The influence of chronic stress on anxiety-like behavior and cognitive function in different human GFAP-ApoE transgenic adult male mice

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Abstract

The apolipoprotein E (ApoE) ε4 allele (ApoE4) is an important genetic risk factor for the pathogenesis of Alzheimer’s disease (AD). In addition to genetic factors, environmental factors such as stress may play a critical role in AD pathogenesis. This study was designed to investigate the anxiety-like behavioral and cognitive changes in different human glial fibrillary acidic protein (GFAP)-ApoE transgenic adult male mice under chronic stress conditions. On the open field test, anxiety-like behavior was increased in the non-stressed GFAP-ApoE4 transgenic mice relative to the corresponding GFAP-ApoE3 (ApoE ε3 allele) mice. Anxiety-like behavior was increased in the stressed GFAP-ApoE4 mice relative to non-stressed GFAP-ApoE3 mice, but was unexpectedly decreased in the stressed GFAP-ApoE4 mice relative to non-stressed GFAP-ApoE4 mice. On the novel object recognition task, both GFAP-ApoE4 and GFAP-ApoE3 mice exhibited long-term non-spatial memory impairment after chronic stress. Interestingly, short-term non-spatial memory impairment (based on the novel object recognition task) was observed only in the stressed GFAP-ApoE4 male mice relative to non-stressed GFAP-ApoE4 transgenic mice. In addition, short-term spatial memory impairment was observed in the stressed GFAP-ApoE3 transgenic male mice relative to non-stressed GFAP-ApoE3 transgenic male mice; however, short-term spatial memory performance of GFAP-ApoE4 transgenic male mice was not reduced compared to non-stressed control mice based on the Y-maze task. In conclusion, our findings suggested that chronic stress affects anxiety-like behavior and spatial and non-spatial memory in GFAP-ApoE transgenic mice in an ApoE isofrom-dependent manner.

Keywords

Alzheimer’s disease, anxiety, apolipoprotein E, chronic stress, cognition, transgenic

Introduction

Alzheimer’s disease (AD), a common neurodegenerative disease, is associated with cognitive impairment and is accompanied by mood disorders including anxiety and depression. The prevalence of dementia is <1% among individuals aged 60–64 years; however, dementia afflicts >24–33% of those >85 years of age (Ferri et al., 2005; Querfurth & LaFerla, 2010). Environmental factors play an important role in the pathogenesis of AD and the behavioral disturbances associated with AD (Moceri et al., 2001; Raiha et al., 1998). Stress, which stems from major life events and from the activities of daily life, is an important factor that affects neural plasticity (Kim & Diamond, 2002), neurogenesis (Gould et al., 1998), and spatial learning and memory (Conrad, 2010). The effects of physical and psychosocial stressors on the hypothalamic–pituitary–adrenal (HPA) axis may be involved in AD onset and progression (Peskind et al., 2001; Swanwick et al., 1998). Stress is also associated with anxious behavior, which has been recently considered as a key factor in AD pathogenesis (Garcia-Alberca et al., 2012; O’Donovan et al., 2013; Zhang et al., 2012).

Apolipoprotein E (ApoE) is a 34-kDa protein that plays an important role in lipoprotein metabolism due to its association with members of the low-density lipoprotein (LDL) receptor family. ApoE signaling is implicated in cognition and synaptic plasticity (Raber et al., 2000b; Ruiz et al., 2005; Shi et al., 2014). Three isoforms of ApoE (ε2, ε3 and ε4), distinguished by their cysteine/arginine content at two polymorphic sites, have been identified in humans. While both ApoE ε2 alleles (ApoE2) and ApoE ε3 (ApoE3) are neuroprotective against AD, the ApoE ε4 allele (ApoE4) is a genetic risk factor for AD pathogenesis (Corder et al., 1993; Farrer et al., 1997; Meng et al., 2011). Anxiety, a very common phenomenon among AD patients, inversely correlates with deficiencies in the activities of daily living (de Toledo et al., 2004; Porter et al., 2003).

The effects of the ApoE genotype on anxiety-like behavior and cognition have been modeled in mice lacking murine ApoE and expressing a human ApoE isoform [e.g. in astrocytes under the control of the glial fibrillary acidic protein (GFAP) promoter, in neurons under the control of the...
neuron-specific enolase (NSE) promoter]. Compared with GFAP-ApoE3 mice, GFAP-ApoE4 mice exhibit increased anxiety-like behavior (Siegel et al., 2012). Consistent with the transgenic mouse data, AD patients carrying with ApoE4 exhibit higher anxiety scores than ApoE3-carrying subjects (Pritchard et al., 2007; Robertson et al., 2005). ApoE transgenic mouse studies have strongly suggested that ApoE genotype is implicated in cognition, as spatial memory retention is impaired in GFAP-ApoE4 female mice compared with age-matched GFAP-ApoE3 female mice (Raber et al., 1998; van Meer et al., 2007). Additionally, ApoE knockout mice express higher basal glucocorticoid levels than wild-type mice (Grootendorst et al., 2004; Raber et al., 2000a). The influences of chronic stress on anxiety-like behavior and cognitive function within different ApoE genotypes, however, have not yet been demonstrated. This study aimed to investigate the differences in behavior and cognition resulting from different ApoE genotypes in the presence or absence of chronic stress using human GFAP-ApoE transgenic adult male mice.

**Materials and methods**

**Animals and housing**

Human ApoE3 (stock number: 004633) and ApoE4 (stock number: 004631) transgenic mice were obtained from The Jackson Laboratory (Bar Harbor, ME). The ApoE transgene was under the control of a GFAP promoter, and specific expression of ApoE in astrocytes was confirmed. The mice were maintained under a 12-h light–dark cycle (lights on at 7:00 am) at a temperature of 22 ± 1°C. Food and water were available *ad libitum*. All of the experiments were performed in accordance with the Animal Care and Use Committee of the University of Science and Technology of China in Anhui, P.R. China.

**Groups and chronic restraint stress**

Three-month-old male GFAP-ApoE transgenic mice were randomly assigned to the control or stress groups for each examined ApoE genotype [ApoE3/3-CON (3 C, \( n = 13 \)), ApoE4/4-CON (4 C, \( n = 14 \)), ApoE3/3-stress (3 S, \( n = 9 \)) and ApoE4/4-stress (4 S, \( n = 8 \)]. The restraint procedure was performed during the light period of the light–dark cycle. Control animals were left undisturbed, while stressed animals were subjected to 4 h/day of immobilization stress in a mouse stress tube (2.8 cm in inner diameter × 9 cm long) for 5 months within their home cages. Mice were stressed at irregular times during the light period 4 days/week to prevent habituation. The restraint stress was continued for 5 months, and the mice were then subjected to the behavioral tests at 8 months of age.

**Behavioral tasks**

All the behavioral tasks were performed during the light phase of the light–dark cycle in a test room similar to the housing room. Before each test, animals were provided with a 20-min adaptation period to acclimate to the test room. All behaviors of the transgenic mice were videotaped, and video tracking was performed using EthoVision XT 5 software (Wageningen, The Netherlands). Figure 1 shows the schedule of the experimental design. We scheduled 9-day intervals between short-term test (1-h interval) and long-term test (24-h interval) to limit adaptation to the tests. In addition, objects (familiar and novel objects) used in the novel object recognition task and the arms (novel and familiar arms) used in the Y-maze test were alternated from the first task to the second task. We also rotated the apparatus to face a different direction between the two tasks.

**Open field test**

An open field (OF) test was designed to detect spontaneous locomotive activity, exploration and anxiety-like behavior according to previous studies (Chen et al., 2004; Meng et al., 2011). An open wooden box (50 cm × 50 cm) with 25-cm walls and a smooth surface was used for this test. The OF chamber was painted with water-resistant, odorless white paint. For each trial, an animal was placed in one of the four corners, facing the corner, and was permitted to explore the environment for 5 min. The OF test was lasted for three consecutive days.

**Elevated plus maze test**

The elevated plus maze (EPM) test was performed as described by Walf & Frye (2007). The maze (made of Plexiglas) consisted of two opposing closed arms (30 cm × 6 cm) that were enclosed by walls (15 cm in height) and two opposing open arms (30 cm × 6 cm, without walls) that formed a plus shape containing a central arena (6 cm × 6 cm). The entire apparatus was elevated 80 cm above the floor. Each mouse was placed in the central arena of the maze facing an open arm and was allowed to explore the maze for 5 min.

**Y-maze test**

Spontaneous alternation behaviors were measured during the Y-maze task according to previous studies with some modifications (Ladurelle et al., 2000). The Y-maze apparatus consisted of three Plexiglas arms (45 cm × 15 cm × 30 cm),...
defined as placing arm, the novel arm and the familiar arm. The Y-maze task included two test sessions. During the first session, the novel arm was closed, and each mouse was placed at the end of the placing arm facing the wall and was allowed to freely explore the maze for 15 min. The second session was performed either 1 or 24 h later. During the second session, the novel arm was opened, and the mice were allowed to freely explore the three arms for 5 min. Total locomotive activity was recorded using a video camera, and the rearing times in the novel arm \( (T_N) \) and in the familiar arm \( (T_F) \) were calculated. Preference for the novel arm \( (P_N) \) was calculated as follows: \[ P_N = T_N/(T_N + T_F) \].

Novel object recognition test

The novel object recognition test was designed according to previous studies with some modifications (Bevins & Besheer, 2006). A plastic OF chamber \( (47 \text{ cm} \times 36 \text{ cm} \times 20 \text{ cm}) \) was employed to assess the cognitive function of the mice in the present study. This test contained three sessions: chamber familiarization, identical object recognition and novel object recognition. The day before the task, each subject was allowed to explore the chamber for 5 min. During the identical object recognition session, two identical objects were placed in the corners 8 cm from the wall on the longest side. Mice were then placed facing the wall in the midpoint of the other side of the chamber, and were allowed to acclimate for 15 min. One of the familiar objects was then replaced with a novel object either 1 or 24 h later. Subsequently, the mice were placed in the chamber, and the rearing times with the novel object \( (T_N) \) and with the familiar object \( (T_F) \) were monitored for 5 min. The novel object preference \( (P_N) \) was defined as the rearing time with the novel object divided by the total rearing time with any object as follows: \( (T_N + T_F) \).

Statistical analysis

Data are presented as the means ± standard error of the mean (SEM). The measurements were analyzed using a repeated measures ANOVA containing one between-subject factor (groups, either the ApoE allele or subjection to stress) and one within-subject factor (days) during the 3 days of the OF test. A two-way ANOVA was used to calculate the significance of the differences between the daily mean values on the OF, EPM, novel object recognition and Y-maze tests. The following levels of significance are indicated: * \( p < 0.05 \), ** \( p < 0.01 \) and *** \( p < 0.001 \).

Results

Anxiety-like behavior in the GFAP-ApoE transgenic mice after chronic stress

The OF and EPM tests were used in the current study to evaluate the effect of chronic stress on the anxiety-like behavior of the GFAP-ApoE transgenic mice.

On the OF test, we found that GFAP-ApoE4 transgenic mice exhibited decreased rearing (Figure 2B, \( F(1,41) = 1391.72, \ p < 0.001 \)) and locomotive activity (Figure 2C, \( F(1,41) = 439.50, \ p < 0.001 \)) compared with GFAP-ApoE3 transgenic mice across the three testing days. Similarly, chronic restraint stress induced changes in rearing (Figure 2B, \( F(1,41) = 381.71, \ p < 0.001 \)) and locomotive activity (Figure 2C, \( F(1,41) = 1199.14, \ p < 0.001 \)) in GFAP-ApoE4 transgenic mice compared with GFAP-ApoE3 transgenic mice after chronic stress.
transgenic mice. During the 3-day OF test, the GFAP-ApoE4 transgenic mice exhibited reduced rearing (Figure 2B, OF1, \( p < 0.01 \); OF3, \( p < 0.05 \)) and locomotive activity (Figure 2C, OF1, \( p = 0.01 \); OF3, \( p < 0.05 \)) compared with GFAP-ApoE3 transgenic mice. The GFAP-ApoE4 transgenic mice subjected to chronic stress exhibited higher rearing (Figure 2B, OF1, \( p = 0.01 \); OF3, \( p < 0.05 \)) and locomotive activity (Figure 2C, OF3, \( p < 0.05 \)) relative to non-stressed GFAP-ApoE4 transgenic mice. Furthermore, the GFAP-ApoE3 transgenic mice subjected to chronic stress exhibited decreased rearing (Figure 2B, OF2, \( p = 0.001 \)) and locomotive activity (Figure 2C, OF1, \( p < 0.05 \); OF3, \( p < 0.05 \)) compared with non-stressed GFAP-ApoE3 transgenic mice.

Both ApoE genotype and chronic stress contributed to anxiety-like behavior of the mice based on the parameters of the OF test, including the inner zone frequency (Figure 2D, ApoE, \( F(1,41) = 147.56, p < 0.001 \); stress, \( F(1,41) = 116.77, p < 0.001 \)), inner duration (Figure 2E, ApoE, \( F(1,41) = 116.77, p < 0.001 \); stress, \( F(1,41) = 111.15, p < 0.001 \)) and inner distance (Figure 2F, ApoE, \( F(1,41) = 124.75, p < 0.001 \); stress, \( F(1,41) = 117.76, p < 0.001 \)). On the OF test, anxiety-like behavior was increased in non-stressed GFAP-ApoE4 transgenic mice relative to non-stressed GFAP-ApoE3 transgenic mice, including a higher frequency of entering the inner area (Figure 2D, OF1, \( p < 0.05 \); OF2, \( p = 0.01 \)), a longer duration in the inner area (Figure 2E, OF2, \( p < 0.01 \)) and a longer distance traveled (Figure 2F, OF2, \( p < 0.01 \)). Conversely, the GFAP-ApoE3 mice displayed less anxiety-like behavior in response to the chronic stress treatment relative to non-stressed GFAP-ApoE4 transgenic mice, including a higher frequency of entering the inner area (Figure 2D, OF2, \( p = 0.001 \); OF1, \( p < 0.01 \) and a shorter distance traveled in the inner area (Figure 2F, OF1, \( p = 0.001 \); OF2, \( p < 0.01 \)). Furthermore, similar results were observed for the percentage of time spent in the inner area (Figure S1).

On the EPM test, ApoE genotype or chronic stress treatment affected neither open arm duration [Figure 3A, ApoE, \( F(1,40) = 0.19, p = 0.665 \); stress, \( F(1,40) = 0.002, p = 0.961 \)] nor open arm distance traveled [Figure 3B, ApoE, \( F(1,40) = 1.87, p = 0.18 \); stress, \( F(1,40) = 0.88, p = 0.373 \)]. However, the locomotive speed in the open arms was affected by an ApoE × stress interaction [Figure 3C, \( F(1,40) = 6.78, p < 0.05 \)]. The non-stressed GFAP-ApoE4 transgenic mice traveled slower relative to non-stressed GFAP-ApoE3 transgenic mice (Figure 3C, \( p < 0.01 \)). The GFAP-ApoE4 transgenic mice subjected to chronic stress

Figure 3. The activity and anxiety-like behavior of the GFAP-ApoE3 and GFAP-ApoE4 transgenic male mice after chronic stress based on the EPM test. The duration (A), the frequency (B) and the velocity (C) in the open arms indicated the anxiety-like behavior of the non-stressed and stressed GFAP-ApoE transgenic male mice. The total distance traveled (D) indicated the locomotor activity of the GFAP-ApoE transgenic mice on the EPM test. The data are expressed as the means ± SEM. *\( p < 0.05 \), **\( p < 0.01 \) and ***\( p < 0.001 \); ApoE3/3-CON (3 C, \( n = 13 \)); ApoE4/4-CON (4 C, \( n = 14 \)); ApoE3/3-stress (3 S, \( n = 9 \)) and ApoE4/4-stress (4 S, \( n = 8 \)).
traveled faster than non-stressed GFAP-ApoE4 transgenic mice (Figure 3C, \( p < 0.01 \)).

Two-way ANOVA revealed that the ApoE genotype (Figure 3D, \( F(1,40) = 36.55, p < 0.001 \)) and the stress procedure (Figure 3D, \( F(1,40) = 4.10, p < 0.05 \)) contributed to the observed locomotive activity changes during the EPM test. The non-stressed GFAP-ApoE4 transgenic mice displayed lower locomotive activity relative to GFAP-ApoE3 transgenic mice (Figure 3D, \( p < 0.0001 \)). Chronic stress treatment increased locomotive activity of the GFAP-ApoE4 transgenic mice (Figure 3D, \( p < 0.05 \)) but not the GFAP-ApoE3 transgenic mice relative to corresponding non-stressed GFAP-ApoE4 or GFAP-ApoE3 transgenic mice.

**Cognitive function of the non-stressed and stressed GFAP-ApoE transgenic mice**

Non-spatial memory function of GFAP-ApoE transgenic mice was assessed using the novel object recognition task for both short-term (1-h interval) and long-term memory (24-h interval). Two-way ANOVA revealed that the ApoE genotype differently affected performance on the short-term novel object recognition task (Figure 4A, \( F(1,40) = 4.69, p < 0.05 \)). On the long-term novel object recognition test, the preference for the novel object was significantly affected by chronic stress (Figure 4B, \( F(1,40) = 15.88, p < 0.001 \)). At 8 months of age, no significant difference in the novel object preference was observed between the GFAP-ApoE3 and GFAP-ApoE4 transgenic mice during either the short- or long-term tasks (Figure 4A and B). After chronic stress treatment, both the GFAP-ApoE3 and GFAP-ApoE4 transgenic mice exhibited an impairment in long-term memory on the novel object recognition test relative to corresponding non-stressed GFAP-ApoE3 or GFAP-ApoE4 mice (Figure 4B, ApoE3 \( p < 0.01 \); ApoE4 \( p < 0.01 \)). The short-term memory performance of the stressed GFAP-ApoE4 transgenic mice was impaired compared with stressed GFAP-ApoE3 transgenic mice based on the 1-h interval novel object recognition task (Figure 4A, \( p < 0.05 \)). In addition, we analyzed the total exploration time (novel and familiar objects) of GFAP-ApoE transgenic male mice after chronic stress treatment during the novel object recognition task. No significant differences in the total exploration time were observed at either the 1- or 24-h interval (Figure S2).

On the Y-maze test, which was used to test spatial memory, an ApoE \( \times \) stress interaction (Figure 5A, \( F(1,40) = 10.38, p < 0.01 \)) was observed. At 8 months of age, the non-stressed GFAP-ApoE4 transgenic mice exhibited a reduced preference for the novel arms compared with non-stressed GFAP-ApoE3 transgenic mice (Figure 5A, \( p < 0.05 \)). The short-term memory performance of the stressed GFAP-ApoE3 transgenic mice was impaired after chronic stress treatment relative to non-stressed GFAP-ApoE3 transgenic mice (Figure 5A, \( p < 0.05 \)). No significant differences were observed for the 24-h interval Y-maze test based on a two-way ANOVA, which was used to exclude the influence of the distance traveled in the Y maze on the results. No significant difference in the total distance traveled was observed between the different groups (Figure S3).

We reanalyzed the data using Tukey multiple comparison test followed by a three-way ANOVA to further evaluate the effect of ApoE genotypes, stress treatment and the interval between sessions on cognition. This analysis revealed that the novel arm preference (Figure S4, \( p < 0.01 \)), but not the novel object preference (Figure S5, \( p = 0.21 \)), was affected by an ApoE \( \times \) stress interaction.

**Discussion**

Sporadic AD is widely believed to be caused by complex interactions between various genetic and environmental factors. Stress, which is an important environmental factor, may increase the risk of developing AD. In addition, stress influences anxiety-like behavior, which can contribute to cognitive dysfunction (Ferri et al., 2005; Sheffler et al., 2014; Wilson et al., 2003). In the current study, we hypothesized that chronic stress may play an important role in the effects of different ApoE genotypes on anxiety-like behavior and cognitive function.

In our study, we investigated the effects of chronic stress on anxiety-like behavior of different human GFAP-ApoE transgenic adult male mice. Anxiety-like behavior was increased in 8-month-old GFAP-ApoE4 transgenic male mice relative to matched GFAP-ApoE3 mice, which was
ApoE3 transgenic mice exhibited long-term non-spatial long-term chronic stress, both GFAP-ApoE4 and GFAP-ApoE3 transgenic (Grootendorst et al., 2005; Raber et al., 2002). However, after females, but not males, exhibited non-spatial memory deficits 6- to 8-month-old NSE-ApoE and GFAP-ApoE transgenic was consistent with previous findings demonstrating that GFAP-ApoE3 transgenic male mice in our study. This result between the 8-month-old non-stressed GFAP-ApoE4 and the short- or long-term non-spatial memory was observed memory (Rampon et al., 2000). No apparent difference in for the assessment of short- and long-term non-spatial function using GFAP-ApoE transgenic adult male mice. Additionally, we found that the locomotive speed of GFAP-ApoE3 transgenic mice was not altered by chronic stress treatment; however, the stressed GFAP-ApoE4 transgenic mice traveled faster compared to non-stressed GFAP-ApoE4 transgenic control mice. These results indicated that this increase in locomotive speed likely resulted from the decreased anxiety-like behavior of the GFAP-ApoE4 transgenic male mice after chronic stress treatment. This conclusion was supported by a study demonstrating that a lower velocity was associated with altered by chronic stress treatment; however, the stressed GFAP-ApoE4 transgenic mice traveled faster compared to non-stressed GFAP-ApoE4 transgenic control mice. These results indicated that this increase in locomotive speed likely resulted from the decreased anxiety-like behavior of the GFAP-ApoE4 transgenic male mice after chronic stress treatment. This conclusion was supported by a study demonstrating that a lower velocity was associated with increased anxiety-like behavior relative to corresponding non-stressed GFAP-ApoE4 or GFAP-ApoE3 transgenic adult male mice. Additionally, we found that the locomotive speed of GFAP-ApoE3 transgenic mice was not altered by chronic stress treatment; however, the stressed GFAP-ApoE4 transgenic mice traveled faster compared to non-stressed GFAP-ApoE4 transgenic control mice. These results indicated that this increase in locomotive speed likely resulted from the decreased anxiety-like behavior of the GFAP-ApoE4 transgenic male mice after chronic stress treatment. This conclusion was supported by a study demonstrating that a lower velocity was associated with high anxiety-like conditions (Farlow et al., 2004). Thus, our results suggested that chronic stress may regulate the effects of ApoE4 on anxiety-like behavior.

Previous studies of humans and experimental animals have shown that both genetic (such as ApoE4) and environmental factors (such as stress) appear to account for cognitive decline (Pritchard et al., 2007; Robertson et al., 2005). In the current study, we explored the influence of chronic stress on changes in cognitive function using GFAP-ApoE transgenic adult male mice. The novel object recognition task is a popular method for the assessment of short- and long-term non-spatial memory (Rampone et al., 2000). No apparent difference in short- or long-term non-spatial memory was observed between the 8-month-old non-stressed GFAP-ApoE4 and the GFAP-ApoE3 transgenic male mice in our study. This result was consistent with previous findings demonstrating that 6- to 8-month-old NSE-ApoE and GFAP-ApoE transgenic females, but not males, exhibited non-spatial memory deficits (Grootendorst et al., 2005; Raber et al., 2002). However, after long-term chronic stress, both GFAP-ApoE4 and GFAP-ApoE3 transgenic mice exhibited long-term non-spatial memory impairment relative to corresponding non-stressed GFAP4 or GFAP-ApoE3 transgenic control mice, whereas only stressed GFAP-ApoE4 transgenic male mice exhibited short-term non-spatial memory impairment relative to non-stressed GFAP4 transgenic male mice. Although the role of the hippocampus in non-spatial memory remains controversial, several studies have indicated that both spatial and non-spatial memories are associated with the hippocampus (Cohen et al., 2013; Ross & Eichenbaum, 2006). In our study, the impaired long-term non-spatial memory of the stressed GFAP-ApoE4 and GFAP-ApoE3 transgenic male mice likely involved the hippocampus, given its involvement long-term non-spatial memory (Bruel-Jungerman et al., 2005; Cohen et al., 2013). This result suggests that chronic stress altered the effect of ApoE4 on both short- and long-term non-spatial memory, but only altered long-term non-spatial memory in the ApoE3 line.

On the Y-maze test, which is a task that is used to assess spatial memory, we found that the GFAP-ApoE4 transgenic male mice exhibited impaired spatial memory compared with GFAP-ApoE3 transgenic male mice at 8 months of age; this result was consistent with those of previous studies (Grootendorst et al., 2005; van Meer et al., 2007). Interestingly, short-term spatial memory impairment was observed in the stressed GFAP-ApoE3 transgenic male mice relative to non-stressed GFAP-ApoE3 transgenic male mice; however, short-term spatial memory performance of GFAP-ApoE4 transgenic male mice was not reduced compared to non-stressed control mice based on the Y-maze task. This finding might be partially attributed to factors such as the stressors, the stress duration, the subjects and their age. Notably, however, stress does not always exert adverse effects (Joels et al., 2007), and a few studies have reported that chronic stress enhanced learning and memory abilities (Bartolomucci et al., 2002; Li et al., 2007). Additionally, some evidence has suggested that stressful events play a critical role in the early development of cognitive symptoms in AD. However, negative effects of stress appear to depend on the presence of other risk factors for AD (Scullion et al., 2013). We propose that decreased anxiety-like behavior may have also contributed to the protection from the chronic
stress-induced exacerbation of spatial memory impairment in the GFAP-ApoE4 transgenic adult male mice (Robinson et al., 2013). However, the relationship between memory decline and the presence of ApoE4 was consistent with the results of neuroimaging studies that showed an association between ApoE4 and hippocampal atrophy (den Heijer et al., 2002; Mueller & Weiner, 2009). One limitation of the present study was that we did not examine whether the modulatory effects of chronic stress on anxiety-like behavior and cognition were associated with the hippocampus.

In summary, our findings indicated that the varying responses of different ApoE genotypes to chronic stress affects anxiety-like behavior and cognitive function, providing new insight into the role of ApoE in AD. Therefore, chronic stress should not be ignored when considering ApoE4 as a potential risk factor for the development of AD.

Declaration of interest

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References

Bartolomucci A, de Biurrun G, Czeh B, van Kampen M, Fuchs E. (2002). Selective enhancement of spatial learning under chronic psychosocial stress. Eur J Neurosci 15(11):1863–6.
Bevis RA, Besheer J. (2006). Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study ‘recognition memory’. Nat Protoc 1(3):1306–11.
Bruel-Jungerman E, Laroche S, Rampon C. (2005). New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. Eur J Neurosci 21(2):513–21.
Chen GH, Wang YJ, Zhang LQ, Zhou JN. (2004). Age- and sex-related disturbance in a battery of sensorimotor and cognitive tasks in Kunming mice. Physiol Behav 83(3):531–41.
Cohen SJ, Munchow AH, Rios LM, Zhang G, Asgeirsdottir HN, Chen GH, Wang YJ, Zhang LQ, Zhou JN. (2004). Age- and sex-related characteristics of anxiety among patients with Alzheimer’s disease and related dementia. J Neuropsychiatry Clin Neurosci 15(2):180–6.
Moceri VM, Kukull WA, Emanuall I, van Belle G, Starr JR, Schellenberg GD, McCormick WC, et al. (2001). Using census data and birth certificates to reconstruct the early-life socioeconomic environment and the relation to the development of Alzheimer’s disease. Epidemiology 12(4):383–9.
Mueller SG, Weiner MW. (2009). Selective effect of age, Apo e4, and Alzheimer’s disease on hippocampal subfields. Hippocampus 19(6):558–64.
O’Donovan A, Slavich GM, Epel ES, Nefsalan TC. (2013). Exaggerated neurobiological sensitivity to threat as a mechanism linking anxiety with increased risk for diseases of aging. Neurosci Biobehav Rev 37(1):96–108.
Mueller & Weiner, 2009). One limitation of the present study was that we did not examine whether the modulatory effects of chronic stress on anxiety-like behavior and cognition were associated with the hippocampus.

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Garcia-Alberca JM, Cruz B, Lara JP, Garrido V, Lara A, Gris E. (2012). Anxiety and depression are associated with coping strategies in caregivers of Alzheimer’s disease patients: results from the MALAGA-AD study. Int Psychogeriatr 24(8):1325–34.
Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E. (1998). Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci USA 95(6):3168–71.
Grootendorst J, Baur A, Vogel E, Kelche C, Sullivan PM, Dordart JC, Bales K, Mathis C. (2005). Human apoE targeted replacement mouse lines: h-apol4E and h-apol3E mice differ on spatial memory performance and avoidance behavior. Behav Brain Res 159(1):1–14.
Grootendorst J, Enthoven L, Dalm, S, de Kloet ER, Oitzl MS. (2004). Increased corticosterone secretion and early-onset of cognitive decline in female apolipoprotein E-knockout mice. Behav Brain Res 148(1–2):167–77.
Joels M, Karst H, Krugers HJ, Lucassen PJ. (2007). Chronic stress: implications for neuronal morphology, function and neurogenesis. Front Neuroendocrinol 28(2–3):72–96.
Kim JY, Diamond DM. (2002). The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 3(6):453–62.
Ladurette N, Eychenne B, Denton D, Blair-West J, Schumacher M, Robel P, Baulieu E. (2000). Prolonged intracerebroventricular infusion of neurosteroids affects cognitive performances in the mouse. Brain Res 858(2):371–9.
Li XH, Liu NB, Zhang MH, Zhou YL, Liao JY, Liu XQ, Chen HW. (2007). Effects of chronic multiple stress on learning and memory and the expression of Fyn, BDNF, TrkB in the hippocampus of rats. Chin Med J (Engl) 120(8):669–74.
Meng FT, Ni RJ, Zhang Z, Zhao J, Liu YJ, Zhou JN. (2011). Inhibition of oestrogen biosynthesis induces mild anxiety in C57BL/6J ovariecetomized female mice. Neurosci Bull 27(4):241–50.
Moeric VM, Kukull WA, Emanuall I, van Belle G, Starr JR, Schellenberg GD, McCormick WC, et al. (2001). Using census data and birth certificates to reconstruct the early-life socioeconomic environment and the relation to the development of Alzheimer’s disease. Epidemiology 12(4):383–9.
Porter VR, Buxton WG, Fairbanks LA, Strickland T, O’Connor SM, Rosenberg/Thompson S, Cummings JL. (2003). Frequency and characteristics of anxiety among patients with Alzheimer’s disease and related dementia. J Neuropsychiatry Clin Neurosci 15(2):180–6.
Pritchard AL, Harris J, Pritchard CW, Coates J, Haque S, Holder R, Bentonham P, Lendon CL. (2007). The effect of the apolipoprotein E gene polymorphisms and haplotypes on behavioural and psychological symptoms in probable Alzheimer’s disease. J Neurol Neurosurg Psychiatry 78(2):123–6.
Querfurth HW, LaFerla FM. (2010). Alzheimer’s disease. N Engl J Med 362(4):329–44.
Raber J, Akana SF, Bhatnagar S, Dallman MF, Wong D, Mucke L. (2000a). Hypothalamic-pituitary-adrenal dysfunction in ApoE(-/-) mice: possible role in behavioural and metabolic alterations. J Neurosci 20(5):2064–71.
Raber J, Wong D, Yu GQ, Buttini M, Mahley RW, Pittas RE, Mucke L. (2000b). Apolipoprotein E and cognitive performance. Nature 404(6776):352–4.
Raber J, Bongers P, LeFevour A, Buttini M, Mucke L. (2002). Androgens protect against apolipoprotein E4-induced cognitive deficits. J Neurosci 22(12):5204–9.
Raber J, Wong D, Buttini M, Orth M, Bellosta S, Pittas RE, Mahley RW, Mucke L. (1998). Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: increased susceptibility of females. Proc Natl Acad Sci USA 95(18):10914–19.
Raiha I, Kaprio J, Koskenvuo M, Rajala T, Sourander L. (1998). Environmental differences in twins pairs discordant for Alzheimer’s disease. J Neuro Metabol Psychiatry 65(5):785–7.
Rampon C, Tang YP, Goodhouse J, Shimizu E, Kyin M, Tsien JZ. (2000). Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. Nat Neurosci 3(3):238–44.

Robertson J, Curley J, Kaye J, Quinn J, Pfankuch T, Raber J. (2005). apoE isoforms and measures of anxiety in probable AD patients and Apoe-/- mice. Neurobiol Aging 26(5):637–43.

Robinson OJ, Vytal K, Cornwell BR, Grillon C. (2013). The impact of anxiety upon cognition: perspectives from human threat of shock studies. Front Hum Neurosci 7:203. doi:10.3389/fnhum.2013.00203.

Ross RS, Eichenbaum H. (2006). Dynamics of hippocampal and cortical activation during consolidation of a nonspatial memory. J Neurosci 26(18):4852–9.

Ruiz J, Kouiavksaia D, Migliorini M, Robinson S, Saenko EL, Gorlatova N, Li D, et al. (2005). The apoE isoform binding properties of the VLDL receptor reveal marked differences from LRP and the LDL receptor. J Lipid Res 46(8):1721–31.

Scullion GA, Hewitt KN, Pardon MC. (2013). Corticotropin-releasing factor receptor 1 activation during exposure to novelty stress protects against Alzheimer’s disease-like cognitive decline in AbetaPP/PS1 mice. J Alzheimers Dis 34(3):781–93.

Sheffler J, Moxley J, Sachs-Ericsson N. (2014). Stress, race, and APOE: understanding the interplay of risk factors for changes in cognitive functioning. Aging Ment Health 18(6):784–91.

Shi L, Du X, Zhou H, Tao C, Liu Y, Meng F, Wu G, et al. (2014). Cumulative effects of the ApoE genotype and gender on the synaptic proteome and oxidative stress in the mouse brain. Int J Neuropsychopharmacol 17(11):1863–79.

Siegel JA, Haley GE, Raber J. (2012). Apolipoprotein E isoform-dependent effects on anxiety and cognition in female TR mice. Neurobiol Aging 33(2):345–58.

Swanwick GR, Kirby M, Bruce I, Buggy F, Coen RF, Coakley D, Lawlor BA. (1998). Hypothalamic-pituitary-adrenal axis dysfunction in Alzheimer’s disease: lack of association between longitudinal and cross-sectional findings. Am J Psychiatry 155(2):286–9.

van Meer P, Acevedo S, Raber J. (2007). Impairments in spatial memory retention of GFAP-apoE4 female mice. Behav Brain Res 176(2):372–5.

Walf AA, Frye CA. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2(2):322–8.

Wilson RS, Evans DA, Bienias JL, Mendes de Leon CF, Schneider JA, Bennett DA. (2003). Proneness to psychological distress is associated with risk of Alzheimer’s disease. Neurology 61(11):1479–85.

Zhang LF, Shi L, Liu H, Meng FT, Liu YJ, Wu HM, Du X, Zhou JN. (2012). Increased hippocampal tau phosphorylation and axonal mitochondrial transport in a mouse model of chronic stress. Int J Neuropsychopharmacol 15(3):337–48.

Supplementary material available online.
Supplementary Figures S1–S5.