



Are neuroticism and extraversion related to morning cortisol release in healthy older people?



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ABSTRACT

The cortisol awakening response (CAR) is a discrete component of the hypothalamic-pituitary-adrenal axis (HPA-axis) function that has been widely related to both health and some personality traits. There is evidence that neuroticism and extraversion affect health and well-being and play a damaging or protective role, respectively. In this study, we aimed to explore the relationship between these personality dimensions and morning cortisol concentrations in people aged 55 or older. To do so, morning saliva samples were collected on two consecutive weekdays from a total of 160 older men and women. Neuroticism and extraversion were assessed using the Eysenck Personality Questionnaire-Revised, short form (EPQ-RS). Our results showed that neuroticism was negatively related to overall morning cortisol concentrations (AUC_G) (i.e., area under the curve with respect to the ground in cortisol levels), but not to the CAR. When we explored sex as a moderator, neuroticism was related to a CAR of increased magnitude in women, although this relationship was not significant in men. No significant relationships were found between extraversion and CAR or AUC_G , regardless of sex. In conclusion, neuroticism – but not extraversion – was related to HPA-axis function in older adults, highlighting its potential relevance in health alterations associated with HPA-axis functioning.

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1. Introduction

There is a physiological response to waking characterized by an increase in cortisol concentrations, peaking between 30 and 45 min post-awakening (Stalder et al., 2016; Fries et al., 2009). This response is considered to be an indicator of cortisol rhythm regulation, as a part of normal healthy human circadian physiology. The sharp increase in cortisol after awakening has been defined as the dynamic of a post-awakening increase in cortisol levels (the cortisol awakening response, hereinafter CAR), usually measured through the area under the curve with respect to the increase (AUC_I) or through the difference in cortisol concentrations between the moment of awakening and 30–45 min later. Additionally, the overall morning cortisol production combines information about the cortisol levels just after awakening and the CAR, which can be assessed through the area under the curve with respect to the ground (hereinafter, AUC_G) (see Pruessner et al., 2003).

Some brain structures, such as the hippocampus, amygdala and prefrontal cortex, contribute to the regulation of the HPA-axis activity due to the high levels of expression of Glucocorticoid receptors there (Fries et al., 2009; Herman et al., 2005; Patel et al., 2000). Interestingly, neuroticism and extraversion have been considered important moderators of the age-related loss of volume and structural connectivity in the

prefrontal cortex in older people, with higher neuroticism and lower extraversion being related to a greater age-related decline (Jackson et al., 2011).

For instance, higher neuroticism has been related to disorders and diseases associated with HPA-axis dysregulation, such as mild cognitive impairment (Kuzma et al., 2011), depressive and anxiety disorders (Ormel et al., 2013), Alzheimer's disease (Dar-Nimrod et al., 2012), chronic pain (Ramirez-Maestre and Esteve, 2013), and diabetes and metabolic syndrome (Mommersteeg and Pouwer, 2012). Given the importance of personality traits for health and their relationship with HPA-axis functioning (Lahey, 2009), several studies have investigated the role of neuroticism and extraversion in CAR and AUC_G , showing mixed results. Hauner et al. (2008) reported a reduced AUC_G in adolescents with high neuroticism and introversion compared to those with low neuroticism and introversion. Hill et al. (2013) reported that higher extraversion predicted heightened AUC_G in people from 18 to 78 years old, whereas van Santen et al. (2011) found that a reduced CAR was associated with higher extraversion. However, no significant relationships were found between neuroticism and the CAR in the Hill et al. (2013) and van Santen et al. (2011) studies. By contrast, Portella et al. (2005) reported heightened CAR and AUC_G in people from 21 to 57 years old with high neuroticism compared to those with low neuroticism. These results agree with Mangold et al. (2012), who observed that people with low neuroticism and acculturation showed increased CAR compared to other groups (high neuroticism and acculturation, high neuroticism and low acculturation, low

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neuroticism and high acculturation) in adults from 18 to 38 years old. Finally, other studies reported no relationships between neuroticism or extraversion and CAR or AUC_G (e.g., Chan et al., 2007; Munafò et al., 2006; Laceulle et al., 2015). Methodological issues may have contributed to these discrepancies in the results. None of the aforementioned studies used electronic devices to control for adherence in the cortisol measurements (Kudielka et al., 2003). Nor did they consider the variation in cortisol profiles (increase or decrease in cortisol levels immediately after awakening) (Almela et al., 2012; Thorn et al., 2006) or measure the CAR and AUC_G for at least two days, as recommended (Stalder et al., 2016). Thus, the lack of control over adherence to the protocol could result in a non-reliable CAR measurement, affecting the results (Stalder et al., 2016; Clow et al., 2010a, 2010b; Kudielka et al., 2003). Recently, no significant relationships between neuroticism and AUC_G and CAR were found in healthy young people when an electronic device was used to control saliva sampling times (García-Banda et al., 2014). Another aspect to be considered is that these studies investigated the relationship between personality and CAR in adolescents, young people and/or samples with a broad age range (i.e., including young adults and older people). Important changes have been reported in both neuroticism and extraversion (Eysenck, 1988) and CAR in older ages (see Fries et al., 2009; Clow et al., 2010a, 2010b); thus, age differences might affect the relationship between neuroticism, extraversion and CAR. However, no previous studies have analyzed this relationship specifically in older people.

Therefore, the present study aimed to investigate how neuroticism and extraversion traits are related to CAR and AUC_G in people aged 55–78 years old. To do so, 160 older participants collected three saliva samples during the first 45 min after awakening on two consecutive weekdays, and they completed the Eysenck Personality Questionnaire-Revised, short form (EPQ-RS). Based on previous literature, we expected to find positive relationships between AUC_G, CAR and extraversion (Hill et al., 2013; Hauner et al., 2008). Regarding neuroticism, we did not have any specific directional hypotheses, due to the contradictory results found by previous studies (García-Banda et al., 2014; Hill et al., 2013; Hauner et al., 2008; Chan et al., 2007; Portella et al., 2005). Additionally, we aimed to explore the importance of sex in the relationship between neuroticism, extraversion and HPA-axis function (CAR and AUC_G), in order to add evidence to the reported data on sex differences (DeSoto and Salinas, 2015; Fries et al., 2009; Lynn and Martin, 1997).

2. Material and methods

2.1. Participants

People aged 55–78 years old were recruited through informative advertisements. The exclusion criteria were: smoking >10 cigarettes a day, consuming drugs of abuse, having surgery under general anesthesia during the past year, the presence of neurological or psychiatric disorders, the use of drugs that affect cognitive or emotional functions, or that influence HPA function (e.g. glucocorticoids, benzodiazepines). All the female participants were postmenopausal and not receiving hormonal replacement therapy.

The final sample was composed of 160 native Spanish speakers (81 men) from 55 to 78 years old (Total sample: $M = 64$, $SD = 4.464$; Men: $M = 64$; $SD = 4.975$; Women: $M = 64$; $SD = 3.899$) with a medium subjective socioeconomic status (measured using the MacArthur Scale of Subjective Social Status; Adler et al., 2000; from 1: lowest, to 10: highest; Total sample: $M = 5.99$, $SD = 1.197$; Men: $M = 6.11$, $SD = 1.331$; Women: $M = 5.87$, $SD = 1.036$). Most of them had an educational level beyond high school (84.4%) and were retired (88.8%). Regarding marital status, 66% were married, 10.1% single, 11.3% divorced and 12.6% widowed.

2.2. Procedure

The study was performed according to the Declaration of Helsinki, and the Ethics Committee of the University approved the protocol. All the participants received verbal and written information about the study and signed an informed consent.

Participants completed the Spanish version of the Eysenck Personality Questionnaire short form (EPQ-RS; Eysenck and Eysenck, 1997) to obtain scores on neuroticism and extraversion. Moreover, they provided 3 saliva samples on two consecutive weekdays. The samples were taken immediately after awakening (0) and 30 min (+30) and 45 min (+45) post-awakening. Additionally, they recorded in a log their awakening time and the time of each saliva collection. The participants were thoroughly instructed about how to provide the saliva samples, and they were also given detailed written instructions (for more details, see Almela et al., 2012).

2.3. Measures

2.3.1 Eysenck Personality Questionnaire-Revised (Eysenck and Eysenck, 1975)

We used the Spanish version of the Eysenck Personality Questionnaire-Revised, short form (EPQ-RS; Eysenck and Eysenck, 1997). The EPQ-RS comprises a total of 48 items to which participants are asked to respond “yes” or “no”. It makes it possible to obtain scores for the three personality dimensions: neuroticism, extraversion and psychoticism. The scales range from 0 to 12, with higher scores indicating more neuroticism, extraversion or psychoticism. The alpha values range from 0.65 to 0.82 for men, and from 0.67 to 0.82 for women.

2.3.2 Cortisol analysis

Saliva was centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity. After that, the saliva was stored at -80°C until the assay was performed in duplicate by competitive solid phase radioimmunoassay (tube coated) with the commercial kit Spectria Cortisol RIA from Orion Diagnostica (Espoo, Finland). All the samples from each subject were analyzed in the same assay and in duplicate, with within- and inter-assay variation coefficients below 8%.

2.4. Data management and statistical analyses

Cortisol data were log transformed because they did not show normal distribution. The areas under the curve with respect to the ground (AUC_G): $((S_{2+30} + S_1) \times \text{time}_{S_2-S_1}) / 2 + (((S_{3+45} + S_{2+30}) \times \text{time}_{S_3-S_2}) / 2)$; and with respect to the increase (AUC_I): $AUC_G - (S_1 \times \Sigma \text{time})$; were calculated as measures of the AUC_G and CAR, respectively (see Pruessner et al., 2003).

Previous studies have reported the importance of ensuring the accuracy of CAR sampling, with it being crucial to take the first saliva sample right after awakening (Stalder et al., 2016; Clow et al., 2010a). Additionally, it has been indicated that self-reported sampling accuracy cannot be relied upon (Almela et al., 2012; Broderick et al., 2004; Kudielka et al., 2003), and that a lack of increase in cortisol levels after awakening might be due to a delay in the first saliva sample (Thorn et al., 2006), although undiagnosed pathologies unknown to the participant might also contribute to CAR disruptions (for more details see Stalder et al., 2016; Clow et al., 2010a). As the exclusion of participants with suspected inaccurate saliva sampling could result in a selection bias that would reduce the generalization of the results (Stalder et al., 2016), we considered the possibility of confirming the results for the complete sample in a subsample of participants who showed a positive CAR (>0) on both days, following the method described in Almela et al. (2012) and Thorn et al. (2006). A total of 98 participants (45 men) showed a positive CAR (AUC_I > 0) on two days (the 2-Day CAR group), 49 participants (26 men) showed a positive CAR on only one day, and 13 participants (10 men) did not show a positive CAR on any day (the

Table 1

Mean and S.D. for S1, +30, +45, AUC_I and AUC_G values for 2-Day CAR group, 0 or 1-Day CAR group, and total sample. Values reported are expressed in raw data.

	S1	+30	+45	AUC _I	AUC _G
2-Day CAR	7.46 (0.46)	15.21 (0.58)	14.87 (0.52)	224.83 (128.09)	561.89 (212.43)
0 or 1-Day CAR	11.56 (0.57)	12.18 (0.73)	10.91 (0.65)	13.406 (98.45)	543.43 (227.29)
Total sample	9.10 (5.10)	13.94 (6.01)	13.22 (5.57)	143.72 (156.17)	557.77 (217.76)

1 or 0-Day CAR group). ANOVAs were used to test age, SES, BMI, extraversion, neuroticism, and time of awakening between groups (2-Day CAR and 0 or 1-Day CAR). ANOVA for repeated measures was used to analyze the cortisol profile, with Time (0, +30 and +45) as a within-subject factor and group (2-Day CAR and 0 or 1-Day CAR) as between-subject factor. The average for each day was performed before analyzing the cortisol profiles through ANOVA for repeated measures. We used the Greenhouse-Geisser procedure because the requirement of sphericity in the ANOVA for repeated measures was violated. Post-hoc comparisons were performed using Bonferroni adjustments.

We tested the relationship between neuroticism and extraversion and awakening time through correlation analyses with (partial correlations) and without (bivariate correlations) controlling for sex and age. Linear regressions were conducted to explore whether neuroticism and extraversion traits were related to awakening cortisol concentrations (S1), CAR and AUC_G average of the two sampling days, with and without covariates. In step 1 we added sex, age and time of awakening as covariates due to their influence on the CAR (Fries et al., 2009), and in step 2 we added neuroticism or extraversion. In step 3 we added the interaction terms of neuroticism × sex or extraversion × sex in order to explore the possible moderator effect of sex in the relationship between neuroticism or extraversion and S1, CAR or AUC_G. With the aim of reducing multicollinearity, the regression analyses were performed separately for each personality dimension. Additionally, following Thorn et al. (2006) and Almela et al. (2012), the regression analyses were repeated in the subsample of participants who showed the CAR on both sampling days (2-Day CAR).

For analyses with AUC_G, two women (one in the 2-Day CAR group and one in the 0 or 1-Day CAR group) were excluded due to values >3 SDs above the mean. Regarding the CAR, one man in the 0 or 1-Day CAR group was excluded due to values >3 SDs above the mean. Using G*Power 3.1.9.2 (Faul et al., 2007, 2009), an optimal sample size of at least $n = 152$ was calculated for a power 0.80 and for small-to-medium effect size (e.g., Hill et al., 2013; Garcia-Banda et al., 2014). All p -values reported are two-tailed. SPSS 22 was used to perform the statistical analyses.

3. Results

3.1. Cortisol awakening response profiles

There were no differences in age (2-Day CAR: $M = 64.39$, $SD = 4.39$; 0 or 1-Day CAR: $M = 64.11$, $SD = 4.64$), SES (2-Day CAR: $M = 5.91$, $SD = 1.16$; 0 or 1-Day CAR: $M = 5.95$, $SD = 1.28$), BMI (2-Day CAR: $M = 27.10$, $SD = 3.99$; 0 or 1-Day CAR: $M = 27.26$, $SD = 3.42$), extraversion (2-Day CAR: $M = 6.28$, $SD = 1.65$; 0 or 1-Day CAR: $M = 6.36$, $SD = 1.82$) or neuroticism (2-Day CAR: $M = 3.84$, $SD = 1.97$; 0 or 1-Day CAR: $M = 4.17$, $SD = 1.85$) between the 2-Day CAR and 0 or 1-Day CAR groups (all $p > 0.254$). Time of awakening was similar in the 2-Day CAR group on the two sampling days (Day 1 $M = 7:13$, $SD = 0:55$; Day 2 $M = 7:16$, $SD = 0:54$) and in the 0 or 1-Day CAR (Day 1 and 2 $M = 7:19$, SD for Day 1 = $1:03$ and for SD Day 2 = $1:02$). There were no differences in time of awakening between the 2-Day CAR group and the 0 or 1-Day CAR group on any of the sampling days (both $p > 0.540$).

ANOVA showed Time ($F_{(1,4, 232.6)} = 144.691$, $p < 0.001$) and Time × Group ($F_{(1,4, 232.6)} = 133.133$, $p < 0.001$) effects. In the 2-Day CAR group, cortisol increased from awakening to 30 min later

($p < 0.001$) and maintained its levels 45 min after awakening (+30 vs. +45: $p > 0.999$). In the 0 or 1-Day CAR group, there were no significant differences between awakening cortisol (S1) and 30 min or 45 min after awakening (all $p > 0.292$), but there was a significant cortisol decrease from the +30 to +45 saliva samples ($p < 0.001$). In the 0 or 1-Day CAR group, S1 showed larger cortisol concentrations than in the 2-Day CAR group ($p < 0.001$), whereas in the +30 and +45 samples, the cortisol concentrations were lower than in the 2-Day CAR group (both $p < 0.001$) (see Table 1).

3.2. Relationships between personality traits and cortisol awakening response

Neuroticism and extraversion were not significantly related to time of awakening on any sampling day, with or without controlling for age and sex, for both the entire sample and the 2-Day CAR group alone (all $p > 0.212$).

Results of linear regression analyses with neuroticism and extraversion as predictors of S1, AUC_I (CAR) and AUC_G after controlling for age and sex, as well as the moderator effect of sex in these relationships, are reported in Table 2.¹

3.2.1. Awakening cortisol (S1)

Regression analyses without controlling for covariates showed a negative relationship between neuroticism and S1 that was statistically significant for the entire sample ($\beta = -0.160$, $p = 0.044$) and marginally significant for the 2-Day CAR group ($\beta = -0.198$, $p = 0.051$). After controlling for covariates, this negative relationship is marginally significant for both the entire sample ($p = 0.061$) and the 2-Day CAR group ($p = 0.071$) (see Table 2). Sex did not moderate these relationships (all $p > 0.313$) (see Table 2). Regression analyses did not show a significant relationship between extraversion and S1, with and without controlling for covariates, in the entire sample or in the 2-Day CAR group (all $p > 0.213$), and sex did not moderate this relationship (all $p > 0.424$) (see Table 2).

3.2.2. CAR

Regression analyses with the entire sample and with the 2-Day CAR group showed no significant relationships between neuroticism, extraversion and the CAR (total sample: all $p > 0.295$; 2-Day CAR: all $p > 0.245$). These relationships remained non-significant after controlling for age, sex and time of awakening covariates (all $p > 0.318$) (see

¹ Additional regression analyses were performed in a sample composed of people who showed a positive CAR (AUC_I > 0) on both days (average of two days) and those who showed a positive CAR only one day (only with data of the day with positive CAR) ($n = 147$). These analyses were performed in order to reduce the bias of completely removing the suspected non-adherent saliva samplings (Stalder et al., 2016). Regression analyses with 2-Day CAR and 1-Day CAR (only with AUC_I > 0 data) showed that higher extraversion was marginally related to higher cortisol concentrations at S1 ($\beta = 0.145$, $p = 0.077$), whereas higher neuroticism was marginally related to lower cortisol at S1 ($\beta = -0.158$, $p = 0.053$). Sex did not moderate these relationships (both $p > 0.142$). As in results performed with the total sample and with the 2-Day CAR group, higher neuroticism was related to lower AUC_G regardless of sex ($\beta = -0.219$, $p = 0.007$), but extraversion was not related to AUC_G ($\beta = 0.128$, $p = 0.121$). Sex did not moderate these relationships ($p = 0.435$). Regarding CAR, neuroticism was not related to CAR ($\beta = 0.036$, $p = 0.662$), whereas higher extraversion was related to reduced CAR ($\beta = -0.184$, $p = 0.024$). Sex did not moderate the relationship between extraversion and CAR ($p = 0.717$), and although the Neuroticism × sex interaction was significant ($p = 0.049$), in post hoc analyses it was not (both $p > 0.096$).

Table 2
Regression analyses with neuroticism and extraversion as predictor of AUC_C and CAR for the total sample and only for the 2-Day CAR group. $p < 0.05$ are marked in bold.

		S1		AUC _C		CAR	
		Total sample (n = 160)	2-Day CAR (n = 98)	Total sample (n = 158)	2-Day CAR (n = 97)	Total sample (n = 159)	2-Day CAR (n = 98)
Step 1	Awakening time	β 0.239	β 0.125	β 0.195	β 0.089	β -0.203	β -0.166
	Sex	p 0.002	p 0.222	p 0.015	p 0.389	p 0.010	p 0.106
	Age	-0.164	0.270	0.686	0.692	0.052	0.317
Step 2	Extraversion	$R^2 = 0.098$	$R^2 = 0.043$	$R^2 = 0.055$	$R^2 = 0.021$	$R^2 = 0.073$	$R^2 = 0.041$
		$\Delta R^2 = 0.009$	$\Delta R^2 = 0.013$	$\Delta R^2 = 0.006$	$\Delta R^2 = 0.006$	$\Delta R^2 = 0.006$	$\Delta R^2 = 0.001$
		$F_{(1,155)} = 4.210$	$F_{(1,93)} = 1.035$	$F_{(1,153)} = 2.233$	$F_{(1,92)} = 0.491$	$F_{(1,154)} = 3.033$	$F_{(1,93)} = 0.987$
Step 3	Sex × Extraversion	β 0.095	β -0.115	β 0.079	β -0.081	β -0.078	β -0.038
		p 0.215	p 0.266	p 0.318	p 0.438	p 0.318	p 0.711
		$R^2 = 0.098$	$R^2 = 0.049$	$R^2 = 0.057$	$R^2 = 0.021$	$R^2 = 0.078$	$R^2 = 0.046$
Step 2	Neuroticism	$\Delta R^2 < 0.001$	$\Delta R^2 = 0.007$	$\Delta R^2 = 0.001$	$\Delta R^2 < 0.001$	$\Delta R^2 = 0.005$	$\Delta R^2 = 0.006$
		$F_{(1,154)} = 3.361$	$F_{(1,92)} = 0.954$	$F_{(1,152)} = 1.820$	$F_{(1,91)} = 0.396$	$F_{(1,153)} = 2.755$	$F_{(1,92)} = 0.895$
		$p = 0.794$	$p = 0.424$	$p = 0.643$	$p = 0.848$	$p = 0.357$	$p = 0.462$
Step 3	Sex × Neuroticism	β 0.118	β -0.023	β 0.039	β -0.102	β -0.158	β -0.122
		p 0.308	p 0.879	p 0.735	p 0.503	p 0.177	p 0.428
		$R^2 = 0.109$	$R^2 = 0.63$	$R^2 = 0.104$	$R^2 = 0.065$	$R^2 = 0.067$	$R^2 = 0.049$
Step 2	Men	$\Delta R^2 = 0.020$	$\Delta R^2 = 0.033$	$\Delta R^2 = 0.055$	$\Delta R^2 = 0.051$	$\Delta R^2 < 0.001$	$\Delta R^2 = 0.010$
		$F_{(1,155)} = 4.764$	$F_{(1,93)} = 1.569$	$F_{(1,153)} = 4.420$	$F_{(1,92)} = 1.610$	$F_{(1,154)} = 2.767$	$F_{(1,93)} = 1.193$
		β -0.144	β -0.185	β -0.235	β -0.228	β -0.006	β 0.099
Step 3	Women	p 0.061	p 0.071	p 0.003	p 0.028	p 0.934	p 0.337
		$R^2 = 0.113$	$R^2 = 0.074$	$R^2 = 0.104$	$R^2 = 0.066$	$R^2 = 0.092$	$R^2 = 0.112$
		$\Delta R^2 = 0.004$	$\Delta R^2 = 0.010$	$\Delta R^2 < 0.001$	$\Delta R^2 = 0.001$	$\Delta R^2 = 0.025$	$\Delta R^2 = 0.063$
Step 2	Neuroticism	$F_{(1,154)} = 3.940$	$F_{(1,92)} = 1.461$	$F_{(1,152)} = 2.925$	$F_{(1,91)} = 1.287$	$F_{(1,153)} = 3.090$	$F_{(1,92)} = 2.324$
		$p = 0.410$	$p = 0.313$	$p = 0.780$	$p = 0.806$	$p = 0.043$	$p = 0.012$
		β -0.072	β -0.054	β -0.211	β -0.197	β -0.186	β -0.225
Step 3	Sex × Neuroticism	p 0.531	p 0.744	p 0.071	p 0.233	p 0.114	p 0.164
		β -0.200	β -0.269	β -0.255	β -0.069	β 0.135	β 0.308
		p 0.052	p 0.043	p 0.016	p 0.806	p 0.196	p 0.019

Table 2). Sex moderated the relationship between neuroticism and the CAR in both the total sample ($p = 0.043$) and the 2-Day CAR sample ($p = 0.012$). Regardless of the significance of the interaction, in the total sample, post hoc analyses did not show a significant relationship between neuroticism and the CAR in women ($p = 0.196$) or men ($p = 0.114$) (see Table 2). However, in the 2-Day CAR sample, neuroticism was positively related to CAR in women ($p = 0.019$), but not in men ($p = 0.164$). Regarding extraversion, sex did not moderate the relationship with CAR in the total sample or in the 2-Day CAR sample (all $p > 0.357$).

3.2.3. AUC_C

Results showed a negative relationship between neuroticism and AUC_C in the entire sample and only in the 2-Day CAR group, without controlling for covariates (all $p < 0.025$). These relationships remained significant when the analyses were performed controlling for covariates (both $p < 0.028$) (see Table 2). Sex did not moderate any relationship between neuroticism and AUC_C in the total sample or in the 2-Day CAR group (all $p > 0.780$). Finally, extraversion was not related to AUC_C in the entire sample or the 2-Day CAR group analyses, with or without controlling for covariates (all $p > 0.356$). Sex did not moderate any relationship between extraversion and AUC_C in the total sample or the 2-Day CAR group (all $p > 0.643$) (see Table 2).

4. Discussion

In the present study, we aimed to investigate the relationships between neuroticism, extraversion, the CAR and AUC_C in healthy older people. Our results showed that neuroticism was negatively related to cortisol concentrations just after awakening (S1) and to AUC_C, regardless of sex, but not to the CAR. However, after exploring the possible moderator effect of sex, our results showed a positive relationship between neuroticism and the CAR in women, but not in men. Finally, no significant relationships were found between the CAR or AUC_C and extraversion, where sex did not moderate any relationship.

Our results showed that higher neuroticism was associated with decreased AUC_C regardless of sex/gender, but not to the CAR. Agreeing with Pineles et al. (2013), neuroticism was also related to reduced cortisol concentrations immediately after awakening (S1). Thus, the relationship between neuroticism and decreased AUC_C is supported by the negative correlation between neuroticism and cortisol concentrations just after awakening, with these lower cortisol concentrations in the first sample driving the association between neuroticism and AUC_C. Thus, beginning with lower concentrations and maintaining a standard increase would result in a reduced AUC_C. In this line, Hauner et al. (2008) found decreased AUC_C in male adolescents with high neuroticism. Thus, the current results extend these findings to older people and are consistent, regardless of whether participants showing a negative CAR on one or both days were included in the analyses. In contrast to our study, a positive relationship was reported between neuroticism and AUC_C in people from 21 to 57 years old (Portella et al., 2005) and in adults from 18 to 38 years old, mediated by acculturation (Mangold et al., 2012). Together, these results may indicate that the association between neuroticism and AUC_C is negative in adolescents and older people, but positive in young-middle adults. This could be due to important changes in the prefrontal cortex in more extreme periods of the life-cycle, but not at young and middle ages. In adolescence, the prefrontal cortex is still developing, while at older ages the prefrontal cortex is declining (for reviews, see Raz and Rodrigue, 2006; Sisk and Zehr, 2005). Specifically, we consider that age-related changes in the brain may explain the negative relationship between AUC_C and neuroticism observed in older people. Recent research has shown that a higher age-related decline in prefrontal regions is associated with greater neuroticism in middle-aged and older people (Jackson et al., 2011). Thus, because the prefrontal cortex is a key structure in HPA and CAR regulations (Fries et al., 2009), a greater age-related prefrontal cortex decline in older people with higher neuroticism could be related to a different regulation of cortisol secretion after awakening, that is, an overall lower cortisol secretion.

Coinciding with previous studies (Garcia-Banda et al., 2014; Hill et al., 2013; Laceulle et al., 2015), we did not observe a significant relationship between neuroticism and the CAR for men and women together. However, after considering sex as a possible moderator in this relationship, our results showed that neuroticism was positively related to the CAR in women, but not in men. In additional results performed with those who showed a positive CAR on two days and on only one day, sex also moderated the neuroticism and CAR relationship, but this relationship was only close to significance in women. Previous studies linked an enhanced CAR to increased physical and mental health problems, such as the rate of healing (Ebrecht et al., 2004), some types of depression (Dedovic and Ngiam, 2015), the progression of subclinical atherosclerosis (Eller et al., 2005) or coronary artery disease (Bhattacharyya et al., 2008). Taking all of the above into account, sex may moderate the damaging relationship between neuroticism and HPA functioning, with this relationship accentuated in women. Agreeing with DeSoto and Salinas (2015), our results confirmed a different sex pattern in the relationship between neuroticism and HPA functioning. It has been proposed that differences in HPA functioning between men and women could occur because of the sex differences in the negative feedback loop, which is stronger in women, and due to the possible interference of testosterone and estrogen in HPA functioning (DeSoto and Salinas, 2015). Moreover, sex-related differences in the relationship between neuroticism and steroid hormones may also contribute to these differences. Along these lines, higher neuroticism has been related to lower testosterone in men (Obmiński et al., 2016), but higher concentrations in women (Barry et al., 2011). However, this relationship has not always been observed (Ekholm et al., 2014; Sellers et al., 2007; Conrad et al., 2002). Further research is clearly needed to better understand the neuroendocrinological mechanisms that underlie sex differences in the way neuroticism is related to HPA axis functioning.

In line with previous literature (Garcia-Banda et al., 2014; Munafo et al., 2006; Laceulle et al., 2015), in our study extraversion was not associated with the CAR or AUC_C for the complete sample or for the 2-Day CAR group. However, these results contrast with other data (Hill et al., 2013; Hauner et al., 2008). Two possible explanations may account for discrepancies in the literature. First, none of the previous studies focused specifically on older people (Hill et al., 2013; Hauner et al., 2008; Chan et al., 2007; Munafo et al., 2006; Laceulle et al., 2015; Portella et al., 2005); and age-related changes in the brain, HPA axis regulation (Clow et al., 2010b; Fries et al., 2009) and personality (Eysenck, 1988) could affect these associations. Another possible explanation is related to methodological differences. These studies measured cortisol levels only one day, an issue that could affect CAR measurements (Hellhammer et al., 2007). Along this line, a negative relationship between CAR and extraversion is observed in our sample when the analyses are performed including the CAR data only for days with positive CARs (2-Day CAR and 1-Day CAR¹), in which, for part of the sample, only the data of one day of CAR measurement is included. However, this relationship did not remain significant for the complete sample or for the 2-Day CAR group. Thus the relationship observed between extraversion and CAR in our study and previous studies may be due to state confounders in CAR measurements (Hellhammer et al., 2007; Stalder et al., 2016). Moreover, our results for the complete sample and, especially, for the 2-Day CAR group, agree with Garcia-Banda et al. (2014), who employed good strategies for checking participants' adherence by using an objective measurement of the saliva sampling. Although we did not use this procedure in our study, we made an effort to control for possible non-adherence to the salivary sampling protocol and increase the reliability of the CAR measurement. To do so, we employed strategies to increase adherence to the protocol (e.g. flexibility in choosing the days of saliva collection). Based on Almela et al. (2012) and Thorn et al. (2006), we collected saliva samples on two consecutive days (Clow et al., 2010a; Hellhammer et al., 2007), and we controlled for participants' possible non-adherence to the salivary sampling

protocol by analyzing the cortisol secretion profile on both days (Almela et al., 2012; Thorn et al., 2006). Most of these strategies were not used in previous studies (Hill et al., 2013; van Santen et al., 2011; Hauner et al., 2008; Chan et al., 2007; Munafo et al., 2006; Portella et al., 2005). Thus, non-adherence to the protocol may have resulted in a non-reliable CAR measurement that affected the results of these studies (Stalder et al., 2016; Clow et al., 2010a, 2010b).

A limitation of the current study is that we did not use an objective registering of the awakening time. Future studies using electronic devices to objectively register the awakening time can improve the reliability of awakening cortisol measurements and make it possible to delve into their relationship with personality traits, as suggested recently (Stalder et al., 2016). Instead, we employed different strategies that have been recommended in the recently published consensus guidelines to improve CAR measurement reliability. First, we compared the results for the total sample to the results for participants suspected of adhering to the saliva sampling (2-Day CAR), as other studies have done (Almela et al., 2012; Thorn et al., 2006). Although it is true that this exclusion strategy only discards extreme cases of non-compliance (Stalder et al., 2016), it could help reduce the bias. Additionally, to increase adherence to the protocol, we considered a large number of issues recommended in the recent guidelines for CAR assessment (Stalder et al., 2016), i.e. we used a self-report diary on saliva sampling, we tried to engage the volunteers with the goals of the research study, we emphasized the importance of the S1 sampling, we gave detailed oral and written information about saliva sampling, we clearly explained what “the moment of awakening” means, and we provided full oral and written instructions about the sampling days and times and the undesired morning behaviors, among others. The CAR measure is still being clarified, and the physiological function of the CAR remains unclear (Clow et al., 2010b). Both reduced and heightened AUC_C and CAR have been related to health problems (Clow et al., 2010a; Fries et al., 2009), and so we still do not know what a heightened or reduced morning cortisol release really means. However, the development of studies that increase the knowledge about factors affecting the AUC_C and CAR could advance their understanding. In addition, this study is limited by its cross-sectional design; thus, causality cannot be inferred.

In conclusion, our results contribute to providing a clearer picture of the relationship between personality traits and the CAR and AUC_C in older people. Moreover, they support the moderator role of sex in the relationship between CAR and neuroticism, as recently reported (DeSoto and Salinas, 2015), and extend it to the older population. We found that, in this age group, neuroticism, but not extraversion, is related to less AUC_C secretion after awakening, regardless of sex, and to a heightened CAR only in women. These results contribute to furthering the knowledge about how neuroticism is related to HPA-axis functioning, which helps better understand a potential underlying biological mechanism relating neuroticism to health problems in the long term and the short term (Lahey, 2009).

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