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SHORT COMMUNICATION

Differences in Anxiety-Related Behavior Between Apolipoprotein E-Deficient C57BL/6 and Wild Type C57BL/6 Mice

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Summary

The influence of ApoE gene deletion on the anxiety state has not been previously investigated. The elevated plus maze was used in this study to determine differences in anxiety-related behavior between apoE-deficient and wild type C57BL/6 mice. The apoE-deficient mice demonstrated less anxiety on the elevated plus maze by spending more time in the open arms of the elevated plus maze compared to wild type mice ($p<0.001$). Additionally, female apoE-deficient mice visited the open arm of the maze more often than their apoE-deficient male counterpart ($p<0.05$). The anxiety state and/or sex are possible variables to be considered when designing physiological and/or behavioral studies involving mice that are apoE-deficient.

Key words

Apolipoprotein E • Anxiety • Elevated plus maze • Mice

Inactivating the endogenous apolipoprotein E (apoE) gene *via* gene targeting in mouse embryonic stem cells produces apoE-deficient mice (Piedrahita *et al.* 1992). The apoE-deficient mice have been extensively used as animal models of Alzheimer's disease and atherosclerosis (Masliah *et al.* 1994, Hofker *et al.* 1998).

Only a few behavioral studies have been carried out on apoE-deficient mice. The findings of these previous studies have predominantly concentrated on working memory and aggression. Working memory deficits for apoE-deficient mice become apparent when assessed within the Morris water maze (Gordon *et al.* 1995). No studies have been conducted on the effect of apoE deletion on the anxiety state. Anxiety might be responsible for the increased aggression and working memory impairments that have been described in apoE-deficient mice.

The aim of this study was to use the elevated plus maze to determine whether differences in anxiety-related behavior (Pellow *et al.* 1985) between apoE-deficient C57BL/6 and wild type C57BL/6 mice exist. A secondary aim was to explore whether any sex differences could be observed within or between the groups tested.

These experiments conform to the international guiding principles for the care and use of laboratory

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animals. The ethics committee of the National University of Singapore has approved the experimental protocol detailed below.

C57BL/6J wild-type and apoE-KO mice were originally obtained from Jackson Laboratory (West Grove, PA) and were bred within a common animal facility at the National University of Singapore. Eighty mice were used in these studies with an age ranging between 12-14 weeks. A cohort of 23 male and 18 female apoE-deficient mice as well as 20 male and 19 female wild type mice were used. Mice were housed in groups of 5 to 6 per cage under ordinary conditions with 12 h light/dark cycle. Animals had free access to food and water.

The elevated plus maze apparatus consisted of four arms, in the standard shape of a + sign. The center of the maze is open and free access to any of the arms is possible. Two opposite arms are surrounded on three sides by high walls (closed arms 40 x 5 x 15 cm), while the other two arms are open (open arms 40 x 5 cm). The maze is made of stainless steel and the arms extend from a central 5 x 5 cm platform and is raised to a height of 53.7 cm above the floor. The behavior of the mice on the maze was recorded by a video camera. The camera was suspended above the maze and the experimenter was not in close contact to the experimental set-up for the duration of the study. Mice at the start of each trial were placed in the central platform of the maze, facing an open arm. Mice to be tested on the elevated plus maze had no prior experience with the maze and were only tested once. Wild type and apoE-deficient mice were tested in random order in the maze during consecutive afternoons until all mice had been tested.

Analysis of mouse behavior on the elevated plus maze: Over a period of 5 min, the total number and duration of entries made in the open and closed arms were recorded. The behavior observed is analyzed offline by a slow motion playback of video recordings. The criteria for an arm visit, be it a closed or an open arm, is considered only when the mouse decisively moves all its four limbs into the arm. Risk assessment behavior with its two paws stretching into an arm is not considered as an arm visit.

Parameters that were analyzed included the cumulative time spent in a closed arms; total number of arm visits; and the ratio between cumulative time spent in the open arm and the cumulative time spent in both closed and open arms (expressed as percentage time spent in the open arm). The maze was cleaned with 70 % ethanol after each mouse had been tested in the maze.

Statistical Analysis: Behavioral data were analyzed using independent sample Student's t-test, and one-way analysis of variance (ANOVA) when 3 or more groups were simultaneously compared. If ANOVA yielded statistical significance, *post hoc* Tukey multiple comparisons were performed. The 5 % level of statistical significance was chosen *a priori*. Values were given as means ± standard deviation. The SPSS statistical software was used for performing these tests.

Open arm time: Disregarding sex, apoE-deficient mice spent a significantly higher percentage time in the open arm (8.72±13.03 %) as compared to wild type mice (4.03±5.87 %) ($P<0.05$). On the basis of sex, female apoE-deficient mice spent a significantly longer time in the open arm as compared to the wild type female mice ($P<0.05$). No statistical differences were found between male apoE-deficient and wild type mice for open arm time. Mean scores across the various measured parameters on the basis of sex are summarized in Table 1.

Table 1. Behavioral analysis of apoE deficient (male, -/-, n=23 and female, -/-, n=18) mice and wild type on the elevated plus maze (male, +/+, n=20 and female, +/+, n=19) mice.

Strain / Sex	Total Visits	CA time	% OA visits	% OA time
Apo E / Male	9.1 ± 4.6	119.6 ± 53.7	6.9 ± 9.5	5.83 ± 8.13
Apo E / Female	12.6 ± 4.2	116.7 ± 52.4	11.6 ± 15.2	12.42 ± 16.98
Wild type / Male	14.6 ± 4.1	170.9 ± 26.9	6.2 ± 6.5	4.80 ± 6.56
Wild type / Female	8.7 ± 2.9	198.3 ± 39.5	6.5 ± 8.6	3.21 ± 5.09

Data are means ± S.D. Total visits = total number of open and close arm visits, CA time= cumulative close arm time (seconds), % OA visits = number of open arm visits / total no. of arm visits,.% OA time= cumulative time in open arm / cumulative time in closed and open arm.

Closed arm time: Wild type mice showed a significantly higher cumulative close arm time (184.30±52.58 s) as compared to apoE-deficient mice (118.32±52.8 s) ($p<0.001$). With regard to sex male wild type mice spent a significantly higher cumulative close arm time compared to female wild type mice ($P<0.05$). With regard to sex, male wild type mice spent a significantly higher cumulative close arm time compared to female wild type mice ($P<0.05$) (Table 1).

Arm visits: On the basis of sex apoE-deficient female mice made a significantly greater number of total arm visits as compared to apoE-deficient male mice ($p<0.05$). The reverse is true for the wild type mice, with wild type males having a significantly higher number of total arm visits as compared to the females ($p<0.001$) (Table 1). Additionally, female apoE-deficient mice made a greater percentage of visits to the open arm compared to all other sex groups ($p<0.001$) (Table 1).

The results in the present study suggest that apoE-deficient mice display lower levels of anxiety as compared to their wild type counterparts, for example, when exposed to an elevated height. The major determinant for this behavioral finding is that in the elevated plus maze test mice display an unconditioned aversion to heights and open spaces that is characteristic of rodents (Dawson and Tricklebank 1995). The behavioral measure in this test is not related to the amount of activity, but to the ratio between activity in the open and closed arms, and represents a good measure of anxiety levels.

We believe that these findings indicate that the absence of the apoE gene has a certain degree of anxiolytic behavior. Indeed, a reduction of hippocampal synaptic density in apoE-deficient mice has previously been reported (Masliah *et al.* 1995). It is postulated that this abnormality also results in an impaired ability for the transmission of anxiogenic impulses. For example, Kjelstrup *et al.* (2002) observed that ventral hippocampal damage in the rat is associated with more time spent in the open arm on the elevated plus maze and fewer secreted feces and lower corticosterone levels when confined to a brightly lit environment. This suggests that the hippocampus is associated with processing of anxiolytic information.

Additionally, in the present studies, it was interesting to observe that female apoE-deficient mice demonstrate higher exploratory activity with respect to the number of open arm visits when compared to their male apoE-deficient counterparts. It could be postulated that under the influence of sex hormones the apoE gene may partly be responsible for regulation of certain neuronal mechanisms related to exploratory behavior of anxiety provoking environments. Further studies are warranted to examine whether sex and the apoE gene influence the structure and function of the hippocampus.

In conclusion, our studies show that there is statistical variability in anxiety behavior between wild type and apoE deficient mice. Additionally, sex-related behavioral responses to anxiety provoking stimuli were observed between apoE-deficient male and female mice. Hence, such variability should be taken into consideration of when designing experiments that incorporate different sexes of apoE-deficient mice for physiological and/or behavioral research.

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