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The Effects of Prolonged Stress and APOE Genotype on Memory and Cortisol in Older Adults

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Abstract

Background—Chronic elevations in cortisol associated with prolonged stress have been associated with memory loss, as has the APOE-ε4 genotype. The combined effects of stress and APOE status on memory and cortisol in humans have not been studied.

Methods—We used a semi-structured interview with standardized scoring to measure stress level, and univariate ANOVA to assess effects of stress and APOE-ε4 status on memory and salivary cortisol in 91 non-demented subjects (mean age: 78.8 years).

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Results—Low stress subjects performed better than High stress subjects on delayed recall of stories (p=.04), word lists (.02), and visual designs (.04). APOE-ε4 negative subjects obtained better scores than ε4 positive subjects on immediate (<.01) and delayed (<.01) recall of visual designs. Significant stress by APOE-ε4 interaction effects on memory (.03) and cortisol (<.01) resulted from consistently worse memory and higher cortisol concentrations in the High stress, ε4 positive group.

Conclusions—These findings are consistent with a model in which prolonged exposure of older, non-demented individuals to stress in the presence of an ε4 allele leads to memory decline. Further studies will assess whether stress and APOE-ε4 interact to increase the risk of developing Alzheimer's disease.

Keywords

Alzheimer's; APOE; stress; memory; cortisol; hippocampus

INTRODUCTION

As the identification of risk factors for developing Alzheimer's disease (AD) evolves, there is greater emphasis on the interaction of factors that may make disease onset and progression more likely. Two large-scale studies evaluating older twin pairs (1-2) showed that both genetic and environmental factors were important in explaining AD, suggesting that environmental factors could be the focus for interventions to lower disease risk or delay onset. The current study is designed to examine the interaction between an environmental factor (i.e., "real life" stress) and a genetic risk factor for AD (i.e., ε4 allele of the Apolipoprotein E gene (APOE-ε4)) in explaining cognitive performance in non-demented elderly individuals.

Neuropathologic changes of Alzheimer's disease (AD) that begin prior to clinical symptoms (3) typically lead to cognitive deficits that initially appear as relatively circumscribed memory loss associated with degeneration in medial temporal lobe brain regions such as the hippocampus. Any co-morbid factor that concomitantly reduces neurons or synapses or increases neuronal vulnerability in this region has the potential to hasten the onset and progression of the clinical manifestations of AD. In both animals and humans, prolonged psychological stress affects the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the sustained release of glucocorticoids (e.g., cortisol in humans). Studies have shown that in healthy human subjects, prolonged cortisol elevations are associated with hippocampal atrophy (4-5) and a decline in learning and memory (5-9). However, studies demonstrating impaired memory directly related to controlled (i.e., laboratory) measures of stress in human subjects are relatively rare (6,10-11). Furthermore, to our knowledge there is little or no evidence other than anecdotal, that prolonged exposure to "real life" stress is associated with memory loss or with the development of AD in older subjects.

Unlike exposure to stressful events, possession of the APOE-ε4 allele is a well-established risk factor for cognitive decline in the elderly. A recent meta-analysis of 38 studies showed that elderly individuals with at least one APOE-ε4 allele performed significantly worse than those without an ε4 allele on measures of global cognitive functioning, executive functioning, and memory (12). Studies that have focused on memory showed poorer verbal and non-verbal memory in older, non-demented adults with at least one APOE-ε4 allele (13-16) compared to those without an ε4 allele. Furthermore, the presence of an APOE-ε4 allele contributed to the likelihood of converting to AD in non-demented elderly who showed memory decline in the years preceding a dementia diagnosis (17-18). The relationship between memory decline and the presence of an APOE-ε4 allele is consistent with neuroimaging studies that have found an association between the ε4 genotype and hippocampal atrophy (15,19), and has been directly demonstrated in an animal study comparing gene-targeted mice that expressed the human apoE

(h-apoE) isoforms h-apoE3 or h-apoE4. Mice with the h-apoE4 isoform had impaired spatial memory compared to those with the h-apoE3 isoform (20). Taken together, the results of these studies from multiple research groups support the notion that possession of an APOE-ε4 allele is associated with memory decline in older adults.

In addition to its effects on cognition, the APOE-ε4 allele may influence components of the HPA axis. Peskind and collaborators (21) found that concentration of cortisol from CSF (but not from plasma) in non-demented, older adults was higher for those with at least one ε4 allele than those without an ε4 allele, and that higher CSF cortisol levels were associated with lower scores on tests of global cognition. The authors speculated that increased risk of AD in ε4 positive subjects might be related to an effect of APOE genotype on HPA axis activity. These investigators, however, did not examine the relationship between stress and HPA axis activity and how that relationship might be modified by APOE status.

Although little is known about how stress level and APOE status interact to influence glucocorticoid levels and cognition in humans, a number of studies with animals have addressed this question. Gordon et al. (22) found elevations in glucocorticoid (i.e., corticosterone) levels following constraint stress to be markedly lower in APOE-deficient mice than in control wild-type mice. Another group of investigators (23-24) observed a stronger corticosterone response in APOE-deficient mice compared to wild-type controls after repeated restraint stress, and suggested that exposure to chronic stress in APOE-deficient mice may be a causative factor, along with the aging process, in the early development of neurodegeneration. Subsequently, Grootendorst and colleagues (25-26) examined behavioral outcomes in APOE-knockout (APOE 0/0) mice and wild-type mice after repeated exposure to predator stress. Learning in the Morris Water Maze improved in the knockout mice and worsened in the wild type mice after predator stress, abolishing differences between the two groups in cognitive performance and corticosterone concentrations. The same result was obtained when the level of corticosterone was increased in these mice by implanting corticosterone pellets (27). These results suggest that the effect of stress on cognition is mediated by corticosterone, and that this effect is modified by the presence of APOE.

To our knowledge, only one study (28) has examined the relationship between stress and APOE status in humans. In a large sample of non-demented, female caregivers of patients with AD, an increased level of self-reported stress was associated with an increased level of depression only in those caregivers with at least one APOE-ε4 allele. While these results suggest that individuals with an APOE-ε4 allele react differently to stress than those without this allele, the study did not address the possibility that this relationship was mediated by an effect on glucocorticoid (i.e., corticosterone) levels or had an effect on cognition.

In the present study, we examined both separate and combined effects of stress and APOE-ε4 status on baseline measures of memory and salivary cortisol in older, non-demented subjects. Measures of cortisol can be influenced by many factors (e.g., age, gender, activity level, time of awakening) making it difficult to establish group differences (29). We will present our findings concerning cortisol, but as observations requiring further study. Stress was evaluated with the Life Events /Difficulties Schedule (LEDS) (30), a semi-structured interview that allows identification of recent, chronic difficulties and discrete events with short-term effects that are serious enough to cause prolonged threat. Memory was our primary focus due to the emphasis on memory in the stress literature and its salience in the neuropsychological profile of AD. We hypothesized that elderly individuals experiencing prolonged stress would show worse memory performance than those without significant stress, and that these relationships would be more robust in those with at least one APOE-ε4 allele than in those without an APOE-ε4 allele. This hypothesis is based on a model in which significant stress is thought to increase

cortisol levels, which coupled with advanced age and presence of an APOE-ε4 allele, accelerates hippocampal damage that underlies memory loss.

METHODS AND MATERIALS

Subjects

Participants, volunteers over the age of 65 and living independently, were recruited from the University of California, San Diego (UCSD) Shiley-Marcos Alzheimer's Disease Research Center (ADRC) and the UCSD Memory Screening Clinic. Potential subjects were excluded if at baseline they were found to have a significant medical (e.g., end-stage cancer) or psychiatric (e.g., psychosis) condition that would affect cognition. Individuals with moderate to severe depression, those with Post Traumatic Stress Disorder (31) currently or at any point in their medical history, and those using corticosteroid medications that could affect daily cortisol production were also excluded. Subjects using inhalers containing steroids were asked to forgo use of their inhaler for the day preceding and the day of sample collection if they felt comfortable doing so. We permitted use of topical corticosteroids such as hydrocortisone.

We enrolled 91 individuals with a mean age of 78.8 (± 6.0) and mean education of 15.7 (± 3.0). Sixty percent of subjects were female. Eighty-two percent of subjects were Caucasian, 12%, Hispanic, 3%, African American, and 2%, Asian. Mean score on a measure of global cognition (Mattis Dementia Rating Scale; DRS) (32) was 136.9 (± 5.0) out of 144 possible points. All subjects were functioning independently in instrumental activities of daily living (ADLs) measured by the Pfeffer Outpatient Disability Scale (PODS) (33). Based on extensive neurological and neuropsychological examinations, no subject met criteria for dementia as defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM), and none met NINCDS- ADRDA criteria for AD (34). We rated subjects on performance in five neuropsychological domains (i.e., memory, attention, executive functions, visuospatial ability, and language). Fifty-seven subjects performed normally in all areas of cognitive functioning, while the remaining 34 subjects showed deficits on neuropsychological testing. We determined that subjects had Mild Cognitive Impairment (MCI) if they had cognitive deficits but did not meet criteria for dementia (35). We classified 17 subjects as amnestic MCI, 14 as single non-memory domain MCI (11 executive functions, 3 visuospatial), and 3 as multiple domains MCI (35).

Procedure

Each subject received comprehensive medical, neurological, behavioral, and neuropsychological evaluations, as well as APOE genotyping. The LEDS provided a rating of chronic stress and required approximately one hour to complete (30,36). Behavioral measures requiring an additional hour were obtained during the same session. Neuropsychological testing was conducted in a separate session by a trained psychometrist and required two to three hours to complete. A one-hour medical and neurological examination comprised an additional session. A senior neurologist reviewed information from this examination and summary data from the neuropsychological examination in order to determine whether the subject met inclusion and exclusion criteria for the study.

Study personnel obtaining data were blind to subjects' APOE status and level of stress at the time of the evaluation. All subjects agreed to participate by signing a written consent approved by the UCSD Clinical Research Protection Program (CRPP). For subjects with evidence of impairment on neuropsychological testing, we identified "study partners", individuals with ongoing relationships with subjects and knowledge of their day-to-day activities. If present during the LEDS interview, the study partners completed a written informed consent; if

contacted by phone to obtain or verify information, they agreed to a CRPP-approved phone consent.

Assessment instruments were as follows—The LEDS (30) is a semi-structured interview that gathers information on a wide range of stressors and identifies serious chronic difficulties (lasting more than two weeks) or discrete events (lasting two weeks or less) serious enough to cause long-term threat. The subject is asked to identify events (e.g., hospitalization) that occurred during the previous 12 months. A list of specific events in 12 categories (e.g., Residence, Health) derived from the Psychiatric Epidemiology Research Interview (PERI) Life Events Scale (37) is shown to the subject to aid memory. Probes concerning contextual detail (e.g., nature of relationships involved) follow the identified experiences to construct a "story." While general guidelines direct the probes, the interview is designed to resemble conversation. No ratings are made at the time of the interview. Items include negative events, but also events involving transitions (births, promotions), circumstances eliciting emotional reactions, and persisting chronic difficulties. Upon completion, the interviewer verbally delivers the narratives to a professional trained to rate the degree of threat for specific types of events and difficulties according to rules produced for the LEDS by Brown and Harris (30). For each event, the ratings include: 1) degree of threat, 2) probable duration (short- or long-term), and 3) focus (self or other), as well as 4) level of severity of difficulties. According to detailed criteria, the rater determines an overall rating of High or Low stress reflecting the presence or absence of substantial long-term or chronic threat to the subject. Following the methods described by Grant et al. (38) and Brown and Harris (30), we used LEDS procedures to assign participants a High overall stress rating if they experienced one or more high stress event or difficulty.

The PODS (33) was administered to the subject's study partner to measure ADLs. The scale includes ten items (e.g., finances, shopping) with scores ranging from 0 to 2 and a higher score indicating greater difficulty.

Subjects completed the Geriatric Depression Scale (GDS) (39), a 30-item, self-rating questionnaire shown to be reliable and valid for assessment of depression in the elderly. A higher score indicates a greater number of symptoms of depression. Subjects and study partners also completed the Neuropsychiatric Inventory (NPI) (40), a 12-item scale evaluating the presence, severity, and frequency of neuropsychiatric symptoms (e.g., irritability, sleep disturbance). The sum of the products of the severity and frequency for each acknowledged symptom determines the score.

We used the self-report Form Y-2 of the State-Trait Anxiety Inventory (STAI) (41) to measure trait anxiety. According to Spielberger, trait anxiety refers to relatively stable individual differences between people in the tendency to perceive stressful situations as dangerous or threatening. The score ranges from 20 to 80; a higher score indicates a greater level of anxiety.

Tests of memory included the Visual Reproductions and Logical Memory subtests of the Wechsler Memory Scale-Revised (WMS-R) (42), the California Verbal Learning Test (CVLT) (43), and the DRS Memory subscale. The Visual Reproductions subtest consists of immediate and 30-minute delayed recall of four visual designs. The WMS-R Logical Memory subtest requires immediate and 30-minute delayed recall of two stories. The CVLT includes five learning trials for 16 words, free recall of the list after immediate and 30-minute delays, and delayed recognition.

APOE Genotyping

Genomic DNA was prepared from white blood cells using standard measures. The APOE gene was amplified by PCR using oligonucleotide primers (44). After amplification, DNA was

digested with the HhaI restriction enzyme, electrophoresed on 6% nondenaturating polyacrylamide gels, and visualized by ethidium bromide staining (45).

Cortisol Measures

We measured salivary cortisol levels to assess HPA axis function. Participants were given "Salivette" devices (Sarstedt, Rommeldorf, Germany) composed of cotton swabs in a plastic holder fitted inside a centrifuge tube, as well as detailed instructions for producing samples. Subjects collected five saliva samples at home on a day close to the time of the LEDS interview. Samples immediately after morning awakening, 30 minutes later, at 2 pm, at 4 pm, and just before going to bed provided information concerning the individual's circadian periodicity (46). The study partner was asked to help the subject remember each sample if needed. Samples were refrigerated until delivered to the UCSD General Clinical Research Center Core Laboratory for analysis and stored at -80° C.

Commercial salivary cortisol (enhanced range) enzyme immunoassay kits (Cat# 1-3002) were purchased from Salimetrics LLC, State College, PA. Samples were thawed, vortexed and centrifuged at 1500xg (@ 3000 rpm) for 15 minutes to sediment particulate matter. Samples, standards and controls were added to a microplate coated with monoclonal antibodies to cortisol. Cortisol-horseradish peroxidase conjugate was added to all wells and incubated at room temperature for one hour and unbound components were washed using Beckman-Coulter MW96W3 programmable washer. Bound cortisol-peroxidase was measured by the reaction of the peroxidase enzyme on tetramethylbenzidine (TMB) substrate. The reaction (blue color) was stopped by sulfuric acid and the resulting yellow color was read on Spectramax M-5 reader (Molecular Devices Inc., Sunnyvale, CA) at 450nm with a wavelength correction at 690nm. The concentrations of unknowns and controls were determined by using 4-parameter sigmoid minus curve fit (softmax Pro version 5.0). The amount of cortisol-peroxidase activity is inversely proportional to cortisol in sample. The intra and inter assay variations were 0.01-2.5% and 3.0-8.0% respectively. The within plate cv% between duplicates varied from 0.01 to 2.5%.

Statistical analyses

Seven subjects received a high stress LEDS rating based on events (e.g., recent MCI diagnosis) or difficulties (e.g., change in important activities) associated with cognitive dysfunction. All were classified as MCI, amnestic type. We made a decision to exclude them from the statistical analyses, since their inclusion in the high stress group based on cognitive (i.e., memory) decline in analyses in which the dependent variable was memory could lead to circular reasoning and misleading results. That is, placing a subject in the high stress group due to the consequences of memory loss could confound the results when comparing the low and high stress groups on memory. The remaining 84 subjects were divided into four groups on the basis of the LEDS stress rating (high versus low) and APOE-ε4 status (i.e., positive or negative for at least one ε4 allele): 1) High stress, APOE-ε4 positive (High, ε4-pos) (n=15), 2) High stress, APOE-ε4 negative (High, ε4-neg) (n=26), 3) Low stress, APOE-ε4 positive (Low, ε4-pos) (n=14), and 4) Low stress, APOE-ε4 negative (Low, ε4-neg) (n=29).

Since inclusion of subjects with MCI within groups divided by stress and APOE-ε4 could influence measures of cognition, numbers within each group were examined. Considering all subtypes of MCI, there were 8 (29.6%) in the High, ε4-pos group, 7 (25.9%) in the High, ε4-neg group, 5 (18.5%) in the Low, ε4-pos group, and 7 (25.9%) in the Low, ε4-neg group. Differences between the groups were not significant (Chi-square=4.3; p=.23). When only MCI amnestic, single domain and MCI amnestic, multiple domains subtypes were included, there were 4 (30.8%) in the High, ε4-pos group, 4 (30.8%) in the High, ε4-neg group, 2 (15.4%) in the Low, ε4-pos group, and 3 (23.1%) in the Low, ε4-neg group. Of the two subjects with a diagnosis of MCI-multiple domains, 1 was in the Low, ε4-neg group and 1 was in the High,

ε4-pos group. Groups differences in the number of MCI amnestic subjects were not significant (Chi-square=3.0; p=.39).

Univariate analyses of variance (ANOVA) were used to assess main and interaction effects of stress and APOE on measures of memory and cortisol, controlling for age, education, and gender. We used t-tests to determine whether differences between group means were significant. We employed SPSS 11.0 (47) for all statistical analyses. Subjects with cortisol levels greater than three standard deviations above the group mean were excluded for specific analyses; one was excluded for the awakening measure, one for the measure 30 minutes after awakening, one for the 2 PM measure, and two for the bedtime measure. No subjects were excluded for the 4 PM measure. Subjects differed on times of awakening and bedtimes on the day samples were collected, but there were no significant differences between the four groups divided by stress and APOE status on mean time of awakening or bedtime.

Since multiple comparisons run the risk of an increase in the number of type I errors, we considered the results in light of this potential limitation. Given the exploratory nature of the study, however, we kept the significance level at alpha=.05.

RESULTS

Table 1 shows means and standard deviations for demographic and cognitive variables for groups based on level of stress (High, Low) and APOE status (ε4-pos, ε4-neg). The groups did not differ significantly in years of education or global cognitive functioning on the Mattis DRS. A Stress × APOE status ANOVA on age showed a significant main effect of APOE status indicating that those with at least one ε4 allele were younger ($F(1,83)=5.9$; $p=.02$) than those with no ε4 allele. The main effect of Stress and the Stress × APOE interaction effect were not significant.

As shown in Table 2, the groups did not differ in scores on the GDS or the NPI. A Stress × APOE status ANOVA on the trait scores of the STAI showed that those with a High stress rating reported greater anxiety than those with a Low stress rating ($F(1,80)=4.1$; $p=.05$); however, means for both groups were well within normal limits based on normative data for older individuals (41). The main effect of APOE status and the Stress × APOE interaction effect were not significant.

Stress × APOE status ANOVAs on the immediate and delayed recall measures from the WMS-R Logical Memory Test showed significant main effects of Stress (immediate: $F(1,83)=3.9$; $p = .05$, delayed: $F(1,83)=4.6$; $p = .04$) and Stress × APOE interaction effects (immediate: $F(1,83)=5.2$; $p = .03$, delayed: $F(1,83)=5.5$; $p = .02$). As can be seen in Table 3, these results indicate that Logical Memory Test performance was worse in High Stress APOE-ε4 subjects than in any of the other groups. ANOVAs showed that High Stress subjects performed worse than Low Stress subjects on CVLT Long Delay Free Recall ($F(1,83)=6.0$; $p = .02$), and that APOE-ε4-pos subjects made more CVLT Recall Intrusion Errors than APOE-ε4-neg subjects ($F(1,83)=10.2$; $p < .01$). A High stress rating paired with at least one ε4 allele was associated with significantly more false positive errors (Interaction: $F(1,83)=4.3$; $p = .04$) in the CVLT Recognition Recall condition. Stress × APOE status ANOVAs on the immediate and delayed recall conditions of the WMS Visual Reproduction Test showed significant main effects of APOE status in both conditions indicating that APOE-ε4-pos subjects performed worse than APOE-ε4-neg subjects (immediate: $F(1,82)=7.5$; $p < .01$, delayed: $F(1,82)=7.5$; $p < .01$). There was also a significant main effect of stress in the delayed condition indicating that those with High Stress performed worse than those with Low Stress ($F(1,82)=4.4$; $p = .04$). A similar pattern was seen for the Mattis DRS Memory Subscale scores. APOE-ε4-pos subjects performed worse on this measure than APOE-ε4-neg subjects ($F(1,83)=4.3$; $p = .04$), and those

with High Stress performed worse than those with Low Stress ($F(1,83)=3.9$; $p = .05$), but there was no interaction between stress level and APOE status.

Stress × APOE status ANOVAs on each of the five salivary cortisol measures showed no significant main effects of Stress or APOE status. However, there was a significant Stress × APOE interaction effect for cortisol measured 30 minutes after awakening ($F(1,72)=7.4$; $p < .01$). As Table 4 shows, this result indicates that cortisol levels were higher in High Stress subjects than in Low Stress subjects in the APOE ε4-pos subjects, but did not differ in High Stress and Low Stress subjects who were APOE ε4-neg. In addition, cortisol levels 30 minutes after awakening were lower in Low Stress APOE ε4-pos subjects than in Low Stress APOE ε4-neg subjects. No other Stress × APOE interaction effect for cortisol measures was significant.

DISCUSSION

Elderly individuals with High stress due to the impact of recent "real life" events and difficulties had worse memory performance than those with Low stress. This finding is consistent with the results of studies assessing memory in humans exposed to stress in laboratory settings (6, 10-11) and extends the results to more naturalistic causes of stress. In addition, elderly individuals with at least one APOE-ε4 allele performed worse than those without an ε4 allele on several memory measures, consistent with a number of previous studies (13-16). A novel finding from the present study is that stress level and APOE genotype have an interactive effect in that High stress has a detrimental effect on certain aspects of memory performance only in APOE-ε4 positive elderly subjects. This interaction effect was observed in performance on the immediate and delayed conditions of the WMS-R Logical Memory Test and in the number of false positive errors produced on the delayed recognition trial of the CVLT, and is consistent with a model in which chronic stress in the presence of at least one ε4 allele can affect memory in the elderly over and above the effects of either factor alone.

A number of investigators have attempted to explain the mechanism by which memory loss occurs under specific conditions of age, stress level, and APOE status. The authors of a series of animal studies (25,27) have postulated that the effects of stress are mediated by glucocorticoids and depend on the presence of APOE. The effects of APOE may alter susceptibility to environmental factors such as stress, or the threshold at which stress can result in damage to neurons may differ according to which APOE isoforms are present.

Observations concerning cortisol level included a stress by APOE genotype interaction effect on the measure taken 30 minutes after awakening. Although the higher cortisol level in the High ε4-neg group was consistent with our expectations, other cortisol findings were not. For example, for three of the five cortisol measures, the mean level for the Low, ε4-neg group was numerically higher than those for the Low, ε4-pos and High, ε4-neg groups (although these differences were not statistically significant). In fact, in one case (bedtime sample), the level for the Low, ε4-neg group was the highest of the four groups.

The failure to find significant correlations between cortisol level and measures of memory dampens support for the idea that the effects of stress on memory are the result of the influence of cortisol on the hippocampus. While there may be factors that affect these relationships (e.g., mood, level of activity, medical illness) (29), there also may be other ways of interpreting the results. It is possible that Low, ε4-pos subjects in general have lower cortisol levels than the ε4-neg subjects, and that elevations in their cortisol levels occur only when these ε4-pos subjects are exposed to significant stress. There is also support for the possibility that the effects of elevated cortisol are apparent only after these levels are significantly elevated over a prolonged period of time (5). Clearly, further study of how cortisol is involved in the

relationship between stress and cognition, particularly memory, is needed to understand our observations.

As treatments for AD have become available, there have been numerous attempts to identify individuals with cognitive decline prior to a clinical diagnosis of dementia in order to intervene before symptoms progress. The most common approach has been the identification of individuals with MCI (48), a state in which the person has objective evidence of cognitive decline but does not meet criteria for dementia. More recently, however, the emphasis is shifting to the identification of groups vulnerable to AD prior to symptom onset. For example, our findings suggest that an older individual carrying an ε4 allele and experiencing significant stress is more vulnerable to cognitive impairment than subjects without an ε4 allele or without recent stressful events. Because APOE-ε4 status and level of event- and difficulty-based stress can be assessed at any point in time, this approach could have the advantage of earlier identification of cognitive vulnerability in the elderly. Whether this decline represents the earliest stages of AD or some other progressive neurodegenerative disorder awaits the results of longitudinal studies.

Several limitations in the present study should be noted. First, the results involved multiple statistical comparisons running the risk of an increase in Type I errors. If we had taken a more conservative approach and only considered a p-value of .025 as significant, a number of significant findings concerning memory would have remained. Caution, however, should be used in the interpretation of the results. A second limitation concerned the generalizability of the results, since subjects were primarily Caucasian with relatively high levels of education. Future studies with a more diverse subject sample could ensure that the present results are not specific to a particular group or a particular set of stressors. Finally, we did not take into account coping strategies that could influence responses to stress. However, we defined the type of stress we were measuring and assessed potential relationships with depression and anxiety, factors that might accompany stressful experiences. Although the High stress group reported more trait anxiety than the Low stress group, the level for both groups was within normal limits. The High and Low stress groups did not differ in their report of symptoms of depression. The results suggest that High stress is relatively independent of depression but may evoke a mild level of persisting anxiety. Future studies to determine if coping strategies can modify the impact of stress on cognition in individuals with the APOE-ε4 allele are warranted.

Since a portion of our sample was comprised of individuals with a diagnosis of MCI, the question arises as to whether the neuropsychological results attributable to the effects of stress and genotype may be a function of existing AD pathological changes in the MCI participants. Based on data presented by Morris and Price (49), it is likely that these individuals do have some degree of pathology associated with AD. Since the stress /genotype groups are reasonably matched on number of MCI participants, however, it is unlikely that AD pathology in the MCI subjects accounts for the results.

In summary, our study supports the notion that stress level alone can affect memory, as can possession of at least one APOE-ε4 allele. The results also provide evidence that cognitive functioning in older, non-demented individuals who possess at least one APOE-ε4 allele is more vulnerable to the negative effects of stress than those without an ε4 allele. Inexpensive, readily available strategies to reduce harmful responses to stressful experiences may prevent or slow progression of cognitive changes in genetically vulnerable, older individuals. Longitudinal data are needed to address the question of whether stress level and APOE status taken together are good predictors of cognitive decline and conversion to a clinical diagnosis of dementia.

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Table 1

Mean (standard deviation) and significance levels for main effects of demographic and cognitive variables for older, non-demented subjects divided on stress level and APOE-ε4 status.

	GROUPS				p-value	
	High Stress		Low Stress			
	ε4-pos	ε4-neg	ε4-pos	ε4-neg	Stress	APOE
	n=15	n=26	n=14	n=29		
Age (Yrs)	75.8 (4.7)	80.1 (6.3)	76.8 (8.2)	79.1 (4.8)	ns	.02
Education (Yrs)	15.2 (2.4)	16.2 (2.7)	14.4 (2.8)	16.1 (3.6)	ns	ns
Mattis DRS Total	135.3 (6.6)	138.4 (3.5)	137.9 (5.0)	137.3 (4.4)	ns	ns

DRS = Dementia Rating Scale

ε4-pos = ε4-positive

ε4-neg = ε4-negative

ns = non-significant

Table 2

Mean (standard deviation) and significance levels for main effects and interactions on behavioral scales in older, non-demented subjects divided on stress level and APOE-ε4 status.

	Groups				p-value		
					Main Effect		Interaction
	High Stress		Low Stress				
	ε4-pos	ε4-neg	ε4-pos	ε4-neg	Stress	APOE	
Geriatric Depression Inventory	4.9 (2.9)	6.6 (6.7)	5.3 (4.9)	4.3 (4.4)	ns	ns	ns
Trait Anxiety Inventory	32.8 (6.5)	36.9 (10.9)	31.2 (9.5)	30.4 (8.7)	.05	ns	ns
Neuropsychiatric Inventory	2.9 (6.3)	2.0 (3.3)	1.5 (2.8)	.48 (1.3)	ns	ns	ns

ε4-pos = ε4-positive

ε4-neg = ε4-negative

ns = non-significant

Table 3

Mean (standard deviation) and significance levels for main effects and interactions on memory measures in older, non-demented subjects divided on stress level and APOE-ε4 status.

	Groups				p-value		
					Main Effect		Interaction
	High Stress		Low Stress				
	ε4-pos	ε4-neg	ε4-pos	ε4-neg	Stress	APOE	
WMS-R Logical Memory							
Stories A & B - Immediate	19.2 (7.2)	26.2 (8.2)$^{\infty}$	26.4 (10.4)$^{\xi}$	25.6 (7.5)‡	**.05**	ns	**.03**
Stories A & B - Delay	14.1 (9.3)	21.5 (9.4)$^{\infty}$	23.3 (12.7)$^{\xi}$	21.1 (8.5)‡	**.04**	ns	**.02**
California Verbal Learning Test							
Learning Trials 1-5	40.7 (10.8)	39.5 (11.4)	45.8 (15.6)	43.4 (10.1)	.08	ns	ns
Long Delay Free Recall	7.1 (4.3)	6.9 (3.2)	9.5 (4.6)^{f}	8.6 (3.2)	**.02**	ns	ns
Recall Intrusion Errors	15.1 (17.4)	5.6 (6.0)	7.9 (9.1)	5.9 (4.9)	ns	**<.01**	.06
Recognition Discriminability	83.7 (12.7)	90.0 (8.6)	90.4 (12.5)	89.0 (8.2)	ns	ns	.09
Recognition False Positive Errors	4.7 (4.4)	2.0 (2.5)$^{\infty}$	2.7 (4.5)	3.2 (3.3)	ns	.06	**.04**
Wechsler Memory Scale							
Visual Reproduction Immediate	9.3 (3.5)	12.2 (2.9)$^{\infty}$	11.5 (3.4)	12.5 (2.8)‡	.07	**<.01**	ns
Visual Reproduction Delay	5.6 (4.3)	9.7 (4.3)$^{\infty}$	9.1 (4.3)$^{\xi}$	10.3 (3.6)‡	**.04**	**<.01**	ns
Dementia Rating Scale							
Memory Subscale	22.3 (3.2)	23.9 (1.7)	23.9 (2.3)	24.1 (.88)‡	**.05**	**.04**	ns

WMS-R = Wechsler Memory Scale - Revised

ε4-pos = ε4-positive

ε4-neg = ε4-negative

ns = non-significant

$^{\infty}$High ε4+ versus High ε4- p≤.05

‡High ε4+ versus Low ε4- p≤.05

$^{\xi}$Low ε4+ versus High ε4+ p≤.05

^{f}Low ε4+ versus High ε4- p≤.05

$^{\theta}$Low ε4- versus High ε4- p≤.05

Table 4

Mean (standard deviation) and significance levels for main effects and interactions on salivary cortisol concentrations obtained over the course of one day in groups divided by LEDS Stress rating and APOE status.

Cortisol Levels (nmol/L)	Groups				p-value		
					Main Effect		Interaction
	High Stress		Low Stress				
	ε4-pos	ε4-neg	ε4-pos	ε4-neg	Stress	APOE	
Just after awakening	7.8 (5.0)	8.3 (7.5)	6.3 (4.4)	11.2 (8.6)	ns	ns	ns
30 minutes after awakening	11.1 (5.4)	7.8 (6.1)	5.4 (5.2)$^{\xi}$	9.5 (5.9)$^{\partial}$	ns	ns	**<.01**
2:00 PM	4.8 (4.4)	4.5 (4.3)	3.1 (3.6)	3.2 (2.4)	ns	ns	ns
4:00 PM	3.6 (3.4)	4.3 (4.4)	3.7 (3.7)	2.7 (2.3)	ns	ns	ns
Just before Bed	1.8 (1.2)	3.1 (3.1)	2.3 (3.4)	3.7 (4.9)	ns	ns	ns

LEDS = Life Events and Difficulties Schedule

ε4-pos = ε4-positive

ε4-neg = ε4-negative

ns = non-significant

$^{\xi}$Low ε4 pos versus High ε4 pos p<.05

$^{\partial}$Low ε4 neg versus Low ε4 pos p<.05