

MICRO REPORT

Interaction between social behavior and paternal age in offspring of the same paternal mice

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Abstract

Aim: Previous studies reported that advanced paternal age (APA) may increase the risk of autism spectrum disorder (ASD) in offspring. However, effects of APA on behaviors have not been investigated in offspring of the same paternal mice. The present study sought to identify behavioral differences in mouse offspring of the same fathers at different paternal ages.

Methods: We assessed locomotor activity, anxiety-like behavior, and social behavior in male mouse offspring that were born from the same fathers at three different paternal ages (3, 12, and 15 months old).

Results: No differences in locomotor activity or anxiety-like behavior were observed among any of the offspring groups. In the three-chamber test, although the control group (3-month-old paternal age) exhibited significantly higher approach behavior toward the novel mouse compared with the novel object, the APA groups (12- and 15-month-old paternal ages) did not exhibit significant approach toward the novel mouse.

Conclusion: Offspring of 3-month-old fathers but not 12- or 15-month-old APA fathers exhibited social preference behavior. Although the present study was only exploratory, it demonstrated an interaction between social behavior and paternal age in offspring of the same paternal mice.

KEYWORDS

aberrant social behavior, advanced paternal age, anxiety-like behavior, autism spectrum disorder

1 | INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by social deficits and repetitive behavior. Advanced paternal age (APA) has been suggested to be a risk factor for ASD.¹⁻³ Paternal age >35 years was shown to be associated with

a higher risk of ASD.⁴ Furthermore, the risk of ASD in offspring of >40-year-old fathers was reported to be 5.75 times higher compared with <30-year-old fathers.⁵ The offspring of APA mice exhibited decreases in social behavior,⁶⁻⁹ abnormal voice communication,¹⁰ and alterations of DNA methylation^{10,11} and transcriptomic profiles,¹² although one study showed that APA did not affect social behavior

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in offspring.¹³ The present study further characterized behavioral phenotypes of offspring of APA mice as a mouse model of ASD by comparing three different groups from the same paternal mice.

2 | METHODS

2.1 | Animals

Male and female C57BL/6J (B6) mice were obtained from CLEA Japan (Tokyo, Japan). The same adult male B6 mice ($n = 3$) were mated at 3, 12, and 15 months of age with 2-month-old female B6 mice (Figure S1). One female mouse was mated per male mouse at 3 and 12 months of age, and two female mice were mated per male mouse at 15 months of age. From these matings, male offspring (3-month APA [3 M], 12-month APA [12 M], and 15-month APA [15 M] groups) were examined between 12 and 16 weeks of age. We used 3-month APA (3 M) mice as the control group. The animals were housed in groups of 3–6 per cage and maintained on a 12h/12h light/dark cycle (lights on at 8:00 AM and lights off at 8:00 PM) in an environment at $23 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ relative humidity with free access to food and water. The experimental procedures were approved by the Animal Experimentation Ethics Committee of Tokyo Metropolitan Institute of Medical Science (license no. 15021). Animal care was in accordance with institutional animal experimentation guidelines.

We conducted the following behavioral tests sequentially: locomotor test, elevated plus maze, hole-board test, and three-chamber test, with ≥ 1 -week intervals between tests.

2.2 | Behavioral tests

2.2.1 | Locomotor activity test

The apparatus consisted of a chamber (300 mm \times 400 mm, with 250-mm high walls) that was illuminated by a fluorescent light (300 lux). The test mouse was placed in the center of the apparatus and allowed to move freely. Total locomotor activity was measured for 10 min using the Supermex system (Muromachi Kikai, Tokyo, Japan) with a sensor monitor that was mounted above the chamber.

2.2.2 | Elevated plus maze test

The elevated plus maze apparatus consisted of two open arms (297 mm \times 54 mm) and two closed arms (300 mm \times 60 mm, with 150-mm high walls) that were set in a plus configuration. The apparatus was elevated 400 mm above the floor. The test mouse was placed in the center of the apparatus and allowed to freely explore it for 10 min. The time spent on the open arms was recorded by a video tracking system (Muromachi Kikai).

2.2.3 | Hole-board test

The hole-board apparatus consisted of an open field (500 mm \times 500 mm, with 400-mm high walls) that was illuminated by a fluorescent light (300 lux). The apparatus had four holes on its floor (30 mm diameter each). The test mouse was allowed to freely explore the apparatus, and the number of head dips into the holes was recorded for 30 min using a video tracking system (Muromachi Kikai).

2.2.4 | Three-chamber test

The three-chamber apparatus consisted of an opaque rectangular Plexiglas box (600 \times 440 mm, with 400-mm high walls) that was divided into three chambers (200 \times 440 mm). The test consisted of two phases: habituation and social approach (stranger mouse vs. inanimate object). During habituation, the test mouse was first placed in the middle chamber and allowed to explore the entire apparatus freely for 10 min. During the social approach phase, a novel C57BL/6J mouse of the same sex and an object (white plastic container) were placed inside two small wire cages that were then placed in the left and right compartments for 10 min. The sides where the object and the mouse were placed were randomly assigned. The time spent exploring the novel mouse and the object was measured using the ANYmaze system (Muromachi Kikai). The social preference index was calculated according to Karvat and Kimchi¹⁴ with modifications: social preference index = (time with stranger mouse)/(time with stranger mouse + time with inanimate object) \times 100–50.

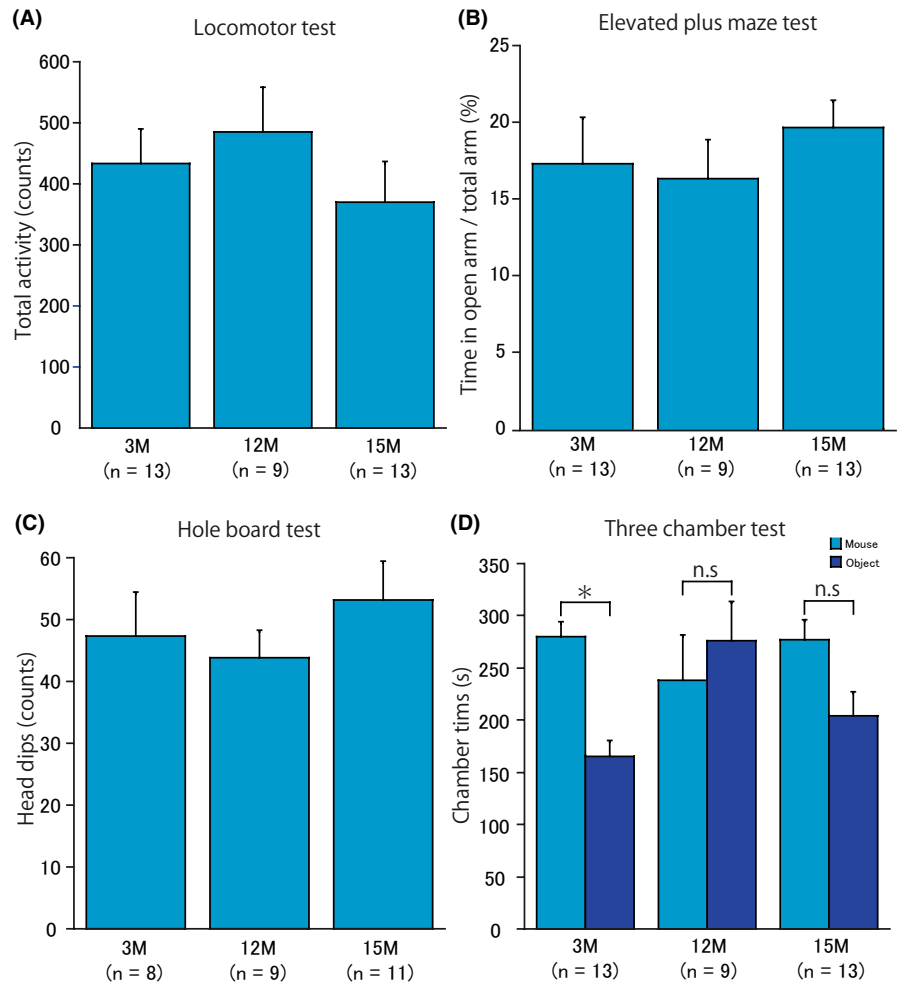
2.3 | Statistical analysis

One-way and two-way analyses of variance (ANOVAs), Bonferroni multiple comparison test, and Student's *t*-test were used for the statistical analyses using SPSS 25.0 software (IBM, Tokyo, Japan). Values of $P < 0.05$ were considered statistically significant. The data are expressed as mean \pm standard error of the mean (SEM).

3 | RESULTS

The APA offspring exhibited no hyperactivity in the locomotor activity test compared with control mice ($F_{2,32} = 0.80$, $P = 0.45$, one-way ANOVA; Figure 1A). We conducted the elevated plus maze test and hole-board test to assess anxiety-like behavior. In the elevated plus maze test, no differences were found in the time spent on the open arms between APA offspring and control mice ($F_{2,32} = 0.50$, $P = 0.61$, one-way ANOVA; Figure 1B). In the hole-board test, no differences were found in the number of head dips between APA offspring and control mice ($F_{2,25} = 0.70$, $P = 0.50$, one-way ANOVA; Figure 1C). These results suggest that APA offspring did not exhibit anxiety-like behavior.

FIGURE 1 Behavioral alterations in APA offspring. (A) Locomotor activity time in the locomotor activity test. (B) Time spent on the open arms in the elevated plus maze test. (C) Number of head dips in the hole-board test. (D) Social interaction time over 10 min in the social approach phase in the three-chamber test. The data are expressed as mean \pm SEM. The numbers in parentheses indicate the number of animals. 3M, 12M, and 15M indicate offspring from the same fathers at 3, 12, and 15 months of paternal age, respectively. * $P < 0.05$, one-way ANOVA followed by Bonferroni post hoc test (a-c) and two-way ANOVA followed by Bonferroni post hoc test (d)



We then evaluated social behavior in the three-chamber test. The time spent in each chamber was not significantly different in any of the APA offspring during the habituation phase (control: $t_{12} = 0.39$, $P = 0.70$; 12M: $t_8 = -1.59$, $P = 0.15$; 15M: $t_{12} = 2.17$, $P = 0.050$; paired t-test; data not shown), indicating that there was no preference bias between chambers. During the social approach phase, however, a significant stimulus (novel object or novel mouse) \times APA interaction was found ($F_{2,64} = 5.29$, $P = 0.0075$; main effect of APA: $F_{2,64} = 1.07$, $P = 0.35$; mouse vs. object: $F_{1,64} = 6.85$, $P = 0.011$, two-way ANOVA; Figure 1D). Bonferroni's multiple-comparison post hoc test showed significantly higher approach behavior toward the novel mouse compared with the novel object in control mice ($P = 0.0012$; Figure 1D), but no such difference was found in APA offspring (12M: $P = 0.87$; 15M: $P = 0.059$; Figure 1D). A significant difference was also found in the social preference index between APA offspring and control mice ($F_{2,32} = 3.67$, $P = 0.037$, one-way ANOVA; data not shown). The social preference index in the 12M group (-4.9 ± 7.1) but not 15M group (8.0 ± 3.9) significantly decreased compared with control mice (12.9 ± 2.6 ; $P = 0.035$, Bonferroni's multiple-comparison post hoc test; data not shown). These results indicated that APA offspring exhibited aberrant social behavior.

4 | DISCUSSION AND CONCLUSION

In the present study, offspring of APA mice did not exhibit social preference behavior. No differences in locomotor activity or anxiety-like behavior were found between APA offspring and control mice, suggesting that aberrant social behavior in APA offspring was not secondary to deficits in locomotor activity or anxiety-like behavior.

To minimize potential confounding factors, we used the same fathers at different ages to obtain offspring for the tests. However, our experimental design resulted in a relatively limited number of fathers. Moreover, the experiments were not conducted blindly, and the timing of the test differed between the offspring groups. Interestingly, the season of the year during which birth occurs has been shown to influence the prevalence of ASD.^{15,16} Although the environment of the animal facility was constant during the present study and although we evaluated the influence of APA during the same season in offspring of 3- and 15-month-old fathers, seasonal issues still could have affected the results. Additional experiments are necessary to test offspring at the same time to confirm reproducibility of the present results.

In summary, when the same paternal mice were used for mating, offspring of 3-month-old fathers but not 12- or 15-month-old APA



fathers exhibited social preference behavior, with no differences in locomotor activity or anxiety-like behavior among groups.

AUTHOR CONTRIBUTIONS

MT and KI conceived and designed the study. MT performed the experiments. MT, AS, HK-M, HK, SI, and YK analyzed the data. MT, SI, and KI wrote the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supplementary Material of this article.

DATA REPOSITORY

The data that support the findings of this study are available in the [Supplementary Material](#) of this article.

APPROVAL OF THE RESEARCH PROTOCOL BY AN INSTITUTIONAL REVIEWER BOARD

n/a

INFORMED CONSENT

n/a

REGISTRY AND REGISTRATION NO OF THE STUDY/TRIAL

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ANIMAL STUDIES

All of the experimental procedures were approved by the Animal Experimentation Ethics Committee of Tokyo Metropolitan Institute of Medical Science (license no. 15021).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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