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Hair cortisol and cognitive performance in healthy older people



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Summary Worse cognitive performance in older people has been associated with hypothalamic–pituitary–adrenal axis dysregulation (in particular, higher cortisol levels). Analysis of hair cortisol concentrations (HCC) is a novel method to measure long-term cortisol exposure, and its relationship with cognition in healthy older people has not yet been studied. We investigated whether HCC (measured in hair scalp) and diurnal salivary cortisol levels (awakening, 30 min after awakening, and evening, across two days) were related to cognitive performance (assessed with the Trail-making Test A and B, Digit Span Forward and Backward, word list-RAVLT and Stories subtest of the Rivermead) in 57 healthy older people (mean age = 64.75 years, SD = 4.17). Results showed that lower HCC were consistently related to worse working memory, learning, short-term verbal memory (RAVLT first trial and immediate recall) and long-term verbal memory. In contrast, higher mean levels and higher diurnal area under the curve of diurnal salivary cortisol were related to worse attention and short-term verbal memory (immediate story recall), respectively. Interestingly, a higher ratio of mean levels of diurnal salivary cortisol over HCC were related to worse performance on working memory and short-term verbal memory, suggesting that those individuals with lower long-term cortisol exposure might be more vulnerable to the negative effect of HPA-axis dysregulation on these cognitive processes. Our findings suggest that both low long-term cortisol exposure and a possible dysregulation of the diurnal rhythm of the HPA-axis may account, at least in part, for the inter-individual variability in cognitive performance in healthy older people.

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1. Introduction

Aging is associated with a decrease in many cognitive functions (Silver et al., 2012). However, the pattern and magnitude of this decline are highly variable and depend on several factors. Stress and cortisol, the end product of the hypothalamic–pituitary–adrenal axis (HPA-axis), have been proposed as potential mediators of this age-related cognitive change. Cortisol can affect cognitive performance acutely through the activation of receptors located in the prefrontal cortex, hippocampus and amygdala (for review see: Lupien et al., 2007). But more interestingly, HPA-axis activity has been linked to cognitive performance in older people, since a marked increase in basal cortisol levels with age has been associated with cognitive decline and a reduction in hippocampal volume (Lupien et al., 1998; Li et al., 2006). In addition, previous studies have shown that patients with Alzheimer's disease and Mild Cognitive Impairment have heightened basal cortisol levels (Arsenault-Lapierre et al., 2010; Venero et al., 2013).

Similarly, most of the studies that have investigated the cross-sectional relationship between basal HPA-axis activity and cognitive performance in healthy older people have shown that HPA-axis dysregulation (particularly, higher cortisol release) is related to worse cognitive performance (e.g. Hodgson et al., 2004; Karlamangla et al., 2005; MacLulich et al., 2005; Li et al., 2006; Kuningas et al., 2007; Lee et al., 2007, 2008; Beluche et al., 2010; Comijs et al., 2010; Evans et al., 2011; Franz et al., 2011; Gerritsen et al., 2011; Johansson et al., 2011), although other studies have not found any relationship between cortisol and cognition (Peavy et al., 2009; Köhler et al., 2010; Schrijvers et al., 2011). In all of these studies, salivary, blood or urinary samples have been used to measure HPA-axis activity. These biological samples are useful to obtain information about HPA-axis dynamics, as repeated samples allow researchers to measure variations in cortisol levels across a given period by comparing different points, and they can also be used to determine day-to-day variations in cortisol levels.

These biological samples reflect point measures (plasma or saliva) or integral cortisol levels over a few hours (urine). Therefore, a large number of samples would be required to measure cortisol exposure over an interval of months. Additionally, cortisol levels measured in saliva, blood or urine may be highly variable, as they are likely to be affected by several factors that may occur shortly before sampling (Stalder and Kirschbaum, 2012). Thus, although these measures have contributed greatly to understanding the short-term relationship between HPA-axis activity and cognitive performance, much more research is needed to explore the relationship between long-term endogenous cortisol exposure (months) and cognitive performance.

The measurement of cortisol levels in hair, a recently developed and more stable way to measure basal cortisol exposure over months than salivary, blood or urine samples, appears to be a good candidate for use in this context. Hair cortisol concentrations (HCC) have been considered an integrated measure of cortisol exposure over a period of up to several months (for more details, see: Russell et al., 2011; Stalder and Kirschbaum, 2012). Previous studies have shown that HCC might be unaffected by circadian rhythmicity and

situational context and have a high degree of intra-individual stability (Skoluda et al., 2012; Stalder et al., 2012b; but see also Sharpley et al., 2012). However, as it is a relatively new technique, some questions still remain unanswered, such as the physiological mechanisms through which cortisol gets into hair (Meyer and Novak, 2012). At the moment, several studies support the idea that HCC may be a useful technique in investigating long-term exposure to cortisol in young and older people. These studies have shown, for example, higher HCC in individuals with diseases or conditions that typically show higher cortisol levels, such as Cushing's syndrome (Thomson et al., 2010), coronary artery disease (Pereg et al., 2011), chronic pain (Van Uum et al., 2008), diabetes mellitus in older people (Feller et al., 2014) and hydrocortisone replacement therapy in young and older people (Gow et al., 2011). Moreover, higher HCC have been found in endurance athletes (Skoluda et al., 2012) and long-term unemployed individuals (Dettenborn et al., 2010).

The aim of the present study was to investigate the relationship between cognitive performance in healthy older people and cortisol exposure in the previous months determined by measuring cortisol in hair. We carried out a neuropsychological assessment (learning, short- and long-term memory, attention and executive function) of healthy older people, and we took samples of their hair to measure total cortisol exposure in the previous three months. Additionally, diurnal cortisol levels were measured using salivary samples, in order to compare the results with the findings in hair samples. Based on previous studies with salivary, blood and urine cortisol samples, we expected higher HCC to be associated with worse cognitive performance.

2. Method

2.1. Participants

As a part of a larger study designed to investigate the effects of stress and cortisol on cognitive performance in older people (Mneme Project), we recruited a healthy subgroup of older people to participate in this study. Participants belonged to a study program at the University of Valencia for people older than 55 years of age. We recruited subjects to participate in the present study in the classes of this study program. Two hundred twenty-two individuals volunteered to participate, and these volunteers were interviewed telephonically to determine whether they met the study prerequisites. In order to avoid a large number of potentially confounding factors that could interfere with the study, we selected a homogeneous healthy sample. The exclusion criteria and the number of volunteers excluded for these reasons were the following: smoking more than 10 cigarettes a day ($n = 2$), alcohol abuse (we asked the participants how many glasses and what kind of alcoholic beverages they drank per week; following the UK National Health Service definitions, only lower-risk drinkers were allowed to participate; www.nhs.uk/livewell/alcohol) or other drug abuse ($n = 1$), visual or hearing problems (except wearing glasses) ($n = 2$), presence of an endocrine ($n = 9$), neurological ($n = 6$) or psychiatric ($n = 6$) disease, using any medication directly related to emotional or cognitive function or medication that was able to influence hormonal levels, such as

glucocorticoids, anti-diabetic medication, antidepressants, anticoagulants, β -blockers, benzodiazepines or psychotropic substances ($n = 33$) (vitamins and sporadic use of painkillers were allowed), having been under general anesthesia once or more in the past year ($n = 9$), and the presence of a stressful life event during the last year (volunteers were asked about the occurrence of any important event considered stressful that changed their life in a negative way; e.g. widowhood) ($n = 8$). After this interview, 63 volunteers decided not to enroll in the study due to the demands of the study protocol (neuropsychological assessment and two days of salivary sampling at home). In addition, 26 volunteers had to be excluded because they did not have enough hair for biochemical cortisol analyses (3 cm).

Finally, 57 participants (14 men and 43 women), from 56 to 77 years old, met the requisites to participate in this study. All the participants scored more than twenty-eight on the MEC (Spanish version of the Mini-Mental Status Examination; Lobo et al., 1999), indicating the absence of cognitive impairment, and none of the participants met the criteria for dementia, as defined by the NINCDS-ADRDA criteria for Alzheimer's disease, or the criteria for Mild Cognitive Impairment, as defined by the European Consortium on Alzheimer's Disease (Portet et al., 2006). All female participants were postmenopausal; they had had their last menstrual period more than 2 years before the testing time, and none of them were taking estrogen replacement therapy. Thirteen participants (4 men and 9 women) were taking anti-hypertensive medication (none of them were taking β -blockers). Nevertheless, the inclusion of these participants did not change the statistical results and conclusions of this study. None of the participants had any other kind of cardiovascular disease.

2.2. Procedure and neuropsychological assessment

Participants were asked to attend a neuropsychological assessment between 1000 h and 1200 h in a laboratory at the Faculty of Psychology. Previously, 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, take any stimulants (such as coffee, cola, caffeine, tea or chocolate), or brush their teeth at least 1 h prior to the session. All participants provided written informed consent for their participation in the study, which was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Research Ethics Committee of the University of Valencia.

Upon arrival, the height and weight of the participants were measured to calculate the Body Mass Index (BMI). In order to avoid the possible effects of fatigue and/or a testing-related stress effect (Goldstein and McNeil, 2004), the neuropsychological assessment was designed to last no longer than 1.5 h. Moreover, the selection of the cognitive tasks was based on previous studies by our group and others that have investigated the relationship between cortisol and cognitive performance (MacLulich et al., 2005; Lee et al., 2007; Evans et al., 2011; Almela et al., 2012). The Spanish version of the Rey Auditory Verbal Learning Test (RAVLT; Miranda and Valencia, 1997) and

the Story Recall subtest of the Spanish version of the Rivermead Behavioral Memory Test (Wilson et al., 1999) were used to measure learning and verbal memory. On the RAVLT, the experimenter reads out a list of 15 words (list A) that the participant is asked to reproduce, and this procedure is repeated five times. In trial six, participants were asked to reproduce a new 15-word list (interference list); after that, participants had to recall list A without hearing it again. Three outcomes were used in subsequent analyses: (i) first trial: total number of words recalled on the first trial; (ii) total learning: total number of words recalled on the first five trials; (iii) immediate recall: total number of words recalled after the interference trial. On the Story recall subtest of the Rivermead, participants had to recall as many "ideas" as possible from two brief stories read aloud by the experimenter. Recall was scored according to the manual. From this test, two outcomes were used in the subsequent analyses: (i) immediate story recall: total number of "ideas" recalled from the two narratives immediately after having heard them; (ii) delayed story recall: percentage of the total number of "ideas" recalled from the two narratives after 20 min, compared to the number of ideas recalled on the immediate recall trial. The Trail Making Test form A (TMT-A) was used to assess general psychomotor speed and attention, and the Trail Making Test form B (TMT-B) was used to assess executive function (Reitan, 1992); the outcome of each part was the time (seconds) needed to perform the test. Finally, the Digit Span Forward Subtest of the Wechsler Memory Scale III (Wechsler, 1997) was used as a task related to attentional processes, and the Digit Span Backward subtest was used to assess working memory (Conklin et al., 2000).

2.3. Cortisol measurements

2.3.1. Hair cortisol

At the end of the neuropsychological session, 3-cm hair samples (~3 mm diameter) were carefully cut with fine scissors as close as possible to the scalp from a posterior vertex position. Based on a hair growth rate of 1 cm/month (Wennig, 2000), these segments are assumed to reflect hair grown over the three-month period prior to the respective sampling points. Hair samples were prepared and analyzed in the laboratory of Prof. Kirschbaum (Department of Psychology, Technische Universität Dresden, Germany), following the laboratory protocol described in detail in Kirschbaum et al. (2009). Hair samples were incubated in 1800 μ l methanol for 18 h at 45 °C (see Stalder et al., 2012b, for a more detailed description), and then analyzed by liquid chromatography mass spectrometry/MS.

2.3.2. Salivary cortisol

Participants provided one salivary sample at the beginning (pre) and one at the end (post) of the neuropsychological session, by using salivettes (Sarstedt, Nümbrecht, Germany). Two indices were calculated from the salivary cortisol samples during the session: (i) Mean_{CortNeuro}: mean cortisol levels during the neuropsychological assessment and (ii) cortisol change (Change_{CortNeuro}: cortisol levels pre-session minus cortisol levels post-session).

In order to measure diurnal cortisol levels, at home participants collected 3 saliva samples per day for 2

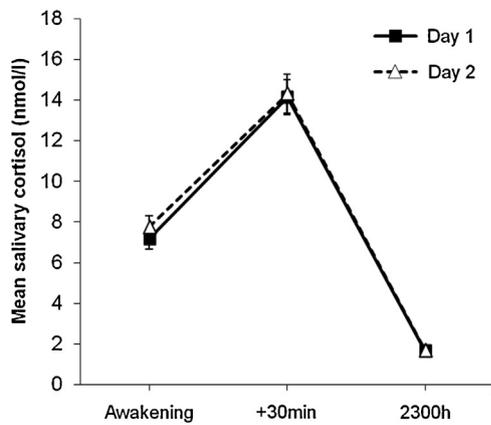


Figure 1 Means of diurnal salivary cortisol levels during both days of sampling.

consecutive days using salivettes. There was a mean of 9 days (± 1.5) between the neuropsychological assessment and the measurement of the diurnal cortisol. No samples were provided over the weekend, and participants were instructed to drink only water, and not eat, smoke or brush their teeth, at least 1 h prior to each saliva sample. The samples were provided immediately after waking, 30 min post-awakening and at 2300 h. In order to objectively verify participant adherence, salivettes were stored in MEMS T TrackCap containers (MEMS 6 TrackCap Monitor, Aardex Ltd., Switzerland), and participants wrote down the exact sampling times in a diary. There were no differences between the salivary cortisol levels at home across days ($p = .297$) (Fig. 1). Two indexes were calculated from these salivary cortisol samples: (i) $\text{Mean}_{\text{CortDay}}$, mean of cortisol levels in the three samples and (ii) $\text{AUC}_{\text{CortDay}}$, averaged area-under-the-curve with respect to the ground (Pruessner et al., 2003). Salivary samples provided during the session and at home were stored and analyzed as described in detail in Almela et al. (2012).

2.4. Statistical analysis and data management

Because hair and salivary cortisol values did not show normal distributions, they were square root transformed (Sqrt). We performed regression analyses to investigate the relationship between cortisol outcomes (HCC, $\text{Mean}_{\text{CortDay}}$ and $\text{AUC}_{\text{CortDay}}$) and cognitive performance, and several covariates were included to control for possible confounder effects. Age and body mass index (BMI) were included because of their effects on both cognitive performance and HPA-axis activity (Cournot et al., 2006; Dettenborn et al., 2012; Silver et al., 2012; Stalder and Kirschbaum, 2012). Subjective Socio-Economic Status (SES, measured using the MacArthur Scale of Subjective Social Status; see Adler et al., 2000) was also included as covariate because it is related to HPA-axis activity (Wright and Steptoe, 2005; Cohen et al., 2006) and health (Adler et al., 2000; Singh-Manoux et al., 2005; Demakakos et al., 2008). We included SES but not educational level as covariate, given that SES is related to educational level ($r = .320$, $p = .015$) and has a stronger relationship with HPA-axis activity than educational level (Wright and Steptoe,

2005; Cohen et al., 2006).¹ $\text{Mean}_{\text{CortNeuro}}$ and $\text{Change}_{\text{CortNeuro}}$ were also included as covariates to control for the possible acute effect of cortisol on cognition (Almela et al., 2011; Hidalgo et al., 2012, 2014) and the stressfulness of the testing situation (Sindi et al., 2013).

In the regression analyses testing the relationship between $\text{AUC}_{\text{CortDay}}$ and cognitive performance, we also included as covariate the mean of the cortisol levels in the first saliva sample (immediately after awakening) to control for differences in the awakening time that could produce differences in cortisol concentrations (Clow et al., 2010).

Moreover, for the Digit Span Backward and TMT-B, we included the Digit Span Forward and TMT-A, respectively, as covariates, in order to specifically pinpoint the executive function measure of these two tasks (working memory for Digit Span Backward and set-shifting ability for TMT-B).

The design of the regression analyses was as follows: in step 1, we included the covariates and Sex (0 = Women; 1 = Men). In step 2, we added HCC or $\text{MEAN}_{\text{CortDay}}$ or $\text{AUC}_{\text{CortDay}}$. Based on Aiken and West (1991), we performed a moderator regression analysis to investigate whether sex was a moderator. Therefore, in step 3, we included the interaction between HCC or $\text{Mean}_{\text{CortDay}}$ or $\text{AUC}_{\text{CortDay}}$ and Sex. Correlation analyses revealed that there were no significant associations among the covariates included (all $p > .118$). Effect sizes (f^2) were reported for the regression analyses (Cohen, 1988). When not otherwise specified, values are mean \pm standard error of mean (SEM).

3. Results

3.1. Description of the sample

Fifty-seven participants were assessed in this study; however, the number of participants used for each analysis varies, given that some of the participants were not included in some of the analyses for the following reasons: (i) 1 woman did not provide a large enough salivary sample in the neuropsychological session; (ii) 2 women and 1 man did not provide a large enough salivary sample at home; (iii) 2 women and 1 man were outliers for salivary cortisol data at home (+3SD); (iv) 2 women were outliers for HCC (+3SD). Thus, the sample available for the analyses was composed of: HCC = 54 participants (14 men and 40 women; reasons for participants' exclusion i and iv); Salivary cortisol data = 50 participants (12 men and 38 women; reasons for participants' exclusion i, ii and iii). Finally, specifically for the TMT, four participants (2 men and 2 women) did not perform this test because they were not wearing their glasses, and they needed them to take the test (HCC: $n = 50$; Salivary data: $n = 46$).

The mean age of the sample was 64.75 years (from 56 to 77 years old). Half of the participants (52.6%) had an educational level beyond high school and their SES was medium

¹ The inclusion of educational level instead of SES as a covariate in the regression analyses does not change the statistical conclusion of the analyses performed with HCC and $\text{MEAN}_{\text{CortDay}}$, but it reduces the significance of the relationship between $\text{AUC}_{\text{CortDay}}$ and immediate story recall, approaching a non-significant association ($\beta = -.267$, $p = .124$).

Table 1 Unadjusted correlation analyses between the variables included in the regression analyses.

	Cortisol					Age	BMI	SES ^a	TMT-A	TMT-B ^b	DSF	DSB ^c	First trial RAVLT	Total learn RAVLT	Imm. recall RAVLT	Rivermead	
	HCC	M _{CD}	AUC _{CD}	M _{CN}	C _{CN}											Imm. recall	%Del. recall
HCC																	
Mean _{CortDay}	.270[#]																
AUC _{CortDay}	-.147	.878^{**}															
Mean _{CortNeuro}	.121	.239[#]	.193														
Change _{CortNeuro}	.237[#]	.188	.199	.108													
Age	.074	-.053	-.053	-.104	-.185												
BMI	.389[*]	-.129	-.170	-.118	-.154	.121											
SES ^a	.026	.016	-.068	-.177	.058	-.229 [#]	-.023										
TMT-A	-.018	-.045	.077	-.127	.021	.331[*]	-.171	-.069									
TMT-B ^b	-.204	-.102	-.009	.225	-.122	.228	.003	-.84	.441[*]								
DSF	.187	-.137	-.070	-.108	.136	-.305 [*]	-.026	-.116	-.212	-.248 [#]							
DSB ^c	.305[*]	-.072	-.028	-.180	.039	-.071	-.109	-.120	-.166	-.514 ^{**}	.542^{**}						
First trial RAVLT	.275[*]	.055	.088	-.153	-.263 [#]	.048	.062	.123	.199	-.001	-.059	.097					
Total learning RAVLT	.260[#]	-.079	-.049	-.252 [#]	-.322 [*]	-.038	.560	.108	.072	-.134	.044	.097	.675^{**}				
Imm. recall RAVLT	.146	-.164	-.126	-.196	-.153	.084	.057	.006	-.116	-.033	-.181	.078	.343[*]	.649^{**}			
Imm. recall Rivermead	.274[*]	.062	-.118	-.140	-.102	-.041	-.040	-.080	-.132	-.227	.425[*]	.447^{**}	.145	.186	.044		
%Delayed recall Rivermead	.290[*]	.124	< .001	-.158	-.004	-.062	.043	.118	-.221	-.214	.041	.345[*]	.169	.201	.174	.360[*]	

M_{CD}: Mean_{CortDay}; AUC_{CD}: AUC_{CortDay}; M_{CN}: Mean_{CortNeuro}; C_{CN}: Change_{CortNeuro}; BMI: Body Mass Index; SES: Subjective socio-economic status; TMT: Trail Making Test; DSF: Digit Span Forward; DSB: Digit Span Backward; RAVLT: Rey Auditory Verbal Learning Test.

^a Unadjusted correlation analyses for educational level show a significant association with SES ($r = .320, p = .015$).

^b Adjusted correlations for TMT-B show a significant association with Digit Span Backward ($r = -.474, p < .001$), and the association with MeanCortNeuro was $r = .289 (p = .058)$.

^c Adjusted correlations for Digit Span Backward also show a significant association with delayed story recall ($r = .302, p = .046$). None of the other correlation analyses for educational level, adjusted TMT-B, or adjusted Digit Span Backward were significant ($p > .108$).

[#] $p < .10$.

^{*} $p < .05$.

^{**} $p < .001$.

($M = 5.48$, $SD = .972$). Student t -tests showed that there were no sex differences in age (Men: $M = 64.50$, $SD = 5.34$; Women: $M = 64.84$, $SD = 3.78$), body mass index (Men: $M = 27.02$, $SD = 2.69$; Women: $M = 25.99$, $SD = 3.72$), SES, educational level, $Mean_{CortNeuro}$ or $Change_{CortNeuro}$ (all $p > .262$). Men showed higher HCC than women ($t(53) = 2.06$, $p = .044$; Men: $M = 3.37$, $SD = 2.48$; Women: $M = 2.07$, $SD = 2.07$), but there were no sex differences in $Mean_{CortDay}$, $t(49) = 1.40$, $p = .165$; Men: $M = 8.69$, $SD = 2.41$; Women: $M = 7.50$, $SD = 2.59$) or the $AUC_{CortDay}$ ($t(49) = 1.32$, $p = .192$; Men: $M = 44.60$, $SD = 41.59$; Women: $M = 41.59$, $SD = 7.01$).

3.2. Unadjusted correlation analyses

Correlation analyses with HCC and separate cortisol measures show that associations between HCC and +30 min and evening cortisol were not significant ($p > .113$), and with the awakening sample, the association was $r = .273$ ($p = .057$).

Table 1 shows unadjusted correlations among all variables included in the regression analyses. HCC was associated with BMI ($r = .389$, $p = .003$), unadjusted Digit Span Backward ($r = .305$, $p = .024$) (Fig. 2A), first trial of the RAVLT ($r = .275$, $p = .042$) (Fig. 2B), immediate story recall ($r = .274$, $p = .043$), and delayed story recall ($r = .290$, $p = .033$) (Fig. 2C). $Mean_{CortDay}$ was associated with $AUC_{CortDay}$ ($r = .878$, $p < .001$). Additionally, there were significant intercorrelations among cognitive test outcomes. These significant associations range from $r = .343$ between the first trial and immediate recall on the RAVLT ($p = .009$), to $r = .675$ between the first trial and total learning on the RAVLT ($p < .001$).

3.3. Regression analyses.

3.3.1. Relationship between cortisol outcomes (HCC, $Mean_{CortDay}$ and $AUC_{CortDay}$) and cognitive performance

In the first step of the regression analyses, we included as covariates: age, BMI, SES, $Mean_{CortNeuro}$, $Change_{CortNeuro}$, Sex (0 = Women; 1 = Men), cortisol levels in the first saliva sample at home (for analyses with $AUC_{CortDay}$), TMT-A scores (for analyses with TMT-B), and Digit Span Forward (for analyses with Digit Span Backward). Table 2 shows the second step of the regression analyses with HCC, $Mean_{CortDay}$ and $AUC_{CortDay}$ as predictors, and cognitive test outcomes as dependent variables.

The results show that higher HCC were related to better performance on the adjusted Digit Span Backward ($\beta = .271$; $p = .050$), the first trial of the RAVLT ($\beta = .514$; $p = .001$), total learning on the RAVLT ($\beta = .591$; $p < .001$), Immediate recall on the RAVLT ($\beta = .489$; $p = .001$), and delayed story recall ($\beta = .390$; $p = .022$). In addition, higher $Mean_{CortDay}$ was related to worse performance on the Digit Span Forward ($\beta = -.250$; $p = .031$), and higher $AUC_{CortDay}$ was related to worse performance on Immediate story recall ($\beta = -.430$; $p = .008$).

3.3.2. Relationship between the ratio of diurnal salivary cortisol over HCC and cognitive performance

Given that regression analyses showed an opposite relationship between salivary cortisol and HCC and some cognitive

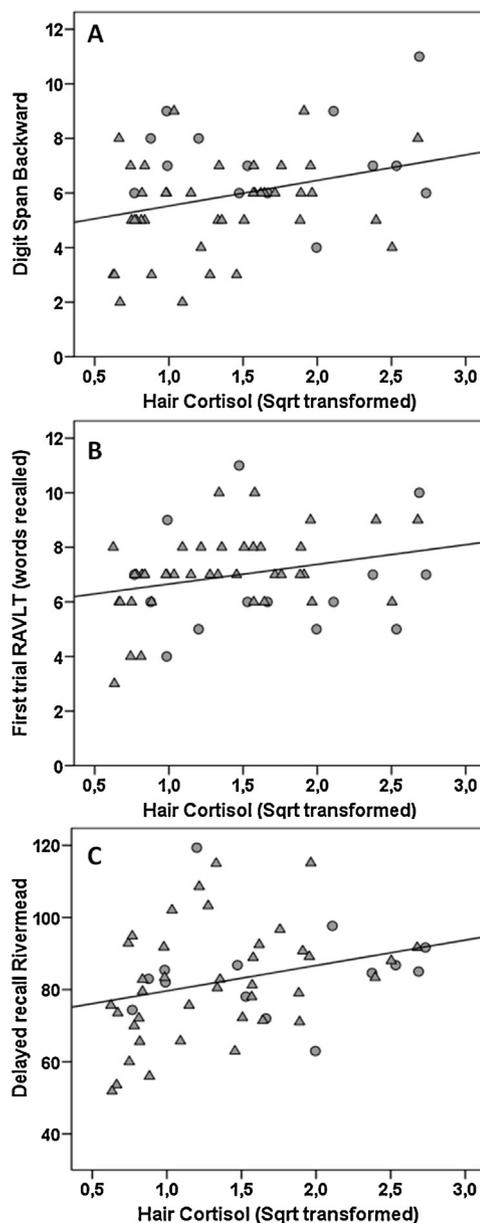


Figure 2 Scatter plots for men (circles) and women (triangles) for the correlations between hair cortisol and (A) unadjusted Digit Span Backward ($r = .305$, $p = .024$), (B) First trial on the RAVLT ($r = .275$, $p = .042$), and (C) Delayed recall on the Rivermead ($r = .290$, $p = .033$).

outcomes, we performed a set of analyses to explore whether the relationship between diurnal salivary data and cognitive performance can be interpreted in relation to baseline long-term cortisol levels (i.e. HCC). To do so, we performed the same regression analyses, but using the ratios of diurnal salivary cortisol over HCC ($Mean_{CortDay}/HCC$ and $AUC_{CortDay}/HCC$) as a predictor.

Table 3 shows the regression analyses with $Mean_{CortDay}/HCC$ and $AUC_{CortDay}/HCC$ as predictors. The results show that a higher $Mean_{CortDay}/HCC$ ratio was related to worse performance on the adjusted Digit Span Backward ($\beta = -.308$; $p = .029$), the first trial of the RAVLT ($\beta = -.538$; $p < .001$), total learning on the RAVLT

Table 2 Step 2 of the regression analyses with cortisol outcomes (HCC, Mean_{CortDay} and AUC_{CortDay}) as predictors.

		First Trial RAVLT	Total Learning RAVLT	Immediate recall RAVLT	Immediate recall Rivermead	Delayed recall Rivermead
HCC	Adj R^2	.202	.378	.286	.160	.039
	β	.514	.591	.489	.251	.390
	p	.001	<.001	.001	ns	.022
	f^2	.264	.449	.267	.059	.124
Mean _{CortDay}	Adj R^2	.036	.018	.077	.058	.042
	β	.131	.058	-.056	-.015	.163
	p	ns	ns	ns	ns	ns
	f^2	.015	.001	.005	<.001	.010
AUC _{CortDay}	Adj R^2	.013	-.043	.038	.236	-.095
	β	.171	.070	-.015	-.430	-.160
	p	ns	ns	ns	.008	ns
	f^2	.023	.003	.001	.191	.017
		TMT-A	TMT-B (adjusted)	Digit Span Forward	Digit Span Backward (adjusted)	
HCC	Adj R^2	.016	.288	.455	.336	
	β	-.036	-.267	.069	.271	
	p	ns	.074	ns	.050	
	f^2	.001	.081	.006	.089	
Mean _{CortDay}	Adj R^2	-.001	.176	.462	.225	
	β	.037	-.064	-.250	-.064	
	p	ns	ns	.031	ns	
	f^2	.002	.005	.111	.004	
AUC _{CortDay}	Adj R^2	.023	.161	.443	.203	
	β	.252	.067	-.128	-.050	
	p	ns	ns	ns	ns	
	f^2	.052	.004	.022	.002	

Adj: Adjusted; RAVLT: Rey Auditory Verbal Learning Test; TMT: Trail Making Test.

Step 1 (covariates) = In the first step of the regression analyses, we included as covariates: age, BMI, SES, Mean_{CortNeuro}, Change_{CortNeuro}, Sex (0 = Women; 1 = Men), mean cortisol levels in the first saliva sample at home (for analyses with AUC_{CortDay}), TMT-A scores (for analyses with TMT-B) and Digit Span Forward (for analyses with Digit Span Backward). *Step 3 (Sex Interactions)* = Digit Span Forward: HCC _ Sex, $p = .088$; *Post Hoc*: Men, $\beta = .313$, $p = .096$; Women, $\beta = -.086$, $p = .565$. Immediate recall Rivermead: Mean_{CortDay} _ Sex, $p = .086$; *Post Hoc*: Men, $\beta = -.584$, $p = .107$; Women, $\beta = .100$, $p = .534$. None of the other sex interactions were significant ($p > .100$).

($\beta = -.506$; $p = .001$), Immediate recall on the RAVLT ($\beta = -.384$; $p = .012$), and delayed story recall ($\beta = -.367$; $p = .032$). Importantly, MEAN_{CortDay}/HCC shows stronger associations with the adjusted Digit Span Backward and the first trial of the RAVLT than the association between the performance on these cognitive tasks and each of these two cortisol outcomes considered separately.

Additionally, a higher AUC_{CortDay}/HCC ratio was related to worse performance on the first trial of the RAVLT ($\beta = -.469$; $p = .003$), total learning on the RAVLT ($\beta = -.463$; $p = .003$), immediate recall on the RAVLT ($\beta = -.348$; $p = .025$), immediate story recall ($\beta = -.348$; $p = .024$) and delayed story recall ($\beta = -.379$; $p = .030$). None of these associations were stronger than the one observed between the performance on these cognitive tasks and each of these cortisol outcomes considered separately.

4. Discussion

We investigated the relationship between HCC and diurnal cortisol secretion and cognitive performance in healthy older people. We observed that lower long-term cortisol exposure

(i.e. the previous three months), measured in scalp hair, was consistently related to worse performance on: working memory (Digit Span Backward), learning (total learning on the RAVLT), short-term verbal memory (first trial and immediate recall on the RAVLT), and long-term verbal memory (delayed story recall). Additionally, higher diurnal salivary cortisol was related to worse performance on attention (association between MEAN_{CortDay} and Digit Span Forward) and short-term verbal memory (association between AUC_{CortDay} and Immediate story recall). Finally, the MEAN_{CortDay}/HCC ratio showed a stronger negative relationship with working memory (Digit Span Backward) and short-term verbal memory (first trial of the RAVLT) than the association observed between the performance on these cognitive tasks and each cortisol outcome considered separately.

It is important to emphasize that, given the correlational nature of this study, we cannot endorse causal relationships. However, our findings are noteworthy, as they provide consistent evidence for an association between low long-term cortisol exposure, measured in scalp hair, and worse executive function (working memory) and verbal memory (learning and short and long-term verbal memory). This association is

Table 3 Regression analyses with ratio outcomes (Mean_{CortDay}/HCC and AUC_{CortDay}/HCC) as predictors.

Step 2		First Trial RAVLT	Total Learning RAVLT	Immediate recall RAVLT	Immediate recall Rivermead	Delayed recall Rivermead
Mean _{CortDay} /HCC	Adj R ²	.272	.225	.226	.131	-.051
	β	-.538	-.506	-.384	-.298	-.367
	<i>p</i>	<.001	.001	.012	.061	.032
	<i>f</i> ²	.369	.307	.176	.095	.128
AUC _{CortDay} /HCC	Adj R ²	.210	.189	.183	.200	.009
	β	-.469	-.463	-.348	-.348	-.379
	<i>p</i>	.003	.003	.025	.024	.030
	<i>f</i> ²	.261	.248	.138	.141	.133
		TMT-A	TMT-B (adjusted)	Digit Span Forward	Digit Span Backward (adjusted)	
Mean _{CortDay} /HCC	Adj R ²	.006	.224	.406	.325	
	β	.018	.238	.014	-.308	
	<i>p</i>	ns	ns	ns	.029	
	<i>f</i> ²	<.001	.064	<.001	.131	
AUC _{CortDay} /HCC	Adj R ²	-.016	.206	.435	.283	
	β	.087	.226	.002	-.261	
	<i>p</i>	ns	ns	ns	.069	
	<i>f</i> ²	.006	.059	<.001	.091	

Adj: Adjusted; RAVLT: Rey Auditory Verbal Learning Test; TMT: Trail Making Test.

Step 1 (covariates) = In the first step of the regression analyses, we included as covariates: age, BMI, SES, Mean_{CortNeuro}, Change_{CortNeuro}, Sex (0 = Women; 1 = Men), mean cortisol levels in the first saliva sample at home (for analyses with AUC_{CortDay}), TMT-A scores (for analyses with TMT-B) and Digit Span Forward (for analyses with Digit Span Backward). *Step 3 (sex interactions)* = None of the sex interactions were significant ($p < .098$).

at odds with our hypothesis, because it has the opposite direction from the one observed previously with salivary, blood or urine data (e.g. Lee et al., 2007; Comijs et al., 2010; Evans et al., 2011; Franz et al., 2011). In our study, higher salivary cortisol output was also related to worse cognitive performance, but this relationship was only found for two cognitive tasks (Digit Span Forward and Immediate story recall). It is likely that the inclusion of only three samples, although on two consecutive days, would account for the weaker results. Nevertheless, our results for acute (salivary) cortisol measurements are in the same direction as previous studies that associated higher daily cortisol output with worse cognitive performance.

In our opinion, the apparently contradictory results observed with HCC and salivary cortisol can be explained by differences in the information provided by hair and salivary samples. While hair cortisol measurement serves as a biomarker of integrated HPA activity over months, salivary samples measure cortisol levels at certain times of the day and, therefore, provide information about the regulation of HPA-axis circadian rhythmicity (Meyer and Novak, 2012).

Consistent with our results for HCC, animal studies have shown that low long-term cortisol exposure may have a negative effect on cognition, an effect that has been related to a low occupation of the mineralocorticoid receptors in the central nervous system (Sloviter et al., 1993; Stienstra et al., 1998; Wossink et al., 2001; Berger et al., 2006). These receptors are located especially in the hippocampus and prefrontal cortex and, under healthy basal cortisol levels, are almost saturated (cortisol occupation of approximately 70–80%). These results suggest that a low cortisol occupation

would be detrimental to these brain structures (for a review, see: de Kloet et al., 1999).

It is possible that, in our healthy sample, higher HCC in those participants with better cognitive performance may reflect the exposure to more activating and stimulating situations every day. Along this line, it has been shown that activities such as physical exercise, cognitively challenging activities, and social interactions can trigger intermittent hormonal activation during the day (Cadore et al., 2008; Kukolja et al., 2008; van der Meij et al., 2010). The repetition of these types of activities every day would reflect, in the long run, higher long-term cortisol exposure, which might produce a higher occupation of mineralocorticoid receptors and, thus, the maintenance of brain structures involved in better cognitive performance. Supporting this idea, several studies have shown that living in an enriched environment increases basal cortisol levels and enhances the cognitive performance of young and aging animals (e.g. Kempermann et al., 2002; Marashi et al., 2003; Moncek et al., 2004; Sampedro-Piquero et al., 2013). Of course, the direction of this relationship could also be the opposite. That is, healthy older people with better cognitive performance might be more interested in highly stimulating activities that would provoke higher long-term cortisol levels. The direction of these relationships should be addressed in future research. In any case, individuals' lifestyles and long-term activities would have an impact on cortisol concentrations measured in hair, but much less in salivary, blood or urine samples.

In the case of salivary samples, higher daily cortisol output would reflect a dysregulation of the diurnal rhythm of the HPA-axis, which would be related to worse cognitive

performance. It is likely that a flatter salivary cortisol slope will result in higher total salivary cortisol output on the sampling days (Beluche et al., 2010). Both factors, i.e. having a flatter daily cortisol slope and higher daily cortisol output, have been consistently related to cognitive impairment in older people (e.g. Lee et al., 2007; Evans et al., 2011; Franz et al., 2011; Stawski et al., 2011) and to health problems such as cancer and chronic fatigue (Abercrombie et al., 2004; Nater et al., 2008). A dysregulation in HPA-axis rhythmicity can be measured with salivary, blood or urine samples, but not with HCC. This translates into a low association between HCC and salivary cortisol levels in our study and in others (Meyer and Novak, 2012).

Interestingly, we observed that the MEANCortDay/HCC ratio had a stronger negative relationship (lower p value and higher effect size) with working memory (Digit Span Backward) and immediate verbal memory (first trial of the RAVLT) than the association of these tasks with each of these two cortisol outcomes considered separately. This result was also observed for the immediate story recall task, although this relationship did not reach statistical significance ($p = .061$). These are interesting findings, as they show a link between our results in HCC and salivary samples, indicating that those individuals with higher long-term basal cortisol levels (which might represent a higher cortisol occupation of the mineralocorticoid receptors) would be less vulnerable to the detrimental effect of a dysregulation of the HPA-axis on cognition. This effect was observed specifically in working memory and short-term verbal memory; however, further studies using more salivary samples might reveal whether the same associations could be observed with other cognitive tasks. Additionally, these results support Walton et al. (2013), who, investigating patients with acute trauma, suggested that hair-normalized salivary cortisol might be a better biomarker of HPA-axis activity than salivary cortisol alone. Future studies could benefit from using this kind of analysis.

The findings reported in this study are noteworthy, as they contribute to the knowledge about the relationship between HCC and cognition in healthy older people. To exclude unknown interactions between HCC and possible confounds, we used very restrictive exclusion criteria, and our sample consisted of healthy individuals with cortisol levels in the normal range. As a result, our sample was small; therefore, future studies with larger samples should confirm our findings, especially in men. Furthermore, the exclusion criteria for our study can affect the generalizability of our results to older people with some age-related diseases. For example, patients with coronary artery disease have shown both high HCC and memory impairments (Vinkers et al., 2005; Pereg et al., 2011; Saleem et al., 2013), suggesting that higher HCC levels than those observed in our study, which may be reflecting unhealthy levels, can also be related to worse cognitive performance. In the same sense, the use of a homogeneous sample could affect our results, as it could cause insufficient variance in cognitive performance, reducing the number of significant associations (e.g., the relationship between HCC and immediate story recall or TMT-B). Future studies may benefit from including older people with age-related diseases.

A limitation of the study is that we did not control the frequency of hair washing and hair treatments, which have

been observed to affect HCC in some studies (Sauve et al., 2007; Manenschijn et al., 2011), but not in others (Dowlati et al., 2010; Dettenborn et al., 2012; Stalder et al., 2012a). Furthermore, due to characteristics of the technique, bald people and/or people with hair shorter than 3 cm could not participate in the study. Results of previous studies suggest the HCC can be used in the older population (Gow et al., 2011; Pereg et al., 2011; Feller et al., 2014); however, the interpretation of timing in HCC (three months) should be considered with caution, as the rate of hair growth might be reduced in some older individuals (Van Neste, 2004). Finally, it has been proposed that a small quantity of local cortisol can be synthesized in hair follicles and might provoke transitory changes in HCC (see Sharpley et al., 2012). However, direct and indirect validation studies support the notion that the bloodstream would be the principal source of the cortisol concentrations in hair (for reviews see: Gow et al., 2010; Meyer and Novak, 2012; Russell et al., 2011; Sharpley et al., 2012; Stalder and Kirschbaum, 2012; Staufenbiel et al., 2012; Wosu et al., 2013). Nevertheless, future research can explore whether this *peripheral* cortisol might also contribute to the observed relationship between HCC and cognition.

In summary, the current study presents the first evidence of a relationship between low long-term cortisol exposure, measured in scalp hair, and worse cognitive performance (working memory, learning, and short and long-term verbal memory) in healthy older people. We also replicated, at least in part, results of previous studies showing that higher daily salivary cortisol output is related to worse cognitive performance. Additionally, we observed that those individuals with lower long-term cortisol exposure would be more vulnerable to the negative effect of HPA-axis dysregulation on cognition. Taken together, our results suggest that differences in cognitive performance in normal aging are related to inter-individual variability in both long-term cortisol exposure and cortisol variation during the day.

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Conflicts of interest

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

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