Impairment of nesting behavior in APPswe/PS1dE9 mice

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Abstract: Background The studies on nesting behavior in AD transgenic mice are inconsistent. The aim of this study was to systematically observe nesting behavior of APPswe/PS1dE9 double transgenic mice and their littermate wild-type mice. **Methods** We chose three age-groups (2-3months,6-8months,10-12months) of male APPswe/PS1dE9 transgenic (Tg) and littermate wild-type (WT) mice to observe their nest-building behaviors with chip shavings and paper towels. The differences in nesting scores of each group were evaluated afterwards. **Results** We found a main effect of genotype ($F(1,51)=7.003$, $p<0.05$) and age ($F(2,51)=4.238$, $P<0.05$) and interaction between genotype and age ($F(2,51)=7.003$, $p<0.05$) on chip shaving nest construction. There were no significant differences between Tg and WT mice in chip shaving nest construction until 10–12months ($p<0.01$). The results of nest-building with paper towels showed a significant effect of genotype ($F(1,51)=13.15$,$p<0.001$) and a main effect of age ($F(2,51)=3.78$,$P<0.05$), but no interaction between genotype and age ($F(2,51)=2.186$,$p>0.05$).Although at 6-8months,the difference between Tg and WT mice did not reach statistical significance($p>0.05$), the downward trend was obvious. **Conclusion** The APPswe/PS1dE9 double transgenic mice show a genetic and age-dependent impairment of nesting behavior. Nesting material also impacts the nesting score.
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Introduction

Alzheimer's disease (AD) is the major cause of cognitive dysfunction in the elderly, which poses a challenge to Medicine and Sociology. Its pathogenesis is still not entirely clear. The pathological features of AD include β-amyloid (Aβ) deposition of the formation of senile plaques, neurofibrillary tangles and neuronal loss and so on. Aβ neurotoxicity hypothesis has been at the core of the pathogenesis of AD [1] .

Progression of cognitive dysfunction is a prominent clinical manifestations of AD, but it is not the only one. AD patients also show decreased activities of daily living (ADL) as well as behavioral and psychological symptoms of dementia (BPSD), which bring patients and their families a lot of trouble even hurt, poor quality of life, a heavy financial and emotional burden[2] .

Laboratory research of behaviors in AD transgenic mice may provide great help for the study on the mechanism of abnormal behaviors in AD patients and possible treatment. However, in the past, researchers paid more attention to cognitive behaviors of AD transgenic mice, such as learning and memorizing, than to non-cognitive behaviors which have began to attract researchers' attention in recent years. Nowadays, the appearance of nesting behavior and other new experimental methods makes it possible to evaluate AD transgenic mice's behaviors beyond spatial cognition. Nest building is a common behavior in mice, and has been reported to simulate ADL in AD transgenic mice[3] .While results in different studies of nesting behavior in AD transgenic mice are inconsistent, more researches are needed to make it clear. Not a systematic nesting behavior study of APPswe/PS1dE9 double transgenic mice has been reported yet. This study is going to systematically observe the nesting behavior of APPswe/PS1dE9 double transgenic mice.

Materials and methods

Transgenic mice

APPswe /PS1dE9 double transgenic mice were purchased from Jackson Laboratories [B6C3-Tg(APPswe,PSEN1dE9)85Dbo/Mmjax]. They were bred at Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology reproducing to 6-7 generations. All mice are group-housed in standard Plexiglas cages (28x12x16cm) with chip shavings for bedding material provided with free intake of food and water, 22±2℃, humidity 50–60% and a 12h light: dark cycle. To exclude gender impact, we have chosen male APPswe /PS1dE9 transgenic and their littermate wild-type mice in our study. Three age-groups of mice were used: 2-3 months, n=10 Tg, n=11 WT;6-8 months, n=9 Tg, n=8 WT; 10-12 months, n=9 Tg, n=10 WT. Animal care and experimental procedures conform to the guidelines

established by the Institutional Animal Care and Use Committee at Tongji Medical College, Huazhong University of Science and Technology.

Nest-building with chip shavings

First, the group-housed APPswe/PS1dE9 transgenic and their littermate wild-type mice were individually housed in Plexiglas cages, which were contained with 300±10 ml of chip shavings for bedding material for at least 24 hours. Then, 240±10 ml of chip shavings were provided as additional nesting material 2 hours prior to the onset of the dark phase of the lighting cycle. All nest scores were based on Broida and Svare's [4] four-point scale on the following morning: (1) there was no nest, i.e. the shavings were scattered about the cage; (2) saucer-shaped nests, which had coarse shavings in a circular or semicircular area; (3) a nest with raised sides; (4) a fully enclosed nest.

Nest-building with paper towels

One week after chip shaving nest-building test, the chip shavings were replaced with clean ones. Two hours prior to the onset of the dark phase of the lighting cycle, 16 pieces of paper towels (5x5cm) were introduced in the home cage to create conditions for nesting. The nests were scored the following morning along a 4-point system[5]: (1)no biting/tearing with random dispersion of the paper,(2)no biting/tearing of paper with gathering in a corner/side of the cage,(3)moderate biting/tearing on paper with gathering in a corner/side of the cage, and (4) extensive biting/tearing on paper with gathering in a corner/side of the cage .

Statistics

All of the data were expressed as mean±standard error(means±SEM) and analyzed by GraphPad Prism software. Data analysis was performed with ANOVAs for independent groups followed by the Bonferroni post hoc test. P <0.05 was defined as statistically significant.

Results

Nest-building with chip shavings

To assess the influence of age and gene on nesting behavior when supplied familiar material, we first explored nest construction with chip shaving material. Results are shown in Figure 1.We found a main effect of genotype ($F(1,51) = 7.003$, $p < 0.05$) and age($F(2,51)=4.238$,$P<0.05$) and interaction between genotype and age ($F(2,51)=7.003$, $p <0.05$) on chip shaving nest construction . There were significant differences between Tg and WT age-matched mice in chip shaving nest construction at 10–12months($p<0.01$),while at 2–3months($p>0.05$) and 6-8months ($p>0.05$)were not. Overall there was no change across age in WT mice ($F(2,25) =0.084$, $p>0.05$), among Tg mice alone nest construction was significantly influenced by age ($F(2,25) = 5.461$, $p < 0.05$).

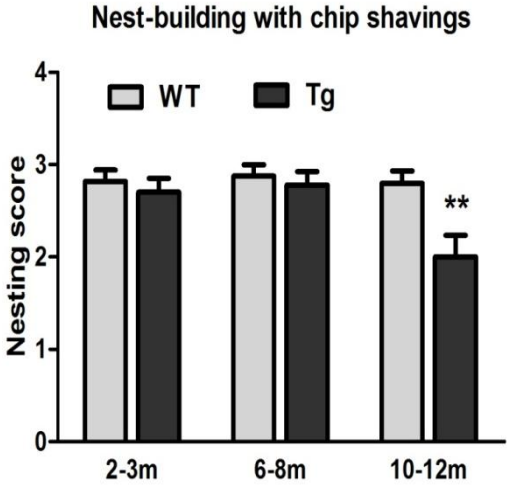

Figure 1. Effects of genotype and age on chip shaving nest construction. Bar graph showing the results from chip shaving nest construction experiments. Tg mice build nests of poorer quality at 10-12months ($p<0.01$).

Nest-building with paper towels

Next, to assess the influence of unfamiliar material on nesting behavior, we tested Nest-building with paper towels. The examples of nests with paper towel were shown in Figure 2A.The results are roughly the same as above and are shown in Figure 2B.We found a significant effect of genotype ($F(1,51) = 13.15$, $p < 0.001$) and a main effect of age ($F(2,51)=3.78$,$P<0.05$) but no interaction between genotype and age ($F(2,51) = 2.186$, $p >0.05$) on nest construction. Although at 6-8months ($p>0.05$), the difference between Tg and WT mice did not reach statistical significance, the downward trend was obvious. Till 10–12months, there were significant difference($p<0.01$). ANOVA showed a main ageeffect in Tg mice ($F(2,25)=4.719$, $p<0.05$) but not in WT mice ($F(2,26)=0.3453$, $p>0.05$).

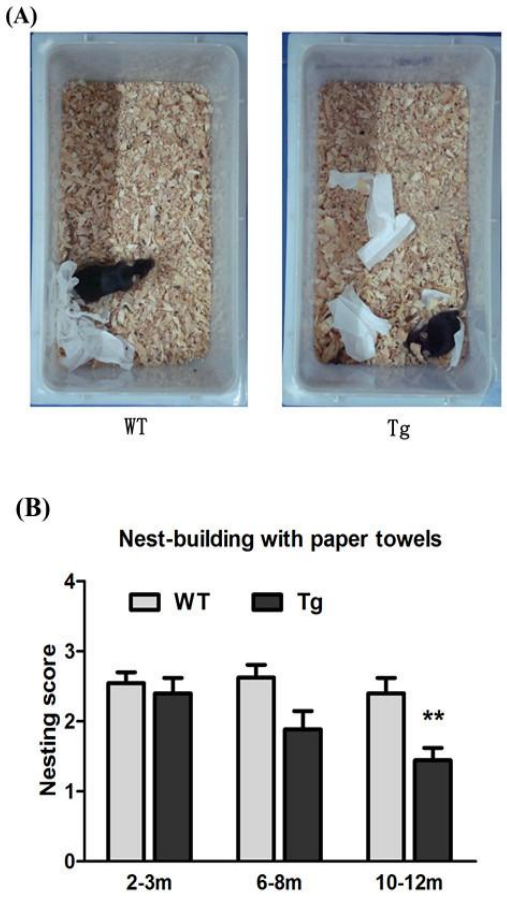

Figure 2. Effects of genotype and age on paper towel nest construction. (A) Representative pictures of paper towel nest construction in 10–12months old WT and Tg mice. (B) Bar graph showing the results from paper towel nest construction experiments. APPswe/PS1dE9 (Tg) mice (black bars) build nests of poorer quality. The deficit starts at 6-8 months of age. Although the difference did not reach statistical significance, the downward trend was obvious. Till 10–12months, there were significant difference ($p<0.01$).

Discussion

Nest building is a common behavior in mice, at the same time it is a kind of maternal behavior and a social behavior, associated with maintaining body temperature. Injuries in the medial prefrontal cortex, ventral tegmental area, hippocampus ,etc. of the mice, changes of sex hormone level, maternal experience [6-8] and other factors can affect nesting behavior. In order to avoid influence of changes of estrogen levels with the menstrual cycle and maternal experience of female mice on the results, we chose male mice as the research objects in this experiment.

There are a few researches on AD transgenic mice and the results are contradictory. Some of the studies have found that decrease in nesting behavior of AD transgenic mice was positively correlated with Aβ burden in brain [7] . But the conclusions are not the same in different experiments, which may be related to different experimental methods or genetic background or other factors. Our study found that APPswe/PS1dE9 mice's nesting behavior reduced along with age, paralleling with the increase of Aβ burden with age. It was reported that the hippocampus and the medial prefrontal cortex damage in mice can lead to reduced nesting material consumption and nest quality[9, 10] , indicating that the impairment of nesting behavior in APPswe/PS1dE9 mice may be caused by toxic injury of Aβ in related brain areas. Some studies using nesting behavior to evaluate the therapeutic effect of AD found that some drugs in reducing Aβ load in related brain regions can significantly improve nesting behavior at the same time. For instance: bexarotene ,a drug that can rapidly clear Aβ in the brain,a novel brain permeable monoamine oxidase inhibitor and iron chelating-radical scavenging drug,M30,and a histone deacetylase inhibitor MS-275[11-13] .These studies have added to the conclusion that Aβ toxicity in brain regions affects nesting function of mice.

Previous reports of APPswe/PS1A246E mice using cotton Nestlets as nesting material showed that the significant impairment of nesting behavior in these double transgenic mice appeared at the age of 6 months [14] . We also found our male APPswe/PS1dE9 mice showed nesting quality decline compared with wild type when using paper towels as nesting material, although there was no significant statistically differences, which may be due to our relatively small experimental sample size. In addition, another reason for the above phenomenon may be that in contrast to the aforementioned reports based on experiments of mixed mice group, this study only selected a single gender of male mice as the research object. There are many reports claiming manifesting female APP/PS1 mice's pathological progress significantly faster than male mice [15, 16]. Therefore gender factors and their interaction with the gene may have effect on nesting behavior of transgenic mice.

3xTgAD mice, with cotton as the nesting material, showed no difference with the wild type, but with paper towel as the nesting material, they performed damage of nest-building behavior [3] . We observed similar phenomenon in APPswe/PS1dE9 mice in our study: 6-8 months old Tg mice showed no difference compared to WT mice with chip shavings as the nesting material, while lower nest quality with paper towels. Although that difference did not reach statistical significance, the downward trend was obvious, suggesting nesting material preferences of mice and degree of difficulty may affect the results. In this experiment, the mice were exposed to chip shavings from birth, and they can learn from their

mothers or other adult mice to use shavings bedding to build nest. On the contrary, the mice have never contacted paper towels before the experiment leading to appear nesting dysfunction earlier, which implied that nesting material has obvious influence on the results, and the nesting behavior may reflect the episodic memory related to daily living ability, and emotional factors such as anxiety, neophobia may affect nesting behavior. Because we also observed some Tg mice staying in a corner of the cage, repeating washing and grooming when we put the paper towel into the cage.

In a word, the APPswe/PS1dE9 double transgenic mice show a genetic and age-dependent impairment of nesting behavior, but the causes and pathophysiological mechanisms need further study to reveal.

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References:

[1] Hardy, J. and D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science, 2002. 297(5580): 353-6.
[2] Etters, L., D. Goodall and B.E. Harrison, Caregiver burden among dementia patient caregivers: a review of the literature. J Am Acad Nurse Pract, 2008. 20(8): 423-8.
[3] Torres-Lista, V. and L. Gimenez-Llort, Impairment of nesting behaviour in 3xTg-AD mice. Behav Brain Res, 2013. 247: 153-7.
[4] Bond, T.L., et al., Nest building in nulligravid, primigravid and primiparous C57BL/6J and DBA/2J mice (Mus musculus). Physiol Behav, 2002. 75(4): 551-5.
[5] Morales-Corraliza, J., et al., Immunization targeting a minor plaque constituent clears beta-amyloid and rescues behavioral deficits in an Alzheimer's disease mouse model. Neurobiol Aging, 2013. 34(1): 137-45.
[6] Deacon, R.M., Assessing nest building in mice. Nat Protoc, 2006. 1(3): 1117-9.
[7] Wesson, D.W. and D.A. Wilson, Age and gene overexpression interact to abolish nesting behavior in Tg2576 amyloid precursor protein (APP) mice. Behav Brain Res, 2011. 216(1): 408-13.
[8] Broida, J. and B. Svare, Strain-typical patterns of pregnancy-induced nestbuilding in mice: maternal and experiential influences. Physiol Behav, 1982. 29(1): 153-7.
[9] Deacon, R.M., A. Croucher and J.N. Rawlins, Hippocampal cytotoxic lesion effects on species-typical behaviours in mice. Behav Brain Res, 2002. 132(2): 203-13.
[10] Holson, R.R., Mesial prefrontal cortical lesions and timidity in rats. III. Behavior in a semi-natural environment. Physiol Behav, 1986. 37(2): 239-47.
[11] Cramer, E., et al., ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models. Science, 2012. 335(6075): 1503-6.
[12] Kupershmidt, L., et al., The novel multi-target iron chelating-radical scavenging compound M30 possesses beneficial effects on major hallmarks of Alzheimer's disease. Antioxid Redox Signal, 2012. 17(6): 860-77.
[13] Zhang, Z.Y. and H.J. Schluesener, Oral administration of histone deacetylase inhibitor MS-275 ameliorates neuroinflammation and cerebral amyloidosis and improves behavior in a mouse model. J Neuropathol Exp Neurol, 2013. 72(3): 178-85.
[14] Filali, M. and R. Lalonde, Age-related cognitive decline and nesting behavior in an APPswe/PS1 bigenic model of Alzheimer's disease. Brain Res, 2009. 1292: 93-9.
[15] Howlett, D.R., et al., Cognitive correlates of Abeta deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. Brain Res, 2004. 1017(1-2): 130-6.
[16] Hirata-Fukae, C., et al., Females exhibit more extensive amyloid, but not tau, pathology in an Alzheimer transgenic model. Brain Res, 2008. 1216: 92-103.

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