Supplementary Materials: Not aplicable.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Departament de Medi Ambient i Habitatge, Generalitat de Catalunya (CEEAH 3588/DMAH 9452) the 8th of March 2019

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Informed Consent Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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