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Memory performance is related to the cortisol awakening response in older people, but not to the diurnal cortisol slope

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ABSTRACT

There are large individual differences in age-related cognitive decline. Hypothalamic-pituitary-adrenal axis (HPA-axis) functioning has been suggested as one of the mechanisms underlying these differences. This study aimed to investigate the relationships between the diurnal cortisol cycle, measured as the cortisol awakening response (CAR), and the diurnal cortisol slope (DCS) and the memory performance of healthy older people. To do so, we assessed the verbal, visual, and working memory performance of 64 participants (32 men) from 57 to 76 years old who also provided 14 saliva samples on two consecutive weekdays to determine their diurnal cortisol cycle. The CAR was linearly and negatively associated with verbal (significantly) and visual (marginally) memory domains, but not with working memory. Sex did not moderate these relationships. Furthermore, no associations were found between the DCS and any of the three memory domains assessed. Our results indicate that the two components of the diurnal cortisol cycle have different relationships with memory performance, with the CAR being more relevant than DCS in understanding the link from HPA-axis activity and regulation to different types of memory. These results suggest that the CAR is related to memory domains dependent on hippocampal functioning (i.e., declarative memory), but not to those that are more dependent on prefrontal cortex functioning (i.e., working memory).

1. Introduction

Cognitive decline stands out among the main negative changes associated with aging. However, there is great variability in the way people experience these age-related changes (Christensen et al., 1999). While some people maintain their cognitive abilities intact or show few changes, others experience important cognitive problems such as dementia. It has been suggested that hypothalamus-pituitary-adrenal axis (HPA-axis) functioning can explain these differences, at least in part. Along these lines, HPA-axis dysregulation has been related to poorer cognitive performance (Lupien et al., 2007, 2009). The HPA-axis would exert its effects on cognitive performance through the action of cortisol, the main glucocorticoid in humans, which binds to receptors (i.e., mineralocorticoid and glucocorticoid receptors) especially distributed in the hippocampus, prefrontal cortex and amygdala (Lupien and Lepage, 2001; Lupien et al., 2009; Roozendaal, 2000). Different mechanisms might underlie the negative effect of an HPA-axis dysregulation on cognitive performance. Several studies have shown that both long-term high and low cortisol levels may produce neurogenesis suppression, dendritic atrophy, synaptic and spine loss, a synaptic transmission reduction, and loss of neuronal integrity, especially in the hippocampus and prefrontal cortex, affecting cognitive performance (Berger et al., 2006; Lupien et al., 2005, 2009; Sloviter et al., 1993; Stienstra et al., 1998; Wossink et al., 2001). In addition, a dysregulation of the HPA-axis has been related to different physical and psychiatric disorders that could explain, at least in part, the inter-individual differences in cognitive performance. Thus, some authors have proposed that a dysregulation of the HPA-axis may be the key to the pathophysiology behind depression in later life and/or the diagnosis of metabolic syndrome, producing a negative effect on cognitive performance (Kuehl et al., 2015; Lupien et al., 2009; Pariante and Lightman, 2008). In this vein, memory is one of the main cognitive processes that have been related to HPA-axis functioning because these brain structures are key brain areas for learning and memory processes (for review see: Lupien et al., 2007).

Most studies on the relationship between HPA-axis functioning and memory performance have been carried out in acute stress situations, and only a few have studied the diurnal cortisol cycle. It is worth noting that the dynamic nature of the cortisol cycle makes

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the study of HPA-axis functioning difficult. In non-stress conditions, the secretion of cortisol follows a circadian pattern characterized by higher cortisol levels in the morning and lower cortisol levels during the last hours of the day. Generally, two components are distinguished in the diurnal cortisol cycle: (i) the cortisol awakening response (CAR; a sharp rise in cortisol that occurs from 30 to 45 min after awakening), and (ii) a steeper decrease in cortisol levels secreted throughout the rest of the day (Adam and Kumari, 2009). It seems that the regulatory mechanism underlying the CAR is independent from the rest of the diurnal cycle (Edwards et al., 2001). Given that these are considered two independent components of HPA-axis activity, some authors have indicated that they deserve to be analyzed independently (Fries et al., 2009; Clow et al., 2010a,b). However, most of the cross-sectional studies investigating the relationship between the HPA-axis functioning and cognitive performance in healthy older people have not jointly considered both of the discrete components of the diurnal HPA-axis activity. In addition, results have been obtained by analyzing a limited and different number of samples and memory tests (MacLullich et al., 2005; Li et al., 2006; Kuningas et al., 2007; Lee et al., 2007, 2008; Comijs et al., 2010; Seeman et al., 1997; Souza-Talarico et al., 2010; Pulopulos et al., 2014). Moreover, the relationship between memory and cortisol has been studied employing different indices, such as the awakening cortisol (O'Hara et al., 2007; Singh-Manoux et al., 2014), the area under the curve with respect to the ground, AUCg (Almela et al., 2012; Franz et al., 2011), mean cortisol levels (Abercrombie et al., 2004; Evans et al., 2011; Singh-Manoux et al., 2014) and bedtime cortisol (Singh-Manoux et al., 2014). However, to our knowledge, only a few studies have investigated the specific contribution of the two dynamic components of the diurnal cortisol cycle (i.e., CAR and cortisol secreted the rest of the day) to memory performance in older people.

Regarding the CAR, most of the studies failed to find a clear association with memory performance (Evans et al., 2011, 2012; Franz et al., 2011; Singh-Manoux et al., 2014; Stawski et al., 2011). For instance, Evans et al. (2012) showed that, in 50 older individuals (60–91 years old), the CAR was positively related to executive function, but not to memory performance (Evans et al., 2012). Along these lines, it has been also observed that the CAR was not related to episodic memory, working memory (Stawski et al., 2011) or short-term verbal memory (Singh-Manoux et al., 2014). Studying only older men (51–60 years old), Franz et al. (2011) did not find significant relationships between the CAR and visual spatial memory and working memory when they controlled for several covariates. By contrast, we found a significant but different relationship between the CAR and memory performance depending on the type of memory studied. Thus, while a higher CAR was negatively related to verbal memory in both men and women, it was positively related to spatial working memory, but only in men (Almela et al., 2012).

For the cortisol secreted during the rest of the day, different measures and indices have been used. One of those most frequently employed is the diurnal cortisol slope DCS, cortisol index, which indicates the decline in cortisol levels during the day, frequently calculated by regressing cortisol values on each sample collection time (Sephton et al., 2000). Poor memory performance has been related to both a steeper (O'Hara et al., 2007) and a flatter DCS (Abercrombie et al., 2004; Gerritsen et al., 2011). Finally, a lack of significant relationships has also been found (Beluche et al., 2010; Stawski et al., 2011; Singh-Manoux et al., 2014; Fiocco et al., 2006). When other indices were employed, mixed results were also reported. For example, the average diurnal cortisol decline was positively related to overall cognitive performance, executive function and verbal fluency tasks (Evans et al., 2011), while the cortisol AUCg (an index of total hormonal output throughout the day) was negatively related to visual spatial memory, executive functions, and processing speed (Franz et al., 2011). Therefore, in light of the inconclusive results, the need to obtain more evidence about this issue seems clear.

It is worth noting that, among the studies that have investigated the specific contribution of the CAR and the cortisol secreted during the rest of the day to differences in the cognitive performance in healthy older people, most of them only assessed one type of memory: visual (Beluche et al., 2010), verbal (Abercrombie et al., 2004; Evans et al., 2011, 2012; Gerritsen et al., 2011; O'Hara et al., 2007; Singh-Manoux et al., 2014) or working and declarative memory (Almela et al., 2012; Fiocco et al., 2006; Stawski et al., 2011). To our knowledge, only Franz et al. (2011) used several memory tasks (two tests for verbal and one test for spatial working memory) and short and delayed recall (two tests for verbal and one test for visual memory). However, this study with a younger sample of only men focused on a more limited age range (51–59 years old) compared to the other studies, which could explain the lack of association between cortisol and most of the memory tasks used. Therefore, more research is needed to investigate whether the two components of the HPA-axis activity may be related to different types of memory tasks in healthy older people.

With all this in mind, the aim of the present study was to investigate whether the components of the cortisol diurnal cycle (i.e., the CAR and the DCS) were related to declarative and working memory assessed with several tasks in older men and women. To do so, we assessed the performance on different memory tests of 64 older people who provided fourteen saliva samples on two consecutive weekdays, in order to obtain the CAR and the DCS. Based on previous results (Almela et al., 2012) and findings about the relationship between general life stress and increased CAR (Chida and Steptoe, 2009), as well as the well-known effect of long-term stress on memory (Lupien et al., 2005, 2009), we expected a CAR of increased magnitude to be associated with poorer performance on memory tasks that are dependent on hippocampal functioning, and at the same time, with better performance on memory tasks that are dependent on prefrontal cortex functioning (Almela et al., 2012; Evans et al., 2012). Moreover, we hypothesized that a flatter DCS would be associated with poorer memory performance (Abercrombie et al., 2004; Evans et al., 2011; Franz et al., 2011; Gerritsen et al., 2011). Finally, because some results suggest that sex moderates the relationship between CAR and working memory (Almela et al., 2012), especially stress-induced cortisol and memory performance (Seeman et al., 1997; Wolf et al., 1998; Almela et al., 2011), we also investigated possible sex differences.

2. Methods

2.1. Participants

The sample was composed of 64 participants (32 men and 32 women) from 57 to 76 years old (Men: $M = 64.47$, $SD = 4.295$; Women: $M = 64.84$, $SD = 3.886$). There were no sex differences in age or educational level (both $p > 0.586$), but men had a higher body mass index (Men: $M = 28.35$, $SD = 3.79$; Women: $M = 25.73$, $SD = 4.18$, $p = 0.011$) and reported slightly higher subjective socioeconomic status (SES) than women (Men: $M = 6.63$, $SD = 1.24$; Women: $M = 5.97$, $SD = 1.09$, $p = 0.028$). SES was measured using the MacArthur Scale of Subjective Social Status (Adler et al., 2000). In this scale, participants rated themselves on a scale ranging from 1 (people with the lowest education, income and worst jobs) to 10 points (people with the best education, income and jobs) according to their subjective socioeconomic status.

Participants belonged to a study program at the University of Valencia for people over 55 years of age. They completed a general questionnaire to check whether they met the study prerequisites. The criteria for exclusion were as follows: smoking

more than 5 cigarettes a day; alcohol or other drug abuse; dental, visual or hearing problems; presence of cardiovascular, endocrine, neurological, or psychiatric disease. Participants who were using any medication directly related to emotional or cognitive functioning or able to influence cortisol levels (e.g., glucocorticoids, anti-diabetic medication, antidepressants, benzodiazepines, and psychotropic substances) were excluded from participation. None of the participants met the criteria for dementia, as defined by the NINCDS-ADRDA criteria for Alzheimer's disease. Vitamins and sporadic use of painkillers were allowed. All the women were postmenopausal, and none of them were receiving hormone replacement therapy.

2.2. Procedure and neuropsychological assessment

Participants meeting the criteria were contacted by telephone and asked to attend a neuropsychological assessment, which took place in a laboratory at the Faculty of Psychology (University of Valencia). They 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 since the night before the session. Additionally, they were instructed to drink only water, and not eat, smoke or take any stimulants (e.g., coffee, cola caffeine, tea or chocolate) two hours prior to the session, or brush their teeth at least one hour prior to the session. All the participants received verbal and written information about the study and signed an informed consent form. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct were approved by the Ethics Research Committee of the University of Valencia.

The neuropsychological assessment was conducted between 10.00 h and 12.00 h and lasted no more than 1.5 h. Participants performed a total of 6 tests that assessed different memory domains: verbal memory, visual memory and working memory. All the tests were extracted from the Spanish version (Pereña et al., 2004) of the Wechsler Memory Scale III (WMS-III, Wechsler, 1997) and administered following the order proposed in the scale manual.

Verbal memory was assessed with the Logical Memory and Verbal Paired Associates tests. For Logical Memory, participants had to recall as many memory units or "ideas" as possible from two brief narratives immediately after the experimenter had read them. After a 30 min delay, participants were again asked to recall as many "ideas" as possible from the two narratives. Participants' answers were audio recorded and later corrected by an expert who followed the instructions provided in the test manual. From this test, three outcomes were used in the analyses: (i) Immediate Recall: total "ideas" recalled from the two narratives immediately after having heard them; (ii) Delayed Recall: total "ideas" recalled from the two narratives after a 30 min delay; and (iii) Retention Rate: percentage of the total number of "ideas" recalled from the two narratives after 30 min, compared to the number of "ideas" recalled on the immediate recall trial (Delayed Recall/Immediate Recall × 00). For the Verbal Paired Associates, the experimenter read aloud eight word pairs (e.g., horse-glass) across four trials. The word-pair list was the same across the different trials, but it was presented in a different order in each trial. In each trial, after reading the eight word pairs, the experimenter read the first word in the pair (e.g., horse), and participants had to recall the other word in the pair (e.g., glass). After a 30 min delay, the experimenter again read the first word in each pair, and participants had to recall the second word. We calculated three outcomes from this test: (i) Immediate Recall: total number of words recalled on the first four trials; (ii) Delayed Recall: total number of words recalled after a 30 min delay; and (iii) Retention Rate: percentage of the total number of words recalled after a 30 min delay, compared to the total number of words recalled on the first four trials (Delayed Recall/Immediate Recall × 100).

Visual memory was assessed with the Family Pictures test. Participants were shown 4 pictures presented consecutively and for 10 s each, and then they were asked to recall as much information as possible about them. Each picture represented a different family scene with different family members appearing in it. Once the pictures had been presented, participants were asked (i) which family member appeared in each picture, (ii) where they were situated in the picture, and (iii) what they were doing. After a delay of 30 min, participants had to answer the same questions again. Participants' answers were audio recorded and later corrected by an expert who followed the instructions provided in the test manual. The outcomes used in the analyses were: (i) Immediate Recall: total number of correct answers about the 4 pictures immediately after having seen them; (ii) Delayed Recall: total number of correct answers about the 4 pictures after a 30 min delay; and (iii) Retention Rate: percentage of the total number of correct answers about the 4 pictures after a 30 min delay, compared to the total number of correct answers recalled in the immediate recall trial (Delayed Recall/Immediate Recall × 100).

Working memory was evaluated with two verbal tests: Letter-Number Sequencing (LNS) and Digit Span (DS), and a spatial test: Spatial Span (SS). For the LNS, participants listened to a sequence of alternating digits (from 0 to 9) and letters (from A to Z) of increasing length. Immediately after that, they first had to repeat the digits in numerical order and then the letters in alphabetical order. The length of the sequences increased from two to eight items and, for each set length, three attempts were given to solve it. One point was assigned for each correctly recalled attempt, and the task ended only when the participant had failed the three attempts for the same set length. As an outcome measure, we used the total number of correctly recalled attempts. On the DS, which had two parts, the Digit Span Forward (DS Forward) and the Digit Span Backward (DS Backward), participants were read a series of numbers (from 0 to 9) with increasing length (from two to nine digits). Participants had to repeat the numbers in the same (DS Forward) or reverse (DS Backward) order as their presentation. For each set length, two attempts were given to solve it, and the task ended only when the participant had failed the two attempts of the same set length. 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. Finally, for the SS, which had two parts, the Spatial Span Forward (SS Forward) and the Spatial Span Backward (SS Backward), participants were presented with a set of 10 cubes on a board. The experimenter touched the cubes in a specific order, and the participants had to repeat the sequence in the same (SS Forward) or reverse (SS Backward) order. The length of the sequences increased from two to nine cubes, and for each sequence length, two attempts were given to solve it. One point was assigned for each correctly recalled attempt, and the task ended only when the participant had failed the two attempts of the same sequence length. Two outcomes were obtained: (i) SS Forward: total number of correctly recalled attempts in the same order, and (ii) SS Backward: total number of correctly recalled attempts in the reverse order.

2.3. Salivary cortisol

To measure the diurnal cortisol cycle, participants provided 7 saliva samples per day on 2 consecutive weekdays using salivettes (Sarstedt, Nümbrecht, Germany) at their home. To check for adherence to the sampling times, we stored the salivettes in MEMS TrackCap containers (MEMS 6 TrackCap Monitor, Aardex Ltd. Switzerland), which recorded the exact time the participants opened the container to provide a sample. Additionally, the participants wrote down the exact sampling times in a diary. After a demonstration by the experimenter in the lab about how to provide

the saliva sample, participants received written instructions and were advised to drink only water, and not eat, smoke or brush their teeth at least 1 h prior to each saliva sample. The saliva samples were provided immediately after awakening, 30 and 45 min post-awakening, and at 12.00 h, 16.00 h, and 20.00 h and immediately before bedtime. The mean number of days between the neuropsychological assessment and the collection of the saliva samples was 14.47 days (±1.74). Participants were instructed to store their samples in their fridge and bring them to the University as soon as possible, never exceeding three days after completion. In order to control the cortisol concentrations in the neuropsychological assessment, participants provided two additional saliva samples (at the beginning and end of the neuropsychological session).

In the lab, the samples were centrifuged at 4000 rpm for 15 min to obtain a clear supernatant that was stored at −80° C until the analyses were performed in the Central Research Unit of the Faculty of Medicine, University of Valencia (Spain). Cortisol concentrations were determined by radioimmunoassay using the commercial kit Spectria Cortisol RIA from Orion Diagnostica (Espoo, Finland). Assay sensitivity was 0.8 nmol/L., and the within- and inter-assay variation coefficients were all below 8%. Each subject's samples were analyzed in the same trial.

2.4. Statistical analyses and data management

Student's *t*-tests were used to investigate sex differences in the demographic variables. As salivary cortisol values did not have a normal distribution, they were log transformed. An ANCOVA for repeated measures with Time (Awakening, 30 min, 45 min, 12 h, 16 h, 20 h, Bed) as within-subject factors, and Sex (men, women) as a between-subject factor was performed to investigate differences across days and between men and women in cortisol levels at home; because sex differences were observed in SES and BMI, these variables were included as covariates. We used Greenhouse-Geisser because the requirement of sphericity in the ANCOVA for repeated measures was violated. *Post-hoc* planned comparisons were performed using Bonferroni adjustments for the *p* values. According to Franz et al. (2011), DS Backward and LNS were adjusted for DS Forward, and SS Backward was adjusted for SS Forward. To calculate these adjusted indexes, we saved the standardized residual scores from the regression analyses, using the forward condition as a predictor (i.e., DS Forward or SS Forward), and the backward condition (i.e., DS Backward or SS Backward) or LNS as the dependent variable.

Our aim was to investigate the relationship between the dynamic components of the diurnal cortisol cycle and memory performance. To do so, we focused on two cortisol indices: (i) the CAR, to reflect the post-awakening measure of cortisol secretion (Clow et al., 2010a,b), and calculated by the cortisol area under the curve with respect to the increase (AUCi; see Pruessner et al., 2003) from the 0, +30 and +45 min cortisol samples; and (ii) the diurnal cortisol slope (DCS): to reflect the decline in cortisol levels during the day, and calculated by regressing cortisol values (except +30 min and +45 min samples to avoid biasing the slope with the CAR) at each sample collection time individually for each participant (Sephton et al., 2000; Smeets et al., 2007; Singh-Manoux et al., 2014). A larger β value was interpreted as a flatter slope, reflecting a slower cortisol decline, while a smaller β value was interpreted as a steeper slope, reflecting a more rapid diurnal decline. Finally, for the cortisol levels during the neuropsychological assessment, the mean of the two values obtained from the pre and post saliva samples was calculated.

Regression analyses were performed to investigate the relationship between CAR, DCS and memory performance. In addition, moderator regression analyses were carried out to examine whether sex was a moderator in these relationships, based on Aiken and West (1991). Scatterplots showing the relationship between cortisol and memory outcomes (i.e., correlation analyses) were checked to investigate linear or curvilinear relationships. These scatterplots suggested that some relationships could be curvilinear, and so we added a curvilinear term in the regression analyses.

Two participants were excluded from the analyses, one woman because her cortisol concentrations differed by more than 3 SD from the CAR mean and DCS mean samples, and one woman who had three missing values for the DCS samples from day 1. All *p* values reported here are two-tailed. When not otherwise specified, the results shown are means ± standard error of mean (SEM). We used SPSS 22.0 to perform the statistical analyses. In order to aid the interpretation of the figures, the values represented are raw values and not square root transformed values.

3. Results

3.1. Preliminary analyses: adherence to the salivary sampling protocol

Studies have indicated if the first saliva sample is not collected immediately after awakening, the reliability of the measurement of the CAR is compromised. Thus, based on Thorn et al. (2006), and in line with previous research by our group (Almela et al., 2012), we explored the cortisol profile of the participants to identify any participants who might be suspected of non-adherence to the protocol. In order to control this issue, participants were divided into two groups, following the method suggested by Thorn et al. (2006): (i) those who had a positive CAR (i.e., cortisol AUCi > 0) on both days (2 Day-CAR group), and (ii) those who had a positive CAR on only one day or none (1 or 0 Day-CAR group). Of the total sample, 57.8% of the participants showed a positive CAR on both days (15 men and 22 women), 35.9% of the participants showed a positive CAR on only one day (13 men and 10 women), and the other 6.3% of the participants did not show a positive CAR on either of the two days (4 men). No significant differences in age, educational level, SES, BMI or memory performance were found between the 2 Day-CAR and 1 or 0 Day-CAR subgroups (all $p > 0.699$). Moreover, we compared the sampling times recorded in the MEMs with the times written in the logs by subjects. No differences were found across two days between the MEMs and the self-reported time in the log: (i) The 2 Day-CAR group: $t(33) = 1.570$, $p = 0.126$; Day 1: Mean = 3.32 min, SEM± 1.42; Day 2: −0.0882 min, SEM ± 1.78, (ii) Participants with only 1 day with positive CAR: $t(17) = -1.289$, $p = 0.215$; Day 1 (adherent day): Mean 1.33 min, SEM ± 2.39; Day 2 (non-adherent day): 4.11 min, SEM ± 1.78, and (iii) Participants without any day with positive CAR ($t(17) = -0.648$, $p = 0.563$; Day 1: Mean = 1.00 min, SEM ± 1.68; Day 2: Mean = 5.50 min, SEM ± 7.33). Fig. 1 represents the differences in the CAR profiles between the 2 Day-CAR and 1 or 0 Day-CAR subgroups (Time x CAR groups: $F(1.701, 95.241) = 55.613$, $p < 0.001$). While the 2 Day-CAR subgroup showed a steeper rise from awakening to 30 min later ($p < 0.001$), the 1 or 0 Day-CAR subgroup showed a flatter rise, given that cortisol levels were higher in the awakening sample (Awakening: 2 Day-CAR vs. 1 or 0 Day-CAR, $p = 0.009$). Forty-five min later, cortisol concentrations started to decrease; however, they were similar to awakening levels only in the 1 or 0 Day-CAR subgroup (Awakening vs. +45 min: $p = 0.999$). The Sex factor was not significant ($p = 0.542$); nor were its interactions with other factors (all $p > 0.285$). Repeated measures ANOVAs were used to analyze the differences across days in the salivary cortisol levels. For the 2 Day-CAR group, no significant differences were found (Day, $F(1, 36) = 2.618$, $p = 0.114$). However, for the 1 or 0 Day-CAR group, the main effect of Day ($F(1, 24) = 7.869$, $p = 0.010$) was significant. Importantly, the awakening saliva sample (i.e., the key saliva sam-

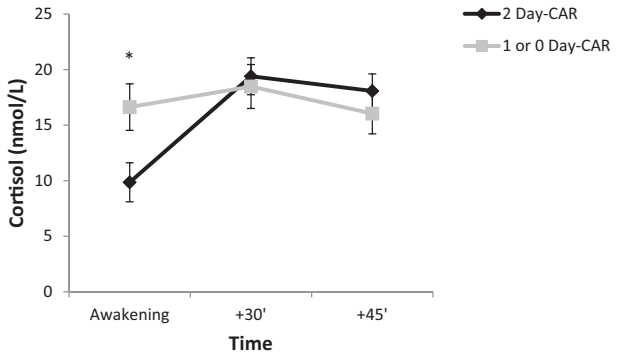

Fig. 1. CAR profiles of the 2 Day-CAR and 1 or 0 Day-CAR subgroups. In the first saliva sample, on awakening, participants suspected of being non-adherent had higher cortisol concentrations than participants suspected of being adherent (*$p = 0.004$). Depicted values are means, and error bars represent the SEM.

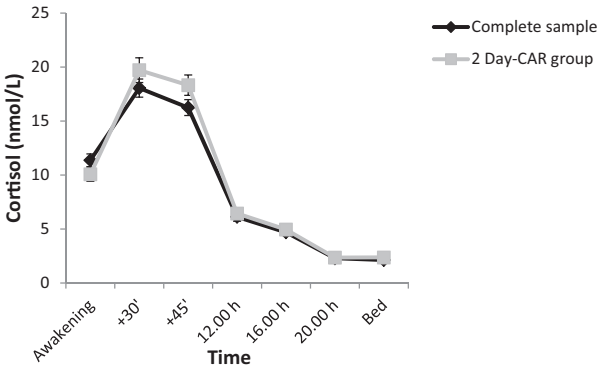

Fig. 2. CAR and diurnal pattern of cortisol for complete sample and 2 Day-CAR group. Depicted values are means, and error bars represent the SEM.

ple in adherence to the protocol) correlated across days in the 2 Day-CAR group ($r = 0.375$, $p = 0.022$), but it did not correlate in the 1 or 0 Day-CAR group ($r = 0.281$, $p = 0.173$) (see Table 1).

Together, these results indicate that there were no differences in demographic characteristics between groups, or between the time reported by the participants and the time registered by the MEMs, regarding the salivary sampling time. However, the differences in the pattern of cortisol levels suggest that participants with negative CAR might not have collected the first salivary sample immediately after awakening, although other explanations (e.g., unreported diseases) cannot be excluded. Considering all this, we calculated the mean cortisol level for each salivary sample across days to compute the CAR. Additionally, and in order to control for a possible confounder effect of non-adherence to the protocol, we repeated all the analyses, excluding those participants who were suspected of being non-adherent (0 or 1 Day-CAR). This approach was proposed by Thorn et al. (2006) and used in our previous study (Almela et al., 2012).

3.2. Sex differences in the diurnal cortisol cycle

For the complete sample, the repeated-measures ANCOVA showed a main effect of Time ($F(4.437, 257.349) = 6.329$, $p < 0.001$). As Fig. 2 shows, participants presented the CAR because their cortisol levels increased from awakening to 30 min later ($p < 0.001$) and then decreased over time, reaching the lowest levels in the last saliva sample ($p < 0.001$). The Sex factor was not significant ($p = 0.231$), nor were its interactions with other factors (all $p > 0.148$).

When the same analyses were performed in the 2 Day-CAR group, the results for Time (F (3.759, 124.060) = 5.955, $p < 0.001$) were replicated. Again, the Sex factor was not significant ($p = 0.130$) nor were its interactions with other factors (all $p > 0.219$).

Table 1
Pearson's correlations between each saliva sample across days for each group.

Saliva sample (Day 1 vs. Day 2)	2 Day-CAR group		1 or 0 Day-CAR group	
	r	*p*	*r*	*p*
Awakening	0.375	0.022	0.281	0.173
30′	0.276	0.098	0.382	0.059
45′	0.270	0.106	0.349	0.087
12.00 h	0.630	<0.001	0.233	0.263
16.00 h	0.457	0.004	0.549	0.004
20.00 h	0.495	0.002	0.242	0.244
Bed	0.491	0.002	0.111	0.596

3.3. Relationship between diurnal cortisol cycle and memory performance

Hierarchical regression analyses were performed to investigate the relationship between the two components of the diurnal cortisol cycle (CAR and DCS) and memory performance. Separate analyses were conducted for each memory outcome. First, we examined these relationships with simple regression analyses, adding the CAR or DCS in Step 1. Additionally, to investigate curvilinear relationships between cortisol and memory outcome, we added the square of the CAR or DCS in Step 2. Results of these regression analyses are presented in Tables 2–5 .

Results for the complete sample, without controlling for possible confounder effects, showed that a higher CAR was associated with worse performance (i.e., negative linear relationship) on the immediate recall ($p = 0.018$) and, as a trend, delayed ($p = 0.060$) recall trials of the Verbal Paired Associates test. Additionally, there were significant curvilinear relationships (i.e., inverted U-shaped) between the CAR and performance on the Logical Memory test: immediate ($p = 0.002$) and delayed recall ($p = 0.004$), the Family Pictures test: immediate ($p = 0.036$) and delayed recall trials ($p = 0.034$), and, as a trend, Digit Span Backward Adjusted ($p = 0.090$). These curvilinear associations indicated that both a larger CAR and a lower CAR were related to worse performance on these tests, while a moderate CAR was related to better performance. None of the other associations were significant (all $p > 0.084$). Moreover, there was only a curvilinear relationship (i.e., U-shaped) between DCS and immediate recall on the Family Pictures test ($p = 0.038$).

Results for only the 2 Day-CAR group showed a significant negative linear relationship between the CAR and performance on the Logical Memory test: immediate ($p = 0.015$) and delayed recall ($p = 0.005$), and on the Verbal Paired Associates test: immediate ($p = 0.052$) and delayed recall ($p = 0.047$), and, as a trend, the retention rate on the Family Pictures test ($p = 0.075$) and Spatial Span Backward adjusted ($p = 0.077$). None of the other associations were significant (all $p > 0.123$).

Second, we examined these relationships controlling for possible confounder effects. Thus, the following covariates were included in the analyses: (i) Age and (ii) BMI, due to their relationships with cognitive and HPA-axis functioning (Cournot et al., 2006; Dettenborn et al., 2012; Silver et al., 2012; Stalder and Kirschbaum, 2012); (iii) SES because it is related to both HPA-axis functioning (Wright and Steptoe, 2005; Cohen et al., 2006) and health status (Adler et al., 2000; Singh-Manoux et al., 2005; Demakakos et al., 2008); (iv) The mean of the cortisol levels during the neuropsychological assessment to control for the acute effects of cortisol levels on memory performance (Sindi et al., 2013; Coluccia et al., 2008; Pulopulos et al., 2015); and (v) The mean of the awaken-

Table 2
Regression analyses (without and with covariates) with CAR as predictor and verbal and visual memory outcomes as dependent variables.

			Logical Memory			Verbal Paired Associates			Family Pictures		
			Immediate recall	Delayed recall	Retention rate	Immediate recall	Delayed recall	Retention rate	Immediate recall	Delayed recall	Retention rate
Without											
Total Sample	Linear	Adj$^c R^2$	−0.016	−0.008	−0.009	0.074	0.041	−0.008	0.013	0.008	−0.008
		β	−0.027	−0.093	−0.088	−0.298	−0.239	−0.092	0.169	0.153	−0.092
		P	0.832	0.469	0.493	**0.018**	**0.060**	0.478	0.185	0.230	0.472
	Curvilinear	Adj$^c R^2$	0.122	0.109	−0.025	0.105	0.070	0.007	0.068	0.064	−0.024
		β	−18.937	−17.605	0.898	−10.357	−10.155	8.646	−12.896	−13.020	1.348
		P	**0.002**	**0.004**	0.887	**0.084**	**0.096**	0.172	**0.036**	**0.034**	0.831
2Day-CAR	Linear	Adj$^c R^2$	0.134	0.183	−0.010	0.078	0.083	−0.029	0.006	0.040	0.062
		β	−0.397	−0.453	−0.133	−0.322	−0.329	0.019	−0.184	−0.258	−0.296
		P	**0.015**	**0.005**	0.434	**0.052**	**0.047**	0.912	0.275	0.123	**0.075**
	Curvilinear	Adj$^c R^2$	0.168	0.201	−0.040	0.062	0.079	−0.039	−0.022	0.012	0.047
		β	−19.055	−16.159	−1.191	−8.150	−11.812	10.975	1.579	−1.437	−8.965
		P	0.128	0.186	0.931	0.534	0.365	0.418	0.908	0.915	0.498
With											
Total Sample	Linear	Adj$^c R^2$	−0.059	−0.057	−0.074	0.164	0.077	−0.050	0.078	0.071	0.097
		β	−0.025	−0.050	−0.057	−0.250	−0.193	−0.148	0.110	0.098	−0.076
		P	0.866	0.733	0.695	**0.057**	0.158	0.310	0.420	0.474	0.570
	Curvilinear	Adj$^c R^2$	0.074	0.060	−0.093	0.182	0.092	−0.028	0.129	0.110	0.094
		β	−18.520	−17.489	1.392	−8.708	−8.484	9.624	−12.351	−11.244	5.696
		P	**0.005**	**0.008**	0.838	0.144	0.176	0.151	**0.047**	**0.072**	0.361
2Day-CAR	Linear	Adj$^c R^2$	0.135	0.234	−0.009	0.131	0.040	−0.105	0.017	0.009	−0.078
		β	−0.470	−0.557	−0.171	−0.352	−0.306	0.029	−0.292	−0.340	−0.247
		P	**0.012**	**0.002**	0.371	**0.054**	0.107	0.886	0.127	**0.079**	0.215
	Curvilinear	Adj$^c R^2$	0.158	0.250	−0.046	0.101	0.034	−0.125	−0.017	−0.027	−0.077
		β	−17.116	−15.372	−0.187	−3.172	−12.377	10.578	3.202	−0.573	−14.763
		P	0.192	0.214	0.990	0.812	0.375	0.471	0.822	0.968	0.317

Values in bold represent significant or marginal *p*.

Table 3
Regression analyses (without and with covariates) with CAR as predictor and working memory outcomes as dependent variables.

			Letter-Number Sequencing		Digit Span		Spatial Span	
			Non-adjusted	Adjusted	Forward	Adjusted	Forward	Adjusted
Without								
Total Sample	Linear	Adj$^c R^2$	−0.011	−0.011	−0.016	0.010	0.001	0.028
		β	−0.071	−0.070	−0.015	0.160	0.132	−0.208
		P	0.581	0.586	0.904	0.211	0.304	0.102
	Curvilinear	Adj$^c R^2$	−0.005	−0.003	−0.033	0.041	−0.004	0.050
		β	−7.360	−7.703	−0.410	−10.506	−5.159	−9.424
		P	0.242	0.221	0.949	**.090**	0.411	0.125
2Day-CAR	Linear	Adj$^c R^2$	−0.007	0.000	−0.029	−0.009	−0.028	0.061
		β	−0.146	−0.165	0.003	−0.136	0.009	−0.295
		P	0.389	0.328	0.986	0.421	0.958	**.077**
	Curvilinear	Adj$^c R^2$	−0.023	−0.004	−0.054	−0.039	0.000	0.033
		β	−9.141	−12.569	5.383	−0.174	18.979	1.104
		P	0.504	0.356	0.698	0.990	0.166	0.934
With								
Total Sample	Linear	Adj$^c R^2$	0.110	0.068	0.003	−0.027	−0.021	0.015
		β	0.007	−0.022	0.081	0.195	0.163	−0.125
		P	0.957	0.873	0.565	0.178	0.256	0.375
	Curvilinear	Adj$^c R^2$	0.098	0.060	−0.013	0.001	−0.037	0.021
		β	−3.445	−4.576	2.455	10.470	−2.705	−7.437
		P	0.579	0.470	0.709	0.119	0.684	0.252
2Day-CAR	Linear	Adj$^c R^2$	0.271	0.274	−0.137	−0.075	−0.034	0.153
		β	0.004	−0.025	0.071	−0.186	0.010	−0.114
		P	0.978	0.875	0.727	0.348	0.959	0.514
	Curvilinear	Adj$^c R^2$	0.252	0.266	−0.172	−0.110	0.028	0.126
		β	−6.496	−9.883	5.846	4.681	22.889	4.431
		P	0.595	0.416	0.702	0.753	0.108	0.737

Values in bold represent significant or marginal *p*.

ing time across two days because it is related to the CAR (Thorn et al., 2006). To do so, in step 1, we included these control variables and sex (0 = women, 1 = men). In step 2, we included the CAR or DCS to investigate a linear relationship between each component of the diurnal cortisol cycle and each memory outcome. In step 3, we included the square of the CAR or DCS to investigate a curvilinear relationship between each component of the diurnal cortisol cycle and each memory outcome. The significant curvilinear relationships were interpreted as a concave upward relationship (U-shaped form) where the value of β is positive, and a concave downward relationship (inverted U-shaped form) where the value of β is negative. Results of the analyses performed for the complete sample

Table 4
Regression analyses (without and with covariates) with DCS as predictor and verbal and visual memory outcomes as dependent variables.

			Logical Memory			Verbal Paired Associates			Family Pictures		
			Immediate recall	Delayed recall	Retention rate	Immediate recall	Delayed recall	Retention rate	Immediate recall	Delayed recall	Retention rate
Without	Linear	Adj$^c R^2$	−0.014	−0.008	−0.013	−0.007	−0.015	−0.004	−0.001	0.004	−0.012
		β	−0.048	0.094	−0.064	0.096	−0.040	−0.113	0.125	0.141	0.065
		P	0.712	0.469	0.622	0.456	0.759	0.384	0.333	0.275	0.616
	Curvilinear	Adj$^c R^2$	−0.020	−0.002	−0.018	−0.019	−0.032	−0.015	0.054	0.028	0.002
		β	−7.873	−11.098	−7.918	5.343	−1.436	−5.718	19.637	14.878	−13.021
		P	0.417	0.249	0.414	0.581	0.883	0.549	**0.038**	0.119	0.177
With	Linear	Adj$^c R^2$	−0.048	−0.037	−0.074	0.101	0.054	−0.070	0.083	0.075	0.102
		β	0.100	0.155	0.079	0.041	−0.127	−0.088	0.094	0.106	0.053
		P	0.488	0.282	0.586	0.759	0.356	0.552	0.488	0.433	0.692
	Curvilinear	Adj$^c R^2$	−0.032	−0.023	−0.078	0.097	0.040	−0.078	0.086	0.068	0.091
		β	−14.647	−14.312	−9.836	8.677	4.643	−8.663	11.095	8.230	−6.203
		P	0.183	0.191	0.379	0.397	0.365	0.438	0.282	0.428	0.545

Values in bold represent significant or marginal *p*.

Table 5
Regression analyses (without and with covariates) with DCS as predictor and working memory outcomes as dependent variables.

			Letter-Number Sequencing		Digit Span		Spatial Span	
			Non-adjusted	Adjusted	Forward	Adjusted	Forward	Adjusted
Without	Linear	Adj$^c R^2$	−0.016	−0.015	−0.015	−0.015	−0.006	0.016
		β	0.023	0.039	−0.040	0.038	0.101	0.179
		P	0.859	0.766	0.760	0.767	0.437	0.164
	Curvilinear	Adj$^c R^2$	−0.001	0.002	−0.032	−0.019	−0.017	0.021
		β	−13.147	13.593	1.004	8.326	5.885	−10.875
		P	0.173	0.159	0.918	0.390	0.543	0.253
With	Linear	Adj$^c R^2$	0.113	0.081	−0.016	−0.063	−0.042	0.067
		β	0.125	0.144	−0.032	0.056	0.117	0.263
		P	0.348	0.287	0.822	0.700	0.417	**0.057**
	Curvilinear	Adj$^c R^2$	0.128	0.090	−0.030	−0.082	−0.061	0.050
		β	13.848	12.678	5.735	2.919	2.888	−2.117
		P	0.171	0.219	0.599	0.794	0.794	0.840

Values in bold represent significant or marginal *p*.

and for the 2 Day-CAR group are shown in Tables 2–5. Because none of the associations were moderated by Sex ($p > 0.1$), only the linear and curvilinear associations for men and women together are shown.

3.3.1. CAR and memory performance

Results for the complete sample showed that a higher CAR was associated, as a trend, with worse performance on the immediate recall trial of Verbal Paired Associates (i.e., negative linear relationship) ($p = 0.057$). Additionally, there were significant curvilinear relationships (i.e., inverted U-shaped) between the CAR and performance on the Logical Memory test: immediate ($p = 0.005$) and delayed recall ($p = 0.008$), the Family Pictures test: immediate ($p = 0.047$) and delayed recall trials (marginally, $p = 0.072$). These curvilinear associations indicated that both a larger CAR and a lower CAR were related to worse performance on these tests, while a moderate CAR was related to better performance. None of the other associations were significant (all $p > 0.119$).

If the analyses are performed only with the 2 Day-CAR group, results show a significant negative linear relationship between the CAR and performance on the Logical Memory test: immediate ($p = 0.012$) and delayed recall ($p = 0.002$), the Verbal Paired Associates test: immediate recall (marginally, $p = 0.054$), and the Family Pictures test: delayed recall (marginally, $p = 0.079$). None of the other associations were significant (all $p > 0.107$). Table 6 summarizes the main results of the regression analyses for the complete sample and for the 2 Day-CAR group.

Table 6
Summary of the regression analyses with covariates between memory test outcomes and CAR for the complete sample and for the 2 Day-CAR group.

Memory Domain and Tests	Outcome	Total sample	2 Day-CAR
Verbal Memory			
Logical Memory	Immediate	Inverted U-shaped	Negative linear
	Delayed	Inverted U-shaped	Negative linear
	Retention	–	–
Verbal Paired Associated	Immediate	Negative linear*	Negative linear***
	Delayed	–	–
	Retention	–	–
Visual Memory			
Family Pictures	Immediate	Inverted U-shaped	–
	Delayed	Inverted U-shaped**	Negative linear****
	Retention	–	–
Working Memory			
LN Sequencing	Non-adjusted	–	–
	Adjusted	–	–
Digit Span	Forward	–	–
	Adjusted	–	–
Spatial Span	Forward	–	–
	Adjusted	–	–

* $p = 0.057$.
** $p = 0.072$.
*** $p = 0.054$.
**** $p = 0.079$.

3.3.2. DCS and memory performance

Regression analyses controlling for possible confounder effects showed, as a trend, a positive curvilinear relationship between the DCS and the Spatial Span Backward adjusted performance

($p = 0.057$). None of the other associations were significant ($p > 0.171$). Sex did not moderate any of these relationships ($p > 0.1$).

4. Discussion

The present study examined the relationship between diurnal HPA-axis functioning and memory performance in older men and women. To do so, we studied how two components of the diurnal cortisol cycle, the CAR and the DCS, were related to several verbal, visual and working memory tasks. For the complete sample, after controlling for the confounding effects, the CAR was negatively associated with tasks that are dependent on hippocampal functioning (in a linear form: immediate recall on the Verbal Paired Associates test (marginally); in a curvilinear form: immediate and delayed recall on the Logical Memory and Family Pictures tests). When the same analyses were performed with only those participants who showed CAR on both days (2 Day-CAR group), a CAR of increased magnitude was related to worse performance on immediate and delayed recall on the Logical Memory test. The same relationship was observed with immediate recall on the Verbal Paired Associates test and delayed recall on the Family Pictures test, but with marginally significant results. No significant relationships were observed for tasks that are more related to prefrontal cortex functioning (i.e., Letter and Number Sequencing, Digit Span and Spatial Span) (see Table 6). By contrast, DCS only showed a marginal positive curvilinear relationship with one outcome of working memory (spatial span backward adjusted). Finally, the sex factor did not moderate any relationships between the components of the diurnal cortisol cycle and memory performance.

We assessed verbal, visual and working (verbal and spatial) memory through six different tasks. Memory performance is one of the main cognitive domains related to HPA-axis functioning in response to acute stress (for a review see: Schwabe, 2013). However, few studies have investigated how the CAR and DCS are associated with memory performance assessed by several memory tasks. A main finding of the current study is that the CAR was negatively related to performance on hippocampus-related memory tasks. These relationships were observed in those participants who showed CAR on both days (2 Day-CAR group), both before and after controlling for the possible effects of covariates in the regression analyses. This result is in line with most of the previous cross-sectional studies showing that a dysregulation of the HPA-axis (measured in urine, blood and saliva samples) is related to worse memory performance (Seeman et al., 1997; MacLullich et al., 2005; Li et al., 2006; Kuningas et al., 2007; Lee et al., 2007, 2008; Comijs et al., 2010; Souza-Talarico et al., 2010; Pulopulos et al., 2014). Specifically, in the current study, and in the 2 Day-CAR group after controlling for the covariates, a higher CAR was associated with worse performance on the Logical Memory test (immediate and delayed recall), and marginally on the Verbal Paired Associates (immediate recall) and Family Pictures (delayed recall) tests. It is important to note that for the complete sample, the relationships with the Logical Memory and Family Pictures tests were quadratic (inverted U-shaped form). As previously discussed, the fact that some people in the total sample had a shifted CAR measurement (i.e., higher cortisol level in the awakening sample, similar to what would be expected 30 or 45 min after awakening), and the possibility that these people were on the left side of the inverted U curve, could explain these different results (Almela et al., 2012). In the same study, we showed, as in the present findings, that a greater CAR was related to poorer verbal memory performance assessed with paragraph recall (Almela et al., 2012). The current study confirms this previous result and extends these findings to memory performance on other verbal (i.e., association task) and visual tasks. It is possible that the marginally significant relationship with the Verbal Paired Associates and visual memory tasks would be significant with a larger sample size. The performance on these tasks is especially related to hippocampal functioning; thus, we consider that there is a consistent negative relationship between the CAR and performance on memory tasks related to hippocampal functioning, and this association would be especially relevant for verbal tasks. This result is not surprising, given that the hippocampus, a key brain area for declarative memory (Scoville and Milner, 1957), plays a central role in the regulation of the CAR (Clow et al., 2010b). Results from previous studies support the link between the hippocampus and CAR, although the observed relationship is not always in the same direction. Studies performed in people with amnesia have observed that when the hippocampus is damaged, the CAR is absent (Buchanan et al., 2004; Wolf et al., 2005). Moreover, larger hippocampal volume has been related to a greater CAR and to worse memory performance in 13 young men (Pruessner et al., 2007). Despite the limitations of these studies (e.g., relatively small sample size), these results may suggest a link between the CAR, the hippocampus, and memory performance. However, it is possible that this relationship is not the same in clinical populations with hippocampal damage and young adults as it is in older people. Along these lines, it has been proposed that the association between hippocampal volume and memory performance is negative for young adults, but positive for older people (Van Petten, 2004). Further studies are needed to systematically investigate whether the age of the participants and their clinical conditions can moderate the relationship between CAR and memory performance.

We found relationships between the CAR and immediate and/or delayed recall (i.e., 30 min later) for Logical Memory, Verbal Paired Associates and Family Pictures tests. However, we failed to find any relationship with the retention rate. One possible explanation for these results is that the retention rate scores were high in most of our participants, and so a ceiling effect (e.g. mean retention rate: Logical Memory: 87.47%, Verbal Paired Associates: 100.67%, and Family Pictures: 99.37%) might hinder a possible relationship between the CAR and this index. Further studies may benefit from using a longer delay between immediate recall and delayed recall.

It is noteworthy that some studies have reported no relationship between the CAR and memory performance (Evans et al., 2011, 2012; Franz et al., 2011; Singh-Manoux et al., 2014). Methodological differences could explain the contradictory results. In contrast to the current study and our previous study (Almela et al., 2012), Franz et al. (2011) and Singh-Manoux et al. (2014) used only two saliva samples (immediately after awakening and +30 min), which could have affected the CAR measurement (Clow et al., 2010b). Another explanation could be the type of memory task used. Evans et al. did not observe any relationship between CAR and verbal memory performance using a word list learning test (Evans et al., 2011, 2012). Importantly, the same result was observed in our previous study using a similar memory task (Almela et al., 2012), suggesting that this test may be less sensitive to associations with the CAR than other tests.

For the results with working memory, we did not find a significant relationship between the CAR and the three working memory tests used after controlling for the confounding effects. This result coincides with previous studies that failed to find a relationship between CAR and verbal fluency (Franz et al., 2011; Singh-Manoux et al., 2014) or semantic fluency (Evans et al., 2011) tasks, two cognitive measures that are also believed to encompass frontal cortex-related functioning. In contrast to the results observed in the current study, two previous papers reported an association between the CAR and spatial working memory performance (Almela et al., 2012; Moriarty et al., 2014). Although still not fully understood, it has been indicated that the frontal cortex may play a part in the dynamics and magnitude of the CAR (for a review see: Clow et al., 2010b). Thus, a possible relationship between work-

ing memory and the CAR could be expected. However, for the time being, the results observed in our studies and others do not offer a clear explanation of what the direction is, or even if there is a consistent relationship between them. Further studies are needed to investigate this association more in-depth, using other kinds of working memory tasks that might be more sensitive to changes in CAR and diurnal cortisol levels.

Regarding the association between the DCS and cognitive performance, our results, after controlling for confounding factors, showed that this index of the diurnal cortisol cycle was not related to most of the memory tasks. Only a marginal positive curvilinear association was observed between DCS and performance on the Spatial Span Backward adjusted. The lack of association with most of the memory tasks agrees with previous studies in older people that failed to find a relationship between the DCS and declarative memory (Fiocco et al., 2006), visual memory (Beluche et al., 2010), short-term memory (Singh-Manoux et al., 2014) and working and declarative memory (Stawski et al., 2011), in samples of very different sizes. By contrast, other studies have reported that both a flatter (Abercrombie et al., 2004; Evans et al., 2011; Gerritsen et al., 2011) and a steeper DCS (O'Hara et al., 2007) are associated with poorer memory performance. Methodological differences, such as the number of participants studied, the cognitive domains assessed, the method used to calculate the DCS, or a combination of them, can explain these inconsistent findings. Taken together, a systematic examination of the involvement of these factors in the relationship between this component of the diurnal cortisol cycle and memory performance will help to better understand this link.

It is important to note that some relationships were significant when no covariates were included in the regression analyses; however, after controlling for covariates, they were no longer significant (among the most important, CAR for the 2 Day-CAR group: delayed recall on the Verbal Paired Associates test, retention rate on the Family Picture test and Spatial Span Backward Adjusted; DCS data: delayed recall on the Family Pictures test) or became marginally significant (among the most important, CAR for the 2 Day-CAR group: delayed recall on the Family Picture test; DCS data: Spatial Span Backward Adjusted). These results could be explained by the possible confounder role of the variables controlled in our study. Along these lines, it has been shown that some factors not controlled in this study, such as stressful life events in childhood, personality and/or genetic factors, may account for variability in the HPA-axis activity and, consequently, affect the relationship between cortisol and cognitive performance (Suzuki et al., 2014; Wingenfeld and Wolf, 2011; Wüst et al., 2004). Thus, it could be important to investigate whether these factors can also moderate the relationship observed here.

Some limitations should be considered. First of all, it is important to note the correlational nature of the results, which means that we cannot endorse causal relationships. Second, given that we performed the task following the order suggested in the WMS-III manual, the order of the administration was not counterbalanced. Third, although we used strategies to check for adherence to the sampling times (i.e., diary logs and electronic monitoring containers), we still do not know the exact time when the participants awoke and, therefore, whether the first saliva sample (i.e., awakening) was collected correctly. As previous studies have shown, self-reported adherence cannot be relied on (Broderick et al., 2004; Kudielka et al., 2003). Moreover, the post-awakening period is related to deficits in alertness and psychomotor and cognitive performance (i.e., sleep inertia effects) (Balkin et al., 2002; Lubin et al., 1976) that make it more difficult to collect the saliva sample correctly immediately upon waking. To control this, we performed the same statistical analysis, first for the complete sample and then for the 2 Day-CAR subgroup. Thus, for the complete sample, most of the associations followed an inverted U-pattern, while for the 2 Day-CAR subgroup, these associations changed to a negative linear form. These results highlight the importance of paying attention to protocol adherence to avoid confounding conclusions. Finally, because the participants were selected based on their good physical and psychological health, our results may not be representative of the older population with age-related diseases (i.e., diabetic or hypertensive older people) are clearly warranted.

On the whole, the present study suggests that different aspects of the HPA-axis function have different impacts on memory performance. Thus, the CAR is negatively related to declarative memory performance (especially for verbal memory), which is more dependent on hippocampal functioning, but not to working memory performance, which is more dependent on the prefrontal cortex. Additionally, the DCS does not appear to be related to declarative, visual, and working memory performance in healthy older people.

Conflict of interest

The authors state that there are no conflicts of interest associated with the research.

Contributors

Author Vanesa Hidalgo participated in the acquisition and interpretation of the data, managed the literature search, undertook the statistical analyses and, with Alicia Salvador, designed the study and wrote the manuscript. Author Mercedes Almela participated in the acquisition of the data, and, with Matias M. Pulopulos, interpreted the data and revised the manuscript. All the authors contributed to and approved the final manuscript.

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