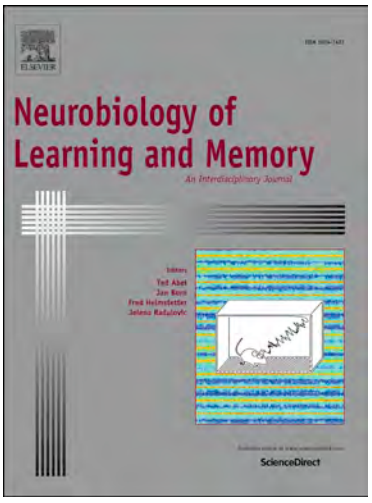

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PII: S1074-7427(18)30197-7
DOI: https://doi.org/10.1016/j.nlm.2018.08.013
Reference: YNLME 6923

To appear in: *Neurobiology of Learning and Memory*

Received Date: 8 January 2018
Revised Date: 2 August 2018
Accepted Date: 17 August 2018

Please cite this article as: Montoliu, T., Hidalgo, V., Pulopulos, M.M., Luis Ivorra, J., José Martínez, M., Salvador, A., The relationship between cortisol and cognitive function in healthy older people: the moderating role of ApolipoproteinE polymorphism, *Neurobiology of Learning and Memory* (2018), doi: https://doi.org/10.1016/j.nlm.2018.08.013

The relationship between cortisol and cognitive function in healthy older people: the moderating role of ApolipoproteinE polymorphism

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Abstract

The Apolipoprotein E4 (ApoE-ε4) allele has been suggested as the main risk factor for late onset Alzheimer's disease (AD), whereas the ApoE-ε2 allele has been proposed as a protective factor. These proposals have increased the interest in the effect of the ApoE genotype in healthy people. Additionally, high cortisol levels have been related to negative effects on cognition. However, few studies have investigated the relationship between cognitive performance and cortisol, taking into account the different ApoE alleles. For this reason, the aim of this study was to evaluate different cognitive domains (declarative and working memory, attention, and executive function) and their relationship with cortisol, considering the ApoE-ε2, ApoE-ε3, and ApoE-ε4 alleles in healthy older people (55-77 years old). Two saliva samples were collected during the neuropsychological session to obtain cortisol levels and the ApoE genotype. Results showed an association between the ApoE genotype and declarative memory, specifically learning ability, where ApoE-ε2 group performed better than ApoE-ε4 and ApoE-ε3 groups. No differences in cortisol levels were obtained considering the ApoE genotype. In addition, higher mean cortisol levels were related to a worse performance on declarative memory, for the whole sample, and when considering the three allelic variation, for the ApoE-ε4 group. On the contrary, an increase of cortisol levels during the neuropsychological session was associated to a better performance on declarative memory for the whole sample, and for the ApoE-ε3 group when considering the three alleles. Besides, ApoE-ε3 group also showed an association between higher mean cortisol levels and a better attention performance. Therefore, our results suggest that carrying the ApoE-ε4 allele may be a vulnerability factor in the adverse effects of Hypothalamic-Pituitary-Adrenal (HPA axis) dysregulation on cognition during aging, while ApoE-ε3 allele could be associated to a more adaptive HPA axis response.

Keywords: Apolipoprotein E, Cortisol, Cognition, Memory, Older people

1. Introduction

The increase in life expectancy has aroused interest in the biological mechanisms underlying cognition and its decline during aging (Payton et al., 2005). The Apolipoprotein E (ApoE) polymorphism has been proposed as a mediator of this age-related cognitive impairment (Helkala et al., 1996).

The ApoE gene has three common allelic variations (Bertram, Lill, &Tanzi, 2010), being the ApoE-ε3 the most common (78%), and the ApoE-ε2 and ApoE-ε4 alleles are less frequent (8% and 14%, respectively) (Menzel, Kladetzky, &Assmann, 1983). While the ApoE-ε4 allele presents the main risk factor for late-onset Alzheimer's disease (AD) (Bertram et al., 2010), the ApoE-ε2 allele has been associated with a reduced risk (Verghese, Castellano, & Holtzman, 2011). These findings have created interest in the role ApoE variants play in the cognitive functioning of healthy people. Although two meta-analyses reported that the ApoE-ε4 allele would have adverse effects on cognitive function in healthy elderly people (Small, Rosnick, Fratiglioni, & Bäckman, 2004; Wisdom, Callahan & Hawkins, 2011), a recent study on mid-adulthood supported comparable cognitive performance between ApoE-ε4 carriers and non-carriers (Lancaster, Tabet & Rusted, 2017). However, Lancaster et al. (2017) concluded that the cognitive performance profile of ApoE- ε4 carriers remains elusive. It is worth noting that there are some important differences among the empirical studies, such as the inclusion of preclinical dementia cases, the cognitive domains assessed, the age range of the participants, or the way of grouping the various types of allelic variations. Most of the studies group the participants as ApoE-ε4 carriers or non-carriers, without

considering the ApoE-ε2 allele independently, which could lead to an overestimation of ApoE-ε3 performance and an underestimation of ApoE-ε4 performance.

In addition to the effects of the ApoE, cognitive function is also affected by the Hypothalamic-Pituitary-Adrenal (HPA) axis function through the influence of its end product, cortisol. Several studies in healthy older people have reported an association between higher cortisol levels and worse memory performance at basal levels (Almela, van der Meij, Hidalgo, Villada, & Salvador, 2012; Hidalgo, Almela, Pulopulos, & Salvador, 2016) and in response to stress (Almela et al., 2011; Hidalgo, Almela, Villada, & Salvador, 2014). Furthermore, increased cortisol levels are a well-established feature of AD, although the mechanism responsible for HPA-axis hyperactivity is unknown (Gil-Bea et al., 2010; Peskind, Wilkinson, Petrie, Schellenberg, & Raskind, 2001). This fact, along with the evidence that ApoE-ε4 is a risk factor for developing AD (Bertram et al., 2010), suggests that the ApoE gene may affect the association between the HPA-axis and cognitive function (Peavy et al., 2007), as animal studies have shown (de Kloet, Grootendorst, Karssen & Oitzl, 2002; Grootendorst et al., 2002; Grootendorst, Enthoven, Dalm, de Kloet & Oitzl, 2004). Moreover, similar to preclinical brain changes related to AD, structural and functional abnormalities of the hippocampus have been found in healthy ApoE-ε4 carriers (Lu et al., 2011).

Nonetheless, studies exploring the associations among the ApoE genotype, cognitive function, and the HPA-axis in non-demented older people are scarce and report mixed results. Whereas some studies found an association in the ApoE-ε4 allele between an HPA-axis alteration and cognitive decline (Gerritsen, Comijs, Deeg, Penninx, & Geerlings, 2011; Lee et al., 2008; Peavy et al., 2007; Singh-Manoux et al., 2014), others failed to find this association (Berteau-Pavy, Park & Raber, 2007; Fiocco, Poirier, Joober, Nair & Lupien, 2008; Lara et al., 2013; Li et al., 2006). These different

findings could at least partly be explained by some methodological differences. Thus, participants with different age ranges (i.e. mean age over 55 (Fiocco et al., 2008), 60 (Fiocco et al., 2008; Lee et al., 2008; Singh-Manoux et al., 2014), 75 (Gerritsen et a., 2011; Li et al., 2006; Peavy et al., 2007), and 80 (Berteau-Pavy et al., 2007) years old) have been included in the studies. Another difference is the cognitive domain assessed, with declarative memory being studied the most (Fiocco et al., 2008; Gerritsen et al., 2011; Lee et al., 2008; Li et al., 2006; Peavy et al., 2007; Singh-Manoux et al., 2014), and only a few studies carried out on object recognition and spatial navigation (Berteau-Pavy et al. (2007) or attention and executive functioning (Lee et al., 2008; Li et al., 2006). In addition, most studies employed basal cortisol measures, and only two studies measured cortisol levels during the neuropsychological evaluation, with different results. Berteau-Pavy et al. (2007) found no association between ApoE alleles, cortisol levels, and cognition, whereas Lee et al. (2008) reported that, although higher cortisol was associated with lower cognitive performance, the slopes were steeper in the ApoE-ε4 group. However, these studies included different cognitive tests and inclusion criteria. More importantly, none of these studies considered the ApoE-ε2 allele. Therefore, more research is needed on the associations among the ApoE genotype, cognitive functioning, and cortisol levels during cognitive testing in healthy elderly people.

With all this in mind, we aimed to examine: (i) the differences in several cognitive domains (i.e. declarative and working memory, attention, and executive function) and cortisol levels during the neuropsychological assessment in different ApoE groups and (ii) the relationship between cortisol and cognitive performance, taking into account the ApoE groups, in non-stressed, healthy older people from 55 to 77 years old. To do so, a neuropsychological battery was administered to assess a wide

range of cognitive functions. Based on previous literature, we hypothesized that the ApoE-ε4 group would show worse cognitive performance, whereas the ApoE-ε2 group would show better performance (see Wisdom et al., 2011). We also hypothesized that there would be higher cortisol levels in the ApoE-ε4 group, compared to the ApoE-ε2 and ApoE-ε3 groups (Peskind et el., 2001). Finally, we expected to find an association between higher mean cortisol levels, as well as an increase of cortisol levels during the session, and a worse cognitive function, especially on declarative memory, and this association would be more pronounced in the ApoE-ε4 group (Gerritsen et al., 2011; Lee etal.,2008).

2. Methods

2.1. Participants

The final sample was composed of 84 participants of both sexes (40 men and 44 woman), ranging in age from 55 to 77 years (*M*= 65.18, *SD*= 4.631). Most of the participants had an educational level beyond high school (79.8%), and their subjective socioeconomic status was medium (*M*= 5.51, *SD*= 1.086) (subjective SES scale: Adler et al., 2000).

The total sample was categorized in three groups: ApoE-ε2 (n= 9; E2/E2=1 and E2/E3=8), ApoE-ε3 (E3/E3=59), and ApoE-ε4 (n=16; E4/E4=2 and E4/E3=14). Due to the small number of participants who were homozygous for the E2 and E4 alleles (E2/E2=1 and E4/E4=2), both the homozygous and heterozygous participants were grouped in the broad category of ApoE-ε2 and ApoE-ε4, respectively. The ApoE group frequencies were 10.7 % for ApoE-ε2, 70.2 % for ApoE-ε3, and 19 % for ApoE-ε4. There were no significant differences among the ApoE-ε2, ApoE-ε3, and ApoE-ε4

groups on sex, age, Body Mass Index (BMI), SES, or educational level (all $p > 0.115$) (Table 1).

	N (%)	Women (%)	Age (*M*)	(*SD*)	SES (*M*)	(*SD*)	BMI (*M*)	(*SD*)	Educational level
Total	84 (100%)	52.4%	65.18	4.631	5.51	1.086	27.34	3.248	79.8%
ApoE-ε2	9 (10.7%)	55.6%	62.78	3.383	5.67	.707	27.81	2.693	77.8%
ApoE-ε3	59 (70.2%)	49.2%	65.27	4.634	5.62	1.040	27.35	3.405	81.4%
ApoE-ε4	16 (19%)	62.5%	66.19	4.996	5.00	1.317	27.09	3.050	75%
		$p = .625$	$p = .203$		$p = .115$		$p = .881$		$p = .627$

Table 1. Descriptive data for the total sample and for each ApoE group (ApoE-ε2, ApoE-ε3, and ApoE-ε4). Age, SES (subjective socioeconomic status), and BMI (body mass index) are represented as mean values (*M*) and standard deviation (*SD*). Educational level is represented as the percentage of participants with an educational level beyond high school (%). Differences between ApoE status were analyzed with ANOVAs (age, SES, and BMI) and chi-square (sex and educational level) analyses. No differences in sex, age, SES, BMI, or educational level were found depending on the ApoE group (all $p \geq .115$).

All the women were postmenopausal and had their last menstrual period more than 3 years before the testing time. None of the participants scored less than 28 on the MEC (Spanish version of the Mini-Mental Status Examination; Lobo et al., 1999), indicating the absence of cognitive impairment.

Participants were Caucasian, and they were recruited from a study program at the University of Valencia for people over 55 years of age. Two hundred and twenty volunteers were interviewed by telephone in order to check whether they met the study prerequisites. Exclusion criteria were: smoking more than 10 cigarettes a day, alcohol or other drug abuse, visual or hearing problems, diabetes, neurological or psychiatric disease, using any medication directly related to emotional or cognitive functioning or able to influence hormonal levels such as glucocorticoids, psychotropic substances, or

sleep medications, having been under general anesthesia once or more than once in the past year, and the presence of a stressful life event during the past year.

One hundred twenty-eight individuals participated in the study, of whom 37 were eliminated because they did not meet the inclusion or exclusion criteria: 20 participants for using medication related to emotional or cognitive functioning or able to influence hormonal levels, such as glucocorticoids, psychotropic substances, or sleep medications; 7 participants for having diabetes; 4 participants due to severe and uncorrected visual or hearing problems; 4 participants for having a stressful life event during the past year; 1 for alcohol abuse; and 1 participant for having been under general anesthesia in the past year. Of the remaining 91 participants, the ApoE polymorphism of three participants could not be genotyped, and one participant was excluded for being ApoE-ε2/ε4. Finally, after dividing the sample according to the ApoE genotype (ApoE-ε2, ApoE-ε3 and ApoE-ε4), three participants in the ApoE-ε3 group were excluded from the analyses, two participants because their cortisol concentrations differed by more than 2.5 SD from the mean, and one participant because his age differed by more than 2.5 SD from the mean.

2.2. Procedure

Participants who met the criteria were asked to attend one session that took place from 10:00 to 12:00 hours in a laboratory at the Faculty of Psychology. Before the session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume alcohol from the night before the first session. They were also instructed to drink only water, and not eat, smoke, take any stimulants (such as coffee, cola, caffeine, tea or chocolate), or brush their teeth at least 1 hour prior to the session. All participants provided written

informed consent to participate in the study, which was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Research Ethics Committee of the University of Valencia. In this session, a neuropsychological battery was administered. Additionally, participants were asked to provide two saliva samples, from which the ApoE genotype and cortisol levels were extracted. The first saliva sample was collected at the beginning of the neuropsychological evaluation (pre-test cortisol), which took place 15 minutes after the participant's arrival. The second saliva sample was collected at the end of the neuropsychological evaluation (post-test cortisol), which took place 1 hour and 25 minutes after the beginning of the neuropsychological evaluation.

2.3. Neuropsychological tests

2.3.1. Declarative memory

2.3.1.1. Rey Auditory Verbal Learning Test (RAVLT). The Spanish version of the RAVLT (Miranda & Valencia, 1997) was used. This task consists of a target list (List A) of 15 neutral words repeated five times by the experimenter (trials I–V: Total Learning) that participants had to learn. Then, an interference list (List B) was presented only once, and participants had to repeat it. Participants were asked to recall the target list again immediately after the interference list (trial VI), and again after a delay of 20 min (trial VII). Three outcomes were used in subsequent analyses: (i) RAVLT Total Learning: total number of words recalled on the first five trials (trial I to V); (ii) RAVLT Immediate Recall: percentage of total number of words recalled after the interference trial compared to the number of words recalled on trial V (trial VI/trial V x 100); and (iii) RAVLT Delayed Recall: percentage of total number of words recalled

after the 20-min delay compared to the number of words recalled on the immediate recall trial (trial VII/trial VI x 100).

2.3.1.2. Rivermead Stories Subtest. The Story recall subtest from the Spanish version of the Rivermead Behavioral Memory Test (Wilson, Cockburn, &Baddeley, 1985) was used. The experimenter read aloud two short stories, and participants had to recall as many memory units or ‘‘ideas’’ as possible immediately after their oral presentation and after a 20-min delay. Participants’ answers were audio recorded and corrected by an expert, and the sum of the correctly recalled ‘‘ideas’’ from the two stories was calculated. From this test, two outcomes were used for the subsequent analysis: (i) Rivermead Immediate recall: total “ideas” recalled from the two stories immediately after the oral presentation and (ii) Rivermead Delayed recall: total “ideas” recalled from the two stories after 20 min, compared to the number of “ideas” recalled from the two stories immediately after the oral presentation (Delayed recall/ Immediate recall x100).

2.3.2. Working memory (WM)

2.3.2.1. Digit Span (DS). The Spanish version of the Wechsler Memory Scale III was administrated (Wechsler, 1997). The experimenter read aloud a series of numbers (from 0 to 9) of increasing length (from 2 to 9 digits) at a rate of one digit per second. The participant had to repeat the numbers, first in the same order (DS-Forward) and then in reverse order (DS-Backward). Each set length was tested twice. The test was finalized when the participant failed two consecutive trials of the same length. For each correctly repeated digit set, one point was given. Two outcomes were obtained: (i) DS-Forward: total number of correctly recalled attempts in the same order and (ii) DS-Backward: total number of correctly recalled attempts in the reverse order. DS-Forward was used as a measure of the attention and memory span component of WM, whereas

DS-Backward was used as a measure of the executive component of WM (Conklin, Curtis, Katsanis, Iacono, 2000).

2.3.2.2. Letter-number sequencing (LNS). The Spanish version of the Wechsler Memory Scale III was administrated (Wechsler, 1997). The experimenter read aloud a series of mixed numbers (from 0 to 9) and letters (from A to Z) of increasing length (from 2 to 8 items). The participant had to repeat the series, ordering the numbers in ascending order and the letters in alphabetical order. Each set length was tested three times. The test was finalized when the participant failed three consecutive trials of the same length. One point was given for each correctly recalled attempt. One outcome was obtained: LNS (total number of correctly recalled attempts).

2.3.3. Executive Function

2.3.3.1. Trail-Making Test (TMT). The TMT (Reitan, 1992) consists of two trials, TMT-A and TMT-B, each composed of 25 circles distributed on a white sheet of paper. In TMT-A, the circles were numbered from 1 to 25, and the participant was asked to trace a line connecting the circles in numerical sequence as quickly as possible. TMT-B included numbers from 1 to 13 and letters from A to L, and the participant was instructed to alternate between numbers and letters in ascending sequence. The score obtained was the number of seconds required to finish each trial. Errors were pointed out instantly by the examiner and contributed to the score, due to the additional time needed for corrections. Two outcomes were obtained: (i) TMT-A: total number of seconds required to finish the TMT-A, and (ii) TMT-B: total number of seconds required to finish the TMT-B. The TMT-A was used to assess attention and general psychomotor speed, whereas the TMT-B was used to evaluate attention-switching performance.

2.3.3.2. Stroop Color-Word Interference test. Golden's version of the Stroop Color-Word Interference Test (Golden, 1978) was administered. The test is composed of three trials. In each trial, participants had to name as many words as possible in 45 seconds. In the first trial, participants had to read the written word (W), which was red, blue, or green. In the second trial, participants had to name the printed color (C), red, blue, or green, of the XXX letters. In the third trial, participants had to name the color of the printed word (red, blue, or green), which was different from the written word (red, blue, or green) (WC), for example, the word green printed in red color. Afterwards, the WC' was calculated (WC' = (W x C) / (W + C)). Finally, the Stroop Interference outcome was obtained (Stroop Interference = WC – WC'), which is a measure of the ability to inhibit automatic responses.

2.4. Biochemical analyses

Participants provided two saliva samples by using salivettes (Sarstedt, Nümbrecht, Germany), the first one at the beginning of the neuropsychological assessment, and the second one at the end of the session. Participants were instructed to keep the cotton swab in their mouths for exactly 2 min, not chew the cotton, and move the swab around in a circular pattern to collect saliva from all salivary glands. The samples were centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity that was stored at -80^{o}C until the analyses of cortisol and ApoE genotype determination were performed.

2.4.1. Salivary Cortisol

The activity of the HPA-axis was measured by analyzing the salivary cortisol levels. Each sample was measured in duplicate, and each participant's samples were analyzed in the same trial. The salivary cortisol samples were analyzed by a competitive

solid phase radioimmunoassay (tube coated), using the commercial kit Spectria Cortisol RIA (cat. Nu 06119) from Orion Diagnostica (Espoo, Finland). Assay sensitivity was 0.8 nmol/L, and the within- and inter-assay variation coefficients were all below 8%. Salivary cortisol levels were determined in the Central Research Unit of the Faculty of Medicine, University of Valencia (Spain).

2.4.2. ApoE genotype determination

To determine the ApoE genotype, the genomic deoxyribonucleic acid (DNA) was isolated from the saliva by using a standard commercial extraction method (KIT REALPURE "SSS"). The genotype of each ApoEpolymorphism was amplified by the polymerase chain reaction (PCR) using two primers: Forward (ACAGAATTCGCCCCGGCCTGGTACAC) and Reverse (TAAGCTTGGCACGCCTGTCCAAGGA). Subsequently, DNA was digested with the *Hhal* restriction enzyme. Next, DNA electrophoresis in 2% agarose gel was performed. Finally, after ethidium bromide staining, the DNA-banding patterns (ApoE-ε2, ApoE-ε3 and ApoE-ε4) were visualized under the ultraviolet lamp and recorded for further analysis. The ApoE genotype was determined at the Department of Genetics of the University of Valencia (Spain).

2.5. Statistical Analysis

Participants' characteristics were described using percentages or means (standard deviation, SD) when appropriate, according to the ApoE groups (ApoE-ε2, ApoE-ε3, and ApoE-ε4).

To investigate whether there were differences between the ApoE groups in age, BMI, and SES, one-way analyses of variance (ANOVAs) were performed. Pearson's Chi-square test was used to assess differences in sex and education level.

Before statistical analyses were performed, cortisol data were checked for normal distribution and homogeneity of variance using Shapiro-Wilks and Levene's test. These analyses revealed significant deviations in cortisol values; therefore, cortisol data were logarithm 10 (Log10) transformed. The *mean cortisol* index was obtained averaging pre-test and post-test cortisol levels and the *delta cortisol* index by subtracting post-test cortisol minus pre-test cortisol levels.

To investigate whether there were differences in cognitive performance and cortisol levels among the ApoE groups, we performed one-way ANOVAs. As dependent variables, we used the following outcomes from the (i) RAVLT: RAVLT Total Learning, RAVLT Immediate recall, and RAVLT Delayed recall, (ii) Rivermead stories: Rivermead Immediate recall and Rivermead Delayed recall, (iii) DS: DS-Forward and DS-Backward, (iv) LNS, (v) TMT: TMT-A and TMT-B, (vi) Stroop interference, and (vii) mean cortisol. *Post-hoc* comparisons were performed using Bonferroni adjustments for the *p* values.

In addition, to analyze the change in cortisol levels during the neuropsychological assessment an ANOVA for repeated measures analysis was performed including Time (pre-test and post-test cortisol) as a within-subject factor and ApoE group as an between-subject factor. These analyses were performed unadjusted and adjusted for the covariates age, sex, SES and IMC.

Next, to investigate whether an association exists between cortisol levels and the different cognitive domain outcomes, linear regressions were performed, unadjusted and adjusted for covariates, as previous studies showed that cortisol levels and/or cognition could be affected by sex (Almela et al., 2011), age (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Van Hooren et al., 2007), SES (Cohen, Doyle, & Baum, 2006), level of education (Sattler, Toro, Schönknecht & Schröder, 2012), and

BMI (Champaneri et al., 2013; Cournot et al., 2006). Thus, separate analyses were performed for each cognitive domain as dependent variable. For unadjusted analyses, we included cortisol indexes (mean or delta) in step one. For adjusted analyses, we included sex, age, BMI, SES, and educational level as covariates in step one, following stepwise analysis. In step 2, cortisol indexes (mean or delta) were included. Finally, in order to investigate whether the relationships between the cortisol levels and the different cognitive domain outcomes were different depending on the ApoE group, these analyses were repeated with the sample divided into ApoE groups.

All *p* values were two-tailed, and the level of significance was taken as $p<0.05$. To perform these statistical analyses, version 22.0 of SPSS was used.

3. Results

3.1. ApoE group differences in cognitive function

One-way ANOVAs revealed significant effects of the ApoE groups on Total Learning from the RAVLT test ($F(2, 81) = 4.191$, $p = .019$). Post hoc analysis revealed significant differences between the ApoE-ε2 and ApoE-ε4 groups ($p = .015$), showing that the ApoE-ε2 group obtained higher scores than the ApoE-ε4 group. A marginal difference was also observed, where the ApoE-ε2 group obtained higher scores than the ApoE-ε3 group ($p = .065$) (Figure 1). None of the other cognitive domain outcomes showed significant differences among the ApoE groups. For the rest of the tests that evaluated declarative memory, no significant differences were found between the ApoE groups on immediate ($F(2, 81) = 1.298$, $p = .279$) and delayed recall ($F(2, 81) = 2.552$, $p = .084$) from the RAVLT test, or immediate ($F(2, 80) = .034$, $p = .967$) and delayed recall ($F(2,80) = .791$, $p = .457$) from the Rivermead test. Likewise, no significant differences were found between the ApoE groups on the tests that evaluated working

memory, such as the DS-Forward (F (2, 81) = 1.113, *p*= .333), DS-Backward (F (2, 80) = .228, *p* = .796), and LNS (F (2, 81) = .694, *p* = .503). Finally, no significant differences were found between the ApoE groups on the tests that assessed executive function attention, such as TMT-A (F (2, 80) =1.212, *p* = .303), TMT-B (F (2, 80) = .329, *p* = .720), and Stroop interference (F (2, 78) = .360, *p* = .699).

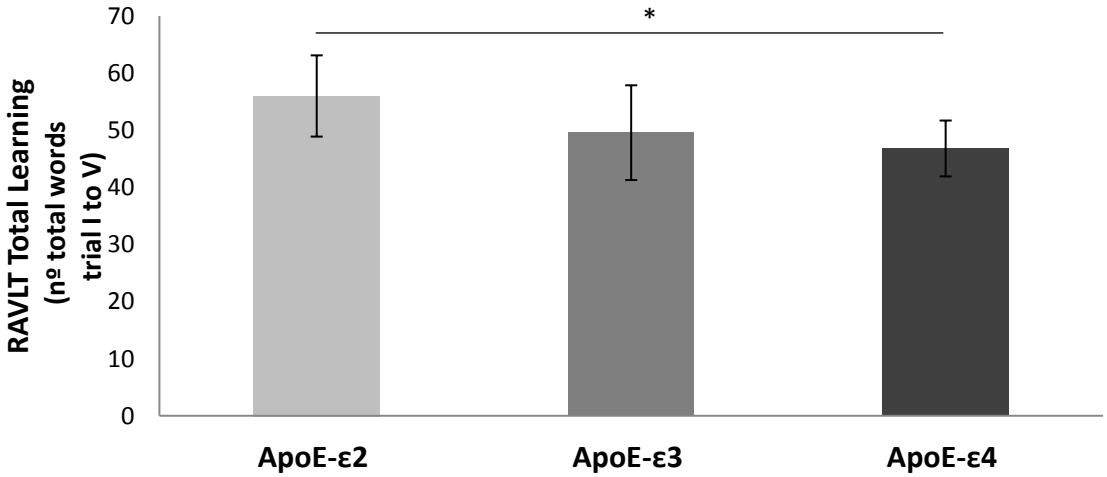

Figure 1. Mean performance on RAVLT Total Learning according to APOE group. One-way ANOVAs showed significant differences in declarative memory performance between ApoE groups (*p*=.019). Post hoc analysis showed better performance in the ApoE-ε2 group compared to ApoE-ε4 (**p*=. 015). Although without reaching significance, a trend was also observed where the ApoE-ε2 group performed better than the ApoE-ε3 group (*p*=.065).

3.2. ApoE group differences in cortisol levels

The results showed no significant differences among the ApoE groups in mean cortisol levels (F (2, 80) = 1.351, *p*= .265) (Table 2).

	Total		**ApoE-ε2**		**ApoE-ε3**		**ApoE-ε4**	
	M	*SEM*	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
Mean cortisol	5.747	.286	6.437	.651	5.733	.317	5.406	.887

Table 2. Cortisol data (nmol/L) for the total sample and for each ApoE group (ApoE-ε2, ApoE-ε3, and ApoE-ε4) are represented as mean values (*M*) and standard error of mean (*SEM*).

The ANOVA for repeated measures showed an effect of Time (F (1, 80) = 5.483, p =.022), where cortisol levels decreased along the session. On the contrary, no interaction Time*ApoE group (F (2, 80) = .579, p=.563) was observed. These results were similar after controlling for age, sex, SES and BMI (Time: F (1, 74) = 4.396, p =.039; Time*ApoE group: F (2, 74) = 1.607, p =.207).

3.3. Relationship between cortisol levels and cognitive function

Unadjusted regression analyses showed, for the complete sample, a negative association between mean cortisol levels and the delayed recall on the RAVLT test (p = .009), and marginally with immediate recall on the Rivermead story test (p = .077). Besides, a negative association was observed between mean cortisol levels and time performing the TMT A test (p = .037). In addition, delta cortisol was positively associated with time performing the TMT B (p = .038) and, marginally, with delayed recall on the RAVLT test (p = .055).

After repeating these analyses considering the ApoE groups, only the ApoE-ε4 group showed a negative association between mean cortisol levels and delayed recall on the RAVLT (p = .021) and immediate recall on the Rivermead story test (p = .012). On the other hand, only the ApoE-ε3 group showed a negative association between mean cortisol levels and time performing the TMT A (p = .019). Also, in this group a positive association between delta cortisol and delayed recall on the RAVLT (p = .023) and time performing the TMT B (p = .039), as well as a negative association between delta cortisol and DS-Forward performance (p = .049) were found.

Adjusted regression analyses showed that, for the complete sample, higher mean cortisol levels were related to worse performance on delayed recall on the RAVLT test (p = .009) and immediate recall on the Rivermead story test (p = .026). In turn, a trend

was observed where higher cortisol levels were associated with lower times performing the TMT A (p = .060) and, therefore, better performance. Regarding the delta index, an increase of cortisol levels during the session was related to a better performance on delayed recall on the RAVLT test (p = .028) and, marginally, to learning ability on the RAVLT test (p = .052).

After repeating these analyses considering the ApoE groups, only the ApoE-ε4 group showed a negative association between mean cortisol levels and performance on delayed recall on the RAVLT test (p = .015) and immediate recall on the Rivermead story test (p = .008). In addition, for the ApoE-ε3 allele it was observed a negative association between mean cortisol levels and time performing the TMT A (p = .024). Besides, only the ApoE-ε3 group showed a positive association between delta cortisol and performance on delayed recall on the RAVLT test (p = .007), as well as a trend with performance on learning ability on the RAVLT test (p = .093). None of the other associations were significant (all p> .110) (Table 3 and 4).

		RAVLT Total Learning	RAVLT Immediate Recall	RAVLT Delayed Recall	Rivermead Immediate Recall	Rivermead Delayed Recall	DS-Forward	DS-Backward	LNS	TMT-A	TMT-B	Stroop Interfe-rence
Unadjusted analyses												
Total	AdjR2	.004	-.007	**.069**	**.026**	-.012	-.012	-.012	-.012	**.041**	-.011	-.012
	Beta	-.126	.069	**-.284**	**-.196**	-.006	.020	.006	-.010	**-.230**	.033	.024
	p	.256	.533	**.009**	**.077**	.956	.858	.958	.928	**.037**	.768	.830
ApoE-ε2	AdjR2	-.069	-.142	-.086	.164	-.125	-.081	-.053	.092	-.142	-.141	-.141
	Beta	-.254	-.020	.223	-.518	-.126	.233	.281	-.454	-.024	-.036	.044
	p	.510	.960	.564	.153	.747	.546	.464	.220	.951	.926	.910
ApoE-ε3	AdjR2	.028	-.017	.017	-.017	.005	.009	-.006	-.017	**.079**	-.017	-.014
	Beta	-.212	-.019	-.185	-.027	-.150	.163	.111	.026	**-.309**	-.028	-.070
	p	.110	.885	.165	.841	.265	.222	.409	.847	**.019**	.837	.611
ApoE-ε4	AdjR2	-.056	.034	**.277**	**.325**	.024	.073	.111	-.065	-.071	.068	.083
	Beta	-.119	.314	**-.570**	**-.609**	.298	-.368	-.413	-.075	-.003	.361	.379
	p	.661	.237	**.021**	**.012**	.263	.161	.112	.783	.990	.170	.147
Adjusted analyses for covariates												
Total	AdjR2	.003	-.005	**.072**	**.227**	-.013	.364	.163	.285	**.085**	.193	-.012
	Beta	-.125	.085	**-.289**	**-.224**	-.016	-.015	-.022	-.030	**-.206**	.062	.033
	p	.267	.452	**.009**	**.026**	.888	.867	.828	.751	**.060**	.544	.771
ApoE-ε2	AdjR2	-.160	-.153	-.121	-.047	-.071	-.111	-.127	.551	-.088	-.167	-.124
	Beta	-.073	.109	.197	-.320	-.287	.218	.185	-.394	.260	-.003	.192
	p	.863	.798	.639	.440	.491	.605	.662	.189	.535	.995	.649
ApoE-ε3	AdjR2	**.032**	-.018	.019	.102	.009	.390	.180	.376	**.074**	.056	-.015
	Beta	**-.221**	-.004	-.190	-.086	-.163	.091	.124	-.038	**-.302**	.015	-.063
	p	**.098**	.974	.156	.505	.229	.394	.314	.723	**.024**	.909	.653
ApoE-ε4	AdjR2	.161	.034	**.548**	**.605**	.261	.640	.280	-.065	.190	.068	.083
	Beta	-.032	.314	**-.491**	**-.518**	.221	.010	-.250	-.075	-.096	.361	.379
	p	.895	.237	**.015**	**.008**	.343	.956	.303	.783	.690	.170	.147

Table 3. Regression analyses with mean cortisol as predictor and cognitive outcomes as dependent variables, unadjusted and adjusted for covariates. Values in bold represent significant or marginal *p* values.

		RAVLT Total Learning	RAVLT Immediate Recall	RAVLT Delayed Recall	Rivermead Immediate Recall	Rivermead Delayed Recall	DS-Forward	DS-Backward	LNS	TMT-A	TMT-B	Stroop Interfe-rence
Unadjusted analyses												
Total	AdjR2	.013	-.009	**.033**	-.008	-.002	.014	-.010	.014	-.011	**.041**	-.013
	Beta	.158	.058	**.211**	-.065	-.100	-.162	.049	-.162	.038	**.230**	.014
	p	.154	.604	**.055**	.564	.371	.143	.661	.143	.736	**.038**	.904
ApoE-ε2	AdjR2	-.135	-.085	-.012	-.098	-.045	-.143	-.143	-.003	-.116	-.083	-.142
	Beta	.084	-.225	.339	-.198	-.293	-.001	.012	-.305	.154	.228	.022
	p	.830	.560	.372	.609	.444	.997	.976	.356	.693	.555	.955
ApoE-ε3	AdjR2	.011	-.007	**.073**	-.017	-.016	**.051**	-.018	.024	-.014	**.059**	-.019
	Beta	.168	.105	**.299**	-.030	-.042	**-.260**	.024	-.204	.064	**.275**	-.002
	p	.207	.433	**.023**	.825	.757	**.049**	.860	.125	.636	**.039**	.991
ApoE-ε4	AdjR2	-.071	-.066	-.057	-.064	-.018	-.071	-.054	-.068	-.071	-.059	-.070
	Beta	-.006	.071	-.116	-.081	-.224	-.023	.126	.057	-.020	.109	.039
	p	.983	.794	.669	.765	.404	.932	.642	.835	.941	.689	.886
Adjusted analyses for covariates												
Total	AdjR2	**.035**	-.013	**.048**	.182	-.007	.365	.190	.284	.042	.191	-.013
	Beta	**.216**	.008	**.245**	.083	-.074	.035	.165	.009	-.017	.045	-.004
	p	**.052**	.945	**.028**	.425	.514	.700	.112	.927	.876	.666	.973
ApoE-ε2	AdjR2	-.094	-.136	-.044	-.167	.026	-.166	-.160	.418	-.009	-.085	-.152
	Beta	.249	-.162	.324	.009	-.407	-.031	-.077	-.231	.367	.265	.112
	p	.551	.702	.434	.983	.318	.943	.885	.460	.371	.527	.792
ApoE-ε3	AdjR2	**.033**	-.017	**.107**	.103	-.018	.383	.197	.375	-.018	.075	-.018
	Beta	**.224**	.034	**.351**	.092	.016	-.035	.182	-.008	.015	.137	-.041
	p	**.093**	.799	**.007**	.480	.910	.750	.146	.938	.915	.300	.769
ApoE-ε4	AdjR2	.161	-.066	.304	.306	.248	.669	.247	-.068	.183	-.059	-.070
	Beta	.027	.071	-.156	-.041	-.189	.202	-.186	.057	-.054	.109	.039
	p	.909	.794	.483	.851	.415	.349	.480	.835	.820	.689	.886

Table 4. Regression analyses with delta cortisol as predictor and cognitive outcomes as dependent variables, unadjusted and adjusted for covariates. Values in bold represent significant or marginal *p* values.

Finally, to investigate whether the significant relationships between mean or delta cortisol and the cognitive outcomes were statistically different for the three groups, we conducted regression models using PROCESS (v2.13.6). To do this, separately regression models with each cognitive measure tested as the dependent variable (i.e., RAVLT delayed recall, Rivermead Immediate recall or TMT A), the cortisol indexes (mean or delta) as the independent variables, the ApoE group (ApoE-ε2, ApoE-ε3, and ApoE-ε4) as the moderator variable, and age, sex, BMI, SES and educational level as covariates were performed.

In order to decompose the significant interactions between ApoE and the regression estimates, we ran a second model with the two ApoE dummy varibles as moderator variables (ApoE-ε2 vs ε3 and ApoE-ε2 vs ε4; ApoE-ε3 vs ε2 and ApoE-ε3 vs ε4; ApoE-ε4 vs ε2 and ApoE-ε4 vs ε3, depending on the allele where the significant relationship had been observed), and age, sex, BMI, SES, and educational level as covariates. The regression model for RAVLT delayed recall revealed a main effect of mean cortisol (p=.013), a main effect of ApoE (p=.012), and a significant mean cortisol*ApoE interaction (p=.013). By decomposing this interaction for RAVLT delayed recall, we did not obtain a significant mean cortisol*ApoE-ε4 vs ε2 (p=.156) interaction, or a mean cortisol*ApoE-ε4 vs ε3 (p=.085) interaction, although a trend was observed in the latter. By contrast, we did not find a significant mean cortisol*ApoE interaction for Rivermead Immediate recall (p= .464) or the TMT A (p=.640), nor a significant delta cortisol*ApoE interaction for RAVLT delayed recall (p=.360) (Table 5). It is important to note that, given our sample size and the different proportions of participants in each ApoE group, there may not be enough statistical power to detect some interactions. Therefore, these exploratory results should be interpreted with caution.

	RAVLT delayed recall			Rivermead immediate recall			TMT A		
	Beta	p	95% CI	Beta	p	95% CI	Beta	p	95% CI
Mean cortisol	**110.126**	**.043**	3.481, 216.771	7.680	.688	-30.330, 45.691	-33.542	.421	-116.301, 49.216
ApoE	**31.608**	**.012**	7.051, 56.165	3.949	.371	-4.800, 12.699	-3.172	.740	-22.230, 15.885
Mean cortisol*ApoE	**-41.062**	**.013**	-73.459, -8.665	-4.252	.464	-15.793, 7.288	5.916	.640	-19.225, 31.057
Mean cortisol	**-49.719**	**.004**	-83.242, -16.196						
ApoE-ε4 vs ε2	-52.174	.188	-130.610, 26.262						
ApoE-ε4 vs ε3	**-30.050**	**.037**	-58.328, -1.773						
Mean cortisol*ApoE-ε4 vs ε2	69.679	.156	-27.400, 166.758						
Mean cortisol*ApoE-ε4 vs ε3	**34.897**	**.085**	-4.986, 74.781						
Delta cortisol	59.664	.183	-28.856, 148.185						
ApoE	3.425	.288	-2.953, 9.804						
Delta cortisol*ApoE	-13,667	.360	-43.248, 15.914						

Table 5. Regression analyses with cortisol (mean or delta), ApoE, and cortisol (mean or delta) *ApoE as predictors, and cognitive outcomes (RAVLT delayed recall, Rivermead immediate recall and TMT A) as dependent variables, adjusted for covariates. Decomposition of significant mean cortisol*ApoE interactions were performed with regression analyses with mean cortisol, ApoE-ε4 vs ε2 and ApoE-ε4 vs ε3, and mean cortisol*ApoE-ε4 vs ε2 and mean cortisol*ApoE-ε4 vs ε3 as predictors, and RAVLT delayed recall, as dependent variable, adjusted for covariates. Values in bold represent significant or marginal p values.

4. Discussion

We aimed to examine the differences between the ApoE groups in cognitive performance and cortisol levels during the neuropsychological assessment in healthy older people. Our second aim was to analyze the association between cortisol levels and cognitive performance, taking into account the three ApoE groups. To do so, we explored these possible relationships, first in the complete sample and then for each ApoE group.

Regarding our first aim, our results showed significant differences among the ApoE groups only on declarative memory, where, consistent with previous results, ApoE-ε2 allele carriers performed better than the other allele groups (Wisdom et al., 2011); specifically, Helkala et al. (1995; 1996) also observed better learning ability in the ApoE-ε2 group, but in a sample of older elderly people (mean age 74 years old). However, contrary to what was reported by Wisdom et al. (2011) in their meta-analysis, we found no differences in declarative memory between the ApoE-ε3 and ApoE-ε4 groups. It is worth noting that, more recently, Lancaster et al. (2017) did not report differences in cognitive function between ApoE-ε4 carriers and non-carriers in mid-adulthood (mean age between 35 and 60 years). In addition, in our study, all participants underwent very restrictive criteria (28 on the MEC), indicating no cognitive impairment, whereas it has been reported that cognitive deficits related to the ApoE-ε4 allele could be due to the inclusion of preclinical cases of AD (Batterham, Bunce, Cherbuin, & Christensen, 2012).

No differences were found among the ApoE groups in mean cortisol levels, coinciding with a lack of association between serum or salivary cortisol levels and the ApoE genotype in AD and healthy controls (Fiocco et al., 2008; Lara et al., 2013; Li et

al., 2006). However, an association between higher CFS cortisol levels and the ApoE-ε4 allele was also found in AD patients (Gil-Bea et al., 2010; Peskind et al., 2001) and healthy elderly people (Peskind et al., 2001). As cortisol secretion follows a diurnal rhythm, with a peak after awakening and a steady decline throughout the day (Edwards, Evans, Hucklebridge, & Clow, 2001), in our study cortisol levels decreased during the session. However, we did not observe an effect of ApoE group, suggesting that no differences between groups occur in cortisol secretion. However, it is important to note that this result may also be due to the relatively small sample size of the groups, and more research is needed.

Regarding our second aim, for the complete sample, higher mean cortisol levels were associated with worse declarative memory performance, which is consistent with previous studies (Lee et al., 2007; Li et al., 2006; MacLullichet al., 2005; Seeman, McEwen, Singer, Albert, & Rowe, 1997; Vedhara, Hyde, Gilchrist, Tytherleigh, & Plummer, 2000; Wright, Kunz-Ebrecht, Iliffe, Foese, & Steptoe, 2005). In addition, a trend was observed where higher mean cortisol levels were associated with less time performing the TMT-A and, therefore, better attention. Glucocorticoid receptors are widely distributed in the hippocampus and prefrontal cortex, which are involved in declarative memory and attention and executive function, respectively (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). An inverted-U pattern has been observed in the association between cortisol and cognition, suggesting that very low or high glucocorticoids levels would worsen cognitive performance, while medium levels would facilitate it (de Kloet, Oitzl, & Joëls, 1999; Lupien et al., 2007). Therefore, our results suggest that the point at which cortisol levels begin to impair cognitive performance would be different for declarative memory and attention, justifying the fact that higher cortisol levels worsened declarative memory but improved attention. On the

other hand, although higher mean cortisol levels worsened declarative memory performance, it was also observed that a progressive increase along the session improved it. Hence, these results would also support the inverted-U pattern, and highlights the complex dynamics that exist in the relationship between cortisol levels and cognitive function.

When we analyze the groups categorized by the ApoE allele, the association between higher mean cortisol levels and worse declarative memory performance, both immediate and delayed recall, was only observed in the ApoE-ε4 group. In addition, we found a significant interaction between ApoE and mean cortisol levels for delayed recall, but not for immediate recall. Although few studies have explored cognitive function, the HPA-axis, and ApoE polymorphism together, previous results showed an association between the ApoE-ε4 allele, a faster decline in verbal fluency, and a flatter diurnal slope, that is, higher evening cortisol and lower morning cortisol (Gerritsen et al., 2011; Singh-Manoux et al., 2014). As mentioned above, two studies explored these associations by measuring cortisol levels during the cognitive assessment (Berteau-Pavy et al., 2007; Lee et al., 2008). Berteau-Pavy et al. (2007) found that in people from 62 to 92 years old, the ApoE-ε4 allele was associated with worse performance on the object recognition and spatial navigation test, but they did not find a relationship with cortisol levels. By contrast, Lee et al. (2008) observed that, whereas higher cortisol was associated with lower cognitive performance, the slopes were steeper in the ApoE-ε4 group. This study was carried out in participants with a similar age range (50-70 years), and it assessed similar cognitive domains to those assessed in our study. However, in Lee et al. (2008), subjects were not clinically assessed for dementia, and they were not excluded due to drug or medication use, which could have altered cognition or diseases such as diabetes (19.3% of the sample) despite it has been observed a dysregulation in

the HPA-axis function in responses to stress (Steptoe et al., 2014). In addition, these authors grouped the participants only as ApoE-ε4 allele carriers and non- carriers. Thus, in our study, we consider the three allelic variations (ApoE-ε2, ApoE-ε3 and ApoE-ε4), and cortisol was obtained while a wide range of cognitive functions were assessed. The findings support the idea that, in healthy non-demented older people from 55 to 77 years old, ApoE-ε4 carriers might be more vulnerable to potential detrimental effects of HPA-axis dysfunction on verbal memory performance.

On the contrary, only the ApoE-ε3 group showed an association between an increase of cortisol levels during the neuropsychological assessment and a better declarative memory performance, suggesting a more adaptive response to a cognitive challenge of the HPA axis in this group. What is more, the association between higher mean cortisol levels and better TMT A performance, was only observed in ApoE-ε3 group. Therefore, our findings would also support lower vulnerability in ApoE-ε3 to the detrimental effects of cortisol on attention and eye-hand coordination. Recently, Piskunowicz et al. (2017) reported that ApoE-ε3 allele carriers performed better on TMT B than ApoE-ε2 and ApoE-ε4 allele carriers. Therefore, our results show that the ApoE genotype modulates attention and eye-hand coordination, and the ApoE-ε3 allele would confer an advantage compared to the other alleles. Finally, it is worth noting that we found no association between cortisol levels and cognition for the ApoE-ε2 allele, despite it has been related to an increased risk of developing post-traumatic stress disorder (Kim et al., 2013), susceptibility to stress-induced impairments in memory (Freeman, Roca, Guggenheim, Kimbrell, & Griffin, 2005) and HPA axis dysregulation (Johnson et al., 2015).

Some limitations should be considered. First, it is important to note the correlational nature of the results, and so we cannot claim causal relationships. In

addition, due to the unequal distribution of the three ApoE alleles and the low frequency of the ApoE-ε2 and ApoE-ε4 alleles in the population, in our sample the number of participants in each ApoE group differed, and the sample size for the ApoE-ε2 and the ApoE-ε4 groups was small. Therefore, it is possible that, due to the small sample sizes, some of the hypothesized results were not observed. Thus, a larger sample size would be necessary to increase the statistical power. Nevertheless, it is important to highlight the effort that has been made in this study to separate and compare the three allelic variations and study their relationship with a wide range of cognitive domains and the HPA axis. In addition, the strict exclusion criteria make it possible to obtain a healthy older sample and control the effect of confounding variables. Moreover, due to the sample size, sex differences were not taken into account, although several studies with healthy elderly people have shown that women outperform men on verbal memory (Zhang, Zhou, Wang, Zhang & Study, 2017; Munro et al., 2012). Furthermore, there is evidence that women are at higher risk of AD than men (Farrer et al, 1997), and so it seems necessary to study sex differences in the influence of the ApoE gene on cognitive function. Thus, future studies with larger samples are needed to shed light on this issue.

In summary, our results show that, in healthy older people, the ApoE-ε2 allele may have a protective effect on declarative memory, specifically learning ability and no association between cortisol levels and cognition was observed for this allelic group. In addition, we did not find differences in cortisol levels between the ApoE groups. Additionally, although the ApoE-ε4 allele did not show a negative effect on cognitive function compared to ApoE-ε3, high cortisol levels would be especially detrimental to declarative memory in this group, compared to the ApoE-ε2 and ApoE-ε3 groups. Thus, the ApoE-ε4 allele could add greater vulnerability to the adverse effects of HPA axis

dysregulation on declarative memory during aging. On the other hand, the ApoE-ε3 allele could be related to a more adaptive stress response, where higher cortisol levels would improve attention and declarative memory.

5. Acknowledgements

This research study was supported by the Spanish Education and Science Ministry (grant no. PSI2013-46889; PSI2016-78763), by the Generalitat Valenciana (grants no. PROMETEOII2015-020), and by the Universitat de València (UV-PREDOC16F1-383576). The authors wish to thank Ms. Cindy DePoy for the revision of the English text and to Mr. Tiago Paiva for his helpful suggestions.

6. References

Almela, M., Hidalgo, V., Villada, C., Espín, L., Gómez-Amor, J., & Salvador, A. (2011). The impact of cortisol reactivity to acute stress on memory: sex differences in middle-aged people. Stress, 14(2), 117-127.doi:10.3109/10253890.2010.514671

Almela, M., Meij, L. V., Hidalgo, V., Villada, C., & Salvador, A. (2012). The cortisol awakening response and memory performance in older men and women. *Psychoneuroendocrinology, 37*(12), 1929-1940.doi:10.1016/j.psyneuen.2012.04.009

Batterham, P. J., Bunce, D., Cherbuin, N., & Christensen, H. (2012).Apolipoprotein E ε4 and Later-Life Decline in Cognitive Function and Grip Strength. *American Journal of Geriatric Psychiatry,* 1.doi:10.1097/jgp.0b013e318266b1ee

Berteau-Pavy, F., Park, B., & Raber, J. (2007). Effects of sex and APOE ε4 on object recognition and spatial navigation in the elderly. Neuroscience, 147(1), 6-17. Doi: 10.1016/j.neuroscience.2007.03.005

Bertram, L., Lill, C. M., &Tanzi, R. E. (2010). The Genetics of Alzheimer Disease: Back to the Future. *Neuron, 68*(2), 270-281.doi:10.1016/j.neuron.2010.10.013

Champaneri, S., Xu, X., Carnethon, M. R., Bertoni, A. G., Seeman, T., Desantis, A. S., . . . Golden, S. H. (2013). Diurnal salivary cortisol is associated with body mass index and waist circumference: The multiethnic study of atherosclerosis. Obesity, 21(1). doi:10.1002/oby.20047

Cohen, S., Doyle, W. J., & Baum, A. (2006). Socioeconomic status is associated with stress hormones. Psychosomatic medicine, 68(3), 414-420.doi: 10.1097/01.psy.0000221236.37158.b9

Conklin, H. M, Curtis, C. E., Katsanis, J., & Iacono, W. G. (2000). Verbal Working Memory Impairment in Schizophrenia Patients and Their First-Degree Relatives: Evidence From the Digit Span Task. *American Journal of Psychiatry, 157*(2), 275-277.doi:10.1176/appi.ajp.157.2.275

Cournot, M. C. M. J., Marquie, J. C., Ansiau, D., Martinaud, C., Fonds, H., Ferrieres, J., & Ruidavets, J. B. (2006). Relation between body mass index and cognitive function in healthy middle-aged men and women. Neurology, 67(7), 1208-1214. doi: 10.1212/01.wnl.0000238082.13860.50

de Kloet, E. R., Grootendorst, J., Karssen, A. M., & Oitzl, M. S. (2002). Gene× environment interaction and cognitive performance: animal studies on the role of corticosterone. *Neurobiology of learning and memory*, *78*(3), 570-577. doi: 10.1006/nlme.2002.4079

de Kloet, E. R., Oitzl, M. S., & Joëls, M. (1999). Stress and cognition: are corticosteroids good or bad guys?. Trends in neurosciences, 22(10), 422-426. doi:10.1016/s0166-2236(99)01438-1.

Edwards, S., Evans, P., Hucklebridge, F., & Clow, A. (2001). Association between time of awakening and diurnal cortisol secretory activity. Psychoneuroendocrinology, 26(6), 613-622. doi:10.1016/s0306-4530(01)00015-4

Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., ... & Van Duijn, C. M. (1997). APOE and Alzheimer Disease Meta Analysis Consortium Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. Jama, 278(16), 1349-1356. doi: 10.1001/jama.278.16.1349

Fiocco, A. J., Poirier, J., Joober, R., Nair, N., &Lupien, S. J. (2008).Acute and long-term associations between ApoE genetic polymorphism, cortisol levels, and declarative memory performance in older adults. *Psychoneuroendocrinology, 33*(5), 625-633.doi:10.1016/j.psyneuen.2008.02.002

Freeman, T., Roca, V., Guggenheim, F., Kimbrell, T., & Griffin, W. S. T. (2005). Neuropsychiatric associations of apolipoprotein E alleles in subjects with combat-related posttraumatic stress disorder. The Journal of neuropsychiatry and clinical neurosciences, 17(4), 541-543. doi:10.1176/appi.neuropsych.17.4.541

Gerritsen, L., Comijs, H. C., Deeg, D. J., Penninx, B. W., &Geerlings, M. I. (2011).Salivary cortisol, APOE-ε4 allele and cognitive decline in a prospective study of older persons. *Neurobiology of Aging,32*(9), 1615-1625. doi:10.1016/j.neurobiolaging.2009.09.007

Gil-Bea, F. J., Aisa, B., Solomon, A., Solas, M., Mugueta, M. D., Winblad, B., . . .Ramírez, M. J. (2010). HPA Axis Dysregulation Associated to Apolipoprotein E4 Genotype in

Alzheimers Disease. *Journal of Alzheimers Disease, 22*(3), 829-838.doi:10.3233/jad-2010-100663

Golden, C.J., 1978. A Manual for the Clinical and Experimental Use of the Stroop Color and Word Test. Stoelting, Wood Dale, IL.

Grootendorst, J., Kempes, M. M., Lucassen, P. J., Dalm, S., De Kloet, E. R., & Oitzl, M. S. (2002). Differential effect of corticosterone on spatial learning abilities in apolipoprotein E knockout and C57BL/6J mice. *Brain research*, *953*(1), 281-285. doi: 10.1016/s0006-8993(02)03399-1

Grootendorst, J., Enthoven, L., Dalm, S., de Kloet, E. R., & Oitzl, M. S. (2004). Increased corticosterone secretion and early-onset of cognitive decline in female apolipoprotein E-knockout mice. Behavioural brain research, 148(1-2), 167-177. doi:10.1016/S0166-4328(03)00188-8

Johnson, L. A., Zuloaga, D. G., Bidiman, E., Marzulla, T., Weber, S., Wahbeh, H., & Raber, J. (2015). ApoE2 exaggerates PTSD-related behavioral, cognitive, and neuroendocrine alterations. Neuropsychopharmacology, 40(10), 2443-2453. doi: 10.1038/npp.2015.95

Helkala, E., Koivisto, K., Hänninen, T., Vanhanen, M., Kervinen, K., Kuusisto, J., . . . Riekkinen, P. (1996). Memory functions in human subjects with different apolipoprotein E phenotypes during a 3-year population-based follow-up study. *Neuroscience Letters, 204*(3), 177-180. doi:10.1016/0304-3940(96)12348-x

Helkala, E., Koivisto, K., Hänninen, T., Vanhanen, M., Kervinen, K., Kuusisto, J., . . .Riekkinen, P. (1995). The association of apolipoprotein E polymorphism with memory: a population based study. *NeuroscienceLetters, 191*(3), 141-144. doi:10.1016/0304-3940(95)11575-h

Hidalgo, V., Almela, M., Villada, C., & Salvador, A. (2014). Acute stress impairs recall after interference in older people, but not in young people. *Hormones and behavior*, *65*(3), 264-272. doi: 10.1016/j.yhbeh.2013.12.017

Hidalgo, V., Almela, M., Pulopulos, M. M., & Salvador, A. (2016). Memory performance is related to the cortisol awakening response in older people, but not to the diurnal cortisol slope. *Psychoneuroendocrinology, 71*, 136-146.doi:10.1016/j.psyneuen.2016.05.019

Kim, T. Y., Chung, H. G., Shin, H. S., Kim, S. J., Choi, J. H., Chung, M. Y., ... & Cho, H. S. (2013). Apolipoprotein E gene polymorphism, alcohol use, and their interactions in combat- related posttraumatic stress disorder. Depression and anxiety, 30(12), 1194-1201. doi: 10.1002/da.22138

Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004). HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. Psychoneuroendocrinology, 29(1), 83-98. doi:10.1016/S0306-4530(02)00146-4

Lara, V. P., Caramelli, P., Teixeira, A. L., Barbosa, M. T., Carmona, K. C., Carvalho, M. G., ...& Gomes, K. B. (2013). High cortisol levels are associated with cognitive impairment no-dementia (CIND) and dementia. *Clinica Chimica Acta*, 423, 18-22.doi:10.1016/j.cca.2013.04.013

Lancaster, C., Tabet, N., & Rusted, J. (2017). The Elusive Nature of APOE ε4 in Mid-adulthood: Understanding the Cognitive Profile. Journal of the International Neuropsychological Society, 23(03), 239-253. doi:10.1017/s1355617716000990

Lee, B. K., Glass, T. A., Mcatee, M. J., Wand, G. S., Bandeen-Roche, K., Bolla, K. I., & Schwartz, B. S. (2007). Associations of Salivary Cortisol With Cognitive Function in the Baltimore Memory Study. *Archives of General Psychiatry, 64*(7), 810-818.doi:10.1001/archpsyc.64.7.810

Lee, B. K., Glass, T. A., Wand, G. S., Mcatee, M. J., Bandeen-Roche, K., Bolla, K. I., & Schwartz, B. S. (2008).Apolipoprotein E Genotype, Cortisol, and Cognitive Function in Community-Dwelling Older Adults. *American Journal of Psychiatry, 165*(11), 1456-1464.doi:10.1176/appi.ajp.2008.07091532

Li, G., Cherrier, M. M., Tsuang, D. W., Petrie, E. C., Colasurdo, E. A., Craft, S., . . . Wilkinson, C. W. (2006). Salivary cortisol and memory function in human aging. *Neurobiology of Aging, 27*(11), 1705-1714. doi: 10.1016/j.neurobiolaging.2005.09.031

Lobo, A., Saz, P., Marcos, G., Día, J. L., de la Cámara, C., Ventura, T., et al. (1999). Revalidación y normalización del mini-examen cognoscitivo (primera versión en castellano del Mini-Mental Status Examination) en la población general geriátrica. *Medicina Clínica*, 112 (20), 767–774.

Lu, P.H., Thompson, P.M., Leow, A., Lee, G.J., Lee, A., Yanovsky, I., Parikshak, N., Khoo, T., Wu, S., Geschwind, D., Bartzokis, G. (2011). Apoliprotein E genotype is associated with temporal and hippocampal atrophy rates in healthy older people adults: a tensor-based morphometry study. *Journal of Alzheimer's disease*, 23(3), 433-42.doi: 10.3233/JAD-2010-101398

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., &Schramek, T. E. (2007).The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition.*Brain and cognition*, 65(3), 209-237.doi: 10.1016/j.bandc.2007.02.007

Menzel, H. J., Kladetzky, R. G., &Assmann, G. (1983). Apolipoprotein E polymorphism and coronary artery disease. *Arteriosclerosis,* 3(4), 310-315. doi:10.1161/01.atv.3.4.310

Miranda, J. P., & Valencia, R. R. (1997).English and Spanish versions of a memory test: Word-length effects versus spoken-duration effects. *Hispanic Journal of Behavioral Science*, 19(2), 171–181.doi: 10.1177/07399863970192005

Munro, C. A., Winicki, J. M., Schretlen, D. J., Gower, E. W., Turano, K. A., Muñoz, B., ... & West, S. K. (2012). Sex differences in cognition in healthy elderly individuals. Aging, Neuropsychology, and Cognition, 19(6), 759-768. doi:10.1080/13825585.2012.690366

Payton, A., Gibbons, L., Davidson, Y., Ollier, W., Rabbitt, P., Worthington, J., . . . Horan, M. (2005). Influence of serotonin transporter gene polymorphisms on cognitive decline and cognitive abilities in a nondemented elderly population.*Molecular Psychiatry*, 10(12), 1133-1139.doi:10.1038/sj.mp.4001733

Peavy, G. M., Lange, K. L., Salmon, D. P., Patterson, T. L., Goldman, S., Gamst, A. C., . . .Galasko, D. (2007).The Effects of Prolonged Stress and APOE Genotype on Memory and Cortisol in Older Adults.Biological Psychiatry, 62(5), 472-478.doi:10.1016/j.biopsych.2007.03.013

Peskind, E. R., Wilkinson, C. W., Petrie, E. C., Schellenberg, G. D., &Raskind, M. A. (2001). Increased CSF cortisol in AD is a function of APOE genotype. Neurology, 56(8), 1094-1098. doi: 10.1212/WNL.56.8.1094

Piskunowicz, M. T., Linkowska, K., Gołota, S., Grzybowski, T., Kędziora-Kornatowska, K., & Borkowska, A. (2017). The Association of Apolipoprotein E Gene Polymorphism With Cognitive Performance in Nondemented Polish Adults Aged 55 to 75. The International Journal of Aging and Human Development, 009141501772454. doi:10.1177/0091415017724548

Reitan, R.M., 1992. Trail Making Test: Manual for Administration and Scoring. Reitan Neuropsychology Laboratory, (Tucson, AZ).

Sattler, C., Toro, P., Schönknecht, P., & Schröder, J. (2012). Cognitive activity, education and socioeconomic status as preventive factors for mild cognitive impairment and Alzheimer's disease. Psychiatry research, 196(1), 90-95. doi:10.1016/j.psychres.2011.11.012

Seeman, T. E., McEwen, B. S., Singer, B. H., Albert, M. S., & Rowe, J. W. (1997). Increase in Urinary Cortisol Excretion and Memory Declines: MacArthur Studies of Successful Aging. Journal of Clinical Endocrinology & Metabolism, 82(8), 2458-2465. doi:10.1210/jc.82.8.2458

Singh-Manoux, A., Dugravot, A., Elbaz, A., Shipley, M., Kivimaki, M., &Kumari, M. (2014). No evidence of a longitudinal association between diurnal cortisol patterns and cognition. *Neurobiology of Aging, 35*(10), 2239--2245. doi:10.1016/j.neurobiolaging.2014.03.015

Small, B. J., Rosnick, C. B., Fratiglioni, L., &Bäckman, L. (2004).Apolipoprotein E and Cognitive Performance: A Meta-Analysis. *Psychology and Aging, 19*(4), 592-600. doi:10.1037/0882-7974.19.4.592

Steptoe, A., Hackett, R. A., Lazzarino, A. I., Bostock, S., Marca, R. L., Carvalho, L. A., & Hamer, M. (2014). Disruption of multisystem responses to stress in type 2 diabetes: Investigating the dynamics of allostatic load. Proceedings of the National Academy of Sciences, 111(44), 15693-15698. doi:10.1073/pnas.1410401111

Van Hooren, S. A. H., Valentijn, A. M., Bosma, H., Ponds, R. W. H. M., Van Boxtel, M. P. J., & Jolles, J. (2007). Cognitive functioning in healthy older adults aged 64–81: a cohort study into the effects of age, sex, and education. Aging, Neuropsychology, and Cognition, 14(1), 40-54. doi:10.1080/138255890969483

Vedhara, K., Hyde, J., Gilchrist, I., Tytherleigh, M., & Plummer, S. (2000). Acute stress, memory, attention and cortisol. Psychoneuroendocrinology, 25(6), 535-549. doi:10.1016/s0306-4530(00)00008-1

Verghese, P. B., Castellano, J. M., &Holtzman, D. M. (2011).Apolipoprotein E in Alzheimers disease and other neurological disorders. *The Lancet Neurology, 10*(3), 241-252. doi:10.1016/s1474-4422(10)70325-2

Wechsler, D., 1997. WMS-III Administration and Scoring Manual. The Psychological Corporation, Harcourt Brace & Company, San Antonio.

Wilson, B., Cockburn, J., &Baddeley, A. (1985). The Rivermead behavioural memory test. Reading, UK: Thames Valley Text.

Wisdom, N. M., Callahan, J. L., & Hawkins, K. A. (2011). The effects of apolipoprotein E on non-impaired cognitive functioning: A meta-analysis. *Neurobiology of Aging, 32*(1), 63-74. doi:10.1016/j.neurobiolaging.2009.02.003

Wright, C., Kunz-Ebrecht, S., Iliffe, S., Foese, O., & Steptoe, A. (2005). Physiological correlates of cognitive functioning in an elderlypopulation. Psychoneuroendocrinology, 30(9), 826-838. doi:10.1016/j.psyneuen.2005.04.001

Zhang, J., Zhou, W., Wang, L., Zhang, X., & Study, H. A. B. (2017). Gender differences of neuropsychological profiles in cognitively normal older people without amyloid pathology. Comprehensive Psychiatry, 75, 22-26. doi:10.1016/j.comppsych.2017.02.008

Highlights

1. No differences on cognition in ApoE-ε3 and ε4 allele carriers in healthy elderly
2. Protective effect of ε2 allele in learning ability
3. Vulnerability effect of ε4 allele on HPA-axis dysregulation and cognitive decline
4. Adaptive HPA-axis response of ε3 allele to a cognitive challenge
5. Beneficial role of ε3 allele on the effects of cortisol on attention and memory