



Neuroticism and cortisol: Pinning down an expected effect



Gloria Garcia-Banda ^{a,*}, Karin Chellew ^b, Joana Fornes ^c, Gerardo Perez ^d, Mateu Servera ^e, Phil Evans ^f

^a Research Institute on Health Sciences (IUNICS), University of Balearic Islands, Ctra. de Valldemossa, km 7.5, 07122 Palma de Majorca, Spain

^b Department of Psychology, University of Balearic Islands, Ctra. de Valldemossa, km 7.5, 07122 Palma de Majorca, Spain

^c Department of Nursing and Physiotherapy, University of Balearic Islands, Ctra. de Valldemossa, km 7.5, 07122 Palma de Majorca, Spain

^d Clinical Analysis Laboratory, Son Espases University Hospital, Ctra. de Valldemossa, 79, 07120 Palma de Majorca, Spain

^e Research Institute of Health Sciences (IUNICS), University of Balearic Islands, Ctra. de Valldemossa, km 7.5, 07122 Palma de Majorca, Spain

^f Psychophysiology and Stress Research Group, University of Westminster, 309 Regent Street, London W1B 2UW, UK

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ABSTRACT

Background: There are strong theoretical arguments that those high on Neuroticism (N) should normally exhibit higher prevailing levels of the stress-linked hormone cortisol (C), but findings are inconsistent, probably reflecting methodological weaknesses especially in taking account of C's diurnal cycle.

Methods: High and low N students [Total N = 118; mean age = 20.99 years] were recruited and their salivary cortisol measured, ensuring that saliva samples were numerically adequate to assess C's diurnal cycle over two days with objective verification of sample timing.

Results: Cortisol secretion was approximately 20% higher in High N than low N participants in the period of 12 h after awakening ($p < .008$), but no differences in secretion were evident during the first 0.75 of this period, when typically the Cortisol Awakening Response (CAR) rapidly takes cortisol to its daily peak. N effects were thus confined to the 0.75 h–12 h period ($p < .007$). Males had approximately 25% higher cortisol secretion levels than females, also confined to the 0.75 h–12 h period ($p < .003$). No significant differences between N groups were evident for dynamic measures of cortisol change, viz. the magnitude of CAR rise and subsequent diurnal fall. All effects were controlled for cohort date of study entry, age, smoking status, study day and time of awakening.

Discussion: With careful control, it appears that an important theoretically predicted effect exists, and is replicated in different student intake cohorts recruited in different years. Most importantly, findings support several lines of evidence that the period of massive rise in the brief 0–0.75 h CAR period should be seen as quite separate from the rest of the diurnal cycle, underpinned by different control mechanisms, and with potentially different correlates.

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1. Introduction

According to the “wear and tear” model of McEwen and Wingfield (2010), some individuals have to work harder (i.e., expend more energy) to maintain homeostasis. The accumulation of this cost (allostatic load) implies a gradual decrease in the ability to respond to daily stressors. Hypothalamic–pituitary–adrenal (HPA) axis dysregulation (blunted cortisol responses to stress and high background levels of cortisol during the day) can be a consequence of such accumulated load. Cortisol, the HPA end product, is a glucocorticoid that our body naturally secretes according to a pronounced diurnal cycle with increased values evident under particularly stressful conditions. The typical diurnal profile shows a sharp rise upon awakening, which peaks 20–45 min later, and is called the ‘Cortisol Awakening Response’ (CAR). Thereafter there is a steady decline over the rest of the day with lower levels in the evening and night. A derivative measure, called area under the curve (AUC), is

often used to estimate total secretion during a predefined time period (Hansen et al., 2008).

Neuroticism (N), a relatively stable trait, is the predisposition to respond with intense emotional reactions to psychosocial stressors (Lahey, 2009). Individuals high on Neuroticism perceive and have more stressors, respond exaggeratedly to them, and require more time to recover from them (Suls and Martin, 2005). It has generally been assumed therefore that individuals high in N will tend to have an increased magnitude of cortisol secretion during the day, reflecting greater frequency and intensity of HPA stimulation from the psychosocial domain.

A vast swathe of published theoretical statements on both the nature of Neuroticism and cortisol as a stress-responsive hormone appears to underpin such an assumption. It would be very puzzling therefore if the assumption were not at least broadly true, with the usual caveats of other things being equal etc. However, empirical studies, which directly or indirectly inform such a simple but highly plausible theoretical prediction, have been surprisingly inconsistent. Some findings (e.g. Nater et al., 2010) have been positive: higher mean daily AUC levels of cortisol were related to higher cortisol in the adult working population. Individuals with extremely high N scores were found to have greater waking cortisol levels compared to those with extremely

* Corresponding author at: Research Institute of Health Sciences (IUNICS), University of Balearic Islands (UIB), Cra. de Valldemossa, km 7.5, 07122 Palma de Majorca, Spain. Tel.: +34 971 172760; fax: +34 971 173190.

E-mail address: ggbanda@uib.es (G. Garcia-Banda).

low N scores (Portella et al., 2005). High N has been associated with significantly less flexibility of cortisol responding (though not over all cortisol level) when weekend and weekday profiles are compared (Mikolajczak et al., 2010). Flatter diurnal cortisol slope has been linked to higher N but only in male participants (Hauner et al., 2008). However in a majority of studies the predicted link between high N and high basal cortisol has eluded statistical significance (Chan et al., 2007; Ferguson, 2008; Gerritsen et al., 2009; Hill et al., 2013; Riese et al., 2009; Van Santen et al., 2011). Finally, Neuroticism was not associated with differences in CAR magnitude in the meta-analyses done by Chida and Steptoe (2009).

Inconsistency of effects does seem to be a prominent feature of the cortisol psychophysiology literature in general and might be explained by a host of methodological considerations. There are the usual problems in looking for consistency across studies. These can arise from diversity of sample populations, e.g. in regard to major demographics such as age and sex. They may be a product of differences in scales used to measure N as well as choice of continuous scale measurement or comparison of extreme groups. They arise from differing statistical power reflecting diversity in sample sizes, since associations of any kind with one of the broadest of all trait personality measures are likely to involve small effect sizes. However what is often less explicit but potentially crucial for cortisol measurement are factors relating to adequate timing and frequency of salivary cortisol sampling across the day, synchronization (or not) of cortisol sampling times to awakening times, and number of sampling days over which daily levels are averaged to arrive at supposedly valid 'typical' values over time, and last (but by no means least) adequacy of procedures to ensure participants' accurate adherence to protocol required sampling times, if saliva collection is carried out by participants.

The present study was part of a larger research project with the overall aim of examining individual differences and various interventions on diurnal cortisol profiles of university students. In the present study, we sought sufficient methodological rigor to yield robust results for this population concerning theoretically expected associations between N and diurnal cortisol secretion. To do this, we sought to demonstrate replicability of effects over time, and given its pronounced diurnal cycle, we utilized objective checks on the timings of all cortisol samples. Therefore, over several year cohorts of students, we recruited samples with extreme high versus low scores for N (using the NEO-FFI measure), sampled from the diurnal cortisol cycle over two days rather than a single day, and verified timings of samples using MEMS (Track Caps) electronic monitoring device.

We had one simple but clear theoretically derived formal hypothesis, namely that high N participants would *ceteris paribus* display elevated diurnal background levels of cortisol compared to low N participants. We also proposed to examine associations between N and the two pronounced dynamic aspects of the typical diurnal cortisol profile, namely the CAR and the subsequent diurnal fall over the course of the day. In respect of diurnal fall, we had an expectation, that high N might be associated with a less steep (i.e. 'flatter') fall, based on the latter's reported links to a number of stress-associated variables. In respect of the CAR, we did not consider the existing literature to permit of any *a priori* expectation in relation to existence or direction of any effect.

2. Method

2.1. Participants

NEO-FFI personality questionnaire data from first year students (mean age = 20.87; 62% female) from University of Balearic Islands were routinely collected each year for four academic years. From those 2202 (58%) who had expressed a general interest in possibly collaborating in further studies, we selected extreme high and extreme low N participants based on NEO-FFI 15th and 85th female and male percentiles (Costa and McCrae, 1999). As a result, 321 high and 263 low

N female students (N score = 33 and 17) and 164 high and 135 low N male students (N score = 28 and 12) were invited to participate in a demanding two-day protocol involving careful assessment of the diurnal cortisol cycle. Of these, 185 initially agreed to participate in the study, but 67 could not do so for several reasons (time constraint, illness, etc.). This left 118 students who completed the questionnaire batch and the salivary sampling protocol, of whom 5 had missing data for time of awakening. The final sample thus comprised 113 participants.

2.2. Procedure

First year UIB students completed the NEO-FFI personality questionnaire assessing Neuroticism and gave informed consent in their classes. Students who wanted to go on participating in the cortisol sampling provided their e-mail address and mobile phone number. High N and low N volunteers were invited to an initial information session. After explaining the research aims and tasks, students were requested to complete EPQ-R personality questionnaire as a consistency check on the accuracy of the NEO-FFI-N-subscale used to select extreme study groups. At the briefing, students were also instructed on saliva sample collection and asked not to brush the teeth, smoke, eat or consume alcohol or caffeine drinks during the 20 min before each saliva collection.

In line with the MacArthur Network protocol on salivary cortisol measurement (Stewart and Seeman, 2000), five samples were collected synchronized to awakening: at awakening, and at 0.75 h, 2.5 h, 8 h and 12 h after waking, on two days of a specific week (Tuesday and Thursday). Following Adam and Kumari (2009) recommendations to control for covariates, every time students took a saliva sample, they filled the information protocol registering the exact time of each sample including wake-time ("as soon as you open your eyes and before getting up"), eating times, caffeine intake, medication taken, or if they had siesta, did sport, etc. This record provided us with a range of potentially relevant information to check if necessary for confounding effects and was used as well to help assess cortisol sampling time compliance. Participants were not given reimbursement for their participation, although at the end of the study they receive detailed information about their personality and cortisol profiles.

2.3. Personality measures

Trait Neuroticism was measured with the 12-item Neuroticism subscale of the NEO-Five Factor Inventory (NEO-FFI), a 60 item questionnaire measuring five personality domains. Neuroticism subscale scores range from 12 to 60. Participants responded on a 5-point Likert scale from 1 (strongly disagree) to 5 (strongly agree). Internal consistency values for our sample were .83 and for the original values range from .74 to .89 (Costa and McCrae, 1992, 1995).

Participants also completed the 24-item Neuroticism Scale of the Eysenck Personality Questionnaire-Revised (EPQ-R; Eysenck et al., 1985). Participants were required to indicate whether a statement related to them, using a dichotomous yes/no response. Original internal reliability coefficient for the Neuroticism scale was .85 for females and .88 for males. Higher internal Neuroticism consistency values were founded for females (.936) and males (.943) in our subsample ($N = 141$). Cronbach's α reliability (.94) for the whole Neuroticism subscale was considered acceptable according to Nunnally (1978).

2.4. Cortisol assay procedures

Student saliva samples were collected with a cotton swab chewed for 1 min, stored in a capped plastic vial ("Salivette" Sarstedt Inc.), centrifuged at 3000 g for 3 min, and then the filtrates frozen at -80°C until analysis. Samples were thawed, mixed, centrifuged and analyzed without pre-treatment. Salivary cortisol was measured using a modification of the Bayer ADVIA Centaur cortisol assay, a competitive direct chemiluminescence immunoassay that uses a rabbit polyclonal antibody.

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