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Intrinsic (genotype, sex) and extrinsic (environment) factors in the bizarre patterns elicited in the open-field test: Effect of forced isolation in old male and female mice with normal and AD-pathological aging

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INTRODUCTION

- Bizarre behaviors (BB) are elicited in animals during stressful conditions and are behavioral markers of disease/sickness relevant to animal welfare [1,2]. However, these behaviors are disregarded in most experimental screenings due to their extremely low incidence and short duration, despite their translational value mimicking disruptive behaviors associated with neurological / psychiatric diseases [3].
- Despite of it, we previously reported experimental conditions with conspicuous BB incidence in both gold-standard C57Bl/6 and 3xTg-AD mice for Alzheimer's disease. Thus, we could identify four basic categories, namely stereotyped rearing (BB-SR) stereotyped stretching (BB-SS), jumping (BB-J) and backward movements (BB-BM), and also a fifth BB type, the circling behavior (BB-CB). When they were quantified, they were found to be higher 1) in the 3xTg-AD mice for Alzheimer's disease than their wild-type C57BL/6 counterparts; 2) in the open-field test (OF) *vs.* other tests; 3) conspicuous at + 6 months of age; 4) more in female than male sex [4].
- Further studies from our lab have also shown that: 5) BB can be recorded at older ages (+13 months) and 6) in the Morris water maze where male 3xTg-AD mice also exhibit more circling behavior (BB-CB) than control mice [5];
- We have also shown that BB can be modified by intrinsic factors as shown in their enhancement at end-of-life [6] . With regards to extrinsic factors, BB were found 5) reduced by early postnatal handling [4], and 6) enhanced by d-galactose-induced accelerated aging [7,8] and psychostimulants [9].

AIMS

In the present work, we further studied the BB patterns elicited in the OF using a longitudinal design with sixty-six male/female mice with normal/AD-pathological aging under social/isolated housing conditions, aimed to explore the effects of intrinsic (genotype, sex) factors and isolation as extrinsic (environment) factor.

METHODS

For this purpose, males and females with normal (NTg, non transgenic gold standard C57BL/6 strain) and AD-pathological aging (3xTg-AD mice model for AD [6]) mice from the Spanish colonies established at the Universitat Autònoma de Barcelona thanks to MTA with Prof. LaFerla (UCI, California Irvine, USA) were studied.

The longitudinal design was set at middle age (12 months of age) that in the 3xTg-AD mice corresponds to advanced stages of disease [7].

Animals were behavioraly assessed for 5 minutes during the morning (9 a.m. to 1 p.m., in a 12h:12h light: dark cycle starting at 8 a.m.) in the classical open field test at 12 and 13 months of age. The apparatus consisted in a 45 x 45 x 25 cm beige metallic open arena. Presence of bizarre behaviors was recorded by direct observation and 'Videotrack Viewpoint behavior technology' system was also used for further verification and trajectory analysis.

Intrinsic factors were defined as genotype (NTg/3xTg-AD), sex (Male/Female), time of test (test/retest, one month in between) with sample size n=7-8/group.

The extrinsic factor was defined as the social condition as animals were in groups (3-4 animals of the same genotype and sex per cage) or isolated (1 single animal per cage).

The protocol CEEAH 3588/DMAH 9452 was approved the 8th of March 2019 by Departament de Medi Ambient i Habitatge, Generalitat de Catalunya.

Statistical analysis: SPSS 15.0 was used. Results are expressed as mean ± SEM, or incidence. ANOVA (parametric variables) and Chi-square or Fisher's Exact test (incidences) were used. Statistical significance was considered at p<0.05.

RESULTS

1. TIME Leaving the center

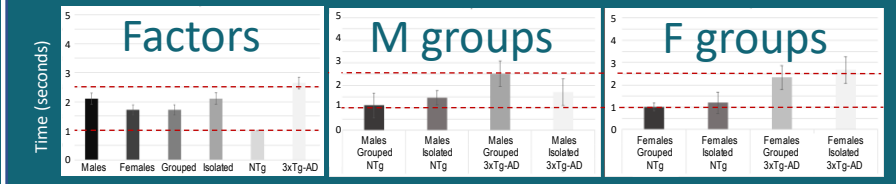

Figure 1. Latency to leave the center N= 14-20 MEAN ± SD, G* $p < 0.05$, S* $p < 0.05$.

1. Incidence

Latency to leave the center of the OF (Figure 1) showed G, genotype and S, Sex effects ($p < 0.05$). The delay was mostly due to presence of freezing (NM: no movement) and the elicitation of Bizarre behaviors (BB: sterotyped rearing, stereotyped stretching, jumping, backward movements and circling behavior) that were recorded in 39% of animals included in this study. However, as the analysis showed, the BB pattern distribution was dependent on intrinsic and extrinsic factors. The direction of the animal's trajectory when leaving the center (Figure 2) was also indicative of forward movement to explore the open field arena (LI) or not (CI).

2. Intrinsic factors

Sex, re-test at an older age, and genotype factors differentiated two BB patterns: higher circling behavior (BB-CB, n=12) in wild-type and males, whereas higher backward movements (BB-BM, n=14) in 3xTg-AD, females, and older age.

3. Extrinsic factors

Isolation increased the incidence of freezing, mostly in 3xTg-AD mice, and exerted a modulatory role in BB, but interaction effects with other factors led to residual significance. (Figure 1, G, genotype and S, sex factors, both $p < 0.05$).

2. DIRECTION Leaving the center

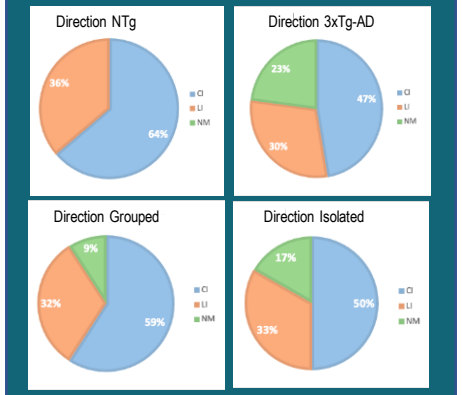

Figure 2. Incidence (%) N= 14-20 G* $p < 0.05$, S* $p < 0.05$.

3. BIZARRE Circling behavior

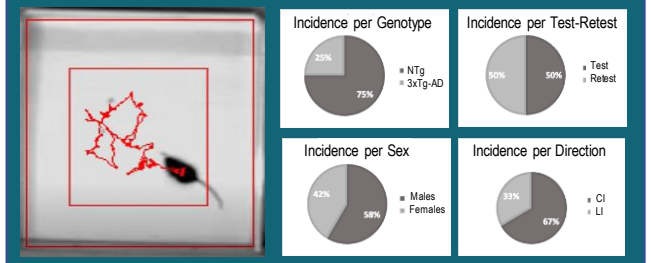

Figure 3. N= 14-20 MEAN ± SD, G*** p< 0.005, S** p<0.01.

4. BIZARRE Backward movement

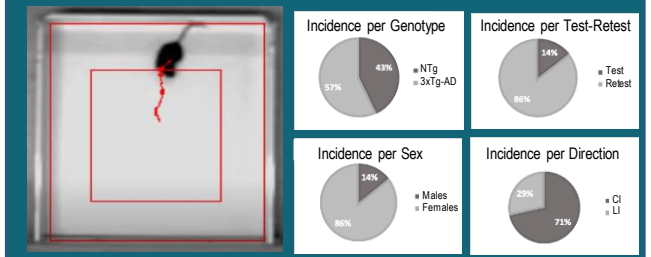

Figure 4. N= 14-20 MEAN ± SD, G* $p < 0.05$, S** $p < 0.01$.

CONCLUSIONS

The results point to BB-BM backward movement as the most pervasive BB pattern in this animal model, the 3xTg-AD mice, at advanced stages of disease. This BB was also found to be sensitive (worsening) to the progress of aging/disease (as measured by the test-retest at 12 and 13 months of age).

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